Direct Observation of $NH_2^\bullet$ Reactions with Oxygen, Amino Acids, and Melanins

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Received: August 9, 2007; In Final Form: November 26, 2007

We report the direct observation of the quenching of the weakly absorbing transient due to the amino radical by oxygen and, hence determine, by a totally direct method, the corresponding rate constant ($k = (1.1 \pm 0.1) \times 10^9$ dm^3 mol^{-1} s^{-1}). We also report the rate constants for the reactions of the amino radical with several amino acids and models of black eumelanin and blond/red phaeomelanin. These reactions lead to a mechanism, based on free radicals, that can explain why ammonia is useful in commercial hair (melanin) bleaching, avoiding excessive amino acid (hair protein) damage.

Introduction

The oxidation of ammonia to amino radicals ($NH_2^\bullet$) via hydroxyl radicals ($OH^\bullet$) is well-known to be of interest both in water-cooled nuclear reactors and atmospheric chemistry. Furthermore, because ammonia is normally used in hair bleaching in the presence of hydrogen peroxide, the formation and fate of $NH_2^\bullet$ may also be of commercial/cosmetic interest. Of course, for *in vivo* hair bleaching, ammonia and hair protein (both present in high concentration) compete for the $OH^\bullet$. In all cases the first step involving ammonia is

$$OH^\bullet + NH_3 \rightarrow H_2O + NH_2^\bullet$$

and the subsequent reaction of $NH_2^\bullet$ with oxygen, amino acids, and melanins are relevant to the balance between hair bleaching and damage.

Early work,[1−4] gave rate constants for the reaction of $NH_2^\bullet$ with oxygen as between 1×10^7 and 3×10^8 dm^3 mol^{-1} s^{-1}.

$$NH_2^\bullet + O_2 \rightarrow NH_2O_2^\bullet$$

More recently, using an indirect method, Laszlo et al.[5] estimated a rate constant of $(1-2) \times 10^9$ dm^3 mol^{-1} s^{-1}. This method involved competition kinetics but did not give linear plots for the rate constant with oxygen concentration, so that there is considerable uncertainty in this important rate constant. Using pulse radiolysis equipment incorporating signal-averaging facilities, we now report a direct measurement of the increased rate of decay of the $NH_2^\bullet$ with increasing oxygen concentrations and, therefore, a non-ambiguous value for this rate constant. We also report directly obtained rate constants for $NH_2^\bullet$ reacting with amino acids, dopa-melanin (DM, a model for naturally occurring black melanin) and cysteinyl-dopa-melanin (CDM, a model for naturally occurring red/blond melanin). These results allow us to speculate on a mechanism for why the presence of ammonia in hair-bleaching formulations leads to melanin bleaching but less (protein) damage than in its absence.

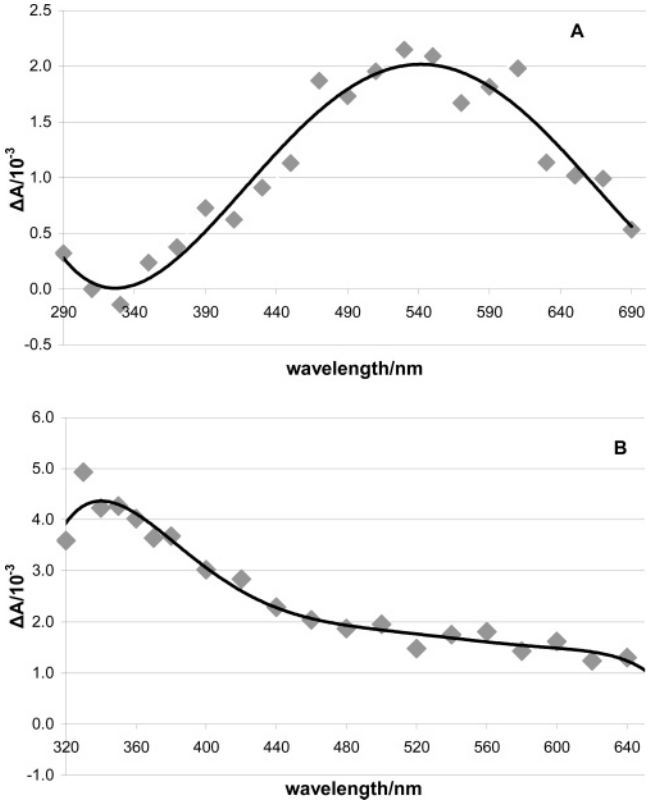

Figure 1. Spectra obtained after pulse radiolysis of (A) 1 mol dm^{-3} ammonia at pH 11.5, N_2O saturated, measured 5 μs after the pulse, and (B) 0.5 mol dm^{-3} ammonia in 20% oxygen + 80% N_2O saturated, measured 4.2 μs after the pulse.

