Pulse Radiolysis Study of Cyclic Peptides in Aqueous Solution. Absorption Spectrum of the Peptide Radical –NHCHCO–

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Abstract: The pulse radiolysis of aqueous solutions of the cyclic dipeptides of glycine, alanine, and sarcosine has been studied. The rate constants for reaction of e_{aq}^- and of OH radicals with these anhydrides were found to be $\sim 2 \times 10^9\ M^{-1}\ sec^{-1}$, independent of pH in the range 5–13. Hydrogen atom abstraction by OH radicals produces characteristic transient absorptions with maxima at ~265 and 365 nm and extinction coefficients of ~8500 and 2500 $M^{-1}\ cm^{-1}$, respectively. These absorption spectra are essentially the same for the three cyclic dipeptides, and are assigned to the peptide radical, *e.g.*, –NHĊHCO–, for glycine anhydride. In alkaline solutions, glycine and alanine anhydrides produce transient spectra which are red shifted. From an examination of the acid–base properties of these radicals, $pK_R = 9.6 \pm 0.2$ was determined. Sarcosine anhydride shows no change in alkaline solution. These results are interpreted to be due to the deprotonation of the peptide hydrogen adjacent to the unpaired electron: $\underline{NH\dot{C}HCONHCH_2CO} + OH^- \rightleftharpoons {}^-\underline{N\dot{C}HCONHCH_2CO} + H_2O$. Ionic strength effects on the second-order decay of this radical were observed. The rate constant of this proton-transfer reaction was found to be $k = 8.0 \pm 2.0 \times 10^9\ M^{-1}\ sec^{-1}$ for glycine anhydride. The reactivity of these peptide radicals with oxygen was measured, $k(R\cdot + O_2) \sim 10^9\ M^{-1}\ sec^{-1}$. The hydrated electron reacts with cyclic dipeptides to produce the corresponding electron adducts: *e.g.*, $\underline{NHCH_2\dot{C}(O^-)NHCH_2CO}$. The rates of electron transfer from these ketyl radicals to sulfhydryl and disulfide acceptors were found to be $\sim 2 \times 10^8\ M^{-1}\ sec^{-1}$, and to aromatic ketones $\sim 2 \times 10^9\ M^{-1}\ sec^{-1}$.

In addition to certain biochemical functions of cyclic peptides, in particular the structure–biological activity of peptide antibiotics, conformational interest in the use of cyclic peptides as models for enzyme active sites and as substrates for enzymes has recently been demonstrated. No studies appear to have been carried out on the reactivity of the functional groups to free-radical attack.

Following our recent studies on the reactions of OH radicals and hydrated electrons with amino acids,[2] amides,[3] and simple peptides,[4,5] we have investigated the radiation chemistry of cyclic dipeptides using the pulse radiolysis technique.

The radiolysis of aqueous solutions of alanine anhydride was studied, and pyrazine derivatives were observed[6] as products. Radiation-induced degradation of peptides was found for cyclic dodecapeptides polymyxin B.[7] The esr of X-irradiated single crystals of glycine anhydride produced[8] a spectrum with principal values for the hyperfine interaction tensor similar to those found in irradiated glycylglycine and acetylglycine.

This paper presents the results obtained on pulse radiolysis of air-free aqueous solutions of glycine, alanine, and sarcosine anhydrides, and the nature of the intermediates produced from the reaction of e_{aq}^- and OH radicals with these cyclic dipeptides. The kinetics for the base-catalyzed proton-transfer reaction of the peptide hydrogen have been studied. The rates of electron transfer from the intermediates to various selected acceptors have also been determined.

Experimental Section

A Febetron (Field Emission Corp.) 705 pulsed radiation source was used. This machine produces single pulses of electrons of 2.3-MeV energy and ~30-nsec duration. Full experimental details have been described elsewhere,[9] on the double monochromator, the pulsed Xenon lamp light source, and the dosimetry. Cells with 2-cm optical paths were used. Reagent grade chemicals were supplied by Cyclo Chemicals, Calbiochem, Nutritional Biochemicals, and Baker and Adamson. Solutions were buffered using perchloric acid, potassium hydroxide, sodium tetraborate (2–5 mM), and potassium phosphates (~3 mM). Owing to the alkaline hydrolysis of diketopiperazines above pH 9.5, the solutions were made alkaline and were used within less than 20 min. Extinction coefficients were derived based on $G(e_{aq}^-) = G(OH) = 2.8$. The experiments were carried out at concentrations of solutes in excess of those reqired for complete scavenging of e_{aq}^- and OH radicals.

