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Intra and Intermolecular Charge Effects on the Reaction of the Superoxide Radical Anion with Semi-oxidized Tryptophan in Peptides and N-acetyl Tryptophan

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The reaction of the superoxide radical anion ($O_2^{-\bullet}$), with the semi-oxidized tryptophan neutral radical ($Trp^{\bullet}$) generated from tryptophan (Trp) by pulse radiolysis has been observed in a variety of functionalized Trp derivatives including peptides. It is found that the reaction proceeds 4–5 times faster in positively charged peptides, such as Lys-Trp-Lys, Lys-Gly-Trp-Lys and Lys-Gly-Trp-Lys-O-tert-butyl, than in solutions of the negatively charged N-acetyl tryptophan (NAT). However, the reactivity of $O_2^{-\bullet}$ with the $Trp^{\bullet}$ radical is totally inhibited upon binding of these peptides to micelles of negatively charged SDS and is reduced upon binding to native DNA. By contrast, no change in reactivity is observed in a medium containing CTAB, where the peptides cannot bind to the positively charged micelles. On the other hand, the reactivity of the $Trp^{\bullet}$ radical formed from NAT with $O_2^{-\bullet}$ is reduced to half that of the free $Trp^{\bullet}$ in buffer but is markedly increased in CTAB micelles. The models studied here incorporate elements of the complex environment in which $Trp^{\bullet}$ and $O_2^{-\bullet}$ may be concomitantly formed in biological system and demonstrate the magnitude of the influence such elements may have on the kinetics of reactions involving these two species.

Keywords: CTAB, SDS, Br_2^-, native DNA, reaction rate constants, microenvironment

INTRODUCTION

Ground state and excited state oxidation of free tryptophan, and of tryptophan incorporated into proteins, have been widely studied for more than two decades.[1,2] This continued interest arises from the key role played by tryptophan residues in important biological processes such as enzymatic reactions, stabilization of nucleic acid–protein interactions,[3] protein–lipid interactions[4] and in the photo-oxidative stress induced by UVA.[5]

It has been demonstrated that the semioxidized Trp radical ($Trp^{\bullet}$), is a reactive intermediate in enzymatic reactions such as those involving mitochondrial cytochrome C peroxidase[6] and

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those involving the photoreactivating enzyme.[7] $Trp^{\bullet}$ is also a key intermediate in type I photodynamic reactions.[8] Recently, it has been suggested that $Trp^{\bullet}$ is formed during the peroxidation of serum low density lipoproteins and constitutes a crucial intermediate in the genesis and development of atherosclerosis.[9]

It has long been known that Trp – the only chromophoric residue of proteins responsive to solar UV radiation – is photo-oxidized by UV light to form $Trp^{\bullet}$. This reaction may occur upon illumination at wavelengths as long as 305 nm.[10] Until recently, the biological effects of solar UV light have been attributed almost exclusively to DNA alteration. However, recent studies[11] point to direct alteration of cytoplasmic membrane targets as an essential primary event in the perturbation of the mitogenic signal which itself is triggered by interaction of growth factors with membrane receptors. Receptors such as the epidermal growth factor receptor found in plasma membranes contain Trp residues. Furthermore, we have recently shown that UV irradiation of human skin systematically alters lipoproteins of the interstitial fluid feeding the human epidermis[12] and that these alterations arise from the photo-oxidation of Trp residues in such lipoproteins.

Under aerobic conditions, the formation of the $Trp^{\bullet}$ radical by direct or sensitized (type I) photoreactions is accompanied by the direct or subsequent production of the superoxide radical anion ($O_2^{-\bullet}$).[8] The $Trp^{\bullet}$ radical can also be produced through Trp oxidation by selective inorganic radical anion (Br_2^-, CNS_2^-) reaction in pulse radiolysis.[1] Using this technique, we have recently shown that $O_2^{-\bullet}$ can react at diffusion controlled rates with $Trp^{\bullet}$. It may be suggested that this reaction can result in recycling of Trp and/or destruction of Trp residues in proteins.[13]

As discussed above, $Trp^{\bullet}$ participation in several biological processes which involve oxidative reactions is well documented.[7–10] Because of the complexity of the environment in which such processes normally occur, we have extended our studies of $Trp^{\bullet}$ in aerobic aqueous solution to several model peptide systems and have determined the recombination kinetics for reaction of $O_2^{-\bullet}$ with $Trp^{\bullet}$ formed on several positively charged Lys-Trp peptides as well as with $Trp^{\bullet}$ formed in negatively charged N-acetyl tryptophan. It may be seen that the rate of recombination is significantly influenced by the strength of the electrostatic field created by the charged Trp derivatives.

In biological systems, of course, these reactions occur in complex environments which provide the possibility of hydrophobic as well as electrostatic interactions between peptide and, for example, membrane components. To examine the effects of such environmental factors at the most elementary level, we have also measured the reaction kinetics of $O_2^{-\bullet}$ with $Trp^{\bullet}$ bearing peptides and N-acetyl tryptophan (NAT) in charged micellar systems. Abundant information from pulse radiolysis studies of charged radical reactions in ionic micelles is available from the literature and can be referred to for the present work.[14–16] Our results suggest that not only electrostatic interactions but also hydrophobic interactions modulate the yield and reaction rate constant in positively or negatively charged micelles. In a further extension of this study we have determined the kinetics of $Trp^{\bullet} + O_2^{-\bullet}$ recombination in DNA. It has been found that this reaction occurs when the positively charged peptides are bound to DNA but at a reduced rate.

