One-Electron Redox Reactions of Water-Soluble Vitamins. III. Pyridoxine and Pyridoxal Phosphate (Vitamin B_6)

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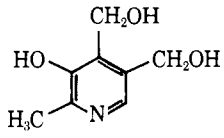
Contribution from the Pioneering Research Laboratory, U.S. Army Natick Laboratories, Natick, Massachusetts 01760. *Received August 2, 1974*

Abstract: The one-electron reduction of pyridoxine (PH) and pyridoxal phosphate (PPH) in water by hydrated electrons, e_{aq}^-, and acetone ketyl radicals, $(CH_3)_2\dot{C}OH$, was studied using the fast-reaction technique of pulse radiolysis and kinetic absorption spectrophotometry. The reaction rate constants of e_{aq}^-, $(CH_3)_2\dot{C}OH$ and OH radicals with PH and PPH were determined at different pH values consistent with the ionization constants of these substrates. The optical absorption spectra and extinction coefficients of the free-radical intermediates produced from the one-electron reduction of pyridoxine and pyridoxal phosphate were obtained at different pH values. From the change in absorbance with pH at fixed wavelengths, the ionization constants of the radicals were derived. For the pyridoxine intermediate, pK_a values of 4.8 and 11.4 were obtained and the species assigned to $\cdot PH_3^+$, $\cdot PH_2$, and $\cdot PH^-$. The rate of protonation of $\cdot PH_2$ to $\cdot PH_3^+$ by protons was $k = 7.8 \pm 1.0 \times 10^9$ M^{-1} sec^{-1}. In the case of pyridoxal phosphate, pK_a values of 3.7 and 6.9 were obtained for the corresponding radicals $\cdot PPH_3^+$, $\cdot PPH_2$, and $\cdot PPH^-$. For both PH and PPH, the first proton loss in the radical is suggested to come from the ring nitrogen, and the second proton loss from the phenolic hydroxyl group. Support for the latter assignment was obtained from the one-electron reduction of 3-methoxypyridoxal phosphate. The properties of the substrates and of the radicals are discussed on the basis of their redox potentials.

Pyridoxal phosphate (vitamin B_6) and related compounds are essential coenzymes for a series of reactions involving α-amino acids, e.g., transamination, racemization, and decarboxylation reactions.[2,3] They are present as Schiff bases bound to the ϵ-amino group of lysine in the protein moiety. Many of these reactions can be brought about nonenzymatically, and the assumption has been made that the mechanisms of these reactions are probably similar.[2,3]

The absorption spectra,[4] ionization constants,[4] proton transfer kinetics,[5] and polarography[6] of pyridoxine (I) and pyridoxal 5-phosphate (II) have been studied in some detail. Except for the polarographic reduction of these compounds, the nature of the intermediates produced from the one-electron redox reactions does not appear to have been studied.



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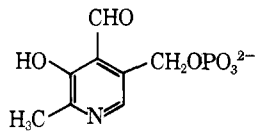


II

The one-electron reduction of compounds I and II by hydrated electrons, e_{aq}^-, and by the acetone ketyl radical, $(CH_3)_2\dot{C}OH$, were investigated in aqueous solution using the fast-reaction technique of pulse radiolysis and kinetic absorption spectrophotometry. The assignment, spectral characteristics, ionization constants, and reactivity of the radical intermediates produced are presented below. The reaction of OH radicals has also been briefly examined.

Experimental Section

The pulse radiolysis technique and experimental set-up used have been described elsewhere.[7,8] Single pulses of 2.3 MeV electrons and ~30 nsec duration were used (Febetron 705 machine).

The radiolysis of water produces $H_2O \rightsquigarrow e_{aq}^-$ (2.8), OH (2.8), and H (0.6), where the numbers in parentheses are the G values (yields per 100 eV of energy absorbed). One-electron reduction of compounds I and II was carried out at ~22° by using two methods: (a) by reaction with e_{aq}^-; (b) by reaction with $(CH_3)_2\dot{C}OH$. For method (a) solutions were irradiated in the presence of argon (1 atm) and ~1.0 M *tert*-butyl alcohol to scavenge the OH radicals produced from the radiolysis of water. The β-carbon radical produced[7] from this alcohol was found not to interfere with the observations reported below. For method (b) solutions were irradiated in the presence of N_2O (1 atm) and ~1.0 M isopropyl alcohol. The reactions occurring in this system are

