Acid–Base Properties of Organic Peroxy Radicals, ·OORH, in Aqueous Solution

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Abstract: The transient optical spectra and extinction coefficients of the peroxy radicals, $\cdot O_2RH$, of lactic and glycolic acids and of lactamide, glycine anhydride, and *N*-acetylglycylglycine (where RH_2 = substrate) have been observed in oxygenated aqueous solutions using the technique of pulse radiolysis. All these peroxy radicals have maxima at or below 250 nm. From the change of the absorbance of the transient spectra at a fixed wavelength with pH, typical titration curves were obtained from which the ionization constants of these peroxy radicals were determined: $\cdot O_2RH \rightleftharpoons \cdot O_2R^- + H^+$. pK_a values of 5.2, 5.4, 5.8, 7.5, and 9.8 for the peroxy radicals from lactate, glycolate, lactamide, glycine anhydride, and *N*-acetylglycylglycine, respectively, were derived. The peroxy radicals from acetate ions and sarcosine anhydride did not show any change with pH and do not ionize. These ionization constants are 10–12 pH units lower than those of the parent compounds and are shown to be due to the dissociation of the OH and NH groups in these molecules. The odd electron primarily localized on oxygen in peroxy radicals appears to affect and interact with these functional groups when attached on the α-carbon atom. The efficiency and rate of electron transfer from these peroxy radicals to *p*-benzoquinone (BQ) were determined by monitoring the formation kinetics of the semiquinone radicals $\cdot BQ^-$ and $\cdot BQ^- - H^+$. The acid–base forms of these peroxy radicals showed characteristic properties. The $\cdot O_2RH$ radicals essentially do not transfer an electron to BQ, whereas the $\cdot O_2R^-$ radicals transfer efficiently leading to the formation of $\cdot BQ^-$ with a $k \sim 3\text{–}10 \times 10^8\ M^{-1}$ sec^{-1}. In the absence of an ionizable functional group in an α position to these peroxy radicals, *e.g.*, from acetate ion and sarcosine anhydride, no semiquinone radical was produced. The importance of these results to oxidation and biochemical reactions is indicated.

Oxidative processes involving reactions of molecular oxygen with organic compounds (RH_2) are of considerable biological and industrial interest, and an extensive literature exists on this subject (see *e.g.*, ref 1). Some of the oxidation processes have been postulated to proceed *via* a free-radical mechanism.[1,2] Direct study of these processes which involve peroxy radicals, $\cdot O_2RH$, is in its infancy due to experimental difficulties in observing and identifying them. The esr spectra of $\cdot O_2RH$ radicals have no hyperfine structure[2] except that due to ^{17}O. The optical absorption spectra,[3–5] on the other hand, are very similar to $\cdot HO_2$ and $\cdot O_2^-$.[6] The existence of α-hydroxyalkyl peroxy radicals,[3,4] $R_1R_2C(OH)O_2\cdot$, has recently been disputed[7] in spite of strong arguments in favor of their existence. It was suggested[7] that these peroxy radicals dissociate rapidly to produce the superoxide radical $\cdot O_2^-$ in neutral aqueous solutions.

Using *p*-benzoquinone, BQ, as an electron acceptor we have recently demonstrated[8] the existence of $R_1R_2C(OH)O_2\cdot$ radicals since these do not transfer an electron to BQ[8] while $\cdot O_2^-$ does.[8,9]

$$\cdot O_2^- + BQ \longrightarrow \cdot BQ^- + O_2 \quad (1)$$

$$R_1R_2C(OH)O_2\cdot + BQ \longrightarrow \cdot BQ^- + R_1COR_2 + H^+ + O_2 \quad (2)$$

In view of these results,[8] the presence of the reported[3,4] pK_a values for α-hydroxyalkyl peroxy radicals is acceptable with more confidence. We now report on the existence and acid–base properties of other peroxy radicals in aqueous solutions which are of considerable interest in biochemical processes.

