Free Radical-Initiated Unfolding of Peptide Secondary Structure Elements

Thesis of the Ph.D. Dissertation by

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Introduction

Cellular damage by free radicals has been studied extensively in recent years, and it has been postulated that free radicals are a causative factor in several pathological conditions, including aging. The aim of this thesis is to determine the implications of hydrogen atom abstraction by free radicals on the structure of peptides and proteins, or more precisely, if this process can initiate peptide unfolding. This is accomplished by computing the changes in the thermodynamic parameters (free energy, enthalpy, and entropy) of hydrogen abstraction from model amino acid residues and peptides using quantum chemical calculations. Force field parameters were developed for a C_{α}-centered radical of the alanyl diamide and were tested in molecular dynamics (MD) simulations of a heptapeptide.

The structure of Gly (*N*-Ac-Gly-NHMe) and Ala (*N*-Ac-Ala-NHMe) residues and pentapeptides (*N*-Ac-GGGGG-NHMe, *N*-Ac-AAAAA-NHMe) were compared to the respective peptide radical to enable the effect of chirality on the reactivity and stability of the residues to be determined. The effect of peptide secondary structure was determined by studying the peptides as helices and as extended chains. Hydrogen abstraction reaction barrier heights were computed with the Gly and Ala transition state structures in different conformations and the pre-reaction and post-reaction van der Waals complexes were identified by minimizing the transition state structures along the intrinsic reaction coordinate. It will be shown how free radicals can initiate the unfolding of a peptide as depicted in **Figure 1.**

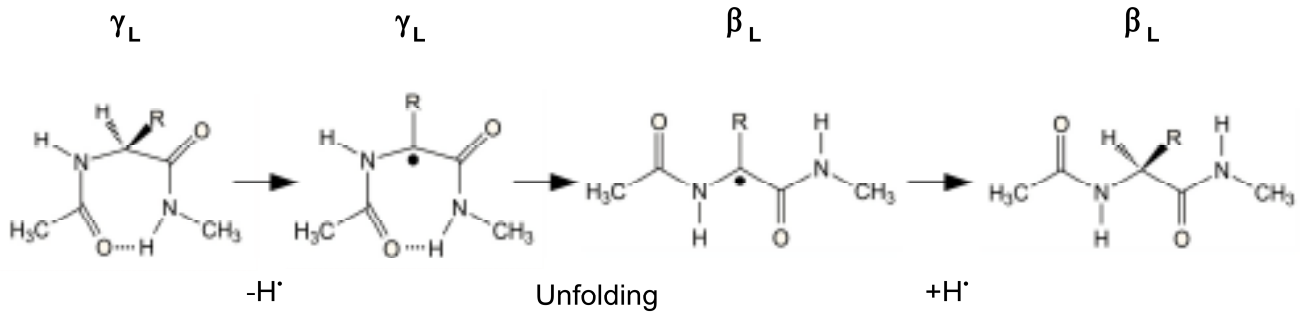

Figure 1. Free radical-initiated unfolding of a peptide from the γ_L to the β_L conformations, showing the loss and subsequent gain of the hydrogen atom.

Methods, Results and Discussion

Hydrogen abstraction from Gly and Ala residues

The Gly and Ala residues were modeled as diamides, which enabled the C_{α} to be studied in between neighboring amide bonds, with a terminal methyl group representing the C_{α} of adjacent residues. The ϕ and ψ angles of the diamides were rotated in 30° increments from 0° to 360°

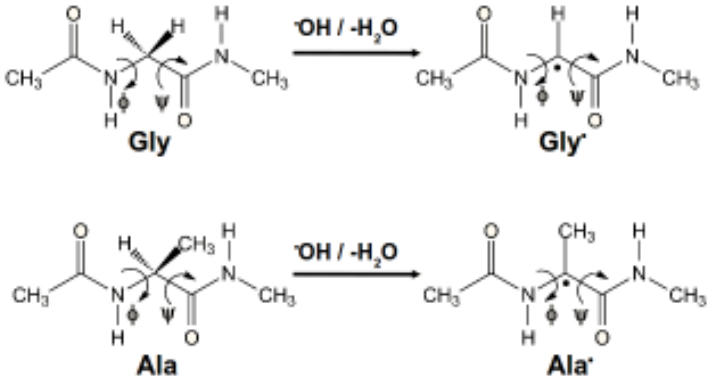

Figure 2. The Gly, Gly•, Ala and Ala• diamide structures. The ϕ and ψ angles which were the dependent variables for the potential energy surfaces are also shown.

and the energy of each structure was plotted as a function of the ϕ and ψ angles in each potential energy surface (PES). The PES showed that the conformers of the Gly• and Ala• peptide radicals were much different than the closed-shell Gly and Ala residues. The relative energy values of the conformers are much closer to each other in the case of the Gly and Ala than in Gly• and Ala•, the latter two of which are also constrained to the β_L or 'extended' conformation by the high energy 'barrier', as shown in **Figure 3**.

