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## Interaction of Hydrated Electrons with the Peptide Linkage

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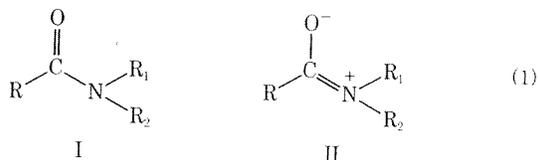
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The reaction rate constants  $k$  of  $e_{aq}^-$  with a wide range of peptides having terminal  $-NH_3^+$  and  $N$ -acetyl groups have been determined in aqueous solutions, using the technique of pulse radiolysis. These rates were shown to be markedly dependent on the state of ionization of the terminal  $-NH_3^+$  groups, the number of peptide linkages, and the overall charge on the peptide molecules. Amino groups in a  $\beta$  (or  $\delta$ ,  $\gamma$ ) position are significantly less reactive than the corresponding  $\alpha$ -amino peptides. The inductive effects on the peptide hydrogen  $-CONH-$  affect the reactivity toward  $e_{aq}^-$ , supporting previous suggestions that  $e_{aq}^-$  interacts with the carbonyl group. The rate constants  $k$  have been shown to be directly proportional to base-catalyzed rates for ionization of the peptide hydrogen of the same peptides, as determined by nmr. A similar correlation has been found between  $k$  and the ionization constants of the carboxylic acids of the corresponding peptides. Transient optical absorption spectra have been observed from the reaction of  $e_{aq}^-$  with the  $N$ -acetyl derivatives of triglycine, hexaalanine, and trisarcosine. These spectra are dependent upon pH and are suggested to be formed from the interaction of  $e_{aq}^-$  with the carbonyl group of the peptide linkage. The ketyl radicals formed can undergo acid-base equilibration  $-\dot{C}(O^-)NH- + H^+ \rightleftharpoons -\dot{C}(OH)NH-$ , with  $pK_a(\text{radical}) \geq 12.0$ . These ketyl radicals have very low (negative) redox potentials and are strong reducing agents.

### Introduction

The principal linkage between the various amino acids making up a protein is the peptide bond  $-CONH-$ . Considerable experimental and theoretical studies have therefore been carried out on this linkage. The conductivity and electron transfer properties of proteins is also of particular importance, and hence the interest in studying the interaction of electrons with the peptide bond.

Nmr studies (see ref 2 for review up to 1969) have indicated the partial double-bond character of the amide bond



As a result<sup>2,3</sup> of this electronic delocalization, the linkage is planar and long-range spin coupling from R to  $R_1$  and  $R_2$  can be expected. The peptide hydrogen ( $R_1 = H$ ) can undergo both base- and acid-catalyzed exchange reactions (see, e.g., ref 4-6). Sheinblatt<sup>4</sup> has determined by nmr the rates of the base-catalyzed exchange, reactions 2 and 3, and established an acidity scale for the peptide hydrogen (see more below)



or, more generally



The  $k_2$  rates were found<sup>4</sup> to be strongly dependent on the nature of R and  $R_1$  (when  $R_2 = H$ ).

The reaction rate constants for the interaction of hydrated electrons,  $e_{aq}^-$ , with amino acids and peptides were correlated by Braam<sup>7</sup> with the ionization constants of the terminal  $-NH_3^+$  groups. This reactivity has since been correlated<sup>8-12</sup> with the presence and the number of carbonyl groups (i.e., the number of peptide linkages). The cooperative effect of the peptide linkage(s), the amino and carbonyl groups, and the overall charge on the molecule have been suggested<sup>11</sup> to contribute to the reactivity toward  $e_{aq}^-$ .

