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The review of the work by Marta Teresa Ignasiak entitled „Study of the mechanism of radiation- and photo-induced oxidation of methionine containing peptides" submitted to the Scientific Board of the Faculty of Chemistry at Adam Mickiewicz University in Poznan, Poland as Ph.D. Thesis.

General remarks

The work concerns studies of oxidation of peptides containing methionine. The structure of the investigated peptides was chosen in order to give background for further studies of much more complex structures as human kinins, Met-Lys-Bradykinin and Neurokinin B, playing an important role in regulating blood pressure or in pregnancy, respectively. Products of methionine oxidation have been identified in organisms of patients with Alzheimer or prion diseases. There are many factors leading to the generation of oxidizing radicals *in vivo*, oxidizing stress connected with various diseases, exogeneous factors connected with pollution, ionizing radiation (medical diagnostics) or sunlight. Additionally the Author has included into her studies sulisobenzone and oxybenzone, benzophenone derivatives that are components of commercially used sunscreens. The photochemical stability of these two compounds is of great importance as their excited states may cause oxidative damage to methionine containing peptides and also proteins.

Taking the above into account the topic of the Thesis is well in line with priorities of contemporary science. The results are important for understanding both basic chemistry and biochemistry problems and may be useful for biological studies *in vivo*. Thus the reviewed work can be regarded as interdisciplinary.

The Author has used two different oxidizing agents hydroxyl radicals, $^{\bullet}OH$, and 3-carboxybenzophenone triplet state, $^{T}(3CB)^{*}$. The oxidizing species were generated by pulse radiolysis or laser flash photolysis, respectively to study transient products of methionine oxidation. Steady-state methods, gamma radiolysis or photolysis, respectively, were used to characterize stable products. The transients were observed by time resolved absorption spectrophotometry, whereas stable products were analyzed with a variety of advanced methods like RP-HPLC, IEC, MS with EI, CI or ESI ionization and different methods of detection (e.g. quadrupole, ion trap, TOF, FTICR) as well as IRMPD-MS.

The methods were chosen adequately to the problems to be solved. It should be underlined that the Author effectively took advantage of the collaboration with several laboratories like University of Paris-Sud, Institute of Nuclear Chemistry and Technology in Warsaw, Poland, and Radiation Laboratory of the University of Notre Dame, Notre Dame, Indiana, USA. Ms Ignasiak was an active collaborator in two grants founded by the Polish National Science Center and has benefited from one of the COST Actions. She is the co-author of three scientific papers (the fourth one has been submitted) published in journals of high international impact.

Editorial remarks

The Thesis contain 191 numbered pages and is divided into six chapters, I. Introduction and aim of the work (37 pages), II. Photochemistry (14 pages), III. Radiation chemistry (10 pages), IV. Other methods (9 pages), V. Results and discussion (67 pages), VI. Conclusions (5 pages). Each chapter contains References section, what I find rather strange as it causes that a number of references is doubled. It is disappointing that the list of references does not contain the names of all the authors of the cited papers. Additionally, there is no information on the number of the journal's volume in the list of papers by Ms Ignasiak. The whole is preceded by the short abstracts in English and in French, astonishingly not included into the list of content. The main part of the Thesis is followed by the Supplementary materials (Figures and Tables), List of Tables, List of Figures and Schemes, an extended abstract in Polish, List of Abbreviations and the List of papers published by the Author of the Thesis – the last three position are not included into the list of content.

I find the organization of the Thesis unusual. Chapters II – IV contain information on the experimental methods applied by the Author and should be rather merged into one chapter entitled Experimental methods. The exclusion of the abstracts from the numbered part of the work suggests that the abstracts were treated as a marginal part of the Thesis.

I regret to underline that the Author has not edited her Thesis with sufficient care. Symbols are used inconsistently, e.g. $^{\bullet}$OH and OH$^{\bullet}$ or $^{T}(3CB)^{*}$ and $^{3}(3CB)^{*}$, and also titles of chapters are inconsistent. There can be found numerous printing and numbering errors. Some phrases in English and also in Polish need improvements as they could be misunderstood. Some examples are included as a supplement to this review.

Comments and questions

Chapter I. Introduction and aim of the work

This chapter presents a concise review of the state-of-art knowledge of the mechanisms and products of oxidation of methionine and some other amino acids as well as of chosen sunscreens. Herein is mentioned the fundamental work of Professor K.-D.Asmus on the formation and stabilization of S-X three electron bonded species. The chapter contains useful schemes, figures and physico-chemical data gathered in tables and is followed by the list of 139 references, that are adequately chosen. The critical remarks concerning this chapter are as follows. Correlations between the text and schemes are not consequently shown. In some cases the processes described in the text are followed by the respective numbers shown in the schemes, but in some cases there lack such connections. The Author is wrong when writing that the reactivity of phenoxyl radicals towards biomolecules is

unknown. MS and HPLC are not the methods appropriate for reactivity studies, they serve to identify the stable products.

Chapter II. Photochemistry

Here one finds also a very brief description of basics of photochemistry including useful information on methodology and equipment used in photochemistry. However, in some cases the Author tried to be too brief. For example one would be disappointed trying to find some information on the basics of Marcus theory in the subchapter II.1.3.b.

