Reaction of the superoxide radical with the N-centered radical derived from *N*-acetyltryptophan methyl ester

Xingwang Fang,[a,b] Faming Jin,[a] Haofang Jin[a,b] and Clemens von Sonntag *,[a]

[a] *Max-Planck-Institut für Strahlenchemie, PO Box 101365, D-45413 Mülheim an der Ruhr, Germany*

[b] *Department of Technical Physics, Peking University, Beijing 100871, China*

Hydroxyl radicals, solvated electrons and H atoms are generated by pulse radiolysis in aqueous solutions of *N*-acetyltryptophan methyl ester (AM-Trp). The solvated electrons are converted with N_2O into further OH radicals and the latter with azide into N_3 radicals which oxidize AM-Trp to its N-centered radical (AM-TrpN$^{\bullet}$). It is characterized by a strong absorption at 510 nm (ε = 1830 dm^3 mol^{-1} cm^{-1}). The bimolecular decay of the radicals ($2k = 7.3 \times 10^8$ dm^3 mol^{-1} s^{-1}) is not affected by O_2 [k(AM-TrpN$^{\bullet}$ + O_2) < 10^5 dm^3 mol^{-1}; 2×10^3 dm^3 mol^{-1} s^{-1} deduced from other data]. In the presence of O_2, and when the majority of the OH radicals are converted with formate into superoxide radicals, $O_2^{\bullet -}$, decay of the AM-TrpN$^{\bullet}$ radicals follows first-order kinetics [k(AM-TrpN$^{\bullet}$ + $O_2^{\bullet -}$) = 1.2×10^9 dm^3 mol^{-1} s^{-1}]. In O_2-saturated azide-containing solutions [G(AM-TrpN$^{\bullet}$) = 2.9×10^{-7} mol J^{-1}; $G(O_2^{\bullet -}) = 3.3 \times 10^{-7}$ mol J^{-1}] AM-Trp is consumed with a G value of $(2.9 \pm 0.5) \times 10^{-7}$ mol J^{-1}, *i.e.* a restitution of AM-Trp by electron transfer from $O_2^{\bullet -}$ to AM-TrpN$^{\bullet}$, although thermodynamically possible [$E^7(O_2^{\bullet -}) = -0.33$ V; E^7(TrpN$^{\bullet}$) = +1.0 V], must be of very little importance compared to an addition. This has been supported by a product study. The major products are the corresponding *N*-formylkynurenine ($G = 1.4 \times 10^{-7}$ mol J^{-1}) and two hydroperoxides (total $G = 0.7 \times 10^{-7}$ mol J^{-1}) which to a large extent convert upon standing at room temperature into 1-acetyl-2-methoxycarbonyl-3-hydroxy-1,2,3,8,8-hexahydropyrroloindole (AM-HIP). The same products and product ratios are also formed, when singlet oxygen (from the irradiation of Rose Bengal with visible light) is reacted with AM-Trp suggesting that these two processes lead to the same (short-lived) hydroperoxidic intermediate.

In general, carbon-centered radicals react readily with molecular oxygen yielding the corresponding peroxyl radicals. The rates of these reactions are usually close to diffusion-controlled [k(R$^{\bullet}$ + O_2) ≈ 2×10^9 dm^3 mol^{-1} s^{-1}] and irreversible at room temperature.[1,2] An exception is the hydroxycyclohexadienyl radical (generated by the addition of the OH radical to benzene) which shows not only a lower rate of oxygen addition but also reversibility ($k_r = 3.1 \times 10^8$ dm^3 mol^{-1} s^{-1}; $k_r - 1.2 \times 10^4$ s^{-1}).[3–5] Some substituted hydroxycyclohexadienyl radicals react with O_2 even more slowly (*e.g.* the OH-adduct radicals of terephthalic acid: $k_f = 1.6 \times 10^7$ dm^3 mol^{-1} s^{-1}, $k_r = 3.3 \times 10^3$ s^{-1}).[6]

Phenoxyl-type radicals have a considerable spin density at carbon, and, for example, the tyrosine-derived phenoxyl radicals react rapidly with one another ($2k = 4.5 \times 10^8$ dm^3 mol^{-1} s^{-1}) yielding mainly 2,2′-bityrosyl.[7] However, these radicals are unreactive towards oxygen.[7,8] This also holds for some other phenoxyl-type radicals.[9,10] There are, however, exceptions such as the monoanion of the phenoxyl radical derived from phloroglucinol.†[11]

The superoxide radical, $O_2^{\bullet -}$, has a reduction potential of only −0.33 V,[12] and hence it has been assumed[8,13,14] for a long time that the high reactivity of the TyrO$^{\bullet}$ radical ($E^7 = +0.93$ V) towards the $O_2^{\bullet -}$ radical ($k \approx 1.5 \times 10^9$ dm^3 mol^{-1} s^{-1})[7,14] is due to an electron transfer reaction. However, we have recently shown that in fact these two radicals mainly react by addition, and electron transfer is, at most, a minor process.[7] Other phenoxyl-type radicals are substantially reduced by $O_2^{\bullet -}$ to the phenoxide ion, and it has been suggested that electron transfer must be occurring in competition with an addition reaction.[10] However, recent data from out laboratory indicate that an addition is likely to precede electron transfer reaction.[10]

† Phloroglucinol = 1,3,5-benzenetriol.

