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Pulse radiolysis of aromatic amino acids – State of the art

Review Article

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Summary. The pulse radiolysis method as well as the primary processes of water radiolysis and the spectroscopic characteristics of H, OH, HO_2/O_2^- and e_{aq}^- are briefly presented. Subsequently, kinetic and spectroscopic data of the transients resulting from the resolved multi site attack on aromatic amino acids are discussed. The reactivity of H and e_{aq}^- with the same substrates, as well as the effect of oxygen on the major radiolytic processes are reviewed. Finally, the formation of tryptophan radical cation is mentioned shortly. The presented radiation mechanisms are the fundamentals for radiolytic processes occurring in proteins, enzymes and hormones in the living cells.

Keywords: Amino acids – Pulse radiolysis – Aromatic amino acids – Reaction mechanisms – Free radicals

Introduction

The basic building blocks of proteins are 20 amino acids. Most of them form peptide bonds as the α-carbonyl group of one amino acid reacts with the α-amino group of the next one forming a peptide chain. It folds spontaneously in a manner determined by the sequence of the amino acids participating in the chain. In addition to the common 20 amino acids there are further hundreds of rare amino acids, mostly isolated from plants and microorganisms. In addition to their function as peptide constituents aromatic amino acids are included in hormones and in enzymes.

The reactivity of the primary products of water radiolysis (e_{aq}^-, H, OH) with the peptides is rather high (10^7 to 10^{10} $dm^3mol^{-1}s^{-1}$) and is depending on the amino acids included in the chain. In general, the aromatic and the sulfur-containing amino acids react with e_{aq}^- and H-atoms with higher rate constants

($k = 10^8$ to 10^{10} dm^{3}mol^{-1}s^{-1}) than the aliphatic ones ($k = 10^6$ to 10^7 M^{-1}s^{-1}). However, all units of the protein chain are attacked by OH-radicals with $k = 10^8$ to 10^{10} dm^{3}mol^{-1}s^{-1} resulting in strand breaks which are leading to various kinds of final products.

In the frame of this paper a short review concerning the reaction mechanisms of the multisite OH-attack on aqueous aromatic amino acids as investigated by pulse radiolysis in combination with computer assisted modelling will be discussed. In addition the reactivity of H-atoms and e^-_{aq} towards the same substrates, the effect of oxygen and finally the formation of tryptophan radical cation will be mentioned. Firstly, a brief description of the pulse radiolysis technique as well as the primary products of water radiolysis and their spectroscopic and kinetic characteristics is introduced.

Pulse radiolysis method

The pulse radiolysis technique was first realized in several laboratories in 1960 [1–3]. Very soon it has become essential to the elucidation of fundamental processes in chemistry and later on for studies in the field of biology, medicine, pharmacy and for solving environmental preservation problems and many others.

A typical pulse radiolysis setup is given in Fig. 1. The electron accelerator (EA) is providing very short electron pulses (10^{-6} to 10^{-12} s duration) which are passing the quartz cell (Q) containing e.g. the substrate solution. The produced

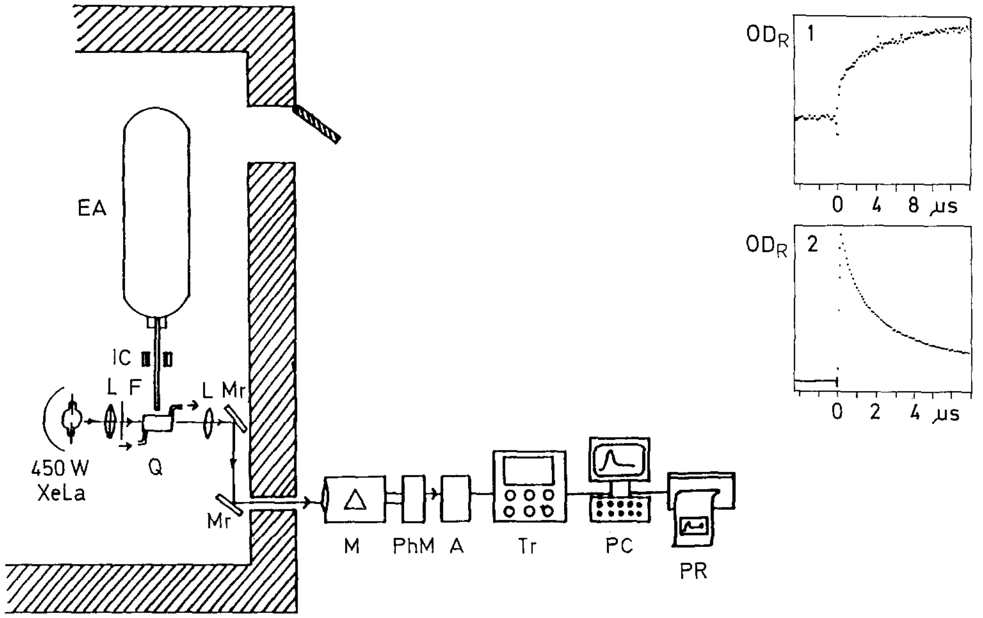
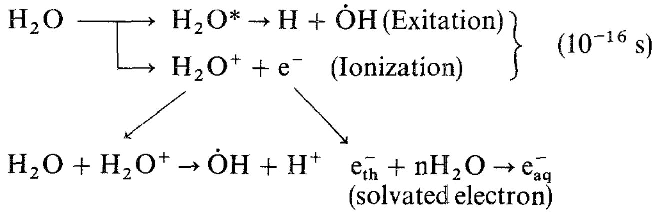


Fig. 1. Block scheme of pulse radiolysis equipment. Insert: Kinetic traces. *1* Formation and *2* Decay of transient

EA Electron accelerator; *IC* Ionization chamber; *Q* Quarz cuvette; *L* Lens for light beam focussing; *F* Filter; *XeLa* Xenon lamp; *Mr* Mirror; *M* Monochromator; *PhM* Photomultipler; *A* Amplifier; *Tr* Transient recorder; *PC* Computer; *PR* Printer short-lived species are usually registered by measuring their specific absorption. The analyzing light from a Xe-lamp (XeLa) is focused and after passing the cell (Q) is directed to a monochromator (M), where at given wavelength (λ, nm) the transmitted light is converted by a photomultiplier into a signal. This signal is amplified and displayed on a transient recorder (Tr) as a function of time (see inserts 1 and 2, Fig. 1). The data can be stored and evaluated on a computer (PC) and finally printed (PR). By means of appropriate variation of the experimental conditions the formation and decay kinetics can be studied exactly. The transient absorption is obtained by measuring the optical density (OD) at various wavelengths. Using a more sophisticated set up (e.g. OMA-system) the entire transient absorption spectrum can be obtained by just one single electron pulse. For further details see e.g. references [4–7]. The formation and decay of charged species can be also measured using the same set up. In this case the cell is fitted out with two Pt-electrodes connected with an appropriate equipment for registration of the voltage-time signal of the charged species [8–11].