EXPERIMENTAL

Chemicals

Racemic tryptophan, N-acetyl tryptophan (NAT) and calf thymus native DNA (type I) were purchased from SIGMA. The peptides Lys-Trp-Lys (KWK), Lys-Gly-Trp-Lys (KGWK) and Lys-Gly-Trp-Lys O-t-butyl (KGWKOtBut) were provided by Bachem. Sodium dodecyl sulphate (SDS) and cetyl tetramethyl ammonium bromide (CTAB) of the purest grade were supplied by

Fluka. Phosphate buffers of various pH and ionic strength were prepared in pure water obtained with a milli/Q system provided by Millipore. For complete solubilization, stock DNA solutions (4 mM) were prepared in pH 6.4, 20 mM phosphate buffer the day before experiment. The DNA concentration was estimated assuming molar absorbance of $6{,}500\,M^{-1}\,cm^{-1}$ at 260 nm.[17] Unless otherwise specified the peptide concentration used in these experiments was 100 μM whereas the NAT concentration used was 500 μM. Solutions were saturated with pure N_2O or O_2 prior to pulse radiolysis.

Pulse Radiolysis

Pulse radiolysis measurements were carried out with the Notre Dame Radiation Laboratory 8-MeV linear accelerator, which provides 5-ns pulses of up to 20 Gy. The doses used here were between 2 and 10 Gy. The principles of the detection system have been previously described.[18] A Corning O-51 optical filter, removing all wavelengths shorter than 350 nm, was placed in the analyzing light beam to avoid direct Trp photochemistry.

Radical concentrations calculated from transient absorption data are referenced to CNS_2^- dosimetry.[19] The extinction coefficient for CNS_2^- is taken to be $7580 \pm 60\,M^{-1}\,cm^{-1}$ at 472 nm, and the G value for $OH^\bullet$ in N_2O saturated solution has been measured as 6.13 ± 0.09.[19] The G value is the number of radicals generated per 100 eV of absorbed energy, and such numbers may be recast as radical concentrations per unit radiation (e.g., a G value of 6.1 corresponds to a concentration of 6 μM/10 Gy). Values given here for $Trp^\bullet$ have been determined by comparison of its measured transient absorbance at 520 nm to the peak absorbance of CNS_2^- determined under the same experimental conditions of cell geometry and dose.

The solutions for pulse radiolysis were prepared either in pH 6.4 or pH 7 phosphate buffers or in buffered solutions of SDS and CTAB. The SDS and CTAB concentrations were 50 and 10 mM, respectively, well above the critical micellar concentration involved, allowing one to assume that most detergent be in micellar form.

RESULTS AND DISCUSSION

Reactivity $O_2^{-\bullet}$ with the $Trp^\bullet$ Radical Formed in Charged Trp Derivatives

NAT and the Trp peptides, KWK, KGWK and KGWKOtBut, provide good model systems for determining the effects of peptide charge on the $Trp^\bullet + O_2^{-\bullet}$ reaction. The charge on NAT is -1 whereas the overall charges on the peptides at pH 6.4 are +2, +2 and +3, respectively, Trp itself is uncharged.[20] The kinetic reference for this study is the reaction of molecular $Trp^\bullet$ with $O_2^{-\bullet}$ in dilute soltition which occurs at the diffusion controlled rate ($k = 2.3 \times 10^9\,M^{-1}\,s^{-1}$).[13]

During pulse radiolysis, $Trp^\bullet$ and $O_2^{-\bullet}$ are generated simultaneously in aerated solutions containing sufficient concentrations of Br^- (conc. ≥ 10 mM). Consider the reaction

$$2Br^- + OH^\bullet \rightarrow Br_2^- + OH^- \qquad (1)$$

The Br_2^- radical initially produced through oxidation of Br^- by $OH^\bullet$, subsequently oxidizes Trp to give $Trp^\bullet$, i.e.

$$TrpH + Br_2^- \rightarrow Trp^\bullet + Br^- + H^+ \qquad (2)$$

The $O_2^{-\bullet}$ superoxide radical anion is formed through reaction of O_2 with hydrated electron, e_{aq}^-

