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Effect of solvent viscosity, polarity and pH on the charge transfer between tryptophan radical and tyrosine in bovine serum albumin: a pulse radiolysis study

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Abstract

The effect of viscosity, solvent polarity and pH of the medium on the reaction of a protein, bovine serum albumin (BSA), with organohalo-peroxyl radical in aqueous solution has been studied using pulse radiolysis technique. Unlike in dilute aqueous solution, electron transfer from tyrosine to tryptophan radical in BSA has been clearly observed at a viscosity of 7.7 centiPoise (cP). The oxidation of BSA, tryptophan and tyrosine in different media has also been compared with those taking place in dilute aqueous solution. The effect of solvent characteristics on the observed charge transfer has been discussed.

Keywords: Bovine serum albumin; Charge transfer; Solvent effects; Pulse radiolysis

1. Introduction

The main components of mammalian cells are water (70%) and proteins (~20%), while DNA accounts for less than 1% of the cell weight. Therefore, most of the radical reactions occur with proteins in the first step, causing modifications and/or damage to protein molecules. Furthermore, radiation-induced modification of the proteins is not only due to the reaction with primary radicals but also with secondary radicals, which are formed from various solutes by scavenging of primary radicals. These secondary radicals react with proteins depending upon their redox potential, surrounding chemical environment, etc., causing their oxidation or reduction. In the reaction of proteins with oxidizing radicals one-electron oxidized radical of tryptophan and/or tyrosine are generally observed. The reaction of oxidizing radical with tyrosine ($pK_a = 10.47$) forming one-electron oxidized tyrosine radical ($TyrOH^{\bullet +}$, $pK_a < 0$) is pH dependent with rate constant increasing with pH. On the other hand, reaction of oxidizing radical with tryptophan ($pK_a = 9.4$) forming one-electron oxidized tryptophan radical ($TrpH^{\bullet +}$, $pK_a = 4.3$) is pH independent [1]. Therefore, at neutral pH one-electron oxidized radical of tryptophan and tyrosine exist as $Trp^{\bullet}$ and $TyrO^{\bullet}$, respectively. In some

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cases, radical transformation involving charge transfer from tyrosine to tryptophan radical in aqueous solution of simple model peptides [2–4] and proteins [4–8] has been observed to take place. This charge transfer is affected by the distance, difference in the redox potential between the donor and the acceptor, solvent structural reorganization accompanying the transfer, etc. Charge transfer from tyrosine to the tryptophan radical also depends on the three-dimensional structure of the protein molecule, which in turn is affected by the solvent. In erabutoxin-b, tryptophan radical does not transform into tyrosine radical until the –S–S– bonds are broken despite the close proximity of tryptophan and tyrosine [4]. This shows that peptide bonds do not provide a channel for electron transfer and a direct contact between the two reaction centres by some means is a prerequisite.

The reaction kinetics of various proteins and enzymes (hemoglobin, cytochrome, serum albumin, papain, trypsin, lysozyme, etc.) with free radicals in the dilute aqueous solution has been well reported and compiled [9,10]. The reactions that take place in cellular environment are expected to be different compared to that in dilute aqueous solution as biological systems have high viscosity and self-assembly which increases efficiency of the biological processes [11]. For example, viscosity of human blood plasma is $\geqslant 4.5$ times that of distilled water [12] and biological membrane is a clear example of restricted environment where lipids, proteins and carbohydrates coexist in a specific arrangement producing compartmentalization [13]. The hydrophobic interactions of protein molecules with lipid membrane and surfactants affect their three dimensional structure, thereby altering their reactions [14,15]. The studies of such reactions are also of significance, since it is recognized that charge migration can facilitate transport of radical-centre away from the initial site. Thus harmful cross-links can be formed at amino acid residues far away from the site where the primary reaction occurs. The effect of solvent characteristics, like polarity and viscosity, on the free radical induced oxidation reactions of protein, though very important, has not been reported. In earlier studies, we have shown that reduction reaction of bovine serum albumin (BSA) in viscous 2% polyvinyl alcohol solution and w/o microemulsion follows kinetics and mechanism different from that in the aqueous solution [16,17]. However, the study of free radical induced oxidative damage of protein in such medium faces inherent experimental difficulties, which arise because of the reactivity of the substrates used (alcohol, surfactant, etc.) in high concentration for mimicking such viscous and micro-heterogeneous environment with the oxidizing species.

