University of Miskolc

Theoretical Investigations of Glutathione – A Unique Antioxidant

Dissertation Presented

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Theoretical Investigations of Glutathione – A Unique Antioxidant

Abstract

Glutathione (γ-L-glutamyl-L-cysteinyl-glycine, GSH) is a linear tripeptide which has a crucial role in free radical regulation (Figure 1).

Figure 1: Chemical Structure of Glutathione (GSH). Pharmacophore Groups are Indicated as Follows: Red - CO$_2^{-}$, Blue - NH$_3^{+}$, Gold - SH, Grey - Peptide Bond.

It is essential in a number of biochemical processes in living organisms, including repair of oxidative damage and defense of the central nervous system against free radicals. It has also a role in apoptosis, signal transduction and gene expression.

The research presented here is focused on different aspects of glutathione which were studied by means of computational chemical tools. The results along with the
corresponding methodologies presented in three separate chapters (Chapter 2, 3 and 4).

In Chapter 2, the radical forming ability of GSH in a thermodynamic sense is determined by means of quantum chemical calculations (Figure 2).

![Figure 2](image_url)

**Figure 2:** Competing Radical Forming Sites in Glutathione (GSH).

Furthermore, the radical scavenging ability of the neutral and anionic GSH is compared.
Chapter 3 is a combined molecular dynamics and quantum chemical study to explore the radical scavenging mechanism of glutathione. To overcome the limitation of the large flexibility of GSH, structures for further ab initio calculations could be determined by non-reactive molecular dynamics (MD) simulations. Therefore, we set a long lasting, comparative MD simulation for the solvated GSH and GSH/HO\(^*\) as model systems. Based on the MD trajectories the different interactions between GSH and HO\(^*\) were characterized (Figure 3).

**Figure 3:** Radical Scavenging Mechanism of Glutathione: Catching and Steering.

Moreover, the non-reactive MD trajectories combined with ab initio calculations allow us to describe a detailed free radical recognition and radical scavenging process (Figure 3).
Chapter 4 describes the evolutionary aspects of the unique structure of GSH (Figure 4). By the study of this simple tripeptide, it can provide us hints about why the peptide bonds in natural peptides and proteins involve exclusively the $\alpha$-carboxyl group of the amino acids. Furthermore, the energetics of the formation of GSH and its $\alpha$ analogue, L-glutamyl-L-cysteinyl-glycine (ECG) can throw some light on the thermodynamics of the regular ($\alpha$-) and isopeptide bond formation.

**Figure 4:** Competition Between $\alpha$- and $\gamma$-peptide Bond Formations in the Case of Glutathione.

Using quantum chemical methods, we intend to contribute to the elucidation of the factors determining the strength of peptide bonds that may explain the preference of the $\alpha$-peptide bond in proteins.
Szeretteimnek.
"Ford!" he said, "there's an infinite number of monkeys outside who want to talk to us about this script for Hamlet they've worked out."

— Douglas Adams,  
The Hitchhiker’s Guide to the Galaxy

1 Introduction

1.1 Radical History - History of Radicals

The science is full of surprises. Ever since the “radical” concept in chemistry was introduced by Lavoisier in 1789 [1] it was a story of successive rises and decline. In the subsequent century, numerous discoveries have been reported, which convinced the scientists that radicals exist, or are preposterous. Thus, it is not surprising that after a contradictory century, Moses Gomberg’s announcement about the synthesis of the first-ever organic radical, triphenylmethyl radical (Figure 5) in 1900 [2] should have been greeted with disbelief and disinterest.

In that time, at the beginning of the 20th century nobody would have thought what a great significance of the radicals have. The definition of the “radicals” is also
Introduction

Figure 5: Structure of the First Synthetic Organic Radical Triphenylmethyl.

changed over the centuries, while it is reached its present form: “Free radicals can be defined as molecules or molecular fragments containing one or more unpaired electrons in atomic or molecular orbitals” \[3\]. This unpaired electron(s) usually gives a considerable degree of reactivity to the free radicals. The last hundred years was the breakthrough in the radical chemistry and many new things have come to light which indicates that the radicals play an important role in a number of biological processes (defense against infectious agents, ageing, oxidation processes, signaling etc.) \[4–6\]. Besides their role in biology, the radicals are important in combustion, polymerization and atmospheric chemistry as well \[7–9\]. From a biochemical point of view the radicals have a kind of duality: in addition to their positive attributes, they can be harmful. The free radical-induced oxidative or nitrosative stress is a significant factor in various diseases such as Alzheimer’s disease, Parkinson’s disease and carcinogenesis as well \[10–12\]. Most of the free radicals found in living organisms can be divided into two major groups: reactive oxygen species (ROS, e.g. hydroxyl radical, \(\text{HO}^*\)) and reactive nitrogen species (RNS, e.g. nitric oxide, \(\text{NO}^*\)). The ROS, as well as RNS, are products of normal cellular metabolism; therefore, free radicals are integral parts of life. For this reason, in living organisms there is a so-called redox homeostasis, a number of mechanisms to regulate the amount of radicals \[13, 14\] to avoid adverse procedures. The negative effects (oxidative or nitrosative stress) of free radicals appear due to the imbalance in the redox homeostasis: when the ROS/RNS are overproduced on one side and a deficiency of enzymatic and non-enzymatic free radical regulators occurs on the
Introduction

other [15]. ROS represent the most important class of radical species generated in living organisms [16]. Since, molecular oxygen (dioxygen) has a unique electronic configuration and it is also a radical, the aerobic life is inseparable from radicals. For this reason, although free radicals are present in Science only 225 years ago from a historical point of view, but their presence in the living organisms is as old as the evolution of aerobic life. To maintain the balance in all respects is indispensable for life. Therefore, the redox homeostasis is as old as the aerobic life and the free radical regulators are appeared in the early stage of aerobic life.
Introduction

1.2 Radicals vs Glutathione

A crucial biomolecule in free radical regulation is the linear tripeptide glutathione (γ-L-glutamyl-L-cysteinyl-glycine), denoted as GSH (Figure 6).

![Figure 6: Glutathione (GSH) and its Building Blocks (Glutamic Acid, Cysteine and Glycine).](image)

It is essential in a number of biochemical processes in living organisms, including repair of oxidative damage \[17\] and defense of the central nervous system against free radicals \[18, 19\]. It has also a role in apoptosis, signal transduction, and gene expression \[20, 21\]. Fluctuating or decreased glutathione concentration leads to neurological diseases, such as Parkinson’s or Alzheimer’s disease \[22, 23\]. The glutathione/glutathione disulfide (GSH/GSSG) system is one of the most important intracellular oxidation-reduction buffers, providing protection against carcinogenic diseases, radical agents of oxidative stress, and lipid peroxidation \[24–27\]. Glutathione detoxifies a variety of compounds by forming glutathione conjugates \[28, 29\]. It also regulates the oxidation state of the SH group in proteins.
Introduction

and other biological systems containing thiol groups via homo- and heterodisulfide formation equilibria [30]. GSH can be found in high concentration in the cytosol (1-11 mM), nuclei (3-15 mM), and mitochondria (5-10 mM) of cells [31]; furthermore, GSH is the major soluble antioxidant in these cell compartments.
Introduction

1.3 Perspectives & Aims

The research presented here is focused on the different properties of glutathione which were studied by means of computational chemical tools (Figure 7).

First, the radical forming ability of glutathione in a thermodynamic sense is determined by means of quantum chemical calculations (Figure 7, lightblue).

![Diagram of GSH]

**Figure 7:** The Studied Properties of Glutathione.

Thereafter, the radical scavenging mechanism of glutathione was studied by combining molecular dynamics with quantum chemical calculations in order to shed light on the detoxification process (Figure 7, green). To overcome the limitation of the large flexibility of GSH, structures for further *ab initio* calculations were determined by non-reactive molecular dynamics (MD) simulations.

The evolutionary aspects of the unique structure of glutathione was also studied using quantum chemical methods. By this, we intend to contribute to the elucidation of the factors determining the strength of peptide bonds that may explain the preference of the α-peptide bond in proteins (Figure 7, gray).
2

Radical Scavenging Ability of Glutathione

2.1 Introduction

Glutathione exhibits antioxidant, radical scavenging activity by its electron donating ability and by the possibility to form stable, unharmful glutathione radicals \[32, 33\]. Therefore, it is important to know which parts of the molecule are more susceptible to form radicals.

The pH dependence of microscopic protonation states of GSH was studied both experimentally \[34\] and computationally \[35\], but the radical-forming ability of

these solvated states of GSH has not yet been investigated. Experimental conformation analyses have been carried out over the whole pH range \([36, 37]\). Interestingly, the stability of radicals formed from neutral GSH in the gas phase has been examined extensively \([32, 38]\), although glutathione anion (GSH\(^{-}\)) is found to be dominant at physiological pH. In the case of GSH\(^{-}\), L-glutamic acid predominantly exists in its zwitterionic form, while the carboxyl group of the glycine fragment prefers to be deprotonated \([39]\). Herein, we determine the radical-forming ability of the biologically important GSH\(^{-}\) in a thermodynamic sense. Furthermore, the radical-scavenging ability of GSH and GSH\(^{-}\) will also be compared.
2.2 Methodology

In previous attempts, the conformational space of GSH was explored by molecular dynamics simulations, and it was found that GSH is very flexible and does not adopt a strongly preferred conformation at any pH \([35]\). Therefore, we assumed that the intramolecular interactions are not strong enough to trap the structure in a stable conformation. Thus, the extended structures of GSH are considered in this work which contains the least intramolecular interactions. Since accurate and robust quantum chemical calculations are still overly demanding for tripeptides like GSH, a simplification of the whole system (analogous to previous attempts \([40–42]\)) seems to be necessary. Therefore, the GSH molecule was split into three (overlapping) fragments: \((2\,S)-2\text{-amino-4-carbamoylbutanoic acid (L-glutamine, Q)}, (R)-2\text{-formamido-3-mercapto} \text{propanamide (N-formyl-L-1-cysteine-amide, C)},\) and \(2\text{-formamidoacetic acid (N-formyl-glycine, G)}\). Similar to neutral glutathione, water-soluble ionic GSH\((-)\) can also be partitioned into three pieces: zwitterionic Q\((\pm)\), C, and anionic G\((-)\) (Figure 8).

Due to the overlapping fragmentation, L-glutamine (Q) replaces L-glutamic acid (E) in the nomenclature; however, glutathione can be synthesized from the amino acids L-cysteine (C), L-glutamic acid (E), and glycine (G). Being a radical scavenger, tripeptide glutathione can function as a hydrogen atom donor. In order to characterize the site preference of radical formation, the \(X–H\) (where \(X\) can be \(\text{C, N, O, or S}\)) homolytic bond dissociation energies (BDEs at 0 K) of GSH and the fragments mentioned above were calculated:

\[
R - X - H \rightarrow R - X^* + H^* \tag{2.1}
\]

where \(R\) can be Q, C, or G. The homolytic bond dissociation energy (BDE) can indicate the stability of the radicals which are formed from homolytic bond dissociation: it is the energy required to separate the ground state molecule into ground state fragments \([43]\). In our case, these fragments are a free radical and a hydrogen
Figure 8: Nomenclature Used with Glutathione (GSH) and its Fragments. Q, C, and G are the Overlapping Fragments of GSH in the Gas Phase. In the Aqueous Phase Q is Zwitterionic (Protonation on the Amine and Deprotonation on the Carboxyl group, Q(±)) and G is Anionic (Deprotonation on the Carboxyl Group, G(-)). The Protonation and Deprotonation are Indicated with Blue "+" and "-", Respectively.
Radical Scavenging Ability of Glutathione

atom. Products of the homolytic X–H bond dissociation reactions are denoted systematically. For instance, \( Q_N \) corresponds to the hydrogen at position 1 abstracted from a nitrogen in fragment Q or the equivalent part in the whole GSH (see Figure 8). There are special cases when superscripts are used to separate the different species. For instance, \( Q_{N_{1,2}}^{a,b} \) corresponds to the same N–H bond cleavage (and its neighbor) as was detailed above, with the "b" and "a" indicating that after the cleavage of the N–H bond consecutive decarboxylation does or does not occur, respectively. Indices "a" and "b" also refer to the closer and further hydrogens of the protonated amine group from the carboxyl group in fragment Q. BDE values as measures of bond strength are comparable in a range of various chemical species. If BDE values obtained in the same molecule are compared, it is worth using relative BDEs (\( \Delta \text{BDEs} \)), which are analogous to the radical stabilization energy (RSE) \([44]\). If the reference reaction is the homolytic dissociation of the S–H bond of GSH (\( C_S \)), the hydrogen atom abstraction potentials of the radicals formed can be obtained by calculating the energetic change in the following reaction:

\[
R - X^* + C - S - H \rightarrow C_S^* + R - X - H \quad (2.2)
\]

where \( C_S^* \) is the S-centered radical in the fragment C. The energetic properties of the X–H bond dissociation determine the antioxidant effect of glutathione. In current literature, the S–H bond is identified as one of the main factors responsible for that effect. Therefore, the BDE of the S–H bond was used as the reference for the calculation of \( \Delta \text{BDEs} \). A positive \( \Delta \text{BDE} \) indicates that the bond is weaker than the S–H of GSH (\( C_S \)). The G3MP2B3 method can provide accurate thermochemical properties such as heat of formation or BDE \([45]\) for radical systems. This method \([46]\) has been chosen as a reference for calculating the BDEs and relative stabilities of the radicals formed from the previously mentioned fragments in gas and aqueous phases (Figure 8). As the first step in the G3MP2B3 procedure, all of these geometry optimizations were carried out using the B3LYP functional \([47]\) as implemented in the Gaussian packages \([48, 49]\) with the 6-31G(d) basis.
Radical Scavenging Ability of Glutathione

