Theoretical Investigations of

Radical-Mediated Protein Oxidation

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Te kai rapu, ko ia te kite
Tama tu, tama ora, tama moe, tama mate.
Takoto kau ana te whanau a Tane.
He manga wai koia kia kore e whitikia

Declaration

The work described in this thesis, to the best of my knowledge, is original and does not contain material, except where appropriately referenced, that has been published or presented by any other person, nor has it been submitted for a degree or diploma at any other University or College.

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Acknowledgements

The following is an excerpt from "Tout est Bien", an article in Voltaire's Philosophical Dictionary, which has hung over my workspace for the last three years. It reminds me to persevere; that things have an order and by design will come to natural fruition. A contradiction I hear you say! However, think of why the contrary choice is made and you will find yourself in an infinitely turning circle.

> *"Here we have a clear and fixed order among every kind of animal. There is order everywhere. When a stone is formed in my bladder it is by means of admirable mechanics: calculous juices pass little by little into my blood, they filter into the kidneys, pass through the ureters, deposit themselves in my bladder, and assemble there by an excellent Newtonian attraction; the stone is formed, gets bigger, I suffer pains a thousand times worse than death, by the most elegant arrangement in the world. A surgeon, having perfected the art invented by Tubalcain, comes to thrust a sharp and cutting iron into the perineum, and takes hold of my stone with his pincers. It breaks under his efforts by a necessary mechanism; and by the same mechanism I die in frightful torments. All this is good, all this is the evident consequence of inalterable physical principles. I agree with them, and I knew it as well as you did."*

I have crossed the Rubicon! This journey has had its share of vicissitudes and I have needed good friends and excellent guidance to arrive where I am.

Firstly and foremostly, I would like to thank my supervisor, Professor Leo Radom. He has provided me with the problem, the tools, and the solution. Throughout the last four years, he has passed on his singular wisdom and direction to the extent that I have been able to combine all three of these aspects together.

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List of Publications

Parts of this thesis have been published in peer-reviewed journals:

(1) Wood, Geoffrey P. F.; Henry, David J.; Radom, Leo. **Performance of the RB3-LYP, RMP2, and UCCSD(T) Procedures in Calculating Radical Stabilization Energies for NHX Radicals.** *Journal of Physical Chemistry A* **2003**, *107*, 7985–7990.

(2) Wood, Geoffrey P. F.; Moran, Damian.; Jacob, Rebecca,; Radom, Leo **Bond Dissociation Energies and Radical Stabilization Energies Associated with Model Peptide-Backbone Radicals.** *Journal of Physical Chemistry A* **2005**, *109*, 6318–6325.

(3) Wood, Geoffrey P. F.; Rauk, Arvi; Radom, Leo **Modeling beta-scission Reactions of Peptide Backbone Radicals: Backbone C–C Bond Fisson** *Journal of Chemical Theory and Computation*, **2005**, *1*, 889–899.

(4) Wood, Geoffrey P. F.; Barnes, Ericka C.; Petersson, George A.; Radom, Leo; Frisch, Michael J.; Montgomery Jr., John A. **Formulation and Evaluation of the ROCBS-QB3 Procedure** *Journal of Chemical Physics,* Submitted.

(5) Wood, Geoffrey P. F.; Easton, Christopher J.; Rauk, Arvi; Davies, Michael J. Radom, Leo **The Effect of Side Chains on Competing Pathways for β-Scission Reactions of Peptide-Backbone Alkoxyl Radicals** *Journal of Physical Chemistry A*, Submitted.

In addition, a number of parallel projects were carried out associated with the work presented in this thesis:

(6) Coote, Michelle L.; Wood, Geoffrey P. F.; Radom, Leo. **Methyl Radical Addition to C:S Double Bonds: Kinetic versus Thermodynamic Preferences.** *Journal of Physical Chemistry A* **2002**, *106*, 12124–12138.

(7) Moran, Damian; Jacob, Rebecca; Wood, Geoffrey P. F.; Coote, Michelle L.; Davies, Michael J.; O'Hair, Richard A. J.; Easton, Christopher J.; Radom, Leo **Rearrangements in Model Peptide-Type Radicals via Intramolecular Hydrogen-Atom Transfer** *Helvetica Chimica Acta,* Accepted.