$$e_{aq}^- + N_2O \longrightarrow N_2 + OH + OH^- \quad (1)$$

$$OH + (CH_3)_2CHOH \longrightarrow (CH_3)_2\dot{C}OH + H_2O \quad (2)$$

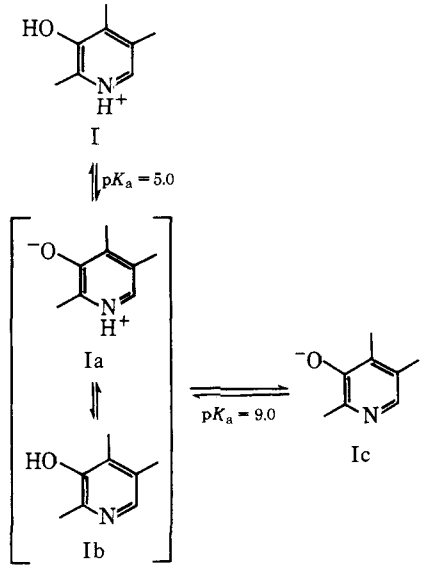
where $k_1 = 8.7 \times 10^9$ M^{-1} sec^{-1} (ref 9) and $k_2 = 2.0 \times 10^9$ M^{-1} sec^{-1} (ref 9). Under these conditions all the e_{aq}^- (>95%) reacted with N_2O and none with the substrates.

The chemicals used were the best research grade commercially available and were obtained from Calbiochem and Sigma Chemicals. Solutions were buffered with perchloric acid, potassium hydroxide, borate, and phosphates. Due to the sensitivity to light of the substrates,[10] the solutions were prepared just prior to use and kept in the dark. Exposure to the monitoring light from a pulsed Xenon lamp was kept to a minimum by using a synchronized shutter (open for ~5–10 msec). Fresh solutions were used for each pulse.

The transient optical spectra obtained were corrected for depletion of the substrate at the appropriate wavelength and pH. The extinction coefficients given were derived[7] on the basis of the G values given above.

Results and Discussion

Pyridoxine. Pyridoxine (PH) is present in aqueous solution at intermediate pH in two forms[4,5] with a proton bound to the phenolic hydroxyl group or to the ring nitrogen. The



forms Ia and Ib (referred to as PH for simplicity) are very reactive to e_{aq}^-, with $k = 2.2 \times 10^{10}$ M^{-1} sec^{-1}, at pH 6.8;

Table I. Reaction Rate Constants of e_{aq}^-, OH, and $(CH_3)_2COH$ Radicals with Pyridoxine and Pyridoxal Phosphate in Aqueous Solutions

Substrate[d]	e_{aq}^-[a] pH	Ionic form	k, M^{-1} sec^{-1}	OH[b] pH	Ionic form	k, M^{-1} sec^{-1}	$\cdot(CH_3)_2\dot{C}OH$[b,c] pH	Ionic form	k, M^{-1} sec^{-1}
Pyridoxine, PH (5.0, 8.97)				3.6	PH_2^+	4.3×10^9		*e*	
	6.8	PH	2.2×10^{10}	7.2	PH	6.3×10^9			
	11.0	P^-	2.5×10^9	10.5	P^-	7.4×10^9			
Pyridoxal phosphate, PPH (<2.5,[f] 4.14, 6.2,[f] 8.7)							1.0	PPH_2^+	5.8×10^8
	6.3, 7.3	PPH	1.6×10^{10}				5.6	PPH	1.3×10^8
	11.2	PP^-	6.1×10^9				10.0, 13.3	PP^-	2.9×10^8

[a] Determined in the presence of 0.1 *M* *t*-BuOH by monitoring decay kinetics of e_{aq}^- at 700 nm. [b] Determined by monitoring formation kinetics of transient species in solutions saturated with N_2O. [c] In the presence of 1.0 *M* isopropyl alcohol. [d] Numbers in parentheses are the pK_a values of the substrate. [e] $k \ll 10^7 M^{-1}$ sec^{-1} at all pH values 0–13.6. [f] Dissociation of phosphate group.