Solvent effects were taken into account in optimizations at the same level of theory by applying the conductor-like polarizable continuum model (CPCM) \([51, 52]\) (SCRF = CPCM, Solvent = Water). The solute cavity is built up using radii from the UFF force field, and the standard value of the dielectric constant \((\varepsilon)\) is employed for water \((\varepsilon = 78.3553)\). The electrostatic scaling factor (Alpha) for the sphere radius of the atoms was the default value \((\text{Alpha} = 1.1)\). Normal mode analysis was carried out on each vacuum and solvent optimized structure at the B3LYP/6-31G(d) level of theory, and calculated harmonic frequencies were scaled by a factor of 0.97 \([53]\) to obtain thermochemical properties. To increase the accuracy of results, QCISD(FC,T)/6-31G(d) and MP2(FC)/G3MP2Large single point calculations were also performed as part of the G3MP2B3 procedure \([45]\). Since the computationally demanding part of the G3MP2B3 method is the calculation of accurate G3MP2 energy, the method is limited to medium-sized systems, preventing the accurate treatment of a tripeptide (such as GSH). One can overcome this issue with the replacement of the G3MP2B3 calculation with an appropriately accurate functional. In order to find such a functional, results of the six metafunctionals MPWKCIS \([54]\), MPWKCIS1K \([54]\), M06 \([55]\), TPSS1KCIS \([56–58]\), TPSSH \([59]\), and B3LYP, were tested against the G3MP2B3 values. These single point calculations were carried out using the 6-311++G(3df,2p) basis set \([60]\) on structures obtained at the B3LYP/6-31G(d) level of theory, in both vacuum and solvent. Most of these state-of-the-art hybrid functionals are not directly available in the Gaussian packages; these calculations were carried out using the IOp keyword \([54]\). The structural comparisons and the calculation of the root-mean-square-deviation (RMSD) values were carried out using the Molecular Operating Environment (MOE 2010.10) \([61]\).
2.3 Results and Discussion

2.3.1 Geometric Considerations

Fragments vs Fragments

Solvent effects can significantly affect BDE values due to structural changes. In order to compare the optimized geometries of the glutathione fragments in gas and aqueous phases (Q and Q(±)aq, C and Caq, G and G(−)aq), the root-mean-square-deviation (RMSD) values of the non-hydrogen atoms have been calculated, respectively. These values are found to be small: the largest RMSD (RMSD$_{\text{max}}$), is less than 0.15 Å and the average (RMSD$_{\text{ave}}$) is 0.09 Å, indicating that the solvent model does not affect the geometries significantly. The main difference in the above-mentioned structures is due to their protonation states. In the comparative investigation of the gas and aqueous phase geometries of radicals formed in the hydrogen cleavage of the fragments, the RMSD$_{\text{max}}$ value is 1.12 Å and the RMSD$_{\text{ave}}$ is less than 0.30 Å. It is evident that the immersion of the GSH fragment radicals into the implicit water model has significant structural effects in some cases.

Fragments vs Glutathione

To measure the effect of partitioning glutathione into fragments, structural alignments of the heavy atoms in the fragments to those in GSH were carried out. Such alignments in water showed negligible deviation, as indicated by the corresponding RMSD$_{\text{max}}$ and RMSD$_{\text{ave}}$ values of 0.07 and 0.05 Å, respectively. For the radicals, alignment shows somewhat enhanced alteration in the water phase and the RMSD$_{\text{ave}}$ value is 0.16 Å. The alignment of gas phase geometries, GSH radicals vs fragment radicals, resulted in a 3 times larger RMSD$_{\text{ave}}$, 0.49 Å. In GSH, there are intramolecular interactions (hydrogen bonds) which have a significant effect on the structure of the glutathione radicals, but these interactions are absent from the fragments. For instance, the hydrogen bonds between the amide nitrogen and the carboxyl oxygen of the two peptide bonds in GSH (typically within the C fragment part) are not present in single fragments. Nevertheless, under biologically relevant
Radical Scavenging Ability of Glutathione

conditions, treating glutathione as separated fragments has little influence on geometry.

**Gas vs Aqueous Phase**

In the gas phase, the largest structural change is due to homolytic bond dissociation which found in fragment Q for the $Q_0^b$ radical. The cleavage of the H atom from the carboxyl group ($Q_0$) can cause a consecutive C–C bond break, resulting in CO$_2$ dissociation, decarboxylation ($Q_0^b$), while the oxygen-centered radical species is transformed into a carbon-centered one. This complex phenomenon (H cleavage from carboxyl initiates decarboxylation) has not been observed in the aqueous phase since the carboxyl group is deprotonated in this medium, so there is no O–H bond to be broken. However, the hydrogen from the positively charged amino group can be abstracted in water. This may break the adjacent C–C bond, as indicated by the roughly 2 times greater bond length than that in GSH (Figure 9).
Figure 9: Optimized Structures of Glutathione and Its Fragment, with the Optimized Structure of $Q_{N1.2}^b$ Radicals (in the Case of the Whole System, Glutathione) and the Fragment in Aqueous Phase.
In aqueous phase, the largest structural change also caused by homolytic bond dissociation and it is occurred in fragment Q for the Q\textsubscript{1,2} radical. This structural realignment also causes a shift of the radical center from the nitrogen to the carbon. Such cleavage can be interpreted as the breaking N–H bond of the NH\textsubscript{3}\textsuperscript{+} group initiating decarboxylation from the formed radical. Actually, this reaction of the Q fragment produces the radical 4-aminobutanamide (which is a protected γ-aminobutyric acid (GABA) radical) and carbon dioxide. Reaction with similar products can also occur naturally via an ionic mechanism by means of glutamate decarboxylase, when glutamate is converted to GABA \[62\].

2.3.2 Bond Dissociation Energies of the Fragments

The performance of the theoretical models applied can be estimated by direct comparison with experimental data. Bond dissociation enthalpies at 298 K (DH\textsubscript{298}) \[43\] were also calculated and compared to experimental data. Bond dissociation enthalpies were used for validation of our calculations, and the collected results are shown in Table 1.

<table>
<thead>
<tr>
<th>bond type</th>
<th>phase</th>
<th>compound</th>
<th>DH\textsubscript{298} (exp)</th>
<th>ref</th>
<th>fragment</th>
<th>DH\textsubscript{298} (calc)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S–H</td>
<td>water</td>
<td>cysteine</td>
<td>353.1</td>
<td>52</td>
<td>C\textsubscript{S}</td>
<td>356.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(cys-S-H)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>O–H</td>
<td>gas</td>
<td>CH\textsubscript{3}COO-H</td>
<td>468.6 ± 12.6</td>
<td>53</td>
<td>Q\textsubscript{G} (G\textsubscript{O})</td>
<td>460.9 (483.7)</td>
</tr>
<tr>
<td>C–H</td>
<td>gas</td>
<td>H-CH\textsubscript{2}CHO</td>
<td>394.5 ± 9.2</td>
<td>31</td>
<td>Q\textsubscript{C6,7}</td>
<td>394.9 ± 0.8</td>
</tr>
<tr>
<td>N–H</td>
<td>gas</td>
<td>CH\textsubscript{3}NH-H</td>
<td>418.4 ± 10.5</td>
<td>54</td>
<td>Q\textsubscript{N1,2}</td>
<td>420.9</td>
</tr>
</tbody>
</table>

"No consecutive decarboxylation after the cleavage of the X–H bond.

DOI: 10.14750/ME.2017.009
In the case of the S–H bond, the G3MP2B3 method reproduces the experimental bond dissociation enthalpy measured in the aqueous phase within chemical accuracy ($\Delta H_{\text{298}}^{\text{calc-exp}} = 3.1 \text{ kJ/mol}$). The calculated BDE values of the C–H and N–H bonds are in good agreement with the experimental ones in the gas phase. Although the deviation is more enhanced for the O–H bond in the gas phase, it is still moderate and probably arises from the absence of the primary amine groups in the measured structure (acetic acid), which are close to the O–H bonds in the fragments. It is also important to point out that the standard deviation of the gas phase experimental values is also very large for both cases (10 kJ/mol). Therefore, it was concluded that the G3MP2B3 model chemistry is able to provide reasonable bond dissociation enthalpies for glutathione fragments.

The analysis of BDE values calculated with the G3MP2B3 method shows that there are distinguishable geometric classes corresponding to the bond lengths of the X–H bonds, where X can be C, N, O, and S. These classes are also separated by the appropriate BDE range (Figure 10).

The data collected in Table 2 show that the BDE values of the C–H bonds can vary from 343 to 403 kJ/mol in the gas phase. The hydrogen atoms bonded to the α-carbons ($Q_{C_3}, C_{C_{11}}, G_{C_{17}},$ and $G_{C_{18}}$) were found to be the most weakly bound among the C–H bonds with BDEs from 343.9 to 357.2 kJ/mol. The aqueous medium makes these bonds stronger by 11-42.9 kJ/mol. The medium has the largest influence (42.9 kJ/mol) on C–H BDE in the case of glutamine α-carbon ($Q_{C_3}$). This may be due to the zwitterionic structure of glutamine [63]. The N–H bonds in the aqueous form of glutathione are stronger, though, as indicated by the corresponding gas phase BDE values (415-465 kJ/mol). The O–H bonds are in the same BDE range as the N–H ones. Although the S–H bond is known to be the weakest, Figure 10 and Figure 11 clearly demonstrate that S–H, C–H, N–H, and O–H can have quite similar BDEs in some cases.

The $\Delta$BDE values are used to measure the relative strength of X–H bonds com-
Radical Scavenging Ability of Glutathione

**Figure 10:** X–H Bond Dissociation Energies (BDE) Against Bond Lengths in Gas Phase (Open Squares) and Aqueous Phase (Filled Squares). The Black Ellipses Separate the Different Bond Types which belong to Different X–H Bonds, and the Green Ellipse Includes the Consecutive Decarboxylations (b).

**Figure 11:** Relative Bond Dissociation Energies (ΔBDEs) in Aqueous and Gas Phases Calculated with the G3MP2B3 Method. The Reference is the BDE of the S–H Bond in the Fragment C (C₅ Radical Formed by the Bond Dissociation of S–H).
Table 2: G3MP2B3 Bond Dissociation Energies (BDE) (0 K, kJ/mol), Their Relative Values (ΔBDEs) and the Standard Entropies (S, 298.15 K, J/molK) in Gas and Aqueous Phases.

