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A kinetic study on the interaction of deprotonated purine radical cations with amino acids and model peptides

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Abstract

By use of pulse radiolysis techniques, the radical cations of purine nucleotides have been successfully produced by the $SO_4^{\cdot-}$ ion oxidation. Time-resolved spectroscopic evidence is provided that the one-electron-oxidized radicals of dAMP and dGMP can be efficiently repaired by aromatic amino acids (including tyrosine and tryptophan) via electron transfer reaction. As a model peptide, Arg-Tyr-AcOH was also investigated with regard to its interaction with deprotonated purine radical cations. The rate constants of the electron transfer reactions were determined to be $(1 \sim 5) \times 10^8$ dm^3 mol^{-1} s^{-1}. These results suggest that the aromatic amino acids in DNA-associated proteins may play some role in electron transfer reactions through DNA.

Keywords: Oxidative damage; Purine radical cation; Electron transfer; Pulse radiolysis

1. Introduction

It is well known that the action of ionizing radiation, UV light [1,2] and a variety of chemical agents [3,4] on DNA can generate radical cations of nucleic acid bases. According to their oxidation potentials [5], purine nucleobases, especially guanine (G), are relatively easier oxidizable than pyrimidine nucleobases. Indeed, a study of electron transfer between monomeric nucleic acid bases and dinucleotides [6] is consistent with these results, and definitive evidence for migration of this oxidative damage to G and/or multiple G sites in models for DNA has been reported [7–10]. When the electron loss centers ultimately end up at G in naked DNA, most of them will be converted into 7,8-dihydro-8-oxo-2′-deoxyguanosine (8-OxoG) [11], a promutagenic lesion in DNA.

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Having relatively higher one-electron redox potential than G, significantly fewer oxidative lesions are observed at adenine (A). However, in some cases, 7,8-dihydro-8-oxo-2′-deoxyadenosine (8-OxoA), a major decomposition product from an A radical cation, has been found [2,12], which means that A might be a secondary sink for positive hole transfer in DNA.

However, in eukaryotic cells nuclear DNA is associated with proteins (mainly histone), this gives the possibility that reactions occurring in naked DNA may be different from those in vivo. The migration of radical anions generated in histone to DNA has been demonstrated using electron spin resonance (ESR) [13]. From the data reported, the one-electron oxidation potential of A and G nucleosides at pH 7.0 (1.42 and 1.29 V vs. NHE, respectively [5]) is higher than that of some amino acids (Tyr 0.94, Trp 1.05 V vs. NHE [14,15]), which means that the migration of the electron loss centers in DNA to a DNA-associated protein is thermodynamically feasible. Indeed recently evidence on the interaction of guanine radical cation in double stranded DNA with DNA-bound tripeptides has been provided by Wegenknecht et al. [15], using the flash-quench technique. In this paper, a systematic study on the reaction of the deprotonated radical cations of both G and A with aromatic some amino acids and model peptides was undertaken.

2. Materials and methods

2.1. Materials

Tyrosine, Tryptophan, dAMP (2′-deoxyadenosine-5′-monophosphate) and dGMP (2′-deoxyguanosine-5′-monophosphate) were all obtained from Sigma and used as received. Arg-Tyr-AcOH (ATA) were commercial samples from Serva Feinbiochemica GmbH & Co. of the highest available grade. *tert*-Butyl alcohol (*t*-BuOH) was distilled before use. Sodium persulfate ($K_2S_2O_8$, analytical grade reagent) was recrystallized from triple-distilled water. All solutions were prepared using triple-distilled water, buffered with phosphate (2×10^{-3} mol dm^{-3}, pH 7.0). All experiments were carried out at room temperature.

2.2. Methods

Pulse radiolytic experiments were conducted using a linear accelerator providing 8 MeV, 8 ns electron pulses. The dosimetry was determined by irradiating 10^{-2} mol dm^{-3} KSCN solution saturated with nitrous oxide, taking $\varepsilon^{\bullet-}_{(SCN)2} = 7600$ dm^3 mol^{-1} cm^{-1} at 480 nm. Detailed descriptions of the pulse radiolysis equipment and experimental conditions have been given elsewhere [16]. In this work, the dose per electron pulse was approximately 10 Gy.

