Spectroscopic and Kinetic Properties of the Radical Zwitterion and Related Intermediates in the One-Electron Oxidation of *p*-Aminobenzoic Acid†

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Abstract: The time-resolved resonance Raman spectra and acid−base properties of the transient prepared on the submicrosecond time scale by $N_3^•$ oxidation of aqueous *p*-aminobenzoic acid (PABA) in near-neutral solutions identify it as the charge-neutral 4-^-O_2C-aniline$^{•+}$ radical. This zwitterion intermediate undergoes slow thermal dissociation ($k \sim 2.4 \times 10^3$ s^{-1}) by intramolecular electron transfer and CO_2 elimination. It reacts with common bases, such as OH^-, N_3^-, and HPO_4^{2-} ions, at the rate constants of 1.9×10^{10}, 2.7×10^6, and 2.2×10^8 M^{-1} s^{-1}, respectively, and converts into the nondissociative anilino radical form (pK_a 6.7). In the $^•OH$ oxidation, formation of the zwitterion radical occurs via OH adducts (hydroxycyclohexadienyl radicals) of *p*-aminobenzoate anion, at a rate of 1.4×10^5 s^{-1} which is independent of the base concentration. In strongly acidic solutions the OH adduct of *p*-aminobenzoic acid reacts with H^+, at a rate constant of 4.7×10^8 M^{-1} s^{-1}, to form the 4-HO_2C-aniline cation radical (pK_a 1.1). The benzidine cation radical, observed as one of the transient secondary products in Raman experiments, results from the reactions of *p*-aminophenyl radical produced on dissociation of the 4-^-O_2C-aniline$^{•+}$ radical. The marked pH dependence of the nature and yields of the photoproducts of *p*-aminobenzoic acid, observed in several recent studies, is explained in terms of the dissociative properties of the zwitterion radical and its base-catalyzed conversion into the anilino radical. A two-state model has been developed for the explanation of the intramolecular electron transfer and bond dissociation in the radical intermediates with structurally stable ground electronic state.

Introduction

p-Aminobenzoic acid (PABA) is widely distributed in plant and animal cells in free and bound forms, and is recognized for its medicinal values.[1,2] It plays an important physiological role as metabolic inhibitor and has been accorded vitamin status restricted to microorganisms where it is utilized in the synthesis of folic acid compounds.[2] In its commercial application as an active ingredient of sunscreens this chemical prevents photocarcinogenesis, but it also increases photosensitivity toward DNA damage in which a role for free radicals has been implicated.[3−8] In the mechanistic studies of molecular oxygen consumption by the reduced form of *p*- hydroxybenzoate hydroxylase, *p*-aminobenzoic acid acts as a substrate that undergoes a hydroxylation reaction in the enzyme,[9] presumably via a free radical path.[10,11] Information on the spectroscopic and kinetic properties of the redox states of unbound *p*-aminobenzoic acid in solution is essential for modeling the intermediate chemical steps in these various processes.

The redox reactions of *p*-aminobenzoic acid are of considerable interest even from a purely chemistry perspective. It is a relatively simple chemical system in which strongly electron-donating ($-NH_2$) and electron-accepting ($-CO_2H$) groups are bound to a single phenyl ring. Therefore, it can be readily oxidized or reduced. This prototype of the aromatic amino acids has been the subject of numerous studies in recent years, mostly focused on stable radiation products and photoproducts, with and without nucleic acid bases in solution, and on transients produced in the hydroxylation reactions in flavoenzymes.[3−9,12−15] In spite of the chemical, biochemical, and photobiological significance of the reaction properties of *p*-aminobenzoic acid, there is very little direct information available on the absorption, kinetic, and structural characteristics of its short-lived radical intermediates in solution. Previously, ESR spectroscopy has

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been used to examine radicals produced on $^{\bullet}$OH oxidation of *p*-aminobenzoic acid in basic aqueous solutions, and detection of 4-carboxyanilino and 2-amino-5-carboxyphenoxyl anion radicals has been reported.[16] In spin-trapping studies of the transient photoproducts in the presence of 2-methyl-2-nitrosopropane, formation of 4-carboxyphenyl radical has also been demonstrated.[17] In the mechanistic formulation of the reaction sequences leading to various products, it is generally assumed that the oxidized radical states of *p*-aminobenzoic acid should exhibit reaction properties analogous to other para-substituted aniline radicals,[18] although the nature of the products, such as benzidine,[12] does not support this assumption. It has been suggested that the oxidation may involve electron transfer from the CO_2^- group,[12] but there has been no experimental evidence to show that it actually happens. It has been recognized previously that the reaction mechanism of the oxidized radical state of *p*-aminobenzoic acid in mildly acidic solutions should be drastically different from that in basic solutions.[4,5,12] However, the molecular properties responsible for this behavior are not understood.

In this paper, one-electron oxidation of *p*-aminobenzoic acid in aqueous solution has been studied by optical absorption and resonance Raman spectroscopy. The latter technique provides structural identification of the transient absorbing species, and is valuable for relating the structure with chemistry. We present here vibrational spectroscopic evidence of a zwitterion radical intermediate which undergoes thermal dissociation to produce benzidine, via intermediary formation of *p*-aminophenyl radical. This dissociative property of the zwitterion radical makes its reactive behavior very different from that of the other structurally similar aniline cation derivatives,[18] with profound chemical and biochemical implications.

Experimental Section

The one-electron oxidation of *p*-aminobenzoic acid (pK_a 2.50 and 4.90)[19] in aqueous solution was achieved by pulse radiolysis. Electron irradiation of oxygen-free water produces e_{aq}^- (2.6), hydroxyl radical (2.7), and H atom (0.6) (numbers in parentheses are *G* values, i.e., yields of radicals per 100 eV of energy absorbed)[20] on the ~100-ns scale. In moderately acidic and basic solutions, the e_{aq}^- is converted into $^{\bullet}$OH radical by saturating aqueous solutions by N_2O.[20] The secondary oxidants $N_3^{\bullet}$ and $Br_2^{\bullet -}$ are prepared by reaction of $^{\bullet}$OH with an excess of NaN_3 and KBr.[21,22] In acidic solutions, the e_{aq}^- reacts with H^+ ($k \sim 2.3 \times 10^{10}$ M^{-1} s^{-1})[20] to form H$^{\bullet}$ atom. Therefore, the subsequent reaction is primarily by $^{\bullet}$OH and H$^{\bullet}$. The $^{\bullet}$OH radical is scavenged by *tert*-butyl alcohol (*t*-BuOH), in order to identify the transient absorption due to the H$^{\bullet}$ reaction only. In basic N_2-saturated solutions containing *t*-BuOH, the e_{aq}^- is the primary reducing species, and is frequently used to prepare electron and hydrogen adduct radicals for transient absorption, ESR, and, more recently, Raman studies.[23−26]

The optical absorption and resonance Raman methods applied here to study transient chemical species produced by pulse radiolysis in solution have been described in detail in several previous publications from this Laboratory.[27−29] Radiolysis by ~8 MeV, ~5 ns electron pulses from a linear accelerator, which produced a radical concentration of ~3 × 10^{-6} M per pulse, was used for optical absorption measurements. In the Raman experiments, 2 MeV, ~100 ns electron pulses, delivered by a Van de Graaff accelerator, at a dose rate sufficient to product ~3 × 10^{-4} M radical concentration per pulse, were applied. In both experiments, a flow system was used to refresh solution between consecutive electron pulses.

Formation of Radical Zwitterion on Oxidation of PABA

It is generally accepted that the $N_3^{\bullet}$ radical reacts with organic molecules by electron transfer. In several recent studies, this radical has been used to prepare para-substituted aniline cation and phenoxyl radicals, to measure their redox potentials.[21,22] In aqueous aniline, conclusive evidence of initial cation radical (pK_a ~ 7) formation by $N_3^{\bullet}$ oxidation, even in basic solutions, has come from Raman spectroscopy.[29−31] In *p*-aminobenzoate anion, electron transfer from two distinct sites, i.e., ring-NH_2 and CO_2^-, has been suggested.[12] In order to visualize the effect of solvation on the oxidation reaction, it would be logical to consider the resulting one-electron-deficient states as a combination of two electronic configurations, i.e., ^-O_2C-aniline$^+$ and O_2C-aniline. The hydration will favor and stabilize the dipolar zwitterion configuration. However, it is difficult to predict the relative contributions of the two configurations to the ground electronic state, based on this qualitative argument. Since much of the chemistry depends on the nature of the oxidation state, it is important to ascertain it by a structure-sensitive method.

