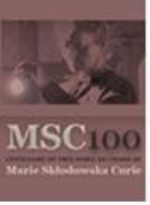

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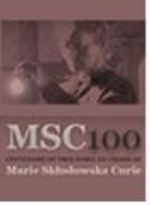
POLAND

From retinal polyenes to peptides and proteins: radiation chemistry approach



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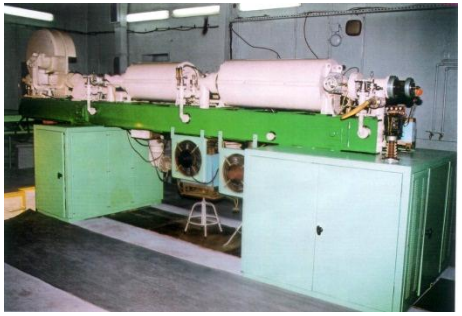
ENTRANCE TO THE FIELD….

1971 – 1972 M.Sc. Thesis

Warsaw University & Institute of Nuclear Research

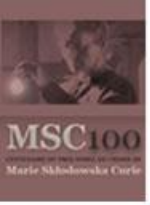
INFLUENCE OF OXYGEN ON THE KINETICS OF RADIATION-INDUCED CROSSLINKING OF POLYISOPRENE IN NATURAL RUBBER LATEX



1972 - 1976 PhD Thesis

Institute of Nuclear Research

OZONIDE ION AND CONCOMITANT SPECIES STUDIED BY PULSE RADIOLYSIS IN CLASSIC VERSION AND WITH USING SELF-ABSORPTION OF THE CHERENKOV LIGHT



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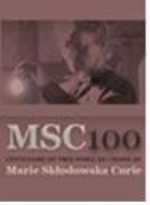
1979 - 1981

POSTDOCTORAL FELLOWSHIP
AT THE NOTRE DAME RADIATION LABORATORY



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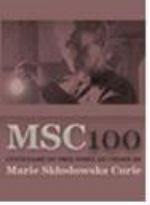
FROM….

RETINAL POLYENES



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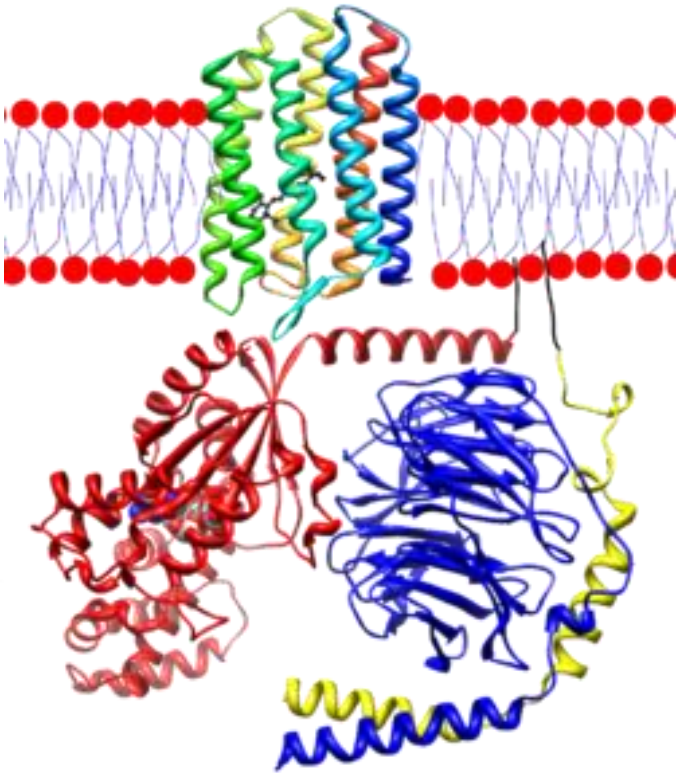
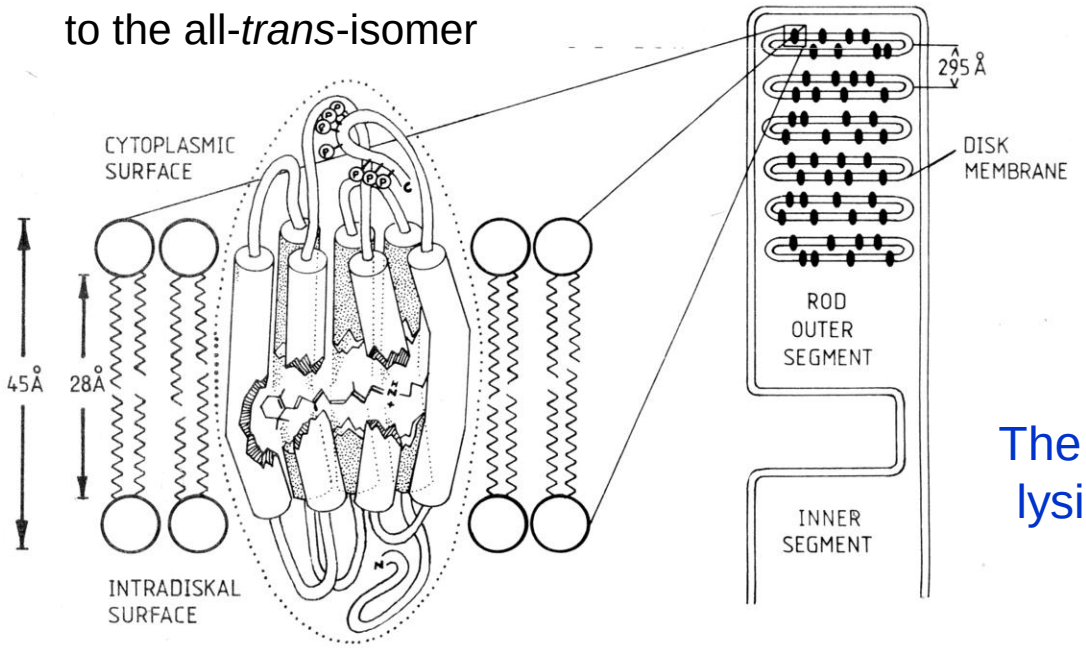
Rhodopsin is the photoreceptor protein of rod cells in the vertebrate retina

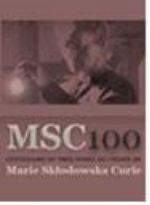
Rhodopsin is composed of the protein opsin in combination with the 11- *cis* isomer of vitamin A aldehyde (retinal)

Vision is initiated when a photon strikes rhodopsin and causes isomerization of the bound 11-*cis* retinal to the all-*trans*-isomer





The retinal is bound to a specific lysine via a Schiff base linkage

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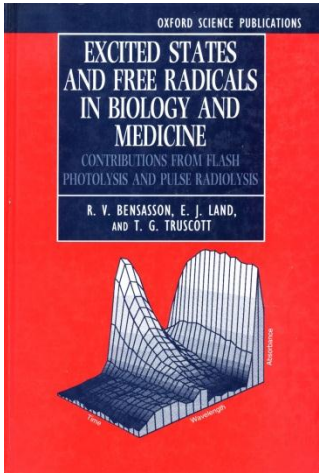
RADICAL IONS IN RETINAL POLYENES

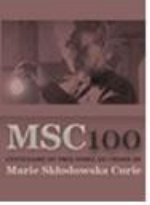


with

Paritosh Kumar DAS

Charge transfer interaction between the retinyl moiety and suitable donor groups such a sulfhydryl and indole in the protein (opsin) might be operative in the early stages of vision

The systems with extended π electrons may mediate the transport of charge across a biological membrane either through electron tunnelling or by a „give-and-take” mechanism



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Photochemistry and Photobiology Vol. 47, No. 1, pp. 73–83, 1988
Printed in Great Britain.

0031-8655/88 $03.00+0.00

J. Am. Chem. Soc. **1981**, *103*, 4569–4573

Transient Phenomena in the Pulse Radiolysis of Retinyl Polyenes. 1. Radical Anions[1]

N. V. Raghavan, P. K. Das,* and K. Bobrowski†

Contribution from the Radiation Laboratory, University of Notre Dame, Notre Dame, Indiana 46556. *Received October 10, 1980*

Abstract: The spectra and kinetics of formation and decay of radical anions of a number of retinyl polyenes have been studied in methanol and 2-propanol at room temperature, using pulse radiolysis and kinetic spectrophotometry. The bimolecular rate constants for the attachment of solvated electrons, e^-_{MeOH}, to the retinyl polyenes are in the diffusion-controlled limit (8.6×10^9–1.6×10^{10} M^{-1} s^{-1}). The radical anions of retinol and retinol acetate have their spectral maxima at 370–390 nm, and undergo decay very slowly with second-order kinetics. On the other hand, the radical anions of retinal, retinal *n*-butylamine Schiff base, and retinoic acid/ester have spectral maxima at 430–510 nm, and decay by first-order kinetics in methanol with rate constants in the range 1×10^4–1×10^6 s^{-1}. The decay rates of radical anions of retinal and retinoic acid/ester become considerably longer on going from methanol to less acidic alcohol, 2-propanol, suggesting that protonation by solvent is the major mode of their decay in protic media. In the case of retinal Schiff base, an additional slow process with bimolecular rate constant 9.0×10^7 M^{-1} s^{-1} in methanol is observed for the formation of radical anion and is ascribed to the electron-transfer reaction from the methanol radical, $\cdot CH_2OH$.

TRANSIENT PHENOMENA IN THE PULSE RADIOLYSIS OF RETINYL POLYENES—7. RADICAL ANIONS OF VITAMIN A AND ITS DERIVATIVES

K. Bhattacharyya,* K. Bobrowski,† S. Rajadurai and P. K. Das‡
Radiation Laboratory, University of Notre Dame, Notre Dame, IN 46556, USA

(*Received* 8 *January* 1987; *accepted* 20 *May* 1987)

Abstract—Upon e^--pulse irradiation in nonprotic solvents, all-*trans* retinol (ROH) and retinylmethyl ether (ROMe) form transient species (τ = 0.5–7 μs, λ_{max} = 575–590 nm) identifiable as radical anions. Similar species are also formed upon laser pulse photoexcitation of these retinyl derivatives in the presence of N,N-dimethylaniline in acetonitrile. In contrast, electron transfer or attachment to all-*trans* retinyl acetate (ROAc) and palmitate (ROPa) results in 'instantaneous' loss of carboxylate anions from electron adducts giving the retinylmethyl radical (R·, λ_{max} = 395 nm, τ_1 > 100 μs); the radical anions in these cases are too short-lived to be detected by nanosecond pulse radiolysis. The lifetimes of radical anions of ROH and ROMe are very sensitive to water and alcohols (e.g. $k_q = 10^7$ M^{-1} s^{-1} with methanol as quencher for $ROH^{\cdot-}$ in tetrahydrofuran). Based on these findings, the spectral dissimilarity of the one-electron reduction products from ROH and ROAc in alcohols and aqueous micelles becomes explainable in terms of fast formation of protonated radical anions ($\dot{R}H(OH)$, τ_1 > 100 μs, λ_{max} = 370–375 nm) in the case of ROH and of retinylmethyl radical *via* loss of AcO^- from radical anion in the case of ROAc. In tetrahydrofuran, the complexation of $ROH^{\cdot-}$ with cations such as Na^+ and Bu_4N^+ affects the relative importance of its major decay modes, namely, protonation and dehydroxylation, the latter process being significantly enhanced by the presence of Na^+.

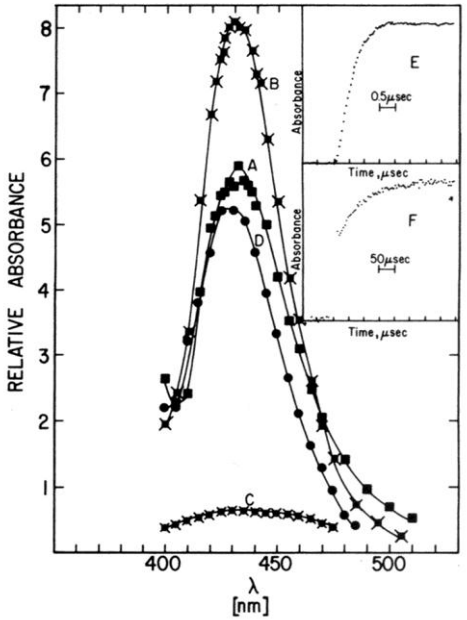
$$e^-_{aq} + RSB \rightarrow RSB^{\bullet -}$$

$$k = (8.6 - 16) \times 10^9\ M^{-1}s^{-1}$$

$$^{\bullet}CH_2OH + RSB \rightarrow RSB^{\bullet -}$$

$$RP^{\bullet -} + ROH \rightarrow {}^{\bullet}RPH + RO^-$$

$$RP^{\bullet -} + ROH_2^+ \rightarrow {}^{\bullet}RPH + ROH$$



polyene	λ_{max}
retinal	445 (460)
retinol	370 (370)
retinyl acetate	390 (390)
retinoic acid	480 (505)
methyl retinoate	480 (510)
retinyl Schiff base	430 (435)

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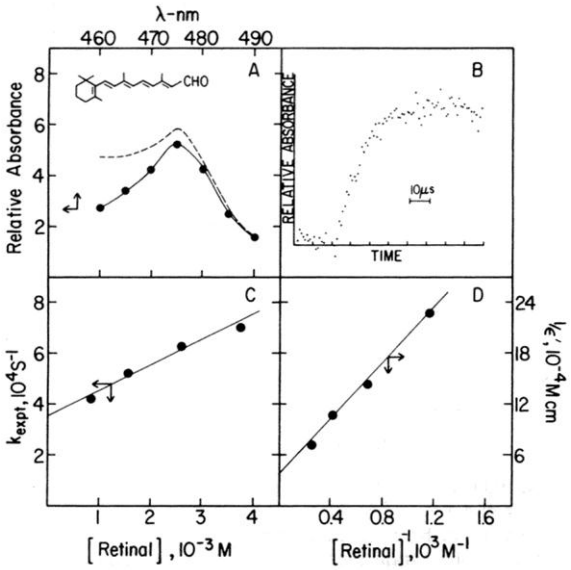
J. Am. Chem. Soc. **1982**, *104*, 1704–1709

Transient Phenomena in the Pulse Radiolysis of Retinyl Polyenes. 2. Protonation Kinetics[1]

K. Bobrowski† and P. K. Das*

Contribution from the Radiation Laboratory, University of Notre Dame, Notre Dame, Indiana 46556. *Received July 27, 1981*



Abstract: The results of a study are presented concerning the kinetics of reactions of various retinyl derivatives with hydrogen ions released pulse radiolytically in aerated 2-propanol containing 0.5 M acetone and 0.2 M carbon tetrachloride. For retinal and polyene Schiff bases, their protonated forms absorb at much longer wavelengths than the unprotonated substrates and are monitored by kinetic spectrophotometry. In the cases of retinol and retinyl acetate, the initial H^+ adduct undergoes loss of water and acetic acid, respectively, producing the retinyl carbonium ion (λ_{max} 585 nm), which, in turn, decays with first-order kinetics ($\tau_{1/2} = 10 \pm 3$ μs). The rate constants for protonation are in the range 1×10^7–4×10^9 M^{-1} s^{-1}. In the cases of polyene Schiff bases, the protonation rate constant increases slightly with increasing polyene chain length and is higher for *11-cis*-retinal Schiff base than for its all-trans counterpart. The absorption spectral maxima of protonated polyene Schiff bases observed in the early stages of protonation in the pulse radiolysis are red-shifted by ~10 nm relative to those of the protonated forms obtained by adding dilute hydrochloric acid to Schiff base solutions in 2-propanol. This is explained by the lack of ion pairing in the protonated species seen in the pulse radiolysis.