The reaction of  $e_{aq}^-$  with simple amino acids<sup>8,13</sup> and oligopeptides<sup>9,11,14,15</sup> has been shown to result in reductive deamination



The  $\dot{\text{C}}\text{H}_2\text{CONHR}$  radicals have been identified by pulse

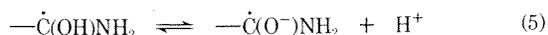
TABLE I: Rate Constants for the Reaction of  $e_{aq}^-$  with Peptides

Peptide	Ionic form	pH	$k(e_{aq}^- + \text{peptide}),$ $M^{-1} \text{sec}^{-1}$ <sup>a</sup>
<i>N</i> -Acetylglycine	Ac-Gly-O <sup>-</sup>	11.5	$2.6 \times 10^{6b}$
<i>N</i> -Acetyldiglycine	Ac-Gly <sub>2</sub> O <sup>-</sup>	11.2	$6.4 \times 10^{7b}$
<i>N</i> -Acetyltriglycine	Ac(Gly) <sub>3</sub> O <sup>-</sup>	9.2	$4.4 \times 10^8$
<i>N</i> -Acetylalanine	Ac-Ala-O <sup>-</sup>	7.0	$1.2 \times 10^7$
<i>N</i> -Acetyltrialanine	Ac(Ala) <sub>3</sub> O <sup>-</sup>	9.2	$6.8 \times 10^8$
<i>N</i> -Acetylhexaalanine	Ac(Ala) <sub>6</sub> O <sup>-</sup>	9.2	$8.2 \times 10^8$
<i>N</i> -Acetylsarcosine	Ac-Sar-O <sup>-</sup>	12.5	$9.0 \times 10^6$
<i>N</i> -Acetyltrisarcosine	Ac(Sar) <sub>3</sub> O <sup>-</sup>	9.0	$3.9 \times 10^8$
Glycine	<sup>+</sup> H <sub>2</sub> -Gly-O <sup>-</sup>	6.4	$8.2 \times 10^{6c}$
Alanine	<sup>+</sup> H <sub>2</sub> -Ala-O <sup>-</sup>	7.4	$1.2 \times 10^7$
$\beta$ -Alanine	<sup>+</sup> H <sub>2</sub> - $\beta$ Ala-O <sup>-</sup>	6.9	$4.2 \times 10^6$
Sarcosine	<sup>+</sup> H <sub>2</sub> -Sar-O <sup>-</sup>	7.0	$1.6 \times 10^7$
Glycylglycine	<sup>+</sup> H <sub>2</sub> (Gly) <sub>2</sub> O <sup>-</sup>	6.4	$3.7 \times 10^{8b}$
	H(Gly) <sub>2</sub> O <sup>-</sup>	13.1	$4.9 \times 10^{7b}$
Glycylsarcosine	<sup>+</sup> H <sub>2</sub> -Gly-Sar-O <sup>-</sup>	6.4	$6.9 \times 10^8$
	H-Gly-Sar-O <sup>-</sup>	11.3	$1.0 \times 10^8$
Sarcosylglycine	<sup>+</sup> H <sub>2</sub> -Sar-Gly-O <sup>-</sup>	5.8	$8.8 \times 10^8$
	H-Sar-Gly-O <sup>-</sup>	12.2	$7.7 \times 10^7$
Glycyl- $\beta$ -alanine	<sup>+</sup> H <sub>2</sub> -Gly- $\beta$ Ala-O <sup>-</sup>	6.4	$6.5 \times 10^8$
	H-Gly- $\beta$ Ala-O <sup>-</sup>	11.3	$6.3 \times 10^7$
Glycyl- $\beta$ -alanine amide	<sup>+</sup> H <sub>2</sub> -Gly- $\beta$ Ala-NH <sub>2</sub>	6.0	$1.4 \times 10^9$
	H-Gly- $\beta$ Ala-NH <sub>2</sub>	12.0	$3.3 \times 10^8$
$\beta$ -Alanyl- $\beta$ -alanine	<sup>+</sup> H <sub>2</sub> - $\beta$ Ala- $\beta$ Ala-O <sup>-</sup>	5.8	$1.2 \times 10^8$
	H- $\beta$ Ala- $\beta$ Ala-O <sup>-</sup>	12.2	$4.5 \times 10^7$
Prolylglycine	<sup>+</sup> H <sub>2</sub> -Pro-Gly-O <sup>-</sup>	6.2	$9.6 \times 10^8$
	H-Pro-Gly-O <sup>-</sup>	10.8	$4.7 \times 10^7$
$\delta$ -Valylglycine	<sup>+</sup> H <sub>2</sub> -Val-Gly-O <sup>-</sup>	6.2	$1.3 \times 10^7$
$\gamma$ -Aminobutyric glycine	<sup>+</sup> H <sub>2</sub> - $\gamma$ AmBut-Gly-O <sup>-</sup>	6.0	$3.4 \times 10^7$
$\beta$ -Alanyldiglycine	<sup>+</sup> H <sub>2</sub> - $\beta$ Ala(Gly) <sub>2</sub> O <sup>-</sup>	6.4	$2.8 \times 10^8$
	H- $\beta$ Ala(Gly) <sub>2</sub> O <sup>-</sup>	12.2	$7.1 \times 10^7$
Diglycyl- $\beta$ -alanine	<sup>+</sup> H <sub>2</sub> (Gly) <sub>2</sub> $\beta$ Ala-O <sup>-</sup>	5.5	$1.4 \times 10^9$
	H(Gly) <sub>2</sub> $\beta$ Ala-O <sup>-</sup>	12.2	$2.3 \times 10^8$
Triglutamic acid	<sup>+</sup> H <sub>2</sub> (Glu) <sub>3</sub> O <sup>-</sup>	6.3	$2.3 \times 10^9$
	H(Glu) <sub>3</sub> O <sup>-</sup>	9.6	$5.8 \times 10^8$
Tetraglycine	<sup>+</sup> H <sub>2</sub> (Gly) <sub>4</sub> O <sup>-</sup>	5.9	$2.6 \times 10^9$
	H(Gly) <sub>4</sub> O <sup>-</sup>	10.2	$3.9 \times 10^8$
Pentaglycine	<sup>+</sup> H <sub>2</sub> (Gly) <sub>5</sub> O <sup>-</sup>	6.1	$4.0 \times 10^9$
	H(Gly) <sub>5</sub> O <sup>-</sup>	11.2	$5.6 \times 10^8$