Results

The radiation chemistry of water and aqueous solutions produces the free radicals e_{aq}^-, OH, and H: $H_2O \sim\!\!\sim\!\!\rightarrow e_{aq}^-$, OH, H, H_2, and H_2O_2. The reactions of these free radicals can be studied separately under well-established conditions. The OH radical reactions can be followed on saturation of the solution with N_2O ($\sim 2.5 \times 10^{-2}\ M$) when the electrons are converted (>98%) to OH radicals

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

with $k_1 = 6 \times 10^9\ M^{-1}\ sec^{-1}$. The e_{aq}^- reactions were followed in the presence of excess *tert*-butyl alcohol to scavenge the OH radicals. The *tert*-butyl alcohol

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(8) W. C. Lim and C. A. McDowell, *Can. J. Chem.*, **41**, 9 (1963).
(9) M. Simic, P. Neta, and E. Hayon, *J. Phys. Chem.*, **73**, 3794 (1969); E. Hayon, *J. Chem. Phys.*, **51**, 4881 (1969); J. P. Keene, E. D. Black, and E. Hayon, *Rev. Sci. Instrum.*, **40**, 1199 (1969).

Table I. Rates of Reaction of OH Radicals and e_{aq}^- with Cyclic Dipeptides in Aqueous Solution

Cyclic peptide	k(OH + S), M^{-1} sec^{-1} [a]	k(e_{aq}^- + S), M^{-1} sec^{-1} [b]
Glycine anhydride	$1.2 \pm 0.1 \times 10^9$	$1.7 \pm 0.1 \times 10^9$
Alanine anhydride	$1.8 \pm 0.1 \times 10^9$	$2.0 \pm 0.1 \times 10^9$
Sarcosine anhydride	$2.6 \pm 0.1 \times 10^9$	$2.0 \pm 0.2 \times 10^9$

[a] Determined *vs.* the CNS^- method, taking $k(OH + CNS^-) = 1.1 \times 10^{10}$ M^{-1} sec^{-1}. Identical rates of k(OH + S) were obtained at pH 5.0 and 11.0 for each peptide. [b] Determined at pH 9.2, in the presence of 0.2 *M tert*-butyl alcohol to scavenge the OH radicals.

The rates of reactions of e_{aq}^- with the cyclic dipeptides were determined by following the decay rate of the hydrated electron at 700 nm. These runs were done at pH 9.2 (using borate buffer) in the presence of *tert*-BuOH to scavenge the OH radicals, in order to eliminate the back reaction of e_{aq}^- + OH. These rates are given in Table I.

Optical Absorption Spectra of Intermediates. The transient optical spectra produced from the reaction of OH radicals with glycine anhydride are shown in Figure 1. At pH 5.0, a spectrum with λ_{max} 265 and 365 nm is observed, with high extinction coefficients of 8500 and 2800 M^{-1} cm^{-1}, respectively; see Table II. In alkaline solutions, a similar spectrum is observed, only it is shifted to the red by ~25 nm. The insert in Figure 1 shows the change of absorbance at 300 nm with pH. From this curve a pK can be derived (see below) of 9.6 ± 0.2. These transient species decay by second-order kinetics at both pH 5.0 and 11.2. The decay rate at high pH is by a factor of 2–3 lower than at pH 5.0 (Table II). In all cases (including alanine and sarcosine anhydrides), the decay rates of the second absorption bands at longer wavelengths are identical with those of the more intense bands in the far-uv region, indicating the presence of only one transient species.

Table II. Absorption Maxima, Extinction Coefficients, Decay Kinetics, and pK Values of Transient Species Produced from the Reaction of OH Radicals with Cyclic Dipeptides in Aqueous Solution

Cyclic peptide	pH	λ_{max}, nm	ϵ, M^{-1} cm^{-1}	$2k$, M^{-1} sec^{-1} [a]	pK_R	Suggested radical [b]
Glycine anhydride	5.0	265, 365	8500, 2800	1.3×10^9 (1.3×10^9)	9.6 ± 0.2	NHĊHCONHCH$_2$CO ⇅ $^-$NĊHCONHCH$_2$CO
	11.2	290, 390	9300, 2500	5.0×10^8 (7.3×10^8)		
Alanine anhydride	5.5	260, 360	7900, 2300	1.4×10^9 (1.3×10^9)	9.6 ± 0.2	NHĊ(CH$_3$)CONHC(CH$_3$)CO ⇅ $^-$NĊ(CH$_3$)CONHC(CH$_3$)CO
	12.7	290, 385	8400, 2000	4.3×10^8 (6.3×10^8)		
Sarcosine anhydride	6.2	265, 365	8300, 2200	1.4×10^9 (1.4×10^9)		N(CH$_3$)ĊHCON(CH$_3$)CH$_2$CO
	12.8	265, 365	8300, 2200	1.3×10^9 (1.3×10^9)		N(CH$_3$)ĊHCON(CH$_3$)CH$_2$CO

[a] Decay constant values given in parentheses were obtained in presence of 0.2 *M* K_2SO_4, to check for ionic strength effects on the decay kinetics of the intermediates observed. [b] See text for discussion on the nature of the intermediates in alkaline solution.

radicals do not essentially absorb above ~280 nm, and corrections were made in all cases for their absorption below 280 nm.