Water radiolysis



$$H_2O \rightarrow H_2O^* \rightarrow H + \dot{O}H \text{ (Exitation)}; \quad H_2O \rightarrow H_2O^+ + e^- \text{ (Ionization)} \quad (10^{-16}\ s)$$

$$H_2O + H_2O^+ \rightarrow \dot{O}H + H^+ \qquad e^-_{th} + nH_2O \rightarrow e^-_{aq}$$

(solvated electron)

Primary products of water radiolysis (airfree solution)

$$H_2O \longrightarrow e^-_{aq}, H, \dot{O}H, H_2, H_2O_2, H^+_{aq}, OH^-_{aq}$$

$$e^-_{aq} + H^+ \rightarrow H \quad (k = 2.3 \times 10^{10}\ dm^3mol^{-1}s^{-1})$$

$$H + OH^- \rightarrow e^-_{aq} \quad (k = 2.5 \times 10^7\ dm^3mol^{-1}s^{-1})$$

$$\dot{O}H \rightleftharpoons O^{\cdot-} + H^+ \quad (pK = 11.9)$$

$$H_2O_2 \rightleftharpoons HO_2^- + H^+ \quad (pK = 11.6)$$

In the presence of oxygen

$$H + O_2 \rightarrow H\dot{O}_2 \quad (k = 1.9 \times 10^{10}\ dm^3mol^{-1}s^{-1})$$

$$e^-_{aq} + O_2 \rightarrow \dot{O}_2{}^- \quad (k = 2.0 \times 10^{10}\ dm^3mol^{-1}s^{-1})$$

$$H\dot{O}_2 \rightleftharpoons H^+ + \dot{O}_2^- \quad (pK = 4.8)$$

In the presence of N_2O

$$e^-_{aq} + N_2O \rightarrow \dot{O}H + OH^- + N_2 \quad (k = 1 \times 10^{10}\ M^{-1}s^{-1})$$

In the presence of H_2

$$\dot{O}H + H_2 \rightarrow H_2O + H \quad (k = 3.5 \times 10^7\ M^{-1}s^{-1})$$

Fig. 2. Pattern of water radiolysis and some major reactions of the primary radicals

As a sequence of radiation absorption in 10^{-16} s the water molecules can become excited (H_2O^*) or ionized: formation of H_2O^+ (radical cations) and secondary electrons (e^-). The latter are loosing energy by interaction with the surrounding molecules (formation of further H_2O^*, H_2O^+ and e^-) and thermalizing to e^-_{th} which finally become solvated (e^-_{aq}) within about 3×10^{-12} s. Some major primary processes of water radiolysis are compiled as a set of reactions in Fig. 2.

The absorption spectra of H, OH, $HO_2^{\cdot}$, $O_2^{\cdot -}$ and H_2O_2 are shown in Fig. 3, the spectrum of e^-_{aq} is given in Fig. 4. Obviously, the transients can be identified by their characteristic absorption maxima (λ max) and molar extinction coefficients (ε, $dm^3mol^{-1}cm^{-1}$). Since H and OH radicals absorb in the same region an appropriate scavenger is used to remove one of them in order to study the reactivity of the other.

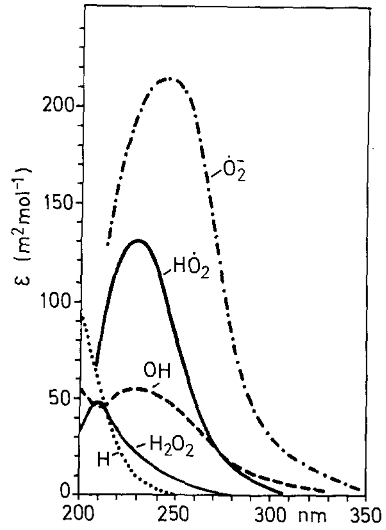


Fig. 3. Absorption spectra of H, OH, HO_2^- species as well as of H_2O_2

It is further of importance to mention that the (G-value)* of the primary products of water radiolysis depends upon the pH of the aqueous solution because of secondary reactions. This pH-dependence is illustrated by Fig. 5.

For a more extensive study of the water radiolysis fundamentals are recommended e.g. [12–15].

In the following the present knowledge concerning the multisite OH-attack on three important aromatic amino acids: phenylalanine (Phe), tyrosine (Tyr) and tryptophan (Try) in aqueous solutions is discussed. For resolving the superimposed kinetics and absorption spectra of the transients produced in fast

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* G-value: number of molecules produced or decomposed per 100 eV absorbed energy. For conversion in Si-units multiply the given G-values by 0.10364 to obtain $\mu mol . J^{-1}$.

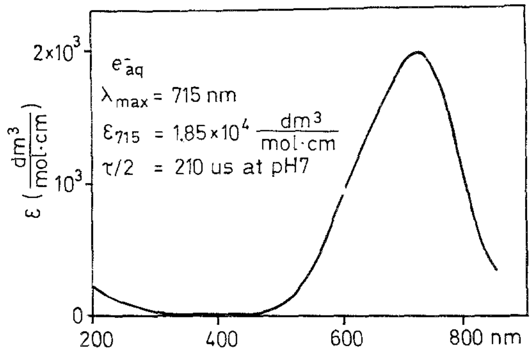


Fig. 4. Absorption spectrum of solvated electron (e^-_{aq})

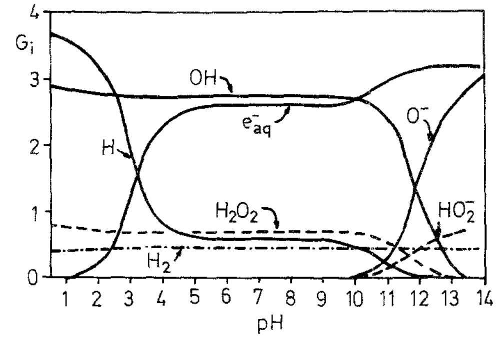


Fig. 5. *G*-values of the primary products of water radiolysis as a function of pH

complex chemical processes a special computer modelling procedure has been developed and applied [16, 17].

The reactivity of H-atoms and e^-_{aq} with the same aromatic amino acids in airfree aqueous solutions will be also briefly mentioned.

Reaction of phenylalanine with OH-radicals

Carefully performed investigations in neutral aqueous solutions on this subject have been reported by Solar [18 and ref. therein]. In order to convert e^-_{aq} into OH-radicals the substrate solution has been saturated with N_2O (2.8×10^{-2} mol.dm^{-3}; $k(e^-_{aq}+N_2O) = 0.91\times10^{10}$ dm^{3}mol^{-1}s^{-1} [19]). The $G(OH)_{total} = G(OH) + G(e^-_{aq}) = 5.5$. Using 3 MeV electrons with pulse length of 0.4 μs a total absorption spectrum is observed with a strong absorption maximum at 320 nm and a rather weak one at 540 nm. A total rate constant, $k(OH + Phe) = 6.7\times10^9$ dm^{3}mol^{-1}s^{-1} was determined [18]. The entire spectrum is shown in Fig. 6, A. By means of an appropriate combination of pulse radiolysis and the above mentioned computer assisted modelling method [16, 17] the individual sites of the OH-attack on the Phe-molecule have been elucidated [18]. The resolved spectra resulting from 50% OH-attack on the o-position of the benzene ring (R_1 species), 14% on meta (R_2) and 30% on the *p*-site (R_3) are pictured in

Fig. 6, B. The rest of the OH-radicals (6%) react with the alanine group of the Phe-molecule ($k = 9 \times 10^8$ dm^{3}mol^{-1}s^{-1}).

