Reactions of Dichloride Anion Radicals with Aliphatic Peptides in Aqueous Solutions

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Rate constants for the reactions of dichloride anion radicals with aliphatic peptides were determined by a conventional flash photolysis of 1 M solutions of potassium chloride containing aliphatic peptides. The rate constants were found to increase with increasing number of C-H bond. Partial rate constants were assigned to each C-H bond of the aliphatic peptides, as estimated for the reactions of hydroxyl radicals with the same substrates.

Trend in the partial rate constants and the effect of a protonated amino group on the reactivity indicate a reaction mechanism, hydrogen atom abstraction, similar to hydroxyl radicals.

INTRODUCTION

Dichloride anion radical (Cl⁻₂) is a transient oxidant produced in photolysis or in radiolysis of aqueous chloride and its reactions with biomolecules have been investigated from the viewpoint of high reactivity toward aromatic amino acids, in particular, tryptophan.1-3) On the other hand, the reaction rates with aliphatic compounds were found to be much slower than those for aromatic compounds.4)

In a previous study,5) we determined the rate constants for reactions of dichloride anion radicals with aliphatic amino acids in aqueous neutral solutions by a conventional flash photolysis and discussed them in comparison with the reactivity of hydroxyl radicals toward the same substrates. In the reactions of hydroxyl radicals, partial rate constants were assigned satisfactorily to each C-H bond of the aliphatic amino acids.6) However, we failed to estimate the partial rate constants for the reactions of dichloride anion radicals. The failure is due possibly to the usage of relatively dilute solutions of potassium chloride (0.1 M). The concentration was chosen by consideration of a probable application of this method to proteins. However, the dilute aqueous potassium chloride does not afford a sufficient amount of dichloride anion radicals, especially in
the presence of a substrate, under the condition of flash lamp with 200 J of input energy. A weak absorption of small amount of dichloride anion radicals causes a fluctuation in analysis of the decay process.

Another reason may be attributed to the use of the Regular Falsh method for the calculation of parameter, $k[RH]$, because the method requires a close approximation of the initial values, which sandwich a true solution.

In the present study, these problems were almost solved by raising the concentration of chloride up to 1 M and by replacing the Regular Falsch method by a best fit method. The aliphatic peptides were subjected to the determination of rate constants for the reactions with dichloride anion radicals and an attempt was made to estimate the partial rate constants assigned to each C-H bond of the aliphatic peptides. The determined rate constants indicate an increase in reactivity of the aliphatic peptides toward the anion radicals with increasing number of C-H bond, as already observed in the reactions of the hydroxyl radicals with the same substrates. The values were found to be two orders of magnitude smaller than those for the reactions of the hydroxyl radicals. The estimated partial rate constants, the suppressive effect of a protonated amino group on the reactivity of neighboring C-H bond, and a mechanism of the reaction will be discussed.

**MATERIALS AND METHOD**

Peptides were purchased from Nutritional Biochemicals Corp. and used without further purification. Aqueous solutions of potassium chloride (1 M) containing various concentrations of a peptide were prepared with triply distilled water and adjusted to pH 7 with aqueous potassium hydroxide, if necessary. Before irradiation, the solutions were bubbled with nitrous oxide for 20 minutes.

A conventional flash photolysis apparatus was operated with 200 J of input energy and 10 microseconds of 1/e duration. Other conditions were described in detail elsewhere.

Calculation for kinetic analysis was carried out with the aid of a microcomputer PC-8001 of Nihon Electric Co. The molar absorption coefficient of dichloride anion radical at 350 nm was taken as $7.6 \times 10^3$ M$^{-1}$ cm$^{-1}$.