Rates of Reaction of e_{aq}^- and OH Radicals. The rates of reaction of OH radicals with glycine, alanine, and sarcosine anhydrides were determined using the thiocyanate method. From the decrease in the absorption at 500 nm of the $(CNS)_2^-$ radical anions at various $[CNS^-]/[$cyclic dipeptide$]$ ratios, the rates k(OH + S) were derived based on $k(OH + CNS^-) = 1.1 \times 10^{10}$ M^{-1} sec^{-1}.[10] Identical k values were observed at pH 5.0 and 11.0 for each cyclic dipeptide.

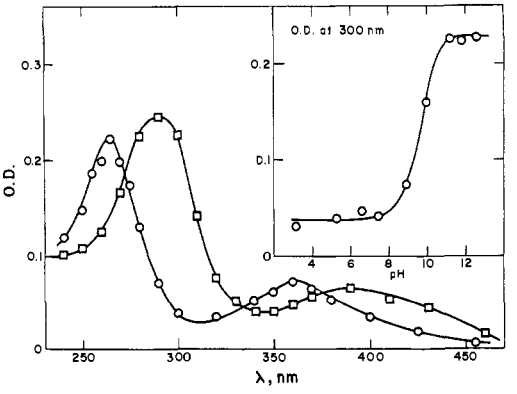


Figure 1. Transient absorption resulting from the reaction of OH radicals with glycine anhydride (10 mM; N_2O (1 atm): pH 5.0, ○; pH 11.2, □. Insert: OD_{300} *vs.* pH curve of transient. Total dose ~2.4 krads/pulse.

(10) J. H. Baxendale, P. L. T. Bevan, and D. A. Stott, *Trans. Faraday Soc.*, 64, 2389 (1968).

The intermediates produced from the reaction of OH radicals with alanine anhydride at pH 5.5 and 12.7 are similar to those observed for glycine anhydride; see Figure 2. The absorption maxima, extinction coefficients, effect of pH, pK of transient (9.6 ± 0.2), and decay kinetics are all close to the values obtained for glycine anhydride; see Table II.

While the reaction at pH 6.2 of OH radicals with sarcosine anhydride also produces a transient with absorption maxima at 265 and 365 nm and similar extinction coefficients, no effect of pH up to pH 12.8 was observed; see Figure 3. This is in marked contrast to the results obtained for glycine and alanine anhydrides. The extinction coefficients and decay rates of the intermediates at pH 6.2 and 12.8 are given in Table II.

Reaction of e^-_{aq} with Cyclic Dipeptides. The transient optical spectra of the intermediates produced

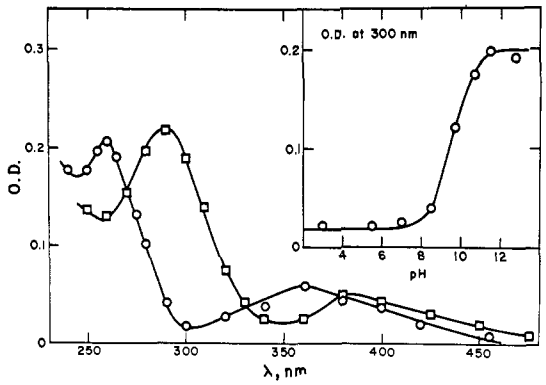


Figure 2. Transient absorption resulting from the reaction of OH radicals with alanine anhydride (10 m*M*); N_2O (1 atm): pH 5.0, ○; pH 12.7, □. Insert: OD_{300} *vs.* pH curve of transient. Total dose ~2.4 krads/pulse.

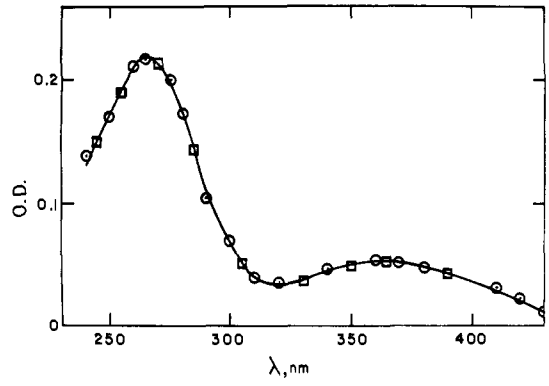


Figure 3. Transient absorption resulting from the reaction of OH radicals with sarcosine anhydride (4 m*M*); N_2O (1 atm): pH 5.5, ○; pH 12.4, □. Total dose ~2.4 krads/pulse.

from the reaction of hydrated electrons with glycine, alanine, and sarcosine cyclic dipeptides are shown in Figure 4. These were obtained in 1.0 *M tert*-BuOH solutions in order to scavenge all the OH radicals. The spectra were corrected for the absorption due to the *tert*-butyl alcohol radical by subtracting one-half the absorbance obtained in presence of N_2O (1 atm) from that obtained in presence of argon (1 atm). The absorption maxima appear to lie below 240 nm in all cases. The extinction coefficients and decay kinetics are given in Table III.