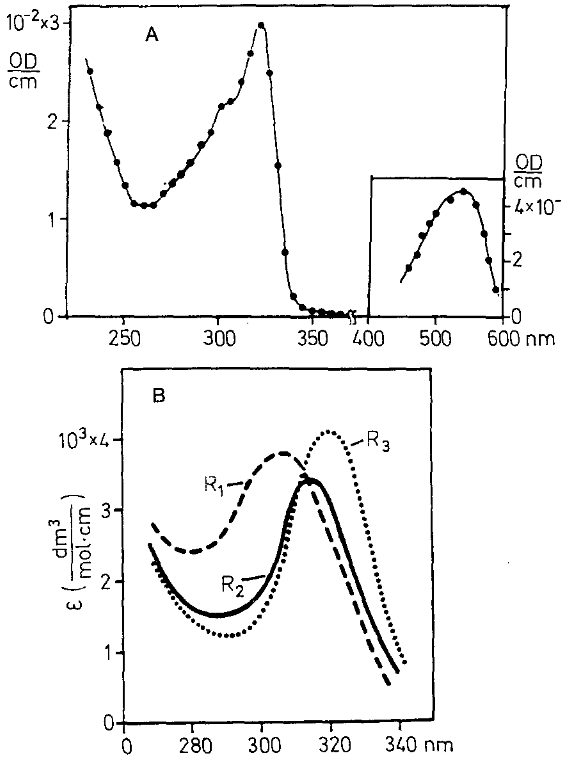
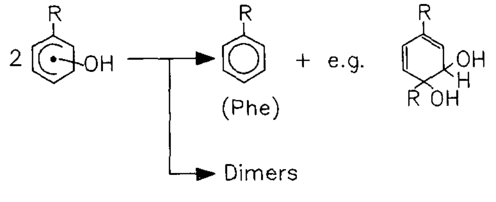


Fig. 6. **A** Measured total absorption spectrum (15 μs after pulse) of transients produced by OH-attack on 10^{-4} mol.dm^{-3} phenylalanine, 2.8×10^{-2} mol.dm^{-3} N_2O, pH 7.5; dose 17 Gy/0.4 μs pulse. **B** Resolved absorption spectra of transients: R_1, R_2 and R_3 formed by OH reaction with phenylalanine [18]

The reaction mechanisms as well as the formation and decay kinetics of the individual transients (R_1, R_2 and R_3) and their spectroscopic characteristics are presented in Fig. 7. Each of the formed OH-adducts can react with its own kind as well as with the others leading to the formation of various products. Thereby the Phe-molecule can be regenerated by disproportionation of two OH adducts (reaction 1a) or resulting in tyrosine and hydroxylated products as well as form dimers (reaction 1b):



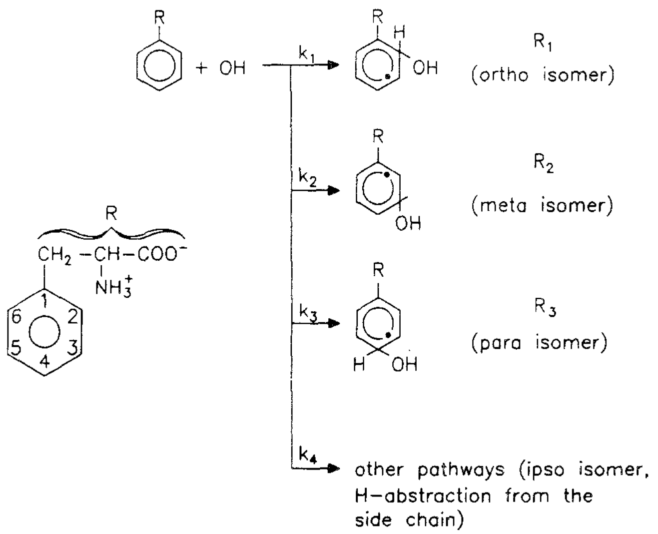
(1a)

(1b)

The radiolysis of the phenylalanine results also in the formation of ammonia, CO_2 and other final products. In the case that the reacting phenylalanine

Multisite OH-attack on phenylalanine (pH = 7.5)

$k(\mathrm{OH} + \mathrm{Phe})_{\mathrm{total}} = 6.7 \times 10^9\ \mathrm{dm^3mol^{-1}s^{-1}}$



Rate constants (k) for formation and decay as well as spectroscopic characteristics of transients formed by OH-attack on phenylalanine in neutral aqueous solutions (pH = 7.5)

	k (dm^{3}mol^{-1}s^{-1})		Absorption characteristics	
Transients	Formation	Decay	$\lambda_{\max}$ (nm)	ε (dm^{3}mol^{-1}cm^{-1})
R_1 (50%)	$k_1 = 3.5 \times 10^9$	$2k_7 = 1.0 \times 10^9$	305	380
R_2 (14%)	$k_2 = 1.0 \times 10^9$	$2k_8 = 1.3 \times 10^9$	315 540	350 32
R_3 (30%)	$k_3 = 2.2 \times 10^9$	$2k_9 = 1.5 \times 10^9$	320	410

Fig. 7. OH-attack phenylalanine as well as kinetic and spectroscopic characteristics of the resulting transients

molecule is contained in a peptide chain the produced transients can initiate reactions with units of neighbouring chains giving rise to a number of processes.

Reactions of tyrosine with OH-radicals

The total rate constant of the OH-attack on various sites of a Tyr-molecule is found to be very high: $k(\mathrm{OH} + \mathrm{Tyr}) = 0.42 \times 10^{10}\ \mathrm{dm^3mol^{-1}s^{-1}}$ [20] and $k = 1.4 \times 10^{10}\ \mathrm{dm^3mol^{-1}s^{-1}}$ [21 and ref. therein]. Using tyrosine solutions saturated with N_2O a total absorption spectrum representing various tyrosine radicals was obtained at pH 7.7 (Fig. 8, IA; 8 μs after electron pulse [21]). It shows several absorption maxima (260, 310, 330 and 540 nm). At 80 μs after pulse end an additional absorption peak occur at 560 nm (Fig. 8, IB) and at ~150 μs an