<table>
<thead>
<tr>
<th>radical</th>
<th>BDE</th>
<th></th>
<th></th>
<th></th>
<th></th>
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<tr>
<td></td>
<td>gas</td>
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*No consecutive decarboxylation after the cleavage of the X–H bond.*

*Consecutive decarboxylation after the cleavage of the X–H bond.*
pared to that of the S–H bond in glutathione and its fragments. As Figure 11 shows, the ΔBDEs for $C_{C11}, G_{C17}, G_{C18},$ and $Q_{C3}$ are only around 10 kJ/mol stronger than that for the S–H bond. These hydrogens connected to α-carbons constitute the weakest bonds in the gas phase. The weakest one corresponds to $C_{C11}$, with $\Delta BDE = -3.6 \text{ kJ/mol}$ compared to the S–H bond. It is clear from Table 2 that the O–H bond ($Q_{bO}$) dissociation energy is one of the smallest in the gas phase, with its slightly negative $\Delta BDE (-7.9 \text{ kJ/mol})$ being comparable with that for the α-C–H bonds, making this O–H also somewhat harder to break than the S–H bond in the gas phase. The BDE value of $Q_{C3}$ increases in the aqueous phase and becomes comparable with that of the non-α-C–H bonds. The corresponding $\Delta BDE$ is $-48.1 \text{ kJ/mol}$, which is smaller than any relative C–H bond dissociation energy except those of $Q_{C4}$ and $Q_{C5}$ ($\Delta BDE = -59.0 \text{ kJ/mol}$). This means that $Q_{C3}$ is the strongest C–H bond in the aqueous phase after the β-C–H bonds ($Q_{C4,5}$) of fragment Q. This change in the BDE value can be attributed to the steric effects of the nearby protonated amine group. The C–H bonds of the α-carbons in fragments $G (G_{C17,C18})$ and $C (C_{C11})$ are as weak as the thiol bond in the aqueous phase. The deviation between the S–H and C–H BDE values for $G_{C17,C18}$ is only $11 \text{ kJ/mol}$. Furthermore, the hydrogen abstraction from the α-carbon in fragment C is more preferable than from G. This bond ($C_{C11}$) is only $3.8 \text{ kJ/mol}$ stronger than the S–H bond. Nevertheless, the N–H bond belonging to $Q_{bN_{1,2}}$ is $2.4 \text{ kJ/mol}$ weaker than the S–H bond, and this is the weakest bond due to the consecutive decarboxylation from the radical after the hydrogen atom cleavage in the aqueous phase. These can be immediately seen from Figure 11, and such results raise doubts about the established view on S–H and α-C–H hegemony in the antioxidant properties of glutathione. The maximal entropy belongs to $Q_{bO}$ in the gas phase and to $Q_{bN_{N1,2}}$ in the aqueous phase (see the last two columns in Table 2). This indicates that the entropy contribution to the Gibbs free energy of the bond dissociation might further increase the preference of the cleavage of the hydrogen over breaking the S–H bond. Comparing gas and aqueous phase $\Delta BDE$ values, no dramatic difference was found (all were less than $10 \text{ kJ/mol}$) except in the cases where large structural changes were recognized. The case of O–H bonds were excluded, since car-
boxyl groups are deprotonated in the aqueous phase, so O–H bond dissociation is not possible. $Q_{C_3}$ shows moderate change (31.2 kJ/mol) by alteration of the surrounding medium. $\Delta$BDE values of $Q_{N_{1,2}}^\lambda$ in gas and aqueous phases differ by as much as 77.1 kJ/mol. This is the largest difference between the gas and aqueous phase $\Delta$BDE values. In order to calculate the bond dissociation properties for glutathione as a whole, a density functional has to be selected with the capacity of reproducing the G3MP2B3 results. Accordingly, the bond dissociation energies are recalculated using B3LYP, MPWKCIS, MPWKCIS1K, M06, TPSS1KCIS, and TPSSh functionals with the 6-311++G(3df,2p) basis set on B3LYP/6-31G(d) geometries (see Table 3) for both media.
Table 3: Bond Dissociation Energies (BDE) of the Glutathione Fragments (0 K, kJ/mol) in Gas and Aqueous Phases, Based on B3LYP/6-31G(d) Optimized Geometries (Basis Set 6-311++G(3df,2p) was Selected for All Methods).

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*No consecutive decarboxylation after cleavage of the X—H bond. †Consecutive decarboxylation after cleavage of the X—H bond.
Figure 12 shows the calculated bond dissociation energies obtained from different density functional computations against those of G3MP2B3 in the gas phase (top panel) and in the aqueous phase (bottom panel).

These graphs include the best linear fits, and the corresponding equations are also given. In both media, the G3MP2B3 BDE values are best approximated by MPWKClS1K/6-311++G(3df,2p)//B3LYP/6-31G(d) calculations out of the B3LYP, MPWKClS, MPWKClS1K, M06, TPSS1KCIS, and TPSSh results. In the gas phase, the linear fit between MPWKClS1K and G3MP2B3 values is almost perfect (slope = 0.987 and $R^2 = 0.980$). The fit is somewhat less good for aqueous results (slope is 0.984 and $R^2 = 0.972$), but still acceptable. Interestingly, those strong N–H bonds which have a BDE greater than 450 kJ/mol proved hard to calculate with most density functionals tested here in either phase. It is worth pointing out that M06 also provides better correlation with G3MP2B3 than B3LYP, MPWKClS, TPSS1KCIS, and TPSSh. However, MPWKClS1K yields slightly better overall results than M06. The selected MPWKClS1K functional has a much smaller computational demand than G3MP2B3, and it is likely to give the most accurate results for GSH.
Figure 12: Comparisons of Bond Dissociation Energies (BDEs) Obtained Using Several Density Functionals to those of the G3MP2B3 Method, Calculated in Gas (Top) and Aqueous (Bottom) Phases. MPWKCIS1K Provides the Best Fit to G3MP2B3, in Both the Gas and Aqueous Phases. Coefficients for the Lines of the Best Fit are also Given.
2.3.3 Radical Scavenging Potential of Glutathione

Experience obtained from the study of glutathione fragments needs to be extended to the glutathione species. For this reason, the bond dissociation energies were calculated using the selected density functional, MPWKCIS1K, in both phases. These results are summarized in Table 4.

It was found that energy values obtained for fragments are consistent with those calculated for the entire glutathione. In the gas phase there are four bonds (where H is connected to the α-carbons \( Q_{C_3}, G_{C_{17}}, G_{C_{18}} \) and the carboxyl oxygen \( Q_{bO} \) weaker than S–H mainly due to electron delocalization (see Figure 13).

![Figure 13: GSH Radicals Classified into Four Types Based on Electron Delocalization. There are Zero, Three, Seven, and Nine Delocalized Electrons in the S-Centered (C_S), C-Centered (Q_{bN_1;2}), and α-Carbon-Centered Radicals (G_{C17,C18} and C_{C11}), Respectively.](image-url)
Table 4: BDEs and Relative BDEs in the Case of the Whole Glutathione, Calculated by B3LYP and MPWKCIS1K Functionals in Gas and Aqueous Phases (0 K, kJ/mol), Based on B3LYP/6-31G(d) Optimized Geometries.

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</table>

$^a$ No consecutive decarboxylation after cleavage of the X−H bond. $^b$ Consecutive decarboxylation after cleavage of the X−H bond.
Radical Scavenging Ability of Glutathione

$Q_{C_3}$ and $G_{C_{17,18}}$ ($\Delta BDE = 12.7$ and $16.6 \text{ kJ/mol}$, respectively) are weaker than the $C_S$ bond. Only the $C_{C_{11}}$ (the $\alpha$-carbon-H in fragment C) is somewhat stronger than the $S-H$ bond ($\Delta BDE = -4.3 \text{ kJ/mol}$). Furthermore, the gas phase relative bond dissociation energy for the O–H bond in fragment Q is $7.3 \text{ kJ/mol}$, which makes this also more preferable than S–H (because of decarboxylation). The aqueous phase results for glutathione and its radical species follow the trend observed for the fragments. The BDE values increase due to the structural influence of the aqueous medium. There are three preferred positions (the thionyl group and the $\alpha$-carbons) to lose an H-atom while GSH transforms into different types of radicals. The thionyl group (S–H bond) itself is rather weak and needs no conjugation to have a small energy difference between GSH and its sulfur-centered radical ($C_S$). In contrast, the cleavage of the bonds between $\alpha$-carbons and H-atoms ($C_{C_{11}}, G_{C_{17,18}}$) allows for delocalized electrons stabilizing the radical product. These radicals are similar to each other, and the number of electrons involved in the delocalization is proportional to the stability of the conjugated species. The $Q_{N_{1,2}}$ position is also preferred, but this radical dissociates immediately. The fast rearrangement of the electron system results in an unsaturated carbon-centered radical (for fragments, the 4-aminobutanamide radical is produced; for GSH, the product is the decarboxylated glutathione (dGSH) radical) (see Figure 9). The bond strength of $Q_{C_3}$ changes (with a $4.5 \text{ kJ/mol}$ change in BDE) in the same way as in the case of the fragments, and in water the S–H bond is preferable ($\Delta BDE = -32.5 \text{ kJ/mol}$). The BDE of $G_{C_{17,18}}$ is also high, but not as much as in the case of the $\alpha$-carbon-H bond in fragment Q. It remains comparable with the bond dissociation energy of $C_S$. The BDE of the $\alpha$-C-H in fragment C decreased, and the breaking of fragment Q’s N–H bond in the NH$_3^+$ group initiates the decarboxylation from the formed radical in the aqueous phase (see Figure 9). Thus, $Q_{N_{1,2}}^b$ and $C_{C_{11}}$ are weaker and hydrogen cleavages are more preferred from here than from the S–H bond ($\Delta BDE = 2.5$ and $6.5 \text{ kJ/mol}$, respectively) under biologically relevant conditions. The geometric and energetic behavior of the GSH radicals and the glutathione fragment radicals shows that electron delocalization is one of the most important factors of radical stability. In the aqueous phase four
Radical Scavenging Ability of Glutathione

particularly important electron delocalization types were found for the radicals (see Figure 13). As opposed to earlier views which assumed the dominance of S–H and $\alpha$-C–H bonds in the antioxidant properties of glutathione, it turns out that there are several almost energetically equivalent sites to form radicals. The description suggested here for the GSH system seems to be more complex than what was previously thought. We hypothesize that the effectiveness of glutathione as a water-soluble radical scavenger is likely due to its multiple radicalization sites. The formed radicals have the ability to transform rapidly into one another as dictated by the environment.
2.4 Conclusions

Bond dissociation energies, BDEs, have been calculated in both gas and aqueous phases for all X–H bonds (where X can be C, N, O, and S) of glutathione, and their values have been compared. According to our results, the O–H bond dissociation of the carboxyl group has the smallest bond dissociation energy in a vacuum. In the aqueous phase, the weakest X–H bond is the N–H bond of the glutamine residue (QbN12) in the vicinity of the negatively charged carboxyl group. In both cases, CO₂ formation (decarboxylation) can accompany the breaking of the X–H bond, making the dissociation energetically more favorable than the cleavage of the S–H bond. The N–H bond is stronger than the C–H bond in the gas phase, but protonation significantly weakens it. Furthermore, C–H bonds with α-carbons are found to be about as weak as the sulfhydryl bond of the cysteine residues. Electron delocalization was found to have an important influence on the antioxidant potential. As clearly evident from our results, glutathione has five possible positions to scavenge a free radical in biologically relevant conditions. From a methodological perspective, the study of these hydrogen cleavages from glutathione has proven that the G3MP2B3 composite method provides results consistent with the experimental values. In order to replace the G3MP2 energy with accurate single point calculations, six density functionals, namely, MPWKCIS, MPWKCIS1K, M06, TPSS1KCIS, TPSSh, and B3LYP, have been tested against G3MP2 for obtaining accurate bond dissociation energies. Single point calculations were carried out on the fragment structures obtained at the B3LYP/6-31G(d) level of theory in both vacuum and solvent. The MPWKCIS1K/6-311++G(3df,2p)//B3LYP/6-31G(d) level of theory provides the best correlation with the G3MP2B3 method for BDE in both phases, and therefore, this method is recommended for similar calculations.
Erwin with his psi can do Calculations quite a few. But one thing has not been seen: Just what does psi really mean?

— Felix Bloch’s memories

3

Radical Scavenging Mechanism of Glutathione

3.1 Introduction

Molecular recognition – the interaction between a larger host and smaller guest molecules – is one of the most important biochemical processes [64]. This complex mechanism can take place during intra- and intercellular communication, the induction of the immune system and the response to external stimuli etc. [65].

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The cell itself evolves its own defensive mechanism against external actions, which can damage cells or cellular organelles, and this mechanism has been investigated for a long time. Free radical initiated oxidation is one of these external actions and one of the most important antioxidants [66], used as a defensive agent, is a small tripeptide, glutathione (γ-L-glutamyl-L-cysteinyl-glycine, GSH, Figure 14).

![Figure 14: The Chemical Structure of GSH, with the Atomic Naming Scheme (Indicated with Green). The Three Amino Acid Residues, γ-Glutamic Acid, Cysteine and Glycine are Indicated in Blue, Gold and Black, Respectively. The Non-Regular γ-Peptide Bond between the Glutamic Acid and the Cysteine Residues is also Labelled.]