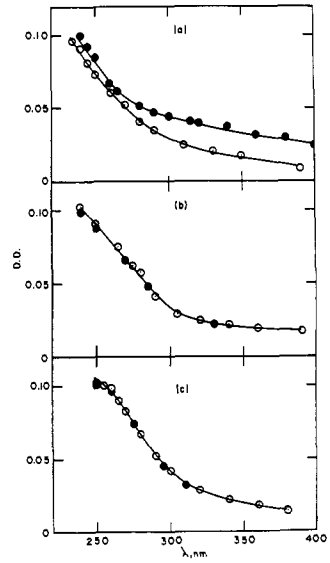


Figure 4. Transient absorption resulting from the reaction of e_{aq}^- with cyclic dipeptides in the presence of 1.0 *M tert*-butyl alcohol; 1 atm of argon; total dose ~2.4 krads/pulse: (a) 4 m*M* glycine anhydride at pH 4.9, ○, and pH 11.4, ●; (b) 4 m*M* alanine anhydride at pH 5.3, ○, and pH 12.5, ●; (c) 4 m*M* sarcosine anhydride at pH 5.1, ○, and pH 11.6, ●. These spectra are corrected in the far-uv region by taking the OD in argon *minus* $^1/_2$OD in N_2O, for the same solutions (see text).

Table III. Absorption Maxima, Extinction Coefficients, and Decay Kinetics of Transient Species Produced from the Reaction of e_{aq}^- with Cyclic Peptides in Aqueous Solution

Cyclic peptide	pH	λ_{max}, nm	ϵ, M^{-1} cm^{-1}	$2k$, M^{-1} sec^{-1} [a]
Glycine anhydride	4.9	<240	~3500[b]	1.4×10^9 (1.6×10^9)
	11.4	<240	~3500[b]	1.5×10^9 (1.4×10^9)
Alanine anhydride	5.3	<240	~3500[b]	9.2×10^8
	12.5	<240	~3500[b]	9.2×10^8
Sarcosine anhydride	5.1	<250	~3500[c]	1.5×10^9 (1.6×10^9)
	11.6	<250	~3500[c]	9.0×10^8 (9.0×10^8)

[a] Decay constant values given in parentheses were obtained in the presence of 0.2 *M* K_2SO_4, to check for salt effects in the decay kinetics of the transient species. [b] At 240 nm. [c] At 250 nm.

Discussion

OH Radical Reactions. The reaction of OH radicals with glycine anhydride (2,5-diketopiperazine) is considered to lead to a hydrogen atom abstraction with the formation of a single radical

$$OH + \underline{NHCH_2CONHCH_2CO} \longrightarrow \underline{NH\dot{C}HCONHCH_2CO} + H_2O \quad (2)$$

The absorption of this radical has a characteristic two-band spectrum with maxima at ~265 and ~365 nm; Figure 1. Its spectrum (the two bands decay with the same rate constant, indicating the presence of only one species), extinction coefficients, and the ratio of $\epsilon_{265}/\epsilon_{365}$ are all closely similar to those of the intermediates produced from the reaction of OH radicals with various amides[3] and simple linear peptides.[4,5] It can now therefore be established that these absorption spectra are characteristic of the -NHĊHCO- (or, more generally, the -NHĊRCO-) radicals. These radicals have been termed "peptide radical" in the literature.

Similar intermediates have been observed from the reaction in neutral air-free aqueous solutions of alanine and sarcosine cyclic dipeptides; see Figures 2 and 3.

$$OH + \underline{NHCH(CH_3)CONHCH(CH_3)CO} \longrightarrow \underline{NH\dot{C}(CH_3)CONHCH(CH_3)CO} + H_2O \quad (3)$$

$$OH + \underline{N(CH_3)CH_2CON(CH_3)CH_2CO} \longrightarrow \underline{N(CH_3)\dot{C}HCON(CH_3)CH_2CO} + H_2O \quad (4)$$

On irradiation of these cyclic peptides in alkaline solution, significant differences are observed in the nature of the transient optical absorption spectra of the radicals produced from glycine and alanine an-