<sup>a</sup> Determined in  $\sim 1.0 M$  aqueous *tert*-butyl alcohol solutions by monitoring the decay kinetics of  $e_{aq}^-$  at 700 nm; values better than  $\pm 10\%$ . <sup>b</sup> From ref 11. <sup>c</sup> From ref 24.

radiolysis<sup>9-11</sup> and confirmed by esr.<sup>14,15</sup> The *initial* site of reaction of  $e_{aq}^-$  is probably the peptide linkage.

The site of attack of  $e_{aq}^-$  with amides, imides, and peptides in aqueous solution has been found<sup>12,16,17</sup> to be the amide or peptide linkage. Characteristic transient optical absorption spectra have been observed (in the microsecond time scale) using the fast-reaction technique of pulse radiolysis. These spectra are pH dependent and were shown<sup>17</sup> to be a result of ionization of the radical, *e.g.*



with  $pK_a$  (radical) values ranging from 3-13, dependent on the nature of R, R<sub>1</sub>, and R<sub>2</sub> in structure I.

In this work, the reaction rate constants of  $e_{aq}^-$  with a number of oligopeptides including *N*-acetyl derivatives and  $\beta$ -alanine derivatives have been determined. These data have been correlated with certain physicochemical properties of these molecules, as determined in nmr studies. The ionization of the  $-\dot{C}(\text{OH})\text{NH}-$  radical, reaction 6, has also been demonstrated.



