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The Carbonate Radical: Its Reactivity with Oxygen, Ammonia, Amino Acids, and Melanins

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The carbonate radical ($CO_3^{\bullet-}$) is of importance in biology and chemistry. We used pulse radiolysis to generate the $CO_3^{\bullet-}$ radical and show there is no reaction with oxygen. However, in the presence of ammonia the $CO_3^{\bullet-}$ radical is removed by $NO^{\bullet}$, which itself arises from the scavenging of $NH_2^{\bullet}$ by oxygen, and the mechanism of this process is reported. The $CO_3^{\bullet-}$ radical shows complex decay patterns in the presence of ammonia, which can be understood as a balance between the radical−radical reaction $CO_3^{\bullet-} + CO_3^{\bullet-}$ and $CO_3^{\bullet-} + NH_2^{\bullet}$ (the amino radical). Also, we report reactivity with glycine and alanine and with melanin models. The $CO_3^{\bullet-}$ reacts with both dopa-melanin (DM, a model of black eumelanin) and with cysteinyl-dopa-melanin (CDM, a model of red/blond phaeomelanin). However, the reaction rate constant is much higher with CDM than with DM.

Introduction

The carbonate radical ($CO_3^{\bullet-}$) is of increasing interest in biology and chemistry with early work, on its reactivity with amino acids,[1−3] being presented more than 30 years ago. For many years, it was assumed that this radical normally exists as the protonated form ($HCO_3^{\bullet}$), but it is now firmly established that above a pH of zero the radical exists in the deprotonated state.[4] Using the pulse radiolysis technique, we report an unusual reactivity of $CO_3^{\bullet-}$ with oxygen in the presence of ammonia but not in its absence; a mechanism is proposed involving nitric oxide ($NO^{\bullet}$) formation and efficient reactivity of $NO^{\bullet}$ with $CO_3^{\bullet-}$. We also investigated the interaction of ammonia with $CO_3^{\bullet-}$ in the absence of oxygen. The reactions of $CO_3^{\bullet-}$ with tryptophan and tyrosine were studied previously with rather high rate constants, between 10^8 and 10^9 dm^3 mol^{-1} s^{-1}, reported in refs 1 and 2. Previous studies of glycine and alanine suggested no detectable reaction between $CO_3^{\bullet-}$ and these simple amino acids.[2] However, we find there are detectable reactions between the carbonate radical and glycine and alanine.

Experimental Methods

The pulse radiolysis equipment at the Free Radical Research Facility, Daresbury, was described previously,[5] the pulse doses being typically 20 Gy. The sodium bicarbonate and the ammonia solution were supplied by Fisher, the mushroom tyrosinase was obtained from Fluka, and the amino acids were obtained from Sigma. The nitric oxide gas was from Aldrich, and all other gases were obtained from British Oxygen Company.

The melanin models used were synthesized “in house”.[6,7]

Results and Discussion

The difference absorption spectrum we obtained for the $CO_3^{\bullet-}$ by pulse radiolysis of aqueous sodium bicarbonate showed a λ_{max} at 600 nm and was comparable to the well-established literature spectrum. Also, in agreement with previous work, there was no effect of pH in the range 8.2−11. The decay of the radical followed predominantly second-order kinetics with a composite rate constant of $2k = 1.7 \times 10^7 \pm 0.3 \times 10^7$ dm^3 mol^{-1} s^{-1}, also in agreement with previously reported work.[2]

Effect of Oxygen on the Carbonate Radical in the Absence and Presence of Ammonia. The kinetics of the carbonate radical decay are not simple, and any effect of oxygen is very small with the second-order rate constant for $CO_3^{\bullet-} + O_2 \leq 1 \times 10^6$ dm^3 mol^{-1} s^{-1}. This is consistent with the claim that there is no reaction between $CO_3^{\bullet-}$ and oxygen.[2] However, we did observe a significant increase in the rate of decay of the carbonate radical in the presence of ammonia and oxygen compared to ammonia alone as shown in Figure 1.

As can be seen, the first half-life of $CO_3^{\bullet-}$ reduces from about 35 to 18 μs in the presence of ammonia and oxygen (i.e., the decay rate is approximately doubled when both ammonia and oxygen are present).