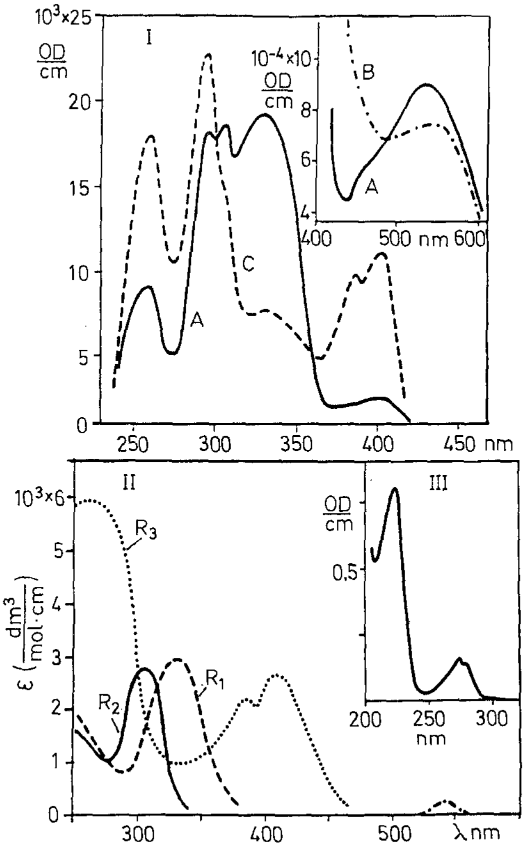


Fig. 8. I Total absorption spectra of transients formed by OH-attack on 10^{-4} mol.dm^{-3}. Tyr, saturated with N_2O, pH = 7.7 at: *A* 8 μs, *B* 80 μs and *C* ~150 μs after pulse; dose/0.4 μs pulse = 10 J.kg^{-1}. **II** Resolved absorption spectra of transients: R_1, R_2 and R_3 formed by OH-attack *k* on tyrosine. **III** Absorption spectrum of 10^{-4} mol.dm^{-3} tyrosine, pH 7 [21]

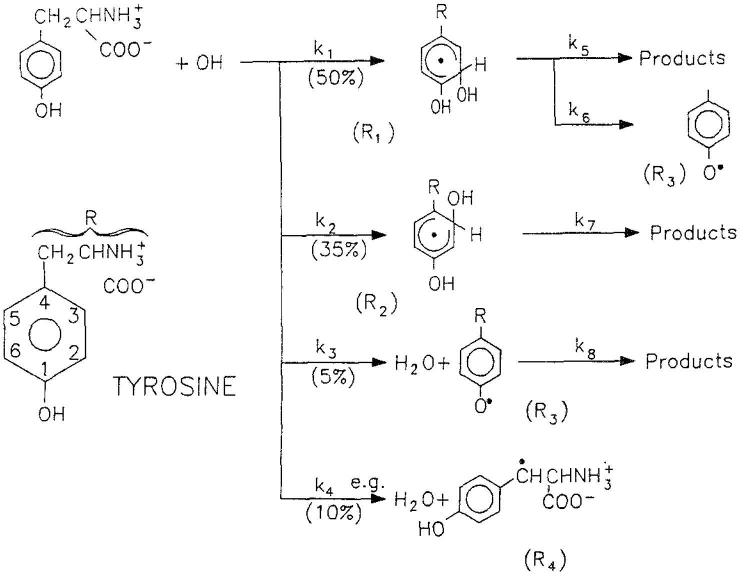
essential change of the spectrum is taking place, indicating the transformation of the primary formed radicals (Fig. 8, IC). The strong absorption maxima are at 260, 300 and 405 nm. The absorption band at 405 nm has been previously assigned to the phenoxyl radical, which is formed by water elimination of the OH-adducts [22]. The phenoxyl radicals can also be produced by a direct reaction of OH-species with the OH-group of the Tyr-molecule [21] as shown below.

By means of pulse radiolysis measurements of the time dependent change of the optical densities (OD-values) of different tyrosine solutions in the time range of 1 to 2000 μs and applying the computer assisted modelling program [16, 17] the superimposed kinetic and spectroscopic characteristics of the individual transients have been resolved [21]. The obtained absorption spectra of the Tyr-species resulting from the OH-attack are presented in Fig. 8, II [21].

Based on the obtained experimental data it has been possible to elaborate a reaction mechanism initiated by the OH-attack on tyrosine, given in Fig. 9. It is established that 50% OH lead primarily to the formation of an OH-adduct (hydroxycyclohexadienyl-radical, R_1) on ortho-position to the OH-group, which

Multisite OH-attack on tyrosine (pH 7.5)

$k(\mathrm{OH} + \mathrm{Tyr})_{\mathrm{total}} = 1.3 \times 10^{10}\ \mathrm{dm^3mol^{-1}s^{-1}}$ [21]



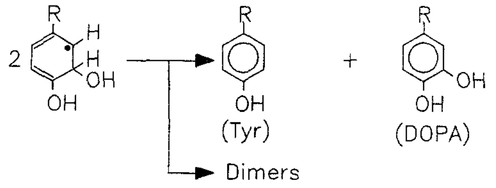
Rate constants for formation and decay as well as absorption characteristics of transients produced by OH-attack on aqueous tyrosine (pH = 7)

	Rate constants (in $\mathrm{dm^3mol^{-1}s^{-1}}$) for:		Absorption characteristics	
Transients	Formation (k)	Decay ($2k$)	$\lambda_{\max}$ (nm)	ε ($\mathrm{dm^3mol^{-1}cm^{-1}}$)
R_1	$k_1 = 7.0 \times 10^9$	$2k_5 = 3.0 \times 10^8$ $k_6 = 1.8 \times 10^4$	330	3000
R_2	$k_2 = 5.0 \times 10^9$	$2k_7 = 2.0 \times 10^9$	305 540	2800 230
R_3	$k_3 = 5.0 \times 10^4$ $^*k_6 = 1.8 \times 10^4$	$2k_8 = 4.0 \times 10^8$	260 405	6000
R_4	$k_4 = 1.4 \times 10^9$	—	—	—

* k in $\mathrm{s^{-1}}$

Fig. 9. Scheme of the reactions of OH radicals with tyrosine as well as kinetic and spectrosopic characteristics of the resulting transients [21]

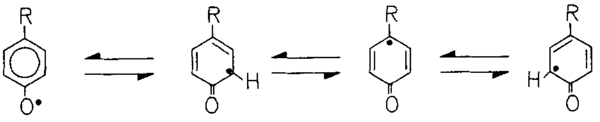
can decay by disproportionation to tyrosine and DOPA (3,4-dihydroxyphenylalanine (eq. 2a), as well as by dimerization (eg. 2b) [23, 24]:



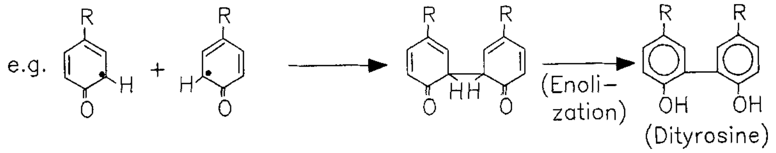
(2a)

(2b)

The phenoxyl radical of Tyr can exist in several resonance structures, each of them can lead to the formation of different final products (see eq. 3 and 4):

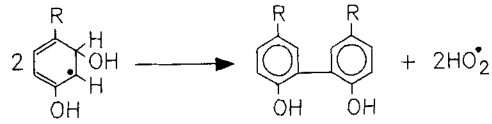


(3)



(4)

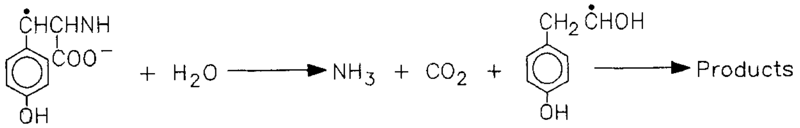
35% OH are producing the OH-adduct R_2 and about 5% are attacking directly the OH-group on Tyr-molecule resulting in phenoxyl radical, R_3. The R_2 species can react with R_1 radicals by disproportionation resulting in Tyr and DOPA, similar to reaction (2a), or R_2 can disappear by dimer formation. Further, two R_2-species can probably combine to form dityrosine [23, 24]:



(5)

The $HO_2^{\bullet}/O_2^{\bullet -}$ radicals are able to attack the Tyr-molecule or react with each other to form hydrogen peroxide. Finally, the remaining 10% OH react with the alanine group of the substrate producing the radicals of the R_4 type. In this respect it should be mentioned that in neutral solution OH-species attack ethylamine with $k = 6.2 \times 10^9$ dm^{3}mol^{-1}s^{-1} on the α- and β-positions of the molecule [25].