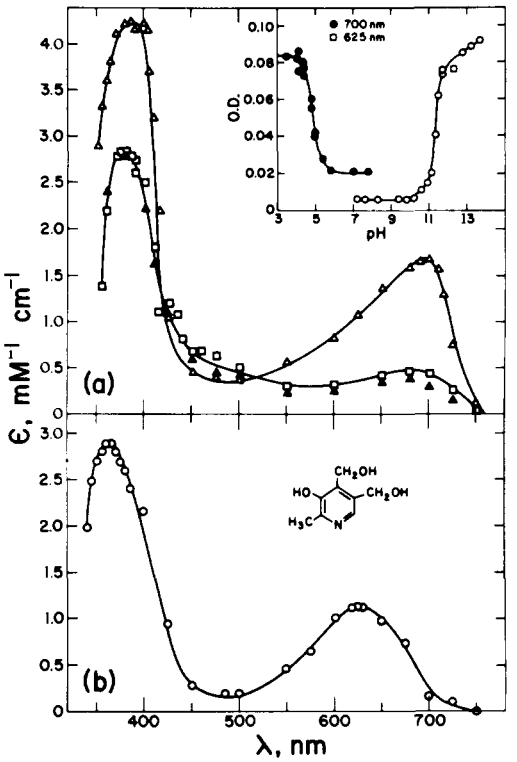


Figure 1. Absorption spectra of intermediates produced from the one-electron reduction by e_{aq}^- of pyridoxine (1–5 m*M*, in the presence of 1.0 *M* *tert*-butyl alcohol): (a) at pH 3.6 (transients T_1 (▲) and T_2 (△) read at ~0.2 μsec and ~3.0 μsec after the pulse, respectively) and at pH 7.0 (□). Total dose ~6 krads/pulse; (b) at pH 13.3 (○). Insert: change in absorbance at 700 and 625 nm with pH.

see Table I. It is interesting to note that this rate is much higher than the rate of e_{aq}^- with pyridine[9] ($3.0 \times 10^9 M^{-1}$ sec^{-1}) or with phenol[9] ($1.8 \times 10^7 M^{-1}$ sec^{-1}). It would appear to suggest that due to the equilibrium the reactivity with form Ia predominates.

In alkaline solutions, form Ic is much less reactive toward e_{aq}^-, and $k = 2.5 \times 10^9 M^{-1}$ sec^{-1} at pH 11.0. This value is close to that of neutral pyridine ($3.0 \times 10^9 M^{-1}$ sec^{-1}). It was, unfortunately, not possible experimentally to determine the rate of e_{aq}^- with form I. It is expected, however, to be somewhat higher than the rate with Ia.

The acetone ketyl radical and its anion were found to be unreactive toward the different forms of pyridoxine at pH 1, 7, and 13.5

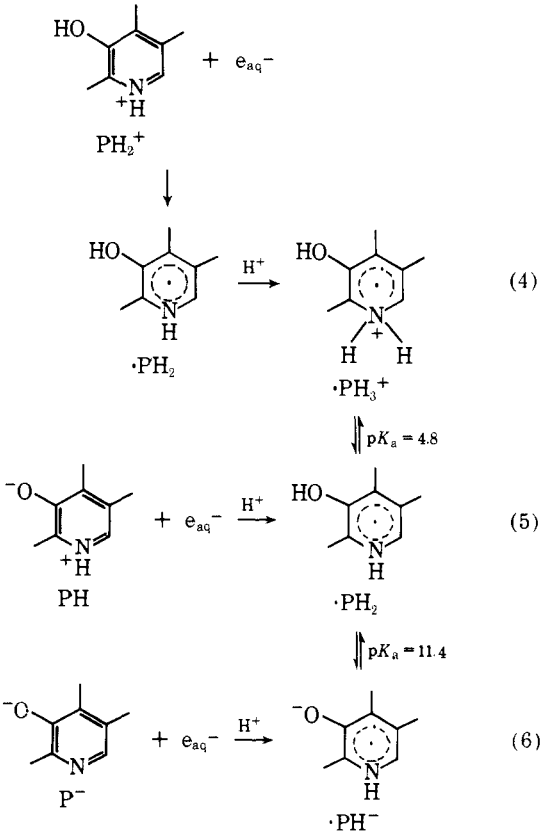
$$(CH_3)_2\dot{C}OH + PH \longrightarrow \cdot PH_2 + CH_3COCH_3 \qquad (3)$$

with $k_3 \ll 10^7 M^{-1}$ sec^{-1}. The redox potential of pyridoxine[6] is $E^{01} = -1.52$ V (at pH 7.0 and 25°C) and this is considerably more negative than the "kinetic" potential[11] of the $(CH_3)_2\dot{C}OH$ radical, $E_k^{01} = -0.82$ V. On the basis of these potentials, it is clear that reaction 3 cannot take place.