The solvent effects are known to play an important role in the reactions of organic compounds with the peroxyl radical [18,19]. It has also been shown that reaction of the hydroxyl radical with gelatin and recombination reaction of the thiocyanate radical, $(SCN)_2^{\bullet -}$ in gelatin is not affected significantly by increase in macro-viscosity of the medium [20]. It was ascribed to small size of the hydroxyl and $(SCN)_2^{\bullet -}$ radicals whose reactions are not affected by triple helixes of gelatin macromolecule. The effects of solvent characteristics on the reactions of benzhydryl cations are also known [21].

To overcome the difficulties mentioned above, reaction of BSA, a carrier protein, with organohalo-peroxyl radical has been studied to investigate solvent effects on the reaction of proteins with oxidizing radicals. The organohalo-peroxyl radicals can be conveniently generated in the aqueous solution containing other organic solutes and is also used as a model peroxyl radical. Glycerol has been added to increase viscosity of the medium. Electron pulse radiolysis system with kinetic spectrophotometry was used to study the fast reactions.

2. Experimental

The pulse radiolysis system using 7 MeV electron pulse has been described elsewhere [22]. The dosimetry was carried out using an air-saturated aqueous solution of 5×10^{-2} mol dm^{-3} KSCN ($G\varepsilon=2.6\times10^{-4}$ m^2 J^{-1} at 475 nm [23]). The kinetic spectrophotometric detection system covered the wavelength range 250–800 nm. The optical path length of the cell was 1.0 cm. The viscosity of the solution used was measured using a DJScientific make Analytical Viscometer AV-

250 and Ostwald viscometer. Dielectric constant of the solvent mixtures has been taken as algebraic sum of mol fraction multiplied with the dielectric constant of each component. *G*-values of the primary radicals of water-radiolysis have been used for the solvent mixture. High dose (corresponding to $\sim 9\times10^{-6}$ mol dm^{-3} peroxyl radical) has been used in this study to have better signal to noise ratio. The bimolecular rate constants were calculated by plotting pseudo-first order rate constant against the respective solute concentrations. BSA (fraction V) from SISCO (India) was used as received. All other chemicals were of AR grade. All aqueous solutions were prepared in nano-pure water (conductivity 0.06 μS cm^{-1}) from Barnstead nano-pure cartridge filtration system. 2-Propanol (3 mol dm^{-3}) was added to aqueous glycerol (45-w/v% corresponding to 4.88 mol dm^{-3}) solution to solubilize CCl_4. Phosphate buffer was used to prepare pH 6.8 and other pHs have been obtained using $HClO_4$ and/or NaOH solutions. All the measurements were carried out at 26 ± 1 °C and the expected error in the measurement of data is $\pm10\%$.

3. Results and discussion

The viscosity and dielectric constant of the solution used are 7.73 cP and 63.66, respectively. The viscosity is $\sim$8.65 times that of the distilled water. $CCl_3O_2^{\bullet}$ radical has been generated as shown by the following reactions.

$$H_2O \rightsquigarrow e_{aq}^-,\ H^{\bullet},\ {}^{\bullet}OH,\ldots \quad (1)$$

$$e_{aq}^- + CCl_4 \rightarrow CCl_3^{\bullet} + Cl^- \quad (2)$$

$$CCl_3^{\bullet} + O_2 \rightarrow CCl_3OO^{\bullet} \quad (3)$$

The hydroxyl radical ($^{\bullet}$OH) and H$^{\bullet}$ atom are scavenged by 2-propanol and glycerol. It has been observed that in aqueous glycerol solution $CCl_3O_2^{\bullet}$ radical has absorption around 300 nm and becomes negligible at 350 nm (not shown here). This suggests that in this medium transient absorption beyond 350 nm is not due to $CCl_3O_2^{\bullet}$ radical. The glycerol and alcohol radicals produced by scavenging $^{\bullet}$OH and H are carbon-centered radicals and did not cause reduction or oxidation of BSA.