$k_{exptl} = k_r + k_f[\text{retinal}]$

$RCH{=}O + (CH_3)CHOH_2^+ \leftrightarrows RCH{=}OH^+ + (CH_3)CHOH$

$K = k_f/k_r$

$K = 270\ M^{-1}$

2-propanol/acetone/CCl_4

$A_{eq}^{-1} = A_\infty^{-1}(1 + K^{-1}[\text{retinal}]^{-1})$

$K = 230\ M^{-1}$

Production of HCl

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Reprinted from The Journal of Physical Chemistry, 1985, *89*, 5079.

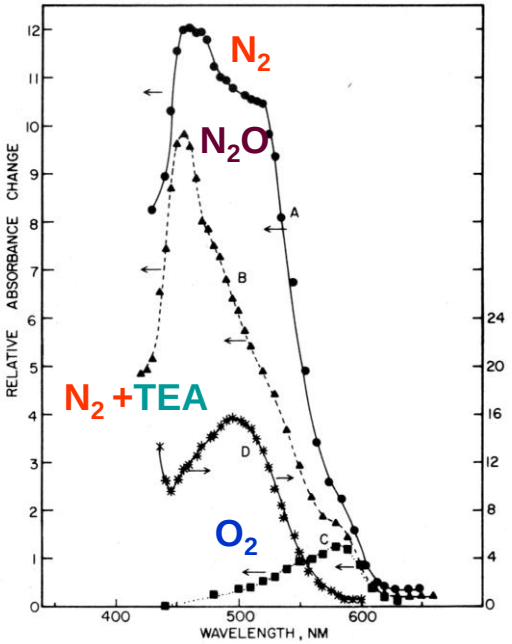
Transient Phenomena in the Pulse Radiolysis of Retinyl Polyenes. 3. Radical Cations[1,2]

K. Bobrowski† and P. K. Das*

Radiation Laboratory, University of Notre Dame, Notre Dame, Indiana 46556 (Received: May 6, 1985)

Results are presented concerning transient absorption phenomena observed upon pulse radiolysis of several retinyl polyenes at submillimolar concentrations in acetone, *n*-hexane, and 1,2-dichloroethane under conditions favorable for radical cation formation. Oxygen insensitive, the polyene radical cations are characterized by intense absorption maxima (575–635 nm) with locations that show little or no dependence on functional groups. In acetone, they undergo decay predominantly by first-order kinetics with lifetimes 4–11 μs. The biomolecular rate constants for radical cation quenching by water, triethylamine, and bromide ion in acetone are in the ranges $(0.8–2) \times 10^5$, $(0.3–2) \times 10^8$, and $(3–5) \times 10^{10}$ M^{-1} s^{-1}, respectively.

RETINAL IN ACETONE



$(CH_3)_2C{=}O \rightarrow {}^3(CH_3)_2C{=}O,\ [(CH_3)_2C{=}O]^{\bullet-},\ [(CH_3)_2C{=}O]^{+\bullet}$

$^3RCH{=}O^*$ $\lambda_{max} = 445$ nm

$[RCH{=}O]^{\bullet-}$ $\lambda_{max} = 495$ nm

$[RCH{=}O]^{\bullet+}$ $\lambda_{max} = 585$ nm

k [$M^{-1}s^{-1}$]

polyene	H_2O	TEA	Br^-
retinal	8.4×10^4	1.8×10^8	5.3×10^{10}
retinol	1.9×10^5	2.7×10^7	4.1×10^{10}
retinyl acetate	1.3×10^5	3.9×10^7	3.1×10^{10}
retinoic acid	3.5×10^5	1.9×10^8	3.9×10^{10}
methyl retinoate	1.6×10^5	1.2×10^8	3.7×10^{10}
retinyl Schiff base	1.5×10^5	3.9×10^7	3.1×10^{10}

Krzysztof Bobrowski
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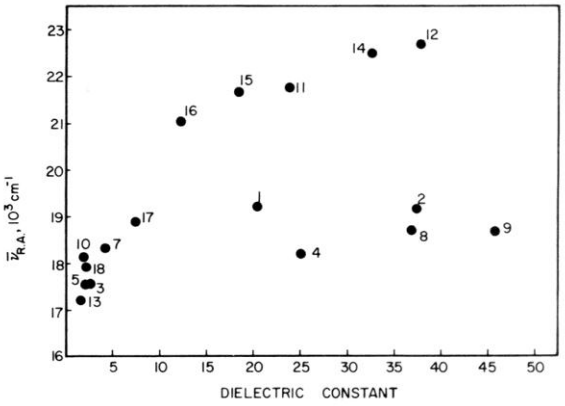
POLAND

Reprinted from The Journal of Physical Chemistry, 1985, *89*, 5733.

Transient Phenomena in the Pulse Radiolysis of Retinyl Polyenes. 4. Environmental Effects on Absorption Maximum of Retinal Radical Anion[1]

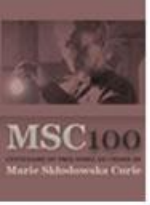


K. Bobrowski† and P. K. Das*

Radiation Laboratory, University of Notre Dame, Notre Dame, Indiana 46556 (Received: June 13, 1985)

The absorption-spectral and kinetic behaviors of radical ions of *all-trans*-retinal in various solvents have been studied by spectrophotometric pulse radiolysis at room temperature. Upon going from nonpolar and polar–nonprotic solvents to polar–protic ones (alcohols), large hypsochromic shifts occur in the absorption maximum (λ_{max}) of retinal radical anion. The anion absorption maxima in the alcohols correlate well with solvent dielectric constants. In a number of solvents, parallelism is noted between the energies corresponding to λ_{max}'s of retinal anion and of solvated electron (the latter at 77 K and room temperature). Transient phenomena associated with the radical anion (λ_{max}'s = 448–460 nm) and the retinyl alcohol radical (λ_{max} = 405 nm) are observed in the course of the pulse radiolysis of retinal in several normal micelles (aqueous). The blue-shifted locations of the anion λ_{max}'s strongly suggest a polar–protic, alcohol-like nature of the micellar region where the polyenal resides.

solvent	ε	λ_{max}	λ_{max}	solvent	ε	λ_{max}	λ_{max}
acetone	20.7	495	580	1,4-dioxan	2.21	545	595
acetonitrile	37.5	520	585	ethanol	24.3	458 (700)	
benzene	2.28	570	600	ethylene glycol	38.0	440 (580)	
benzonitrile	25.2	550	595	hexane	1.88	580	590
cyclohexane	2.023	530	595	methanol	32.6	445 (630)	
1,2-DCE	10.36		595	propanol	18.3	460 (820)	
diethylether	4.4	545		*tert*-butyl alcohol	12.3	475	
dimethylformamide	37	535		tetrahydrofuran	7.5	530	
dimethyl sulfoxide	46.6	535	595	triethylamine	2.44	560	

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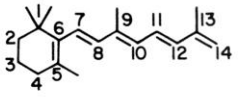
Transient Phenomena in the Pulse Radiolysis of Retinyl Polyenes. 5. Association of Radical Cations with Parent Molecules[1]

K. Bobrowski[†] and P. K. Das*

Radiation Laboratory, University of Notre Dame, Notre Dame, Indiana 46556 (Received: July 12, 1985)

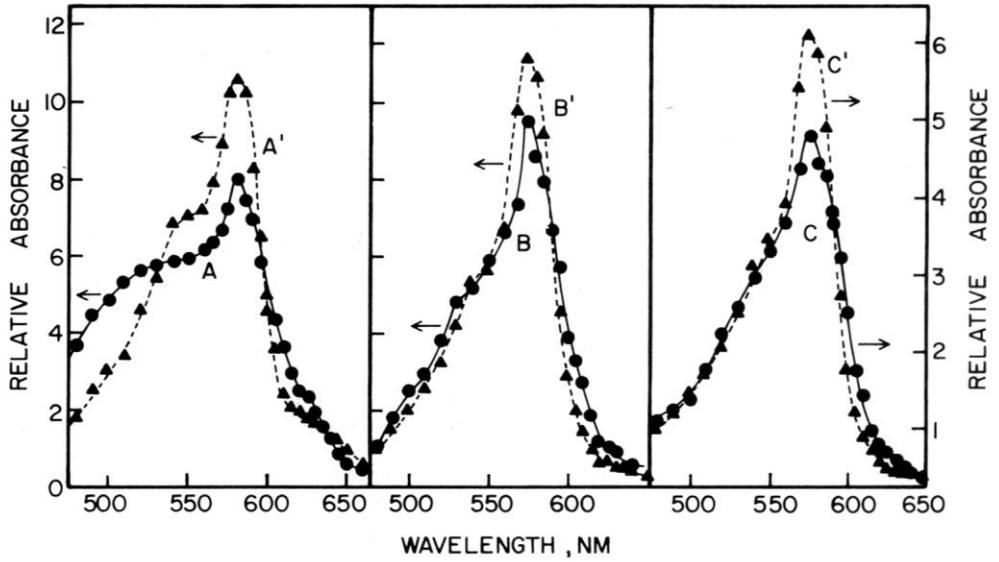
At relatively high concentrations (1–10 mM) in O_2-saturated acetone, pulse radiolysis of *all-trans*-retinal, -retinoic acid, and -methyl retinoate gives rise to fast transient absorption processes that are best explained in terms of association of radical cations with parent polyenes to form dimers. From the concentration dependence of initial decay/formation kinetics, equilibrium constants (K) for monomer/dimer interconversion are measured to be 220–440 M^{-1} (in acetone). On going from acetone to 1,2-dichloroethane, K values for retinal and retinoic acid increase almost by an order of magnitude. For *all-trans*-retinol and -retinyl acetate, radical cation dimer formation appears to be negligible in the concentration range 1–10 mM of the polyene substrates (based on the lack of transient absorption changes seen with retinal and retinoic acid/ester).

CHART I

R ≡ 

1. Retinal, R = 
2. Retinoic acid, R = 
3. Methyl retinoate, R = 
4. Retinol, R =
5. Retinyl acetate, R =

RETINAL RETINOIC ACID METHYL RETINOATE



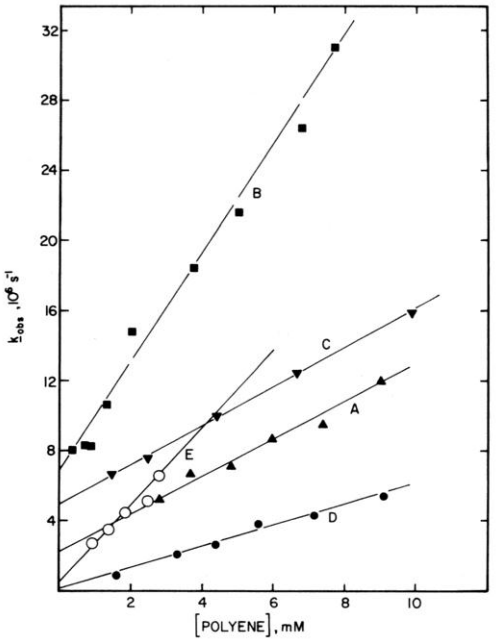
$k_{obsd} = k_b + k_f[\text{Ret}]$

$K = k_f / k_b$

4.3 (29)

4.6 (37)

2.2



$[(CH_3)_2C{=}O]^{+\bullet} + \text{Ret} \rightarrow [(CH_3)_2C{=}O] + \text{Ret}^{+\bullet}$

$\text{Ret}^{+\bullet} + \text{Ret} \leftrightarrows \text{Ret}_2^{+\bullet}$

Krzysztof Bobrowski
INCT, Warsaw
POLAND

Reprinted from The Journal of Physical Chemistry, 1987, *91*, 1210.