We propose the following series of reactions to explain this observation:

$$OH^{\bullet} + HCO_3^{-}/CO_3^{2-} \rightarrow CO_3^{\bullet-} + H_2O/OH^{-} \quad (1)$$

$$OH^{\bullet} + NH_3 \rightarrow NH_2^{\bullet} + H_2O \quad (2)$$

$$NH_2^{\bullet} + O_2 \rightarrow NH_2O_2^{\bullet} \quad (3)$$

$$NH_2O_2^{\bullet} \rightarrow NO^{\bullet} + H_2O \quad (4)$$

$$NO^{\bullet} + CO_3^{\bullet-} \rightarrow \text{Products (maybe } NO_2^{-} + CO_2) \quad (5)$$

with, of course, the last of these reactions accounting for the reduced lifetime of the $CO_3^{\bullet-}$ radical.

Equations 1−3 are well-known, and eq 4 was previously discussed.[8,9] In an attempt to confirm this mechanism, we repeated a study of eq 5 reported by Czapski et al.[10] The rate of decay of the $CO_3^{\bullet-}$ radical in the absence of nitric oxide and in the presence of increasing concentrations of nitric oxide was measured. The results obtained are shown in Figure 2. The slope

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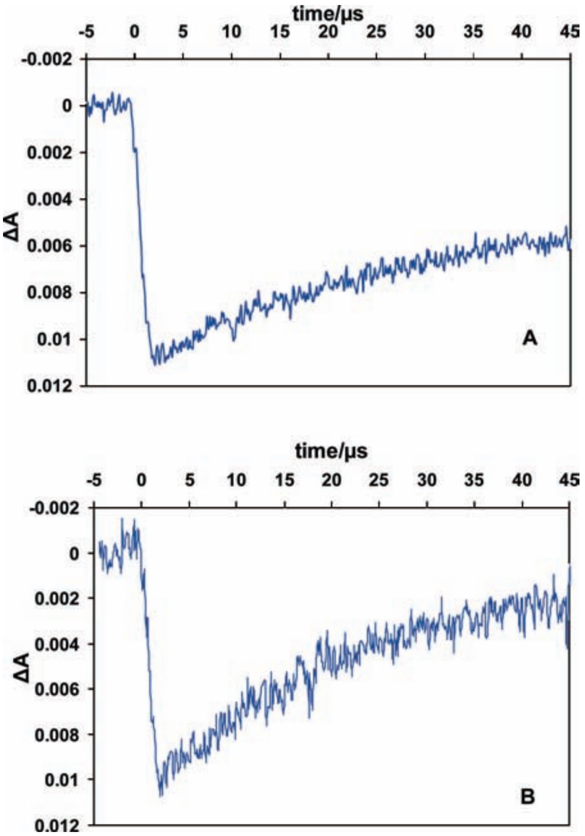

Figure 1. Transients arising from pulse radiolysis of 2 mol dm^{-3} ammonia and 0.1 mol dm^{-3} $NaHCO_3$ in the absence (A) and presence (B) of 20% oxygen, in N_2O, monitored at 600 nm, pH 11.2.

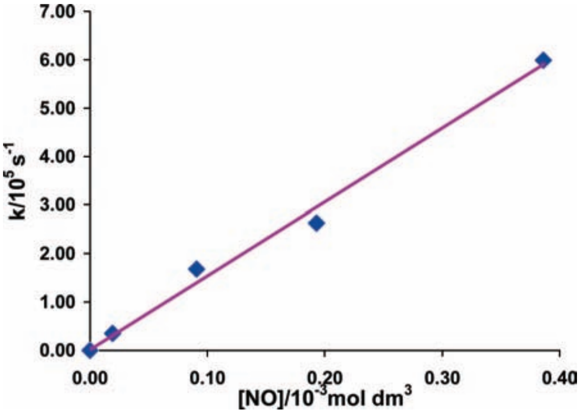

Figure 2. Rate constant for the decay of the carbonate radical as a function of NO concentration at pH 8.2.

of this plot gave a second-order rate constant of 1.6 × 10^9 ± 0.2 dm^3 mol^{-1} s^{-1}. This fast reaction is consistent with the proposed mechanism for the increased decay rate of the $CO_3^{\bullet-}$ radical in the presence of ammonia and oxygen. However, our rate constant is about half that reported previously.[10] We studied the reaction over a somewhat wider NO concentration (100−400 μM, compared to 100−300 μM[10]). It is not easy to understand this difference since the methods used are the same: if we only include our data at lower NO concentrations we do observe a slightly higher rate constant (about 2 × 10^9 dm^3 mol^{-1} s^{-1}).