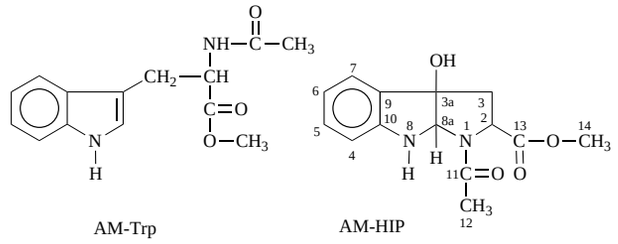
In work related to the radiation chemistry of DNA it has been found that the purine radical cations readily lose protons, thereby yielding heteroatom-centered radicals.[15] Neither of these radicals react with oxygen at an appreciable rate.[15,16] The guanine-derived one, again, reacts with $O_2^{\bullet -}$ at close to diffusion-controlled rates (3×10^9 dm^3 mol^{-1} s^{-1},[17] 4.4×10^9 dm^3 mol^{-1} s^{-1}, ref. 18) with low yields of product formation.[18] On the basis of the above-mentioned observation this 'repair' has been suggested to proceed by an $O_2^{\bullet -}$-addition/O_2-elimination reaction.[18]

Another radical that falls into the category of radicals that do not react with oxygen is the radical derived from tryptophan (Trp, ref. 19) and the related radicals derived from bisbenzimidazole derivatives (DNA-binding fluorescent dyes, *e.g.* Hoechst 33258).[20] The Trp-derived radical [TrpN$^{\bullet}$; pK_a(TrpNH$^{\bullet +}$) = 4.3][19] has a reduction potential of $E^7 = +1.0$ V (at pH 7), very similar to that of the tyrosine-derived phenoxyl radical, TyrO$^{\bullet}$ (+0.93 V).[12] In the present paper we will show that the TrpN$^{\bullet}$ radical also reacts very rapidly with $O_2^{\bullet -}$. However, practically no 'repair' by electron transfer is observed. This points again to addition being the general process in these reactions of strongly oxidizing heteroatom-centered radicals with the reducing $O_2^{\bullet -}$ radical.

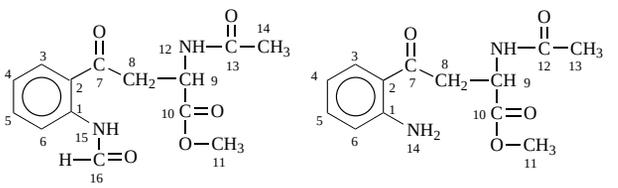
Experimental

General methods

Tryptophan (Trp; Serva), *N*-acetyltryptophan methyl ester (AM-Trp; Fluka), sodium azide (Merck) and sodium formate (Merck) were used as received. Solutions were made up in Milli-Q-filtered (Millipore) water. γ-Irradiations were carried out in a ^{60}Co-γ-source (Nuclear Engineering Ltd.) at a dose rate of 0.16 Gy s^{-1}. The pulse radiolysis set-up has been described recently.[21]



AM-Trp AM-HIP



AM-NFKy AM-Ky

Rose Bengal-sensitized photooxygenation of AM-Trp[22] yields the same products as the reaction of the AM-TrpN$^{\bullet}$ radical with $O_2^{\bullet -}$. Hence this method was used to prepare the products on a preparative scale for comparison and as reference material for quantification. The products were separated by preparative HPLC (Nucleosil-7 C-18, 250 × 20 mm) and isolated fractions were characterized by ^{1}H NMR, ^{13}C NMR (DEPT) (Bruker AM 400) and gas chromatography–mass spectroscopy (GC–MS, Hewlett-Packard 5971A Mass selective detector, coupled with a HP 5890 Ser. II gas chromatograph) after trimethylsilylation [*N*,*O*-bis(trimethylsilyl)trifluoroacetamide, BSTFA, Macherey Nagel] of the sample.[23]