## Experimental Section

The pulse radiolysis experimental set-up used has been

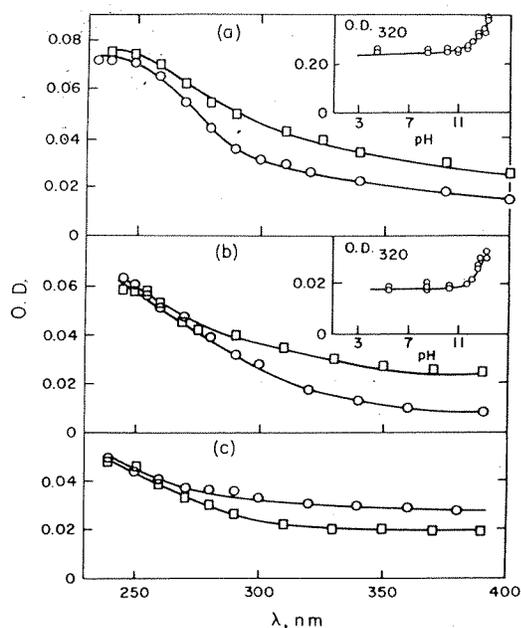
described.<sup>18,19</sup> The reaction rate constants of  $e_{aq}^-$  with peptides were determined in presence of  $\sim 0.1-1.0 M$  *tert*-butyl alcohol (to scavenge the OH radicals produced in the radiolysis of water) by monitoring the decay kinetics of  $e_{aq}^-$  at 700 nm. From the pseudo-first-order-decay of  $e_{aq}^-$ , the second-order reaction rate constants were derived. Solutions were buffered using  $\sim 1 \text{ mM}$  phosphate or borate buffers, and the pH was adjusted with perchloric acid and potassium hydroxide.

The chemicals used were the highest purity research grade commercially available, and were obtained from Cyclochemicals, Miles Laboratory, Fox Chemical Co., and Calbiochem. They were used as received.

Dosimetry was carried out using KCNS solution as described.<sup>18</sup> Extinction coefficients were derived based on  $G(\text{OH}) = G(e_{aq}^-) = 2.8$ .

## Results and Discussion

*Transient Species.* The reaction of  $e_{aq}^-$  with simple peptides having terminal amino groups were shown to lead to reductive deamination,<sup>8,9,11,13</sup> reaction 4. The reaction of  $e_{aq}^-$  with *N*-acetyl derivatives of peptides leads to addition to the carbonyl group of the peptide linkages, with the rate constant increasing with increase in the number of  $-\text{CONH}-$  groups, see Table I. These



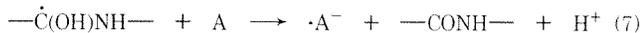
**Figure 1.** Transient optical absorption spectra produced from the reaction of  $e_{aq}^-$  with (a)  $10^{-2} M$  *N*-acetyltryglycine at pH 5.2, O, and pH 13.3, □; (b)  $10^{-2} M$  *N*-acetyltrisarcosine at pH 5.5, O, and pH 13.4, □; (c)  $0.5 mM$  *N*-acetylhexaalanine at pH 6.2, O, and pH 13.3, □. In all cases  $2.0 M$  *t*-BuOH was added to scavenge the OH radicals. Total dose  $\sim 4.0$  krad/pulse. Inserts show change in absorbance at 320 nm with pH. OD measured  $\sim 0.2 \mu\text{sec}$  after electron pulse.

electron adducts have relatively low extinction coefficients and absorption maxima below  $\sim 240$  nm. Figure 1 shows the transient spectra produced from *N*-acetyltryglycine, *N*-acetyltrisarcosine and *N*-acetylhexaalanine. These spectra are similar<sup>12</sup> to those formed by addition of  $e_{aq}^-$  to the cyclic dipeptides of glycine, alanine, and sarcosine.

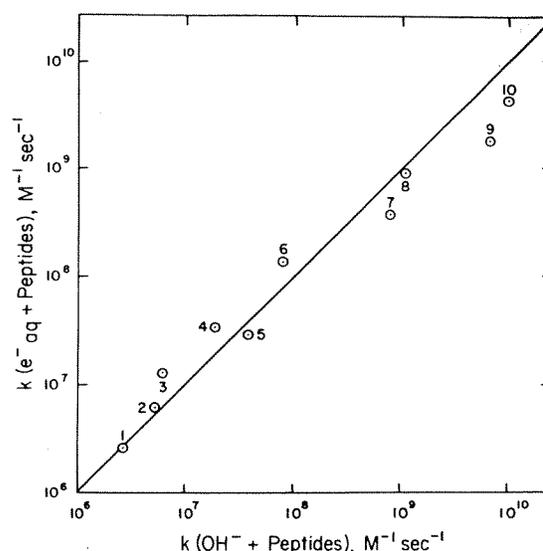
Slight differences with pH are observed in the optical spectra of these transient species, Figure 1, and these are interpreted to be due to the acid-base properties of the radicals, reaction 6. The  $pK_a$  (radical) are  $\geq 12.0$ , indicating that these radicals are very weak acids. Since *N*-acetyltrisarcosine produces similar transient absorptions which are also pH dependent, it is concluded that the peptide hydrogens are not involved in the ionization reaction 6.