3. Results and discussion

3.1. Characterization of the one-electron oxidized intermediates of Tyr and Trp

Fig. 1 shows the transient absorption spectra after pulse radiolysis of air-saturated aqueous solution containing 0.1 M *t*-BuOH, 20 mM $K_2S_2O_8$ and parent amino acids (0.5 mM). The composition of aqueous solution ensures scavenge of OH radicals by *t*-BuOH and selective generation of the strongly oxidizing radical $SO_4^{\bullet-}$ ($E = 2.5$–3.1

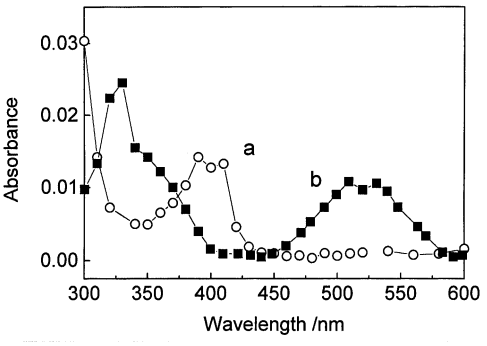


Fig. 1. Transient absorption resulting from the formation of the corresponding radicals at pH 7 by $SO_4^{\bullet-}$-induced oxidation of (a) Tyr, (b) Trp.

V vs. NHE) via the following reaction:

$$e^-_{aq} + S_2O_8^{2-} \rightarrow SO_4^{\cdot -} + SO_4^{2-}$$

The $SO_4^{\cdot -}$ further oxidizes amino acids to give their radical cations at diffusion-controlled rates. Through build-up kinetic analysis of the amino acids radicals at different concentrations (0.1–0.5 mM), the rate constants for Tyr and Trp are determined to be 2.8 and 2.1×10^9 dm^3 mol^{-1} s^{-1}, respectively, similar to the data reported by Redpath and Willson [17]. According to the pK_a given in the literature [18], the radical cations of Tyr and Trp will deprotonate rapidly under our experimental conditions, so the spectra shown in Fig. 1 should be the absorption of their neutral radicals.

3.2. Reaction between one-electron oxidized dGMP radical and amino acids

Fig. 2 shows the transient absorption spectra after pulse radiolysis of a neutral solution containing excess $K_2S_2O_8$, 5 mM dGMP and 0.5 mM Tyr. In its reaction with dGMP, the selectively generated $SO_4^{\cdot -}$ radical has a high preference for interaction with the base part of the molecular rather than with the deoxyribose (phosphate) moiety [19]. On the basis of the reactants concentration in the solution and the rate constant for the reaction of $SO_4^{\cdot -}$ with dGMP (2.3×10^9 dm^3 mol^{-1} s^{-1}) [6], the fraction of primarily generated $SO_4^{\cdot -}$ that reacted with dGMP is calculated to be 0.891. Therefore, the transient spectra recorded at 1 μs after the electron pulse (Fig. 2a) can be predominantly attributed to the absorption of the dGMP radical cation, with little contribution from the phenoxyl radical of Tyr, and this is confirmed by comparison with spectra reported previously [6]. In fact, at pH 7 the radical cation of dGMP (p$K_a = 3.9$) will deprotonate rapidly ($k \approx 3 \times 10^5$ s^{-1}) to give its O-6 centered neutral radical [20–22], which is a strong oxidant. Following the decay of the dGMP radical, a new absorption band with peak at 410 nm appeared. Owing to its identity to the spectra shown in Fig. 1a, it is attributed to the absorption of phenoxyl radical of Tyr, which is produced by one-electron

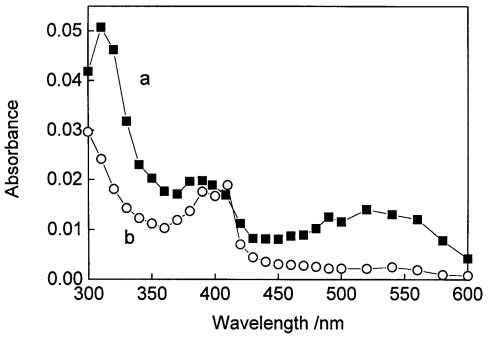


Fig. 2. Transient absorption spectra from pulse radiolysis air-saturated neutral solution containing 0.1 M *t*-BuOH, 50 mM $K_2S_2O_8$, 5 mM dGMP and 0.5 mM Tyr at (a) 1 μs, (b) 35 μs after the pulse.

oxidation of Tyr by the dGMP radical. In the meantime, the dGMP radical was repaired to dGMP after gaining an electron followed by protonation. The mechanism is shown in Scheme 1.