Spectroscopic data

1-Acetyl-2β-methoxycarbonyl-3aα-hydroxy-1,2,3,3aβ,8,8a-hexahydropyrrolo[2,3-*b*]indole (*cis*-AM-HIP). $\delta_H(CD_3CN)$ 7.2 (dd, *J* 7.5, 0.8, 4-H), 7.09 (td, *J* 7.5, 7.6, 1.0, 6-H), 6.72 (td, *J* 7.5 7.5, 0.8, 5-H), 6.59 (dd, *J* 7.6, 0.8, 7-H), 5.46 (d, *J* 3.7, 8-H), 5.39 (d, *J* 3.7, 8a-H), 4.32 (dd, *J* 5.2, 8.5, 2-H), 3.66 (s, 12-H), 2.46 (dd, *J* 8.5, 13.5, 3b-H), 2.37 (dd, *J* 5.2, 13.5, 3a-H), 1.95 (s, 14-H); $\delta_C(CD_3CN)$ 173.4 (C-11), 171.33 (C-13), 149.35 (C-9), 131.38 (C-10), 130.50 (C-6), 123.69 (C-4), 119.47 (C-5), 110.83 (C-7), 87.0 (C-3a), 85.77 (C-8a), 60.44 (C-2), 52.74 (C-12), 41.88 (C-3), 22.39 (C-14).

1-Acetyl-2β-methoxycarbonyl-3aβ-hydroxy-1,2,3,3aα,8,8a-hexahydropyrrolo[2,3-*b*]indole (*trans*-AM-HIP). $\delta_H(CD_3CN)$ 7.24 (dd, *J* 7.5, 0.8, 4-H), 7.15 (td, *J* 7.5 7.5, 1.0, 6-H), 6.8 (td, *J* 7.5, 7.5, 0.8, 5-H), 6.66 (dd, *J* 7.5, 0.8, 7-H), 4.59 (dd, *J* 1.7, 8.9, 2-H), 5.52 (d, *J* 4.0, 8-H), 5.47 (d, *J* 3.7, 8-H), 3.74 (s, 12-H), 2.61 (dd, *J* 1.7, 13.5, 3a-H), 2.38 (dd, *J* 8.9, 13.5, 3b-H), 2.12 (s, 14-H); $\delta_C(CD_3CN)$ 173.41 (C-11), 172.31 (C-13), 149.49 (C-9), 132.04 (C-10), 130.82 (C-6), 124.01 (C-4), 120.45 (C-5), 111.69 (C-7), 89.42 (C-3a), 86.23 (C-8a), 61.63 (C-2), 53.18 (C-12), 41.07 (C-3), 22.60 (C-14); *m*/*z* AM-HIP-TMS (both isomers) (*M*, 420) 420 (2%), 405 (13), 385 (3), 361 (100), 306 (6), 289 (3), 271 (10), 264 (24), 232 (5), 218 (6), 202 (29), 143 (5), 100 (6), 73 (82).

N_a-Formyl-N_b-acetylkynurenine methyl ester (AM-NFKy). $\delta_H(CD_3OD)$ 11.3 (br, H-17), 8.71 (d, *J* 8, H-3), 8.45 (s, H-15), 7.87 (d, *J* 8, H-6), 7.56 (t, *J* 8, 8, H-5), 7.15 (t, *J* 8, 8, H-4), 6.55 (d, *J* 7, H-12), 4.94 (m, H-9), 3.8–3.6 (m, H-8), 3.73 (s, H-11), 2.0 (s, H-14); δ_C 202.2 (C-7), 173.2 (C-10), 173.1 (C-13), 162.4 (C-16), 140.0 (C-2), 135.9 (C-5), 132.1 (C-3), 124.8 (C-6), 124.2 (C-1), 122.8 (C-4), 53.0 (C-11), 49.7 (C-9), 42.5 (C-8), 22.3 (C-14); *m*/*z* AM-NFKy-2TMS (*M*, 436) 436 (1%), 421 (2), 393 (1), 346 (6), 290 (10), 274 (5), 246 (4), 234 (43), 216 (27), 203 (27), 171 (8), 146 (16), 126 (27), 116 (11), 73 (100).

N_b-acetylkynurenine methyl ester (AM-Ky). $\delta_H(CD_3OD)$ 7.71 (dd, *J* 8.5, 1.4, H-1), 7.23 (td, *J* 8.5, 8.5, 1.4, H-3), 6.72 (dd, *J* 8.5, 1.0, H-4), 6.58 (td, *J* 8.5, 8.5, 1.0, H-2), 4.9 (dd, *J* 6.0, 4.5, H-9), 3.71 (s, H-11), 3.57 (dd, *J* 17.5, 6.0, H-8a), 3.46 (dd, *J* 17.5, 4.5, H-8b), 1.96 (s, H-13); $\delta_C(CD_3OD)$ 199.7 (C-7), 173.7 (C-10), 173.2 (C-12), 152.8 (C-6), 135.7 (C-3), 132.0 (C-1), 118.3 (C-4), 118.1 (C-5), 116.2 (C-2), 52.9 (C-11), 49.6 (C-9), 41.4 (C-8), 22.4 (C-13); *m*/*z* AM-Ky-2TMS (*M*, 408), 408 (6%), 393 (2), 354 (2), 334 (4), 262 (100), 246 (3), 218 (80), 202 (35), 174 (6), 160 (11), 126 (10), 73 (77).