> *I. The differences between the potential energy surfaces of Gly and Gly• and between Ala and Ala• suggest that a peptide or protein with a Ca radical would unfold, at least at the residue from which the hydrogen was removed.*

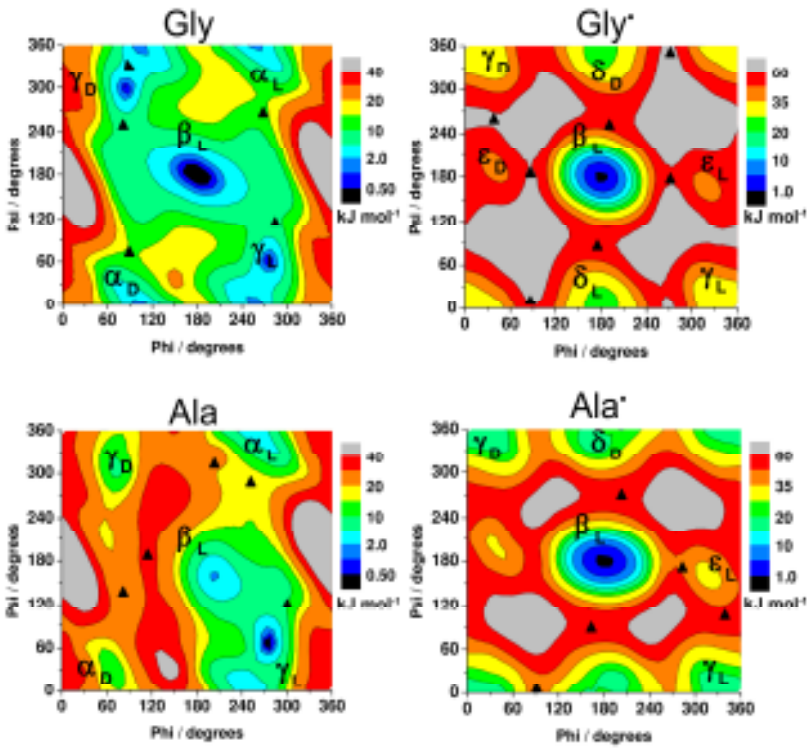

Figure 3. The potential energy surfaces of the Gly, Gly•, Ala, and Ala• amino acid diamides. The minima are labeled by their respective conformation whereas the transition states are labeled with triangles.

The conformers of the Gly and Ala diamides were the starting structures for the optimization of the transition states, pre-reaction van der Waals complexes, and post-reaction van der Waals compexes of the Gly and Ala residues in H abstraction reactions with ˙OH. Subsequent hydrogen abstraction reactions from H_2O_2 by Gly˙ and Ala˙ were used to compute the energy required to convert the peptide radicals back to the respective Gly and Ala residues. It was shown the energy of the system during the subsequent reactions remains approximately 90 kJ mol^{-1} below that of the entrance level, as shown in **Figure 4**.

II. The ˙OH/H_2O_2 abstraction reaction coordinate confirms that the unfolding of Gly or Ala initiated by the H abstraction reactions involving ˙OH/H_2O_2 is energetically favorable. The effect of peptide conformation on the thermodynamics of these reactions is also discussed.