The reaction of e_{aq}^- with pyridoxine at pH 3.6 gave rise to a transient spectrum T_1 with maxima at 380 and 680 nm (Figure 1). At ~3.0 μsec after the electron pulse, an increase in absorbance is observed to give a transient spectrum T_2 with maxima at 390 and 695 nm (Figure 1a). The formation kinetics of T_2 was found to be dependent on $[H^+]$ but independent of phosphate buffer and of pyridoxine concentrations. The rate constant $k(T_1 + H^+ \rightarrow T_2) = 7.8 \pm 1.0 \times 10^9 M^{-1}$ sec^{-1}.

On pulse radiolysis of aqueous solutions of pyridoxine at pH 7.0, a spectrum identical to the T_1 spectrum found at pH 3.6 is observed (Figure 1a). At pH 13.3, when pyridoxine is present as form Ic, the transient spectrum observed has maxima at 360 and 625 nm and is different from that observed at pH 7.0 (Figure 1b). On monitoring the change in absorbance with pH at 625 and 700 nm, two titration curves are obtained from which one derives pK_a values of 4.8 and 11.4 (see Figure 1a).

Based on the above results, the following mechanism is suggested (reactions 4–6). Other equilibria between various forms of the radicals may be occurring.



The reaction of e_{aq}^- with PH_2^+ at pH 3.6 is shown in reaction 4; $\cdot PH_2$ is the initial transient (T_1) produced immediately after the pulse. Its protonation by H^+ to form $\cdot PH_3^+$ (T_2 transient) is kinetically observable with $k = 7.8 \times 10^9$

Table II. Absorption Maxima, Extinction Coefficients, Decay Kinetics, and Ionization Constants of Radicals Produced by One-Electron Reduction of Pyridoxine and Pyridoxal Phosphate in Water

Substrate[a]	pH	λ_{max}, nm	ϵ, mM^{-1} cm^{-1}	$2k$, M^{-1} sec^{-1}	pK_a (radical)	Suggested radical
Pyridoxine, PH	3.6	380,[d] 680[d]	2.85, 0.45			$\cdot PH_2$
		390,[e] 695[e]	4.30, 1.70	4.1×10^8 [c]	4.8	$\cdot PH_3^+$
	7.0	380, 680	2.85, 0.45	3.4×10^8 [c]	11.4	$\cdot PH_2$
	13.0	360, 625	2.90, 1.15	2.5×10^8 [c]		$\cdot PH^-$
Pyridoxal phosphate,	1.0[b]	395, ~480	≥3.4, 1.5	2.8×10^8 [b]		$\cdot PPH_3^+$
PPH	5.5	~395,[d] ~620[d]	7.0, 0.7		3.7	$\cdot PPH^-$ (species A)
		385,[f] 450, 620[f]	6.2, 3.0, 0.7	2.5×10^8 [b]		$\cdot PPH_2$
	13.3	410, 535	8.2, 2.2	8.7×10^6 [b,g]	6.9	$\cdot PPH^-$ (species B)

[a] Intermediates produced by pulse radiolysis of the substrates in the presence of ~1.0 M t-BuOH. [b] Intermediates produced by electron transfer from $(CH_3)_2\dot{C}OH$ radicals (see text). [c] Same decay rate for both bands. [d] Initial transient T_1 measured at "zero" time. [e] Second transient T_2 measured at ~30 μsec after the pulse. [f] Transient T_2 measured at ~10 μsec after the pulse. [g] Decay kinetics was not a perfect second-order process.

Table III. Absorption Maxima, Extinction Coefficients, and Decay Kinetics of Radicals Produced by Reaction of OH Radicals with Pyridoxine in Water[a]

pH	λ_{max}, nm	ϵ, mM^{-1} cm^{-1}	$2k$, M^{-1} sec^{-1}
3.6	325, 395, ~465	2.7, 2.4, 1.1	1.8×10^8
7.2	395, ~475	2.9, 1.3	3.4×10^8
13.3	355	3.1	1.3×10^8 [b]

[a] 5×10^{-4} M pyridoxine solutions saturated with N_2O. [b] Determined at pH 10.5. At pH 13.3 transient decays via mixed kinetics.