The reaction of  $e_{aq}^-$  with polysarcosine (mol wt  $\sim 3000$ ,  $3.0 \times 10^{-4} M$  solutions used in presence of  $0.5 M$  *t*-BuOH) generated similar transient spectra (not shown) which were also dependent on pH. The maxima were below 240 nm, and  $\epsilon_{250} 1300 M^{-1} \text{cm}^{-1}$  at pH 6.3 and  $\epsilon_{250} 1500 M^{-1} \text{cm}^{-1}$  at pH 13.3.

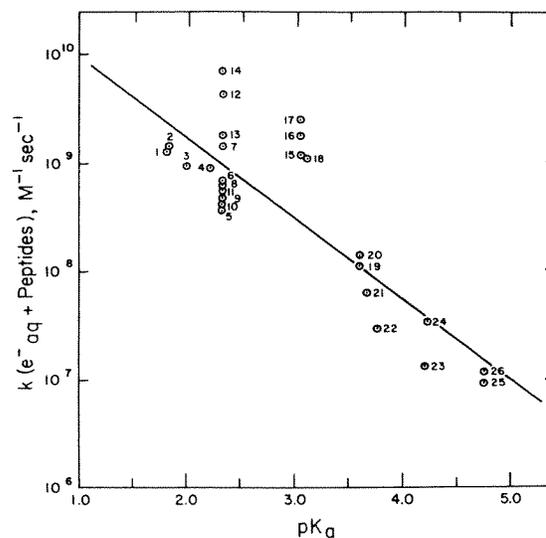
It is interesting to note<sup>20,21</sup> that  $-\dot{C}(\text{OH})\text{NH}-$  and  $-\dot{C}(\text{OH})\text{NCH}_3-$  radicals transfer an electron very efficiently to a range of acceptors, A



with  $k_7$  values close to diffusion-controlled rates.<sup>21</sup> These rates are dependent upon the redox potentials of the acceptor molecules and the donor radicals. The  $-\dot{C}(\text{OH})\text{NH}-$  radicals have been found<sup>22</sup> to have very low redox potentials,  $E^{01} \leq -1.2 \text{ V}$  (where  $E^{01}$  notation is at pH 7.0 and 25°) making these peptide radicals powerful reducing agents. These results support the hypothesis of intramolecular electron transfer processes in proteins and peptides.



**Figure 2.** Correlation between the rate constants of  $e_{aq}^-$  with some peptides and the rate constants for base-catalyzed ionization of the peptide hydrogen of the same peptides as derived by Scheinblatt:<sup>4</sup> 1, *N*-Ac-Gly; 2, *N*-Me-acetamide; 3,  $\delta$ -Val-Gly; 4,  $\gamma$ -Am-But-Gly; 5, *N*-Form-Gly; 6,  $\beta$ -Ala-Gly; 7, Gly-Gly; 8, Pro-Gly; 9, (Gly)<sub>3</sub>; 10, Gly-Gly-NH<sub>2</sub>.



**Figure 3.** Correlation between the rate constants of  $e_{aq}^-$  with peptides and the  $pK_a$  of the carboxyl group of the corresponding acids (see text and ref 23): 1, His-His; 2, Phe-NH<sub>2</sub>; 3, Pro-Gly; 4, Sar-Gly; 5, Gly-Gly; 6, Gly-Sar; 7, Gly- $\beta$ -Ala-NH<sub>2</sub>; 8, Gly- $\beta$ -Ala; 9, Gly-Trp; 10, Gly-Tyr; 11, Gly-Phe; 12, Gly-Gly-NH<sub>2</sub>; 13, (Gly)<sub>3</sub>; 14, (Gly)<sub>3</sub>-NH<sub>2</sub>; 15, Gly-Gly-Phe; 16, Gly-Gly- $\beta$ -Ala; 17, (Gly)<sub>4</sub>; 18, Phe-Gly-Gly; 19,  $\beta$ -Ala- $\beta$ -Ala; 20,  $\beta$ -Ala-Gly; 21, *N*-Ac-Gly-Gly; 22, *N*-Form-Gly; 23,  $\delta$ -Val-Gly; 24,  $\gamma$ -Am-But-Gly; 25, *N*-Ac-Sar; 26, *N*-Ac-Ala.