The obtained transients can disappear according to various reaction paths which are not further discussed here. Though, at least one of them leads to diamination and decarboxylation, probably by reaction (6):



(6)

The deamination of the amino group of tyrosine has been observed at pH 4 and 13 in airfree solutions and in such saturated with N_2O or air. At pH 13 the $G(NH_3)$-values in the given sequence are: 0.12, 0.30 and 0.04 [23 and ref. therein]. These findings strongly support the postulated reaction (6).

The spectroscopic and kinetic characterists of the Tyr-radicals are compiled in the table in Fig. 9 [21]. Summing up, it can be stated that the predominant part of the OH-radicals (85%) are forming OH-adducts on the phenol ring. The reactivity of these transients is rather high ($k = 3 \times 10^8$ to 2×10^9 dm^{3}mol^{-1}s^{-1}) and a number of final products can be formed as shown above.

In addition to the mentioned radiolytic products the formation of three tyrosine and cross-linked molecules occur at higher radiation doses.

Reactions of tryptophan with OH-radicals

The radiolysis of tryptophan has been investigated in deoxygenated [26, 27] as well as in aerated aqueous solutions [28–30] from different points of view. Armstrong and Swallow [26] performed carefully steady state and pulse radiolysis investigations on aqueous tryptophan (pH = 1–9). They determined: $k(\mathrm{OH} + \mathrm{Try}) = 1.25 \times 10^{10}$ $\mathrm{dm^3mol^{-1}s^{-1}}$, $k(\mathrm{H} + \mathrm{Try}) = 7.4 \times 10^9$ $\mathrm{dm^3mol^{-1}s^{-1}}$ and $k(e^-_{aq} + \mathrm{Try}) = 3 \times 10^8$ $\mathrm{dm^3mol^{-1}s^{-1}}$. The total transient absorption spectrum resulting from the OH-attack on the substrate showed several absorption bands in the range from 300 up to 600 nm.

They have been interpreted in terms of various reaction sites by addition of OH on the benzene and indol ring. As final products have been found: $G(\mathrm{NH_3}) = 0.51$, $G(\mathrm{H_2}) = 0.37$, $G(\mathrm{H_2O_2}) = G(\mathrm{ROOH}) = 0.63$ and $G(\text{-Try}) = 0.7$ [26].

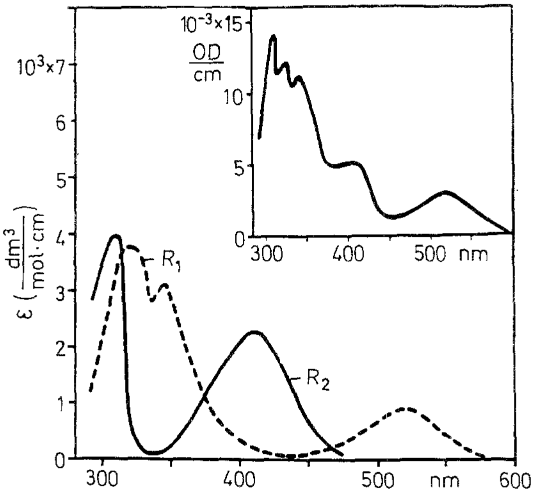
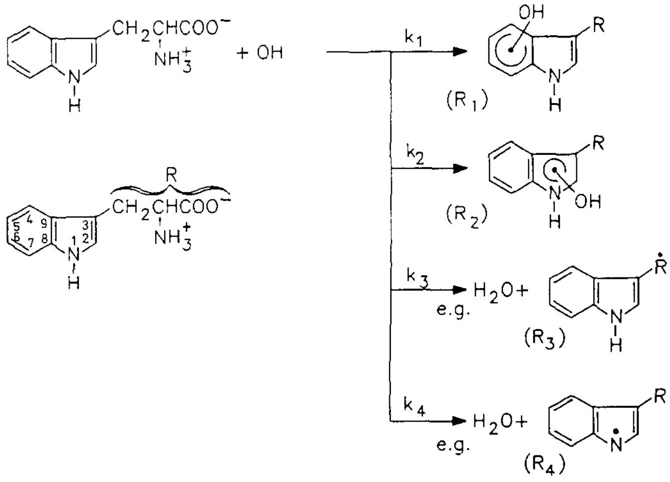


Fig. 10. Resolved absorption spectra of R_1 and R_2 transients produced by the OH-attack on aqueous tryptophan at pH = 6.5 to 8.5 (Solution: 2×10^{-5} mol.dm^3 tryptophan, 2.8×10^{-2} mol.dm^{-3}$\mathrm{N_2O}$; applied dose: 4 to 6 J.kg^{-1}/0.4 μs pulse). Insert: Experimentally measured total absorption spectrum of transients ($R_1 + R_2$) dose/pulse: 10 J/kg [31]

The pulse radiolysis of aqueous tryptophan, saturated with $\mathrm{N_2O}$ (scavenger for e^-_{aq}) has been reinvestigated with regard to resolve the multisite absorption spectrum of the transients resulting from the reaction of OH radicals with tryptophan measured in neutral solution (Fig. 10, insert [31]). It shows several maxima and is very similar to that previously reported by Armstrong and Swallow [26]. From the formation kinetics at the absorption bands a mean value of the total rate constant of the OH with Try-molecule was obtained, $k(\mathrm{OH} + \mathrm{Try}) = 1.3 \times 10^{10}$ $\mathrm{dm^3mol^{-1}s^{-1}}$ [31].

Based on the differences of the decay kinetics at the various maxima it was concluded that the OH-radicals attack the Try-molecule on various sites. By applying the combined method of pulse radiolysis and computer assisted modelling [16,17] it was possible to resolve the spectrum into two major components as presented in Fig. 10. The R_1-transient represents the OH-reaction at different positions on the benzene ring (39% OH), whereas R_2-species (57% OH) are formed as a sequence of the OH-reactions on the pyrrole ring. The rest (~4% OH) is consumed by an attack on the alanine chain of the Try-molecule. By means of MO-calculations according to the method by Fukui [32] it was established that position 2, followed by positions 4 and 3 are the most predestinated ones for reaction with OH-radicals [31]. Hence, both positions, namely C_2 and C_3 of the pyrrol ring are the most involved in the process, leading to two transient isomers. Similar conclusions based on the inductive effect of heterocyclic nitrogen have been made by Armstrong and Swallow [26]. They assigned the absorption band at 325 nm to OH-adduct on C_3-position and that at 345 nm to the C_2-position. It was, however, not possible by means of the