Transient Absorption Spectra. The transient absorption observed 2 μs after electron pulse irradiation of an aqueous solution containing 1 mM *p*-aminobenzoic acid (PABA) and 0.1 M NaN_3 at pH 5.2 is shown in Figure 1a. The absorption peaks are located at 445 ($\epsilon_{max} \sim 2.6 \times 10^3$ M^{-1} cm^{-1}) and 425 nm (shoulder peak, $\epsilon_{max} \sim 2.3 \times 10^3$ M^{-1} cm^{-1}). Temporal evolution of this absorption at substrate concentrations of 0.5 to 10 mM was used to determine the reaction rate constant of $N_3^{\bullet}$ radical with PABA, which was found to be 5 (±0.5) × 10^9 M^{-1} s^{-1}. This rate constant shows little variation between pH 6 and 13. Because of strong overlapping absorption due to

$$^-O_2CC_6H_4NH_2 + N_3^{\bullet} \rightarrow (^-O_2CC_6H_4NH_2)^{\bullet +} + N_3^- \quad (1)$$

products, an accurate determination of the transient decay kinetics was difficult. A similar problem was encountered previously in the case of aniline oxidation.[30,31] The insert in Figure 1 depicts a first-order plot for the decay of the transient absorption in the 445-nm region with time, using background absorption as reference. From this plot the decay rate constant for the 445-nm transient at pH ~ 6 was estimated as ~2.4 × 10^3 s^{-1}.

The absorption spectrum in Figure 1a resembles the spectrum of the aniline cation radical,[30−32] except that it is shifted to the

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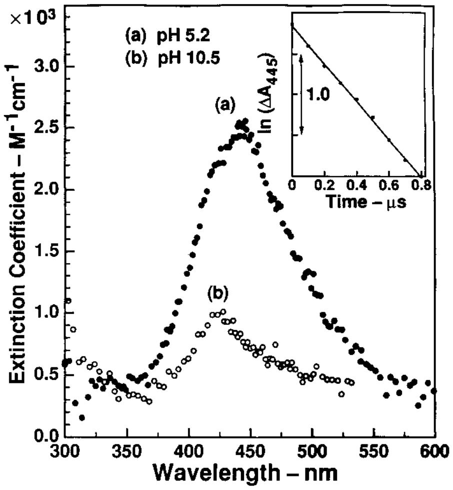

Figure 1. Transient absorption spectra obtained 2 μs after electron pulse irradiation of N_2O-saturated aqueous solution containing 1 mM PABA, 2 mM phosphate buffer, and 0.1 M NaN_3 at pH (a) 5.2 and (b) 10.5. The insert shows exponential decay of the 445-nm absorption peak in Figure 1a with time.

Table 1. Rate Constants for PABA Reactions

reacting species	state of PABA	k ($M^{-1}\ s^{-1}$)[a]	
$N_3^{\bullet}$	$^{-}O_2CC_6H_4NH_2$	5×10^9	
$Br_2^{\bullet -}$	$HO_2CC_6H_4NH_2$	1.3×10^8	
$OH^{\bullet}$	$^{-}O_2CC_6H_4NH_2$	7×10^9	(8.2×10^9)[b] (1.6×10^{10})[c]
$H^{\bullet}$	$HO_2CC_6H_4NH_3^+$	6.7×10^8	
$O^{\bullet -}$	$^{-}O_2CC_6H_4NH_2$	1.7×10^9	
e_{aq}^-	$HO_2CC_6H_4NH_2$	2.8×10^{10}	
e_{aq}^-	$^{-}O_2CC_6H_4NH_2$	1×10^{10}	(2.1×10^9)[d]
OH^-	$^{-}O_2CC_6H_4NH_2^{\bullet +}$	1.9×10^{10}	
N_3^-	$^{-}O_2CC_6H_4NH_2^{\bullet +}$	2.7×10^6	
HPO_4^{2-}	$^{-}O_2CC_6H_4NH_2^{\bullet +}$	2.2×10^8	
H_2O	$^{-}O_2CC_6H_4NH_2^{\bullet +}$	$(2 \times 10^5\ s^{-1})$[e]	
H_2O	$^{-}O_2CC_6H_4NH_2(OH)^{\bullet}$ (OH adduct)	$(1.4 \times 10^5\ s^{-1})$[e]	
H^+	$HO_2CC_6H_4NH_2(OH)$ (OH adduct)	4.7×10^8	

[a] This work. [b] Reference 42. [c] Reference 43. [d] Reference 23. [e] First-order rates.

red by ~15 nm. In basic solutions, the 445-nm transient decays into another species, the spectrum of which is given in Figure 1b. The $Br_2^{\bullet -}$ radical produced by pulse radiolysis of N_2O-saturated aqueous solution containing 0.1 M KBr was also used to oxidize PABA. This reaction was examined only in the pH range 3.5 to 4.7,[21,22] in which the $Br_2^{\bullet -}$ radical reacts with PABA at a rate constant of $1.3 \times 10^8\ M^{-1}\ s^{-1}$. The transient absorption observed 10 μs after the electron pulse was found to be identical to the spectrum displayed in Figure 1a, showing that $N_3^{\bullet}$ oxidation of *p*-aminobenzoate anion and $Br_2^{\bullet -}$ oxidation of *p*-aminobenzoic acid produce the same intermediate. Table 1 summarizes the various rate constants measured in this work.

Resonance Raman Spectra. For structural identification of the reaction intermediates, the Raman spectra of transients produced on pulse radiolytic oxidation of PABA were probed at several excitation wavelengths, between 360 and 470 nm. Raman spectra (600−1800 cm^{-1}) obtained by 440-nm excitation, 0.1 and 10 μs after electron pulse irradiation of a N_2O-saturated solution containing 5 mM PABA, 0.1 M NaN_3, and 2 mM phosphate buffer at pH 5.4, are displayed in Figures 2A and 2B, after proper subtraction of the background Raman signals of the unirradiated solution. The two spectra are quite different and it is clear that they represent two distinct species.

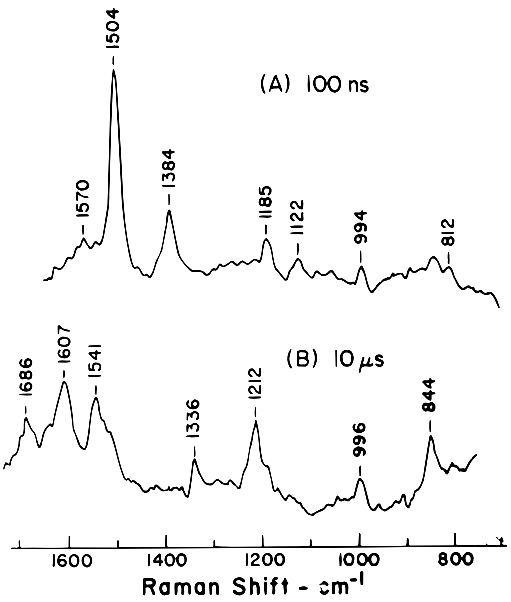

Figure 2. Resonance Raman spectra obtained (A) 0.1 and (B) 10 μs after electron pulse irradiation of N_2O-saturated aqueous solution containing 5 mM PABA, 0.1 M NaN_3, and 2 mM phosphate buffer at pH 5.4. Excitation at 440 nm.