For GC–MS analysis γ-irradiated solutions were extracted with diethyl ether (Merck, redistilled prior to use). The products were quantified by analytical HPLC (for the conditions see the legends of Figs. 2 and 3).

Results and discussion

The radical-generating system

In the radiolysis of water the primary species formed are OH radicals, solvated electrons and H atoms [reaction (1)].[24] The

$$H_2O \xrightarrow[\text{radiation}]{\text{ionizing}} {}^{\bullet}OH,\ e_{aq}^{-},\ H^{\bullet},\ H^{+},\ H_2O_2,\ H_2 \qquad (1)$$

solvated electrons can be converted into further OH radicals [reaction (2)], or in the presence of O_2 they will be converted

$$e_{aq}^{-} + N_2O \longrightarrow {}^{\bullet}OH + N_2 + OH^{-} \qquad (2)$$

into superoxide radicals [reaction (3)]. The H atom is also

$$e_{aq}^{-} + O_2 \longrightarrow O_2^{\bullet -} \qquad (3)$$

scavenged by O_2 and contributes to the superoxide yield [reaction (4)]. In the presence of azide, the OH radical is

$$H^{\bullet} + O_2 \longrightarrow HO_2^{\bullet}\ [pK_a(HO_2^{\bullet}) = 4.8] \qquad (4)$$

converted into the N_3 radical [reaction (5)] which is a selective

$${}^{\bullet}OH + N_3^{-} \longrightarrow OH^{-} + N_3^{\bullet} \qquad (5)$$

oxidant and oxidizes tryptophan and its derivatives to the corresponding N-centered radicals [*e.g.* reaction (6)].[24] Using (a)

$$\text{AM-Trp} + N_3^{\bullet} \longrightarrow \text{AM-TrpN}^{\bullet} + N_3^{-} + H^{+} \qquad (6)$$

N_2O-, (b) N_2O–O_2 (4 : 1)- or (c,d) O_2-saturated aqueous solution of tryptophan (Trp) or its derivative (AM-Trp) (2×10^{-4} mol dm^{-3}) in the presence of azide ions (5×10^{-3} mol dm^{-3}) conditions prevail[25] where essentially only TrpN$^{\bullet}$ (AM-TrpN$^{\bullet}$) radicals (a,b) are generated,[26] either in the absence (a) or in the presence of oxygen (b), or TrpN$^{\bullet}$ (AM-TrpN$^{\bullet}$) and $O_2^{\bullet -}$ in a molar ratio of about 1 : 1 : 2 (c). The yield of $O_2^{\bullet -}$ relative to TrpN$^{\bullet}$ (AM-TrpN$^{\bullet}$) can be considerably enhanced (d) by reacting a part of the OH radicals with formate ions [reaction (7)]

$${}^{\bullet}OH + HCO_2^{-} \longrightarrow OH^{-} + CO_2^{\bullet -} \qquad (7)$$

which are then converted by O_2 into $O_2^{\bullet -}$ [reaction (8)]. For rate constants see refs. 26–28.

$$CO_2^{\bullet -} + O_2 \longrightarrow CO_2 + O_2^{\bullet -} \qquad (8)$$

The radiation-chemical yields (*G* values) for TrpN$^{\bullet}$ (AM-TrpN$^{\bullet}$) are $G(\text{TrpN}^{\bullet}) = G(\text{AM-TrpN}^{\bullet}) = 5.8 \times 10^{-7}$ mol J^{-1} under conditions (a) and (b) and $G(\text{TrpN}^{\bullet}) = 2.9 \times 10^{-7}$ mol J^{-1}, $G(O_2^{\bullet -}) = 3.5 \times 10^{-7}$ mol J^{-1} under condition (c).

Pulse radiolysis

Most of the experiments were carried out with *N*-acetyltryptophan methyl ester (AM-Trp). It is a closer model to Trp embedded in proteins than the free amino acid itself. It also has the advantage that the *N*-acetylation and methylation renders it more amenable to the analysis of the products without changing the essential features of the tryptophan chemistry.