Interaction between Carbonate Radical and Ammonia. No study of the effect of ammonia on the carbonate radical has been previously reported. Figure 3A−H shows transient decays of $CO_3^{\bullet-}$ radical (λ_{max} 600 nm) with ammonia concentrations from 0 to 2.0 mol dm^{-3} at pH 11.

As can be seen, when the concentration of ammonia is varied, the decay of $CO_3^{\bullet-}$ markedly changes. At the highest ammonia concentration (2 mol dm^{-3}; see Figure 3H), the decay follows good second-order kinetics (confirmed by lowering the radiation dose that increased the half-life ($t_{1/2}$) as expected for second-order kinetics). At lower ammonia concentrations, the decays are more complex than simple second-order kinetics.

It can also be seen that the initial transient (carbonate radical absorption) decreases as the ammonia concentration increases (e.g., compare panels A and G of Figure 3) where the initial transient is reduced by a factor of 2. This corresponds to half of the hydroxyl radicals being scavenged by the carbonate and the other half by ammonia in Figure 3G (note that the transient due to the amino radical itself cannot be seen because its molar absorption coefficient is so small [80 dm^3 mol^{-1} cm^{-1}][11]).

The interpretation of the $CO_3^{\bullet-}$ transient decays in the presence of ammonia is complex. At least three processes must be considered even though it is only possible to monitor the transient due to $CO_3^{\bullet-}$:

$$CO_3^{\bullet-} + CO_3^{\bullet-} \rightarrow \text{Products} \quad (6)$$

$$NH_2^{\bullet} + CO_3^{\bullet-} \rightarrow NH_2O^{-} + CO_2 \quad (7)$$

$$NH_2^{\bullet} + NH_2^{\bullet} \rightarrow \text{Products (e.g., } N_2H_4) \quad (8)$$

When there is no ammonia present, only eq 6 applies. As the ammonia concentration increases, first eq 7 and then eq 8 become increasingly important. Indeed, at a sufficiently high ammonia concentration (not reached) no carbonate radical transient would be seen and, because the ammonia radical molar absorption coefficient is so small, virtually no transient at all.

Using Figure 3G, where approximately half of the $OH^{\bullet}$ reacts with bicarbonate and half with ammonia, for the optimum contribution of eq 7, we obtained a second-order rate constant for eq 7 of 8 × 10^9 dm^3 mol^{-1} s^{-1}. A previous estimate by Draganić et al.,[12] involving 5 × 10^{-3} mol dm^{-3} ammonium bicarbonate at pH 7.8, gave a somewhat smaller rate constant of 1.5 × 10^9 dm^3 mol^{-1} s^{-1}.

We used Chemical Kinetics Simulator (version 1.01, IBM 1996) for this scheme to obtain the best fit for the experimental carbonate radical decay at various ammonia concentrations. Certainly, the corresponding rate constant for eq 7 lies in the range 5−10 × 10^9 dm^3 mol^{-1} s^{-1}. There is a range of rate constants for eq 6 in the literature, and using a value of 2 × 10^7 dm^3 mol^{-1} s^{-1} leads to a value of 8 × 10^9 dm^3 mol^{-1} s^{-1} for eq 7, virtually the same as given above.

Overall, the simplest explanation of the effect of ammonia on the $CO_3^{\bullet-}$ transient decay (Figure 3B−G) is that after the initial spike the transient shows the second-order decay of the carbonate radical alone after all of the $NH_2^{\bullet}$ radical has reacted. At earlier times, the spike is reflecting eq 7, which gets more significant as the ammonia concentration increases.

Reactions of the Carbonate Radical with Amino Acids. The reactions of the carbonate radical were studied with two amino acids, namely glycine and alanine. Despite literature claims of no reaction (≤10^3 mol dm^{-3}),[2] we find that a significant reaction does arise.