Multisite OH-attack on aqueous tryptophan (pH = 6.5 to 8.5)
$k(\mathrm{OH} + \mathrm{tryptophan})_{\mathrm{total}} = (1.3 \pm 0.5) \times 10^{10}\ \mathrm{dm^3mol^{-1}s^{-1}}$



Kinetic and absorption characteristics of transients formed by OH-attack on tryptophan (pH6.5–8.5)

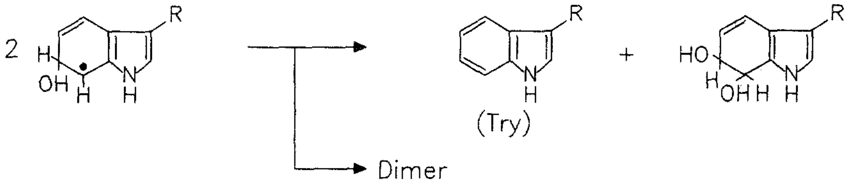
	Rate constants for:		Absorption characteristics	
Transients	Formation (k in $\mathrm{dm^3mol^{-1}s^{-1}}$)	Decay (k in $\mathrm{dm^3mol^{-1}s^{-1}}$)	$\lambda_{\max}$ (nm)	$\varepsilon_{\max}$ ($\mathrm{dm^3mol^{-1}cm^{-1}}$)
R_1	$k_1 = 5.0 \times 10^9$	$k_3 = 2.0 \times 10^9$	310	4000
R_2	$k_2 = 7.5 \times 10^9$	$k_4 = 2.3 \times 10^8$	325	3750
			340	2950
			520	750
$R_3 + R_4$	$k_3 + k_4 = 0.5 \times 10^9$	—	—	—

Fig. 11. Scheme of the reaction of OH radicals with tryptophan as well as kinetic and spectroscopic characteristics of the resulting transients [31]

semilinear optimization procedure to obtain a precise elucidation of the subject matter [31].

Based on their results, Solar et al [31] presented a reaction mechanisms shown in Fig. 11, together with the resolved formation and decay kinetics and spectroscopic data. Obviously, the R_3 and R_4 transients resulting from the 4% OH are of minor interest.

One of the major processes responsible for the decay of the OH-adducts is the reformation of tryptophan, e.g.:



(7a)

(7b)

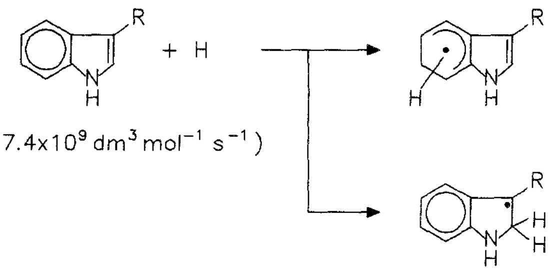
Reactivity of H and e_{eq}^-

The reactions of H-atoms and e_{aq}^- with the aromatic amino acids are only briefly mentioned. The mean values of the corresponding rate constants (k-values) including these of histidine are compiled in the following Table 1. For the sake of completeness the $k(OH + AS)$ are also given.

Table 1. Mean values of rate constants (k) for the reactions of Phe, Tyr, Try and His with OH, e_{aq}^- and H [18, 21, 26, 31, 33]

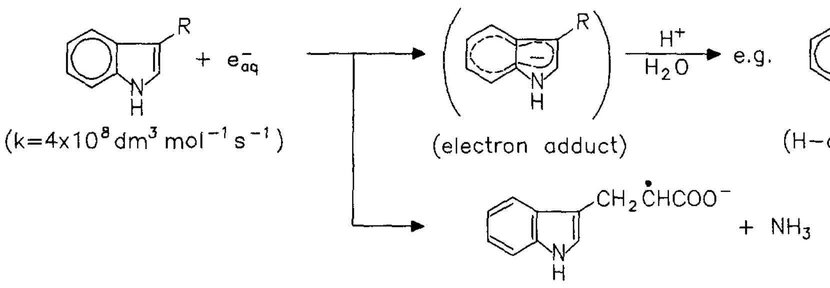
Amino acid	k(dm^{3}mol^{-1}s^{-1} × 10^{-9}) with: OH (pH 7)	e_{aq}^- (pH 7)	H (pH 1)
Phe	6.6	0.15	0.74
Tyr	14.0	0.4	2.0
Try	13.0	0.46	7.4
His	5.0	0.06	0.047

The type of reactions in which the aromatic amino acids are involved with H and e_{aq}^- is illustrated on tryptophan as an example. The H-atoms are forming adducts (eq. 8a and 8b) whereas e_{aq}^- results in electron adduct first which protonates to H-adduct (eq. 9a). Also, e_{aq}^- can attack specifically the $-NH_3^+$ group leading to ammonia production (eq. 9b):



(8a)

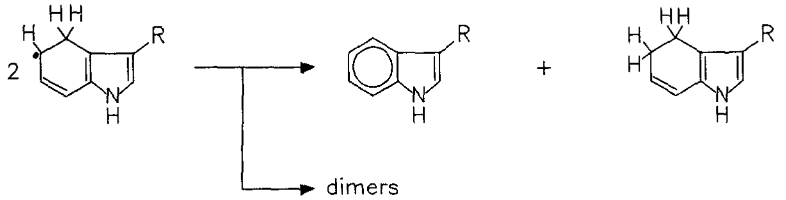
(8b)



(9a)

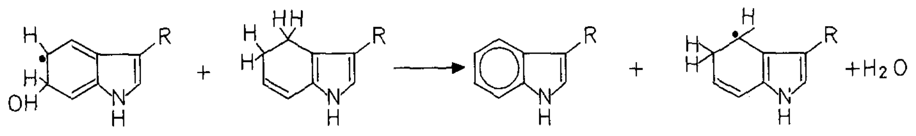
(9b)

In the following reactions (10–12) the major decay possibilities of the try-transients are presented: disproportionation (eq. 10a) leading to reformation of Try, dimerization (eq. 10b and 12b) as well as combination of two different species (eq. 11 and 12a).

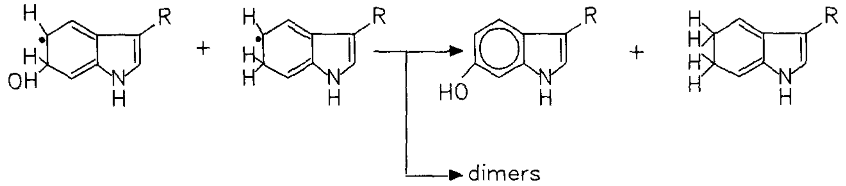


(10a)

(10b)



(11)



(12a)

(12b)

In addition to this, the transients of the aromatic amino acids play an important role in the cross-linking between proteins, enzymes and in some cases of nucleic acids too. This effect explains some of the radiation induced processes in bio-polymers [34]. The attack of OH, e^-_{aq} and H on various sites of an enzyme is also initiating consecutive intramolecular processes in which one electron equivalent radical transfer takes place from one amino acid residue to others involving selectively only aromatic and divalent sulfur components. In simple peptides containing e.g. tyrosine and tryptophan an intramolecular electron transfer occurs between both of them [35, 36].