Table 2. Comparison of the Resonance Raman Frequencies (cm^{-1}) and Band Intensities[a] of the Oxidized Radical States of Aniline[b] and PABA[c]

$C_6H_5NH_2^{\bullet +}$	$^{-}O_2C$-$C_6H_4NH_2^{\bullet +}$	$C_6H_5NH^{\bullet}$	$^{-}O_2C$-$C_6H_4NH^{\bullet}$	assignment[d]
1574 w	1570 w	1560 s	1557 m	8a, ring stretch
1494 vs	1504 vs	1505 m	1504 m	7a, C−N stretch
1380 m	1384 m	1452 w	1463 w	19a, ring stretch
		1324 m	1326 m	14, ring stretch
1175 w	1185 m	1167 w		9a, CH bend
	1121 w			13, $C-CO_2$ stretch
1001 m	994 w	1003 m		18a, CH bend
820 w	812 w	817 w		1, ring breathe

[a] Relative intensities in each spectrum: v = very, s = strong, m = medium, w = weak. [b] References 29 and 32. [c] This work. [d] Approximate mode description in terms of Wilson notations.

The frequencies and relative intensities of the transient Raman signals in Figure 2A are compared in Table 2 with those of the aniline cation radical prepared by pulse radiolytic oxidation of aqueous aniline in mildly acidic solutions.[32] The two spectra appear almost indistinguishable, except for the slight shifts in some peak positions. For example, the most intense band at 1504 cm^{-1} in Figure 2A is at 10 cm^{-1} higher frequency than the corresponding 1494-cm^{-1} band in the 432-nm Raman spectrum of the aniline cation radical. A weak band at 1122 cm^{-1} is an additional feature in the spectrum of Figure 2A which is not observed in the aniline cation radical (see Table 2). Whether this band belongs to the same species as the strong band at 1504 cm^{-1} or to some minor reaction product is a question which relates to the transient identification. Therefore, this aspect was carefully examined. First, the spectrum in the combination and overtone region, 1800−3000 cm^{-1}, was recorded. Combination bands were observed at 2875 (1504 + 1384 = 2888), 2689 (1504 + 1185 = 2689), 2625 (1504 + 1122 = 2626), and 2569 (1384 + 1185 = 2569) cm^{-1}. Since the fundamental frequencies of two distinct species cannot combine, the 1122-cm^{-1} frequency must be attributed to the same transient as the other frequencies in the spectrum of Figure 2A. Secondly, the decay kinetics of the 1122-cm^{-1} band was compared with that of the closeby 1384-, 1185-, and 994-cm^{-1} bands in the spectrum. The kinetic monitoring was difficult, as these bands are quite weak and appear in the vicinity of the product bands which are relatively stronger (see Figure 2). The 1384-, 1185-, and 1122-cm^{-1} bands appear to decay at the same time scale. Only the 994-cm^{-1} band grows slightly in intensity with time, as a stronger product band also occurs at about this frequency (996 cm^{-1}).

The transient Raman signals in Figure 2A appear less than 100 ns after the electron pulse, and the spectrum attains its maximum intensity when excitation approaches ~440 nm, relating it to the same transient as the absorption spectrum in Figure 1a. The transient associated with the Raman spectrum in Figure 2A, although structurally very similar to the aniline cation radical, cannot be identified with the latter species because of the minor differences noted above. The additional 1122-cm^{-1} band suggests that the species observed is a substituted aniline cation radical, with little perturbation of the vibrational structure due to this substitution. In strongly acidic solutions the 1122-cm^{-1} frequency shifts downward by 9 cm^{-1}, suggesting protonation of the substituent group. These facts, along with the chemical conditions used in the observation, identify the 445-nm transient with the CO_2^--substituted aniline cation radical. Because of the CO_2^- group, the vibrational modes containing ring-CO_2^- and symmetric CO_2^- stretching motions, in addition to the modes enhanced in the aniline cation spectrum, become likely candidates for resonance enhancement in the Raman spectrum of the intermediate. The 1122-cm^{-1} band is attributable to the ring-CO_2^- stretch. It is clear that the $N_3^\bullet$ oxidation of PABA in aqueous solution involves electron transfer from the aniline moiety.

The resonance Raman spectra of the aniline cation and related radicals,[29-33] and isoelectronic phenoxyl radicals,[34] have been studied previously, and the spectrum in Figure 2A can be interpreted in a straightforward way by comparison (Table 2). The very strong band at 1504 cm^{-1} represents a vibrational mode which primarily involves the $C-NH_2$ stretching motion mixed with ring vibrations. A 10 cm^{-1} higher frequency for this mode than in aniline cation radical may suggest a slight strengthening of the C−N bond due to the CO_2^- substitution, although the effect must be small. A frequency in the region of 1500 cm^{-1} for the C−N stretching mode in aniline cation radicals (~1280 cm^{-1} in aniline)[35] is an indication of a C−N bond which is close to a double bond (bond order >1.5). The loss of an electron from the NH_2 group would leave an unpaired (p) electron on the nitrogen atom to compete for $\pi-\pi$ bonding with the adjacent ring carbon (p) electron, thus strengthening the CN bond. Therefore, the substantial double bond character of the C−N bond in the radical is an indication of the loss of a large fraction of the electron from the NH_2 group of the parent molecule. The frequency of the 1122-cm^{-1} mode, which primarily represents the $C-CO_2^-$ stretching motion, although somewhat lower, is in the range where the stretching frequencies of the C−C single bonds generally occur.[36] This bond clearly does not have any double bond character which would suggest, although indirectly, that the CO_2 plane is perpendicular to the ring plane in the radical structure.

It is evident from the resonance Raman spectrum of the 445-nm transient that the molecular structure of the species is such that the positive charge is mostly on the amine nitrogen and the negative charge is largely confined on the CO_2^- oxygens. In a less polar structure, one would expect the ring-NH_2 stretching frequency to be significantly lower than in aniline cation radical, and not slightly higher as observed. One would also expect the $C-CO_2$ stretching frequency to be much lower than the observed frequency. Thus, the spectroscopic evidence is fairly conclusive that the 445-nm transient is a radical zwitterion.

The zwitterion structures stabilized by hydration are an important feature of the closed-shell amino acids and their free radical states.[2,36-39] In most closed-shell amino acids, the $-CO_2H$ proton transfers to the $-NH_2$ group on hydration, forming the zwitterion structure which represents a lower energy structure. The zwitterion radical state, ^-O_2C-aniline$^+$, established above as the ground electronic state by Raman spectroscopy, can be thought of as a state arising from transfer of an electron from ring-NH_2 to $-CO_2$ group in the higher energy state, O_2C-aniline (hereafter referred to as the neutral state). The solvent reaction field which stabilizes the zwitterion structure may act as a barrier between the two states. Since the neutral radical state is expected to undergo spontaneous dissociation, direct observation of thermal equilibrium between the zwitterion and neutral states is not possible. However, because of this equilibrium, the zwitterion state will appear to dissociate, at a relatively low first-order rate. Thus, we have a very simple but logical two-state model for intramolecular electron transfer leading to bond dissociation which is applicable not only in the case of the oxidized PABA but also for a variety of radicals, including those derived from aliphatic amino acids (this model is discussed in detail in a later section). It should be emphasized that the ground electronic state of these radicals does not have to possess a dissociative bond. Since the solvent reaction field depends on the dielectric constant (ϵ) $\{2(\epsilon-1)/(2\epsilon+1)\}$,[40] the model predicts a faster dissociation rate in the solvents of low polarity, e.g., $CCl_4(\epsilon = 2.3)$, compared to highly polar solvents, e.g., $CH_3CN(\epsilon = 36)$ and water ($\epsilon = 78$). This prediction is readily verified by the decarboxylation rates measured by Ingold and co-workers for the photooxidized benzoic and *p*-methoxybenzoic acids in CCl_4 and CH_3CN,[41] and the rate measured for the radiolytically oxidized *p*-methoxybenzoic acid in water in this work.