Experimental Section

Pulse radiolysis was used to generate certain specific free radicals in aqueous solution, and the technique employed has been described elsewhere. The radicals, $\cdot RH$, were produced by reaction of OH radicals with various substrates, RH_2, in the presence of 13 m*M* N_2O (to convert e_{aq}^- to OH radicals) and 0.7 m*M* O_2 to produce the peroxy radicals $\cdot O_2RH$ from $\cdot RH$ radicals.

Extinction coefficients were derived based on $G(OH) = G(e_{aq}^-) = 2.8$. Total dose used was ~8 krads/pulse to determine the transient absorption spectra and ionization constants of the peroxy radicals, and ~1.0 krads/pulse when studying the electron transfer properties of those radicals to *p*-benzoquinone. Dosimetry was carried out[3,10] using 0.1 *M* KCNS.

Perchloric acid, potassium hydroxide, phosphates (~1 m*M*), and tetraborate (~1 m*M*) were used as buffers. Chemicals were highest research grade commerically available. Glycine anhydride was recrystallized twice from water, and *p*-benzoquinone was recrystallized from alcohol and resublimed.

Results and Discussion

In order to establish the formation of peroxy radicals under the experimental conditions used in this investigation, the reaction rate constants of $\cdot RH$ radicals with oxygen were measured. These rates were determined

$$\cdot RH + O_2 \longrightarrow \cdot O_2RH \quad (3)$$

by monitoring the decay kinetics of the $\cdot RH$ radicals in the presence of O_2 at the appropriate wavelengths.

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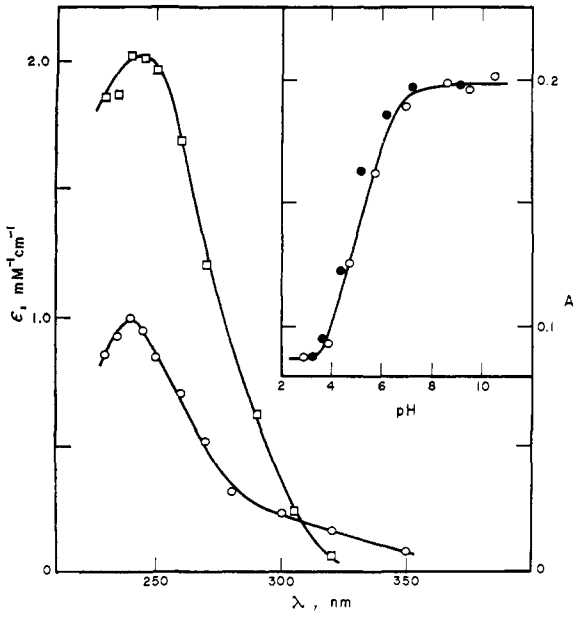

Figure 1. Transient absorption spectra of the peroxy radicals of $CH_3\dot{C}(OH)COOH$ at pH 3.0 (○) and pH 10.5 (□), produced on pulse radiolysis of aqueous solutions of 5 m*M* lactic acid in the presence of 13 m*M* N_2O and 0.7 m*M* O_2. Total dose ~8 krads/pulse. Insert: (a) change in absorbance at 250 nm as a function of pH (○), and (b) change, as a function of pH (●), of the efficiency (expressed as %) of the electron-transfer process from the peroxy radicals of lactic acid (5 m*M*, 13 m*M* N_2O, 0.7 m*M* O_2) to *p*-benzoquinone (20 μM). Experiment monitored at 410 nm, where the semiquinone radicals of *p*-BQ absorbs, and corrected for the pK_a = 4.0 of the semiquinone radical (see text). Total dose 1.0 krads/pulse.