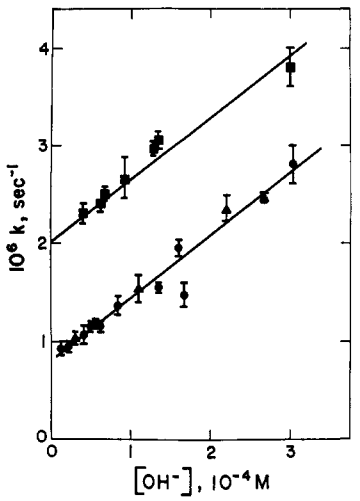


Figure 5. Kinetics of proton-transfer reaction of the peptide hydrogen in base-catalyzed reactions of peptide radicals. Bottom curve, the —NHĊHCO– radical from glycine anhydride (4 mM, in the presence of 3×10^{-4} M, ▲, an 1×10^{-3} M, ● tetraborate buffer) and top curve, the –NHC(CH_3)CO– radical from alanine anhydride (4 mM, in the presence of 1×10^{-3} M tetraborate buffer, ■).

hydrides, but not from sarcosine anhydride (Figures 1–3). By monitoring this change with pH at a fixed wavelength, one obtains an S-shaped curve (see inserts in Figures 1 and 2) similar to acid–base titration curves. That this change, in the case of glycine and alanine anhydrides, is due to an acid–base equilibrium of the intermediates is supported by the following observations and arguments. (a) The apparent dissociation constant of the radicals, $pK = 9.6 \pm 0.2$, which is the same for both alanine and glycine anhydrides, occurs at a pH well below that of the parent molecules. (b) The dissociation constant of OH radicals has $pK = 11.9$

$$OH \overset{pK \sim 11.9}{\rightleftharpoons} O\cdot^- + H^+ \quad (5)$$

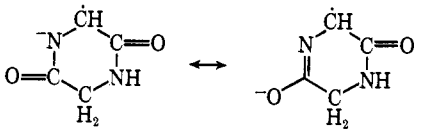
It is, therefore, possible to exclude a change in the site of attack and/or nature of the intermediate produced from the reaction with $O\cdot^-$ radicals. (c) The decay rates of the free radicals from glycine and alanine anhydrides are measurably lower at pH 11–12 compared to pH 5–6, as expected for single charged and neutral species. Furthermore, these decay rates are dependent on the ionic strength of the solution at alkaline pH values, but not at neutral pH (see Table II).

The increased second-order decay rates of the peptide radicals from glycine and alanine with increase in ionic strength indicate the presence of a negative charge on the radical. It is suggested that it is the peptide hydrogen, in an α position to the unpaired carbon atom, which *initially* undergoes proton dissociation in alkaline solution

$$\underline{NH\dot{C}RCONHCHRCO} \overset{pK = 9.6 \pm 0.2}{\rightleftharpoons} \underline{^-N\dot{C}RCONHCHRCO} + H^+ \quad (6)$$

where R = H or CH_3 for glycine and alanine anhydride, respectively. This mechanism is supported by the absence on an acid–base dissociation for the peptide radical $\underline{N(CH_3)\dot{C}HCON(CH_3)CH_2CO}$ from sarcosine anhydride and the absence of an ionic strength effect on the decay kinetics of this radical; see Figure 3 and Table II.

Owing to the electron affinity of the carbonyl peptide group, it could be argued that in alkaline solutions the charge on the peptide nitrogen is not localized and that the intermediate has resonance structures



and other structures.

Kinetics of the Proton-Transfer Reaction of the Peptide Radical. The acidity, and therefore the dissociation constant, of the peptide hydrogen adjacent to an unpaired electron could be expected to be different from that of the parent molecule. The kinetics of the rate of exchange in aqueous solution of the peptide hydrogen adjacent to an unpaired electron was therefore determined. For the peptide radical from glycine anhydride, the following equilibria can be given.

$$\underline{NH\dot{C}HCONHCH_2CO} + H_2O \rightleftharpoons \underline{^-N\dot{C}HCONHCH_2CO} + H_3O^+ \quad (7)$$

$$\underline{NH\dot{C}HCONHCH_2CO} + OH^- \rightleftharpoons \underline{^-N\dot{C}HCONHCH_2CO} + H_2O \quad (8)$$

In addition, a specific catalysis by the buffer (BH) could take place.

$$\underline{NH\dot{C}HCONHCH_2CO} + B^- \rightleftharpoons \underline{^-N\dot{C}HCONHCH_2CO} + BH \quad (9)$$

The rate of deprotonation of the peptide radical was determined on pulse radiolysis of 4 mM solutions of glycine and alanine anhydrides in the presence of N_2O (1 atm). This rate was monitored at 300 nm (see Figures 1 and 2), since at this wavelength the extinction coefficient of the peptide radical (PH·) is lowest, while that of the deprotonated peptide radical ($P\cdot^-$) is near its maximum. Under controlled buffer concentrations, the rate of formation of the deprotonated peptide radical could be observed and was measured in the pH range ~9.2–10.5. In all cases, the kinetics of formation of $P\cdot^-$ was first order. These pseudo-first-order rates are plotted as a function of [OH^-] in Figure 5. Each point on the curves is the average of at least three rate values. Within the fluctuations of the experimental points, a straight line was drawn for the radicals from glycine and alanine anhydrides.