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

Additionally, there is a small contribution to $O_2^{-\bullet}$ yield from scavenging of $H^\bullet$

$$H^\bullet + O_2 \rightarrow O_2^{-\bullet} + H^+ \qquad (3b)$$

While it might be suggested that $HO_2^\bullet$ plays a role in these reactions, the lowest pH used in this study is 6.4. As the pK_a for the equilibrium $HO_2^\bullet \leftrightarrow O_2^{-\bullet}$ is 4.8, the $HO_2^\bullet$ concentration can be no more than 2.5% of $O_2^{-\bullet}$ or negligible in determining the kinetics of reaction. Furthermore, under all conditions used here, $HO_2^\bullet$ is

consumed by Br_2^-.[21] The reactions of interest involving $Trp^\bullet$ are

$$Trp^\bullet + O_2^{-\bullet} \rightarrow \quad (4)$$

$$Trp^\bullet + Trp^\bullet \rightarrow \quad (5)$$

The $Trp^\bullet$ decay data for O_2 saturated solutions in this study were analyzed by least squares fits for the numeric integration of the rate equations describing reactions (4) and (5) using the *Scientist* program from Micromath. In complex systems, component rate constants established independently under identical solution conditions were incorporated into the calculations. The initial concentrations of $Trp^\bullet$ and $O_2^{-\bullet}$ employed for the calculations presented in this study were determined using the equations of La Verne and Pimblott; these equations relate G values of $OH^\bullet$ and e_{aq}^- scavenging intermediates (here $Trp^\bullet$ and $O_2^{-\bullet}$) to the mathematical product of scavenging rate constant and concentrations of scavenger (here Br^- and O_2).[22,23] With 100% O_2 saturation, the ratio of initial $Trp^\bullet$ to $O_2^{-\bullet}$ is 0.97 assuming an oxygen concentration in water of 1.2 mM[24] and a molar extinction coefficient of 1750 $M^{-1}\,cm^{-1}$ at 520 nm for the $Trp^\bullet$ radical[25,26]. There is general agreement that the extinction coefficient is essentially unchanged in proteins and peptides.[27,28]

For reaction (5), the bimolecular reaction rate constants for the $Trp^\bullet$ radical recombination reaction have been obtained with the peptides under similar experimental conditions with N_2O saturation and have been published.[20] The values obtained here are in excellent agreement with those reported values. With NAT, the rate constant for $Trp^\bullet$ recombination was found to be $5.3 \times 10^8\,M^{-1}\,s^{-1}$. As may be seen in Table I, for NAT and the peptides, the rate constants for reaction (5) are lower than that observed with free Trp because of the electrostatic repulsion introduced by the positive and negative charges. Additionally, there may be some inhibition of reaction due to steric hindrance arising from the bulky nature of the peptides.[20]

TABLE I Radiolytic yield of the $Trp^\bullet$ radical ($G(Trp^\bullet)$) formed on Trp, NAT, KWK, KGWK and KGWKOtBut and bimolecular reaction rate constant (k_4) for the reaction $Trp^\bullet + O_2^{-\bullet}$ under various experimental conditions

Compounds	Conditions	$G(Trp^\bullet)$	$k_4(10^9\,M^{-1}\,s^{-1})$
Trp[(a)]	O_2	3.3	2.3
NAT	N_2O	6.1	—
NAT	O_2	3	0.8
NAT	N_2O, CTAB	4	—
NAT	O_2, CTAB	2.1	1.4
KWK	O_2	3.3	3.5
	0.1 M Br^-, O_2	3.2	1.1
	1 M Br^-, O_2	3.6	0.6
	SDS, 0.1 M Br^-, N_2O	1.3	—
	SDS, 0.1 M Br^-, O_2	0.6	N.D. (see Figure 1A)
KGWK	O_2	3.3	3.1
	SDS, N_2O	0.5	—
	SDS, O_2	0.2	N.D. (see KWK)
	N_2O	6.1	—
KGWKOtBut	O_2	3.3	4.1
	N_2O	6.1	—
	SDS, O_2	0.1	N.D. (see KWK)
	CTAB, N_2O	3	—
	CTAB, O_2	0.6	N.D. (see Figure 1B)

(a) Data from Ref. [13] – solutions were prepared in 10 mM phosphate buffer and contained 100 μM peptide (pH 6.4) or 500 μM NAT (pH 7) and 10 mM Br^- unless otherwise specified – [SDS] = 50 mM, [CTAB] = 10 mM. Solutions were saturated with O_2 or N_2O as indicated. N.D.: Not determined.

In an earlier study we established that the reaction $Trp^{\bullet} + O_2^{-\bullet}$ (reaction (4)) was the only reaction of $Trp^{\bullet}$ involving oxygen which occurs within the millisecond time scale of the pulse radiolysis experiment and which can compete with $Trp^{\bullet}$ recombination.[13] As explained earlier,[13] reaction rate constants for reaction (4) from the $Trp^{\bullet}$ decay data for O_2 saturated solutions are obtained from a least squares fit for the numeric integration of the rate equations describing reactions (4) and (5):

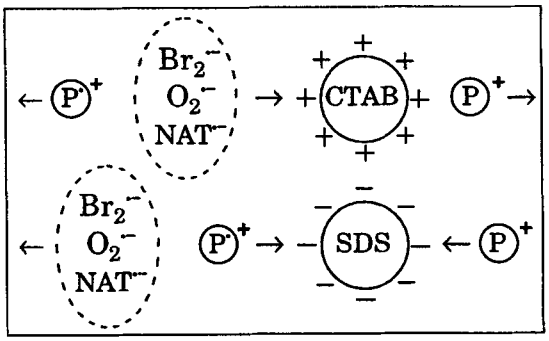
$$\frac{d[Trp^{\bullet}]}{dt} = k_4[Trp^{\bullet}][O_2^{-\bullet}] + k_5[Trp^{\bullet}]^2$$

Examination of Table I shows that the bimolecular reaction rate constant of reaction (4) for NAT is less than half of that for Trp, but is notably higher for the KGWK and KWK peptides at pH 6.4, approximately 1.5 times higher than that obtained with Trp under similar ionic strength conditions. Most notable is the case of KGWKtBut peptide which has the highest positive charge and exhibits an even higher rate constant, twice that of free $Trp^{\bullet}$. Data for KWK in Table I also illustrate the ionic strength effect on the rate constant for reaction (4) expected in a peptide bearing a +2 charge.