The nature and absorption spectra of the ·RH radicals studied had previously been established, *e.g.*, for lactic acid, the $CH_3\dot{C}(OH)COO^-$ radical[11] was monitored at 270 nm, the $\cdot CH_2COO^-$ radical[12] from acetic acid at 350 nm, the $\underline{NH\dot{C}HCONHCH_2CO}$ radical[13] from glycine anhydride at 360 nm, and the Ac-Gly-$NH\dot{C}HCOO^-$ radical[14] from *N*-acetylglycylglycine at 330 nm.

$$OH + CH_3CH(OH)COO^- \longrightarrow CH_3\dot{C}(OH)COO^- + H_2O \quad (4)$$

$$OH + CH_3COO^- \longrightarrow \cdot CH_2COO^- + H_2O \quad (5)$$

$$OH + \underline{NHCH_2CONHCH_2CO} \longrightarrow \underline{NH\dot{C}HCONHCH_2CO} + H_2O \quad (6)$$

$$OH + \text{Ac-Gly-}NHCH_2COO^- \longrightarrow \text{Ac-Gly-}NH\dot{C}HCOO^- + H_2O \quad (7)$$

From the pseudo-first-order decay of these radicals at 2–3 different oxygen concentrations, the second-order rates for reaction 3 were obtained. These values are given in Table I.

Formation and Ionization of Peroxy Radicals. The transient optical absorption spectra of the intermediates produced in the presence of oxygen are distinctly different from those observed in the absence of oxygen. Figure 1 shows the spectrum obtained on pulse radiolysis of aqueous solutions of 5 m*M* lactic acid at pH 3.0 in the presence of 13 m*M* N_2O and 0.7 m*M* O_2. This

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Table I. Reaction Rate Constants of Some Free Radicals with Oxygen in Aqueous Solution

Substrate, RH_2	pH	·RH radical	$k(\cdot RH + O_2)$,[a] M^{-1} sec^{-1}
Lactic acid	7.3	$CH_3\dot{C}(OH)COO^-$	3.5×10^8
Acetic acid	4–9	$\dot{C}H_2COO^-$	5.0×10^8
Glycine anhydride	5.0	$\underline{NH\dot{C}HCONHCH_2CO}$	1.2×10^9 [b]
Sarcosine anhydride	5.2	$\underline{N(CH_3)\dot{C}HCON(CH_3)CH_2CO}$	0.9×10^9 [b]
N-Acetylglycylglycine	5.5	Ac-Gly-$NH\dot{C}HCOO^-$	5.5×10^8

[a] Values to ±15%. [b] From ref 13.

species has a maximum at ~240 nm and $\epsilon_{240} = 1.0 \times 10^3$ M^{-1} cm^{-1}. In the absence of oxygen, the $CH_3\dot{C}(OH)COOH$ radical produced[11] at this pH has a maximum below 240 nm and $\epsilon_{240} > 4 \times 10^3$ M^{-1} cm^{-1}. At pH 10.5 in the presence of oxygen a spectrum somewhat similar to that observed at pH 3.0 is found but with an $\epsilon_{245} = 2.0 \times 10^3$ M^{-1} cm^{-1} (see Figure 1). On monitoring the change in absorbance with pH at a fixed wavelength, a titration-type curve is obtained (insert Figure 1) from which a pK_a value of 5.2 ± 0.2 can be derived. The decay kinetics of these species are completely different from those produced in the absence of oxygen. Acid–base equilibrium takes place before the radicals decay under the experimental conditions used.

These changes are attributed to the following acid–base equilibrium

$$CH_3\dot{C}(OH)COO^- + O_2 \longrightarrow CH_3C(\dot{O}_2)(OH)COO^- \quad (8)$$

$$CH_3C(O_2)(OH)COO^- \underset{pK_a = 5.2 \pm 0.2}{\rightleftharpoons} CH_3C(\dot{O}_2)(O^-)COO^- + H^+ \quad (9)$$

and are compared to

$$CH_3\dot{C}(OH)COO^- \underset{pK_a = 9.8 \pm 0.2}{\rightleftharpoons} CH_3\dot{C}(O^-)COO^- + H^+ \quad (10)$$

Considering the high pK_a[2] $\geq$ 15 for ionization of the hydroxyl proton in lactic acid, it is important to note here the unusually high increase in the acidity of the OH group in the peroxy radical $CH_3C(\dot{O}_2)(OH)COO^-$, leading to a decrease in ΔpK by ~10 units.