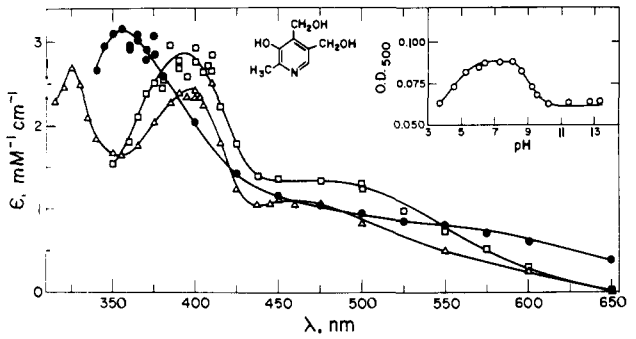


Figure 2. Absorption spectra of intermediates produced from the reaction of OH radicals with pyridoxine (5×10^{-4} M, 1 atm N_2O) at pH 3.6 (△), pH 7.2 (□), and pH 13.3 (●). Insert: change in absorbance at 500 nm with pH. Total dose ~3 krads/pulse.

M^{-1} sec^{-1}. At pH 7.0 (reaction 5), the protonation of the initial species produced from the reaction of e_{aq}^- with PH to give the $\cdot PH_2$ radical is not observed. Protonation by water or the buffer is probably occurring with $k \geq 10^7$ sec^{-1}, but is not observable under our time resolution ($\tau \sim 0.2$ μsec). The spectral characteristics of the $\cdot PH_2$ radical at pH 7.0 are identical to those of the T_1 species formed at pH 3.6; see Figure 1a. In alkaline solutions the protonation of the species produced from the reaction of e_{aq}^- with P^- to give $\cdot PH^-$ was also not observed. The $\cdot PH^-$ radical can be in equilibrium with the proton on either the ring nitrogen or the phenolic OH group (only one structure is shown).

Recent studies[12-14] have shown that the intermediates produced from the one-electron reduction of various aromatic nitrogen heterocyclic compounds by e_{aq}^- or $(CH_3)_2\dot{C}OH$ radicals undergo rapid protonation to give radical cations in neutral solution; e.g., pyrazine (Pz) produces the $\cdot PzH_2^+$ radical with a $pK_a = 10.5$. The relatively slow protonation of $\cdot PH_2$ presumably indicates that the formation of the $\cdot PH_3^+$ radical has some kinetic barriers. The change from $\cdot PH_2$ to $\cdot PH_3^+$ leads to a breakdown of the ring conjugation (as compared to $\cdot PzH \rightarrow \cdot PzH_2^+$), and the radical is no longer planar.

With an odd electron in the ring, the phenolic group can exhibit ketonic properties. The enol-keto tautomerism of the radicals suggested to be formed (reactions 4-6) presumably accounts for the observed absorption bands in the visible region. In the radical $\cdot PH^-$, the negative charge can be localized on the oxygen atom and the electron spin density is delocalized over the conjugated ring.

Support for the above assignments can be derived from the properties of radicals produced from unsaturated aliphatic alcohols.[15,16] Using 1,4-pentadien-3-ol it was found[16] that the $CH_2{=}CH\dot{C}(OH)CH{=}CH_2$ radical can be ionized to $CH_2{=}CH\dot{C}(O^-)CH{=}CH_2$ with a $pK_a = 8.9$. These radical species also absorb in the visible region. In the absence of conjugation the -Ċ(OH)- radicals are much weaker acids[15] and absorb[7] in the far-uv region.

These radicals decay by second-order kinetics (Table II), with quite similar rate constants.

Reaction of OH with Pyridoxine. The reaction rate constants of OH radicals with pyridoxine are dependent on the state of protonation of the molecule; see Table I. Deprotonation of the nitrogen increases the electrophilic properties of the molecule and its reactivity toward OH radicals, as was found for various amines.[17] The rate constants are lower than those found for the reaction of OH radicals with phenolic compounds[18] ($k \geq 10^{10}$ M^{-1} sec^{-1}).

Figure 2 shows the transient optical absorptions observed at pH 3.6, 7.2, and 13.3. The spectra at pH 3.6 and 7.2 appear to be similar and reflect acid-base changes in the radicals produced. The change in absorbance with pH at 500 nm shows two titration curves with pK_a values very close to those of pyridoxine (see insert, Figure 2). The OH radical is thought to: (i) add to pyridoxine, and may eventually lead to dehydration with the formation of a phenoxy type of radical, as was observed for phenolic compounds;[19,18] and (ii) abstract an H atom from the CH_2OH and CH_3 groups. The pyridoxine-ĊHOH radical formed is expected to have spectral and acid-base properties similar to radicals formed from unsaturated aliphatic alcohols.[16]

Since more than one radical is formed, it is not possible to assign the spectral bands observed in Figure 2. Table III gives the spectral characteristics and decay kinetics of these intermediates.