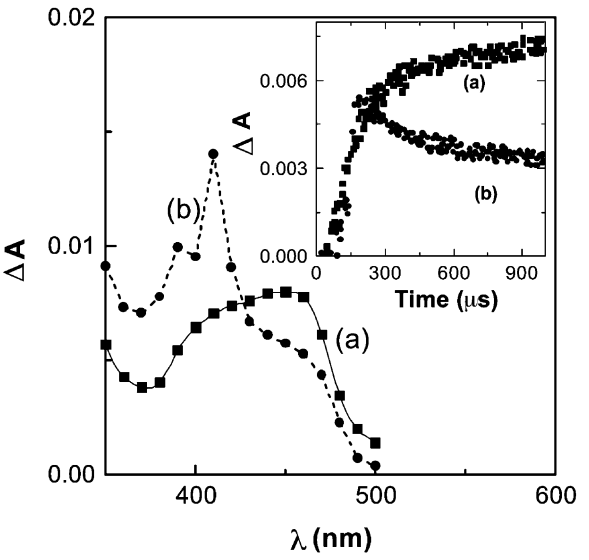


Fig. 1. Transient absorption spectrum obtained from aerated aqueous solution containing BSA (1.0×10^{-4} mol dm^{-3}), glycerol (45-w/v%), 2-propanol (3.0 mol dm^{-3}) and CCl_4 (4.0×10^{-2} mol dm^{-3}) at pH 6.8, (a) 50 μs and (b) 1700 μs after the electron pulse. *Inset:* kinetic traces at (a) 410 nm and (b) 460 nm under similar conditions. Dose 40 Gy.

3.1. Effect of solvent characteristics

The reaction, which has been studied by changing solvent characteristics, can be written as:

$$CCl_3O_2^{\bullet} + \text{Solute (S)} \rightarrow CCl_3O_2^- + S^{\bullet+} \quad (4)$$

Since unionized species are producing ionized species, the kinetics of reaction is expected to be affected by the dielectric constant of the medium [18,19]. The molecular conformation of BSA, a macromolecule, is also known to be affected by characteristics of the medium [15]. Therefore, the effect of different solvent characteristics, namely viscosity, polarity and pH, on the oxidation of BSA can be observed.

3.1.1. Viscosity

Transient absorption spectrum obtained from $CCl_3O_2^{\bullet}$ induced oxidation of BSA in 45-w/v% aqueous aerated glycerol solution at pH 6.8 has been shown in Fig. 1. Its characteristics are different from that observed in the reaction of the same radical with BSA in dilute aqueous solution [24].

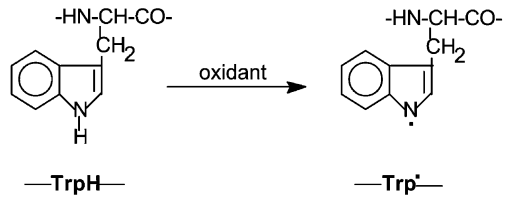
This transient absorption spectrum shows simultaneous decay of 460 nm and growth of 410 nm absorption after the first step of formation of absorption at these two positions. The inset in Fig. 1 shows a delayed formation of transient absorption at 410 nm along with decay at 460 nm, which has not been observed in the dilute aqueous solution [24]. The transient absorption at 410 nm is known to be due to the one-electron oxidized radical of tyrosine but the absorption at 460 nm is new. To identify the transient species observed in the reaction of BSA with $CCl_3O_2^{\bullet}$ radical in this medium, similar studies have been done with tryptophan, tyrosine and dimethyl disulfide (disulfide link), which are more susceptible to oxidation in BSA molecule. The transient absorption spectra obtained for tryptophan, tyrosine and dimethyl disulfide under similar conditions have been shown in Fig. 2A–C, respectively. Fig. 2A–C and the transient absorption maxima of one-electron oxidized radical of tryptophan [25–28], tryosine [29–32] and dimethyl disulfide [33] suggest that the transient absorption in Fig. 1 at 460 nm is due to the one-electron oxidized tryptophan radical ($Trp^{\bullet}$) and that at 410 nm is due to the one-electron oxidized radical of tyrosine ($TyrO^{\bullet}$). It has to be noted that tryptophan and not tyrosine can be oxidized with $CCl_3O_2^{\bullet}$ radical at neutral pH. The transient absorption maximum at 460 nm for tryptophan radical has also been observed in the reaction of casein with $CCl_3O_2^{\bullet}$ radical [8]. It is well known that absorption and emission characteristics of tryptophan are polarity dependent. The blue-shift in absorption maximum of tryptophan radical can be ascribed to the reduction in polarity of the medium. If the oxidation of the disulfide group has taken place, it is either masked by the relatively strong absorption of $TyrO^{\bullet}$ or there is hole migration from one-electron oxidized disulfide group to tyrosine [1]. The oxidation of other amino acids, even if taking place, could not be observed in this complex system or probably gets repaired by hole migration to generate thermodynamically more stable tyrosine radical. The hole migration has been suggested earlier in the case of oxidation of peptides [2] and BSA in aqueous solution [24]. The oxidation of BSA by $CCl_3O_2^{\bullet}$, at pH 6.9, in aqueous solution gives transient absorption at 410 nm along with a broad transient absorption approximately 460 nm [24]. However, no radical transformation was reported in BSA molecule. It is observed from the Fig. 1 that while the transient absorption at 410 nm corresponding to $TyrO^{\bullet}$ increases the absorption at 460 nm corresponding to $Trp^{\bullet}$ decreases with the time, suggesting that electron (or hole) transfer takes place. The formation of tryptophan radical and charge transfer from tyrosine to tryptophan radical in protein are shown in Schemes 1 and 2.