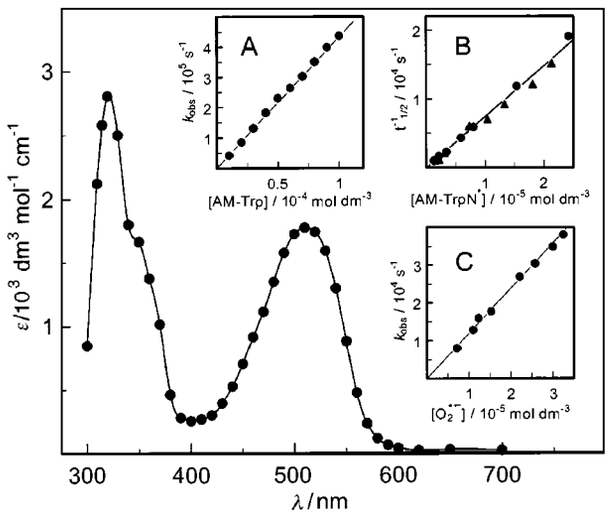


Fig. 1 Pulse radiolysis of N_2O-saturated aqueous solutions of *N*-acetyltryptophan methyl ester (AM-Trp, 2×10^{-4} mol dm^{-3}) in the presence of azide (5×10^{-3} mol dm^{-3}) at pH 10. Spectrum of the AM-TrpN$^•$ radical. Inset A: rate of build-up of the 510 nm absorption as a function of the AM-Trp concentration. Inset B: inverse of the first half-life of the decay of the 510 nm absorption as a function of the AM-TrpN$^•$ concentration (●) and in the presence of 20% O_2 (▲). Inset C: O_2-saturated AM-Trp solutions (2×10^{-4} mol dm^{-3}) containing 3×10^{-3} mol dm^{-3} azide and 5×10^{-2} mol dm^{-3} formate. Rate of decay of the AM-TrpN$^•$ radicals as a functions of the superoxide radical concentration (*i.e.* dose per pulse).

Table 1 Compilation of rate constants measured in this study

Reaction	Rate constant/dm^3 mol^{-1} s^{-1}
$N_3^•$ + Trp → TrpN$^•$	4.1×10^9
$N_3^•$ + AM-Trp → AM-TrpN$^•$	4.4×10^9
2 TrpN$^•$ → Products	4.6×10^8
2 AM-TrpN$^•$ → Products	7.3×10^8
AM-TrpN$^•$ + $O_2^{•-}$ → Products	1.2×10^9
TrpN$^•$ + $O_2^{•-}$ → Products	1.6×10^9
AM-TrpN$^•$ + O_2 → Products	$<10^5$ (pulse radiolysis) $\approx 2 \times 10^3$ (product studies)

TrpN$^•$-type radicals were generated by N_3 radicals in N_2O-saturated AM-Trp solutions containing an excess of azide ions. These radicals are characterized by a strong absorption at 510 nm (Fig. 1). The AM-Trp$^•$ spectrum is close to identical to that reported for the TrpN$^•$ radical.[29]

The rate of reaction of the N_3 radical with AM-Trp was measured at 510 nm at various AM-Trp concentrations. From the slope of the straight line of the k_{obs} *vs.* [AM-Trp] plot (inset A in Fig. 1) $k(N_3^• + \text{AM-Trp}) = 4.4 \times 10^9$ dm^3 mol^{-1} s^{-1} was calculated (for a compilation of rate constants see Table 1). This rate constant is very close to the one obtained for the reaction of $N_3^•$ with tryptophan itself (*cf.* Table 1) which agrees well with the value reported in the literature.[26] The AM-TrpN$^•$ radicals decay by second-order kinetics [$2k = 7.3 \pm 0.4) \times 10^8$ dm^3 mol^{-1} s^{-1}, assuming $G(R^•) = 6.5 \times 10^{-7}$ mol J^{-1}]. In the presence of oxygen [N_2O–O_2 (4:1)-saturated solutions] the decay kinetics did not change (inset B in Fig. 1) indicating that the TrpN$^•$-type radicals do not react with O_2 ($k < 10^5$ dm^3 mol^{-1} s^{-1}, in agreement with a previous report[19]).

However, when O_2-saturated AM-Trp solutions (2×10^{-4} mol dm^{-3}) containing 3×10^{-3} mol dm^{-3} azide and 5×10^{-2} mol dm^{-3} formate, *i.e.* conditions were met where $O_2^{•-}$ is generated in an approximately 12-fold excess over the AM-TrpN$^•$ radicals (as calculated from the absorption at 510 nm), their decay turned into first-order kinetics. As can be seen from inset C in Fig. 1 the observed first-order rate constant increased with increasing dose per pulse, *i.e.* with increasing $O_2^{•-}$ concentration. From this figure a rate constant of k(AM-TrpN$^•$ + $O_2^{•-}$) = 1.2×10^9 dm^3 mol^{-1} s^{-1} is calculated. Similar experiments were carried out with Trp itself. From these data (not shown) $k(\text{TrpN}^• + O_2^{•-}) = 1.6 \times 10^9$ mol^{-1} s^{-1} was calculated. Both values are very close to one another and to the rate constant for the reaction of the tyrosine-derived phenoxyl radical with $O_2^{•-}$ ($k \approx 1.5 \times 10^9$ dm^3 mol^{-1} s^{-1}).[7,14] The rate constants measured in this study are compiled in Table 1.