Figure 4 gives the decay of the $CO_3^{\bullet-}$ radical at glycine concentrations of 0, 3 × 10^{-3}, and 10^{-2} mol dm^{-3}.

Clearly, there is an increased decay of $CO_3^{\bullet-}$ at the two concentrations of glycine studied, corresponding to a mean rate constant of 3.2 × 10^4 dm^3 mol^{-1} s^{-1}. A similar rate constant was obtained at pH 7.5 and a higher value at pH 11 (1.3 ± 0.3 × 10^5 dm^3 mol^{-1} s^{-1}).

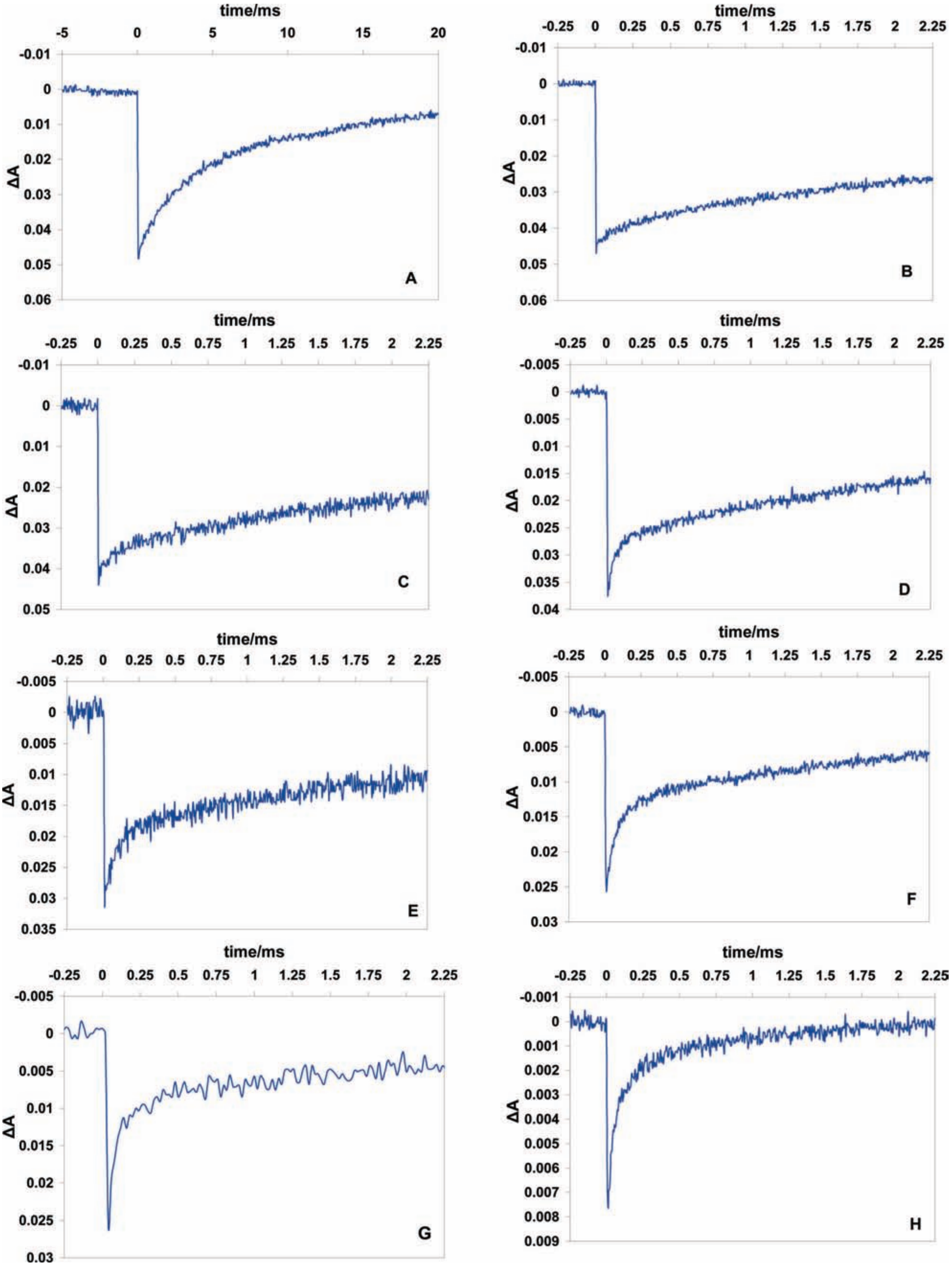

Figure 3. Transients arising from pulse radiolysis of N_2O-saturated aqueous 0.1 mol dm^{-3} sodium bicarbonate in the absence and presence of ammonia at pH 11. (A) 0, (B) 0.05, (C) 0.1, (D) 0.2, (E) 0.4, (F) 0.5, (G) 0.6, and (H) 2 mol dm^{-3} ammonia.