As discussed earlier, a similar type of radical transformation has been observed in the oxidation of aqueous solution of peptides and some proteins [1–8]. In the reaction of $CCl_3O_2^{\bullet}$ with lysozyme in this medium, contrary to that in dilute aqueous solution, no such radical transformation has been observed. The change in observation of radical transformation from $Trp^{\bullet}$ to $TyrO^{\bullet}$ for BSA (582 amino acids) and lysozyme (128 amino acids) macromolecules suggests that some conformational changes of protein molecules take place. The kinetic traces at 410 nm for the oxidation of BSA in water/2-propanol and water/90% (v/v) glycerol mixture (Fig. 3) qualitatively show that solvent viscosity has significant effect on the rate of reaction. Since this phenomenon has been observed for BSA in aqueous glycerol solution and not in dilute aqueous solution it may be due to higher viscosity and/or decrease in polarity of the medium. The reduction in the reaction rate constants has not been found to be in proportion with increase in viscosity of the medium and even the reduction is not the same for different molecules. This suggests that diffusion is not the only factor in this reaction. The calculated dielectric constant of the medium (63.66) used is little lower than that of water (78.54) indicating that polarity of the medium may be exerting some effect.

The rate constants for the reaction of $CCl_3O_2^{\bullet}$ with BSA, tryptophan, tyrosine and dimethyl disulfide measured in the absence and presence of 45-w/v% glycerol have been given in Table 1. The kinetic traces at 410 nm for the formation of $TyrO^{\bullet}$ radical in water/2-propanol, 45-w/v% glycerol and 50 (v/v)% of *tert*-butanol have also been recorded. This comparison and earlier studies [18,19] suggest that the polarity of the medium

affects the rate of oxidation by $CCl_3O_2^{\bullet}$ radical and higher viscosity has significant effect on delayed formation of $TyrO^{\bullet}$.

Similar transient absorption spectrum, with very poor signal to noise ratio, has also been observed in the reaction of BSA with $CHCl_2O_2^{\bullet}$ (not shown here). The rate constants for this reaction estimated at 410 and 460 nm are 1.1×10^8 and 3.0×10^8 $dm^3\,mol^{-1}\,s^{-1}$, respectively.



Scheme 1. Formation of tryptophan radical.