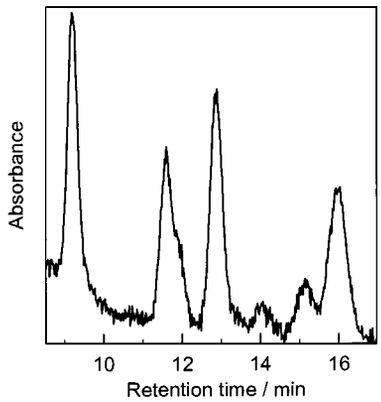


Fig. 2 HPLC chromatogram of γ-irradiated N_2O-saturated aqueous solutions of AM-Trp at pH 10 (10^{-3} mol dm^{-3}, containing 5×10^{-2} mol dm^{-3} NaN_3) on a 250×4.6 mm Nucleosil-5-C_{18} column with 60% methanol in water as eluent. Elution profile of the dimer fraction (retention time of AM-Trp is 7 min under these conditions).

Product studies

When N_2O-saturated AM-Trp solutions containing an excess of azide ions were γ-irradiated four major and two minor products with much longer retention times than AM-Trp itself were detected by HPLC (Fig. 2).

These products are most likely dimers resulting from coupling reactions of the AM-TrpN$^•$ radicals. No products are observed with retention times shorter than AM-Trp. However, when the solutions were saturated with an N_2O–O_2 (4:1) mixture prior to irradiation, the yield of the dimer fraction was considerably reduced. At the dose rate of the γ-irradiation the lifetime of the AM-TrpN$^•$ radicals is 0.2 s (based on the biomolecular decay rate constant of $2k = 7.3 \times 10^8$ dm^3 mol^{-1} s^{-1}, determined above). Thus the lifetime of the AM-TrpN$^•$ radicals is increased by three orders of magnitude compared to their lifetime in the pulse radiolysis experiments. This now allows O_2-addition to compete more successfully with the bimolecular decay of the AM-TrpN$^•$ radicals. From the reduction of the dimer yield (70% remaining under these conditions) a value of $k(\text{AM-TrpN}^• + O_2) \approx 2 \times 10^3$ dm^3 mol^{-1} s^{-1} is obtained. The limit set for this reaction according to the pulse radiolysis experiments was $<10^5$ dm^3 mol^{-1} s^{-1} (see above).

When O_2-saturated solutions of AM-Trp (10^{-3} mol dm^{-3}) containing 5×10^{-2} mol dm^{-3} NaN_3 were pulse- or γ-irradiated AM-TrpN$^•$ and $O_2^{•-}$ radicals are formed side by side in a 1:1.2 ratio. A computer simulation based on the rate constants given in Table 1 shows that 75–80% of the AM-TrpN$^•$ radicals react with $O_2^{•-}$ under these conditions. At ice-bath temperature, G(AM-Trp consumption) was determined at $(2.9 \pm 0.5) \times 10^{-7}$ mol J^{-1} (pulse radiolysis, *cf.* Table 2; the γ-radiolytic value was slightly lower). The major product was AM-NFKy, and four further products were observed (Fig. 3).

By post-column derivatization with Allen's reagent[30] (essentially KI) two of them were characterized as hydroperoxides (hydrogen peroxide coelutes with the azide ion). A further (minor) product was AM-HIP (the two stereoisomers seem not to be separated under these conditions). Upon standing at room temperature, the organic hydroperoxides disappeared and the yield of AM-HIP and that of the unknown product increased. In addition, AM-NFKy releases formic acid, and AM-Ky is formed, which elutes at longer times (*cf.* arrow in Fig. 3). The yields of the products increased linearly with absorbed

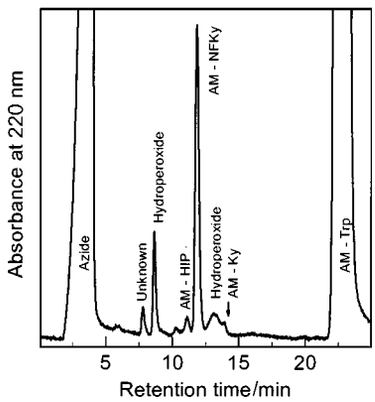


Fig. 3 HPLC chromatogram of pulse-irradiated O_2-saturated aqueous solutions of AM-Trp at pH 10 (10^{-3} mol dm^{-3}, containing 5×10^{-2} mol dm^{-3} NaN_3) on a 250×4.6 mm Nucleosil-5-C_{18} column with 40% methanol in water as eluent

Table 2 *G* values of the products from pulse-irradiated O_2-saturated AM-Trp (10^{-3} mol dm^{-3}) solutions containing 5×10^{-2} mol dm^{-3} azide, pH 10, ice-bath temperature

Product	*G* value/10^{-7} mol J^{-1}
AM-NFKy	1.4
Organic peroxides	0.7
AM-HIP	≈0.1
Unknown	≈0.1
Hydrogen peroxide	0.7
AM-Trp consumption	2.8 ± 0.5

dose. From the yield *vs*. dose plot *G* values were calculated which are compiled in Table 2.