Similar spectra and results were obtained on pulse radiolysis of aqueous solutions of lactamide in the presence of oxygen (see Table II), indicating that the COO^- group is not involved in the pH dependence of the observed transient spectra. Furthermore, the absence of spectral changes with pH (from 3.5 to 11.0) for the $\cdot O_2CH_2COO^-$ radical from acetic acid confirms the presence of equilibrium 9 and the need of an ionizable functional group (OH in this case) on the α carbon. Results with the radicals from glycolic acid are also in complete agreement with the above conclusions (see Table II).

The ionization of the peptide hydrogen in an α position to an odd electron has been demonstrated.[13,14] The peptide radical from glycine anhydride was found[13] to have absorption maxima at 265 and 365 nm with extinction coefficients of 8.5×10^3 and 2.8×10^3 M^{-1} cm^{-1}, respectively. The ionization constant of this cyclic radical is 9.6 ± 0.2 and was shown[13] to be due to the following equilibrium.

Table II. Ionization Constants of Some Organic Peroxy Radicals $\cdot O_2RH$ and Electron-Transfer Processes from These Radicals to *p*-Benzoquinone in Aqueous Solutions

Substrate, RH_2[a]	pH	Suggested donor peroxy radical	pK_a ·RH	pK_a $\cdot O_2RH$	Electron transfer[b] Efficiency, % No-O_2	Efficiency, % O_2	Rate, M^{-1} sec^{-1} No-O_2	Rate, M^{-1} sec^{-1} O_2
Lactic acid	3.5	$CH_3C(\dot{O}_2)(OH)COO^-$	9.8[c]	5.2 ± 0.1	97 (7.0)	<5	6.5×10^9 (7.3)	
	7.3	$CH_3C(\dot{O}_2)(O^-)COO^-$			97 (10.5)	86	$\sim 7.0 \times 10^9$ (10.8)	8.0×10^8
Lactamide	3.7	$CH_3C(\dot{O}_2)(OH)CONH_2$	6.5[d]	5.8 ± 0.1	80 (5.5)	<5	2.0×10^9 (5.0)	
	8.3	$CH_3C(\dot{O}_2)(O^-)CONH_2$			93 (9.2)	85	3.6×10^9 (7.3)	9.5×10^8
Glycolic acid	3.6	$\cdot O_2CH(OH)COO^-$	8.8[c]	5.4 ± 0.1	72 (7.0)	<5	2.2×10^9 (7.0)[e]	
	8.1	$\cdot O_2CH(O^-)COO^-$				~85		
Acetic acid	3.4–9.3	$\cdot O_2CH_2COO^-$	4.5[i]	None[j]	~15	<5		
Glycine[k] anhydride	4.5	$\underline{NHC(\dot{O}_2)HCONHCH_2CO}$	9.6[f]	7.5 ± 0.1	<5 (7.0)	<5		
	9.3	$\underline{NC(\dot{O}_2)HCONHCH_2CO}$			87 (10.5)	>60[g]	2.2×10^9	3.5×10^8
Sarcosine[k] anhydride	5.5–9.3	$\underline{N(CH_3)C(\dot{O}_2)HCON(CH_3)CH_2CO}$	None[j]	None	<10 (7.0)	<10		
N-Acetyl-glycylglycine	5.5	Ac-Gly-$NC(\dot{O}_2)HCOO^-$	~12[h]	9.8 ± 0.1	<10 (7.0)	10		
	11.0	Ac-Gly-$N^-CHC(\dot{O}_2)OO^-$				>50[g]		

[a] 5 m*M*, unless stated otherwise. [b] Values to ±10%; numbers in parentheses refer to pH values. "No-O_2" refers to solutions containing 26 m*M* N_2O, and "O_2" refers to solutions containing 13 m*M* N_2O and 0.7 m*M* O_2. [c] From ref 11. [d] J. Bell, unpublished results from this laboratory. [e] P. S. Rao and E. Hayon, *Nature (London)*, **243**, 334 (1973). [f] From ref 13. [g] % value low due to low rate and incomplete electron transfer. [h] From ref 14. [i] Reference 12. [j] None observable from pH 4 to 11. [k] 2 m*M* used.

$$\underline{HN\dot{C}HCONHCH_2CO} \underset{pK_a = 9.6 \pm 0.2}{\rightleftharpoons} \underline{\bar{N}\dot{C}HCONHCH_2CO} + H^+ \quad (11)$$