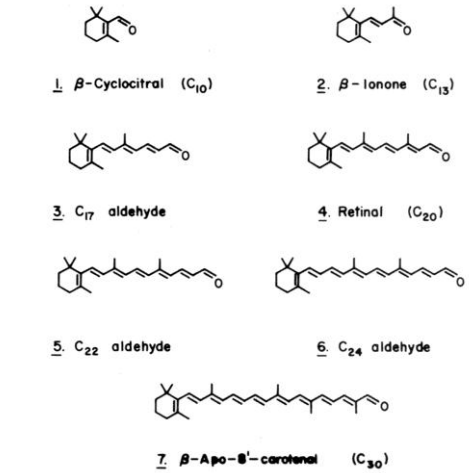
Transient Phenomena in the Pulse Radiolysis of Retinyl Polyenes. 6. Radical Ions of Retinal Homologues[1]

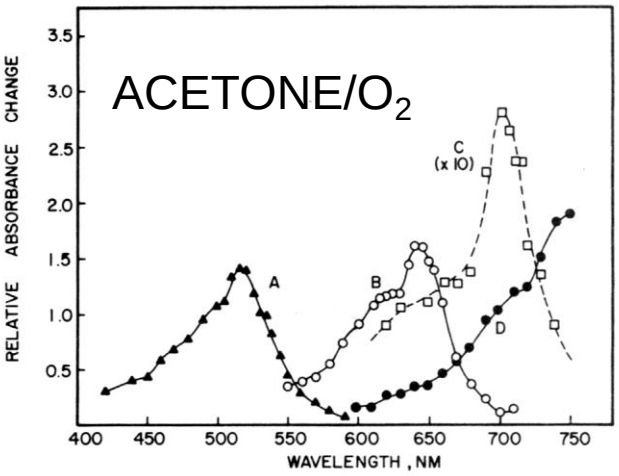
K. Bobrowski† and P. K. Das*

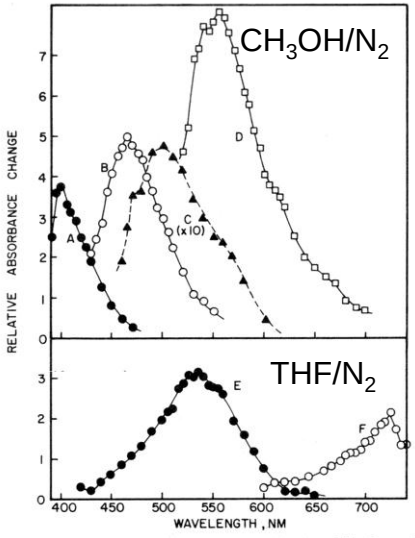
Radiation Laboratory, University of Notre Dame, Notre Dame, Indiana 46556 *(Received: August 6, 1986; In Final Form: September 24, 1986)*

The spectral and kinetic behaviors of radical ions of several retinal homologues possessing varying chain lengths have been examined by pulse radiolysis in acetone, tetrahydrofuran, alcohols, and aqueous micelles. The absorption maxima of radical ions shift progressively to lower energies as the number of double bonds is increased. The rate constants for protonation of radical anions in alcohols, and with 1,1,1,3,3,3-hexafluoro-2-propanol added as a reagent in tetrahydrofuran, show decreasing trends upon increasing the polyene chain length. A similar chain length dependence is also noted in the reactivity of polyenal radical cations with water, triethylamine, and bromide ion. The absorption spectra of C_{30} aldehyde (8′-apo-β-carotenal) radical anion in CTAB and Triton X-100 micelles suggest an alcohol-like nature of the environment probed by the long-chain polyenal. The radical anion decays via protonation by reaction with water, leading to the alcohol radical; the longer lifetime of the anion in CTAB than in Triton X-100 solution suggests that the cation pairing with the head groups in the former stabilizes the anion against its reactivity with water.

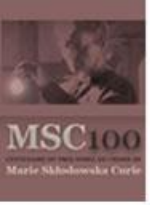
CHART I: *all-trans*-Retinal Homologues under Study







polyene	λ_{max}	λ_{max}
C_{10}	315 (370)	
C_{13}	350 (380)	385
C_{17}	395	515
C_{20}	445 (530)	580
C_{22}	465	640
C_{24}	490	700
C_{30}	555 (725)	>740



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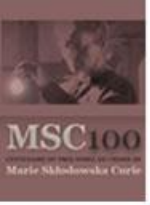
POLAND

TO….

PEPTIDES AND PROTEINS



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LONG-RANGE ELECTRON TRANSFER IN PEPTDES AND PROTEINS

with:

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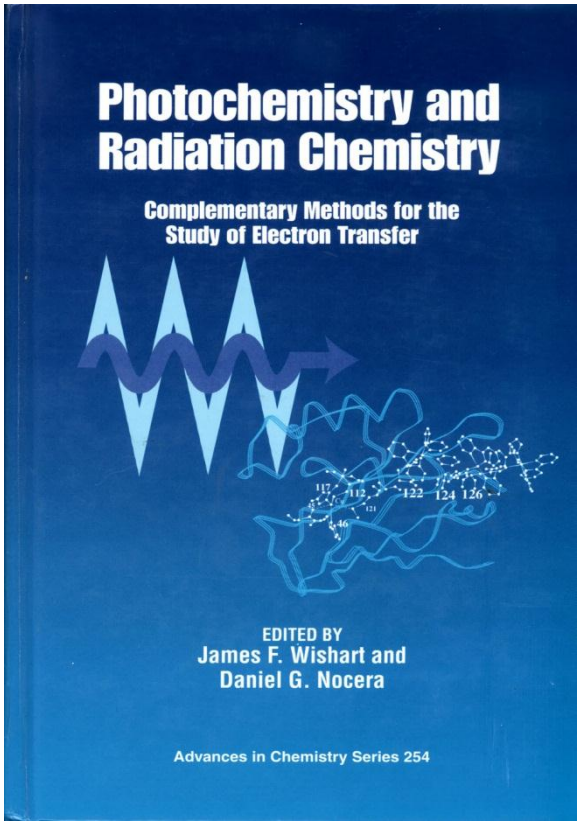

Radiation Chemistry Symposium: Marie Curie's Heritage

Maison de la Chimie - Paris

15th and 16th November 2011

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10036 Reprinted from The Journal of Physical Chemistry, 1992, 96.

Pulse Radiolysis Studies of Intramolecular Electron Transfer in Model Peptides and Proteins. 5. $Trp^{\bullet} \rightarrow Tyr^{\bullet}$ Radical Transformation in H-Trp-$(Pro)_n$-Tyr-OH Series of Peptides

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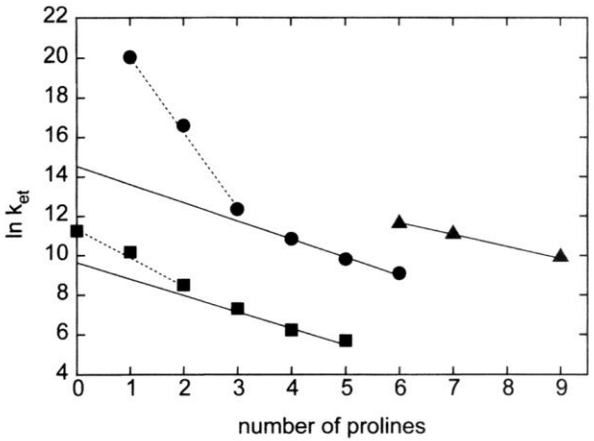
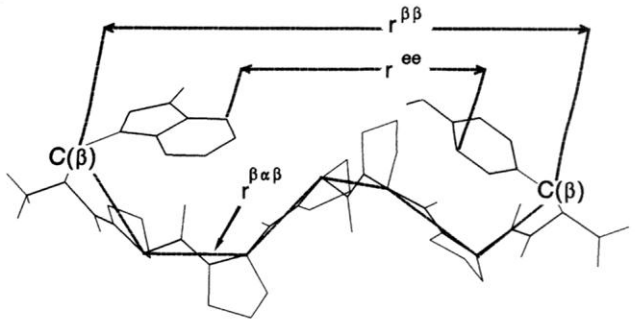
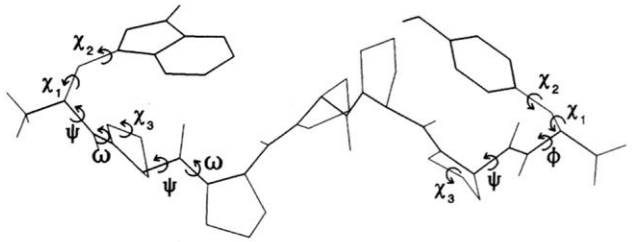
(Received: May 29, 1992; In Final Form: August 13, 1992)



The kinetics of intramolecular long-range electron transfer (LRET) between neutral tryptophan radical and tyrosine in aqueous solution of H-Trp-$(Pro)_n$-Tyr-OH, n = 4 and 5, peptides has been studied by pulse radiolysis over the temperature range 288–328 K. The rate constants, k_1, and thermodynamic activation parameters of LRET thus obtained, together with those for shorter (n = 0–3) peptides of the same series, measured earlier, are analyzed in terms of Marcus nonadiabatic theory of LRET and distributions of donor–acceptor distances and angular orientations determined by the conformational energy calculations. To explain the observed exponential decrease of k_1 with the number of n of Pro residues, simulation of the overall distance dependence of the rate according to models assuming involvement of LRET (i) through-bond (TB), (ii) through-space (TS), and also (iii) through both pathways simultaneously were performed by fitting the calculated mean rate constants, $\langle k^{TB} \rangle$ and $\langle k^{TS} \rangle$, to the experimental k_1 data. The best agreement between the experimental and calculated rates was obtained for a modified version of the last model (iii), according to which competitive electron transfer through the TS pathway occurs only in the conformers exhibiting van der Waals contacts and favorable angular orientation for a large overlap of π and σ orbitals between the indole and phenol rings. The best-fit rate constants obtained indicate that in short-bridged peptides (n = 0–2) electron transfer takes predominantly the TS pathway, while in longer ones (n = 3–5) it occurs mainly by the TB pathway ($\beta^{TB} = 0.28 \pm 0.05$ Å^{-1} at 298 K). Descriptors of electronic ($\beta_{el}^{TB} \approx 0.2$ Å^{-1}) and nuclear ($\beta_n^{TB} \approx 0.1$ Å^{-1}) contributions to the overall distance dependence of the TB-LRET were roughly estimated from the distance dependence of activation parameters, derived from rate constants, $k_{1,cor}$, corrected for an expected thermal longitudinal expansion of the oligoproline bridge.





n	k_{IET} s^{-1}	
0	7.7×10^4	
1	2.6×10^4	
2	3.9×10^4 (t)	4.9×10^3
3	1.5×10^3	
4	5.1×10^2	
5	3.1×10^2	

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J. Phys. Chem. B **1999,** *103,* 10316−10324

Pulse Radiolysis Studies of Intramolecular Electron Transfer in Model Peptides and Proteins. 8. Trp[NH$^{\bullet+}$] → Tyr[O$^{\bullet}$] Radical Transformation in H-Trp-(Pro)$_n$-Tyr-OH, n = 3−5, Series of Peptides

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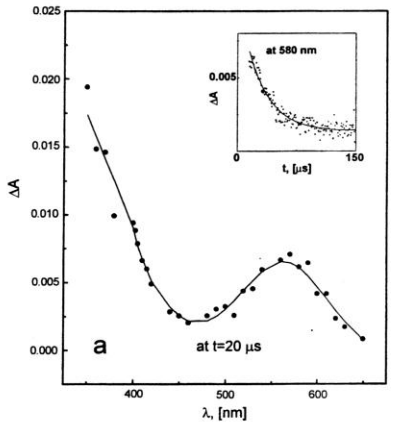
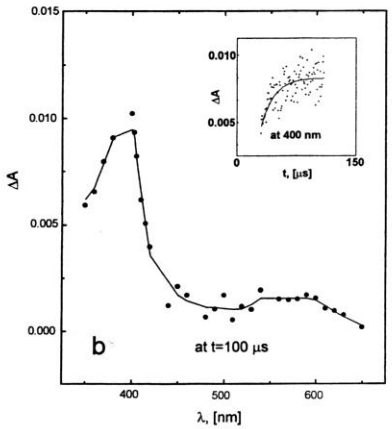
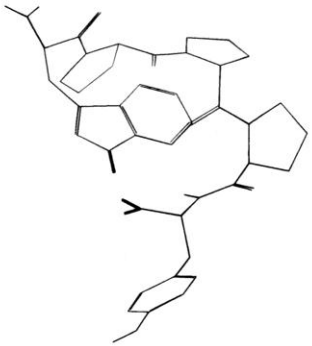
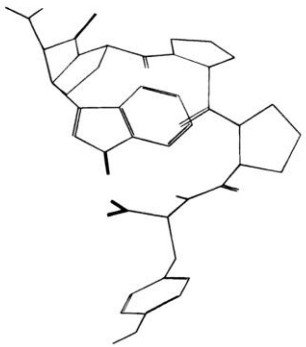
Received: June 28, 1999; In Final Form: September 20, 1999





n	k_{IET} s^{-1}		k_1/k_2
3	1.5×10^3	2.7×10^4	18
4	5.1×10^2	3.0×10^4	59
5	3.1×10^2	1.9×10^4	61