For alanine, rather similar results were obtained at pH 8.2 and 11 but with a somewhat lower rate constant (2−3 times slower) at pH 7.5. Overall, our results show an unexpected reactivity of glycine and alanine with the $CO_3^{\bullet-}$ radical near neutral pH and a further increase in reactivity for the anionic form of the amino acid present at pH 11.

For both these amino acids, we considered other reactions that could lead to the rather low rate constants observed. In

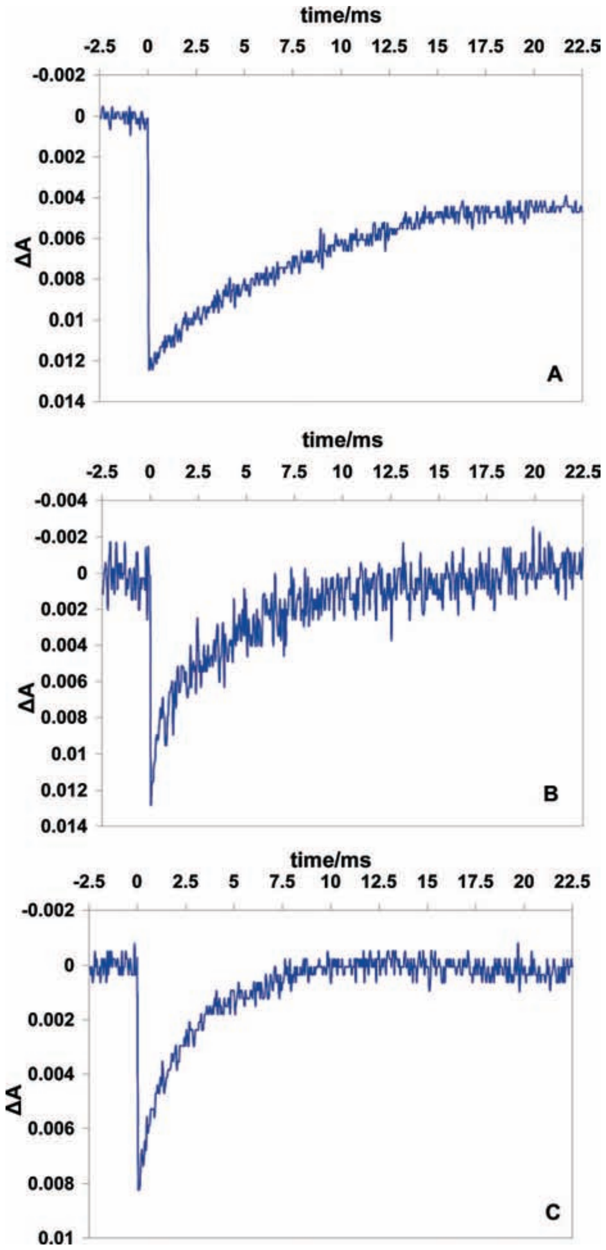

Figure 4. Decay of the carbonate radical from 0.5 mol dm^{-3} sodium bicarbonate at [glycine] = 0 (A), 3 × 10^{-3} (B), and 10 × 10^{-3} (C) mol dm^{-3} using low radiation doses, N_2O saturated, pH 8.2, monitored at 600 nm.

particular, can the products of the amino acid and hydroxyl radical react with the carbonate radical to give the observed effects? However, this is most unlikely taking into account both the concentrations used (0.5 mol dm^{-3} carbonate and 3−10 × 10^{-3} mol dm^{-3} amino acid) and rate constants for the reaction of these amino acids and carbonate with the hydroxyl radical.