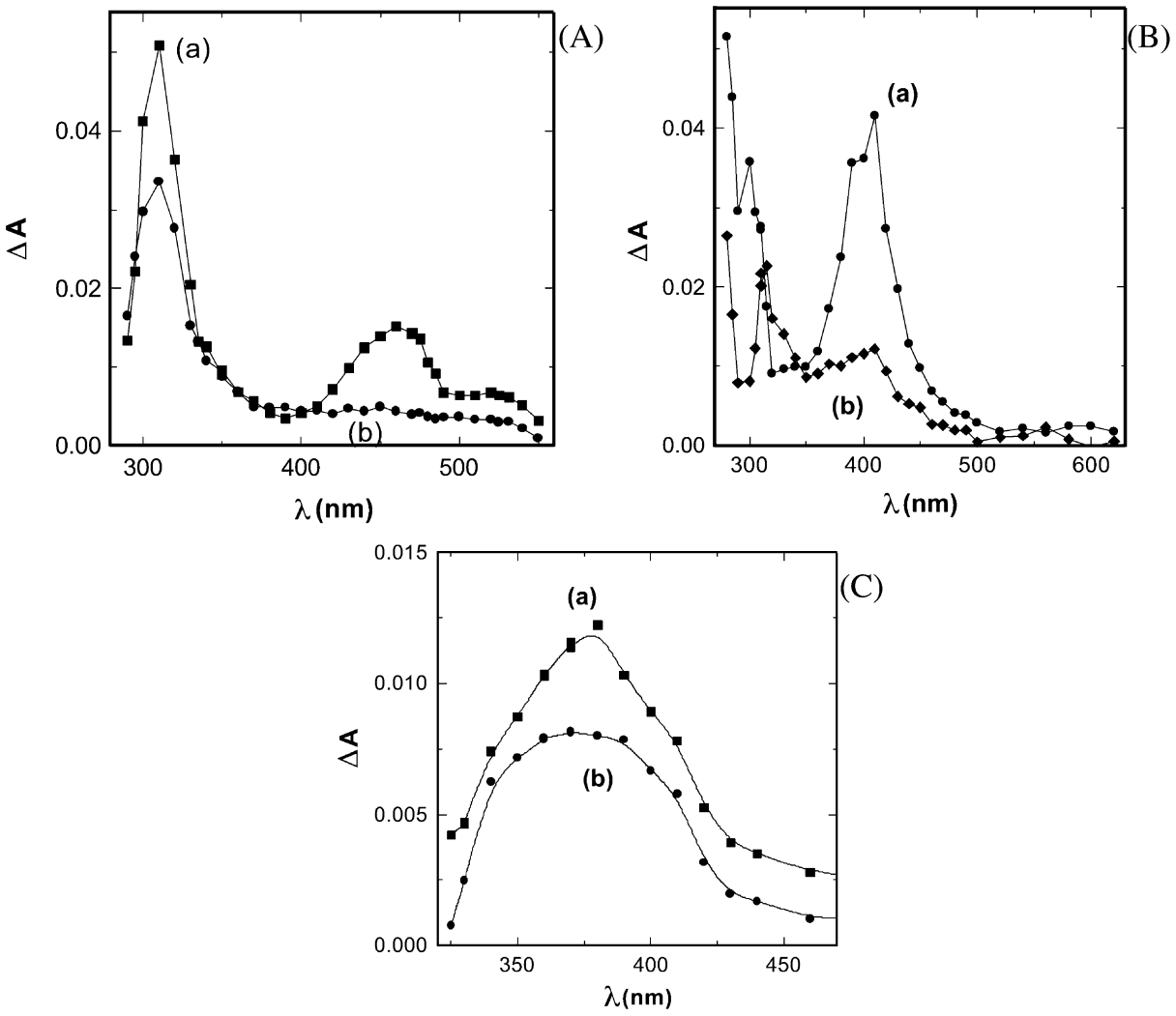


Fig. 2. Transient absorption spectrum obtained from aerated aqueous solution containing glycerol (45-w/v%), 2-propanol ($3.0\ mol\ dm^{-3}$), CCl_4 ($4.0\times10^{-2}\ mol\ dm^{-3}$) and (A) tryptophan ($1.0\times10^{-3}\ mol\ dm^{-3}$) at pH 6.8 (a) 40 μs and (b) 900 μs (B) tyrosine ($1.0\times10^{-3}\ mol\ dm^{-3}$) at pH 10.0 (a) 40 μs and (b) 1600 μs (C) dimethyl disulfide ($2\times10^{-3}\ mol\ dm^{-3}$) at pH 6.8 (a) 10 μs and (b) 40 μs, after the electron pulse. Dose 40 Gy.

The anti-oxidant effect of ascorbic acid was studied in this medium. It was observed that the tyrosine radical ($TyrO^{\bullet}$) of BSA, with absorption maximum at 410 nm, reacted with ascorbic acid with a rate constant of 2.4×10^{6} $dm^3\,mol^{-1}\,s^{-1}$ and thus got scavenged. A similar reaction is known to take place in dilute aqueous solution [24]. This is in accordance with the fact that the reaction of the small-sized radical is unaffected by viscosity of the medium [20].

3.1.2. Polarity

The oxidation of BSA with $CCl_3O_2^{\bullet}$ in 45-w/v% glycerol solution containing 2-propanol (3.0 mol dm^{-3}) is probably affected by reduction in polarity as well as increase in viscosity. However, the transient absorption spectrum obtained for the oxidation of BSA with $CCl_3O_2^{\bullet}$ radical in 50% (v/v) *tert*-butanol solution also shows similar $Trp^{\bullet}$ (460 nm) → $TyrO^{\bullet}$ (410 nm) radical transformation (Fig. 4). This suggests that even reduction in polarity alone can show such radical transformation. This may be again due to some conformational changes of BSA molecule. To see the effect of solvent polarity on kinetics, rate constant for the reaction of BSA with $CCl_3O_2^{\bullet}$ in water/alcohol mixtures having different dielectric constant have been measured and are reported in Table 2. The reaction rate constants for the formation of both tryptophan (460 nm) and tyrosine (410 nm) radicals decrease with decrease in dielectric constant of the medium in water/alcohol mixtures. This is in accordance with the fact that reduction in polarity reduces the rate of formation of charged species [Eq. (4)]. The plot of logarithm of the rate constant of formation at 410 and 460 nm, i.e. log k, against dielectric constant (ε) of the medium (Fig. 5) has been found to be linear for this