The kinetics of intramolecular long-range electron transfer (LRET) between the protonated tryptophan indolyl radical cation Trp[NH$^{\bullet+}$], remaining in equilibrium with its neutral Trp[N$^{\bullet}$] form, and tyrosine in aqueous H-Trp-(Pro)$_n$-Tyr-OH, n = 3−5, peptides (abbreviated **3, 4,** and **5**) has been studied by pulse radiolysis at pH 2−8 at 298 K and at pH 4 over the temperature range 283−328 K. LRET has been found to occur down to pH 2, contrary to expectations based on the electrochemical redox potentials for Trp and Tyr in the form of free amino acids. The first-order rate constants of LRET, k_{obs}, varied sigmoidally with pH, allowing evaluation of pK_{a1}'s for deprotonation of Trp[NH$^{\bullet+}$]: 3.7, 4.1, and 4.3 for **3**, **4**, and **5**, respectively, as well as the intrinsic rate constants, k_2, of LRET involving solely Trp[NH$^{\bullet+}$] radicals. Variation of pK_{a1} was attributed to electrostatic interactions between the indolyl radical and the COOH group of terminal Tyr, expected to decrease in the shown order, and taken as an indication of variation, in the same order, of the electrochemical driving force ΔG° of LRET. In **4** and **5**, k_2 proved to be about 60-fold larger than the corresponding k_1 values characteristic for Trp[N$^{\bullet}$], which indicates that the kinetics of LRET involving Trp[NH$^{\bullet+}$] exhibits similar through-bond distance dependence as that found earlier for the reaction with Trp[N$^{\bullet}$]. For **3** the ratio of k_2/k_1 was found to be about 3-fold lower than for its two longer-bridged analogues, **4** and **5**. Arrhenius activation energies, E_{a2}, of LRET involving Trp[NH$^{\bullet+}$] proved to be rather low for **4** and **5**, 8.6 and 7.2 kJ mol^{-1}, respectively. In the case of **3**, however, k_{obs} varied nonlinearly with temperature. A nonlinear fit of Arrhenius type function to $k_{obs}(T)$ data under the assumption $\delta E_{a2}/\delta T = 0$ and $\delta pK_{a1}/\delta T \neq 0$ gave E_{a2} of 31.6 kJ mol^{-1} and indicated a decrease in pK_{a1} of Trp[NH$^{\bullet+}$] by about one unit in the studied temperature range. Conformational preferences of **3** were thus studied by means of molecular dynamics modeling to understand why the parameters of LRET in this peptide were so different from those of **4** and **5**. It has been shown that a $\beta \rightarrow \alpha$ transition at the central ψ(Pro$_3$) dihedral angle, which could bring NH (indole) and COOH groups into a close contact compatible with formation of a hydrogen bond, may occur in **3** on the time scale of the observed electron-transfer reaction. In longer-bridged analogues two such concerted transitions would be necessary to perturb LRET measurably, an event of extremely low probability. It is thus argued that this transition may explain the kinetic and energetic peculiarities of LRET in **3**. Analysis of thermodynamic parameters indicated that in reactions involving both Trp[NH$^{\bullet+}$] as well as Trp[N$^{\bullet}$] radicals the free energy barrier of activation of LRET, $\Delta G^{\#}$, is for the most part entropic in nature. A more detailed analysis of thermodynamics of these reactions must await experimental determination of ΔG° for both reactions in the peptides studied.

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INT. J. RADIAT. BIOL., 1992, VOL. 62, NO. 5, 507–516

Pulse radiolysis studies of intramolecular electron transfer in model peptides and proteins. IV. Met/S∴Br→Tyr/O· radical transformation in aqueous solution of H-Tyr-(Pro)$_n$-Met-OH peptides

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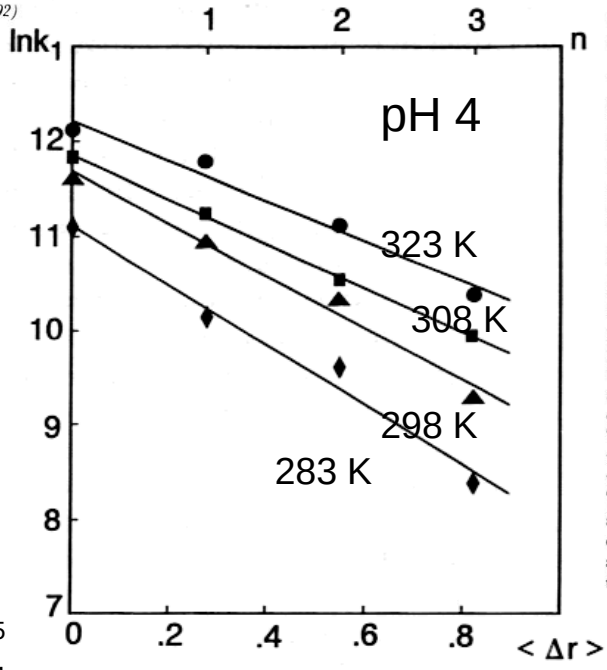
(Received 14 February 1992; revised 6 May 1992; accepted 10 May 1992)

Abstract. The intramolecular radical transformation Met/S∴Br→Tyr/O· in aqueous peptides H-Tyr-(Pro)$_n$-Met-OH, n=0–3, was investigated in the temperature range of 283–328 K by pulse radiolysis. Corresponding first-order rate constants and thermodynamic parameters of activation of electron transfer, E_a and $\Delta S^{\ddagger}$, were determined from kinetic data. The rate constants of the reaction were found to decrease exponentially with the number of Pro units and the distance between C_β atoms of the terminal amino acids, with a correlation coefficient $\alpha = 3{\cdot}2 \pm 0{\cdot}5\ nm^{-1}$ at 298 K. Its value appeared to be temperature dependent suggesting the occurrence of thermally induced conformational changes in the peptides. Analysis of experimental data in terms of known conformational properties of the peptides indicates that apparent values of α, E_a and $\Delta S^{\ddagger}$ are probably complicated functions of conformation and thermodynamic stability of the oligoproline bridge, varying with the number of Pro residues, and of intramolecular hydrophobic interactions between side chains of tyrosine and methionine. Estimation of the relative efficiency of electron transfer pathways through the peptide backbone and through direct and/or water mediated contact between groups bearing radical sites led to the conclusion that partitioning of electron transfer along these pathways is likely to occur.



INT. J. RADIAT. BIOL., 1990, VOL. 57, NO. 5, 919–932

Intramolecular electron transfer in peptides containing methionine, tryptophan and tyrosine: a pulse radiolysis study

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(Received 27 July 1989; revision received 3 November 1989; accepted 10 November 1989)

The kinetics of pulse radiolytically induced intramolecular radical transformations: Trp/N·→Tyr/O·, Tyr/O·→Trp/N·, Met/S∴Br→Trp/N· and Met/S∴Br→Tyr/O· has been investigated at various pH levels and temperatures in model peptides: Trp-(Pro)$_n$-Tyr, Trp-(Gly)$_n$-Tyr, Trp-(Pro)$_n$-Met (n=0–3), Tyr-Phe-Met-Arg-Phe-NH$_2$·2AcOH, Met^5-enkephaline and [D-Ala]^{2}Met^5-enkephaline. The rate constants of the Trp/N·→Tyr/O· transformation at pH 8 were found to decrease exponentially with the distance between Trp and Tyr in the proline peptides, while in the glycine peptides the rate of the transformation is less dependent on the number of bridging glycine residues. The activation energies determined fall into the range 10–20 kJ mol^{-1} irrespective of: (*i*) the ionization state of tryptophyl radical and tyrosine, (*ii*) type of bridging amino acids, and (*iii*) reversal of the direction of the electron transfer upon tyrosine OH group ionization. The activation entropies at 298 K for the peptides of the glycine and proline series are negative and rather high, and fall into the relatively narrow range of −90 to −140 J mol^{-1} deg^{-1}. These activation parameters seem to indicate that a tunnelling mechanism is involved in the electron transfer between strictly oriented aromatic moieties of Trp and Tyr. The variation of the activation parameters with average separation distance in the case of Trp-(Pro)$_n$-Tyr shows a predominance of the electronic factor over the nuclear in determining the distance dependence of the electron transfer rate. The intramolecular Met/S∴Br→Tyr/O· transfer proceeds with the rate constant of $1{\cdot}1 \times 10^5\ s^{-1}$ in Met^5-enkephaline and $5{\cdot}7 \times 10^4\ s^{-1}$ in [D-Ala]^{2}Met^5-enkephaline. The activation parameters for this transformation $E_a = 30\ kJ\ mol^{-1}$ and $\Delta S^{\ddagger}_{298} = -62\ J\ mol^{-1}\ deg^{-1}$ are close to those of the Trp/N·→Tyr/O· transformation, suggesting a similar mechanism for the electron transfer.

Tyr-Met	1.1×10^5
Tyr-Pro-Met	5.8×10^4
Tyr-(Pro)$_2$-Met	3.1×10^4
Tyr-(Pro)$_3$Met	1.1×10^4

Tyr-(Pro)$_n$-Met(S∴Br) → TyrO•-(Pro)$_n$-Met + Br$^-$

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Comparison of k_{IET} in peptides

TyrOH-Met(S ∴ Br) → TyrO•-Met(S) + Br^- + H^+

$$k_{IET} = 1.1 \times 10^5\ s^{-1}$$

K. Bobrowski *et al.*
Int. J. Radiat. Biol., 1992, 62, 507-516

TyrOH-Pro-Pro-Pro-Met(S ∴ Br) →
TyrO•-Pro-Pro-Pro-Met(S) + Br^- + H^+

$$k_{IET} = 1.1 \times 10^4\ s^{-1}$$

K. Bobrowski *et al.*
Int. J. Radiat. Biol., 1992, 62, 507-516

TyrOH-Gly-Gly-Phe-Met(S ∴ Br) →
TyrO•-Gly-Gly-Phe-Met(S) + Br^- + H^+

$$k_{IET} = 1.1 \times 10^5\ s^{-1}$$

K. Bobrowski *et al.*
Int. J. Radiat. Biol., 1990, 57, 919-932

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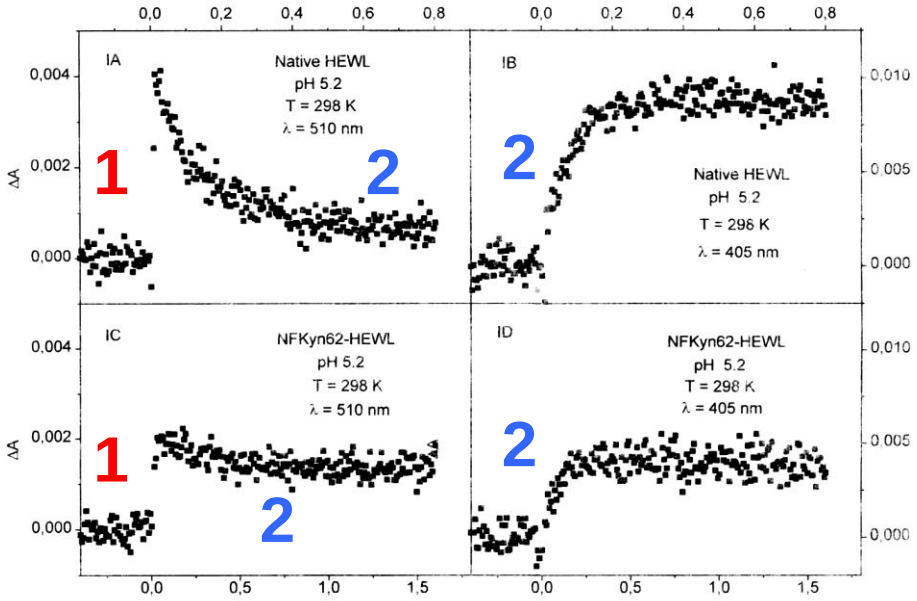
Biophysical Chemistry 63 (1997) 153–166



Pulse radiolysis studies of intramolecular electron transfer in model peptides and proteins. 7. $Trp^{\cdot} \rightarrow TyrO^{\cdot}$ radical transformation in hen egg-white lysozyme Effects of pH, temperature, Trp62 oxidation and inhibitor binding

Krzysztof Bobrowski [a,b], Jerzy Holcman [c], Jaroslaw Poznanski [a], Kazimierz Lech Wierzchowski [a,*]

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[c] Department of Environmental Sciences, Risø National Laboratory, DK-4000 Roskilde, Denmark

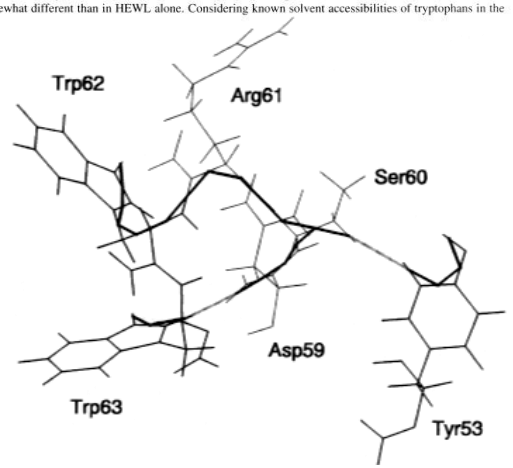
Received 24 June 1996; revised 2 September 1996; accepted 10 September 1996

Abstract

Intramolecular long-range electron transfer (LRET) in hen egg-white lysozyme (HEWL) accompanying $Trp^{\cdot} \rightarrow TyrO^{\cdot}$ radical transformation was investigated in aqueous solution by pulse radiolysis as a function of pH (5.2–7.4) and temperature (283–328 K). The reaction was induced by highly selective oxidation of Trp with $N_3^{\cdot}$ radicals under low concentration of the reactants but at a high HEWL/$N_3^{\cdot}$ molar ratio, so that more than 99% of the oxidized protein molecules contained only a single tryptophyl radical. Synchronous decay of $Trp^{\cdot}$ and build-up of $TyrO^{\cdot}$ conformed satisfactorily to first-order kinetics, indicating that LRET involved either one or more Trp/Tyr redox pairs characterized by similar rate constants. The rate constant of LRET, k_5, increased monotonously with decreasing pH showing the following characteristics: (i) in the pH range 7.4–5.2 the plot of k_5 against pH was sigmoidal in shape, reflecting protonation of Glu35 ($pK_a \approx 6$) and pointing to involvement of conformational control of the kinetics of LRET, (ii) below pH 5.2 a sharp increase in k_5 was observed due to the protonation of $Trp^{\cdot}$ to form $TrpH^{\cdot+}$, which is known to oxidize tyrosine faster than does $Trp^{\cdot}$. Arrhenius plots of the temperature-dependence of k_5 showed that the activation energy of LRET varies both with temperature and the protonation state of the enzyme. The activation energies are in the range 7.6–56.0 kJ mol^{-1} and are similar to those for activation of amide hydrogen exchange in native HEWL below its denaturation temperature. Selective oxidation by ozone of the Trp62 indole side-chain in HEWL to *N'*-formylkynurenine (NFKyn62-HEWL) caused a large drop in the initial yield of $Trp^{\cdot}$ radicals, $G(Trp^{\cdot})_i$. This was accompanied by a relatively small decrease in k_5 but selective oxidation by ozone had a pronounced effect on its temperature-dependence. Taken together these observations indicate that of the six tryptophans present in HEWL Trp62 contributes about 50% to the yield of the observed LRET. In the enzyme–inhibitor complex, HEWL(GlcNAc)$_3$, where Trp62 and Trp63 are completely shielded from the solvent by the bound triacetylchitotriose, $G(Trp^{\cdot})_i$ was lower than in NFKyn62-HEWL, and both the kinetic and energetic characteristics of LRET, observed at pH 5.2, were again somewhat different than in HEWL alone. Considering known solvent accessibilities of tryptophans in the

$$N_3^{\bullet} + {-}Trp^{62}H{-} \xrightarrow{1} {-}Trp^{62}{\bullet} + Tyr^{53}OH + N_3^{-} + H^{+}$$

$$Tyr^{53}OH + {-}Trp^{62}{\bullet} \xrightarrow{2} {-}Trp^{62}H{-} + Tyr^{53}O^{\bullet}$$