Reaction of Zwitterion Radical with Base

The pH dependence of the nature and yields of the oxidation products of PABA[4,5] clearly indicates the critical role of the bases in solution in controlling the chemistry. In aniline cation radical, deprotonation by reaction with OH^- has been investigated,[30] but the role of other bases and water is not well understood. The reactions of the zwitterion radical of PABA with three commonly used bases have been examined. The decay rates of the 445-nm absorption were measured at several concentrations of KOH in solution, and from the observed linear relationship, the rate constant for the reaction of zwitterion radical with OH^- was determined as 1.9×10^{10} M^{-1} s^{-1}. In aniline cation radical, the corresponding rate constant has been

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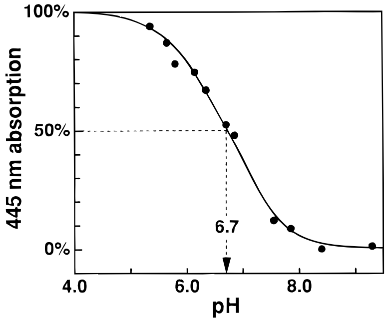

Figure 3. pH dependence of the 445-nm absorption, 20 μs after electron pulse irradiation of N_2O-saturated solution containing 1 mM PABA and 0.1 M NaN_3 (phosphate buffer). The sigmoidal curve corresponds to a simple equilibrium with pK_a = 6.7.

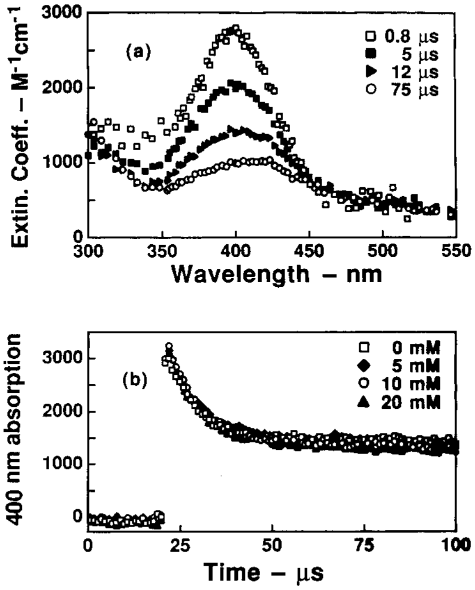

Figure 4. (a) Absorption spectra obtained at different time intervals after electron pulse irradiation of N_2O-saturated solution containing 1 mM PABA and 1 mM phosphate at pH 7.3. (b) Time dependence of the 400-nm absorption in (a) at different phosphate concentrations for N_2O-saturated solution with 10 mM PABA at pH 6.7.

estimated as 2.2×10^{10} M^{-1} s^{-1} by optical absorption, and $\sim 5 \times 10^{10}$ M^{-1} s^{-1} by Raman spectroscopy.[30,31]

The transient formed on reaction of the zwitterion radical with base has a weak absorption at 425 nm (λ_{max}), with ϵ_{max} $\sim 1 \times 10^3$ M^{-1} cm^{-1}, which we attribute to 4-$^{-}O_2C$-anilino radical anion (Figure 1b). The absorption band shape and intensity is similar to that of the anilino radical, but λ_{max} is shifted to the red by 15 to 20 nm. On Raman excitation at 423 nm, 100 ns after the electron pulse, a weakly enhanced Raman spectrum, with bands at 1581, 1557, 1504, 1463, and 1326 cm^{-1}, appears. The 1581-cm^{-1} band gains intensity in slow flow experiments, showing its origin in some radiolytic product accumulated in the Raman cell. Therefore, only the 1557-, 1504-, 1463-, and 1326-cm^{-1} bands can be associated with the 425-nm transient. These frequencies are similar to those reported for the anilino radical[32] (see Table 2). The carboxylate group has a negligible effect on the vibrational structure of the anilino radical, as noted previously for the aniline cation radical. The ESR hyperfine constants of the anilino radical are also not significantly affected by the CO_2^- substitution.[16]

The reactions of the $^{-}O_2C$-aniline$^{\bullet+}$ radical with other bases were examined in near neutral solutions where the OH^- reaction is too slow. From a linear plot of decay rates of the 445-nm absorption at different NaN_3 concentrations, in the presence and absence of phosphate buffer at pH 7.5 and 8.5, the rate constant for the N_3^--catalyzed deprotonation of the zwitterion radical was found to be 2.7×10^6 M^{-1} s^{-1}. The rate of transient formation in the reaction of molecular oxygen with *p*-hydroxybenzoate hydroxylase has been found to depend on the N_3^- concentration.[9] It is likely that the role of azide anion in this complex biochemical system is somewhat similar to its role in aqueous solution, i.e., as a catalyst for proton transfer. This also suggests that the pK_a of the transferable proton is not too high for the N_3^- anion to have perceptible effects on the kinetics.

Phosphate (HPO_4^{2-})-catalyzed deprotonation of the zwitterion radical was examined at pH 8, which gave a rate constant of 2.2×10^8 M^{-1} s^{-1}. There is also an additional decay component ($\sim 2 \times 10^5$ s^{-1}) in the deprotonation reaction which is independent of the substrate concentration. We attribute this rate to the reaction of zwitterion radical with water acting as a weak base.

pK_a of the Zwitterion Radical. As noted above, the 4-$^{-}O_2C$-aniline$^{\bullet+}$ radical undergoes deprotonation in neutral and basic solutions. In the 450-nm region the absorption of the zwitterion radical is very strong compared to that of its conjugate base. Therefore, by monitoring the absorption in this region at different pH, the pK_a of the zwitterion radical can be estimated with reasonable accuracy. Figure 3 shows a titration curve of the 445-nm absorption *vs* pH, which gives a pK_a value of 6.7 (±0.1). The pK_a of the unsubstituted aniline cation radical is reported as 7.05.[30] This shows that the presence of an electron-withdrawing group, such as CO_2^-, at the para position has only a small effect on the proton dissociation constant of the aniline cation radical.

Reaction of $^{\bullet}OH$ Radical with PABA

In addition to the chemical interest in determining the rate constants and understanding the mechanism of $^{\bullet}OH$ radical reactions with PABA, the properties of the transient formed are of relevance to the mechanistic studies of the reaction of oxygen with certain enzymes.[9-11] Since the mode of $^{\bullet}OH$ attack on benzenoid systems is known to be different from that of $N_3^{\bullet}$, comparison can be helpful in determining if electron transfer from the CO_2^- group of PABA is of significance in the oxidation reaction. The reaction of $^{\bullet}OH$ radical with *p*-aminobenzoate anion initially produces a transient absorption with λ_{max} 400 nm and ϵ_{max} $\sim 2.8 \times 10^3$ M^{-1} cm^{-1} (Figure 4). The absorption spectrum of this species is different from the spectra of 4-$^{-}O_2C$-aniline$^{\bullet+}$ and anilino$^{\bullet}$ radicals, depicted in Figure 1. From the growth of the 400-nm absorption the rate constant for the reaction of PABA with $^{\bullet}OH$ was determined as $(7.0 \pm 1) \times 10^9$ M^{-1} s^{-1}. This rate constant is somewhat lower than 8.2×10^9 and 1.6×10^{10} M^{-1} s^{-1}, which were obtained previously by competition studies.[42,43] The $^{\bullet}OH$ radical generally reacts with aromatic molecules by addition.[44-46] We attribute the 400-nm transient to the OH adduct of *p*-aminobenzoate anion. The absorption of this species is shifted to the red by ~35 nm with respect to the OH adduct of *p*-hydroxybenzoate anion ($^{-}O_2CC_6H_4OH$), and toward the blue by ~20 nm with respect to the OH adduct of *p*-oxybenzoate dianion ($^{-}O_2CC_6H_4O^{-}$).[46] These shifts are as expected, as the effect of the NH_2 substitution

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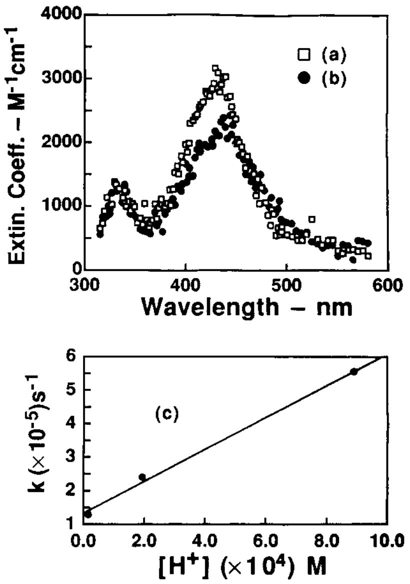