It should be noted that in oxygen-saturated solutions of the peptides, spectra identical to those observed in free Trp solutions are recorded at the beginning and at the end of the reaction of $Trp^{\bullet}$ with $O_2^{-\bullet}$ (data not shown). This result is in agreement with our hypotheses[13] that a possible repair of the $Trp^{\bullet}$ radical by $O_2^{-\bullet}$ and/or a splitting of the benzopyrrole ring of the tryptophan radical may occur during reaction (4), leading to another, but non absorbing, transient in the near UV.

Reactivity of $O_2^{-\bullet}$ with the $Trp^{\bullet}$ Radical in CTAB and SDS Micelles

The above results demonstrate the extent to which rate constants for reaction (4) are enhanced by the electrostatic interaction between the superoxide anion and the overall positive charge born by the Lys residues of the three peptides under investigation but also the extent to which they are decreased with negatively charged NAT. More pronounced attractive or repulsive electrostatic effects on the rate constant of equation (4) may be expected when such molecules interact with positively or negatively charged molecular assemblies such as micelles. The scheme below indicates the manner in which the various species should partition in CTAB and SDS micelles, based on their electrostatic charges (see Scheme 1). In the presence of negatively charged SDS, the situation is complicated by the fact that peptide is partitioned between the aqueous phase and micellar pseudophase. This explains the behavior observed in Figure 1A(c) in which there is an initial rapid growth of $Trp^{\bullet}$ through oxidation of peptide in bulk solution followed by a delayed reaction of Br_2^- with bound peptide. The initial $Trp^{\bullet}$ yield produced by the Br_2^- attack, is subsequently modulated by the inaccessibility of peptide Trp residues bound to the micelles. In positively charged CTAB micelles, which favor localization of the Br_2^- at the micellar surface and, hence, the recombination $Br_2^- + Br_2^-$,[14] the $Trp^{\bullet}$ yields for positively charged peptides are significantly lowered. By contrast, negatively charged NAT does bind to CTAB micelles[29] and the yield of $Trp^{\bullet}$ is $\frac{2}{3}$ of that found in buffer.



SCHEME 1 Partitioning in micellar systems of the various radical and non-radical species used in this study. P represents a positively charged peptide while P• represents the corresponding radical.