The hydroxyl radical is one of the most reactive of the ROS [67] and has a key role in the oxidative stress related events, such as lipid peroxidation [68] and DNA oxidation [69, 70]. The rate constants of the reactions between GSH and radicals, as well as the GSH and hydroxyl radical reaction were studied previously by experimental methods, e.g. by laser photolysis, absorption spectroscopy and pulse radiolysis [33, 71]. The theoretical research were focused on the calculation of the bond dissociation energies of GSH [66], the potential energy surface and the rate constants of elementary steps of the reaction of glutathione with free radicals [72]. Furthermore, conformational analyses of GSH were carried out by NMR spectroscopy [36, 37, 73] and molecular dynamics (MD) [35, 73–75] methods,
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which showed that GSH is flexible and does not adopt a strongly preferred conformation. Recently Machuqueiro et al. performed constant-pH MD simulations for GSH and GSSG (oxidized form of GSH) \[74\]. They concluded that the conformational flexibility of GSH is pH-dependent and it has reduced flexibility at higher pH (pH>10) values. The large flexibility of GSH can be the weak point of the classical potential energy surface calculations, because the most reliable initial conformations are difficult to find. Moreover, the radical scavenging mechanism depends on the interactions formed between GSH and radicals, and the steric properties of collisions and attractive interactions can strongly influence the overall kinetics and the mechanism of this bimolecular reaction. To overcome this limitation, structures for further \textit{ab initio} calculations could be determined by non-reactive molecular dynamics simulations. Therefore, we set a long comparative MD simulation for GSH and GSH/OH\(^*\) systems. The MD trajectories will be able to characterize the different interactions between GSH and OH\(^*\). Moreover, the non-reactive MD trajectories combined with \textit{ab initio} calculations allow us to describe a detailed free radical recognition process.
3.2 Materials and Methods

The glutathione anion is found to be dominant at physiological pH, where the \( \gamma \)-L-glutamic acid predominantly exists in its zwitterionic form, while the carboxyl group of the glycine residue prefers to be deprotonated [75]. For these reasons, to obtain the most reliable theoretical model for GSH in water, its anionic form was considered and is hereinafter referred to as GSH. Five independent molecular dynamics (MD) simulations (5 x 240 ns) were performed for the GSH and GSH/OH\(^*\) systems, respectively. GSH was solvated with TIP3P [76] water molecules and one Na\(^+\) ion was also placed in the box in order to ensure the electroneutrality of the system. The simulation box was cubic (37.3 Å\(^3\)), where the minimum distance between any atom of the GSH and the wall of the box was 12 Å. The simulations were conducted with the Desmond v. 30110 [77] software using the CHARMM22 [78] force field. The short range van der Waals and electrostatic cut-off values were set to 9.0 Å and the long-range electrostatic interaction was calculated via the Particle Mesh Ewald [79] method. The missing bond parameters and charges for the OH radical were calculated with the Force Field Toolkit Plugin [80] implemented in Visual Molecular Dynamics (VMD) [81]. The simulation protocol was as follows: 1) steepest descent minimization (with and without solute restraints); 2) NVT MD (\( T = 10 \) K, \( \Delta t = 12 \) ps) with the Berendsen thermostat [82] \( \tau_T = 0.1 \) ps and restrained solute heavy atoms; 3) NPT MD (\( T = 10 \) K, \( \Delta t = 12 \) ps, \( p = 1 \) bar) with Berendsen thermo- and barostat (\( \tau_T = 0.1 \) ps, \( \tau_p = 50 \) ps, separate coupling for solute and solvent) and no restraints; 4) NPT MD (\( T = 310 \) K, \( \Delta t = 12 \) ps, \( p = 1 \) bar) with Berendsen thermo- and barostat (\( \tau_T = 0.1 \) ps, \( \tau_p = 50 \) ps, separate coupling for solute and solvent) and restrained solute heavy atoms; 5) NPT MD (\( T = 310 \) K, \( \Delta t = 24 \) ps, \( p = 1 \) bar) with Berendsen thermo- and barostat (\( \tau_T = 0.1 \) ps, \( \tau_p = 2.0 \) ps, separate coupling for solute and solvent) and no restraints. 6) NPT MD (\( T = 310 \) K, \( p = 1 \) bar) with Berendsen thermo- and barostat [82] (\( \tau_T = 0.1 \) ps, \( \tau_p = 2.0 \) ps, separate coupling for solute and solvent) and no restraints. The structures were saved every 9.8 ps, which resulted in 25 000 frames for each simulation. The protocol was repeated 5 times with different ran-
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Dom velocities and a total of 1.2 μs of simulations were obtained for each GSH and GSH/OH* system, respectively.

Intra- and intermolecular interactions were identified by the geometric analysis using the following criteria: d(A×××D) < 3.5 Å and α(A××H-D) > 100.0°. The structural analysis was performed with the ptraj module of the AmberTools 1.5 program package and Visual Molecular Dynamics [81] was used to prepare the 3D structures in the figures. To describe these interactions more in detail, electron density analyses (Atoms in Molecules, AIM) [83, 84] were carried out on some geometrically selected structures (highest number of intermolecular interactions) at the B3LYP/6-31G (d) level of theory. The AIM analysis was carried out with the AIM2000 program [85]. Previously, similar refinements were successfully used to improve the analysis of intramolecular interactions in the case of human galactokinase enzyme [86]. Geometry optimization were conducted on these selected GSH/OH* complexes and on the corresponding GSH conformers with the BHandHLYP density functional combined with the 6–31G(d) split valence basis set. To mimic the bulk water, the solvation model "D" (SMD) [87] was used. Normal mode analysis was also carried out at the same level of theory in order to confirm that the structures obtained are minima on the respective potential energy surface. The quantum chemical calculations were carried out using the Gaussian 09 program package [49].

d - distance, α - angle, A - acceptor, D - donor, ××× - non-covalent interaction

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3.3 RESULTS AND DISCUSSION

Interactions between water and radicals have been investigated earlier by classical MD methods [88], wherein the authors used the same van der Waals parameters for the radicals (OH$^\bullet$, HO$_2^\bullet$) as for the water molecules. The obtained free energy profiles through the water slab were in good agreement with experimental studies; therefore we developed new parameters only for the O-H bond term and the O and H partial charges. Atomic point charges of the OH radical were calculated to be 0.444 for H and 20.444 for O and the bond parameters for the O-H bond were found $b_o = 0.9791$ Å and $K_b = 444.8474$ kcal/molÅ$^2$. Other consequences of the above mentioned paper [88] are that a direct reaction between water molecules and radicals is not necessary in order to describe the interaction between them. Therefore, to investigate the interaction patterns between GSH and OH$^\bullet$, we can use classical MD simulations to generate different GSH conformers and identify those parts of the molecule that are the most important molecular recognition sites. The root-mean-square deviation (RMSD) of the heavy atoms was calculated for the glutathione structures obtained with respect to the initial, extended structure of GSH. The RMSD value fluctuated between 1.00 and 3.00 Å, indicating that the GSH was indeed very flexible during the simulations, which is in good agreement with previous studies [35, 73–75]. The compactness of the GSH structure during the simulations was measured by the radius of gyration of heavy atoms ($R_{gyr}$). The $R_{gyr}$ values for most of the GSH structures varied from 3.0 to 4.5 Å both with and without hydroxyl radical. The percentage of the distribution of the structures on the RMSD – $R_{gyr}$ surface was also calculated (Figure 15).

In order to describe the flexibility of GSH from another structural point of view we used the same parameters as Machuqueiro et al. [74]. All possible distances were measured between the GlyOT\textsubscript{1}/OT\textsubscript{2} and GGL.OT\textsubscript{1}/OT\textsubscript{2}/N (for the nomenclature of the different GSH sites see Figure 14) and the minimum value among these 6 distances was used as the head-tail distance (HT). The other parameter was
Figure 15: The Percentage Distribution of the Structures on the RMSD – R$^{gyr}$ (Upper Panel) and HT – CYS-HT Surfaces was Calculated for the GSH and the GSH/OH Systems (Left Panels). Representative Structures from the most Populated Region from the RMSD – R$^{gyr}$ Surface are Shown as well. The Different Origin of the Representative Structures is Indicated by Colored Carbon Atoms (Green – GSH, Brown – GSH/OH). The Differences Between Surfaces were also Calculated (Right Panel).
the minimum distance between CYS.SG and the above mentioned atoms (CYS- HT). Thereafter, using a 0.25 Å bin width, the distribution of the structures on this surface was determined. Similar distributions were obtained for the simulations with and without OH*, which can be seen on the difference surfaces (right panel, Figure 15). The largest deviation obtained was only about ±0.4% and ±0.1% on the RMSD – R_gyr and HT – CYS-HT surfaces, respectively. In case of the HT – CYS-HT surface, the same distribution was obtained also in our case comparing the results of Machuqueiro et al.: the HT values varied between 2 and 12 Å, while the CYS-HT values were between 2 and 8 Å. This indicates that the presence of OH* has no large impact on the dynamic nature of GSH in a non-reactive, classical MD simulation. The most populated regions of the GSH and GSH/OH* systems (12.9% and 13.1% of the structures, respectively) are located in the same [1.75 Å ≤ RMSD <2.00 Å; 3.75 Å ≤ R_gyr <4.00 Å] range of the RMSD – R_gyr surface. The averages of the RMSD and the R_gyr values in this most populated range were calculated and the structures which had the smallest deviation from the averages were selected for comparison as representative structures. The RMSD between the two representative GSH structures is 1.34 Å, which means that the representative structures fit to each other very well (Figure 15). The largest deviation was obtained for the glycine (GLY) residue and the peptide bond between the cysteine (CYS) and GLY residues. In the representative structures we did not obtain any intramolecular hydrogen bonds, except the interaction between the protonated amine and the deprotonated carboxyl group in the γ-glutamic acid (GGL) residue. Furthermore, the representative structures of GSH are in good agreement with the conformations obtained previously by NMR and MD studies [17–21]. Intramolecular hydrogen bond analyses were carried out as well for GSH in both systems for each snapshot from the MD simulations. The intramolecular hydrogen bonds were formed in less than 0.05% of the total number of the MD frames. This also indicates that the structure of glutathione is very flexible, since internal interactions cannot facilitate a preferred conformation. These results are in good agreement with other studies, where the authors concluded that in tripeptides the intramolecular H-bonding interactions do persist in vacuo or in acetonitrile, but
vanish in water \cite{89,90}. These findings suggested that in aqueous phase the solute – solvent interactions are favored instead of intramolecular H-bonds, therefore in the following sections we focused on the analysis of GSH×××OH*/WAT interactions. During the MD simulation, the individual atomic pair distances and distributions can be calculated by the radial distribution function (g(r)) between the oxygen atom of water or OH* and all heavy atoms in GSH (Figure 16).

We considered those heavy atoms as the most probable radical attack points of GSH where the g(r) values show high maxima for OH*. During the g(r) curve analysis and in the further discussion all CA atoms were considered, although in the case of GLY the terminal carboxyl group is responsible for the enrichment around this region. Nevertheless, we were interested in these regions as well, because the α-carbon atoms are highly vulnerable attack points in the proteins \cite{91}. Hydrogen atom abstraction by radicals from these positions can easily happen if the α-carbons are accessible, because the process is favorable from the thermodynamics point of view \cite{92}. In the case of the γ-glutamic acid residue, the OH* enrichment is conspicuous near the α-carbon (GGL.CA), the carboxyl carbon (GGL.C) and both carboxyl oxygens (GGL.OT1/GGL.OT2). The β-carbon (CYS.CB) and the sulfur (CYS.SG) in the cysteine residue are potential radical attractors, as shown by the corresponding g(r) values. The α-carbon (GLY.CA), the carboxyl carbon (GLY.C) and carboxyl oxygens (GLY.OT1 and GLY.OT2) in the glycine residue also have radical control ability. The distribution functions of the GLY.OT1/2 show the same characteristics as the corresponding g(r)s of the GGL residue (see Figure 16). To determine the probability of finding the OH* near GSH, the maxima of the g(r) curves (g(r)max) were analyzed and these values were collected in Table 5 with the corresponding distances.

Based on these g(r)max values, the most probable OH* attack points can be selected. The highest g(r)max values for the OH* belong to the carboxyl oxygens of GLY (8.63 and 8.71). These were followed by the carboxyl oxygens of GGL (5.25 and 6.00), while the lowest value (1.31) was attributed to the carbonyl oxygen of
Figure 16: The Radial Distribution Functions ($g(r)$) were Calculated Between the Oxygen Atom of Water (Blue Curves) or OH* (Black Curves) and All Heavy Atoms in GSH for the GSH/OH* System. The Maximum $g(r)$ Values and the Corresponding Distance for OH* are Indicated in the Lower Right Corner of Each Graph.
Table 5: The Maximum Values of the Radial Distribution Functions \( g(r) \) Between the Oxygen Atom of OH\(^*\) and All Heavy Atoms in GSH.