Chapter III. Radiation chemistry

This chapter contains the short description of water radiolysis, including generation of primary and secondary radicals, information on the sources of ionizing radiation most commonly used in radiation chemistry, and also on dosimetry methods and sample preparation. I could not find any word on the radiolysis of acetonitrile solutions, although the Author did some experiments with benzophenone derivatives dissolved in this medium. Also in this chapter the Author did not avoid some errors. First of all radiation chemistry does not deal with the interaction of ionizing radiation with matter, while this is the domain of physics. Radiation chemistry deals with chemical processes initiated by ionizing radiation. Secondly, electrons ejected from an orbital of the molecule by ionizing radiation indeed have enough energy to cause further ionizations until they, but not parent molecules (as it is written in the text) become thermalized. Another controversial phrase used by the Author is that "products of radiolysis initially separated in spurs start to diffuse and react…", while one should rather consider that the processes proceed simultaneously. The absorption spectrum of hydrated electron extends over almost the whole visible region with a peak in its red fragment (720 nm). The reaction $N_2O + H^{\bullet}$ should be taken into account only at high temperature. I cannot accept the logic of the statement (page 55, line 4 from the bottom) where the Author writes "larger amount of N_2O is consumed by electrons", while I would rather say that "N_2O appears to be the main scavenger of electrons" under described conditions. The absorbed dose is the part of energy delivered by the ionizing radiation which is absorbed by the matter, but not the amount of ionizing radiation absorbed by the system. From which reference are taken the absorption coefficient of dirodanate radical and its radiation yield?

Chapter IV. Other methods.

In this chapter there is given some information on the methods used by the Author to characterize the stable products of methionine-containing peptides. Described are chromatography, mass spectrometry with different methods of ionization, and Infra-Red Multiphoton Dissociation Spectroscopy coupled to MS (IRMPD-MS). The references cited are well chosen.

Here again imprecise sentences can be found, e.g. (p. 68) "The molecules in the gas phase may interact with intense IR lasers yielding collisions less unimolecular dissociation after absorption of IR photons" or (p.69) "The energy required to transition from $\nu = 0$ to 2 requires less energy than in the case…".

Chapter V. Results and discussion

This is the main chapter in which the results of the studies performed by the Author are presented and discussed. It should be underlined that Ms Ignasiak did an imposant number of experiments and was able to find a relatively clear way to present and discuss them. It is worth noting that the work of Ms Ignasiak is a successful continuation of long time investigation run in different laboratories including the one headed by her supervisor, professor Bronisław Marciniak.

The results, discussion and conclusions described in the subchapters V.1.1 – V.1.3. are complete and sound and bring many important new information. As the most significant achievement of this part of the work I would mention the unambiguous identification of α-S radicals as a major intermediate and sulfoxide as a stable end product of the oxidation of Met-containing peptides by $^{\bullet}OH$ radicals or by $^{T}(3CB)^{*}$. These results have been published in two original papers and in one review, additionally the forth paper has been submitted.

The results described and discussed in the subchapter V.1.4. have more speculative character, due to much more complicated system investigated, i.e. kinins. The proposal of structures of some possible products of radiolytic or photolytic oxidation of kinins should be considered a success at this stage of the studies. The Author has paved the way for further studies which might be the subject of the subsequent PhD thesis.

At last, in the subchapter V.2. the Author describes and discusses the results of her studies on oxybenzone and sulisobenzone, that are benzophenone derivatives used as components of sunscreens. It was shown that photolysis of these compounds leads to the formation of phenoxyl radicals, what was proved by the pulse radiolysis method. It appeared that phenoxyl radicals are reactive towards Met-derivatives. Phenoxyl radicals may also form as the result of sunlight interaction with sulisobenzone or oxybenzone. Thus the important message from these part of the Author's studies is that sunscreens might be dangerous due to modifications of proteins induced by phenoxyl radicals.

Chapter V. Conclusions

Here the Author tried to point out the main achievements of her work, what she really did. However, the conclusions are mixed with fragmentary discussion, what makes this chapter not as clear as one would have expected.

Questions

After reading Chapter V several questions to the Author appeared:

1. Does the dose rate influence the mechanism of radiation oxidation Met-containing peptides?
2. Why the Author has chosen $^{\bullet}OH$ radical as an oxidizing species in her investigations instead of other, more selective radicals as for example azide radical, $^{\bullet}N_3$?

3. Could the Author comment on the selectivity of $^{T}(3CB)^{*}$ as an oxidizing agent in comparison with $^{\bullet}OH$ radical? What could be the reason of discrepancies between results obtained by the Author and those by Barata-Vallejo concerning sulfoxide formation upon radiation oxidation of Met amino acids?
4. By what method were analyzed the kinetic traces presented on the Fig.V.2.Right or in Fig.V5.Right? Do the absorption spectra of the observed species overlap?

Reviewer's conclusion

All the above critical remarks do not influence the high scientific level of the Ph.D. Thesis presented by Ms Marta Teresa Ignasiak in order to obtain the degree of doctor of philosophy in chemistry. Ms Ignasiak proofed herself as a talented young scientist able to apply advanced experimental methods, to collaborate with others and having potential for the further scientific work. Therefore I consider the reviewed Thesis to be adequate for the degree of Doctor.

Wniosek końcowy

Zawarte w recenzji uwagi krytyczne nie obniżają wysokiego poziomu naukowego rozprawy przedstawionej przez panią Martę Teresę Ignasiak celem uzyskania stopnia doktora chemii. Pani Marta Teresa Ignasiak udowodniła, że jest zdolnym naukowcem umiejącym wykorzystywać nowoczesną aparaturę badawczą, umie współpracować z innymi badaczami, potrafi efektywnie pracować i ma predyspozycje do dalszej pracy naukowej. Uważam, że praca doktorska przedłożona przez Panią Martę Teresę Ignasiak spełnia wszystkie wymagania przewidziane procedurą uzyskiwania stopnia naukowego doktora opisane w Rozporządzeniu Ministra Nauki i Szkolnictwa Wyższego z dnia 22.09.2011 roku i stawiam wniosek o dopuszczenie Pani Teresy Marty Ignasiak do dalszych etapów przewodu doktorskiego.

Lodz, 05.05.2014