(8) Wood, Geoffrey P. F.; Rintelman, Jamie, M.; Gordon, Mark S; Radom, Leo **Solvation of the Glycyl Radical** *in preparation.*

(9) Wood, Geoffrey P. F; Radom, Leo; Smith, David M. **The Nature of Glycine and its α-Carbon Radical in Aqueous Solution: A Theoretical Investigation** *in preparation.*

Summary

This thesis primarily details the application of high-level *ab initio* quantum chemistry techniques in order to understand aspects of free-radical mediated protein oxidation. Traditionally, product analysis and electron paramagnetic resonance (EPR) spectroscopy are the primary means for elucidating the chemistry of protein oxidation. However, in experiments involving relatively small proteins reacting with a controlled radical-flux, a vast array of compounds can be produced, which are often difficult to analyse. Quantum chemical techniques on the other hand, can calculate the properties of any particular species directly, without suffering from the problems associated with experiment, such as side-reactions and chain processes. The results presented in this thesis are aimed at elucidating mechanistic details of protein oxidation, which might otherwise be difficult to probe experimentally.

Chapter 1 gives an overview of the free-radical hypothesis of disease and ageing. Protein-derived radicals can undergo a variety of reactions, with the particular reaction that occurs depending on numerous aspects. Many types of reactions have been identified through radiolysis experiments of amino acids, and these are detailed in this chapter. In addition, the key reactive species are characterized and their different chemistries explained.

Chapter 2 details the theoretical tools used throughout this thesis. Species with unpaired electrons (radicals) present unique problems for quantum chemistry to handle, thus an appropriate choice of theoretical technique is needed. The approach taken in this thesis is to use high-level compound methods, many of which have been directly formulated to give improved results for radical species, to provide benchmark quality results by which other less demanding techniques can be assessed.

During the course of this study, it became apparent there was a void in the armoury of tools that could be used for the theoretical chemistry calculations. Chapter 3 details the formulation of a new tool in an attempt to fill this gap. Historically, the formulation of this new procedure came after much of the work in this thesis had been carried out. Thus, for the study of many of the reactions of this thesis the new method has not been used. However, it is most appropriate to place its formulation after summarizing the current status of techniques in common use today.

Chapters 4 and 5 detail computations carried out on models of peptides containing backbone carbon- and nitrogen-centered radicals. A number of different theoretical techniques are used in these chapters, ranging from the highly accurate and computationally intensive to the less reliable and less demanding. The highly accurate techniques are used to gauge the accuracy of the other less demanding theoretical techniques so that the latter can be used with confidence in larger systems. Not only is the choice of theoretical technique important but also the judicious choice of model is essential. With this in mind, models are incrementally built until convergence of the particular property of interest is reached.

Chapters 6 and 7 detail the calculations of β-scission reactions of alkoxyl radicals, which are a particular class of reaction known to occur on peptide backbones. Alkoxyl radicals are particularly difficult for theory to describe correctly. Therefore, Chapter 6 extensively assesses and then identifies the theoretical methods needed to portray them. Chapter 7 uses the techniques identified in the previous chapter in order to predict how the preference for a particular type of β-scission reaction changes.

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List of Abbreviations

The following are the most common abbreviations used in this work.

Theoretical Procedures:

HF	Hartree-Fock theory
MP*n*	*n*th order Møller-Plesset perturbation theory
CCSD(T)	Coupled-cluster with singles and doubles and a perturbative treatment of triples
B3-LYP	Becke's three-parameter density functional model (B3), incorporating the Lee, Yang and Parr (LYP) gradient correction to the correlation energy
BMK	Density functional model formulated by Boese and Martin
MPWB1K	A combination of a modified Perdew and Wang exchange functional and the B1K correlation functional formulated by Truhlar and co-workers.
U/R	Each of the methods above have been used with either a restricted (designated by an R prefix) or unrestricted (designated by a U prefix) wave function.
G3X(MP2)-RAD	The RAD variant of the G3X(MP2) compound method
W1	The Weizmann-1 compound method for benchmark calculations
CBS	Complete basis set model chemistries formulated by Petersson et al.
CBS-QB3	The QB3 variant of Petersson's complete basis set methods
U-CBS-QB3	The QB3 variant of Petersson's complete basis set methods excluding the empirical correction for spin-contamination
ROCBS-QB3	The QB3 variant of Petersson's complete basis set methods based on restricted open-shell methods

Amino Acids:

Ala	Alanine	Leu	Leucine
Arg	Arginine	Lys	Lysine
Asp	Aspartate	Met	Methionine
Asn	Asparagine	Phe	Phenylalanine
Cys	Cysteine	Pro	Proline
Gln	Glutamine	Ser	Serine
Glu	Glutamic acid	Thr	Threonine
Gly	Glycine	Trp	Tryptophan
His	Histidine	Tyr	Tyrosine
Ile	Isoleucine	Val	Valine