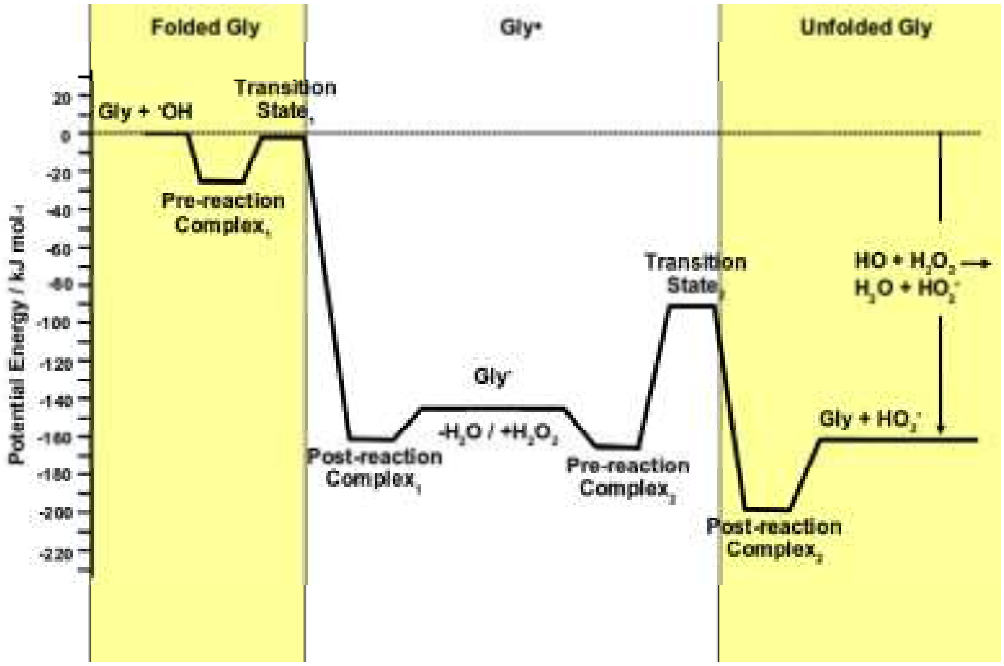

Figure 4. The reaction coordinate diagram showing the E_{pot} of the hydrogen abstraction from Gly in the β_L conformation by ˙OH and the subsequent hydrogen abstraction from H_2O_2 by Gly˙ in the β_L conformation. The energy of the two reactions stays below the energy of the entrance level.

Hydrogen abstraction reactions from Gly and Ala Pentapeptides

Hydrogen abstraction reactions were also studied in extended and helical Gly ($G5_{EXT}$, $G5_{HEL}$) and Ala ($A5_{EXT}$, $A5_{HEL}$) pentapeptides, with ˙OH, HO_2˙ and O_2˙⁻ used as radicals, and the C_α amide N and C_β (for A5 only) of residue 3 were the reaction sites, as shown in **Figure 5**. The changes in potential energy (ΔE^o), free energy (ΔG^o), enthalpy (ΔH^o), and entropy (ΔS^o) were calculated for these reactions.

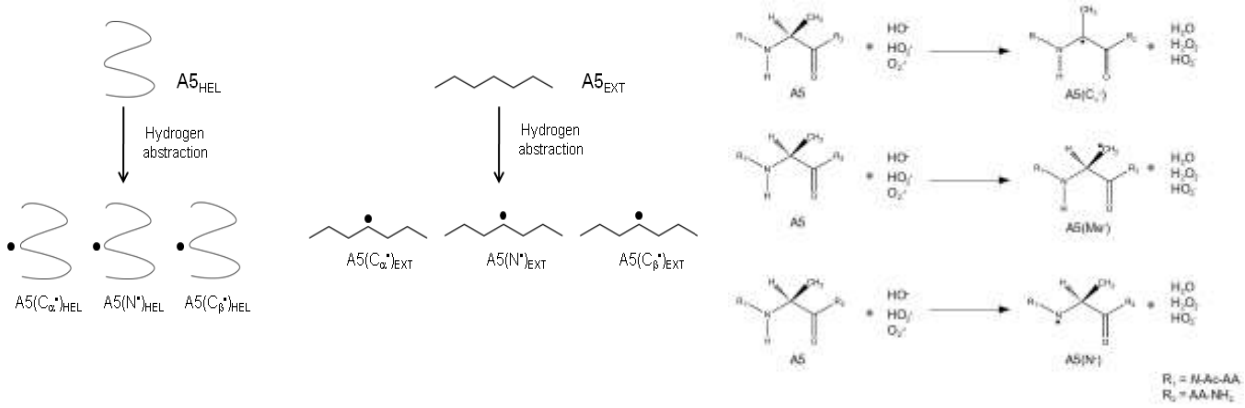

Figure 5. A Schematic representation of the peptides computed in this study. The structures of the $A5_{HEL}$ and $A5_{EXT}$ peptides were compared to the structures of the respective peptide radicals after hydrogen abstraction (shown to the right). The G5, G_{EXT} and G_{HEL} peptides were compared in a similar way.

III. In a comparison of the structures of the peptide radicals were compared to those of the respective G5 and A5 peptides, large deviations between the peptide structures were observed in the case of the amide N radicals, whereas very little was observed in the Cb radicals.

The structural changes are shown in **Figure 6**. The unpaired electron was delocalized in the C_α radicals, as shown by changes in the bond lengths within residue 3.