Experimental Methods

The pulse radiolysis equipment at the Free Radical Research Facility, Daresbury, has been described previously[6] (Supporting Information), the pulse doses being typically 20 Gy. The ammonia solution was supplied by Fisher and the mushroom tyrosinase was obtained from Fluka. All the amino acids were

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10.1021/jp076395r CCC: $40.75
Published on Web 01/24/2008

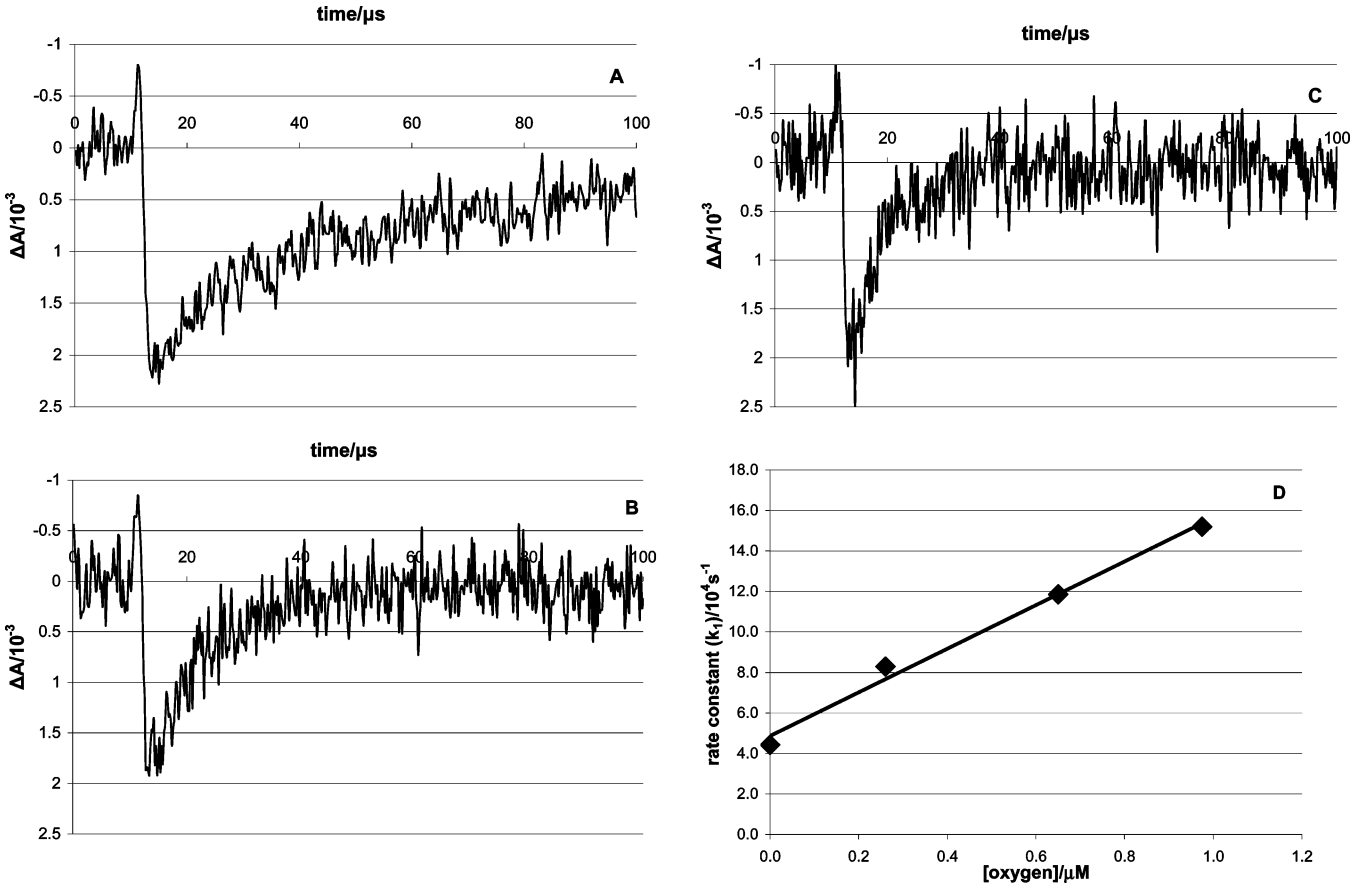

Figure 2. Transients [averaged at least 10 times], generated from pulse radiolysis of 1 mol dm^{-3} ammonia solution, monitored at 530 nm in (A) 100% N_2O (no oxygen), (B) 98% N_2O + 2% oxygen, and (C) 95.2% N_2O + 4.8% oxygen. (D) Stern−Volmer plot of first-order rate constants of the decay at 530 nm after pulse radiolysis of 1 mol dm^{-3} ammonia pH 11.5 with varying oxygen concentrations up to 7.5%. [Each point on the Stern−Volmer plot is the average of at least three distinct experiments.]

purchased from Sigma-Aldrich and all gases were obtained from British Oxygen Company.

The melanin models used were produced "in house"[7,8] (Supporting Information).

Results and Discussion

Reaction of $NH_2^{\bullet}$ with Oxygen. Figure 1a shows the spectrum of $NH_2^{\bullet}$ between 290 and 690 nm with the peak near 530 nm, the accepted λ_{max},[3] and with a molar absorption coefficient around 80 $dm^3\ mol^{-1}\ cm^{-1}$, again consistent with previous results.[3]

The spectrum reported by Laszlo et al.[5] has much more scatter of experimental points but is consistent with our new data. The decay of the $NH_2^{\bullet}$ we observe, in the absence of oxygen, was independent of pH between 7.5 and 11. This decay follows second-order kinetics and corresponds to dimerization of the radical to produce hydrazine.