Pyridoxal 5-Phosphate. Pyridoxal phosphate (PPH) in water also has two dissociation constants associated with the phenolic hydroxyl group and the ring nitrogen, with pK_a values[4] of 4.14 and 8.7. The various forms present in solution are the same as those given above for pyridoxine (forms Ia, Ib, Ic). In addition, the phosphate group ionizes with $pK < 2.5$ and 6.2. Infrared results[20] suggest that hydration of the aldehyde group in PPH is pronounced in acid but not alkaline solutions.

At pH 6.3 and 7.3, the reaction rate constant of e_{aq}^- with PPH is 1.6×10^{10} M^{-1} sec^{-1}, close to that with PH (see Table I). The small difference could be due to the dinegative charge on the phosphate. At pH 11.2, the rate is 6.1 ×

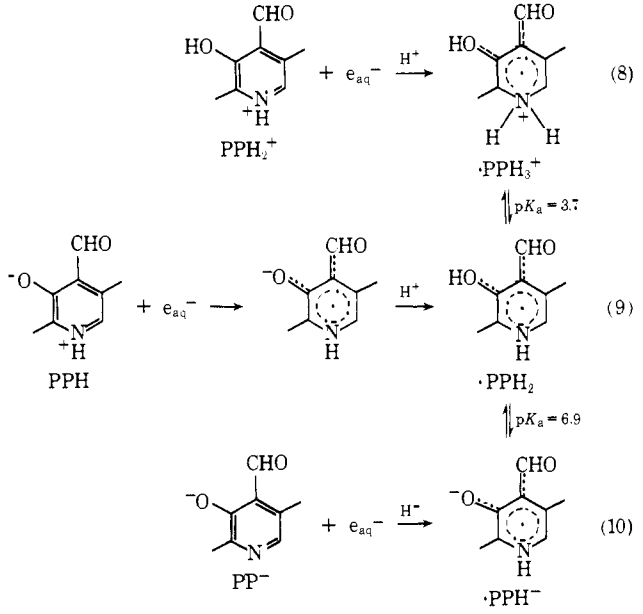
$10^9\ M^{-1}\ sec^{-1}$ and significantly higher than that with pyridoxine ($2.5 \times 10^9\ M^{-1}\ sec^{-1}$). Clearly, the presence of the 4-formyl group in PP^- accounts for this increase. Carbonyl groups are known[21] to be reactive toward e_{aq}^-.

The $(CH_3)_2\dot{C}OH$ radical was found to react with pyridoxal phosphate at pH 1.0, 5.6, and 10.0, with rate constants of $1\text{-}6 \times 10^8\ M^{-1}\ sec^{-1}$; see Table I. These rates were determined from the formation kinetics of the radicals produced from pyridoxal phosphate. The radicals were identical to those formed from the reaction with e_{aq}^-. The redox potential[6] of PPH is $E^{01} = -0.516$ V (at pH 7.0 and 25°), making it a stronger oxidant than PH and hence explains why it can be reduced with almost 100% efficiency by $(CH_3)_2\dot{C}OH$ radicals (see also above). Figure 3a shows the transient absorption spectrum of the radical produced from the reaction of $(CH_3)_2\dot{C}OH$ with PPH_2^+ at pH 1.0.

The reaction of e_{aq}^- with PPH at pH 5.5 produces, immediately after the pulse, a transient (T_1) absorption with maxima at ~395 and ~620 nm; see Figure 3b and Table II. At ~10 μsec later, the spectrum (T_2) changes and one observes maxima at 385, 450, and 620 nm. It was not possible to obtain good kinetic information for the rate of the proton-catalyzed reaction $T_1 + H^+ \rightarrow T_2$. The spectra observed at pH 10.0 and 13.3 are the same but are different from that obtained at pH 5.5; see Figure 3b. From the change in absorbance with pH monitored at 460 nm and at 540 nm, two titration curves are obtained from which pK_a values of 3.7 and 6.9 are derived. The increase in absorbance at pH >11 (insert in Figure 3b) is due to the conversion of H atoms into e_{aq}^-, which in turn react with PP^-.

$$H + OH^- \longrightarrow e_{aq}^- \qquad (7)$$

Based on the above results, the following scheme (reactions 8-10) is tentatively suggested. Other equilibria between various forms of the radicals may also be present.