<table>
<thead>
<tr>
<th>Res.</th>
<th>Atom</th>
<th>( r ) (Å)</th>
<th>( g(r)_{\text{max}} )</th>
<th>Norm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>GGL</td>
<td>CA</td>
<td>4.65</td>
<td>2.06</td>
<td>1.57</td>
</tr>
<tr>
<td></td>
<td>CB</td>
<td>5.35</td>
<td>1.49</td>
<td>1.14</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>3.25</td>
<td>2.91</td>
<td>2.22</td>
</tr>
<tr>
<td></td>
<td>CD</td>
<td>3.75</td>
<td>1.40</td>
<td>1.06</td>
</tr>
<tr>
<td></td>
<td>O</td>
<td>7.55</td>
<td>1.31</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>CG</td>
<td>3.65</td>
<td>1.32</td>
<td>1.01</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>5.35</td>
<td>1.53</td>
<td>1.17</td>
</tr>
<tr>
<td></td>
<td>OT1</td>
<td>2.65</td>
<td>5.25</td>
<td>4.01</td>
</tr>
<tr>
<td></td>
<td>OT2</td>
<td>2.65</td>
<td>6.00</td>
<td>4.58</td>
</tr>
<tr>
<td>CYS</td>
<td>CA</td>
<td>4.25</td>
<td>1.49</td>
<td>1.14</td>
</tr>
<tr>
<td></td>
<td>CB</td>
<td>3.85</td>
<td>1.76</td>
<td>1.34</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>3.95</td>
<td>1.62</td>
<td>1.23</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>5.65</td>
<td>1.39</td>
<td>1.06</td>
</tr>
<tr>
<td></td>
<td>O</td>
<td>7.15</td>
<td>1.47</td>
<td>1.12</td>
</tr>
<tr>
<td></td>
<td>SG</td>
<td>3.45</td>
<td>2.24</td>
<td>1.71</td>
</tr>
<tr>
<td>GLY</td>
<td>CA</td>
<td>3.75</td>
<td>2.33</td>
<td>1.78</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>3.15</td>
<td>4.54</td>
<td>3.47</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>5.15</td>
<td>1.70</td>
<td>1.30</td>
</tr>
<tr>
<td></td>
<td>OT1</td>
<td>2.65</td>
<td>8.63</td>
<td>6.58</td>
</tr>
<tr>
<td></td>
<td>OT2</td>
<td>2.65</td>
<td>8.71</td>
<td>6.65</td>
</tr>
</tbody>
</table>

The normalized values (Norm.) were calculated \( \text{Norm.} = g(r)_{\text{max}}/\min\{g(r)_{\text{max}}\} \) and are also tabulated.
Radical Scavenging Mechanism of Glutathione

GGL (GGL.O). Compared to the probability at the carbonyl oxygen, we obtained more than 1.5 times higher probability values around the α-carbons (GGL.CA and GLY.CA), the carboxyl carbons, oxygens (GGL.C, GLY.C and GGL.OT₁₂, GLY.OT₁₂) and the sulfur (CYS.SG), altogether around 9 atomic positions. Seven of these cases are situated closer than the maximum distance criteria of the hydrogen bonding interaction (<3.5 Å). The high probability of finding of OH⁺ around the carboxyl carbons (GLY.C and GGL.C) is caused by the strong H-bonds between OH⁺ and negatively charged carboxyl oxygen atoms. Therefore we will omit these from the further analysis, since there are no direct interactions between OH⁺ and these carbon atoms. To obtain a more detailed description about the interactions between the hydroxyl radical and the different functional groups of GSH, interaction pattern analyses were carried out. The interactions were determined between the OH⁺ and those heavy atoms of GSH where the g(r) curves showed higher probability of finding it, and/or which were favorable attack points (GGL: CA, N, OT₁, OT₂; CYS: SG, CA, CB and GLY: CA, OT₁, OT₂; see Figure 14 for atom names) based on our previous results (see Chapter 2). All in all, 2305 structures were found where at least one interaction was established between the OH radical and the corresponding parts of GSH. The most frequently occurring interaction (~78%) was present between the OH⁺ and the anionic carboxyl groups of GSH. We determined the structures in which the OH⁺ is interacting with the sulfhydryl group (~4%) as well. In around 36% (838) of the structures, the OH⁺ interacts with at least two heavy atoms of GSH. The distribution of OH⁺ around GSH in such structures was depicted as a volumetric map (see Figure 17).

The volumetric map also demonstrates that the carboxyl oxygens (GGL.OT₁₂ and GLY.OT₁₂) of GSH are dominant in the interactions, and that the OH⁺ is mostly hydrogen-bonded with these atoms of GSH. The thiol (CYS.SG) and the protonated amine group (GGL.N) are also important for intermolecular interactions between GSH and OH⁺. 82% of these structures contained hydrogen bonds where the OH⁺ is interacting with carboxyl oxygens. These structures can be divided into two subgroups, depending on which site of GSH (GLY or GGL) takes...
Radical Scavenging Mechanism of Glutathione

Figure 17: The Volumetric Map was Created for the Radical (OH*) Occurrence Around those 838 Structures where the OH* Interacts with at Least Two Heavy Atoms of the GSH.

part in the interaction (GLY ~48%, GGL ~34%). In 11% of the structures, the hydroxyl radical formed interactions with a carboxyl oxygen and with a hydrogen bonded atom at the same time. The analysis of the intermolecular interactions revealed 12 cases in which three interactions were established between GSH and OH* (see Figure 18), and this is the highest number of such (GSH×××OH*) intermolecular interactions. We assumed that these are the most stable complexes, because of the high number of interactions between the two molecules. AIM analysis was carried out on these structures to describe these interactions and investigate their existence from a molecular electron density point of view (see Figure 18, second and fourth columns). Two main types of interactions can be identified in these cases, zwitterionic and anionic, based on the interacting functional groups of GSH (see Figure 18, I. and II.). The zwitterionic group (-NH4+×××OH*×××-CO2-) contains 4 members, where one of the anchor points is the protonated amine group of GGL (GGL.N) and the other one is a deprotonated carboxyl group. In the anionic group (-CO2-×××OH*×××-X-H) there are 8 structures where the main interaction between OH* and GSH is formed with the carboxyl group of the
Figure 18: The Structures that Contained the Maximum (3) Number of Interactions Between GSH and the OH* Based on the Geometrical Criteria are Depicted (I. – Zwitterionic Group, II. – Anionic Group). The Interactions Based on Geometrical Criteria are Indicated with Blue, Dashed Lines, while those Resulted from the AIM Analyses are Depicted with Green Points (Bond Critical Points, BCPs).
GLY or the GGL residue. Besides this interaction, others were also found in these complexes with the comprising of GGL.CA or GLY.CA and/or CYS.CA/CB/SG. The AIM analysis resulted in bond critical points (BCPs) indicating the existence and the strength of the interactions. The BCPs varied from 0.002 a.u. to 0.058 a.u. (higher electron densities at the BCPs represent stronger interactions). The weakest interactions correspond to geometrically not investigated ones (GLY[-N-HN] × × × OH*), which appeared in two cases (I/4, zwitterionic group and II/7, anionic group). The AIM analysis showed that the strongest interactions (H-bonds) were between the carboxyl oxygens of the glycine and the hydroxyl radical (GLY.OT1,2 × × × OH*). This finding is in good agreement with the g(r)max values calculated.

Geometry optimization and frequency calculations were conducted on selected GSH/OH* complexes and GSH conformers at the BHandHLYP/6-31G(d) level of theory to describe the energy profile of the recognition process. The representative GSH structure from the molecular dynamics simulation of GSH without OH radical was selected as a reference structure. The relative enthalpy of other conformers with respect to this structure is in the range of -27.1 to -5.1 kJ/mol (see Figure 19).

The lowest enthalpy conformer is the 3rd from the zwitterionic group (see Figure 18, right upper corner). The complex formation between the hydroxyl radical and the GSH is highly exothermic, regardless of the molecular motion of GSH, as well as the attack point of OH* on the glutathione. The enthalpy of complex formation is between -42.4 and -27.8 kJ/mol. The relative enthalpy of the formed complexes varies from -67.2 to -24.5 kJ/mol compared to the reference conformer and the hydroxyl radical. These results show that the radical recognition process of GSH (GSH/OH* complex formation) is energetically favorable. In summarizing our results, we outline a possible general radical recognition process as follows. The g(r) curves show that the probability of finding OH* is 4 times larger around the GLY.OT1,2 (g(r)max > 8.6) compared to CYS.SG (g(r)max = 2.2). Additionally, the relative amount of the GLY.OT1,2 × × × OH* H-bonds compared to the
Figure 19: The Relative Enthalpy of the Optimized GSH Conformers and the GSH/OH\(^{\bullet}\) Complexes. The Reference Conformer (Red Line) was the Representative Structure Obtained from the Molecular Dynamics Simulation of GSH without OH\(^{\bullet}\). The Calculations were Carried out at the BHandHLYP/6-31G(d) Level of Theory Combined with the SMD Implicit (Continuum) Solvent Model.
total number of interactions shows us that the terminal GLY may have a double role in the radical recognition: catching and steering. In the first step of the process, the GLY.OT\textsubscript{1,2} most often forms a H-bonded intermolecular complex with OH\textsuperscript{*}, however the GGL.OT\textsubscript{1,2} and/or GGL.N are also capable of this, quasi catching the radical from the bulky solvent phase. In the second step, owing to the high flexibility of GLY, it can further steer the radical in the direction of the CYS or other parts of the GSH. In this step, new interactions are forming, e.g. with the CYS.CB, and the original interactions \textcolor{red}{(GLY.OT\textsubscript{1,2} \times \times \times OH\textsuperscript{*})} also remain. After the radical recognition, the detoxification of OH\textsuperscript{*} by GSH can take place and the OH\textsuperscript{*} abstracts a hydrogen from one of the multiple radicalization points of glutathione (Chapter 2).
3.4 Conclusions

Two, in total 1.2 μs comparative MD simulations were conducted on GSH and GSH/OH* systems to explore the molecular recognition process and identifying the OH radical attractor regions of GSH. The high flexibility of GSH was preserved during the simulation of the GSH/OH* system and this is one of the driving forces of the radical recognition process. Two main steps of the detailed molecular radical recognition process of GSH were assigned, namely catching and steering (Figure 3). In ~78% of all interactions characterized, strong complexes were formed between anionic carboxyl groups and the OH radical. After the catching step, the strong carboxyl-OH* complexes could evolve additional interactions with other parts of GSH, stabilized by both the donor and acceptor features of the OH radical. The glycine residue dominates the steering role in the recognition step, while the glycine-hydroxyl radical complexes could facilitate further interactions with α-, β-carbons (Chapter 2) and with the thiol group via the OH* lone pair electron. The glutamic acid residue does not show this property during the MD simulations. Quantum chemical calculations on selected GSH/OH* complexes revealed exothermic heats of formation between -42.4 and -27.8 kJ/mol, and these strong complexes become the starting configurations of individual bond rearrangement to complete the radical scavenging mechanism.
4

Glutathione & Chemical Evolution

4.1 Introduction

In the biosynthesis of bacterial proteins, the amino acid (AA) polymerization always begins with formation of a peptide bond to the carboxyl group of a modified methionine, N-formylmethionine (fMet). In the first step of bacterial protein synthesis, the amino group of methionine is protected by enzymatic formylation of the NH₂ group so that the next residue can attack only its carboxyl group \(^{[93]}\) (Figure 20).

Glutathione & Chemical Evolution

Figure 20: Initial Steps of the Bacterial Protein Synthesis.

The first amino acid residue that will connect to fMet later will be the N-terminal end of the protein. The peptide chain is then built step by step, each new peptide bond being formed by the carboxyl group of the C-terminus amino acid whose α-amino group is involved in an existing peptide bond. Finally, the methionine is removed from the N-terminus of the protein. In fact, N-formylmethionine acts like a catalyst or an activator: connecting to the amino group, it makes the would-be N-terminal amino acid capable of forming a new peptide bond at the C-terminus. Similar “activation” seems to operate in the biosynthesis of other peptides, too.

For example, in the synthesis of glutathione (γ-L-glutamyl-L-cysteinyl-glycine, GSH, Figure 21, bottom right), in spite of being performed by completely different enzymes in different organisms, the first step is always formation of the peptide bond involving the γ-carboxyl group of glutamic acid and the amino group of cysteine.