Figure 2 shows typically the effect of different oxygen concentrations, for 100% N_2O (0% O_2), 98% N_2O:2% O_2, and 95.2% N_2O:4.8% O_2 and a Stern−Volmer plot of the observed rate constant for the decay of $NH_2^{\bullet}$ against oxygen concentration up to 7.5% oxygen. The slope of this plot gives our direct value of $(1.1 \pm 0.1) \times 10^9\ dm^3\ mol^{-1}\ s^{-1}$ for the rate constant of $NH_2^{\bullet}$ reacting with oxygen. Above 7.5% oxygen the line deviates from linearity and the reason for this is now discussed.

Figure 1b gives the spectrum observed corresponding to 80% N_2O plus 20% O_2 measured 4.2 μs after the pulse. This spectrum is consistent with that reported by Giguere and Herman,[9] with λ_{max} around 350 nm, and is assigned to the amino-peroxyl radical $NH_2O_2^{\bullet}$. As can be seen, this shows some absorption at 530 nm, so that under 20% oxygen, it is this tail of the $NH_2O_2^{\bullet}$ that is observed at 530 nm rather than $NH_2^{\bullet}$. Thus, the apparent kinetics of $NH_2^{\bullet}$ reaction with 20% oxygen (and at all oxygen concentrations above 7.5%) are not valid.

Reactions of $NH_2^{\bullet}$ with Amino Acids. We could detect no reaction between $NH_2^{\bullet}$ and glycine or alanine. For glycine the limit for the rate of reaction is $\leq 2 \times 10^6\ dm^3\ mol^{-1}\ s^{-1}$, for alanine the value is likely to be similar but our experiments were restricted to a lower alanine concentration and a limit $\leq 2 \times 10^7\ dm^3\ mol^{-1}\ s^{-1}$. Furthermore, for cysteine, a strong transient due to the dimer radical anion between 360 and 560 nm precluded measurement of the corresponding rate constant. For tryptophan, tyrosine, cystine and histidine the rate constants are between $5 \times 10^6\ dm^3\ mol^{-1}\ s^{-1}$ and about $1 \times 10^7\ dm^3\ mol^{-1}\ s^{-1}$.