Practically the same products and product ratios are observed when singlet oxygen (formed by the irradiation of Rose Bengal with visible light) was reacted with AM-Trp.

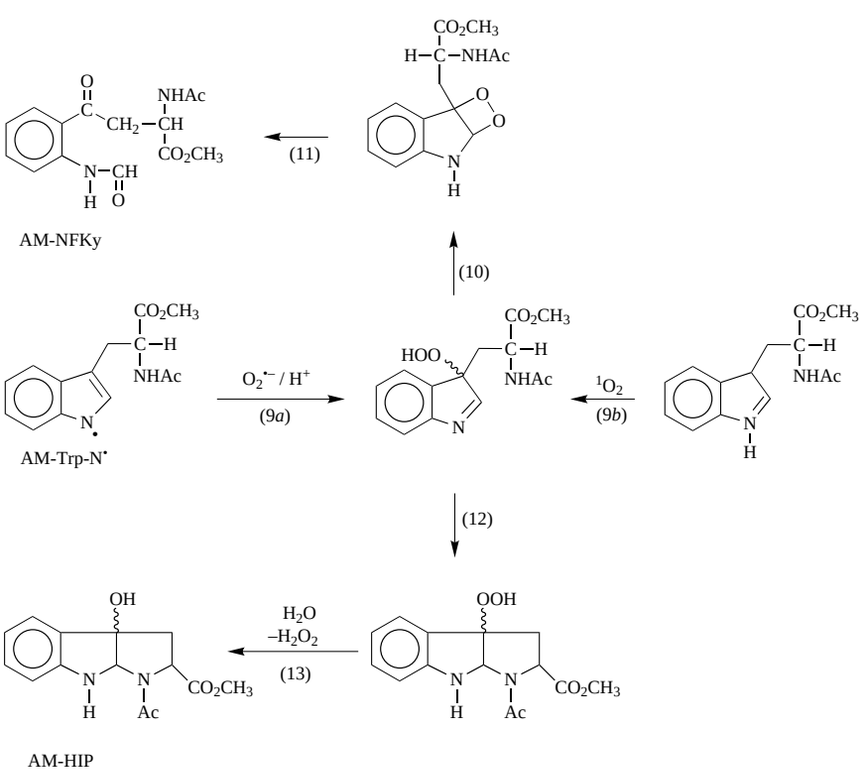
Mechanistic aspects

In O_2-saturated azide-containing solution of AM-Trp at pH 10 AM-TrpN$^\bullet$ and $O_2^{\bullet -}$ are formed in a 1:1.2 ratio. The rate of the self-termination of the AM-TrpN$^\bullet$ radicals is nearly half that of reaction of the AM-TrpN$^\bullet$ radicals with $O_2^{\bullet -}$. The self-termination of the $O_2^{\bullet -}$ radicals can be neglected under these conditions.[27] Thus even under the conditions of pulse radiolysis, where no pool of $O_2^{\bullet -}$ radicals builds up, the majority (75–80%) of the AM-TrpN$^\bullet$ radicals will react in the cross-termination reaction. If the $O_2^{\bullet -}$ radicals did react by electron transfer to the AM-TrpN$^\bullet$ radical (the thermodynamics are in favour of such a reaction, see above) AM-Trp should be largely regenerated, and only small amounts of dimeric products should be formed. However, as can be seen from Table 2, the yield of AM-TrpN-consumption is practically that of the AM-TrpN$^\bullet$ radical yield, and a number of oxidized products are formed. We conclude from this that, like in the case of the reaction of the phenoxyl radical derived from tyrosine,[7] these two radicals mainly react by combination.

In this reaction a hydroperoxide (two stereoisomers) is formed [reaction 9(*a*)]. This short-lived intermediate may close up to form a dioxetane [reaction (10)] or, in competition, closes in a Micheal-type reaction to form the longer-lived hydroperoxide [two stereoisomers, reaction (12)]. The dioxetane will readily break up, and as a final product AM-NFKy is formed [reaction (11)]. At elevated temperatures the *N*-formyl group is hydrolyzed, and the final product is AM-Ky. The relatively stable organic hydroperoxides also decompose upon standing at room temperature and faster at elevated temperature and yield AM-HIP [reaction (13)]. An attempt has been made to follow the formation of the expected corresponding product, hydrogen peroxide. However, this product is not stable in this system for a prolonged period of time, and this correspondence could not be followed.