The ΔE^o, ΔG^o, ΔH^o and ΔS^o of the conversion from $G5_{HEL}$ to $G5_{EXT}$ and from $A5_{HEL}$ to $A5_{EXT}$ indicated that only H abstraction from the C_α increased the propensity of the G5 and A5 peptides to unfold. It was also shown that it is most favorable for $^{\bullet}OH$ to abstract an H from a peptide, followed by the $HO_2^{\bullet}$, and $O_2^{-\bullet}$ radicals.

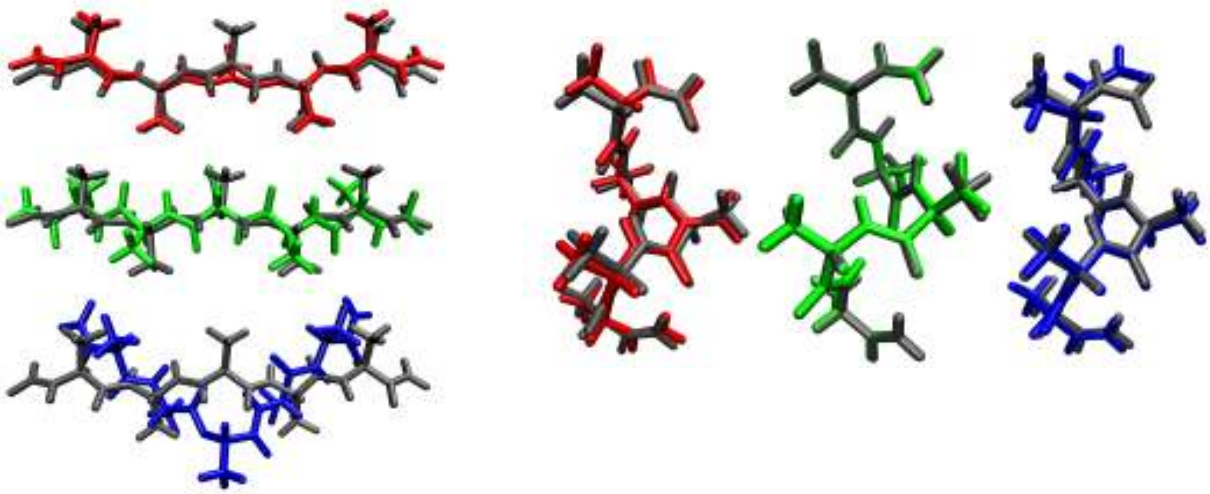

Figure 6. Structural alignment of the A5 peptide and the A5 peptide radicals in the extended (left) and helical (right) conformation. The alignment of A5($C_\alpha^{\bullet}$) peptide is shown in red, that of A5($Me^{\bullet}$) is shown in green and that of A5($N^{\bullet}$) is shown in blue, whereas the A5 peptide is shown in gray. The structures of the G5 peptides were also aligned in this way.

IV. Hydrogen abstraction is more favorable with the G5 and A5 in the extended conformation than in the helical conformation, suggesting that secondary structures protect the peptide from oxidative attack.

The values of the reaction barrier heights of Gly and Ala also suggest that H abstraction occurs fastest in the β conformation.

V. It has been shown that the hydrogen atom 'side chain' of Gly contributes to the greater propensity to Gly residues to unfold.

Molecular dynamics simulations of Ala heptapeptides

VI. Force field parameters for the Ca-centered Ala and Gly radicals were developed for use with the OPLS-AA and OPLS-AA force-fields, respectively.

The parameterization was accomplished by minimizing the sum of squares deviations between the quantum chemical and OPLS-AA energy hypersurfaces. These parameters were used to determine the effect of the C_α-centered Ala radical on the structure of a hepta-alanyl peptide in MD simulations.

A negligible sum-of-squares energy deviation was observed in the stretching parameters, and the newly-developed OPLS-AA torsional parameters showed a good-agreement with the LMP2/cc-pVTZ(-f) hypersurface. The parameterization also demonstrated that force-field-derived equilibrium bond length and bond angle values can deviate from the quantum chemical equilibrium values, and that the improper torsional parameters should be developed explicitly with respect to the coupled torsional parameters.

VII. The MD simulations showed planar conformations of the Ca-containing residue (Alr) are preferred and these conformations increase the formation of g- a- and p-turn structures depending on the position in the turn occupied by the Alr residue.

This feature is shown in the density Ramachandran maps of the Ala and Alr residues (**Figure 7**). The Ramachandran map of the of the central Ala residue of the heptapeptide shows that the $-180^o \leq \phi \leq -30^o$ region of the Ala residue, which are stable for L- amino acid residues, was highly populated, whereas the $-30^o \leq \phi \leq +30^o$ region of the Alr residue, which contains more planar conformers, was highly populated. These maps agree well with the Ala potential surfaces shown in **Figure 3**.