The one-electron reduction of pyridoxal phosphate in acid, near neutral and alkaline solutions, is indicated by reactions 8-10, respectively. The redox potential[6] of PPH is much higher (more positive) than that of PH, indicating that the formyl group has a strong effect on the electron affinity of the molecule. It follows, therefore, that one-electron reduction of the formyl group may be occurring. Alternate structures for the $\cdot PPH_2$ and $\cdot PPH^-$ radical could be written based on the ionization of the $-\dot{C}HOH$ radical. The pK_a of the $Ph\dot{C}HOH$ radical formed from benzaldehyde has been reported to be 8.4[21] or 10.5.[22]

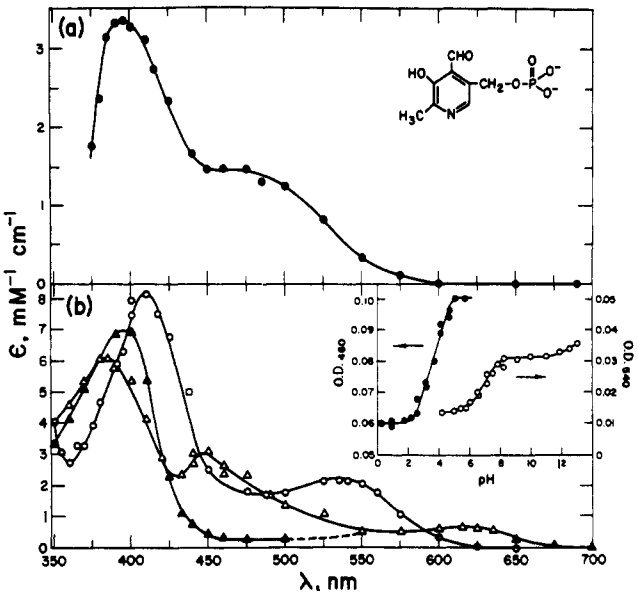
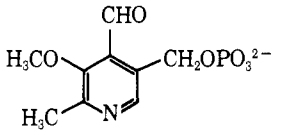


Figure 3. Absorption spectra of intermediates produced from the one-electron reduction of pyridoxal phosphate ($2 \times 10^{-4}\ M$) in: (a) 1.0 M isopropyl alcohol, 1 atm argon, pH 1.0, total dose ~3 krads/pulse; and (b) 1.0 M *tert*-butyl alcohol, 1 atm argon at pH 5.5 (transients T_1 (▲) and T_2 (△) read at "zero" time and ~10 μsec, respectively, after the pulse), and pH 13.3 (○). Insert: change in absorbance at 460 nm (using *i*-PrOH) and at 540 nm (using *t*-BuOH) with pH.

Support for the scheme of reactions represented by reactions 8-10 was, however, obtained from the reaction of e_{aq}^- with 3-methoxypyridoxal phosphate. At pH 5.6 (under ex-



perimental conditions similar to those used for PPH), the transient species observed has an absorption spectrum similar to that observed for PPH at pH 5.5. Between pH 5.5 and 10.2 no change in the absorption spectrum of the transient species produced from 3-methoxypyridoxal phosphate could be observed. This finding would seem to support the mechanism suggested above for the ionization of $\cdot PPH_2$ to $\cdot PPH^-$ with a $pK_a = 6.9$, namely the ionization of the phenolic hydroxyl group.

Conclusions

The one-electron reduction of pyridoxine and pyridoxal phosphate by e_{aq}^- and $(CH_3)_2\dot{C}OH$ radicals in water has been found to be efficient and to form free radicals which have marked acid-base properties. Pyridoxine shows two pK_a values of 4.8 and 11.4, while the values for pyridoxal phosphate are 3.7 and 6.9. The 11.4 and 6.9 values fall in good agreement with the correlation[15,24] of pK_a (radical) vs. redox potential of the parent compound for the ionization of ketyl radicals $-\dot{C}(OH)- \rightleftharpoons -\dot{C}(O^-)- + H^+$. This agreement not only supports the suggested assignment given above for the radical intermediates observed, but provides information on the nature and reactivity of these intermediates. For example, the kinetic potentials[11,25] of $-\dot{C}(O^-)-$ radicals are generally much lower (i.e. more negative) than those of the corresponding $-\dot{C}(OH)-$ radicals. This makes $-\dot{C}(O^-)-$ radicals much more powerful reducing agents in electron transfer processes.