Figure 5. Absorption spectra observed (a) 0.6 and (b) 7.5 μs after pulse irradiating a solution containing 1 mM PABA at pH ~3.5 (N_2O saturated). (c) Dependence of the decay rate of the absorption in (a) on H^+ concentration. The OH adduct of PABA reacts with H^+ at a rate constant of 4.7×10^8 M^{-1} s^{-1}.

on the physicochemical properties of the aromatic systems is generally intermediate between those of the OH and O^- substitutions. The 400 nm transient decays with a rate constant of 1.4×10^5 s^{-1} between pH 7 and 13.5. There is no evidence that the decay is base-catalyzed or that the rate of decay depends on buffer concentration (Figure 4b). Thus, the mechanism of the adduct decay here is quite different from that of the OH adducts of phenols. In basic solutions (pH >8) the product formed is the 4-^-O_2C-anilino$^•$ radical with absorption peak at 425 nm. At pH <8, however, the decay of the 400-nm absorption results in absorption peaks at ~425 and ~450 nm, i.e., both the anilino anion and zwitterion radicals are formed. As can be seen from the 75-μs absorption spectrum in Figure 4a, taken at pH 7.3, the zwitterion absorption at 445 nm is weaker than the absorption of the anilino anion radical at 425 nm, as expected from the acid–base properties of the radicals discussed earlier.

In strongly basic solutions, the $^•$OH radical is converted to $O^{•-}$ in <5 ns (pK_a = 11.9). At pH 13.1, where 94% of the $^•$OH radical converts into $O^{•-}$ in less than 1 ns, the reaction rate constant 1.7×10^9 M^{-1} s^{-1} was found to be approximately three times slower than in the $^•$OH reaction. The transient product seen at 10 μs is very similar in absorption to the ^-O_2C-anilino$^•$ radical formed by $N_3^•$ reaction at pH 11.4. At shorter times, we observe an initial absorption in the 400-nm region which decays at a rate of 1.5×10^5 s^{-1}, i.e., the same as the decay rate of OH adduct in mildly basic solutions, to produce the 425-nm transient. It appears that the $O^{•-}$ reaction is similar in mechanism to the $^•$OH reaction in this system.

In acidic solutions, the OH adduct absorption shifts to higher wavelengths with respect to the absorption observed at pH >7. At pH 3.5, the λ_{max} of the absorption is at 435 nm (ϵ_{max} ~ 3×10^3 M^{-1} cm^{-1}) (Figure 5a). The 435-nm transient is attributed to the OH adduct of the neutral PABA. This transient decays at a rate which depends on H^+ concentration (Figure 5c) from which the H^+-catalyzed decay rate constant was determined as 4.7×10^8 M^{-1} s^{-1}. The transient formed at pH 0.9 by acid-catalyzed loss of water from OH adduct has absorption λ_{max} at 445 nm, and a shoulder at 425 nm, i.e., very similar to the spectrum of the 4-^-O_2C- aniline$^{•+}$ radical in Figure 1a. As discussed later on, this absorption is identified with the 4-HO_2C-aniline cation radical by Raman spectroscopy. In acidic solution, e_{aq}^- is partially converted to H atom, which gives an adduct absorption at 337 nm (ϵ_{max} ~1×10^4 M^{-1} cm^{-1}). The H atom reacts with PABA at pH 0.9, at a rate of 6.7×10^8 M^{-1} s^{-1}.

The 1-μs Raman spectrum obtained by $^•$OH oxidation of PABA at pH ~3 was identical to the spectrum in Figure 2A, although the signal intensity was significantly lower. Since the formation half-period of 4-^-O_2C-aniline$^{•+}$ via H_2O elimination from the OH adduct is ~1.5 μs at this pH, the radical concentration available for detection in Raman experiments is very much reduced due to the loss of the OH adduct and zwitterion radicals by second-order reactions on the microsecond time scale. We do not observe any Raman signals attributable to the adduct radicals, a problem we have encountered in almost every aromatic system. We believe that the adduct radicals generally undergo photodissociation induced by the Raman probe pulse.

Protonation of the Zwitterion Radical. The acid constants, readily measured for the $-NH_2$ proton in aniline radicals by optical absorption, are an important chemical parameter in determining the redox potentials.[21] The measurement of the dissociation constant for the carboxylic proton in PABA radical is not so straightforward. The absorption spectrum of the 4-^-O_2C-aniline$^{•+}$ radical in mildly acidic solutions is not readily distinguishable from the spectrum in strongly acidic solutions. Therefore, protonation of the zwitterion radical is difficult to monitor by optical absorption. Unfortunately, the resonance Raman spectrum also does not change significantly at low pH. This is not unexpected, as carboxyl substitution has very little effect on the aniline cation structure as discussed previously. The only change which is readily noticeable is in the relative intensities of the 1185- and 1122-cm^{-1} bands in the Raman spectra. While the 1185-cm^{-1} band becomes very weak at low pH, the 1122-cm^{-1} band shifts downward to 1113 cm^{-1}. Thus, it is clear that the species observed at very low pH, although structurally similar, is distinct from the species produced in mildly acidic solutions. We attribute the species observed at low pH as 4-HO_2C-aniline cation radical.

The pK_a of the radical was determined by recording the Raman spectra by oxidizing PABA with $N_3^•$ radical at pH 6 (G = 6) (Figure 6a), and by $^•$OH radical at pH 0.5 (G = 2.6) (Figure 6b), and combining them after multiplying by appropriate scaling factors (Figure 6c), in order to simulate the spectrum for the equal concentration of the two radical forms. The composite spectrum was then compared with the spectra recorded at different pH. It was found that the spectrum recorded at pH 1.1 (Figure 6d) matched the composite spectrum (Figure 6c). Since the signals are very weak, this pK_a value should be regarded as approximate, and an error as large as ±0.3 is not unlikely.

Raman Observation of Benzidine Cation Radical as a Transient Product

Because of ~100 times higher radiation dose in Raman experiments than in transient absorption studies, complications may arise due to product formation by second-order reactions on the microsecond time scale. Figure 7 depicts the 440-nm spectra, in the 1250–1700-cm^{-1} region, obtained on electron pulse irradiation of a N_2O-saturated solution containing 5 mM PABA, 0.1 M NaN_3, and 1 mM phosphate buffer at pH 5.5, at different time intervals after the pulse. The spectra are displayed after appropriate subtraction of background Raman signals of unirradiated solution. The spectrum in Figure 7a was obtained

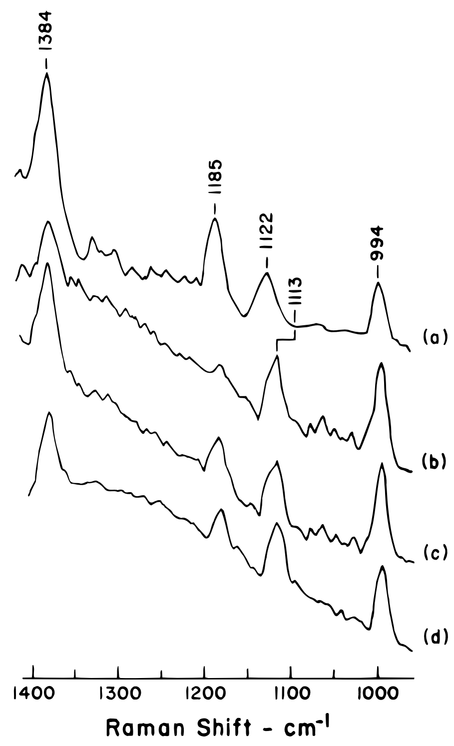

Figure 6. pK_a estimation of the $HO_2CC_6H_4NH_2^{\bullet+}$ radical by Raman spectroscopy. Excitation at 440 nm, 0.1 μs after pulse irradiation of 5 mM PABA solution: (a) 0.1 M NaN_3, 2 mM phosphate added to solution at pH ~6.0, (N_2O saturated); (b) pH ~0.5, N_2 saturated; (c) = (a) + (b); (d) pH ~1.1, N_2-saturated solution. Spectra in (a) and (b) are scaled to reflect equal concentrations of the species.