**RESULTS AND DISCUSSION**

A dichloride anion radical has been established to be produced in UV-photolysis of aqueous chloride solutions.

$$\text{Cl}^- + h\nu \longrightarrow \text{Cl}^- + e^-$$  \hspace{1cm} (1)

$$\text{Cl}^- + \text{Cl}^- \rightleftharpoons \text{Cl}_2$$  \hspace{1cm} (2)
When a substrate is absent, it decays according to second order kinetics.

\[
\frac{2\text{Cl}_2}{2k_3} \rightarrow 2\text{Cl}^- + \text{Cl}_2 \tag{3}
\]

In the presence of a substrate (RH), the dichloride anion radical reacts with the substrate, in addition to reaction 3.

\[
\text{Cl}_2^- + \text{RH} \xrightarrow{k_4} \text{product(s)} + 2\text{Cl}^- \tag{4}
\]

From these reactions, the following rate equation can be derived.

\[
-\frac{d[\text{Cl}_2^-]}{dt} = 2k_3 [\text{Cl}_2^-]^2 + k_4 [\text{Cl}_2^-][\text{RH}] \tag{5}
\]

By integrating equation 5 under the condition of \([\text{RH}] > [\text{Cl}_2^-]\), the following equation is obtained.

\[
\frac{1}{k_{\text{ob}}} \ln \frac{[\text{Cl}_2^-]_0 (2k_3 [\text{Cl}_2^-] + k_{\text{ob}})}{[\text{Cl}_2^-]_0 (2k_3 [\text{Cl}_2^-]_0 + k_{\text{ob}})} = t \tag{6}
\]

where \(k_{\text{ob}}\) is equal to \(k_4 [\text{RH}]\); \([\text{Cl}_2^-]_0\) and \([\text{Cl}_2^-]\) are the concentrations of the dichloride anion radicals at time zero and t, respectively. Time zero was set on 10 microseconds after the start of flash, which corresponds to a peak of the exciting light intensity. The initial concentration \([\text{Cl}_2^-]_0\) can be estimated by extrapolation of \(1/[\text{Cl}_2^-]\) to time zero, because a second-order plot indicates a good linear relation between time and reciprocal of the concentration of the dichloride anion radicals, in the absence of a substrate. In the previous study, the calculation of \(k_{\text{ob}}\) was carried out by the Regular Falsch method with the aid of a computer. In this study, a best fit method was applied to the estimation of \(k_{\text{ob}}\). The method provides a value of \(k_{\text{ob}}\), which minimizes the difference in concentration of dichloride anion radicals between the values observed and calculated from equation 6. The calculation was carried out for the observed values of the concentration at every 10 µs in 50–100 µs after the start of flash. Second-order rate constant, \(k_4\), was obtained from the slope, by plotting \(k_{\text{ob}}\) against the concentration of the substrate.

The rate constants determined for the reactions of dichloride anion radicals with several aliphatic peptides are summarized in Table 1. It is noticed that the reactivity increases with increasing number of C-H bond in peptides, as observed for the reactions of hydroxyl radicals (Fig. 1). The reactivity of aliphatic peptides toward dichloride anion radicals is almost two orders of magnitude smaller than that toward hydroxyl radicals.
Fig. 1. The rate constants for the reactions of dichloride anion radicals with aliphatic peptides and comparison with those of hydroxyl radicals.

1, glycylglycine
2, glycylalanine
3, glycyl-α-aminobutyric acid
4, glycynorvaline
5, glycynorleucine
6, glycylvaline
7, glycilisoleucine
8, glycylleucine

The rate constants for the reactions of hydroxyl radicals have been reported in reference (7).
Table 1. Rate constants for the reactions of dichloride anion radicals with aliphatic peptides.

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Rate constant (M⁻¹ s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>glycylglycine</td>
<td>(8.0 ± 0.9) x 10⁵</td>
</tr>
<tr>
<td>glycylalanine</td>
<td>(1.5 ± 0.3) x 10⁷</td>
</tr>
<tr>
<td>glycyl-α-amino butyric acid</td>
<td>(3.0 ± 0.5) x 10⁷</td>
</tr>
<tr>
<td>glycylnorvaline</td>
<td>(5.1 ± 0.4) x 10⁷</td>
</tr>
<tr>
<td>glycylnorleucine</td>
<td>(5.8 ± 0.9) x 10⁷</td>
</tr>
<tr>
<td>glycylvaline</td>
<td>(4.1 ± 0.5) x 10⁷</td>
</tr>
<tr>
<td>glycylleucine</td>
<td>(5.4 ± 0.1) x 10⁷</td>
</tr>
<tr>
<td>glycylisoleucine</td>
<td>(5.8 ± 0.7) x 10⁷</td>
</tr>
<tr>
<td>glycylglycylglycylglycine</td>
<td>(1.8 ± 0.5) x 10⁷</td>
</tr>
</tbody>
</table>

Table 2. Comparison of partial rate constants for the reactions of dichloride anion radicals with those for the reactions of hydroxyl radicals.