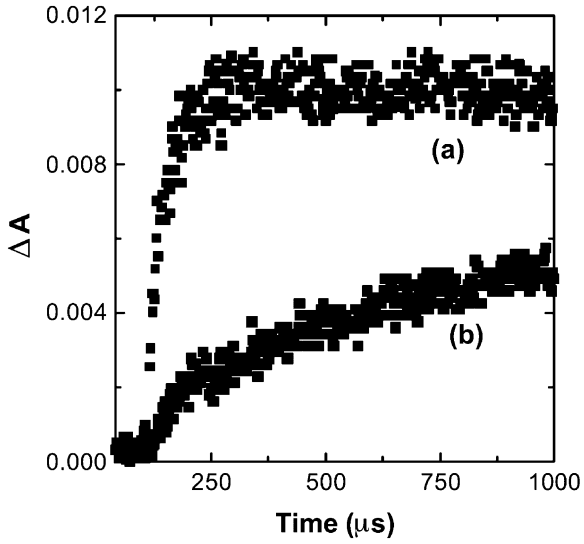
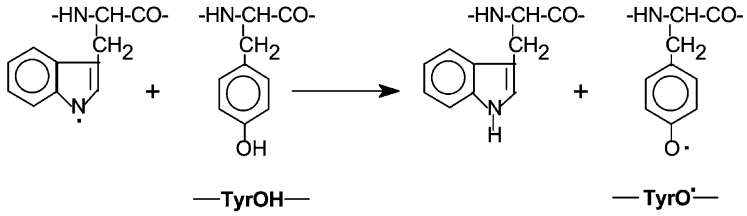


Fig. 3. Kinetic traces at 410 nm for solution containing BSA (1.0×10^{-4} mol dm^{-3}), CCl_4 (4.0×10^{-2} mol dm^{-3}) and (a) 2-propanol (3.0 mol dm^{-3}) (b) 90% (v/v) glycerol, after the electron pulse. Dose 40 Gy.

reaction with $R=0.967$ and 0.978, respectively. However, in aqueous glycerol solution, rate constants have not been found to decrease with the reduction in polarity to a further lower value. This suggests that reaction of BSA with $CCl_3O_2^{\bullet}$ is affected by polarity as well as viscosity of the medium. The decay rate constant of the transient absorbing at 460 nm in various solvents (Table 2) also indicates that this radical transformation is affected by solvent characteristics. The formation traces at 410 nm and the rate constants at 410 and 460 nm in different media (Table 2) suggest that the rate constants for the reaction of $CCl_3O_2^{\bullet}$ with tryptophan, tyrosine, dimethyl disulfide and BSA are strongly affected by the dielectric constant of



Scheme 2. Radical transformation from tryptophan to tyrosine.

the medium and the radical transformation from $Trp^{\bullet}$ (460 nm) to $TyrO^{\bullet}$ (410 nm) is affected by polarity as well as viscosity of the medium. The rate constants (k) for the reaction of BSA with $CCl_3O_2^{\bullet}$ radical, measured at 410 and 460 nm and at pH 6.8 in this medium can be related with the dielectric constant (ε) and the viscosity (η) of the medium by a semi-empirical equation:

$$\log k = A + B\varepsilon + C/\eta.$$

3.1.3. pH

The transient absorption spectrum obtained from the reaction of BSA with $CCl_3O_2^{\bullet}$ in this medium at pH 10, under identical conditions, also shows simultaneous decay of 460 nm absorption and growth of absorption at 410 nm (Fig. 6). This suggests that radical transformation takes place even at pH 10. It has been found that even at pH 10 reaction rate constants in this medium are lower than those in dilute aqueous solution but are still higher than that at pH 6.8. The decay rate of 460 nm absorption has been found to be the same at pH 6.8 and 10, suggesting that it is due to the same process of charge transfer from tyrosine to tryptophan radical at both pHs and is independent of pH. ΔA at 410 nm has been found to increase with increase in pH which is in accordance with the fact that the basic form of the radical has higher molar extinction coefficient than the acidic form.