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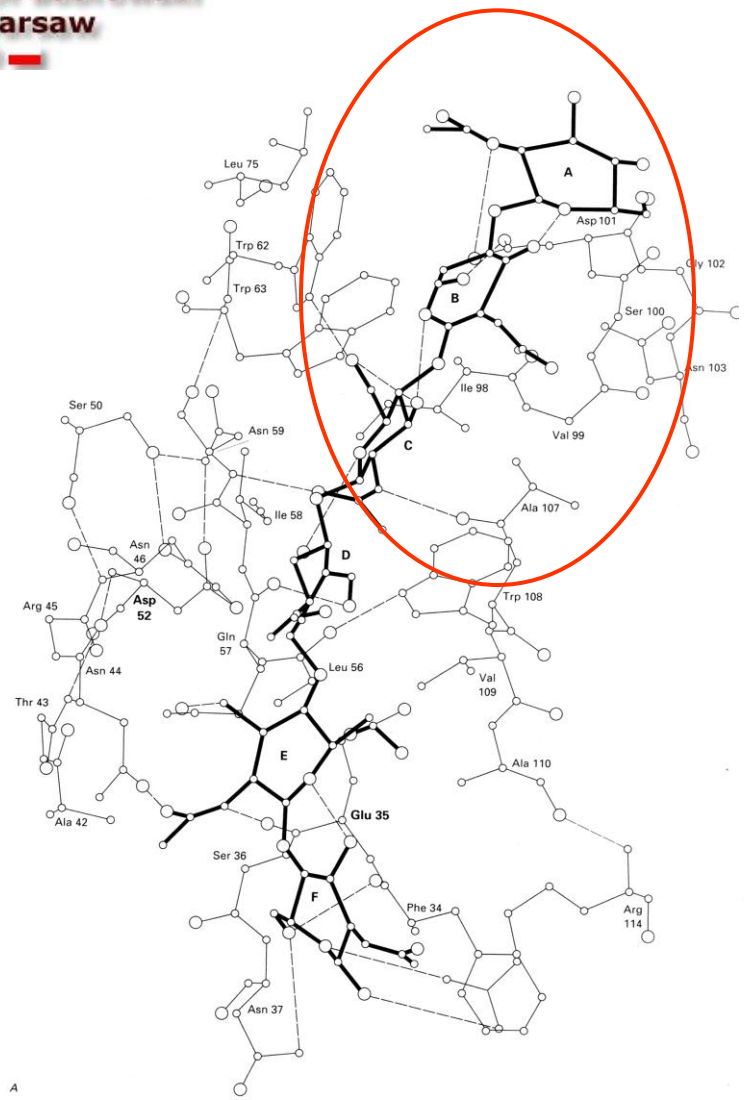
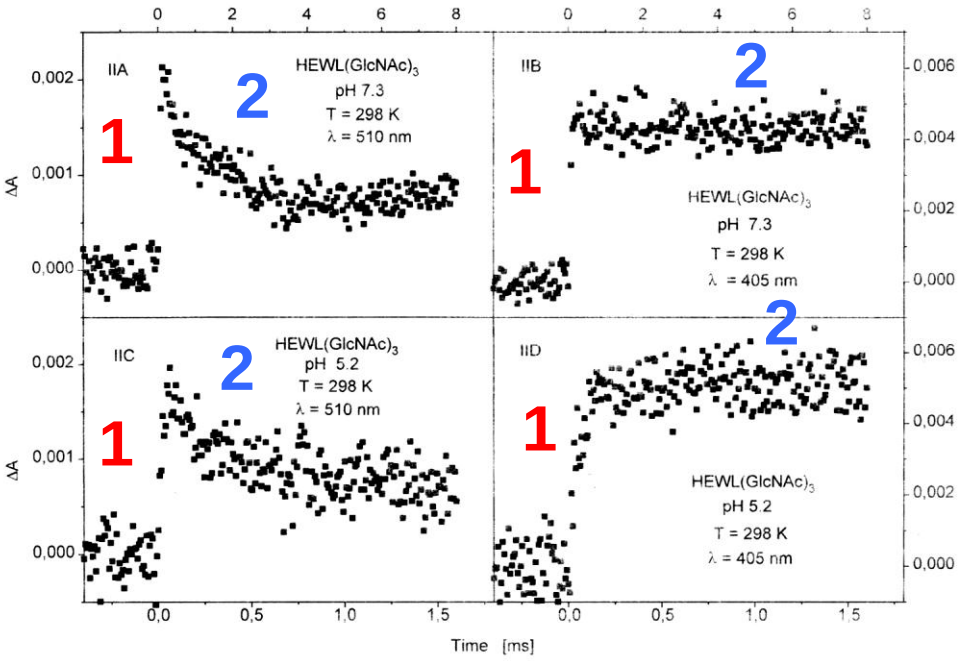
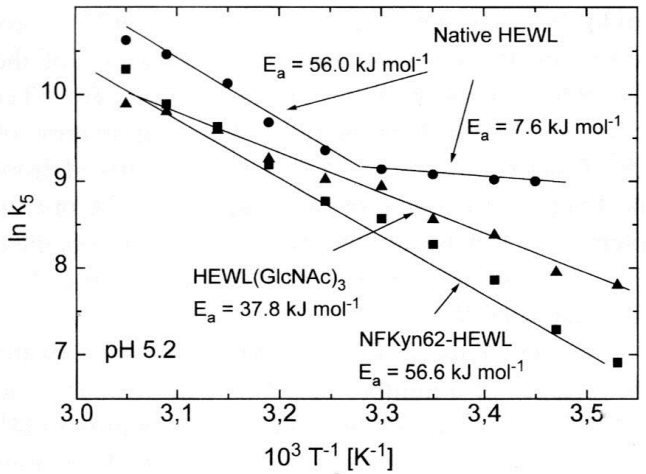
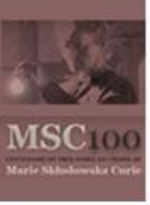


N-Acetyloglucosoamine trimer





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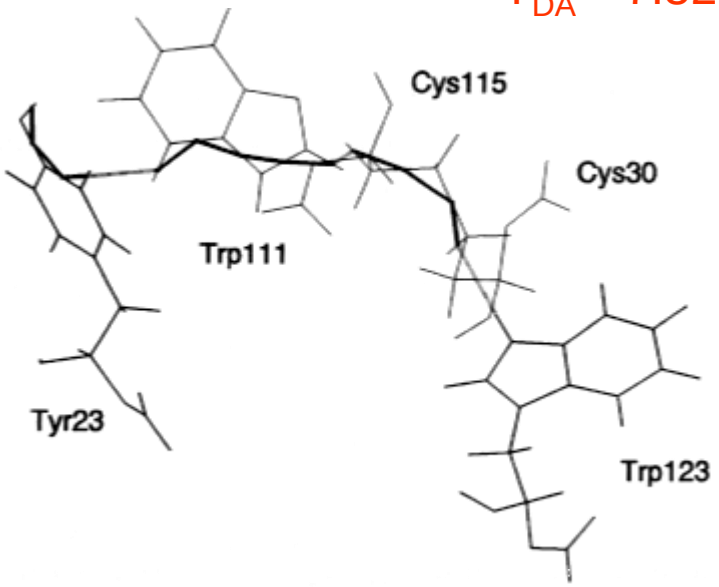
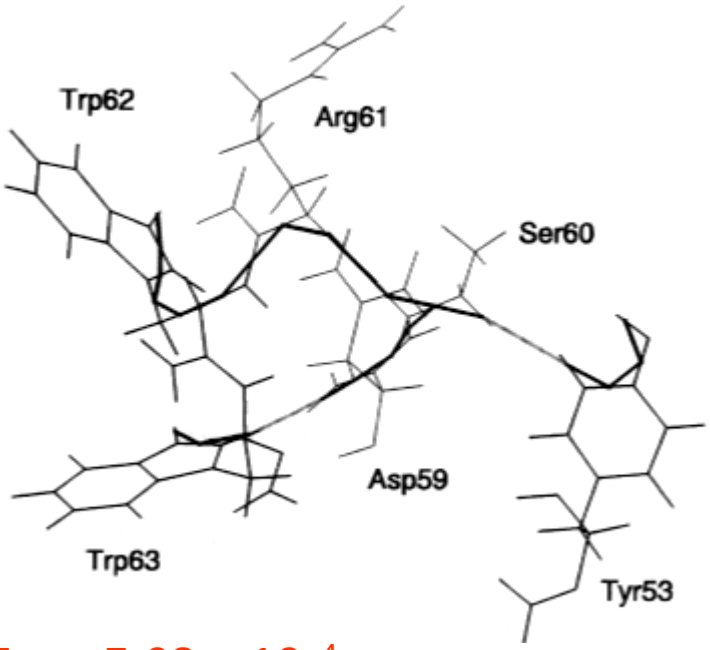
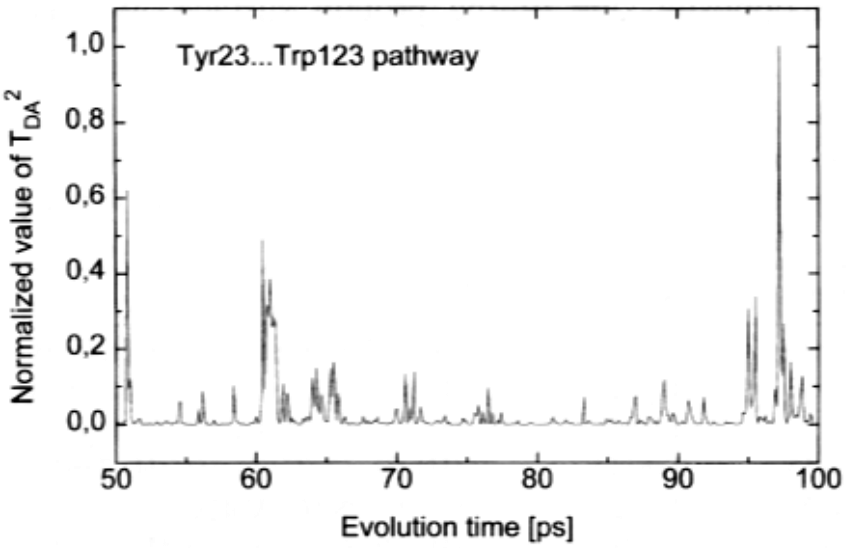
$T_{DA} = 7.52 \times 10^{-5}$

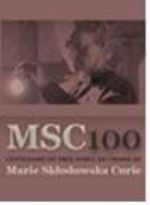


$T_{DA} = 3.69 \times 10^{-4}$



$T_{DA} = 5.68 \times 10^{-4}$





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STABILIZATION OF SULFUR RADICAL CATIONS IN PEPTIDES

with:

Jerzy HOLCMAN
Gordon L. HUG
Bronislaw MARCINIAK
Dariusz POGOCKI
Christain SCHÖNEICH
Pawel WISNIOWSKI

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INT. J. RADIAT. BIOL., 1987, VOL. 52, NO. 1, 139–144

Formation of three-electron bonds in one-electron oxidized methionine dipeptides: a pulse radiolytic study

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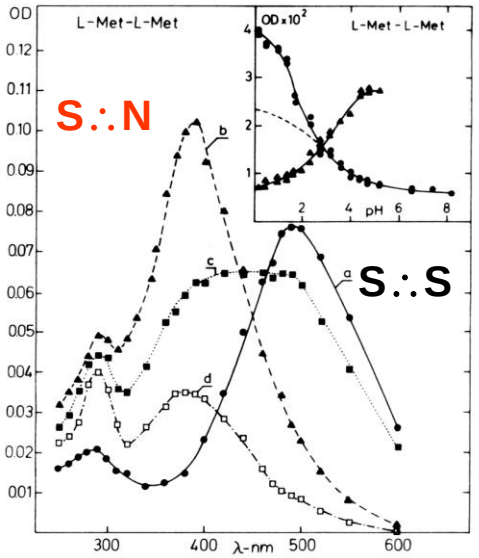
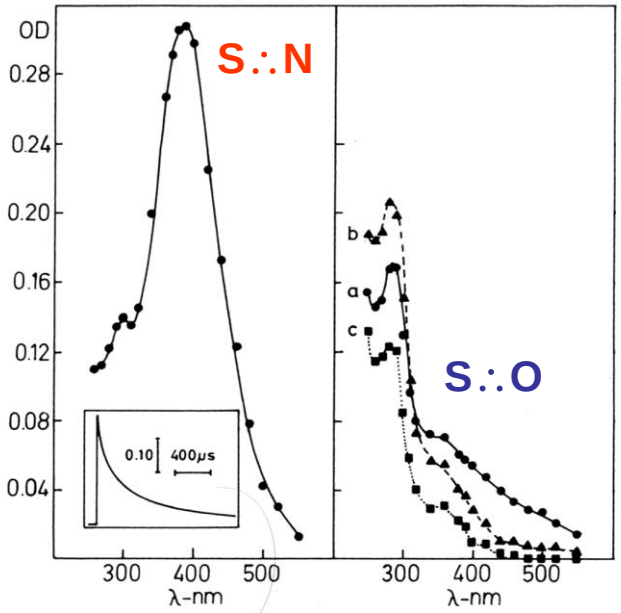
(*Received 22 August 1986; revision received 28 November 1986; accepted 1 December 1986*)

One electron oxidation of methionine dipeptides (Met-X and X-Met, where X = Gly or Ser) was carried out using the pulse radiolysis technique. It was apparent that the mode of oxidative action of OH radicals on methionine dipeptides was governed by the sequence of amino acids. Spectral evidence suggests that an intramolecular three-electron bond between nitrogen and sulphur atoms is not formed if these two atoms are separated by a peptide bond.