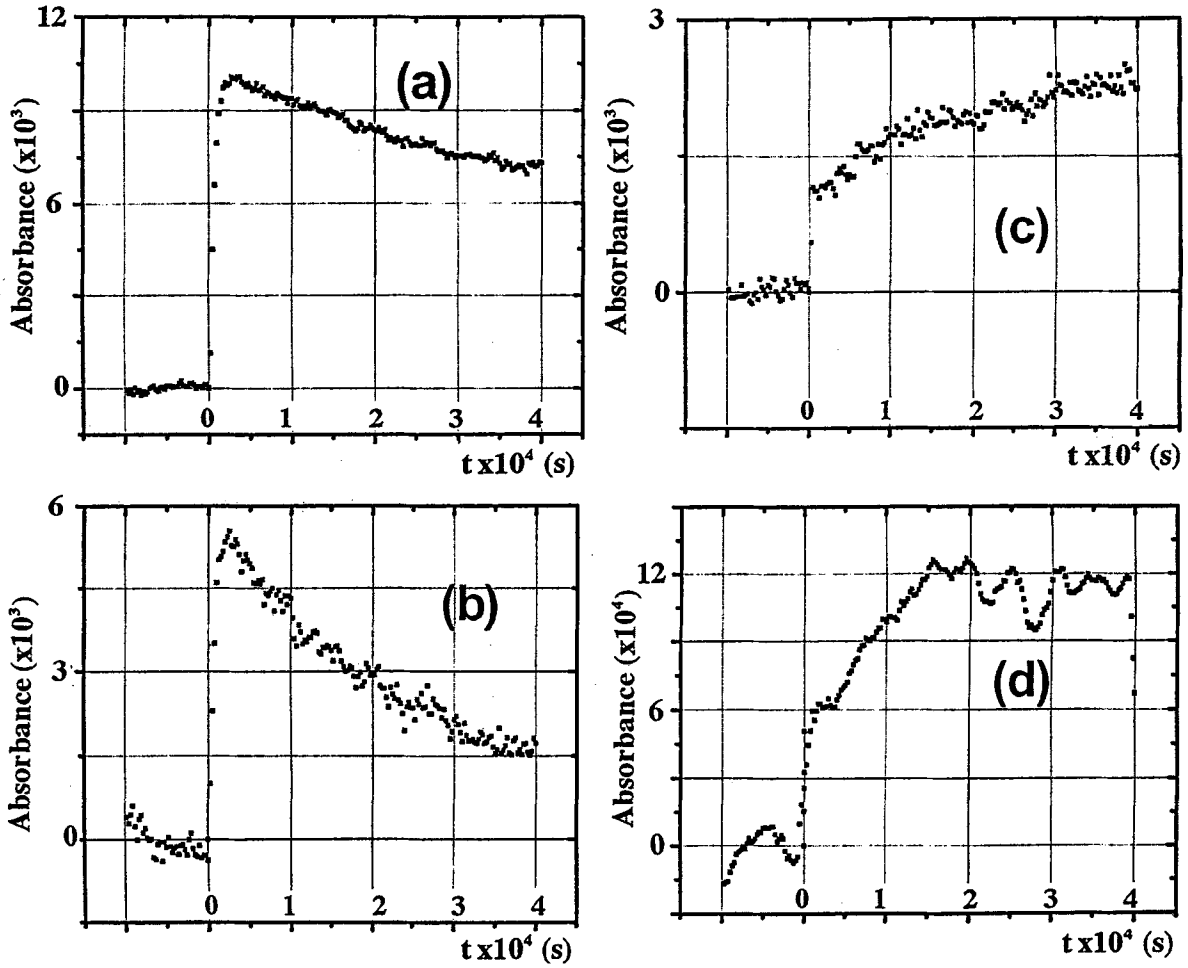


FIGURE 1A Formation and decay of transient absorption at 520 nm obtained by pulse radiolysis of 100 μM KWK in aqueous solution at pH 6.4. (a) Solution contained 10 mM phosphate buffer, 10 mM Br^- and was N_2O-saturated at 22°C; (b) same as (a) but solution was saturated with O_2; (c) solution contained 50 mM SDS and 100 mM Br^-; (d) same as (c) but the solution was saturated with O_2. Dose was 9 Gy. The light path of the optical cell was 1 cm. Ten experiments were averaged to obtain the data in (a) and (b) and twenty for (c) and (d).

It is obvious that the same reasoning applies to the $O_2^{-\bullet}$ radical reaction with the $Trp^\bullet$, enhancing recombination in CTAB and inhibiting it in SDS.

The consequences of the various repulsive or attractive electrostatic interactions on the initial absorbance and decay of the $Trp^\bullet$ transient absorption under several microenvironmental conditions are illustrated in Figure 1 with KWK and KGWKOtBut peptides. It may be seen that in O_2^- saturated solutions low yields of $Trp^\bullet$ radical are formed by the Br_2^- attack on KWK in SDS micelles (Figure 1A and Table I). A much lower yield is obtained with KGWK and KGWKOtBut at low ionic strength (Table I). However, once formed in SDS micelles, the $Trp^\bullet$ radical does not appreciably react with $O_2^{-\bullet}$ although $O_2^{-\bullet}$ itself is formed in a much larger yield (about an order of magnitude at low ionic strength).

The extent to which Trp and $Trp^\bullet$ reactivities toward Br_2^- and $O_2^{-\bullet}$ respectively are inhibited in presence of SDS can be deduced from Figure 1A. Such inhibition is expected since the negatively charged radicals are repelled by the micellar surface. It has previously been demonstrated

through fluorescence quenching studies that positively charged indoles bind to SDS micelles such that the charged moiety of the derivative interacts with the oppositely charged Stern layer of the micelle allowing the aromatic ring to penetrate into the hydrophobic core.[29] A fluorescence study with KWK in SDS micelles at pH 7 have provided data consistent with this model for peptide–micelle interaction (results not shown).

In the case of CTAB, the positively charged peptides do not bind to the micelles. As a result, the reactivity of $O_2^{-\bullet}$ with $Trp^\bullet$ is restored as illustrated with KGWKOtBut in Figure 1B. Under N_2O-saturation, the $Trp^\bullet$ yield itself is halved after addition of 10 mM CTAB to the pH 6.4, buffered aqueous solution of the peptides. This reduction in the G value of the $Trp^\bullet$ radical ($G(Trp^\bullet)$) is, no doubt, due to enhanced Br_2^- recombination at the CTAB micellar surface.[14,15] Furthermore, because Br_2^- is significantly localized by the CTAB micelles, it reacts at a very slow rate with KGWKOtBut which is resident in the aqueous phase. Thus, the bimolecular reaction rate constant determined here by the numerical integration for reaction (2) is $8 \times 10^7\,M^{-1}s^{-1}$ as compared to $5 \times 10^9\,M^{-1}s^{-1}$ in buffer.[20] The initial fast growth of $Trp^\bullet$ occurs in the interval before Br_2^- is fully equilibrated with the micelle.