The effect of oxygen

In the presence of oxygen the H-atoms and e^-_{aq} are converted into peroxy radicals:

$$\mathrm{H} + \mathrm{O}_2 \rightarrow \mathrm{H\dot{O}_2} \quad (k_{13} = 1.9 \times 10^{10}\,\mathrm{dm^3mol^{-1}s^{-1}}\ [37]) \tag{13}$$

$$e^-_{aq} + \mathrm{O}_2 \rightarrow \mathrm{\dot{O}_2^-} \quad (k_{14} = 2 \times 10^{10}\,\mathrm{dm^3mol^{-1}s^{-1}}\ [38]) \tag{14}$$

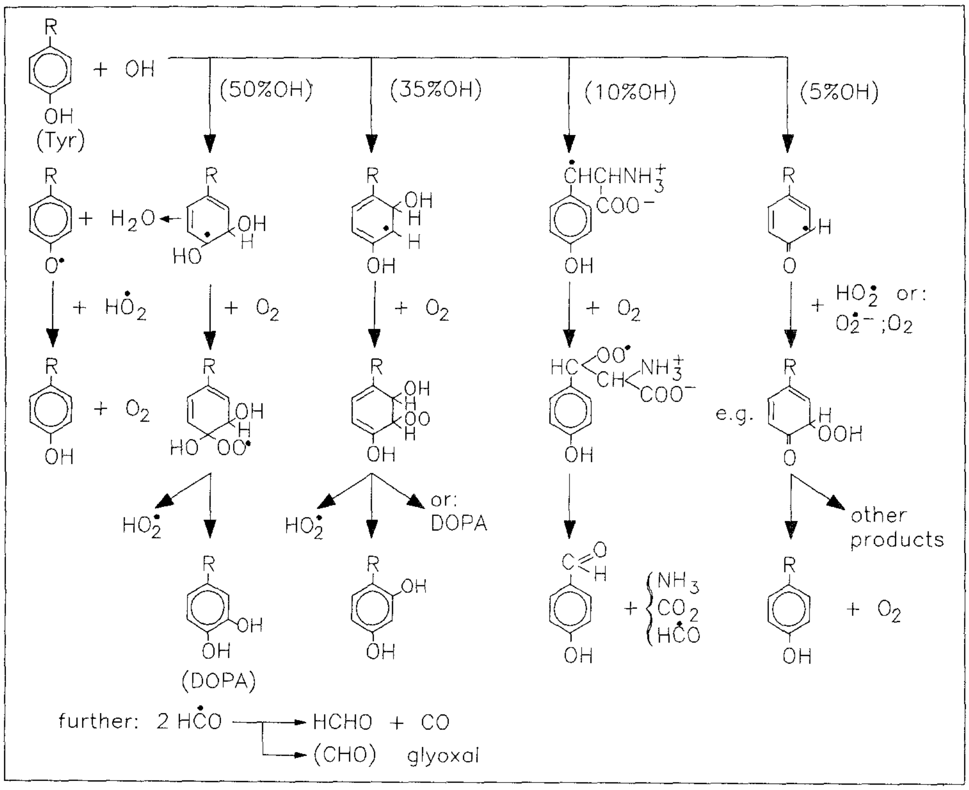
$$\mathrm{H\dot{O}_2} \rightleftharpoons \mathrm{H^+} + \mathrm{\dot{O}_2^-} \quad (\mathrm{pK} = 4.8\ [39]) \tag{15}$$

$$2\mathrm{H\dot{O}_2} \rightarrow \mathrm{H_2O_2} + \mathrm{O_2} \quad (k_{16} = 3.7 \times 10^{6}\,\mathrm{dm^3mol^{-1}s^{-1}}\ [39]) \tag{16}$$

$$2\mathrm{\dot{O}_2^-} \rightarrow \mathrm{O_2^{2-}} + \mathrm{O_2} \quad (k_{17}\ 10\,\mathrm{dm^3mol^{-1}s^{-1}}\ [39]) \tag{17}$$

Similary to these processes also the OH-adducts of the aromatic amino acids are adding oxygen ($k = 5 \times 10^8$ to 10^9 $\mathrm{dm^3mol^{-1}s^{-1}}$) leading to the production of peroxy radicals. These species are unstable and decay by splitting $\mathrm{H\dot{O}_2}$ transients (at pH > 5, $\mathrm{\dot{O}_2^-}$) under formation of hydroxylated products, e.g. phenylalanine forms tyrosine. One of the major radiolytic products of Tyr is DOPA, whereas the oxidation products of Try are N-formylkynurenine and dioles. This subject matter is illustrated by taking Tyr and Try as examples.

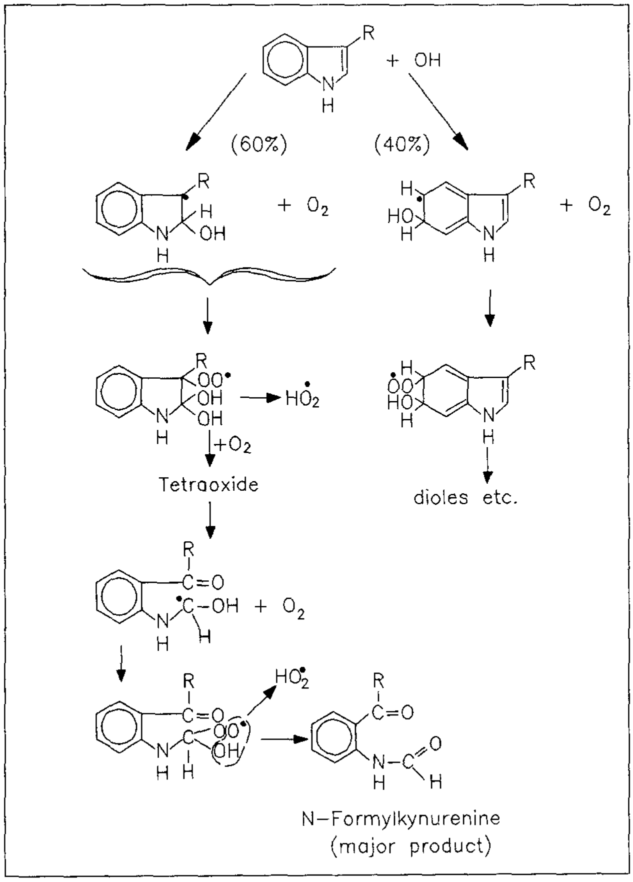
As discussed above the multisite OH-attack on tyrosine is leading to the formation of various OH-adducts. These can react with oxygen very likely according to the following Scheme 1 [40, 41]:



Scheme 1. Radiolyses of tyrosine in the presence of oxygen

It has been found that the tyrosine phenoxyl radical react with $O_2^{\bullet -}$ species with $k = 1.7 \times 10^9$ dm^{3}mol^{-1}s^{-1} leading to reformation of tyrosine [42]. In addition to the tyrosine regeneration also DOPA appears as a final product among other compounds [40, 42]. It should be mentioned that the formation of dityrosine is suppressed in oxygenated acid and neutral tyrosine solutions, but not in alkaline media at pH 12 [23]. The produced $HO_2^{\bullet}$ and $O_2^{\bullet -}$ species (see Scheme 1) can react efficiently with aromatic transients leading to a variety of products [43].

The most important radiation induced reactions of oxygenated aqueous tryptophan solutions are given in Scheme 2 [24, 26].