Met-Gly **Gly-Met**



Formation and Stability of Intramolecular Three-Electron S∴N, S∴S, and S∴O Bonds in One-Electron-Oxidized Simple Methionine Peptides. Pulse Radiolysis Study

K. Bobrowski*

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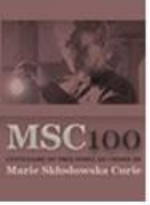
Accelerator Department, Risø National Laboratory, DK 4000 Roskilde, Denmark
(Received: November 16, 1988)

Intramolecular sulfur–sulfur $(S\therefore S)^+$ and sulfur–nitrogen $(S\therefore N)^+$ three-electron-bonded radical cations and sulfur–oxygen (S∴O) radicals have been generated in aqueous solutions of some simple di-, tri-, and tetrapeptides containing methionine units due to oxidation by hydroxyl radicals under pulse radiolysis conditions. All these transient species are formed at the diffusion-controlled rate ($k \geq 10^{10}$ dm^3 mol^{-1} s^{-1}), and they exhibit optical absorptions with the maxima at 390 nm (S∴N- and S∴O-bonded species) and at 490 nm (S∴S-bonded species) with extinction coefficients of 5000–7000 dm^3 mol^{-1} cm^{-1}. In slightly acidic solutions of tri- and tetrapeptides, a protolytic equilibrium between S∴O- and S∴S-bonded species was observed. The position of this equilibrium shifts by approximately 2 pK units when going from L-Met-Gly-L-Met (pK = 3.05) to L-Met-Gly-L-Met-L-Met (pK = 5.15). Conversion of the S∴O-bonded species into the S∴S-bonded species proceeds via kinetically distinct $[H^+]$-dependent ($k = 10^7$–10^8 dm^3 mol^{-1} s^{-1}) and $[H^+]$-independent ($k \simeq 10^4$ s^{-1}) routes. In the pH range 6.0–9.0, a pH- and buffer-concentration-independent conversion of the 490-nm into the 390-nm absorption band was observed. This fast process ($k > 10^5$ s^{-1}) is consistent with the conversion of the S∴S-bonded species into the S∴N-bonded species.



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10224 *J. Am. Chem. Soc.* **2000,** *122,* 10224−10225

Intramolecular Sulfur−Oxygen Bond Formation in Radical Cations of *N*-Acetylmethionine Amide

Christian Schöneich,*,† Dariusz Pogocki,† Pawel Wisniowski,‡ Gordon L. Hug,§ and Krzysztof Bobrowski*,‡

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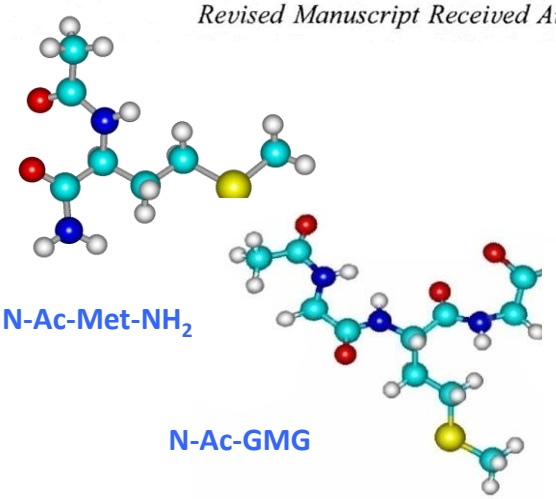
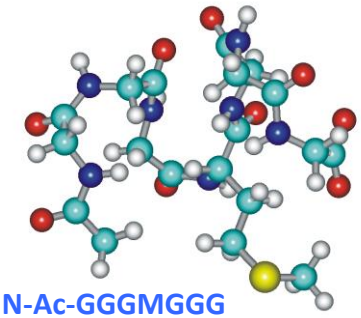
Received April 7, 2000
Revised Manuscript Received August 24, 2000



N-Ac-Met-NH_2

N-Ac-GMG



N-Ac-GGGMGGG

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ARTICLES
Published on Web 10/18/2003

Free Radical Reactions of Methionine in Peptides: Mechanisms Relevant to β-Amyloid Oxidation and Alzheimer's Disease

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Received June 17, 2003; E-mail: schoneic@ukans.edu

Abstract: The pathogenesis of Alzheimer's disease is strongly associated with the formation and deposition of β-amyloid peptide (βAP) in the brain. This peptide contains a methionine (Met) residue in the C-terminal domain, which is important for its neurotoxicity and its propensity to reduce transition metals and to form reactive oxygen species. Theoretical studies have proposed the formation of βAP Met radical cations as intermediates, but no experimental evidence with regard to formation and reactivity of these species in βAP is available, largely due to the insolubility of the peptide. To define the potential reactions of Met radical cations in βAP, we have performed time-resolved UV spectroscopic and conductivity studies with small model peptides, which show for the first time that (i) Met radical cations in peptides can be stabilized through bond formation with either the oxygen or the nitrogen atoms of adjacent peptide bonds; (ii) the formation of sulfur−oxygen bonds is kinetically preferred, but on longer time scales, sulfur−oxygen bonds convert into sulfur−nitrogen bonds in a pH-dependent manner; and (iii) ultimately, sulfur−nitrogen bonded radicals may transform intramolecularly into carbon-centered radicals located on the $^{\alpha}$C moiety of the peptide backbone.

Heteroatoms (oxygen and nitrogen) located in the peptide bonds are involved in stabilization of sulfur radical cations derived from methionine

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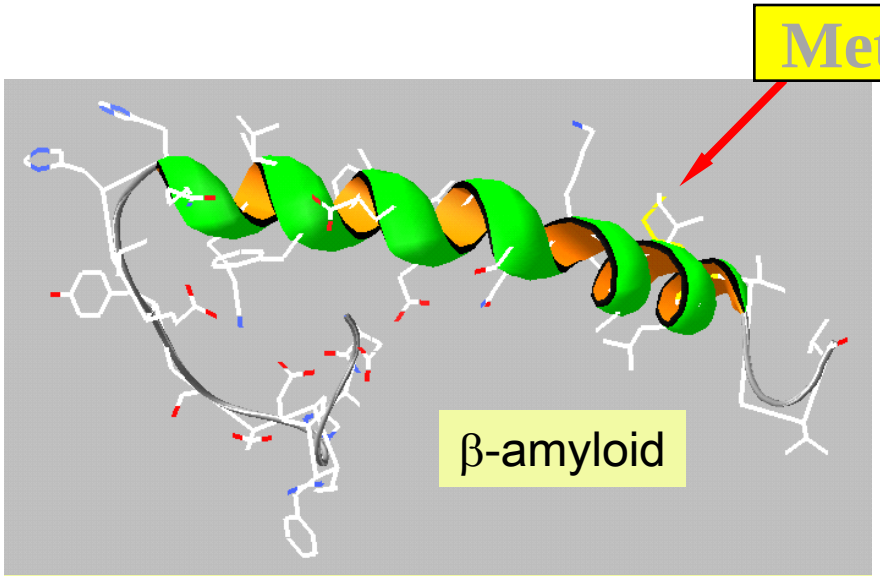
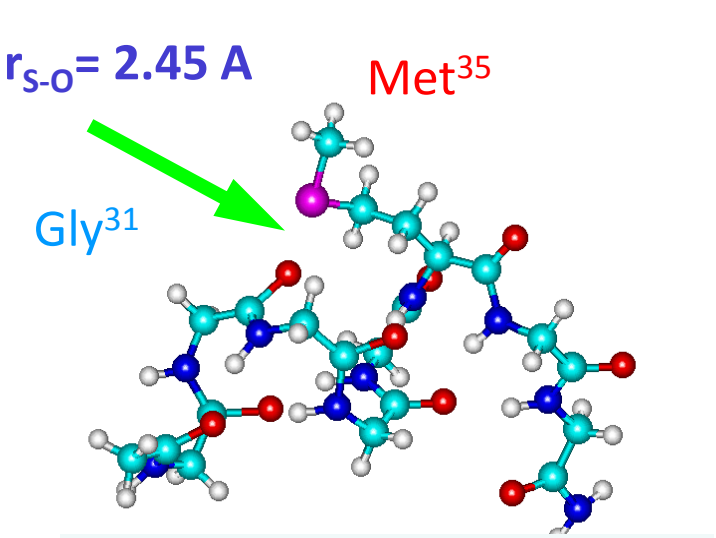
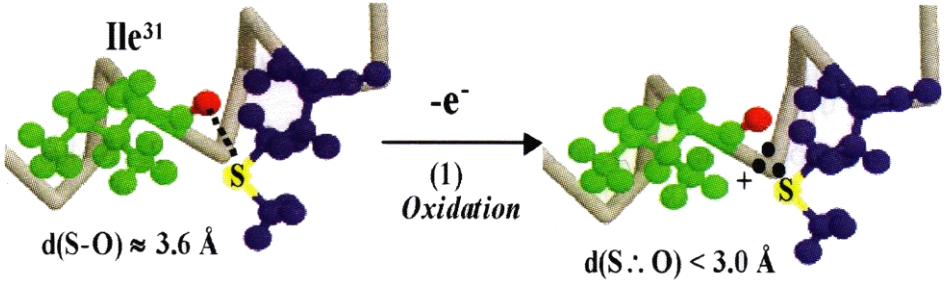
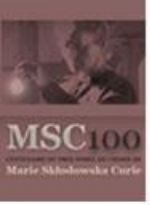
Models for stabilization of sulfur radical cations in oligopeptides and proteins containing simple methionine residue





The SCC-DFTB calculated geometry of the $(S\therefore O)^+$ radical





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CHEMPHYSCHEM

DOI: 10.1002/cphc.200700369

Conformational Influence on the Type of Stabilization of Sulfur Radical Cations in Cyclic Peptides

Gordon L. Hug,*[a, b] Krzysztof Bobrowski,[c] Dariusz Pogocki,[c, d] Gerald Hörner,[b, e] and Bronislaw Marciniak[b]

The free-radical chemistry of two oxidized cyclic dipeptides is investigated using time-resolved optical and conductivity detection. Two cyclic dipeptides, cyclo-Gly-L-Met and cyclo-D-Met-L-Met, are synthesized and irradiated with nanosecond pulses of electrons, which initiate the oxidation of the methionine side chains with hydroxyl radicals from the radiolysis of water. The cyclic peptides are taken to be models for the interior of proteins where there are no terminal groups. This opens up the possibility that neighboring-group effects can be studied directly between the initially formed sulfur radical cations and the heteroatoms associated with the peptide bonds. Such complexation of the sulfur radical cations is observed with the amide nitrogen atoms. In addition, intermolecular stabilization with the unoxidized sulfur atoms on separate cyclic dipeptide molecules is observed. Little or no intramolecular stabilization by the unoxidized sulfur in the neighboring methionine occurs in cyclo-D-Met-L-Met, in contrast to the previously observed intramolecular sulfur stabilization of the sulfur radical cation in the isomer cyclo-L-Met-L-Met. This contrasting behavior is rationalized by conformational differences in the two isomers as seen through molecular-modeling simulations. The implications for the oxidation of the protein calmodulin, which contains multiple residues of methionine, are discussed as having analogous determining factors.

Res Chem Intermed (2009) 35:431–442
DOI 10.1007/s11164-009-0044-6

Factor analysis of transient spectra. Free radicals in cyclic dipeptides containing methionine

Gordon L. Hug · Krzysztof Bobrowski · Dariusz Pogocki · Bronislaw Marciniak · Christian Schöneich · Gerald Hörner

Received: 31 October 2008 / Accepted: 16 January 2009 / Published online: 19 June 2009

Abstract Factor analysis is introduced and applied to resolving puzzling behavior in the free-radical chemistry of the cyclic dipeptide cyclo-(D-Met-L-Met). Previously, spectral analysis of the transient absorption spectra, following the hydroxyl radical-induced oxidation of cyclo-(D-Met-L-Met), failed to match expectations seen when transient conductivity was used to monitor the same reaction sequence. In the current work, factor analysis is used to show that a radical cation is formed via the stabilization of the oxidized sulfur through the formation of two-centered three-electron bonds with the lone pairs on oxygen. This previously undetected radical resolves the discrepancy between transient absorption and transient conductivity observations.

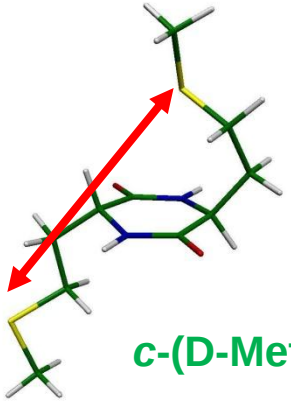
Stabilization of Sulfide Radical Cations through Complexation with the Peptide Bond: Mechanisms Relevant to Oxidation of Proteins Containing Multiple Methionine Residues

Krzysztof Bobrowski,[†] Gordon L. Hug,*[‡,§,∥] Dariusz Pogocki,[†,⊥] Bronislaw Marciniak,[§] and Christian Schöneich[⊗]

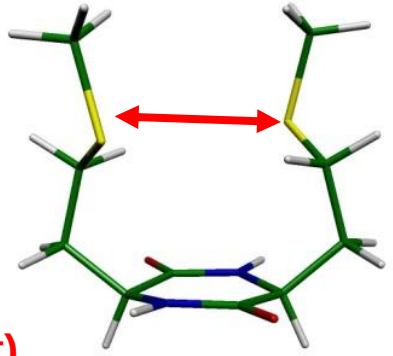
Institute of Nuclear Chemistry and Technology, 03-195 Warsaw, Poland, Radiation Laboratory, University of Notre Dame, Notre Dame, Indiana 46556, Faculty of Chemistry, Adam Mickiewicz University, 60-780 Poznan, Poland, Faculty of Chemistry, Rzeszow University of Technology, 35-959 Rzeszow, Poland, and Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, Kansas 66047.