From the slopes of the lines in Figure 5, the observed rate constant, k, can be expressed as

$$\frac{k}{1 + \dfrac{10^{-14}}{K_{PH}[OH^-]}} = k_7[H_2O] + k_8[OH^-]$$

For glycine anhydride $k_8 \sim 0.8 \pm 0.2 \times 10^{10}$ M^{-1} sec^{-1}, and for alanine anhydride $k_{10} \sim 1.1 \pm 0.2 \times 10^{10}$ M^{-1} sec^{-1}

$$\underline{NH\dot{C}(CH_3)CONHCH(CH_3)CO} + OH^- \rightleftharpoons \underline{^-N\dot{C}(CH_3)CONHCH(CH_3)CO} + H_2O \quad (10)$$

The following points can be made. (a) Within ex-

perimental error, the rate of k_8 was apparently independent of tetraborate buffer concentration (in the range 3×10^{-4}–2×10^{-3} M), glycine anhydride concentration (within a factor of 3), and peptide radical concentration (within a factor of 3). (b) In agreement with Eigen's[11] observed rates for proton-transfer reactions, when the pK difference between HA and HB is largest the reaction should be diffusion controlled in one direction (the proton falls "downhill"), and linearly dependent on ΔpK for the reaction in the opposite direction. The value of k_8 obtained is close to being diffusion controlled. (c) The dissociation constant of the peptide radical, pK = 9.6, can be expressed in terms of the rate constants of the base-catalyzed forward and backward reactions

$$K_R = k_8 K_w / k_{-8}[H_2O] \qquad (11)$$

Taking $k_8 = 8.0 \times 10^9$ M^{-1} sec^{-1}, one obtains a value of $k_{-8} \sim 10^4$ sec^{-1}. (d) The intercepts in Figure 5 could be due to reactions 7 and 9. Surprisingly, however, within the range of buffer concentrations used no significant difference in the intercept was observed. Even more surprising is the experimentally significant difference in the intercept for alanine anhydride compared to glycine anhydride. The only difference between these two radicals is the presence of a $-CH_3$ group in place of an H atom on the carbon carrying the unpaired electron. These observations and the kinetics of proton-transfer reactions of various free radicals are being examined in detail.

It should be mentioned that the rate constants of the base-catalyzed exchange reaction of peptide hydrogen in various peptides have been measured in nuclear magnetic resonance kinetic studies.[12] Sheinblatt found a rate constant $k = 6.7 \times 10^9$ M^{-1} sec^{-1} for triglycin- and 1.1×10^{10} M^{-1} sec^{-1} for diglycinamide.

$$^+NH_3CH_2CONHCH_2CONH_2 + OH^- \longrightarrow {}^+NH_3CH_2\bar{C}ONHCH_2CONH_2 + H_2O \qquad (12)$$

Reactivity of the Peptide Radical. The second-order decay of the radicals produced from the three cyclic dipeptides presumably leads to disproportionation and recombination reactions. Support for disproportionation reactions for glycine and alanine anhydrides can be shown from the product analysis work of Kland-English and Garrison,[6] who observed the formation of pyrazine derivatives.

The rates of reaction of the peptide radicals from glycine, alanine, and sarcosine anhydrides with oxygen were determined (see Table IV). These were carried out in mixtures of N_2O and O_2 under conditions such that all the e_{aq}^- reacted with N_2O and all the OH radicals reacted with the cyclic dipeptides. The decay of the radicals monitored at 360 nm was found to be pseudo first order on the oxygen concentration. Further details on the basis of these measurements and comparison with the rates of other free radicals with oxygen will be presented elsewhere.[13] In alkaline solution, the $^-N\dot{C}HCONHCH_2CO$ radical reacts more slowly with O_2, and no permanent product absorbing at 360 nm is observed. $^-N\dot{C}(CH_3)CONHCH(CH_3)CO$ gives a permanent product with O_2 at 360 nm.

(11) M. Eigen, *Fast React. Primary Processes Chem. Kinet., Proc. Nobel Symp., 1967*, 5, 245 (1967).
(12) M. Sheinblatt, *J. Amer. Chem. Soc.*, 92, 2505 (1970).
(13) E. Hayon, in preparation.