Scheme 2. Major radiolytic processes of tryptophan in oxygenated solutions

In addition to the OH-incorporation on the benzene ring of the aromatic amino acids also C-C-bond splitting can occur in the presence of oxygen. Comparative studies in this respect have been performed on phenol [44] and p-Cl-phenol [45].

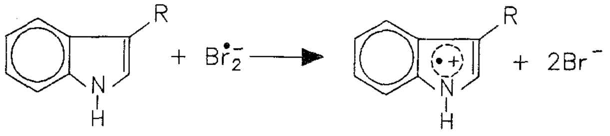
Formation of tryptophan radical cation

In general the radical cation ($RH^{\cdot +}$) of a molecule can be produced by: a) electron transfer from the substrate to a transient (e.g. $X_2^{\cdot -}$) acting as an electron acceptor, as studied by pulse radiolysis [e.g. 46–48]; b) as a sequence of electron ejection from an electronically excited molecule [e.g. 49, 50]. In this case common flash photolysis or pulsed laser light are applicable.

The formation of tryptophan radical cation ($TryH^{\cdot +}$) has been studied by several authors applying flash photolysis as well as pulse radiolysis [51]. For the formation of $TryH^{\cdot +}$ two processes are theoretically possible: electron or hydrogen atom transfer from the N-position of the Try-molecule. On the basis of the equality of the obtained spectroscopic and the kinetic data for formation and decay of $TryH^{\cdot +}$ species in H_2O and D_2O it was proven that electron transfer from the N-atom of Try molecule to the $X_2^{\cdot -}$ is the driving factor of the process [48]. The one electron oxidation of Try was achieved by the following set of reactions, where $X_2^{\cdot -} = Cl_2^{\cdot -}$ $Br_2^{\cdot -}$ $(SCN)^{\cdot -}$ $I^{\cdot -}$ etc.

$$Br^- + OH \longrightarrow Br^{\bullet} + OH^- \qquad (16)$$

$$Br^- + Br^{\bullet} \longrightarrow Br_2^{\bullet -} \qquad (17)$$



(Try) (18)

$$\text{in } H_2O : k = 1.3 \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1} /48/$$

$$\text{in } D_2O : k = 1.2 \times 10^9 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1} /48/$$

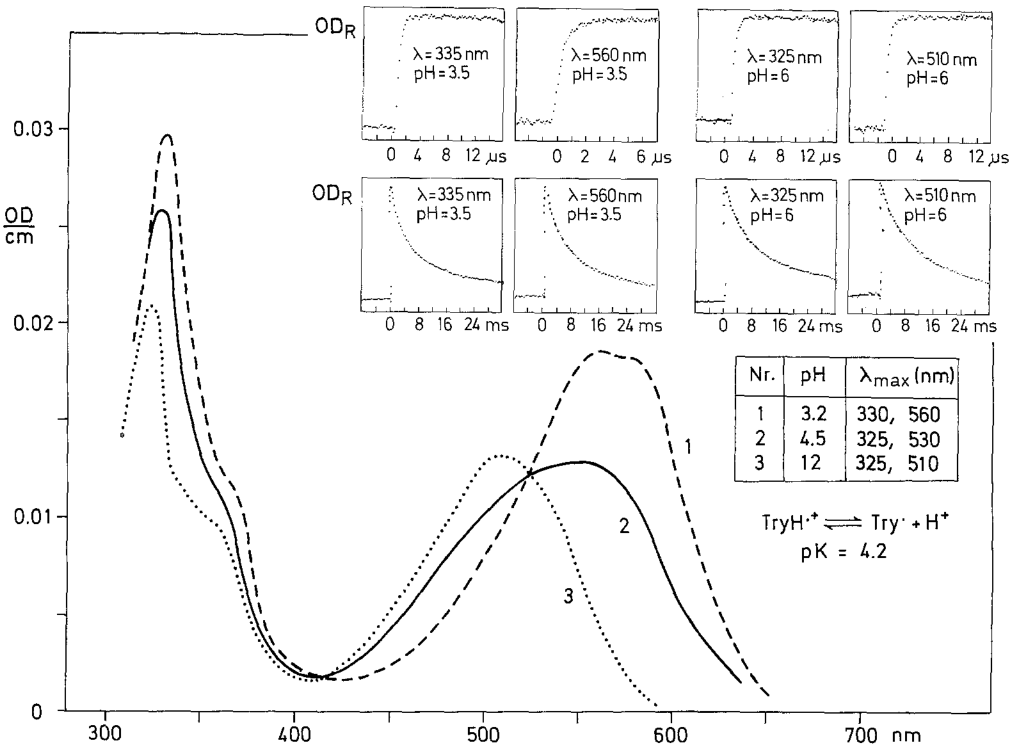
The absorption spectra of tryptophan radical cation measured in the pH-range from 3.5 to 12 is presented in Fig. 12 [52]. Based on the pH-dependence of the optical densities (OD/cm) at 580 nm a pK = 4.2 + 0.1 was determined for the equilibria (19) [48]:

$$TryH^{\cdot +} \rightleftharpoons H^+ + Try^{\cdot} \qquad (19)$$

This value is in excellent agreement with previously reported data (see ref. in [48]). $Try^{\cdot}$ represents a neutral radical on the N-position.

The kinetic traces for the formation and decay measured at the absorption maxima at pH 3.5 and 6 are given for illustration of the subject matter as insert in Fig. 12. The $TryH^{\cdot +}$ species disappear by a bimolecular reaction with $2k = 5.1 \times 10^8$ dm^{3}mol^{-1}s^{-1} at pH 3 and for $Try^{\cdot}$ (pH = 7–10) was found $2k = 4.8 \times 10^8$ dm^{3}mol^{-1}s^{-1} [48].

Not only the radical cation of tryptophan ($TryH^{\cdot +}$), but a number of other such species, resulting from various biological molecules, can play an essential role in the radiation therapy of carcinoms. Hence, the knowledge of their existence, chemical and biological behaviour is of importance for medicine.



Nr.	pH	λmax (nm)
1	3.2	330, 560
2	4.5	325, 530
3	12	325, 510

Fig. 12. Transient absorption spectra of tryptophan radical cation obtained 10 μs after 0.4 μs electron pulse (3 MeV) using 10^{-4} mol.dm^{-3} Try, 10^{-2} mol.dm^{-3} KBr and 2.8 × 10^{-2} mol.dm^{-3} N_2O at various pH. Insert: Kinetic traces of the formation and decay of the transients at the absorption maxima at pH 3.5 and 6 [52]

Conclusion

By means of pulse radiolysis in combination with semi-linear computation method it has been possible to elucidate the multisite fast processes initiated by OH-attack on aromatic amino acids (Phe, Tyr and Try). It became evident that the electron structure of the individual molecule is the determining factor for the distribution of the OH-reaction site. The resulting variety of the transients leads to a rather complicated reaction scheme and cross-linked processes. This complexity is even increased in the presence of oxygen due to the formation of peroxy radicals, splitting of C-C bands and finally degradation of the biological compounds. By studying these processes on individual aromatic amino acids valuable informations were obtained which are the fundamentals for understanding the radiation induced processes of biological systems.

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