Acknowledgment. We are most grateful to Dr. E. Helmreich from the Physiologisch-Chemisches Institut,

Würzburg University, West Germany, for the gift of 3-methoxypyridoxal phosphate.

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Kinetic Studies in Bile Acid Micelles

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Received August 15, 1974

Abstract: Pyrene is solubilized in aqueous solutions of sodium taurocholate (NaTC) micelles and excited by a frequency doubled ruby laser pulse. The kinetics of the pyrene fluorescence decay are studied in the presence of nonionic and ionic quenchers. These data yield information about the permeability of the NaTC micelles with respect to various quenching solute molecules. While a neutral molecule such as oxygen readily penetrates into the micelle, the entry of iodide ions is inhibited by the negative micellar surface charge. Positively charged quenchers such as Cu^{2+} and Tl^{+} are strongly absorbed at the surface of NaTC micelles. This allows for an investigation of the motion of pyrene within a NaTC micelle. The effect of additives such as cholesterol, benzyl alcohol, and Mg^{2+} ions on the permeability of NaTC micelles is also studied. The local viscosity in the interior of NaTC micelles is investigated by fluorescence depolarization measurement using 2-methylanthracene as the photoactive probe. The rotation of this probe within a NaTC micelle is strongly restricted and a microviscosity of 670 cP is derived from the degree of polarization of the emitted light. Mg^{2+} ions, benzyl alcohol, and sodium lauryl sulfate (NaLS) decrease the microviscosity. NaLS exhibits the strongest effect due to efficient comicellization with NaTC. The 347.1 nm laser photolysis of pyrene also produces significant amounts of positive ions and hydrated electrons. While the photoionization cross section in NaTC and NaLS micelles is similar, the lifetime of pyrene cations is much shorter in the former than in the latter type of micelles. This effect may be explained with the destruction of the smaller NaTC micelle by the positive charge. In comicelles of NaLS and NaTC the lifetime of pyrene cations increases rapidly with increasing NaLS content of the micelle. The electrons which are photoejected from the pyrene in the interior of the micelle become hydrated in the surrounding aqueous phase. Hydrated electrons have a low reactivity toward monomer NaTC ($k = 10^8\ M^{-1}\ \text{sec}^{-1}$). The reactivity decreases further upon micellization.

Bile acids participate in many important physiological processes. For example, the salts of these acids are involved in intestinal hydrolysis, and also act as emulsifying and solubilizing agents for neutral fats. The term bile acid covers the several derivatives of cholic acid which differ in the number and position of α-hydroxyl substituents. The acids have a rigid "cholesterol like" ring structure which is solubilized in water by glycine or taurine residues. The latter acids are linked to the ring structure by a peptide bond. The bile acids form aggregates called micelles, when dissolved in water above a certain critical concentration. This parallels the behavior of many other surface active molecules.[2,3] However, the bile acid micelles differ from conventional micelles in the fact that only a few molecules are associated in the micellar assembly. For example, trihydroxycholic acid and its derivatives form micelles with an aggregation number of four to ten.[4] In a conventional micelle formed by surfactant molecules such as sodium lauryl sulfate (NaLS) or cetyltrimethylammonium bromide (CTAB) the aggregation number lies between 50 and 100. The architecture of these micelles has already been discussed,[4] and the effect of micellization on the rate of hydrolysis of fatty acid esters has been studied.[5]

In earlier work[6-8] we have investigated the dynamic and kinetic properties of several micelles by laser photolysis and pulse radiolysis techniques. The laser photolysis method has also been successful in describing various permeability properties of *E. Coli* membranes.[9] The degree of rigidity and the rate of entry and exit of various molecules into and out of the micelle are readily measured by the techniques. Initial work with micelles of the bile acid, sodium taurocholate,[10] showed that the laser photolysis technique can be conveniently applied to a study of these micelles also. The present work is an enlarged and more rigorous study of kinetic processes in sodium taurocholate micelles. Pulse radiolysis is used to investigate the effect of micellization on the rate of reaction of e_{aq}^{-} with the peptide group. Laser photolysis is used to investigate the permeability of the micelles to various small molecules such as oxygen, iodide ion, etc. The effect of additives such as benzyl alcohol and sodium chloride on the permeability is also studied. Wherever possible fluorescence polarization techniques are used to complement the pulsed data.

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