**Reactivity toward  $e_{aq}^-$ .** The reaction rate constants of  $e_{aq}^-$  with a number of peptides and their derivatives have been determined as a function of the state of protonation of the terminal amino groups. The  $pK_a$  of  $-\text{NH}_3^+$  groups are known.<sup>23</sup> These results and a few others from the literature<sup>11,24</sup> are presented in Table I. The following points can be made from this data.

(a) The rate constants decrease with ionization of the terminal amino groups, in agreement with results on other peptides.<sup>7,11,24</sup>

(b) The rate constants increase with the number of peptide linkages. Even in pentaglycine, however, ionization of the terminal  $\text{-NH}_3^+$  group reduces  $k$  from  $4.0 \times 10^9$  to  $5.6 \times 10^8 \text{ M}^{-1} \text{ sec}^{-1}$ .

(c) Some correlation appears between the rate constants and the overall charge on the molecules. The amide or ester derivatives of the peptides are significantly more reactive (Table I and ref 11) than the corresponding  $\text{-COO}^-$  derivatives.

(d) The  $\beta$ -amino derivatives have lower reactivities with  $\text{e}_{\text{aq}}^-$ , particularly when present in the terminal position. With  $\delta$ - and  $\gamma$ -amino peptides, the rate constants decrease further.

(e) Sarcosyl peptides  $\text{-CON(CH}_3\text{)-}$  appear to be slightly more reactive than the corresponding  $\text{-CONH-}$  peptides.

Thus inductive effects on the peptide hydrogen, as well as on substituents on both sides of the peptide linkage (*i.e.*, the nature of  $\text{R}$ ,  $\text{R}_1$ , and  $\text{R}_2$  in structure I), affect the reactivity of the  $\text{-CONH-}$  group to the attack by  $\text{e}_{\text{aq}}^-$ .

Many of the factors mentioned above to explain the reactivity of peptides toward  $\text{e}_{\text{aq}}^-$  appear to affect also the base-catalyzed rate constants,  $k_2$ , as determined by Sheinblatt.<sup>4</sup> The  $k_2$  rates vary by about three orders of magnitude as do the rates for reaction of  $\text{e}_{\text{aq}}^-$  with the same compounds. Indeed a linear correlation can be demonstrated between these rates, and is represented in Figure 2. The agreement is remarkably good and would seem to support the acidity scale suggested<sup>4</sup> for the peptide hydrogen. Such a relationship can be used to predict  $k_2$  and/or  $k(\text{e}_{\text{aq}}^- + \text{peptide})$  rates.

The acidity of the peptide hydrogen has also been correlated<sup>4,5</sup> with the acidity ( $\text{p}K_a$ ) of the carboxylic group of the corresponding peptides. Figure 3 shows a plot of  $k(\text{e}_{\text{aq}}^- + \text{peptide})$  vs. the  $\text{p}K_a(\text{-COOH})$  of the corresponding peptide (*e.g.*, for glycylglycine, the  $\text{p}K_a(\text{COOH})$  of glycine was plotted). This correlation is in agreement with  $k_2$  values as determined<sup>4,5</sup> by nmr.

## Conclusions

This study shows that the inductive effects of substituents on the peptide linkage affect considerably the reactivity of peptides toward  $\text{e}_{\text{aq}}^-$ . Factors affecting the proton transfer<sup>4</sup> reactions of the peptide hydrogen, as can be described by a generalized Brønsted relationship, are the same as those which govern the interaction with  $\text{e}_{\text{aq}}^-$ .

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