Received: February 12, 2007; In Final Form: May 31, 2007

The recent study on the •OH-induced oxidation of calmodulin, a regulatory "calcium sensor" protein containing nine methionine (Met) residues, has supported the first experimental evidence in a protein for the formation of S∴N three-electron bonded radical complexes involving the sulfur atom of a methionine residue and the amide groups in adjacent peptide bonds. To characterize reactions of oxidized methionine residues in proteins containing multiple methionine residues in more detail, in the current study, a small model cyclic dipeptide, *c*-(L-Met−L-Met), was oxidized by •OH radicals generated via pulse radiolysis and the ensuing reactive intermediates were monitored by time-resolved UV−vis spectroscopic and conductometric techniques. The picture that emerges from this investigation shows there is an efficient formation of the Met(S∴N) radicals, in spite of the close proximity of two sulfur atoms, located in the side chains of methionine residues, and in spite of the close proximity of sulfur atoms and oxygen atoms, located in the peptide bonds. Moreover, it is shown, for the first time, that the formation of Met(S∴N) radicals can proceed directly, via H^+-transfer, with the involvement of hydrogen from the peptide bond to an intermediary hydroxysulfuranyl radical. Ultimately, the Met(S∴N) radicals decayed via two different pH-dependent reaction pathways, (i) conversion into sulfur−sulfur, intramolecular, three-electron-bonded radical cations and (ii) a proposed hydrolytic cleavage of the protonated form of the intramolecular, three-electron-bonded radicals {Met(S∴N)/Met(S∴NH)$^+$} followed by electron transfer and decarboxylation. Surprisingly, also α-(alkylthio)alkyl radicals enter the latter mechanism in a pH-dependent manner. Density functional theory computations were performed on the model *c*-(L-Met−Gly) and its radicals in order to obtain optimizations and energies to aid in the interpretation of the experiments on *c*-(L-Met−L-Met).



c-(D-Met-L-Met)



c-(L-Met-L-Met)

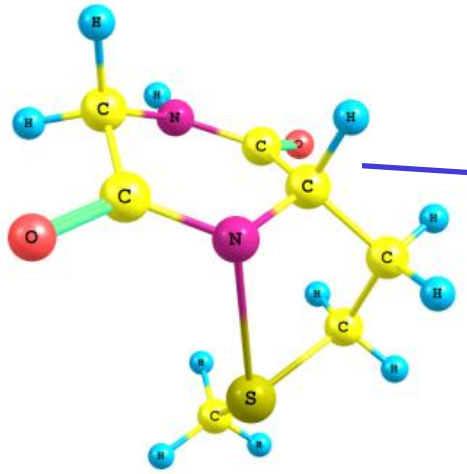
Krzysztof Bobrowski
INCT, Warsaw
POLAND

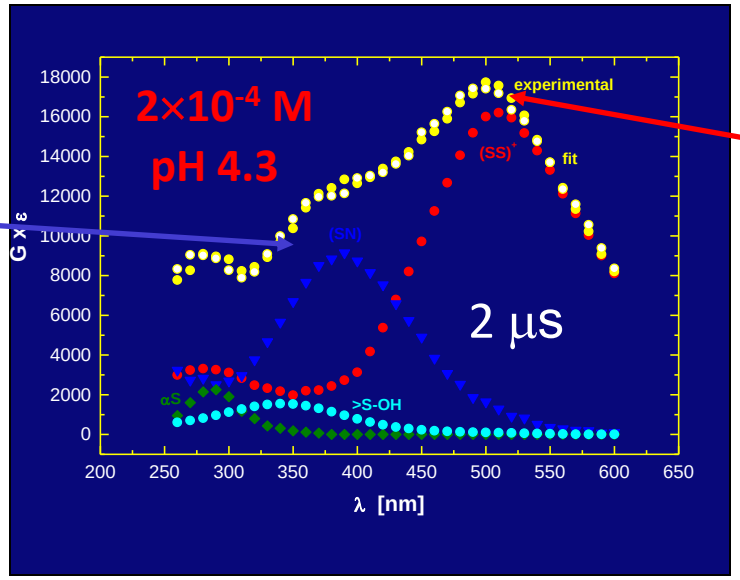
Sulfide radical cation chemistry in cyclic dipeptides containing stereoisomers of methionine

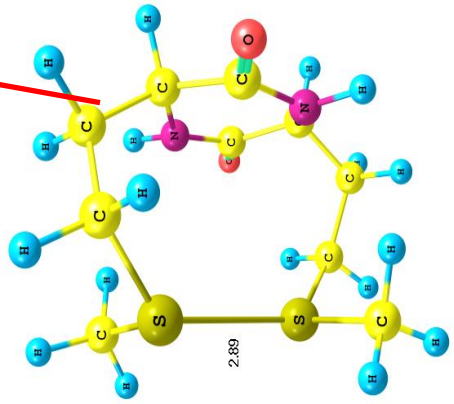
DFT B3LYP/6-31G(d)

c-(L-Met-L-Met)

DFT B3LYP/6-31G(d)







S∴N-bonded radical

S∴S-bonded radical cation

Stabilization of methionine sulfur radical cations in the *c*-(L-Met-L-Met) peptide occurs through intra-molecular bond formation with a sulfur atom of the adjacent methionine residue and with a nitrogen atom of adjacent peptide bonds

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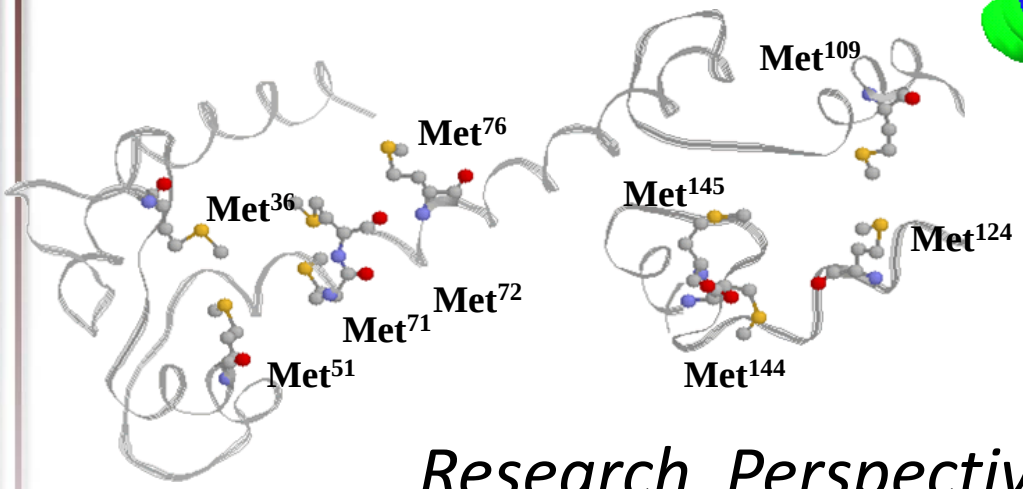
Stabilization of sulfur radical cations in selected proteins containing multiple methionine residues

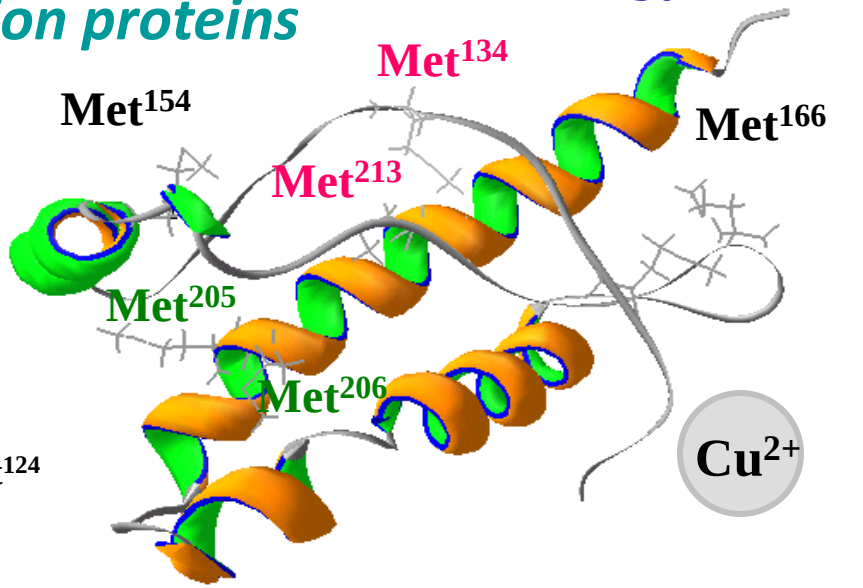
Calmodulin

regulatory calcium protein



Prion proteins

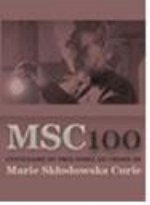
hPrP121-230



hPrP 108-113: Met109 Met112

Research Perspective

Knowledge that can be used in understanding the mechanism of oxidation processes in proteins (calmodulin, prion proteins) containing multiple methionine residues



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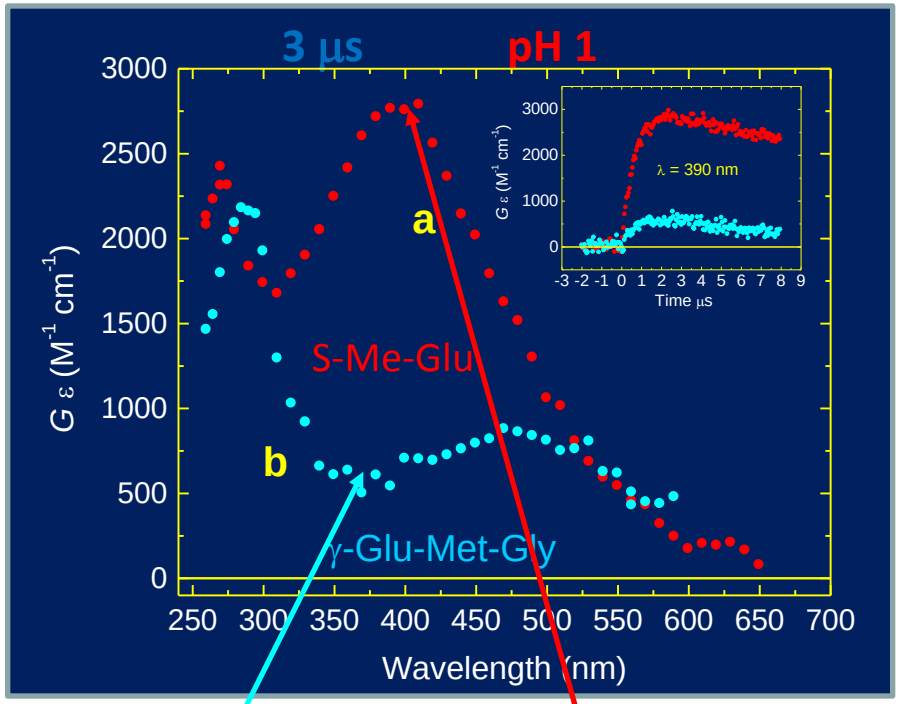
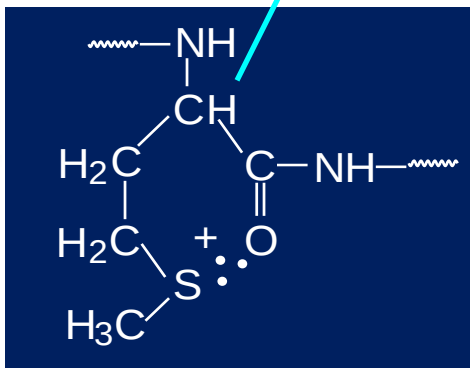
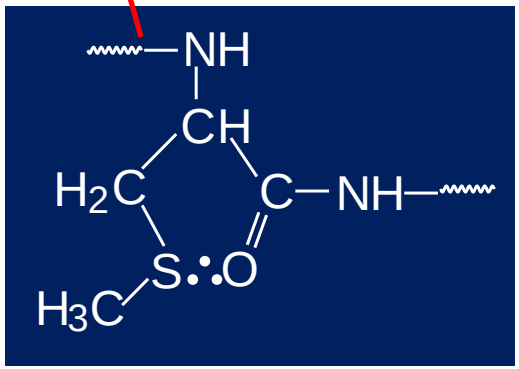
Five- vs. six-membered ring geometry







J|A|C|S
ARTICLES
Published on Web 06/29/2007

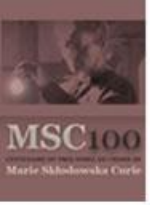
Sulfur Radical Cation–Peptide Bond Complex in the One-Electron Oxidation of *S*-Methylglutathione

Krzysztof Bobrowski,† Gordon L. Hug,*,‡,§,# Dariusz Pogocki,†,|| Bronislaw Marciniak,§ and Christian Schöneich⊥

Contribution from the Institute of Nuclear Chemistry and Technology, 03-195 Warsaw, Poland, Radiation Laboratory, University of Notre Dame, Notre Dame, Indiana 46556, Faculty of Chemistry, A. Mickiewicz University, 60-780 Poznan, Poland, Faculty of Chemistry, Rzeszow University of Technology, 35-959 Rzeszow, Poland, Department of Pharmaceutical Chemistry, University of Kansas, Lawrence, Kansas 66047

Received April 2, 2007; E-mail: hug.1@nd.edu

Abstract: Neighboring group participation was investigated in the •OH-induced oxidation of *S*-methylglutathione in aqueous solutions. Nanosecond pulse radiolysis was used to obtain the spectra of the reaction intermediates and their kinetics. Depending on the pH, and the concentration of *S*-methylglutathione, pulse irradiation leads to different transients. The transients observed were an intramolecularly bonded $[>S\therefore NH_2]^+$ intermediate, intermolecularly S∴S-bonded radical cation, α-(alkylthio)alkyl radicals, α-amino-alkyl-type radical, and an intramolecularly $(S\therefore O)^+$-bonded intermediate. The latter radical is of particular note in that it supports recent observations of sulfur radical cations complexed with the oxygen atoms of peptide bonds and thus has biological and medical implications. This $(S\therefore O)^+$-bonded intermediate had an absorption maximum at 390 nm, and we estimated its formation rate to be $\geq 6 \times 10^7\ s^{-1}$. It is in equilibrium with the intermolecularly S∴S-bonded radical cation, and they decay together on the time scale of a few hundred microseconds. The S∴S-bonded radical cation is formed from the monomeric sulfur radical cation ($>S^{\bullet+}$) and an unoxidized *S*-methylglutathione molecule with the rate constant of $1.0 \times 10^9\ M^{-1}\ s^{-1}$. The short-lived $[>S\therefore NH_2]^+$ intermediate is a precursor of decarboxylation, absorbs at ~ 390 nm, and decays on the time scale of hundreds of nanoseconds. Additional insight into the details of the association of sulfur radical cations with the oxygen atoms of the peptide bonds was gained by comparing the behavior of the *S*-methylglutathione (S∴O⁺-bonded five-membered ring) with the peptide γ-Glu-Met-Gly (S∴O⁺-bonded six-membered ring). Conclusions from experimental observations were supported by molecular modeling calculations.