The common features of these processes indicate that the chemistry, in particular, the thermodynamical characteristics, can be similar. Investigation of the simpler case, the energetics of glutathione formation, can help one to understand how this “activation” works. GSH is accumulated in several cellular compartments such as the cytosol, nucleus, and mitochondria (in as high concentration as 1−11, 3−15, and 5−10 mM, respectively) [31]. Besides many of its other features, it is one of the most important antioxidants [66, 94, 95], and it contributes to amino acid transport through the cell membrane [96, 97]. GSH has an essential role in
Figure 21: Scheme of Peptide Formation and the Steps Leading to \(\alpha\) (Red Channel) and \(\gamma\) (Green Channel) Di- and Tripeptides from Glutamic Acid (GLU, E), Cysteine (CYS, C), and Glycine (GLY, G). The Pharmacophore Groups are Marked by Colors: Blue, Amino; Red, Carboxyl; Yellow, SH; Gray, \(\alpha\)-Peptide Bond; Green, \(\gamma\)-Peptide Bond. The \(\alpha\)-, \(\beta\)-, and \(\gamma\)-Carbons are also Marked.
numerous biochemical processes like cell differentiation, proliferation, apoptosis, signal transduction, and gene expression [20, 21]. A large variety of human diseases like cystic fibrosis, cancer, and neurodegenerative diseases are closely related to the irregular GSH homeostasis [22, 23, 98, 99]. Its omnipresence indicates that it has some structural element that lends it the capability of performing a special function, as well as of surviving and remaining active in drastically different environments. Using quantum chemical methods, we intend to contribute to the elucidation of the factors determining the strength of peptide bonds that may explain the role of an existing peptide bond in the formation of a new one and the energetic factors that contribute to the preference of the α-peptide bond in proteins. In the following, we summarize the known properties of GSH, and then present the results of a comparative theoretical study of the thermodynamics of the formation of the α- and γ-peptide bonds and show the probable factors responsible for the primary accumulation of GSH. Finally, we address the consequences of the relative strength of regular α- vs β- and γ-peptide bonds in the assembly of proteins. Thereafter, the connection between the thermodynamics of peptide and protein formation and the acidity and basicity of amino acids will be discussed.
4.2 Methods

Molecular structures, standard reaction enthalpies ($\Delta rH^\circ$), and reaction Gibbs free energies ($\Delta rG^\circ$) were calculated for the di- and tripeptide formation by quantum chemistry calculations. Since peptides can assume numerous conformations, it is important to decide which molecular geometry is used in the calculations. Conformation analysis of glutathione carried out by NMR spectroscopy and molecular dynamics (MD) [35–37, 73, 100] shows that GSH does not adopt a preferred conformation in solution. This suggests that the solvent–solute interactions are preferred instead of (internal) solute–solute interactions. In order to describe the solvent–solute interactions for different functional groups in a comparable environment, the electronic structure calculations were performed at the optimized geometry of the extended zwitterion forms of the amino acids and peptides. At the extended linear geometry, the intramolecular interactions are small and not specific to the substituents on the peptide backbone. This kind of standardization then allows one to separate local thermodynamic factors such as bond strength from secondary effects such as intramolecular interactions and different solvation due to differences of the environment. The thermodynamics of the peptide formation reaction

\[
\begin{align*}
H_3^+N - Q - COO + H_3^+N - Q - COO \\
\rightarrow H_3^+N - Q - C(O)NH - Q - COO + H_2O
\end{align*}
\] (4.1)

was studied using the G3MP2B3 method [45, 46] and two density functionals, namely, B3LYP, as it is implemented in the Gaussian 09 program package [49], and the hybrid meta-GGA (generalized gradient approximation) functional M05-2X [101], combined with two split-valence basis sets, 6-31G(d) and 6-311+G(d,p). The G3MP2B3 method and each functional–basis-set pair was combined with two implicit water models, the conductor-like polarizable continuum model, CPCM [51, 52], and the continuum solvation model “D”, SMD [87], to mimic the solvent effects of bulk water. The four different DFT levels of the-
ory combined with two solvent models provided very similar molecular geometries. The method dependence of the energy is larger, but each model provides the same qualitative picture. Among the DFT methods, the M05-2X/6-311+G(d,p) level of theory provides energy differences closest to the benchmark G3MP2B3 data. Reaction enthalpies and reaction Gibbs free energies were calculated using the standard rigid rotor–harmonic oscillator (RRHO) approximation. The calculated relative enthalpies realistically reflect the differences in the bond strength of different peptides. The calculation of reaction entropies, however, is more sensitive to anharmonicities and solvent effects on vibrations. In addition, during the formation of a peptide formation in neutral water, one NH$_3^+$ and one carboxylate ion disappear, involving extensive changes in solvation, especially in the first solvation shell that is not considered explicitly by continuum solvation models. As a consequence, the reaction free energies calculated with continuum solvation models and the RRHO approximation cannot be expected to accurately reproduce the available experimental values. In order to provide a more realistic picture, we corrected the reaction Gibbs free energies based on the following reasoning: The experimental free energy change for formation of the zwitterionic form of an amino acid from the neutral is $-30.4$ kJ/mol [102]. The formation of a peptide bond from two zwitterionic amino acids results in a zwitterionic peptide so that one zwitterionic structure disappears. The free energy change then involves the free energy of the peptide bond formation reaction from neutral peptides plus the free energy of annihilation of an ion-pair structure. We correct our calculated peptide formation reaction Gibbs free energies $\Delta G_{pf}$ by $+30.4$ kJ/mol for each new peptide bond (each corresponding to the disappearance of one ion pair), i.e., once for dipeptide formation from two amino acids plus once for the tripeptide formation from a dipeptide and an amino acid. As a test of our correction procedure, we calculated the reaction Gibbs free energy of formation of zwitterionic diglycine from two glycine zwitterions with the G3MP2B3 composite method combined with the SMD solvation model. We obtained $\Delta G_{GG,calc} = -16.3$ kJ/mol. Correction of the calculated value according to our scheme yields $\Delta G_{GG,corr} = 14.1$ kJ/mol. The experimental value is $\Delta G_{GG,exp} = 15.1$ kJ/mol [103], which means that the correction
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reproduces the experimental reaction free energy within 1 kJ/mol. All computations were carried out using the Gaussian 09 program package [49].

4.3 RESULTS AND DISCUSSION

4.3.1 STRUCTURE AND FUNCTIONALITY OF GLUTATHIONE

Let us first investigate the properties of glutathione that indicate its possible prebiotic origin. The most conspicuous in the structure of GSH is the nonregular peptide bond that is formed between the side-chain carboxyl group (located in the γ position, i.e., two carbons away from the α-carbon carrying the amino group) of glutamic acid (E) and the amino group of the cysteine residue (C), a γ-peptide bond (Figure 21). Remarkably, free ECG, the analogue of GSH in which there is an α-peptide bond between E and C (Figure 21), has not been found to appear in living organisms. On the other hand, the antioxidant activity of GSH is retained if the third residue in the tripeptide is changed. In these GSH analogues, glycine is replaced by β-alanine [104–106], serine [105, 106], or glutamate [106]. Furthermore, another GSH derivative, trypanothione, in which two GSH units are connected by a polyamine linker, also acts similarly to glutathione in kinetoplastids [105, 107], just as the dipeptide γ-L-glutamyl-L-cysteine (γ-EC, Figure 21) does in halobacteria [108]. All analogues of glutathione that can perform its functions share a common structural unit, the γ-peptide bond. These analogues operate and perform the same function in rather different organisms, inspiring one to surmise that the γ-EC structural element is an ancient motif and has a special role in living organisms. There is another fact supporting this conjecture. When GSH is synthesized in cells, the first step is always the formation of the γ-peptide bond [109, 110]. However, in different organisms, the synthesis of GSH is facilitated by different enzymes that, while fulfilling analogous functions, do not display any detectable similarity in their structure and sequence [108, 111]. On the basis of these facts, it seems reasonable to assume that the γ-EC motif is more ancient than the proteins synthesizing it. Apparently, once Nature found a small peptide
that performs very well the job of an antioxidant, very probably there were several "attempts" to synthesize them, and under different conditions different pathways were found to be successful. Finally, some of those that proved to be "useful" were retained during evolution. It is curious why Nature relies on the unusual $\gamma$-peptide bond in GSH and its analogues when the standard in biology is the $\alpha$-peptide linkage. Molecular biological studies showed that, if GSH did not contain the nonregular peptide bond, the peptidases would degrade the molecule, thus preventing it from accumulating in concentrations needed to fulfill its function \[108\]. This means that, at the current stage of evolution, it is the $\gamma$-peptide bond that ensures that in living organisms GSH remains in the arsenal of antioxidant agents. Accepting that GSH and its role are ancient, probably prebiotic, it is reasonable to assume that the chemistry of its formation is responsible for its appearance in living organisms in the first place. From the observation that different enzymes synthesize GSH via the same first step, one can assume that the decisive factor is the thermodynamics, not the kinetics of the $\gamma$-peptide bond formation. The most straightforward assumption is that the relative stability of the possible isomers of the key building unit, glutamyl-cysteine, determines the actual reaction mechanism.

4.3.2 Energetics of Peptide Bond Formation

At the early stages of evolution, dipeptides from glutamic acid and cysteine obviously were formed without the assistance of enzymes that would control the relative rate of formation of the possible isomers, because at that time enzymatic catalysis was obviously less developed. In other words, dipeptide "synthesis" took place under thermodynamic control. Thermodynamic control operates when neither of the isomers is formed significantly faster than the other, and the result is that their formation leads to a mixture being in thermodynamic equilibrium. Accordingly, when the formation of both $\alpha$- and $\gamma$-dipeptides (EC and $\gamma$-EC, Figure 21) is possible from their two constituents, their relative stability governs their relative population. The dominance of one of them will then be passed on to the tripeptide formation step. Thus, if $\gamma$-EC and GSH are thermodynamically more stable
compared to EC and ECG, respectively, one can understand why the former were easily available when an SH-group based antioxidant was needed. In quantitative terms, the relative amount of the \( \alpha \)- and \( \gamma \)-peptide products is determined by the equilibrium constant \( K \) of the formal \( \alpha \)- to \( \gamma \)-isomerization:

\[
EC \rightleftharpoons \gamma EC
\]  

We computed \( K \) using quantum chemical methods at 10 different levels of theory. At all theoretical levels, \( K \) is obtained to be over 100, indicating that the formation of the \( \gamma \)-isomer is found to be more favorable than its \( \alpha \) counterpart. The analysis of the energetics of the sequential formation of di- and tripeptides provides a more comprehensive picture. The relative strength of a peptide bond is reflected in the enthalpy of the reaction in which the bond is formed, namely, when the two amino acids react and the peptide is formed, with water as a byproduct (see reaction 4.1). Since the byproduct is always the same, the difference between the reaction enthalpies of two different peptide-formation channels will be the same as the difference between the strengths of the peptide bonds formed in the two processes. (It should be noted that the picture can be refined by considering acidic dissociation and proton take-up, but the leading term remains the relative bond strength.) Figure 22 shows the reaction enthalpies (\( \Delta H_{pf} \)) of all possible steps of tripeptide formation: (a) formation of the EC peptide bond first leading to ECG (red) via EC and GSH (green) via \( \gamma \)-EC and (b) formation of the CG bond first (blue) yielding both ECG or GSH.

The comparison of the pathways shows that the enhanced stability of GSH comes from the larger strength of the \( \gamma \)-peptide bond, the formation of which either in the first or the second step brings the system to lower enthalpy than the corresponding step in ECG formation. \( \Delta H_{pf} \) for \( E + C \rightarrow \gamma \)-EC as well as \( E + CG \rightarrow SH \) is \(-38.8\) and \(-52.7 \) kJ/mol, significantly more negative than \( \Delta H_{pf} \) for the corresponding \( \alpha \)-peptide bond formation steps, \(-23.4\) and \(-34.0 \) kJ/mol, respectively. The reaction Gibbs free energies reflect the same features. For example, \( \Delta G_{pf,corr} \)
Figure 22: Standard Reaction Enthalpy ($\Delta H_{pf}$) and Corrected Gibbs Free Energy ($\Delta G_{pf,corr}$) Profile of Peptide Bond Formation Leading to ECG and GSH, Respectively, Calculated at the G3MP2B3 Level of Theory Combined with the SMD Implicit (Continuum) Solvent Model. Red: Both Steps Form $\alpha$-Peptide Bonds. Blue: First Step is $\alpha$-, Second is $\gamma$- or $\alpha$-Peptide Formation. Green: First Step is $\gamma$-, Second is $\alpha$-Peptide Formation.
for the reaction forming γ-EC is negative, that for EC is not negligibly positive, and, similarly, the formation of GSH from CG is much more favorable than that of ECG. The equilibrium constants for peptide formation, $K_{pf, corr}$, obtained from the corrected Gibbs free energy changes are consistently much higher for the formation of the γ-peptides than for their α counterparts ($K_{pf, corr, \gamma}/K_{pf, corr, \alpha} = 109$ for di- and 34 for tripeptides). Consequently, in thermal equilibrium, the GSH concentration is much higher compared to that of ECG, similarly to the γ-EC−EC pair. Accordingly, at ancient times, very probably more γ-EC than EC and more GSH than ECG was available to fulfill the role of a redox agent. By the time the regular α-peptide bond became standard for peptides and proteins, GSH probably performed its function satisfactorily efficiently not to be replaced later. This is the possible reason why such a curious structure was retained by evolution, and also supports the assumption that GSH is a prebiotic relict.