In the presence of oxygen, completely different transient spectra were observed (see Figure 2A) with maxima below 235 nm and much lower extinction coefficients. From the change in absorbance with pH, a $pK_a = 7.5 \pm 0.1$ was obtained (insert, Figure 2A), showing that the acidity of this peroxy peptide radical is further increased. Support for the assignment of these transient

$$\underline{HNCH(O_2)CONHCH_2CO} \underset{pK_a = 7.5 \pm 0.1}{\rightleftharpoons} \underline{N^-CH(O_2)CONHCH_2CO} + H^+ \quad (12)$$

spectra to the peroxy radicals and their acid–base equilibrium was obtained from the pulse radiolysis of aqueous solutions of sarcosine anhydride in the presence of oxygen. Figure 2B shows the spectra obtained at pH 3.8 and 10.5. The absence of any change with pH is in agreement with the conclusions reached[13] in oxygen-free solutions. The sarcosine anhydride radical $\underline{N(CH_3)\dot{C}HCON(CH_3)CH_2CO}$ does not have a peptide hydrogen and hence cannot undergo an acid–base dissociation.

Similar results were obtained for the radical produced[14] from *N*-acetylglycylglycine. Here again (see Table II) the acidity of the corresponding peroxy radical is ~2 pH units lower than that of the radical.

It is interesting to note that the absorption spectra and extinction coefficients of $R_1R_2C(OH)O_2\cdot$ radicals, where $R_1 = H$ and $R_2 = CH_3$, COO^-, or $CONH_2$, are similar to each other with maxima ~240–250 nm. The $R_1R_2C(NH)O_2\cdot$ radicals, on the other hand, have maxima at lower wavelengths and higher extinction coefficients. The odd electron primarily localized on oxygen in peroxy radicals does, however, affect and interact with OH and NH functional groups attached on the α-carbon atom. Substituents positioned on the β carbon appear to be unaffected by the strong inductive effect of the oxygen molecule in $\cdot O_2RH$ radicals. The availability of excess electrons on nitrogen (in NH and NH_2 group) would seem to reduce somewhat the inductive effect of oxygen, and hence render these groups less acidic.

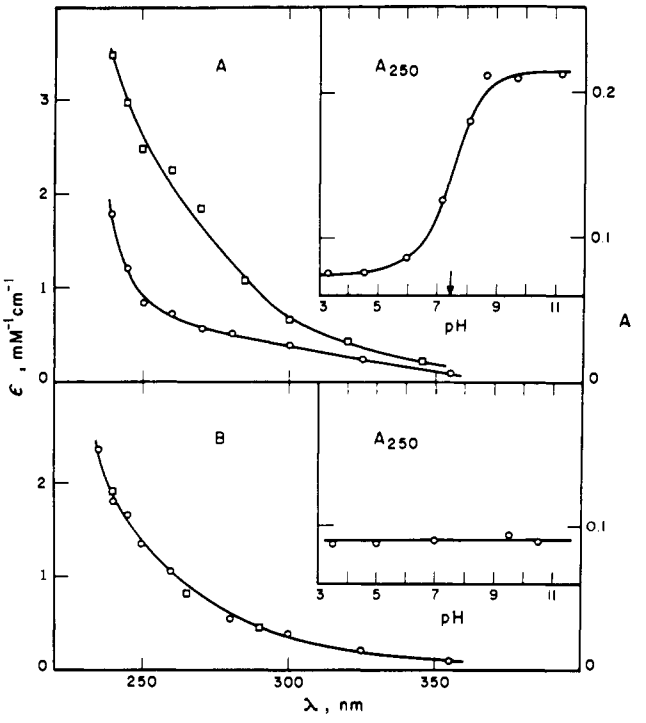

Figure 2. Transient absorption spectra of the peroxy radicals produced at pH 3.8 (○) and pH 10.5 (□) on pulse radiolysis of 2 m*M* aqueous solutions of (A) glycine anhydride and (B) sarcosine anhydride in the presence of 13 m*M* N_2O and 0.7 m*M* O_2. Total dose ~5 krads/pulse. Inserts: change in absorbance at 250 nm as a function of pH.