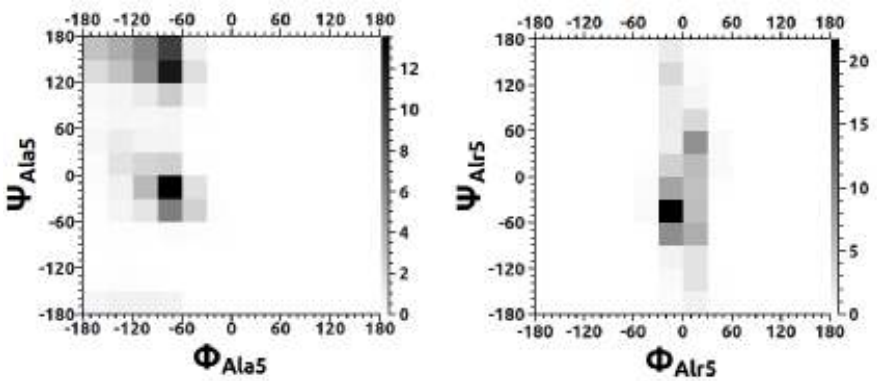

Figure 7. The density Ramachandran maps of the central amino acid (Ala_5 or Alr_5) for the ALA (left) and ALR (right) peptides.

VIII. Higher-ordered structures are destabilized by Alr except when this residue occupies position "i + 1" of the 310-helix.

The results presented in this thesis offer new insight in to the protein-misfolding mechanisms initiated by H-abstraction from the C_α of peptide and protein residues. The implications of this research to protein misfolding diseases such as Alzheimer's, Parkinson's and Creutzfeld-Jakob diseases is also discussed.

Publications related to this dissertation

[1] M. C. Owen, I. Komáromi, R. F. Murphy, S. Lovas. The conformational preference of C^{α}-centered radicals in proteins. J. Mol. Struct. (Theochem) 759, (2006) 117-124. (**IF = 1.495**)

[2] I. Komáromi, M. C. Owen, R. F. Murphy, S. Lovas. Development of glycyl radical parameters for the OPLS-AA/L force field. J. Comput. Chem. 29, (2008) 1999-2009. (**IF = 3.39**)

[3] M. C. Owen, B. Viskolcz and I. G. Csizmadia. Quantum chemical analysis of the unfolding of a penta-alanyl 3_{10}-helix initiated by $HO^{\bullet}$, $HO_2^{\bullet}$ and $O_2^{-\bullet}$ J. Phys. Chem. B 115, (2011) 8014-8023. (**IF = 3.603**)

[4] M. C. Owen, B. Viskolcz and I. G. Csizmadia Quantum chemical analysis of the unfolding of a penta-glycyl 3_{10}-helix initiated by $HO^{\bullet}$, $HO_2^{\bullet}$ and $O_2^{-\bullet}$ J. Chem. Phys. 135, (2011) 035101. (**IF = 2.920**)

[5] M. C. Owen, Milán Szőri, Imre G. Csizmadia and Béla Viskolcz. Conformation-Dependent $\bullet OH/H_2O_2$ Hydrogen Abstraction Reaction Cycles of Gly and Ala Residues: A Comparative Theoretical Study. J. Phys. Chem. B 116, (2012) 1143-1154. (**IF = 3.603**)

[6] M. C. Owen, I. Komáromi, B. Jojárt, I. G. Csizmadia and B. Viskolcz. The development of alanyl radical parameters for the OPLS-AA/L force field. J. Chem. Theory Comput. (*Submitted*) (**IF = 5.629**)

Cumulative impact of accepted journal articles: **15.011**

Other publications

[7] M. C. Owen, M. Szőri, B. Jojárt, B. Viskolcz, I. G. Csizmadia. Conformational and Thermodynamic Analysis of the COXIB Scaffold Using Quantum Chemical Calculations. Int. J. Quant. Chem. 112, (2011) 922-936. (**IF = 1.302**)

[8] N. Y. Palermo, J. Csontos, M.C. Owen, R.F. Murphy, S. Lovas. Aromatic-backbone interactions in model α-helical peptides. J. Comput. Chem. 28, (2007) 1208-1214. (**IF = 4.297**)

[9] T. . Pecora, M. C. Owen, C. N. J. Marai, D. H. Setiadi, G. A. Chass. Bridging the gap between pure science and the general public: comparison of the informational exchange for these extremities for scientific awareness. J. Mol. Struct. (Theochem) 666-667, (2003) 699-706. (**IF = 1.021**)

Cumulative impact of supplementary journal articles: **6.620**