The growth and decay kinetics of transients generated in the solution at certain wavelengths is shown in Fig. 3. The phenoxyl radical of Tyr has no marked absorption at wavelength longer than 420 nm (see Fig. 1), so the trace at 520 nm (curve b) should be the pure decay kinetics of dGMP neutral radical. Differing from this, the trace at 410 nm (curve a) is obviously an overlap of the decay of dGMP neutral radical and the build-up of the phenoxyl radical of Tyr. The ab-

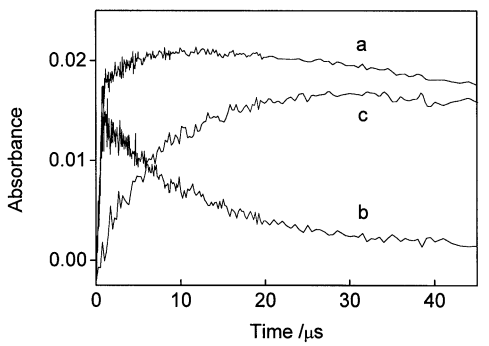
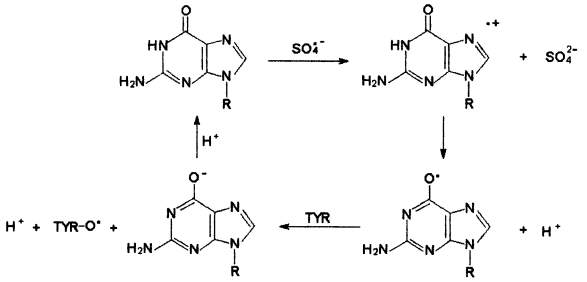


Fig. 3. Traces of the transient intermediates from pulse radiolysis air-saturated neutral solution containing 0.1 M *t*-BuOH, 50 mM $K_2S_2O_8$, 5 mM dGMP and 0.5 mM Tyr at wavelength of (a) 410 nm, (b) 520 nm. (c) growth trace obtained by subtracting trace b from trace a.



Scheme 1. Conversion of the dGMP radical to dGMP by electron transfer from Tyr.

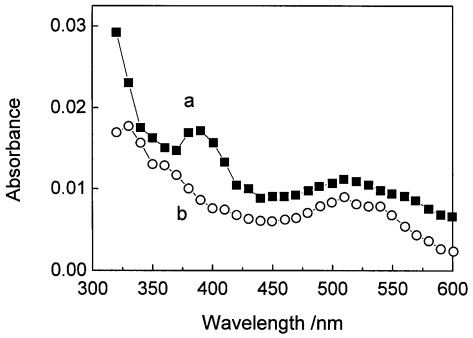


Fig. 4. Transient absorption spectra from pulse radiolysis the neutral solution containing 0.1 M t-BuOH, 50 mM $K_2S_2O_8$, 5 mM dGMP and 0.5 mM Trp at (a) 1 μs, (b) 40 μs after the pulse.

sorbance at wavelength λ (nm) and t (μs) after the pulse is symbolized by A_t^{λ}. According to the subtraction method suggested by Jian et al. [23], we have derived the pure growth trace (curve c) of the phenoxyl radical of Tyr at 410 nm by subtracting the absorbance A_t^{520} at 520 nm multiplied by A_1^{410}/A_1^{520} from A_t^{410} at 410 nm. We can see that the growth occurs exactly in the same time interval as the dGMP radical decay, implying that the dGMP radical is the precursor of the phenoxyl radical of Tyr. By varying the Tyr concentration (0.1–0.5 mM), the rate constant for repair of deprotonated dGMP radical cation by Tyr via electron transfer was determined to be 2.2×10^8 dm^3 mol^{-1} s^{-1}. The transient absorption spectra and the rate constant for Trp in the electron transfer reaction were also obtained (see Fig. 4 and Table 1).

3.3. Reaction between one-electron oxidized dAMP radical and amino acids

The absorption spectra of transient intermediates generated by pulse radiolysis of neutral solutions containing excess $K_2S_2O_8$, 5 mM dAMP and 0.5 mM Tyr are shown in Fig. 5. The spectra at 1 μs after the electron pulse are attributed to the deprotonated radical cation of dAMP in view of the analogy with the literature [6]. At longer times, the characteristic absorption spectra (Fig. 5b) of the Tyr phenoxyl radical appears concurrent with the decay of the dAMP radical. The trace at 410 nm (see the inset), which grows with a first-order rate law, shows the pure build-up of the Tyr phenoxyl radical since the dAMP radical has negligible absorption at this wavelength. By build-up kinetic analysis of the phenoxyl radical at 410 nm at variations of Tyr concentration (0.1–0.5 mM), the rate constant for the repair reaction was determined to be 4.6×10^8 dm^3 mol^{-1} s^{-1}, much faster than for the other amino acids tested (see Table 1).