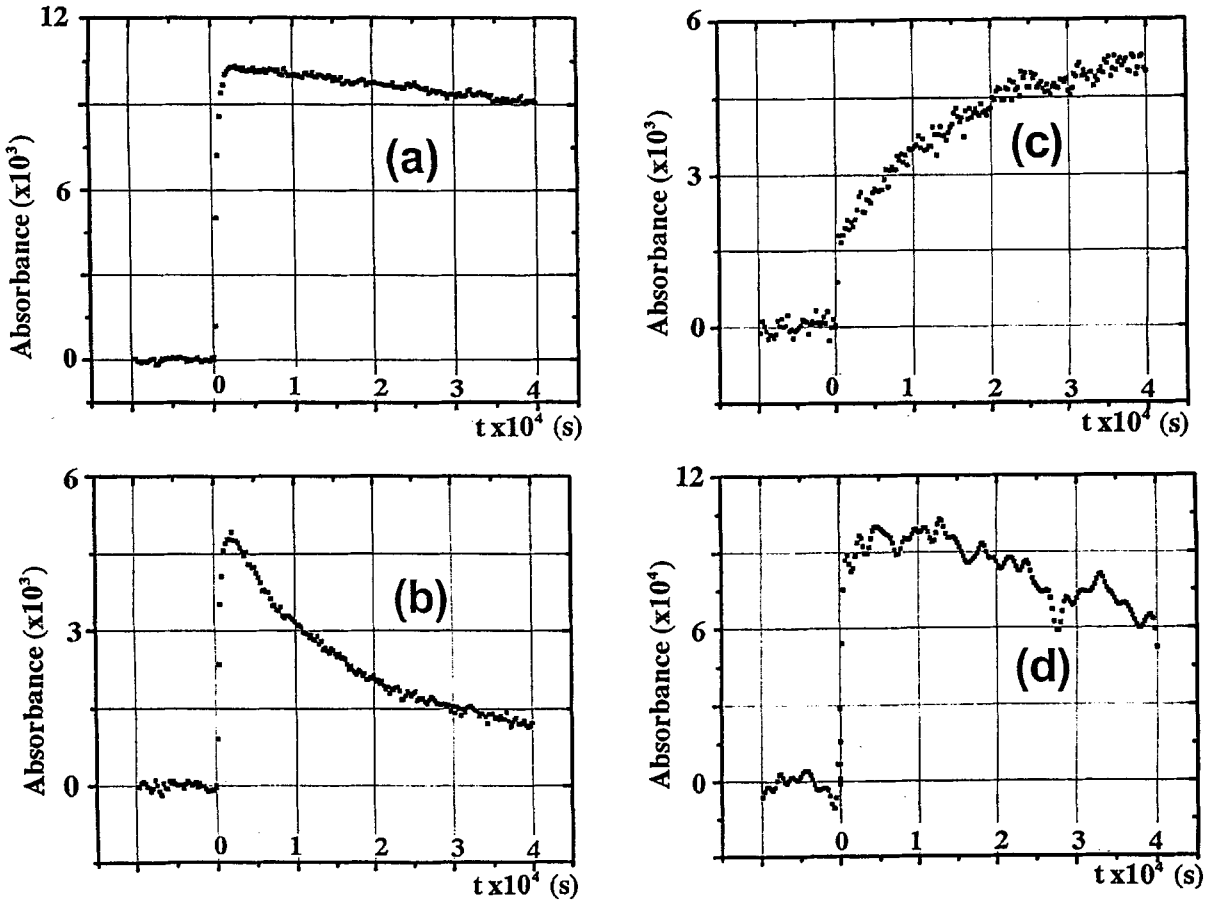


FIGURE 1B Formation and decay of transient absorptions at 520 nm obtained at 22°C by pulse radiolysis of 100 μM KGWK-OtBut in 10 mM phosphate buffer, pH 6.4, containing 10 mM Br^-. (a) N_2O-saturated; (b) O_2-saturated; (c) same as (a) but solution contained 10 mM CTAB; (d) same as (c) but the solution was saturated with O_2. Dose was 9 Gy. The light path of the optical cell was 1 cm. Ten experiments were averaged to obtain the data in (a) and (b) and twenty for (c) and (d).

In O_2-saturated CTAB solution, the apparent $G(Trp^{\bullet})$ from positively charged KGWKOtBut is only $\frac{1}{5}$ that obtained in buffer. It seems thus probable that in the CTAB micelles the peptide $Trp^{\bullet}$ radical is rapidly consumed during its formation by $O_2^{-\bullet}$, which will itself be in equilibrium between the aqueous phase and the micellar surface.[16]

Since Br_2^- does not interact with SDS, the same phenomenon cannot take place in negatively charged micellar systems. In the latter, the expected two-fold decrease in the Br_2^- yield is observed both in micellar and buffer solutions in going from N_2O- to O_2-saturation at low (data not shown) or high ionic strength as evidenced from Figure 1A data on the KWK peptide. Similar results are obtained of course with the KGWK peptide (see Table I).

Negatively charged NAT binds to CTAB micelles.[2] Hence, in contrast to observations made with the peptides, the $Trp^{\bullet}$ yield in the micelles ($G = 4$) is only moderately decreased as compared to the yield in buffer (Table I). As expected from the above discussion concerning the importance of micelle charge on the reactivity of charged radicals, both the the $TrpH + Br_2^-$ (compare $k_2 = 8 \times 10^8\,M^{-1}\,s^{-1}$ in buffer to $k_2 = 1.6 \times 10^9\,M^{-1}\,s^{-1}$ in CTAB) and the $Trp^{\bullet} + O_2^{-\bullet}$ reaction rate constants (Table I) are notably increased.