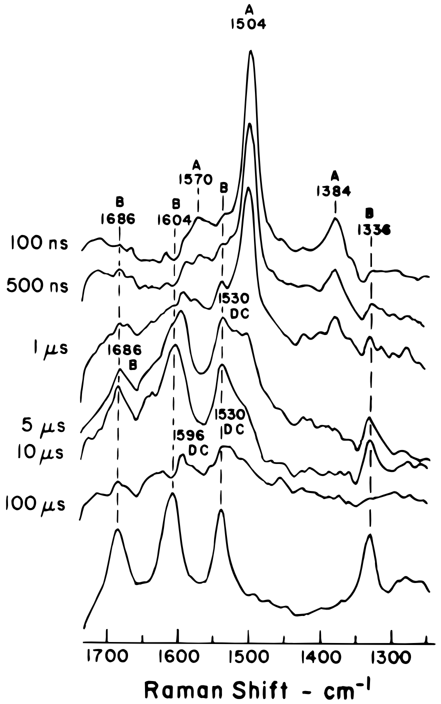

Figure 7. Raman spectra excited at 440 nm and observed (a) 100 ns, (b) 500 ns, (c) 1 μs, (d) 5 μs, (e) 10 μs, and (f) 100 μs after pulse irradiation of a N_2O-saturated 5 mM PABA solution containing 0.1 M NaN_3 at pH ~5.5. (g) Spectrum taken at 1.0 μs after pulse irradiation of a N_2O-saturated aqueous solution of benzidine (<1 mM) and 0.1 M NaN_3 at pH ~5.9.

0.1 μs after the electron pulse (complete spectrum in Figure 2A). The Raman signals of the transient species formed completely by this time are labeled "A". In the spectrum recorded at later times, the intensity of the "A" signals decreases, and signals due to another species labeled "B" start growing. The Raman signals of the transient "B" attain their maximum intensity ~10 μs after the electron pulse (complete spectrum is shown in Figure 2B). In the spectra, taken at longer times, the signals "A" and "B" both decay, and at 100 μs after the electron pulse, both signals become very weak. Experiments performed at very fast flow of the solution through the Raman cell show slight reduction in the intensity of the "B" signals, and complete disappearance of signals marked "D". A signal labeled "C" at ~1500 cm^{-1} can also be seen in the 5-μs spectrum.

The transient "B" spectrum in Figure 7 (and Figure 2B) was compared with the benzidine cation spectrum obtained by $N_3^{\bullet}$ oxidation of benzidine at pH 5.9 (Figure 7g), and also with the published spectrum of the radical.[47] Within experimental error, the Raman spectra of the two species are identical. Therefore, the transient "B" can be assigned to the benzidine cation radical with certainty. It should be noted that the benzidine cation radical has been detected because its absorption is in resonance with the Raman excitation wavelength.[47] It does not necessarily imply that other transient secondary products, with absorption far removed from the excitation wavelength, are not formed in the reaction. In slow flow experiments, we have observed Raman signals of unidentified species (e.g., signals marked "D" in Figure 7) which result from radiolysis of the products formed by previous electron pulses. Benzidine cation radical, observed here, is mostly produced by the reactions initiated by a single electron pulse. It can be seen that the decay of the "A" signals is followed by the growth of "B" signals, establishing the 4-$^{-}O_2C$-aniline$^{\bullet+}$ radical as a precursor of the benzidine cation radical. Signal "C" is attributed to the aniline cation radical.

Mechanism of Benzidine Formation. Benzidine has not been seen as a product of the photolytic oxidation of PABA by every investigator.[4,5,12,13] This is surprising, as most of the proposed reaction schemes start with the 4-$^{-}O_2C$-aniline$^{\bullet+}$ and 4-$^{-}O_2C$-anilino$^{\bullet}$ radicals as the initial transients. An understanding of the mechanism by which benzidine is produced may provide a clue for not only these apparently contradictory observations but also the overall chemistry. We want to look for the reasons why a particular product should or should not be observed under certain chemical conditions. This work confirms that benzidine is indeed a product of PABA oxidation in mildly acidic solutions. Here, we critically examine some reaction mechanisms, proposed previously, for the formation of benzidine in aniline related systems, and suggest improvements based on the experimental data obtained in this work.

Mechanism I: This mechanism is based on a reaction scheme proposed to explain benzidine formation in the photolytic reaction of $B(OH)_3O^{\bullet-}$ radical with PABA.[12] The $N_3^{\bullet}$ radical (see reaction 1) may abstract an electron from the CO_2^{-} group of *p*-aminobenzoate which would result in spontaneous dissociation of CO_2 and formation of *p*-aminophenyl radical. The latter species, by bimolecular reaction, should produce benzidine. Electron transfer from benzidine to 4-$^{-}O_2C$-aniline$^{\bullet+}$, formed by reaction 1, may convert it into the cation radical observed in Raman experiments (reactions 2−4).

$$^{-}O_2CC_6H_4NH_2 + N_3^{\bullet} \rightarrow {}^{\bullet}O_2CC_6H_4NH_2 \rightarrow {}^{\bullet}C_6H_4NH_2 + CO_2 \quad (2)$$

$$^{\bullet}C_6H_4NH_2 + {}^{\bullet}C_6H_4NH_2 \rightarrow (C_6H_4NH_2)_2 \quad (3)$$

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[47] Tripathi, G. N. R.; Schuler, R. H. *Int. J. Radiat. Phys. Chem.* **1988**, *32*, 251.

$$ {}^{-}O_2CC_6H_4NH_2^{\bullet+} + (C_6H_4NH_2)_2 \rightarrow (C_6H_4NH_2)_2^{\bullet+} + {}^{-}O_2CC_6H_4NH_2 \quad (4)$$

The *p*-aminophenyl radical may also react with water to produce aniline which is subsequently oxidized to form its cation radical.

Mechanism II: Bimolecular tail−tail addition of 4-$^{-}O_2C$-aniline$^{\bullet+}$ radicals may lead to coupled product which may undergo intramolecular charge transfer to form benzidine (reaction 5). This mechanism is frequently used to explain benzidine formation in the oxidation of aqueous aniline.[18,29−31,48] However, such a mechanism has been discounted in para-substituted anilines,[48] and in *p*-methoxybenzoic acid it is thought to produce a peroxide (eq 5a), without the step in eq 5b.[41]

$$ {}^{-}O_2CC_6H_4NH_2^{\bullet+} + {}^{-}O_2CC_6H_4NH_2^{\bullet+} \rightarrow ({}^{-}O_2CC_6H_4NH_2^{+})_2 \quad (5a)$$

$$\rightarrow (C_6H_4NH_2)_2 + 2CO_2 \quad (5b)$$

Mechanism III: In this modified version of mechanism I, the 4-$^{-}O_2C$-aniline$^{\bullet+}$ radical is the precursor of the *p*-aminophenyl radical (reaction 6) that produces benzidine by reaction 3.

$$ {}^{-}O_2CC_6H_4NH_2^{\bullet+} \rightarrow {}^{\bullet}O_2CC_6H_4NH_2 \rightarrow {}^{\bullet}C_6H_4NH_2 + CO_2 \quad (6)$$

In a parallel reaction, this phenyl radical abstracts hydrogen from water to produce aniline. Electron transfer from aniline to 4-$^{-}O_2C$-aniline$^{\bullet+}$ forms aniline cation radical which is known to produce benzidine.[18,29−31,48] At high radical concentrations, bimolecular head−tail electron exchange in the 4-$^{-}O_2C$-aniline$^{\bullet+}$ radicals may also produce *p*-aminophenyl radical. The absorption spectrum of this extremely reactive species is not known, but it is certainly not in resonance with the Raman excitation wavelength to make it detectable. The benzidine and aniline formation in this mechanism is essentially a manifestation of the dissociative property of the zwitterion radical. The Raman observation of the benzidine cation radical, depicted in Figure 7, represents a case of high radical concentration. It should also be noted that the absorption of the Raman probe pulse by the zwitterion radical state is likely to increase the yield of the phenyl radical by photochemically populating the neutral radical state.