4.3.3 Enthalpy of Peptide Bond Formation and Acidity / Basicity of the Contributing Amino Acids

The enhancement of the reaction enthalpy, i.e., the larger strength of γ- versus α-peptide bonds, can be traced back to the difference of their environment in the molecule. In α-peptides, the amino group is close to the carboxyl functional group involved in the peptide bond. The inductive effect of the α-NH$_2$ group polarizes the carboxyl group, and one can expect that the strength of the peptide bond it forms will be different from that made by the essentially unpolarized carboxyl in the γ-position to the amino group. The degree of polarization of the carboxyl group will be reflected in other properties, for example, in its acidity. Indeed, the acid dissociation constant $K_a$ corresponding to the γ-carboxyl group in glutamic acid is 2 orders of magnitude smaller than that of the α-carboxyl (Table 6).

The acidic strength is connected to the covalent character of the O−H bond, which is more expressed in weak acids, making the bond relatively strong, less easy to dissociate into ions. One can expect that weak acids would form stronger covalent bonds not only with the H atom but also with other functional groups. The
Table 6: Standard Reaction Enthalpies $\Delta H_{pf}$ (in kJ/mol) of Peptide Bond Formation Involving the $\alpha$- and $\gamma$-Carboxyl Groups of Glutamic Acid, and Other Acids with and without the $\alpha$-Amino Group$^a$.

<table>
<thead>
<tr>
<th>formed peptides</th>
<th>$\Delta H_{pf}$ level of theory</th>
<th>$\Delta H_{pf}$</th>
<th>$pK_a$ (COOH)</th>
<th>N-terminal acids</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M05-2X</td>
<td>G3MP2B3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EC</td>
<td>−14.7</td>
<td>−23.4</td>
<td>2.16</td>
<td>E-$\alpha$-COOH</td>
</tr>
<tr>
<td>$\gamma$-EC</td>
<td>−31.0</td>
<td>−38.8</td>
<td>4.15</td>
<td>E-$\gamma$-COOH</td>
</tr>
<tr>
<td>N-glutaryl-$\alpha$-cysteine</td>
<td>−33.5</td>
<td>−39.7</td>
<td>4.34</td>
<td>glutaric acid I</td>
</tr>
<tr>
<td>DC</td>
<td>−12.7</td>
<td>−22.2</td>
<td>1.95</td>
<td>D-$\alpha$-COOH</td>
</tr>
<tr>
<td>$\beta$-DC</td>
<td>−23.9</td>
<td>−30.3</td>
<td>3.71</td>
<td>D-$\beta$-COOH</td>
</tr>
<tr>
<td>N-succinyl-$\alpha$-cysteine</td>
<td>−34.1</td>
<td>−32.5</td>
<td>4.21</td>
<td>succinic acid I</td>
</tr>
<tr>
<td>AA</td>
<td>−13.5</td>
<td>−21.8</td>
<td>2.33</td>
<td>A-$\alpha$-COOH</td>
</tr>
<tr>
<td>N-propionyl-$\alpha$-alanine</td>
<td>−33.1</td>
<td>−41.4</td>
<td>4.87</td>
<td>propionic acid</td>
</tr>
<tr>
<td>AG</td>
<td>−18.1</td>
<td>−23.7</td>
<td>2.33</td>
<td>A-$\alpha$-COOH</td>
</tr>
<tr>
<td>N-propionyl-glycine</td>
<td>−37.8</td>
<td>−43.6</td>
<td>4.87</td>
<td>propionic acid</td>
</tr>
<tr>
<td>GA</td>
<td>−11.9</td>
<td>−18.1</td>
<td>2.34</td>
<td>G-$\alpha$-COOH</td>
</tr>
<tr>
<td>N-acetyl-$\alpha$-alanine</td>
<td>−33.0</td>
<td>−37.3</td>
<td>4.76</td>
<td>acetic acid</td>
</tr>
</tbody>
</table>

$^a$Results of calculations at the G3MP2B3 and M05-2X/6-311+G(d,p) levels of electronic structure theory combined with the SMD implicit solvent model. The experimental acidity constants of N-terminal acids forming the bond are also shown.$^{36}$ The abbreviations of acid residues are E, $\alpha$-glutamyl; $\gamma$-E, $\gamma$-glutamyl; D, $\alpha$-asparagyl; $\beta$-D, $\beta$-asparagyl; A, alanyl; G, glycyl.
opposite is the expectation for the connection between the base strength of the C-terminus acid and the strength of the peptide bond: strong bases hold the proton more strongly because the covalent character of their N–H bond is more expressed. Overall, the weaker acid is the amino acid that becomes the N-terminus of the peptide, the stronger will be the peptide bond it forms. Similarly, stronger will be the new peptide bond if the C-terminal amino acid is a stronger base. If one of the constituents, either the one that becomes the N-terminal or the one that turns into the C-terminal, is kept the same, one can expect a linear free energy relationship between the reaction enthalpy of peptide formation, \( \Delta H_{pf} \) (and bond strength), and the pK\(_a\) or pK\(_b\) of the respective amino acids. Table 6 shows the acidic dissociation constants of the constituents (in the form of pK\(_a\)) and the calculated reaction enthalpies of the first peptide bond formation steps shown in Figure 22, supplemented by those of peptide or amide bonds formed by a number of related acids. The linearity of the correlation is displayed in Figure 23.

Recalling the general tendencies of changes of acidity with molecular structure, one may attempt to extend the applicability of the rule sketched above. For example, it is known that \( \alpha \)-amino acids are stronger acids than the analogous organic acids lacking the amino group from the \( \alpha \)-carbon atom. To explore the correlation, the reaction enthalpies for peptide/amide formation from a number of amino-acid/amino-acid versus organic-acid/amino-acid pairs were calculated. The results, together with the experimental acid dissociation constants of the N-terminal acids, are listed in Table 6. In the absence of the amino group from the \( \alpha \)-position, the peptide bond is consistently stronger. When the NH\(_2\) group is built into the \( \alpha \)-position of the N-terminal acid, the acid dissociation constant \( K_a \) increases by about 2 orders of magnitude, and \( \Delta H_{pf} \) as well as the strength of the peptide bond decreases by around 20 kJ/mol. For example, glutaric acid, formally obtained when the amino group of glutamic acid is replaced by a hydrogen atom, is a much weaker acid than the \( \alpha \)-carboxyl unit and somewhat weaker than the \( \gamma \)-carboxyl unit in glutamic acid, and forms a peptide bond with cysteine that is about 20 kJ/mol stronger than that in EC. Similar energetic order is observed when the
**Figure 23:** Correlation Between the Standard Reaction Enthalpies of Peptide Bond Formation $\Delta H_{pf}$ (in kJ/mol) and the Experimental Acidity Constants $pK_a$ of the Bond-Forming $N$-Terminal Acids. Results of Calculations at the G3MP2B3 (Green) and M05-2X/6-311+G(d,p) (Blue) Levels of Electronic Structure Theory Combined with the SMD Implicit Solvent Model.
chain length of the N-terminal acid is reduced by a CH$_2$ group. In dipeptides with cysteine as the C-terminal and aspartic acid (aminosuccinic acid, denoted here as D, formally obtained by removing the central carbon of glutamic acid, resulting in "moving" the side-chain carboxyl to the $\beta$-position) as well as succinic acid as the "N-terminal", $\beta$-DC and N-succinyl-L-cysteine, respectively, are more stable than the $\alpha$-dipeptide, DC. This follows the order of acidic strength of the carboxyl group participating in the formation of the peptide/amide bond. A similar correlation was found between $\Delta H_{pf}$ and the basicity of the C-terminal acid when the latter is varied and the N-terminal unit is kept constant. For example, the $\alpha$-peptide bond formed between E and C ($pK_b = 10.78$ [112]) is weaker than that between E and CG ($pK_b = 8.67$ [113]) (Figure 22, blue lines). As Table 6 shows for AG and GA as well as for N-propionyl-glycine and N-acetyl-alanine, the weaker base alanine ($pK_b = 9.87$ [112]) forms weaker peptide/amide bonds than glycine ($pK_b = 9.78$ [112]). The same tendency is obtained with alanine and glycine as with cysteine and glutamic acid. The listed examples confirm that the assumed connection between the acid and base strength and that of the peptide bond is general.

4.3.4  **The Mechanism of Bacterial Protein Synthesis**

Figure 22 and Figure 23 shows a remarkable fact that is also related to the acidic strength of acids participating in peptide bonds. Namely, the formation of the first peptide bond is always less exothermic than that of the second. In other words, the presence of an existing peptide bond in dipeptides makes the new peptide bond stronger. This is not surprising if one recalls that amino acid monomers are always stronger acids and bases than the peptides already containing a peptide bond. The exothermicity of the formation of further peptide bonds is also larger than that of the first (in general, it is similar to that of the second one). Consequently, the formation of the first $\alpha$-peptide bond is the bottleneck in amino acid polymerization. One can expect that, in the biosynthesis of proteins, the formation of the first peptide bond is also the key obstacle because of its relative weakness. The astonishing mechanism of the biosynthesis of bacterial proteins is obviously a smart way of
circumventing the bottleneck. The first peptide bond is formed between a modified methionine, \(N\text{-formylmethionine (fMet)}\), and some other amino acid. The amino group of methionine is not only protected by the enzymatic formylation, ensuring that only its carboxyl group is available for condensation with the next amino acid \([93]\), but since the \(\text{NH}_2\) group of methionine is involved in a peptide bond (in fact, an amide bond), the "dipeptide" fMet will be a weaker acid than methionine. This is an ingenious application of the weak acid–strong peptide bond principle. This facilitates the formation of the first real peptide bond with the next residue. In terms of thermodynamics, the formylation of methionine reduces the Gibbs free energy of the peptide-formation reaction, shifting the equilibrium to a more favorable dipeptide concentration. This way, the bottleneck of the first peptide bond is bypassed. The initiator fMet is later removed, indicating that its only role is the facilitation of the formation of the first peptide bond. The bottleneck of the first peptide bond must have had a role in prebiotic formation of peptides. Since enzymatic catalysis was not yet available, thermodynamics controlled the outcome of chemical reactions. If a reaction was thermodynamically unfavorable, such as the formation of a regular peptide bond, the concentration of its products was very small, giving little chance for AA polymerization. However, if there was a way to bypass the bottleneck, the dimerization step, then the polymerization forming longer peptides became thermodynamically more favored and relatively easy. For example, the first amide bond might have been formed from any weak organic acid. Another alternative is that the acidity of an amino acid was reduced by a "lucky" substitution or by binding to a surface, making the formation of the first dipeptide accessible, which, in a sense, initiated polymerization.