Effect of DNA Binding on the Reaction $Trp^{\bullet} + O_2^{-\bullet}$

Since peptide binding to SDS inhibits the reactivity of both Br_2^- and $O_2^{-\bullet}$ with KWK, KGWK and KGWKOtBut, it can be anticipated that the binding of these peptides to the negatively charged polyphosphate backbone of DNA may also impede the reaction $Trp^{\bullet} + O_2^{-\bullet}$.

We have previously shown that at pH $\leq$ 6.5, low ionic strength (10 mM Br^-) and appropriate concentration, more than 90% of these peptides reversibly bind to native DNA. These interactions give two different types of complexes with nucleic acids: (a) an outside complex having the Lys residue bound to the polyphosphate backbone with the Trp aromatic ring exterior to the DNA chain; this represents about 75% of the bound peptide; (b) a stacked complex in which the remaining Trp residue is intercalated between two nucleic acid bases.[20] In contrast to the case of SDS micelles where the aromatic rings are immersed in the hydrophobic micellar region, the DNA outer complex is susceptible to the Br_2^- attack (Figure 2) resulting in a much higher $G(Trp^{\bullet})$ than that obtained with SDS.[20] As can be seen for N_2O-saturated solutions in Figure 2, the peptide $Trp^{\bullet}$ radical formed on DNA undergoes a major fast decay with a little contribution of reaction (5). The $G(Trp^{\bullet})$ found here in N_2O-saturated solutions confirm those previously reported.[20] In that earlier study the fast decay was found to be first order and was attributed to a fast electron or hydrogen atom transfer from DNA to semi-oxidized Trp.[20]

$$Trp^{\bullet} + DNA \rightarrow \qquad (6)$$

Here, first order rate constants of the order of $5 \times 10^3\,s^{-1}$ are obtained from the data and are consistent with those previously observed.[20] Transient behavior shown in Figure 2 demonstrates that the fast decay process is also enhanced by the presence of oxygen, suggesting that $O_2^{-\bullet}$ can compete with the nucleic bases in reaction with the bound $Trp^{\bullet}$ radical. On the other hand, at high ionic strength (0.5 M Br^-) where peptide–DNA complexes dissociate, the slow second order decay is restored (Table II). Under these conditions, this decay is again responsive to the presence of oxygen in solution due to reaction (5). The bimolecular reaction rate constants for this reaction are also given in Table II for the cases of KGWK and KGWKtBut under conditions of high ionic strength. These are in good agreement with those found in Table I, obtained in the absence of DNA, under similar high ionic strength conditions. Because there is no modification of the $Trp^{\bullet} + O_2^{-\bullet}$ kinetics by unbound DNA, one may conclude that there is no competitive