Reactions of the Carbonate Radical with Synthetic Melanins. Figure 5 shows the decay of the carbonate radical transient monitored at 600 nm alone, in the presence of 9.4 × 10^{-4} mol dm^{-3} dopa-melanin (DM) and 9.6 × 10^{-6} mol dm^{-3} cysteinyldopa-melanin (CDM). These concentrations of synthetic melanins are based on a unit molecular weight of 150[6]. The corresponding second-order rate constants are approximately 3 × 10^{5} dm^{3} mol^{-1} s^{-1} for DM and 2 × 10^{8} dm^{3} mol^{-1} s^{-1} for CDM.

The increased reactivity of CDM with radicals compared to that of DM has been observed previously for the amino

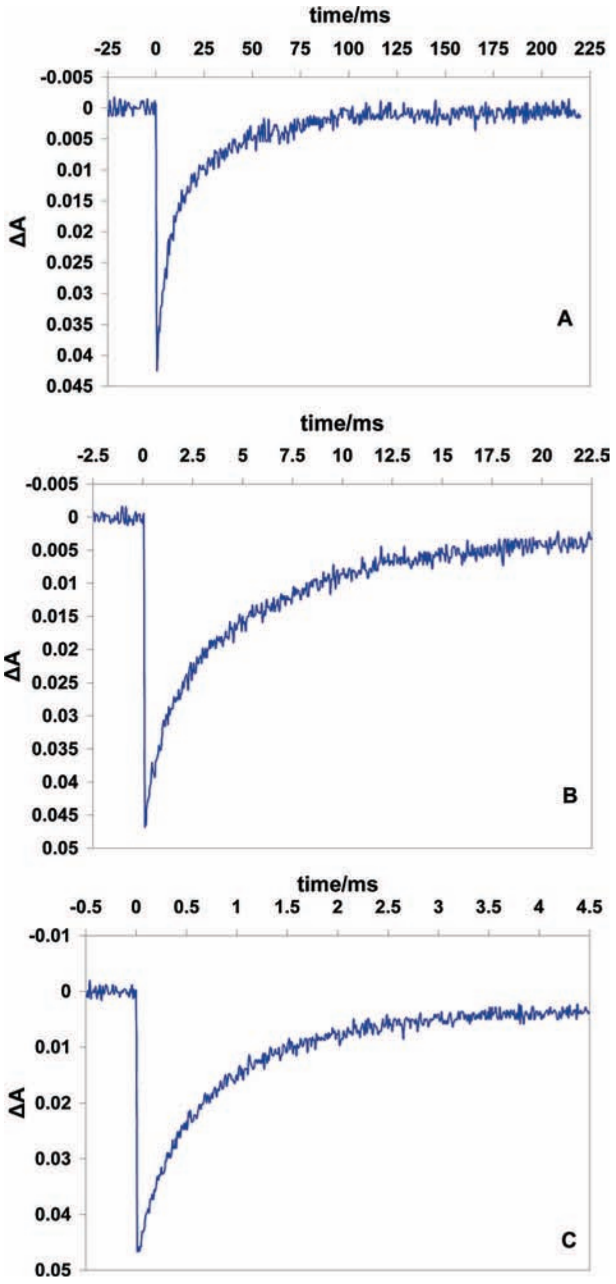

Figure 5. Decay of transient from pulse radiolysis of 0.1 mol dm^{-3} sodium bicarbonate at 600 nm due to the $CO_3^{\bullet -}$ radical (at pH 10). (A) Alone. (B) In the presence of 9.4 × 10^{-4} mol dm^{-3} DM. (C) In the presence of 9.6 × 10^{-6} mol dm^{-3} CDM.

radical.[13] However, the difference between DM and CDM is much more marked for the carbonate radical than the amino radical.[13]

Conclusions

Radiation processes involving bicarbonate[12] may be relevant to the economic and environmental fixation of carbon and to the origins of biologically significant compounds on the primeval earth. Such processes involve the carbonate radical, which is a particularly strong oxidizing agent. We report, for the first time, reactivity with glycine, alanine, and melanin models. Also, we report an unexpected increase in the rate of decay of the carbonate radical in the presence of ammonia and oxygen but not in the presence of only oxygen. We relate this to the formation of nitric oxide via the aminoperoxyl radical.

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