Conclusions

The N-centered radicals derived from tryptophan and compounds that have a tryptophan-like structure such as the DNA-binding fluorescent bisbenzimidazol derivatives (*e.g.* Hoechst 33258) belong to the few radicals which do not react with O_2 at a reasonable rate. The driving force of their reduction by the superoxide radical is 1.3 V, and yet an electron transfer does not occur. Instead, in the present system they react by addition, whereby the same products are formed that are also generated



when $^1O_2$ react with this tryptophan derivative. Thus these two processes must lead to the same short-lived hydroperoxide.

References

1 P. Neta, R. E. Huie and A. B. Ross, *J. Phys. Chem. Ref. Data*, 1990, **19**, 413.
2 C. von Sonntag and H.-P. Schuchmann, in *Peroxyl Radicals*, ed. Z. B. Alfassi, London, Wiley, 1997, p. 173.
3 X.-M. Pan and C. von Sonntag, *Z. Naturforsch.*, *Teil B*, 1990, **45**, 1337.
4 X. Fang, X. Pan, A. Rahmann, H.-P. Schuchmann and C. von Sonntag, *Chem. Eur. J.*, 1995, **1**, 423.
5 X.-M. Pan, M. N. Schuchmann and C. von Sonntag, *J. Chem. Soc.*, *Perkin Trans. 2*, 1993, 289.
6 X. Fang, G. Mark and C. von Sonntag, *Ultrason. Sonochem.*, 1996, **3**, 57.
7 F. Jin, J. Leitich and C. von Sonntag, *J. Chem. Soc.*, *Perkin Trans. 2*, 1993, 1583.
8 E. P. L. Hunter, M. F. Desrosiers and M. G. Simic, *J. Free Radicals Biol. Med.*, 1989, **6**, 581.
9 D. J. Deeble, B. J. Parsons, G. O. Phillips, H.-P. Schuchmann and C. von Sonntag, *Int. J. Radiat. Biol.*, 1988, **54**, 179.
10 N. d'Alessandro, G. Bianchi, X. Fang, F. Jin and C. von Sonntag, unpublished results.
11 D. Wang, G. György, K. Hildenbrand and C. von Sonntag, *J. Chem. Soc.*, *Perkin Trans. 2*, 1994, 45.
12 P. Wardman, *J. Phys. Chem. Ref. Data*, 1989, **18**, 1637.
13 W. A. Prütz, J. Butler and E. J. Land, *Int. J. Radiat. Biol.*, 1983, **44**, 183.
14 I. Cudina and L. J. Josimovic, *Radiat. Res.*, 1987, **109**, 206.
15 S. Steenken, *Chem. Rev.*, 1989, **89**, 503.
16 M. Isildar, M. N. Schuchmann, D. Schulte-Frohlinde and C. von Sonntag, *Int. J. Radiat. Biol.*, 1982, **41**, 525.
17 L. P. Candeias, Ph.D. Thesis, Technical University, Lisbon, 1992.
18 C. von Sonntag, *Int. J. Radiat. Biol.*, 1994, **66**, 485.
19 S. V. Jovanovic and M. G. Simic, *J. Free Radicals Biol. Med.*, 1985, **1**, 125.
20 A. Adhikary, E. Bothe, C. von Sonntag and V. Jain, *Radiat. Res.*, 1997, **148**, 493.
21 C. von Sonntag and H.-P. Schuchmann, *Methods Enzymol.*, 1994, **233**, 3.
22 M. Nakagawa, H. Watanabe, S. Kodato, H. Okajima, T. Hino, J. L. Flippen and B. Witkop, *Proc. Natl. Acad. Sci. USA*, 1977, **74**, 4730.
23 F. Jin, Ph.D. Thesis, Ruhr-Universität Bochum, 1993.
24 C. von Sonntag, *The Chemical Basis of Radiation Biology*, Taylor and Francis, London, 1987.
25 D. J. Deeble, B. J. Parsons and G. R. A. Johnson, *Radiat. Phys. Chem.*, 1990, **36**, 487.
26 G. V. Buxton, C. L. Greenstock, W. P. Helman and A. B. Ross, *J. Phys. Chem. Ref. Data*, 1988, **17**, 513.
27 B. H. J. Bielski, D. E. Cabelli, R. L. Arudi and A. B. Ross, *J. Phys. Chem. Ref. Data*, 1985, **14**, 1041.
28 P. Neta, R. E. Huie and A. B. Ross, *J. Phys. Chem. Ref. Data*, 1988, **17**, 1027.
29 A. Singh, G. W. Koroll and R. B. Cundall, *Radiat. Phys. Chem.*, 1982, **19**, 137.
30 A. O. Allen, C. J. Hochanadel, J. A. Ghormley and T. W. Davis, *J. Phys. Chem.*, 1952, **56**, 575.

Paper 7/06979K
Received 26*th September* 1997
Accepted 4*th November* 1997