Table IV. Absolute Rate Constants for Reaction of Cyclic Dipeptide Radicals with Oxygen in Aqueous Solution

Radical, R	pH	λ monitored, nm	$k(R\cdot + O_2)$, M^{-1} sec^{-1}
$NH\dot{C}HCONHCH_2CO$	5.0	360	$1.2 \pm 0.1 \times 10^9$
$^-N\dot{C}HCONHCH_2CO$	12.0	360	$2.8 \pm 0.3 \times 10^8$
$NH\dot{C}(CH_3)CONHCH(CH_3)CO$	5.4	360	$1.0 \pm 0.2 \times 10^9$
$^-N\dot{C}(CH_3)CONHCH(CH_3)CO$	12.0	360	$1.1 \pm 0.2 \times 10^9$
$N(CH_3)\dot{C}HCON(CH_3)CH_2CO$	5.2	360	$0.9 \pm 0.2 \times 10^9$

A number of organic free radicals undergo a repair mechanism on reaction with sulfhydryl compounds, *e.g.*, with cysteine

$$R\cdot_1 + R_2SH \longrightarrow R_1H + R_2S\cdot \qquad (13)$$

Values of k_{13} up to $\sim 10^9$ M^{-1} sec^{-1} have been obtained.[14] Solutions of 10–20 mM cyclic dipeptides were irradiated in the presence of 1–5×10^{-4} M cysteine and N_2O (1 atm) at pH 6, 10, and 12. Under these conditions, all the OH radicals react with the dipeptides and all the e_{aq}^-'s react with N_2O. No change in the decay rate of the peptide radicals could be observed at 265 nm and no formation of $(RSSR)^-$ was seen at 410 nm. From these observations it would appear that the rate constant of the repair mechanism of these radicals with cysteine is below $\sim 10^7$ M^{-1} sec^{-1}.

e_{aq}^- Reactions. The cyclic dipeptides of glycine, alanine, and sarcosine have relatively high reactivities with hydrated electrons, $k(e_{aq}^- +$ cyclic dipeptide$) \sim 2.0 \times 10^9$ M^{-1} sec^{-1}; see Table I. These rates are significantly higher than those of the corresponding linear[5] dipeptides: $k(e_{aq}^- + {}^+NH_2CH_2CONHCH_2COO^-) = 3.7 \times 10^8$ M^{-1} sec^{-1} and $k(e_{aq}^- + NH_2CH_2CONHCH_2COO^-) = 4.9 \times 10^7$ M^{-1} sec^{-1}. The rates of e_{aq}^- with the linear diglycine esters or amides $^+H_2$–Gly–Gly–OMe and $^+H_2$–Gly–Gly–NH_2 have been found to be $\sim 1.7 \times 10^9$ M^{-1} sec^{-1}, and close to the values for cyclic dipeptides. These higher values probably arise from resonance stabilization of the electron adducts.

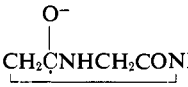
With linear amino acid peptides, the hydrated electron has been shown[4,5] to lead to almost quantitative deamination, *e.g.*

$$e_{aq}^- + {}^+NH_3CH_2CONHR \longrightarrow NH_3 + \dot{C}H_2CONHR \qquad (14)$$

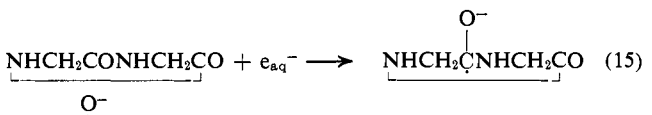
With cyclic dipeptides, the amino group is absent. The primary intermediate is presumably the electron adduct. The transient absorptions of this species are shown in Figure 4. These species have maxima below 240 nm and relatively high extinction coefficients. They decay by an almost[15] perfect second-order process, but no definitive ionic strength effect could be observed; see Table III. It is therefore not possible

(14) G. E. Adams, G. S. McNaughton, and B. D. Michael, *Trans. Faraday Soc.*, 64, 902 (1968); M. Z. Hoffman and E. Hayon, in preparation.
(15) The electron adducts could be decaying by reaction with the *tert*-butyl alcohol radicals.