As stated previously, from a consideration of the nature of the electronic states in the oxidized PABA, that the radicals with zwitterionic ground state are likely to undergo thermal dissociation in solution. The oxidized radical state of *p*-methoxybenzoic acid displays that behavior.[41] The rate of decarboxylation in this radical has been reported as 2×10^5 s^{-1} in CCl_4 and $\leq 2 \times 10^4$ s^{-1} in CH_3CN. The latter rate, although approximate, appears to be qualitatively correct from the trend seen in the first-order decay rates of comparative radicals in the two solvents.[41] We are currently examining the structure and decarboxylation in the zwitterion radical state of *p*-methoxybenzoic acid ($H_3COC_6H_4CO_2^{\bullet}$ in Ingold's designation) in water. The radical is prepared by pulse radiolytic oxidation, as described in ref 49 and the Experimental Section of this paper. Our preliminary results show the transient absorption attributable to the zwitterion radical[41,49] decaying by a pH-independent first-order rate of 1.5 (±0.5) × 10^4 s^{-1}. Because of low radical concentration used in our work second-order reactions, which interfered with the first-order rate determination in CH_3CN by Ingold and co-workers, are of no consequence. From the Onsager expression for the solvent reaction field,[40] which does not include specific solvent interactions like hydrogen bonding, one would generally expect the rate of decarboxylation in water and CH_3CN to be comparable. Hydrogen bonding is essentially a weak electrostatic interaction which would slightly lower the effective negative charge on the carboxylic group, thus reducing the zwitterionic character of the radical structure and weakening the ring−CO_2 bond. Therefore, a slightly higher rate of decarboxylation in water than in CH_3CN is not unexpected. It is interesting to note that the rate of decarboxylation drops by only a factor of $\sim 10^2$, from oxidized benzoic acid radical in CCl_4 to *p*-methoxybenzoic acid radical in water. The NH_2 substitution is known to exert greater stabilizing effect than OCH_3 in radicals, but the difference is far less in comparison to that between H and OCH_3. Therefore, the rate of decarboxylation of the radical zwitterion of *p*-aminobenzoic acid (PABA) in water is expected to be lower than 2×10^4 s^{-1}, but greater than 2×10^2 s^{-1}. Therefore, the first-order decay of PABA zwitterion radical (rate $\sim 2.4 \times 10^3$ s^{-1}), noted earlier, can be safely attributed to decarboxylation. The benzidine, observed as a product in low dose photolysis[12] or pulse radiolysis (this work), is a consequence of the decarboxylation of the PABA radical. It can be shown that benzidine formation does not occur by mechanism I or II.

In mechanism I the $N_3^{\bullet}$ radical undergoes two parallel reactions. Therefore, the radiation yield of 4-$^{-}O_2C$-aniline$^{\bullet+}$, prepared by reaction 1, should be much lower than that of $N_3^{\bullet}$. Comparison of absorbance at 445 nm in the spectra of 4-$^{-}O_2C$-aniline$^{\bullet+}$ radical, prepared by reactions of $N_3^{\bullet}$ with *p*-aminobenzoate anion (Figures 1a) and $^{\bullet}OH$ with *p*-aminobenzoic acid (Figure 5b), shows that the yields are identical, within the experimental uncertainties. Mechanism I is clearly not consistent with these yields which do not change with the state of ionization of the carboxylic group (electron loss from −COOH group cannot occur with the same efficiency as that from the COO^{-} group). It is also inconsistent with the Raman observation of the 4-$^{-}O_2C$-aniline$^{\bullet+}$ radical as a precursor of the benzidine cation radical. Steric hindrance and Coulombic repulsion of the CO_2^{-} groups should greatly reduce the reaction probability at the C(4) site of 4-$^{-}O_2C$-aniline$^{\bullet+}$, in comparison to the other unobstructed reaction sites in the radical. Therefore, benzidine formation in appreciable concentration by mechanism II is improbable.[18] Consistent with this argument, we did not observe 4,4′-biphenoxide monoanion radical (isoelectronic with benzidine cation radical) in the $N_3^{\bullet}$ oxidation of *p*-hydroxybenzoate anion in Raman experiments,[46] although 4,4′-biphenol is a major product in the bimolecular reaction of the unsubstituted phenoxyl radical.[29,48] Similarly, in the Raman studies of the radical−radical reactions of anisole cation radical, 4,4′-dimethoxybiphenyl cation radical appears as a dominant transient product, but not in the reactions of *p*-methylanisole cation radical.[50] In the electrochemical oxidation of para-substituted anilines in aqueous solution, benzidine has not been seen as a product,[18] and in oxidized *p*-methoxybenzoic acid tail−tail addition is thought to produce peroxide,[41] which conforms with these arguments.

Mechanism III explains benzidine formation without involving electron transfer from the CO_2^{-} site of *p*-aminobenzoate to $N_3^{\bullet}$ radical, as in mechanism I, or requiring tail−tail addition of two 4-$^{-}O_2C$-aniline$^{\bullet+}$ radicals, as in mechanism II, both of which are inconsistent with the experimental observations. We

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discuss some interesting chemical consequences of this mechanism in the following section.

Trends in the pH Dependence of the Product Yields

Product formation is the central theme of the recent photochemical and photobiological research on PABA. Several interesting and unexpected results are reported in the literature. Although this study is mainly concerned with the properties of the primary radical intermediates, the mechanism discussed above can be analyzed to make some generalized comments on the pH dependence of the nature and yields of the products observed in photolytic experiments. These photoproducts can be broadly classified into two categories: (1) the products which show negligible yields in near neutral solutions, but their yields increase as the solution becomes more and more basic, and (2) the products which exhibit the opposite trend, i.e., their yields gradually decrease with the increase in the pH, and become negligible at high pH. The products which have been seen by some investigators, but not by others, essentially fall into one of the above two categories. It should be noted that the steady-state photolysis represents a complex chemical situation in which reaction products of primary intermediates can also undergo photolysis and enter into a variety of uncontrolled reactions. We discuss here the behavior expected in the idealized conditions where cross reactions do not occur.

The electronic structures of aniline cation and anilino radicals are qualitatively similar,[32] with C(4), C(2), and amine N sites of high spin population and, therefore, high reactivity toward covalent bonding. As discussed previously, the CO_2H/CO_2^- substitution does not significantly alter the structural properties of the two radicals, except for blocking the C(4) position so that bonding distances are not approached on radical−radical encounter. In the absence of thermally activated dissociation of 4-^-O_2C-aniline$^{\bullet+}$, one would expect addition reactions mainly at the C(2) and N sites of these radicals. The spin population on these atoms and number of equivalent reaction sites (2 for C(2)) should mainly determine the relative yields of products. One does not anticipate a drastic dependence of product yields on pH, or formation of products with relatively high yields by reaction at the C(4) site.[18] These expectations are contrary to the various observations in PABA.

Now let us examine the chemical consequences of mechanism III (reaction 6 in particular) in the formation of products. If the rate of first-order decay of the 4-^-O_2C-aniline$^{\bullet+}$ radical (k) is much faster than the rate of the second-order reactions (i.e., low concentration of radicals produced), PABA radical−radical adducts at the C(2) and N sites will not be observed, as the radical will dissociate prior to reactions at these sites. On the other hand, in highly basic solutions where the zwitterion radical completely converts into its nondissociative anilino anion form, the product yields will reach their optimum values. In the intermediate pH range the yields will be observed to increase with pH. This predicted behavior has been seen recently in the pH-dependence study of the 4-((2′-amino-5′-carboxyphenyl)-amino)benzoic acid and 4-((4′-aminophenyl)amino)benzoic acid (see ref 18 for the formation mechanism of this product) yields in the photolysis of PABA.[4] The yields of these products become negligible at pH between 7 and 8. Considering equilibrium between 4-^-O_2C-aniline$^{\bullet+}$ and its deprotonated anilino$^{\bullet}$ form, attained on the microsecond or faster time scale (see section on reaction rates with base), the decay rate by the dissociative channel is given by fk, where f is the fraction of the zwitterion radical in equilibrium. As f decreases with pH, the dissociative decay rate becomes slower, increasing the yields of the coupled products. At a fairly high pH, when fk becomes negligible in comparison to the initial second-order decay rate, the product yields should reach saturation. Since the second-order decay rate depends on radical concentration, and diminishes with time, a very slow rate of production of PABA radicals can push the pH at which saturation in the product yields is observed to a very high value. On the other hand, when fk is greater than the second-order decay rate (i.e., high f values), the PABA radical−radical adduct yields will be negligible. One can readily estimate fk at any pH from the data on k (2.4×10^3 s^{-1}) and pK_a (6.7) of the zwitterion radical provided in this work.