Table 1
Rate constants (10^8 dm^3 mol^{-1} s^{-1}) of electron transfer from amino acids to the deprotonated radical cations of dGMP (k_G) and dAMP (k_A)

Substrate	Tyr	Trp	ATA
k_G	2.2	1.2	2.1
k_A	4.6	2.5	2.4

3.4. Interaction of purine radicals with model peptides

The addition of ATA enhanced the decay of the deprotonated radical cations of both dGMP and dAMP with rate constants of 2.1 and 2.4×10^8 dm^3 mol^{-1} s^{-1}, similar to free Tyr. Since Tyr is the only component to be easily oxidized in ATA, the absorption spectra appeared after the corresponding purine radical decay, which is almost identical to that observed for the free Tyr, can be assigned to the phenoxyl radical of Tyr in the peptide (see Fig. 6). This indicates that the inter-

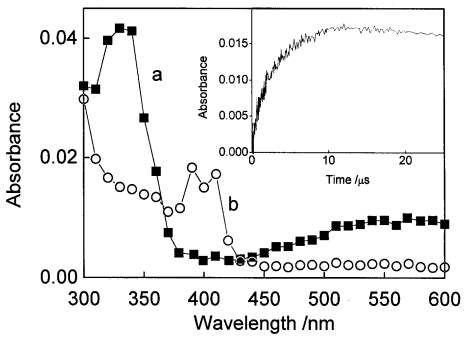


Fig. 5. Transient absorption spectra from pulse radiolysis air-saturated neutral solution containing 0.1 M *t*-BuOH, 50 mM $K_2S_2O_8$, 5 mM dAMP and 0.5 mM Tyr at (a) 1 μs, (b) 12 μs after the pulse. Inset: growth trace at 410 nm.

action of tyrosyl peptides with deprotonated purine radical cations also proceeds via an electron transfer mechanism.

4. Conclusions

The present work provides systematic data on the interaction of aromatic amino acids and model peptides with deprotonated purine radical cations, and the results might shed light on the role of DNA-associated protein in DNA-mediated electron transport. On the other hand, the results indicate that DNA-associated proteins may have some protective effect on DNA oxidative damage, as Jovanovic and Simic [24] explained, although tyrosine radical can also lead to the formation of DNA-protein cross-links [15]. However, our results indicate that DNA-associated protein may be an obstacle on cleaving DNA in cells in controllable ways via an electron transfer mechanism. One focus of this field is the discovery of photonucleases, which offer special advantage as footprinting reagents and for examination of processes such as transcription [25]. Many photonucleases such as substituted anthraquinones [26], naphthalimide derivatives [27] and riboflavin [28] cause cleavage of DNA via electron transfer when irradiated with light. However, the DNA used in these experiments is all naked DNA, not in complex with protein as in cells. So when photonucleases are practically used, there may be less efficiency of DNA cleavage, because the positive 'holes' in DNA primarily generated by them via one-electron oxidation can be transferred, at least partly, to proteins.

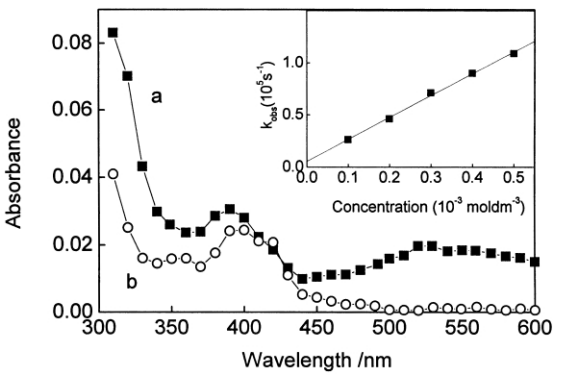


Fig. 6. Transient absorption spectra from pulse radiolysis the neutral solution containing 0.1 M *t*-BuOH, 50 mM $K_2S_2O_8$, 5 mM dGMP and 0.5 mM Ata at (a) 1 μs, (b) 45 μs after the pulse. Inset: plot of the apparent decay rate constant of dGMP radical at 520 nm vs. the concentration of Ata.

It has been recognized that the one-electron oxidation potential of purine bases in a DNA helical array might differ somewhat from the isolated ones. For example, an oxidation potential on the order of 1.10–1.24 V vs. NHE for G in DNA was suggested [4], slightly lower than 1.29 V for the G nucleoside [5]. However, a similar tendency was also observed for amino acids in both peptides [18] and proteins [29], and an oxidation potential of 0.65 V for Trp in protein was suggested, much lower than that for free Trp. These data indicate that electron transfer from proteins to one-electron oxidized DNA is thermodynamically feasible. Therefore, the results we obtained by use of monomeric DNA bases and amino acids should be of significance for understanding the electron transfer reaction in DNA.

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