Electron Transfer Reactions of Peroxy Radicals. The use of *p*-benzoquinone (BQ, $E^0 = 0.699$ V) as an acceptor in electron-transfer reactions was recently shown[8] to be effective in titrating different free radicals. The semiquinone radical and radical anion of *p*-benzoquinone have characteristic absorption bands.[15] From the amounts of $\cdot BQ^-$ and $\cdot BQ^-$–H^+ radicals produced the efficiency (expressed as percentage) of electron-transfer reactions to BQ can be obtained, and from the rate of formation of these radicals the rates of these electron transfer processes can be calculated.

On pulse radiolysis of 2–5 m*M* aqueous solutions of the substrates given in Table II in the presence of 13 m*M* N_2O, 0.7 m*M* O_2, and 20 μM BQ, all the OH radicals produced react with the substrates, all the e_{aq}^- react with N_2O, and essentially none of the e_{aq}^- react with BQ or O_2. At pH 3.0, when all the $\cdot O_2RH$ peroxy radicals are present in their acidic form, no formation of the semiquinone radical $\cdot BQ^-$–H^+ was observed at 400 nm. On increasing the pH, reaction of the ionized forms of the peroxy radicals with BQ was found to occur, and the characteristic absorption due to the semiquinone radical anion $\cdot BQ^-$ was observed. These

$$\cdot O_2RH + BQ \not\longrightarrow \cdot BQ^-\text{–}H^+ + R + O_2 \quad (13)$$

$$\cdot O_2R^- + BQ \longrightarrow \cdot BQ^- + R + O_2 \quad (14)$$

results are given in Table II and present a direct evidence for the existence of $\cdot O_2RH$ radicals. The spectrum of $\cdot O_2RH$ in Figure 1 is also sufficiently different from that of $\cdot O_2H$ to rule out electron transfer to O_2 from $\cdot RH$ radicals. This difference in the behavior of $\cdot O_2RH$ and $\cdot O_2R^-$ radicals toward BQ was used to "titrate" the equilibrium between these two radical forms. The increase in $\cdot BQ^-$ (or $\cdot BQ^-$–H^+) with pH was followed at 410 nm and appropriate corrections for the different extinction coefficient of these two semiquinone forms were made (~15%). The titration curve obtained in this fashion (Figure 1) follows very closely the one obtained through direct changes in absorbances in the absence of BQ.

In addition, the following features in the electron-transfer reactions to BQ are noticeable: (a) $\cdot O_2R^-$ transfers much slower than $\cdot RH$ and $\cdot R^-$ radicals; (b) lactamide, glycolate, glycine anhydride, and *N*-acetylglycylglycine peroxy radicals behave similarly; (c) the peroxy radicals of sarcosine anhydride and acetate ion do not transfer at any pH.

(15) $\cdot BQH \rightarrow \cdot BQ^- + H$, $pK_a = 4.0$: G. E. Adams and B. D. Michael, *Trans. Faraday Soc.*, **63**, 1171 (1967).

Conclusions

The above results clearly indicate that organic peroxy radicals are relatively stable in aqueous solutions and they have characteristic physical and chemical properties, and can undergo specific chemical reactions. The strongly inductive effect of the oxygen perturbs the acid–base properties of the OH and NH functional groups attached to the same α carbon. The peroxy radicals increase the acidity of these functional groups by $\Delta pK_a \sim 10$–12, thus ionizing them at much lower pH values and affecting the nature of the ensuing chemical reactivities.

The acid–base properties of peroxy radicals and their characteristic electron-transfer properties are considered to play an important role in oxidation, autoxidation, and cellular processes. The ability of ionized peroxy radicals to undergo electron-transfer reactions to various acceptors having higher redox potentials, whereas un-ionized peroxy radicals do not, is considered to be most significant, particularly since it would seem that many peroxy radicals have ionization constants in the physiological pH range 5–7. Its biochemical importance and further work will be described elsewhere.