4.3.5 The Strength of the Peptide Bond and the Preference of \(\alpha\)-Amino Acids

As we have seen, the presence of the \(\text{NH}_2\) group next to a peptide bond always reduces the strength of the latter. This factor is in fact missing when the \(\text{NH}_2\) group is two carbon atoms farther away. The difference between the acidic strengths of \(\alpha\)-
versus γ-amino acids is reflected in facts observed in natural peptides. For example, peptide bonds involving the γ-carboxyl group of glutamic acid are very rare in natural peptides and proteins. Obviously, the mechanism of peptide formation and metabolism Nature has developed handles efficiently the "standard" α-peptide bonds. It is not surprising that the individual steps in this "regular" mechanism do not operate for γ-peptides because of their enhanced strength. This is in agreement with GSH being found stable in cells: the large strength of the γ-peptide bond protects it from being degraded by peptidases. This explains why the too strong γ-peptide bond appears only in exceptional cases like GSH. The comparison of the stability of α-peptides and proteins with those based on β- or γ-peptide bonds shows that, if the amino group in amino acids were not next to the carboxyl group but, instead, in the β- or γ-position, the formed peptide bond would be much stronger. However, while such peptides would be easier to make, they would have a strong disadvantage: they would be less easy to transform because the peptide bond would be too strong. This can explain Nature's preference of α-amino acids with respect to β- or γ-amino acids.
4.4 Conclusions

The factors influencing the thermodynamics of the formation of peptide bonds was studied on the example of glutathione and related compounds with quantum chemical methods. A correction procedure is proposed for the calculation of the Gibbs free energy of peptide formation using standard quantum chemical techniques and continuum solvent models. The enthalpy of the peptide formation reaction 4.1, reflecting the strength of peptide bonds, was shown to be linearly related to the acidity and basicity of the N- and C-terminal amino acids. The stronger acid the N-terminal and the weaker base the C-terminal amino acid, the weaker is the peptide bond. Since the γ-carboxyl group of glutamic acid is weaker than the α-COOH, the dipeptide γ-EC and the tripeptide GSH are more stable than their regular, α-peptide isomers. The preference for formation of γ-EC and GSH is due to the enhanced stability of the γ-peptide bond. The correlation between peptide bond strength and acidic strength proves to be a key factor in protein synthesis. Under thermodynamic control, the stronger the peptide bond the larger is the equilibrium concentration of the peptide. The closer the biosynthesis of proteins can follow the thermodynamics of the individual reaction steps the less effort and less smart technology is needed to make and break bonds efficiently. All essential amino acids are relatively strong acids and the α-peptide bonds they form as N-terminal partners are relatively weak. Dipeptides, on the other hand, are weaker acids and make stronger peptide bonds, so that once a peptide bond has been created, the formation of the next one is favored. This explains why the relatively strong γ-peptide bond is formed first in glutathione synthesis. More importantly, by considering the thermodynamics of peptide formation, one can understand why the biosynthesis of bacterial proteins starts with the formation of a peptide bond to the carboxyl group of N-formylmethionine. The role of fMet is similar to a catalyst or rather to an activator. The acidic strength of methionine is reduced by formylation, enabling it to form a stronger peptide bond whose strength is similar to that of α-peptide bonds in a peptide chain. This way the "bottleneck of the first peptide bond" is circumvented and fMet can be released from
the N-terminus. The energetics of peptide bond formation also adds an item to the list why Nature relies on α-amino acids in proteins: β- or γ-peptide bonds are stronger than the α-peptide bond. While the enhanced strength is favorable in the first step of the synthesis, later too strong peptide bonds would be more difficult to manipulate in living organisms. The weak acid–strong peptide bond principle can also be considered as one of the factors why prebiotic relicts like glutathione are still among the chemical agents in living organisms. At the beginning of chemical evolution, the larger exothermicity of the γ-dipeptide formation was probably one of the reasons why GSH has appeared, and when it successfully fulfilled its role as an antioxidant, it was retained in the redox arsenal of cells. Finally, the correlation between the acid and base strength and the reaction enthalpy of peptide bond formation offers the possibility of being utilized in the design of new synthetic pathways for peptide synthesis, allowing one to control the steps of the process by varying the acidity and/or basicity of the amino acids.
As a consequence of our detailed computational study of the different properties of glutathione the following new scientific results were obtained:

1st Thesis Point

The radical forming ability of glutathione in a thermodynamic sense is determined by means of quantum chemical calculations. Bond dissociation energies, BDEs, have been calculated in both gas and aqueous phases for all X–H bonds (where X can be C, N, O, and S) of glutathione, and their values have been compared (Chapter 2).

---

New Scientific Results

- The O–H bond dissociation of the carboxyl group has the smallest bond dissociation energy in vacuum.

- In the aqueous phase, the weakest X–H bond is the N–H bond of the glutamine residue (Q_{N1,2}) in the vicinity of the negatively charged carboxyl group. In both cases, CO_{2} formation (decarboxylation) can accompany the breaking of the X–H bond, making the dissociation energetically more favorable than the cleavage of the S–H bond (Figure 2).

- The N–H bond is stronger than the C–H bond in the gas phase, but protonation significantly weakens it. Furthermore, C–H bonds with \( \alpha \)-carbons are found to be about as weak as the sulfhydryl bond of the cysteine residues.

- Electron delocalization was found to have an important influence on the antioxidant potential. As clearly evident from our results, glutathione has five possible positions to scavenge a free radical in biologically relevant conditions (3 \( \alpha \)-C–H, S–H and N–H, Figure 2).
From a methodological point of view the following results were obtained (Chapter 2):

- It was proved that the calculations using G3MP2B3 composite method provides bond dissociation enthalpy (DH$_{298}$) values consistent with the experimental ones. Therefore, it was concluded that the G3MP2B3 model chemistry is able to provide reasonable bond dissociation enthalpies for glutathione fragments as well.

- In order to reduce the cost of the calculations by the replacement of the G3MP2 energy with accurate single point calculations, six density functionals, namely, MPWKCIS, MPWKCIS1K, M06, TPSS1KCIS, TPSSh, and B3LYP, have been tested against G3MP2 for obtaining accurate bond dissociation energies. These single point calculations were carried out on optimized glutathione fragment structures obtained at the B3LYP/6-31G(d) level of theory in both vacuum and solvent. The MPWKCIS1K/6-311+++G(3df,2p)//B3LYP/6-31G(d) level of theory provides the best correlation with the G3MP2B3 method for BDEs in both phases, and therefore, this method is recommended for similar calculations.

Comparative MD simulations were conducted on GSH and GSH/OH\textsuperscript{*} systems. Based on these, the radical recognition process of GSH was described and the OH radical attractor regions of GSH were identified (Chapter 3).

- Two main steps were identified during the radical recognition process, namely catching and steering (Figure 3). In \( \sim 78\% \) of all interactions characterized, strong complexes were formed between anionic carboxyl groups and the OH radical (catching). After this catching step, the strong carboxyl-OH\textsuperscript{*} complexes could evolve additional interactions with the other parts of GSH, stabilized by both the donor and acceptor features of the OH radical.

- Glycine residue dominates the steering role in the recognition step, while the glycine-hydroxyl radical complexes could facilitate further interactions with the thiol group, \( \alpha \)- and \( \beta \)-carbons of the cysteine residue via the OH\textsuperscript{*} lone pair electron. The glutamic acid residue does not show this property during the MD simulations.

- Additional quantum chemical calculations on appropriately selected GSH/OH\textsuperscript{*} complexes revealed exothermic heats of formations. These complexes, containing two or more intermolecular interactions (see Figure 18), identified as the starting configurations for the hydrogen atom migration to quench the hydroxyl radical and to complete the radical scavenging mechanism \textit{via} different reaction channels.

\[\text{This thesis point is based on the following manuscript: B. Fiser, B. Jójárt, I. G. Csizmadia, B. Viskolcz, "Glutathione - Hydroxyl Radical Interaction: A Theoretical Study on Radical Recognition Process", PLOS ONE, vol. 8 (9), pp. e73652, 2013.}\]
4th Thesis Point

By calculating the formation of glutathione, and its α analogue, L-glutamyl-L-cysteinyl-glycine along with similar systems, the thermodynamics of regular (α-) and isopeptide bond formation was described (Chapter 4).

- The enthalpy of the peptide formation, reflecting the strength of peptide bonds, was shown to be linearly related to the acidity and basicity of the N- and C-terminal amino acids (Figure 23). The stronger acid the N-terminal and the weaker base the C-terminal amino acid, the weaker is the peptide bond. Since the γ-carboxyl group of glutamic acid is weaker than the α-COOH, the dipeptide γ-EC and the tripeptide GSH are more stable than their regular, α-peptide isomers. The preference for formation of γ-EC and GSH is due to the enhanced stability of the γ-peptide bond. Therefore, the weak acid–strong peptide bond principle can also be considered as one of the factors why glutathione is still among the chemical agents of living organisms. At the beginning of chemical evolution, the larger exothermicity of the γ-dipeptide formation was probably one of the reasons why GSH has appeared, and when it successfully fulfilled its role as an antioxidant, it was retained in the redox arsenal of cells.

- Based on the weak acid-strong peptide bond principle the role of formylmethionine (fMet) in the first step of the bacterial protein synthesis can also be viewed in a new way. According to this, fMet act as a catalyst or rather an activator (Figure 20). The acidic strength of methionine is reduced by formylation, enabling it to form a stronger peptide bond whose strength is similar to that of α-peptide bonds in a peptide chain. This way the "bottleneck of the first peptide bond" is circumvented and fMet can be released from the N-terminus.

New Scientific Results

- The correlation between the acid and base strength and the reaction enthalpy of peptide bond formation offers the possibility of being utilized in the design of new synthetic pathways for peptide synthesis, allowing one to control the steps of the process by varying the acidity and/or basicity of the amino acids.
New Scientific Results

5th Thesis Point

By our research, we contributed to the elucidation of the factors determining the strength of peptide bonds that may explain the preference of the α-peptide bond in proteins (Chapter 4).

- The correlation between peptide bond strength and acidic strength proves to be a key factor in protein synthesis. Under thermodynamic control, the stronger the peptide bond the larger is the equilibrium concentration of the peptide. The closer the biosynthesis of proteins can follow the thermodynamics of the individual reaction steps the less effort and less smart technology is needed to make and break bonds efficiently. The energetics of peptide bond formation may also add an item to the list why Nature relies on α-amino acids in proteins: β- or γ-peptide bonds are stronger than the α-peptide bond. While the enhanced strength is favorable in the first step of the synthesis, later too strong peptide bonds would be more difficult to manipulate in living organisms.

6th Thesis Point

A correction procedure is proposed for the calculation of the Gibbs free energy of peptide formation using standard quantum chemical techniques and continuum solvent models (Chapter 4), but further verification necessary to prove its general applicability.

Glutathione (γ-L-glutamyl-L-cysteinyl-glycine, GSH) is essential in a number of biochemical processes in living organisms, including repair of oxidative damage and defense of the central nervous system against free radicals. It has also a role in apoptosis, signal transduction and gene expression.

Several research programs have been conducted to study the different properties of GSH. However, there are still blind spots, which are waiting to be elucidated. We tried to throw some light on these and as a result three papers have been published so far about glutathione (J. Phys. Chem. B, vol. 115(38), pp. 11269-11277, 2011; PLOS ONE, vol. 8(9), pp. e73652, 2013; J. Phys. Chem. B, vol. 119(10), pp. 3940-3947, 2015), which form the basis of my dissertation and the theses presented here.

In our earliest work the radical forming ability of GSH in a thermodynamic sense is determined by means of quantum chemical calculations. Furthermore,
Summary

the radical scavenging ability of the neutral and anionic GSH was compared (J. Phys. Chem. B, vol. 115(38), pp. 11269-11277, 2011).

Thereafter, we tried to shed light on the radical scavenging mechanism of glutathione. To overcome the limitation of the large flexibility of GSH, structures for further \textit{ab initio} calculations were determined by non-reactive molecular dynamics (MD) simulations. A long comparative MD simulation were set for the solvated GSH and GSH/HO* as model systems. Based on the MD trajectories the different interactions between GSH and HO* were characterized. Moreover, the non-reactive MD trajectories combined with \textit{ab initio} calculations allow us to describe a detailed free radical recognition and radical scavenging process (PLOS ONE, vol. 8(9), pp. e73652, 2013). Based on these findings, new better antioxidant molecules can be designed.

The formation of \(\alpha\)- and isopeptide bonds were in the focal point of our most recent work. The stability of GSH and its \(\alpha\) analogue, ECG (L-glutamyl-L-cysteinyl-glycine) and other isopeptide-normal peptide pairs were computed and compared using quantum chemical methods. The obtained results showed that special peptides can be formed before the appearance of living organisms (J. Phys. Chem. B, vol. 119(10), pp. 3940-3947, 2015).
Összefoglaló

Összefoglaló


If I have seen further, it is by standing on ye shoulders of giants.
— Isaac Newton,
Letter to Robert Hooke (1675)
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There are more things in heaven and earth, Horatio,
Than are dreamt of in your philosophy.
— Hamlet, Scene V
List of Publications

Articles Related to the Thesis Points

IF ~ 10.417


List of Publications
Poster and Oral Presentations Related to the Thesis Points


European Conference on Chemistry for Life Sciences (4ECCLS), Budapest, Hungary, 31 August - 3 September 2011.

List of Publications

Articles not Related to the Thesis Points

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For from him and through him and for
him are all things. To Him be the glory
forever! Amen.

Romans 11:36

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Témavezetői Ajánlás

Fiser Béla

Theoretical Investigations of Glutathione – A Unique Antioxidant

című PhD értekezéséhez


A jelölt eddig 3 könyvfejezet és 30 cikk elkészítésében vett részt, melyeket elismert hazai és nemzetközi folyóiratokban tett közé munkatársaival (JPCB, PCCP, JACS stb.). Publikációi összesített impakt faktora 107,4, ami figyelembe véve fiai korát kiemelkedő eredménynek számít. Számos konferencián és workshopon vett részt, melyeken összesen 21 előadást tartott és 16 posztért mutatott be, s további 6 előadás és 50 poszter társzerzőjeként működött közre.

Meggyőződésésem, hogy Fiser Béla doktori diszsertációja és tudományos eredményei is alátámasztják, hogy alkalmas önálló kutatási tevékenység végzésére, s együttal érdemes a PhD fokozat megszerzésére is.


Prof. Dr. Viskolcz Béla