For example, Figure 3 shows the transient at 510 nm arising from pulse radiolysis of tryptophan in the presence of 2 mol dm^{-3} ammonia.

The fast formation of the tryptophyl radical $Trp^{\bullet}$ is due to reaction of tryptophan (TrpH) with $OH^{\bullet}$ ($k = 1.3 \times 10^{10}\ dm^3\ mol^{-1}\ s^{-1}$),[10] and the slower reaction is due to $NH_2^{\bullet}$ oxidation of tryptophan ($k = 7 \times 10^6\ dm^3\ mol^{-1}\ s^{-1}$). However, an alternative explanation could be that there are two routes to $Trp^{\bullet}$ from $OH^{\bullet}$, a direct electron transfer (fast)

$$TrpH + OH^{\bullet} \rightarrow Trp^{\bullet} + OH^- + H^+$$

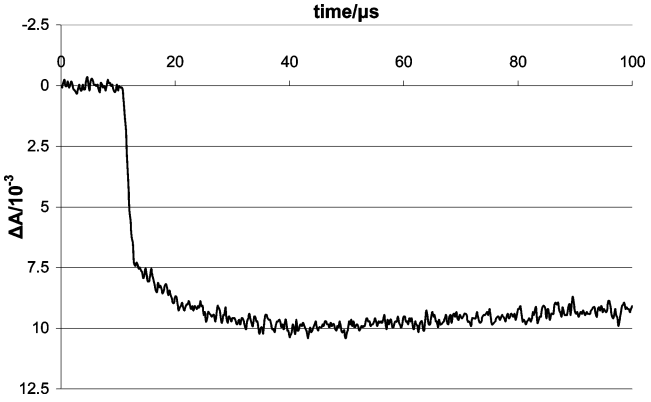

Figure 3. Transient arising from pulse radiolysis of [10^{-2} mol dm^{-3}] tryptophan in 2 mol dm^{-3} ammonia adjusted to pH 11.0 (via HCl) monitored at 510 nm.

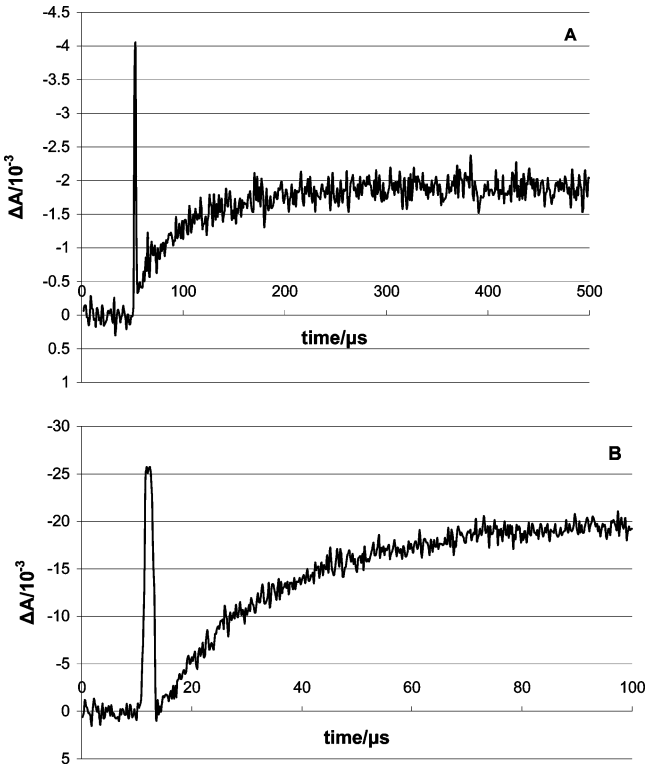

Figure 4. Transients generated from pulse radiolysis of (A) 4×10^{-4} mol dm^{-3} DM monitored at 360 nm and (B) 2×10^{-4} mol dm^{-3} CDM monitored at 530 nm, with 0.25 mol dm^{-3} ammonia at pH 11.

and a radical addition reaction followed by a water elimination (slow).

$$\mathrm{TrpH} + \mathrm{OH}^{\bullet} \rightarrow \mathrm{TrpH\text{-}OH}^{\bullet}$$

$$\mathrm{TrpH\text{-}OH}^{\bullet} \rightarrow \mathrm{Trp}^{\bullet} + \mathrm{H_2O}$$

The water elimination is known to be base catalyzed for phenols.[11] In the present studies of TrpH in the presence and absence of ammonia similar kinetics at pH 11 and 9.5 were observed, suggesting that a water elimination step is unlikely to be important in this system. The spectrum of the product(s) is a composite spectrum of Trp$^{\bullet}$ (formed from OH$^{\bullet}$ and $NH_2^{\bullet}$) and TrpH-OH$^{\bullet}$. We subtracted the spectrum due to the adduct[10] and obtained a spectrum consistent with that of Trp$^{\bullet}$.