The adducts formed by the reaction of *p*-aminophenyl radical should generally show a pH dependence complementary to those of PABA radical−radical adducts, i.e., the yields should decrease as the pH of solution increases past the pK_a of the zwitterion radical (6.7). The yields of these products should vanish as the zwitterion radical completely converts into anilino radical at high pH. Benzidine is one such product which displays this behavior. This is also true about the products formed by the reaction of *p*-aminophenyl radical with other chemicals in solution. The yields of the PABA−thymine and PABA−thymidine adducts have been found to decrease with pH, at pH >6.5.[5] However, if the zwitterion radical is produced in low concentration, the *p*-aminophenyl radical is more likely to react directly with thymine and thymidine than with their radicals. At high concentrations of the zwitterion radical, when the second-order reactions are initially more dominant than the first-order decay of the radical, the product yields will still follow the same pattern as discussed above, although the pH dependence of the yields will be relatively less pronounced. The observation of benzidine by some investigators, but not by others, as a photolytic product of PABA probably reflects the differences in the experimental conditions in which the yields were measured. It should be noted that benzidine is also consumed, at least in part, by oxidation and subsequent formation of more complex products.[18] It can be seen that the benzidine cation Raman signals in Figure 7 decay on the 100 μs time scale. In summary, the trends in the photoproduct yields in various experiments can be readily understood if the dissociative behavior of the zwitterion radical is taken into consideration.

Generalization of the Two-State Model for Intramolecular Electron Transfer

Dissociation of the chemical bonds resulting from intramolecular electron transfer has been observed in a number of hydrated radical species produced on one-electron reduction.[51,52] The dissociation rates and chemistry often depend on the pH of the solution. The experimental and theoretical models used for the description of the chemical events in these systems are frequently based on charge reorganization in the ground electronic state. Since the solvent response to the charge distribution in a molecular system is extremely fast ($<10^{-12}$ s), there is no apparent physical stimulus for the electron to walk slowly ($>10^{-6}$ s) from one molecular site to the other to cause dissociation (model in ref 52), while the system remains in the ground electronic state. Also, the theoretical treatment of the electron as a classical particle in this context has conceptual difficulties. A more satisfactory explanation for this interesting chemical phenomenon in various radical systems can be given by the analogy of the oxidized PABA, where two

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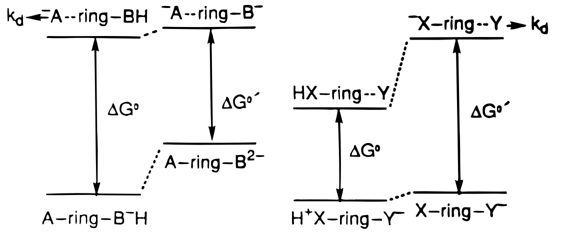
closeby energy states, as discussed earlier, can be readily visualized. To elaborate this point, let us consider k_d as the rate of dissociation of the upper electronic state (US) in a hydrated radical. In the absence of other chemical reactions, the ground state (GS) of the radical will decay by first-order rate (k_{et}) = $k_d \exp(-\Delta G°/RT)$, considering thermal equilibrium between the two states. Here, $\Delta G°$ is the free energy change in the standard state, and k_{et} is the rate ascribed to intramolecular electron transfer (a questionable terminology) in the literature.[51,52] For the sake of numerical illustration, let us assume a value of 10^{12} to 10^{14} s^{-1} for k_d, which is quite reasonable for a dissociative electronic state. For $\Delta G° = 0.42$ ($\pm$10%) to 0.6 ($\pm$10%) eV, k_{et} varies by three orders of magnitude, from $\sim 10^6$ to $\sim 10^3$ s^{-1}. Most of the reported values of k_{et} are in this range. Thus, the observed slow rate of intramolecular electron transfer in a radical can be readily understood in terms of the two-state model. On changing the state of protonation of the radical, the free energy difference changes to $\Delta G°' = \Delta G° + (2.3RT)\Delta pK_a$, $\Delta pK_a = pK_a(US) - pK_a(GS)$, which can drastically affect the magnitude of k_{et}. If ΔpK_a in oxidized PABA is approximated by the pK_a difference between the $-N-H$ and $-N^+-H$ protons in aliphatic amines (i.e., $\sim$25),[53] one estimates k_{et} in the protonated radical to be $\sim 10^{25}$-times faster than in its deprotonated state. Without associating quantitative significance to this factor, used only as an illustration, it can be concluded that decarboxylation is possible only when the oxidized PABA is in its zwitterion radical state, and not in the deprotonated anionic form, contrary to a recent suggestion.[4] On the other hand, in the reduced radical states undergoing intramolecular electron transfer and dissociation, a similar argument leads to $pK_a(GS) \gg pK_a(US)$, and k_{et} several orders of magnitude higher in the proton-ionized state than in the protonated state (if US remains the dissociative state).

The pH dependence of the radical dissociation, in terms of the two-state model, is illustrated in the following diagram. In this diagram, $-BH$ is an electron-accepting group in the reduction of the molecule $A-ring-BH$, and $-XH$ an electron losing group in the oxidation of $HX-ring-Y^-$. In the upper dissociative state of the radicals, the acid property of the $-BH$ or $-XH$ proton is expected to be close to that of the parent molecules. However, in the ground electronic state, the negative charge on $-BH$ will increase the pK_a by several units due to the Coulombic attraction of the proton, and the positive charge on $-XH$ will greatly reduce the pK_a due to the Coulombic repulsion.[53]



Let us examine the effect of protonation of $-A$ on $\Delta G°'$ in the above illustration. In that case, the free energy difference changes to $\Delta G°'' = \Delta G°' - (2.3RT)\Delta pK_a$. If $\Delta G°' < (2.3RT)\Delta pK_a$, the dissociative state, $HA{-}{-}R-B^-$, becomes the ground electronic state (reversal of the state ordering) and spontaneous dissociation of the AH fragment should occur. This special situation arises in aliphatic amino acids where $A = NH_2$ and $B = CO_2$, and ΔpK_a is close to $\sim$25. Thus, $\Delta G°'' = \sim \Delta G°' - 1.5$ eV. It should be noted that the $HA{-}{-}R-B^-$ state is the radical ground state formed by electron addition to the amine group in the $H_3N^+-R-CO_2^-$ structure. Therefore, no intramolecular electron transfer is involved in the radical dissociation. The system reverts to the original state ordering (1.5 eV $\gg \Delta G°' > 0$) when electron is added to the anionic form of the amino acid structures ($H_2N-R-CO_2^-$), and deamination becomes a slower process, consistent with the experimental observations.[38] The ground electronic state is now $H_2N-R-CO_2^{2-}$, i.e., the added electron is mostly on the carboxylic group, and deamination occurs from the upper state, $^-H_2N{-}{-}R-CO_2^-$ (see the diagram). The dissociated fragment NH_2^- should react very fast ($\sim 10^{-11}$ s)[26] with water to form NH_3 ($pK_a \sim 35$).[53] This very simple but probably the only plausible explanation of the intramolecular electron transfer leading to bond dissociation in solvated radicals has been overlooked thus far, to our knowledge. A temperature-dependence study of k_{et} should provide information on kinetic and thermodynamical parameters, such as k_d and $\Delta G°$. Such a study has been undertaken for the oxidized radical states of p-$XC_6H_4CO_2^-$ (X = H, Cl, OCH_3) in CCl_4 by Ingold and co-workers.[41] Their observations fit extremely well with the expectations of the model, with $k_d \sim 10^{12.5}$. The model explains the origin of this Arrhenius parameter and, more importantly, it relates the measured activation energy with the stabilization energy of the ring$-CO_2$ bond in the ground electronic state of the radical by the substituent (X) and the solvent.

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