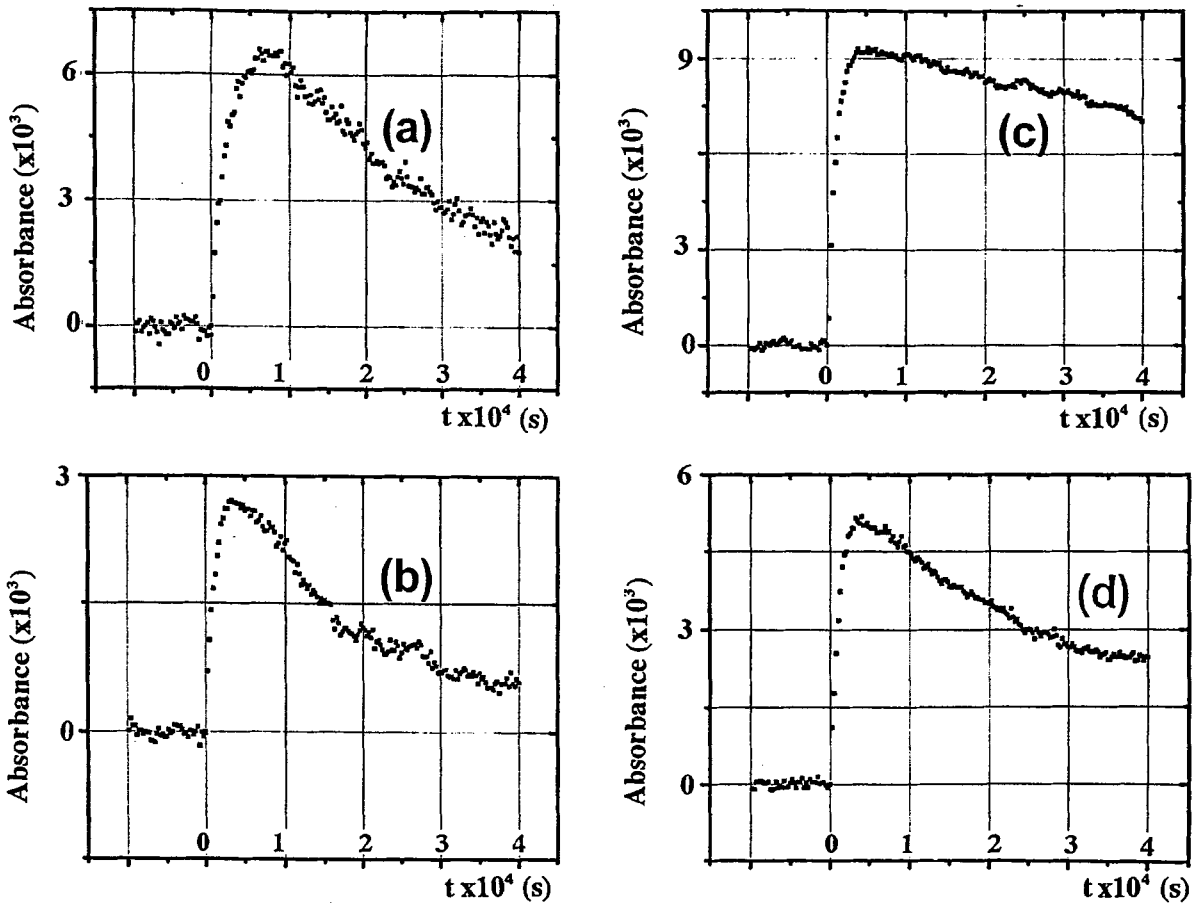


FIGURE 2 Formation and decay of transient absorptions at 520 nm obtained at 22°C by pulse radiolysis of 80 μM KGWKOtBut and 0.4 mM DNA in 10 mM phosphate buffer, pH 6.4. (a) Solution contained 10 mM Br^- and was saturated with N_2O; (b) same as (a) but solution was saturated with O_2; (c) solution contained 0.5 M Br^- and was saturated with N_2O; (d) same as (c) but solution was saturated with O_2. Dose was 9 Gy. The light path of the optical cell was 1 cm. Ten experiments were averaged to obtain the data in all cases.

reaction of $O_2^{-\bullet}$ with redox metal ions probably bound to commercial native DNA. The high reactivity of the positively charged peptide $Trp^\bullet$ radical in KGWKOtBut suggests that such molecules could be used in competition studies to probe the reactivity of $O_2^{-\bullet}$ with other substrates whose study by pulse radiolysis method would otherwise be experimentally difficult.

CONCLUSION

Tryptophan is a key residue in many proteins, where the $Trp^\bullet$ neutral radical and the $O_2^{-\bullet}$ radical anion can be formed by a variety of processes including enzymatic reactions and direct or sensitized photolysis, as emphasized in the introduction. Because it is important to understand the role of $O_2^{-\bullet}$ in the formation of Trp (photo) oxidation products,[13] such studies as these, coupled to determination of the nature of the (photo) oxidation products formed with other active oxygen species such as singlet oxygen[30] should lead – even at the cellular level – to improved understanding of the mechanistic pathways for Trp oxidation in proteins. Using simple but highly relevant model systems here, we have obtained an indication of the extent to

TABLE II Radiolytic yield and decay rate constants of the $Trp^{\bullet}$ radical formed in KGWK and KGWKOtBut in the presence of native DNA under various experimental conditions at pH 6.4

Compounds	Conditions	$G(Trp^{\bullet})$	k_4	k_5	k_6
KGWK	N_2O	6.1	—	0.3	—
	N_2O, DNA	3	—	1	4
	O_2, DNA	1.7	1.9	1	4
	O_2, 0.5 M Br^-, DNA	2.1	1.2	0.4	—
	N_2O, 0.5 M Br^-, DNA	6	—	0.4	—
KGWKOtBut	N_2O	6.1	—	0.1	—
	N_2O, DNA	4.1	—	—	3.4
	O_2, DNA	2	2.8	—	—
	N_2O, 0.5 M Br^-, DNA	6.1	—	0.2	—
	O_2, 0.5 M Br^-, DNA	2.8	1.5	0.2	—

Rate constants k_4 and k_5 are expressed in units of $10^9\,M^{-1}\,s^{-1}$ and k_6 is expressed in $10^3\,s^{-1}$. Solutions were prepared in 2 mM phosphate buffer and contained 80 μM peptide, 0.4 mM native DNA and 10 mM Br^- unless otherwise specified. Solutions were saturated with N_2O or O_2 as indicated.

which the kinetics of processes such as reaction (5) in complex biological environments will be influenced by the nature and extent of interactions between such environments and the reaction components involved. The study demonstrates that the reactivity of $O_2^{-\bullet}$ with the $Trp^{\bullet}$ radical is significantly dependent on the charge in the vicinity of the Trp residue from which it originates and, futher that such reactivity will be significantly influenced by the electrostatic field generated by neighboring residues and/or other charged cell constituents, e.g., DNA and lipids. Furthermore, it may be expected that in multitryptophan proteins undergoing $O_2^{-\bullet}$ oxidation, the nature as well as the yield of the final oxidation products of a Trp residue located in a peculiar domain of the protein will depend strongly on its immediate environment.

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