Similar results were obtained with tyrosine and cystine, i.e., a fast and slow absorption change, with the slow step giving rate constants of 8×10^6 and 1×10^7 dm^3 mol^{-1} s^{-1}, respectively, for the reactions with $NH_2^{\bullet}$.

For histidine, unlike the other amino acids, we were able to observe an increased decay of $NH_2^{\bullet}$ at 530 nm, corresponding to a rate constant of approximately 5×10^6 dm^{3}mol^{-1}s^{-1}.

Reactions of $NH_2^{\bullet}$ with Melanin Models. In the presence of DM [2×10^{-3} mol dm^{-3}] (the concentration is based on monomer units with RMM of 150 and on comparison with previous optical absorption spectra[7,12]) the apparent first-order decay rate constant at 530 nm increases from $(3.5-4.5) \times 10^4$ to $\sim 6 \times 10^4$ s^{-1}. This rather small increase corresponds to a quenching rate constant of $\sim 1 \times 10^7$ dm^3 mol^{-1} s^{-1}, i.e., a slow reaction, but of course, this is related to the size and molecular weight of the DM molecule; i.e., the DM is folded so that many of its constituent monomer units are not accessible to the amino and other radicals.

In the presence of $NH_2^{\bullet}$, bleaching of DM (most notable around 350 and 600 nm) was observed. DM bleaching is slow, occurring over ~ 250 μs (as shown in Figure 4), whereas the $NH_2^{\bullet}$ decay is completed in ~ 60 μs. This means the observed bleaching of DM by $NH_2^{\bullet}$ is due to a slow secondary process such as $NH_2^{\bullet}$ + Mel $\rightarrow$ products $\rightarrow$ bleaching, rather than a direct process: $NH_2^{\bullet}$ + Mel $\rightarrow$ bleaching. We are unable to determine the rate of the faster initial process because we cannot monitor the $NH_2^{\bullet}$ in the presence of DM.

For CDM, a relatively large and fast bleaching, probably due to a direct reaction with $NH_2^{\bullet}$ ($k = 1.5 \times 10^8$ dm^3 mol^{-1} s^{-1}) was observed, as shown in Figure 4b.

In contrast to DM, the CDM bleaching is most prominent at 450−550 nm and is not observed around 360 or 600 nm.

Conclusions

We observe an efficient reaction between the amino radical and oxygen to give the amino-peroxyl radical, rather inefficient reactions between the amino radical and several amino acids, and the bleaching of melanin models initiated by the amino radical. These reactions allow us to suggest an important role of ammonia in bleaching dark hair: $NH_2^{\bullet}$ bleaches the hair eumelanin but reacts only very slowly with amino acids and, therefore, only leads to minor hair (protein) damage. In the absence of ammonia the OH$^{\bullet}$ also bleaches black hair eumelanin but, in addition, now reacts very efficiently with protein amino acids leading to significant hair damage. The ammonia bleaching of CDM is markedly faster than that of DM, and this is consistent with other radical reactions reported for these melanin models.[7] However, bleaching mechanisms involving CDM are of little commercial interest.

Acknowledgment. We thank Drs. Balzer and Laucher of Wella, Darmstadt, Dr. Javet of Cosmital, Switzerland (Proctor and Gamble Plc), and Dr. J. Marsh (Proctor and Gamble Plc, USA), for useful discussions and the STFC Daresbury Laboratory for access to the Free Radical Research Facility (Pulse radiolysis, Station 0.1). We thank Wella (Proctor and Gamble Plc) for financial support.

Supporting Information Available: Description of the pulse radiolysis equipment at Daresbury and of the synthesis of the melanin models, DM and CDM. This material is available free of charge via the Internet at http://pubs.acs.org.

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