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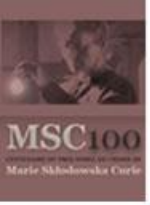


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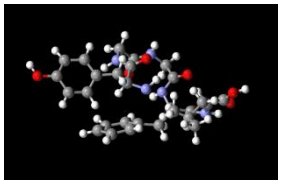
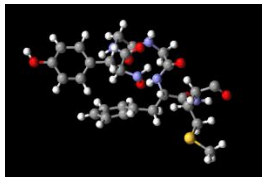
Radiation-induced intramolecular electron transfer in peptides

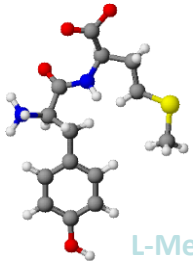
❑ **Intramolecular electron transfer in dipeptides containing tyrosine and methionine residues**

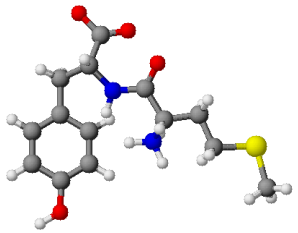
• Influence of aminoacid sequence, pH of the reaction environment, geometry of the molecule, optical isomerizm

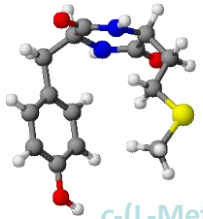
❑ **Intramolecular electron transfer in enkephalins (opiate pentapeptides)**

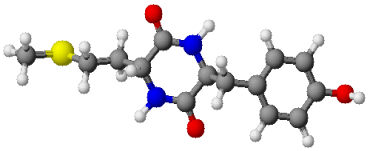
• Influence of replacement of the Leu-residue by the Met-residue

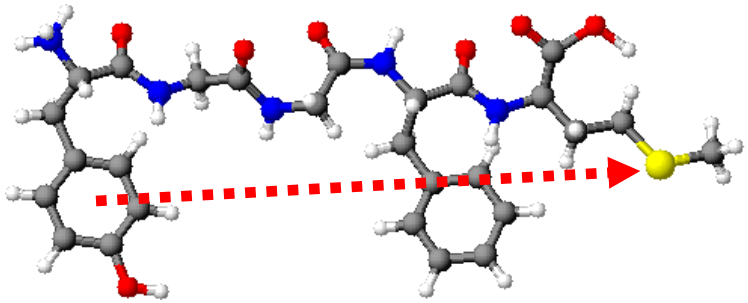




L-Met-L-Tyr


L-Tyr-L-Met

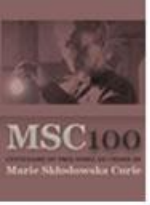

c-(L-Met-L-Tyr)


c-(L-Met-D-Tyr)


L-Tyr-Gly-Gly-L-Phe-L-Met

Research Perspective

Knowledge which can be used in understanding the mechanism of intramolecular electron transfer processes in oligopeptides and proteins containing tyrosine and methionine residues

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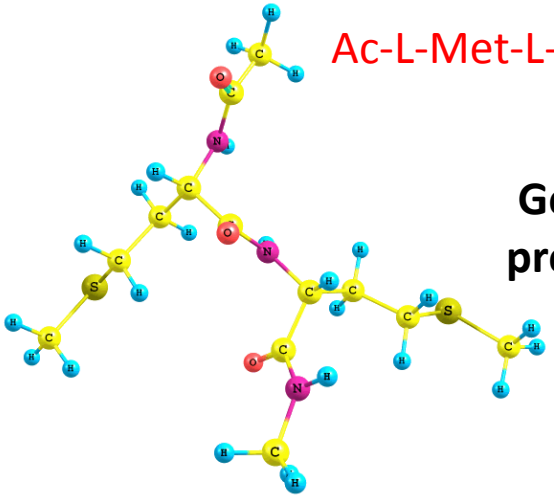
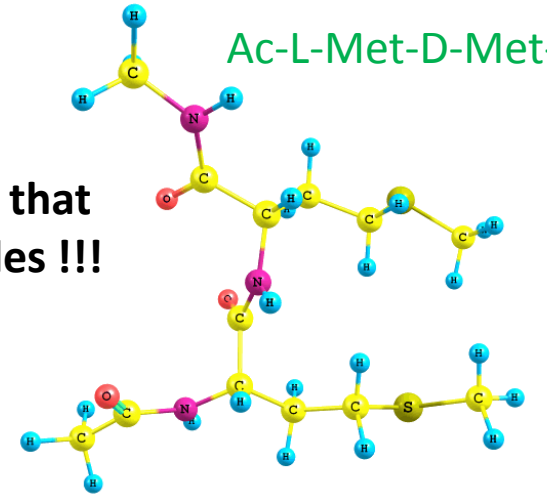
Stabilization of sulfide radical cations in linear (open chain) dipeptides containing stereoisomers of methionine with functionalized amino and carboxyl groups



Ac-L-Met-L-Met-$NHCH_3$

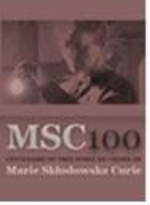
Geometry is reversed to that present in cyclic dipeptides !!!



Ac-L-Met-D-Met-$NHCH_3$

The **side chains of amino acids** residues in **L,L-configured dipeptides** are forced to point **in opposite directions** in space, which makes it more difficult to be in close contact

The **side chains of amino acids** residues in **L,D-configured dipeptides** brings both side chains to **the same side** of the peptide backbone and for this reason close contacts should be enhanced



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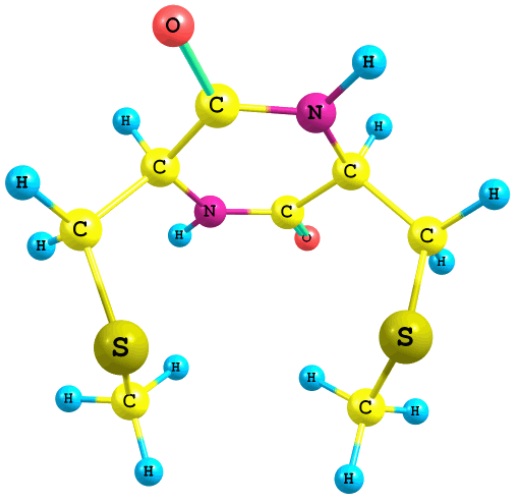
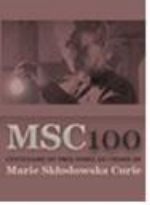
Stabilization of sulfide radical cations in cyclic dipeptides containing S-methylcysteine residues

Does the tendency towards formation of intramolecular S∴S, S∴N and S∴O-bonds change in cyclic dipeptides containing side chains of different length ?



c-(L-S-Me-Cys-L-S-Me-Cys)



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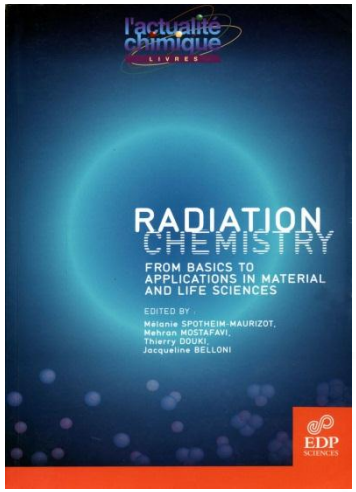

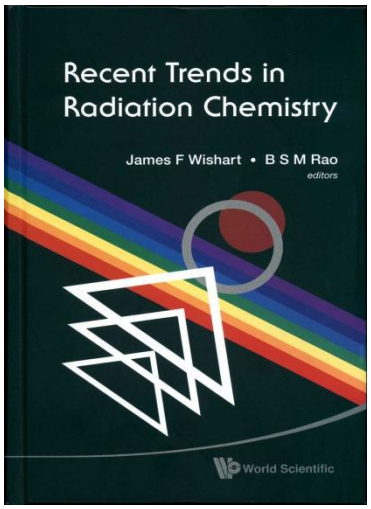

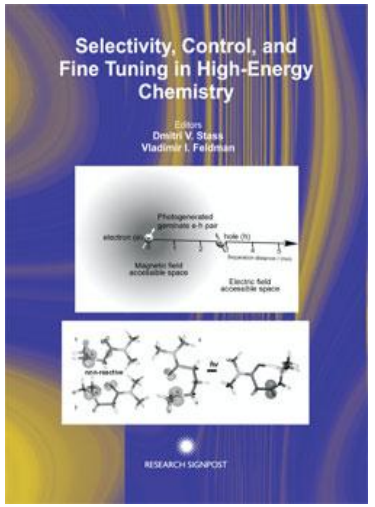

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Chapter 16

Pulse radiolysis studies of free radical processes in peptides and proteins

Chantal HOUÉE-LEVIN and Krzysztof BOBROWSKI

Introduction

The story of free radical reactions in biological systems began many years ago, when Harman postulated that they played a prominent role in ageing [1]. Since the sixties, the relevance to biological as well as industrial processes became more and more clear every year. It is now current to invoke free radicals in ordinary life, in cooking, in prevention of ageing processes, etc. and the scientific knowledge gave a basis to these assertions [2]. It is now beyond doubt that free radicals processes in proteins are involved in all steps of life, going from conception to death induction. They are believed to be part of the cellular defence against oxidative stress and at the same time responsible of severe damage like atherosclerosis [3] Protein free radicals are also enzyme active sites [4].

The studies by the methods of radiolysis provided a wealth of knowledge about the kinetic and thermodynamic controls of radical reactions, the importance of which is no more questioned. Indeed, it is known that the chemical events initiated by ionizing radiation, are the same as those that take place in normal and deleterious events of every day's life. In this review we focus on some of the major knowledge that was acquired by the use of pulse radiolysis and steady-state gamma radiolysis of aqueous solutions of amino acids, peptides and proteins (**Inset**). The potential role of pulse radiolysis (Chapter 2) for studying biomolecules has been acknowledged rather early. In most cases, pulse radiolysis

Chapter 16

Chemistry of Sulfur-Centered Radicals

*Krzysztof Bobrowski**

In the past few years, unprecedented progress has been made in the recognition and understanding of the role and structure, and reaction mechanisms of sulfur-centered radicals. Relevant examples include radical processes connected with repairing and protective mechanisms, oxidative stress, aging, and various diseases.

1. Introduction

Radiation chemistry, and pulse radiolysis in particular, is now a mature subject that is available as a very valuable and a powerful tool by which fundamental problems in free radical reaction mechanisms can be addressed. This chapter is restricted to studies concerning sulfur-centered radicals and radical-ions performed by radiation chemistry techniques in the first eight years of XXI century (2001–2008). Sulfur-centered radicals represent a very interesting class of radicals since they exhibit very interesting redox chemistry, including biological redox processes, and different spectral and kinetic properties as

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Selectivity, Control, and Fine Tuning in High-Energy Chemistry, 2011: 41-68
ISBN: 978-81-308-0432-3 Editors: Dmitri V. Stass and Vladimir I. Feldman

2. Radiation-induced radicals and radical ions in amino acids and peptides

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Abstract. Radiation chemistry methods, i.e., pulse and γ-radiolysis have been successfully applied for generation, identification, and spectral and kinetic characterization of radicals and radical ions in amino acids and peptides. In the past few years of XXI century a further progress has been made in recognition and understanding of the role and structure of the amino acid and peptide radicals and radical ions and their reaction mechanisms. Relevant examples include radical processes connected with repairing and protective mechanisms, enzymatic processes, oxidative stress, aging, and various diseases.

1. Introduction

Radiation chemistry methods, i.e., pulse and γ-radiolysis, are very valuable and powerful tools for solving numerous fundamental problems connected with understanding of radical processes that are of particular interest in biology and life sciences [1,2]. Amino acid and peptide radicals have been implicated in a wide variety of biochemical processes and

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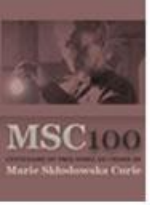

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Thank you very much for your attention