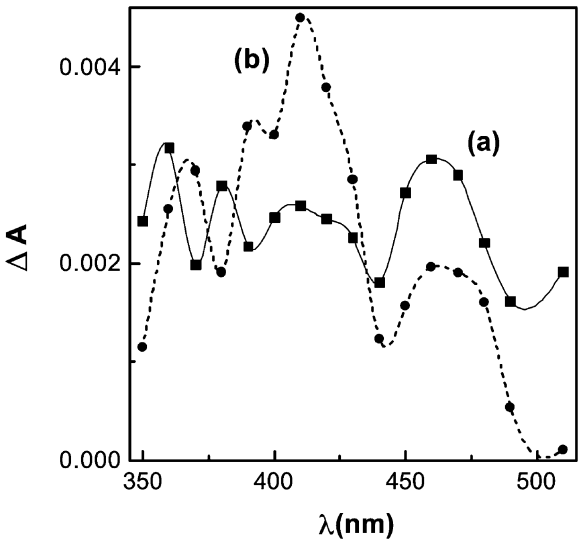


Fig. 4. Transient absorption spectrum obtained from aerated aqueous solution containing BSA (1.0×10^{-4} mol dm^{-3}), *tert*-butanol (50-v/v%), 2-propanol (3.0 mol dm^{-3}) and CCl_4 (4.0×10^{-2} mol dm^{-3}) at pH 6.8, (a) 50 μs and (b) 900 μs after the electron pulse. Dose 40 Gy.

BSA is a globular protein composed of 582 amino acids and has ellipsoidal structure of 141×41 Å^2 dimensions [15]. However, it is diffi-

Table 1
Rate constants for the reaction of different solutes with $CCl_3O_2^{\bullet}$ radical

Solute	pH	λ (nm)	k (dm^3 mol^{-1} s^{-1})$\times10^{-8}$	
			Water	45-w/v% glycerol
Tryptophan	6.8	460	–	2.25
		520	0.85[a](pH ~7)	0.39
Tyrosine	10.0	410	0.71[b](pH 10.6)	0.54
DMDS	6.8	380	42.0	35.0
BSA	6.8	410	4.8	3.3
	6.8	460	5.7	3.2
	6.8	510	13.0	8.6
	10	410	25.0	1.8
	10	460	–	5.4
BSA + $CHCl_2O_2^{\bullet}$	6.8	410	–	1.1
	6.8	460	–	3.1

[a] Ref. [25–28]
[b] Ref. [29–32]

Table 2
Rate constants ($k\times10^{-8}$ dm^3 mol^{-1} s^{-1}) for the reaction of BSA with $CCl_3O_2^{\bullet}$ in different solvents

Solvent[a]	ε (calculated)	At 410 nm	At 460 nm	Decay at 460 nm $k\times10^{-3}$ s^{-1}
2-Propanol (20% v/v)	75.20	4.8	5.7	5.5
Tert-butanol (25% v/v)	74.57	3.0	5.0	13.0
Tert-butanol (40% v/v)	71.05	2.0	1.8	8.3
Tert-butanol (50% v/v)	67.89	1.1	1.1	4.0
Glycerol (45-w/v%) +2-propanol (30% v/v)	63.66	3.3	3.2	9.2

[a] Rest is water.

cult to comment on the distance-dependent charge transfer from tyrosine to the tryptophan radical in solution phase because of conformational changes of the protein molecule with polarity of the medium and protonated state of the constituent amino acids with pH of the solution. The number of possible conformations of BSA (mol. wt. 66700 Da) with change in solvent properties is large. BSA contains two tryptophan and 19 tyrosine units. This gives 38 possible donor–acceptor pairs for charge transfer to take place. However, the conformational changes in protein molecule with solvent properties is an altogether different problem and has not been discussed here.

In aqueous solution of native BSA, tryptophan and not tyrosine is at the molecule–water interface contrary to the fact that tyrosine and not tryptophan is polar in nature [14]. If total reactivities of tryptophan and tyrosine residues of protein molecule are considered, by multiplying the rate constant with the respective number of amino acid present in the protein molecule, tryptophan should react first. This should be followed by a charge

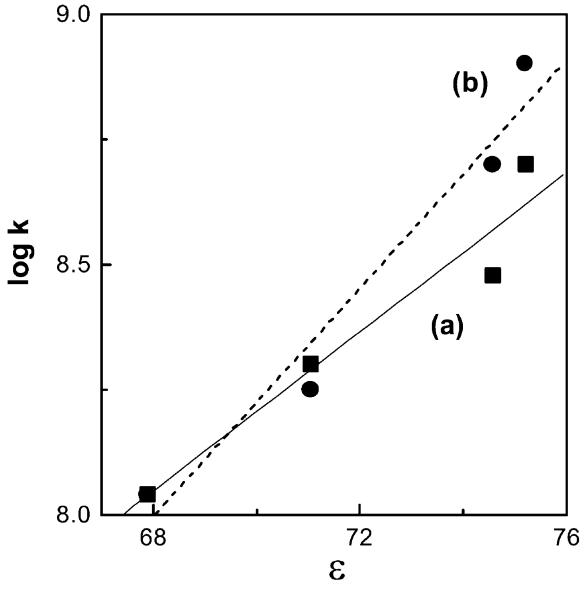


Fig. 5. Log k versus dielectric constant (ε) of the medium at (a) 410 and (b) 460 nm for the reaction of BSA with $CCl_3O_2^{\bullet}$ at pH 6.8.