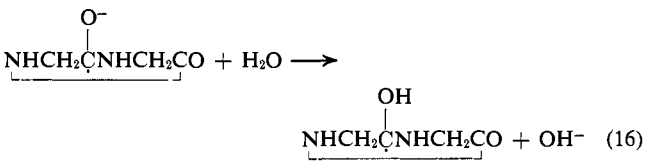
Table V. Rates of Electron Transfer from Cyclic Peptide Radicals to Various Acceptors

Donor	Acceptor	pH	λ monitored, nm	k(electron transfer), M^{-1} sec^{-1}
	Cysteine	6.4	265	$2.1 \pm 0.4 \times 10^8$
	$HSCH_2CH_2COO^-$	5.3, 7.4	265	$3.0 \pm 0.3 \times 10^8$
	Glutathione (RSH)	6.4, 7.4	265	$1.8 \pm 0.3 \times 10^8$
	Cystamine (RSSR)	5.7, 11.0	265	$1.2 \pm 0.3 \times 10^8$
	Glutathione (RSSR)	6.2	275	$4.0 \pm 0.4 \times 10^7$
	Acetophenone	5.2	280	$2.3 \pm 0.3 \times 10^9$
	Acetophenone	12.3	320	$2.0 \pm 0.3 \times 10^9$
	Benzophenone	5.5	330	$2.2 \pm 0.3 \times 10^9$
	Benzophenone	12.3	320	$2.5 \pm 0.4 \times 10^9$
	Cysteine	7.4	265	$1.4 \pm 0.3 \times 10^8$
	Cystamine (RSSR)	5.1, 11.4	265	$1.1 \pm 0.2 \times 10^8$
	Acetophenone	5.2	280	$2.0 \pm 0.3 \times 10^9$
	Acetophenone	12.2	320	$1.5 \pm 0.4 \times 10^9$
	Benzophenone	5.2	330	$1.6 \pm 0.2 \times 10^9$
	Benzophenone	12.2	320	$1.9 \pm 0.3 \times 10^9$
	Cysteine	7.0	265	$1.5 \pm 0.3 \times 10^8$
	Acetophenone	5.2	280	$2.0 \pm 0.2 \times 10^9$
	Acetophenone	12.4	320	$2.1 \pm 0.2 \times 10^9$
	Benzophenone	5.2	330	$2.3 \pm 0.2 \times 10^9$
	Benzophenone	12.2	320	$2.4 \pm 0.2 \times 10^9$

to establish whether the intermediates observed in neutral solution are radicals or radical anions



(15)



(16)

However, from the rates of electron transfer to various acceptors (see below), it could be argued that the species are present as radical anions in neutral solution.

Solutions of 4 mM glycine anhydride and 0.2 M sodium formate in N_2O (1 atm) were pulse radiolyzed at pH 6.2. Under these conditions, all the e_{aq}^-'s react with N_2O and all the OH radicals with $HCOO^-$. No formation of the peptide radical could be observed, indicating that the rate of the electron-transfer reaction from $CO_2\cdot^-$ to the dipeptide is relatively slow, $<10^7$ M^{-1} sec^{-1}.

Rates of Electron Transfer from Peptide Radicals. Inter- and/or intramolecular electron-transfer reactions could be occurring in polypeptides and proteins. Since in some ways cyclic dipeptides are models for the polypeptides (these have one $-NH_3^+$ and one $-COO^-$ group connected by peptide linkages), the absolute rates of electron transfer from the electron adducts to various selected acceptors were determined.

For cysteine, thiopropionic acid, glutathiones (RSH and RSSR), and cystamine, these rates were determined by following the decay rate of the electron adducts (Figure 4) at 265 nm. In all cases, the transients decayed to "base line" and the rates were pseudo first order relative to the acceptor concentration. With acetophenone and benzophenone, these rates were determined by following the rate of formation of the corresponding ketyl radical or radical anion. New intense absorption bands for these ketyl radicals were discovered in the uv region.[16] For example, for acetophenone $PhC(OH)CH_3$ has a maximum at 273 nm and ϵ_{273} 2.9×10^4 M^{-1} cm^{-1} and $PhC(O^-)CH_3$ has ϵ_{314} 2.9×10^4 M^{-1} cm^{-1}. The rates of formation were monitored at these bands.

Table V presents the rates of electron transfer from the electron adducts of the three cyclic dipeptides to certain important functional groups, *viz.*, RSH, RSSR, and aromatic ketones. From these data, it can be seen that electron-transfer rates to sulfhydryl or disulfide groups are the same, with $k \sim 1\text{–}3 \times 10^8$ M^{-1} sec^{-1}. However, transfer to aromatic ketones is one order of magnitude faster. Furthermore, the rate of transfer to various acceptors is the same at pH 6.0 and pH 12.0. These results do not prove but support the argument that the electron adducts are reacting as radical anions. These observations (Table V) are potentially of considerable interest and indicate the possibility of electron-transfer reactions in proteins and enzymes.

Acknowledgment. An interesting discussion with Dr. J. Feitelson and with Dr. E. Grunwald must be acknowledged.

(16) E. Hayon, T. Ibata, N. N. Lichtin, and M. Simic, in preparation.