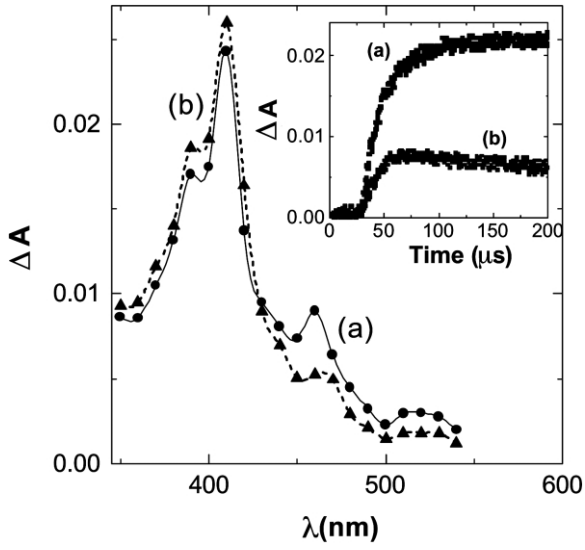


Fig. 6. Transient absorption spectrum obtained from aerated aqueous solution containing BSA (1.0×10^{-4} mol dm^{-3}), glycerol (45-w/v%), 2-propanol (3.0 mol dm^{-3}) and CCl_4 (4.0×10^{-2} mol dm^{-3}) at pH 10 (a) 80 μs and (b) 350 μs, after the electron pulse. *Inset:* kinetic traces at (a) 410 nm and (b) 460 nm under similar conditions. Dose 40 Gy.

transfer from tyrosine to tryptophan radical, as this radical transformation is thermodynamically favoured ($\Delta G^{o} = -8$ kJ mol^{-1}) in the forward direction at neutral pH [34,35]. In dilute aqueous solution of BSA, instead of radical transformation, only tyrosine radical is known suggesting a very fast radical transformation. According to the reactivities of the amino acids tryptophan and tyrosine with $CCl_3O_2^{\bullet}$ radical in this medium, at neutral pH almost 100% of the $CCl_3O_2^{\bullet}$ radical should react with tryptophan and even at pH 10 almost 30% of the radicals should react with tryptophan in the first step followed by the radical transformation. This has been observed in glycerol/water/2-propanol solvent mixture suggesting some changes in three-dimensional structure of protein molecule in this medium bringing $Trp^{\bullet}$ radical and tyrosine close together. The reduction in polarity as well as diffusion of species in this medium presumably results in the observation of radical transformation. Furthermore, the pHs inside and over the macromolecules, like protein, also vary from the bulk resulting in altogether different reactions. For example, the small pockets within and over lysozyme (a protein) are known to have pH far different from the bulk [36]. Even the unfolding–folding of a part of protein molecule cannot be ruled out in solution phase resulting in anomalous behaviour.

However, the reduction in the reaction rate constant with increase in viscosity is as expected. The reduction in reaction rate constant with decrease in polarity is also as expected due to formation of the charged species from the uncharged ones. Since addition of glycerol causes simultaneous change of viscosity and polarity, it is difficult to study the effect of viscosity exclusively. The effect of pH on the state of protonation and ease of oxidation of tyrosine residues is reflected in the reaction of BSA with $CCl_3O_2^{\bullet}$ at pH 10, where higher optical density for the tyrosine radical has been observed.

4. Conclusions

The reaction of BSA with $CCl_3O_2^{\bullet}$ radical in aqueous-glycerol and 50% (v/v) *tert*-butanol shows a clear charge transfer from tyrosine to the tryptophan radical. In this medium $Trp^{\bullet}$ radical has transient absorption maximum at 460 nm unlike at 510 nm in dilute aqueous solution. The rate constants for the reaction of $CCl_3O_2^{\bullet}$ radical with tryptophan, tyrosine and BSA in this medium are lower than those in dilute aqueous solution but not in proportion to the increase in viscosity. The rate constants for the formation of tryptophan and tyrosine radical of BSA decrease with decrease in polarity of the medium. It can be said that charge transfer takes place in physiological environments in those reactions, which is not observed in dilute aqueous solutions. This study suggests that not only kinetic and/or thermodynamic parameters but also three-dimensional structure of the protein molecule affecting the proximity of the donor–acceptor pair is also a governing parameter in charge transfer.

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