<table>
<thead>
<tr>
<th>C-H</th>
<th>Partial rate constant</th>
<th>Ratio of partial k_{OH} to partial k_{Cl₂}</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cl₂⁻ (10⁶ M⁻¹ s⁻¹)</td>
<td>OH* (10⁶ M⁻¹ s⁻¹)</td>
</tr>
<tr>
<td>α-sec</td>
<td>4.0</td>
<td>1.02</td>
</tr>
<tr>
<td>α-tert</td>
<td>9.0</td>
<td>2.04</td>
</tr>
<tr>
<td>β-prim</td>
<td>2.0</td>
<td>0.515</td>
</tr>
<tr>
<td>β-sec</td>
<td>7.1</td>
<td>4.41</td>
</tr>
<tr>
<td>β-tert</td>
<td>16</td>
<td>8.82</td>
</tr>
<tr>
<td>γ-prim</td>
<td>2.3</td>
<td>0.675</td>
</tr>
<tr>
<td>γ-sec</td>
<td>10</td>
<td>7.17</td>
</tr>
<tr>
<td>γ-tert</td>
<td>18</td>
<td>14.3</td>
</tr>
<tr>
<td>δ-prim</td>
<td>2.1</td>
<td>0.763</td>
</tr>
<tr>
<td>δ-sec</td>
<td>4.4</td>
<td>7.17</td>
</tr>
<tr>
<td>ε-prim</td>
<td>2.0</td>
<td>0.763</td>
</tr>
</tbody>
</table>

*Reference (7).*
The rate constant for glycyl moiety in aliphatic peptides can be estimated to be less than $6 \times 10^5$ M$^{-1}$ s$^{-1}$, because the rate constant determined for the reaction of the dichloride anion radicals with glycine is $6 \times 10^5$ M$^{-1}$ s$^{-1}$ and loss of COO$^-$ group is expected to lower the reactivity. The partial reactivity of the glycyl moiety is, therefore, assumed negligible as compared with the whole rate constant of the dipeptides. The partial rate constants estimated from the observed values are listed in Table 2. In the third column of Table 2, the partial rate constants estimated for the reactions of hydroxyl radicals with the same peptides are shown and the ratios of partial rate constants of hydroxyl radicals to those of dichloride anion radicals in the fourth column. As seen from Table 2, the value for δ-secondary C-H is extraordinarily small. In the case of hydroxyl radicals, the value for δ-secondary C-H was assumed as being identical with that for γ-secondary C-H and the assumption led to a reasonable conclusion. If the same assumption is taken here, the rate constant for the reaction with glycynorleucine is $6.9 \times 10^7$ M$^{-1}$ s$^{-1}$, which exceeds an experimental error associated with the observed value. If a kind of folding takes place at a hydrophobic side chain, a steric effect will be greater for a dichloride anion radical than for a hydroxyl radical, because the size of the former radical is approximately two times greater than that of the latter. However, the steric effect is not clear for the δ-primary or ε-primary C-H bond, although a brief increase is noticed in the ratio of partial rate constants between the hydroxyl radicals and the dichloride anion radicals (the fourth column in Table 2). Except for the primary C-H, the partial reactivities increase as C-H bond goes away from the α-position, as shown in Table 2. The trend can be understood in terms of effect of amino group bound to α-carbon, although the degree of suppression is much smaller than a protonated amino group. The increasing tendency of the partial rate constants for hydroxyl radicals is greater than that for dichloride anion radicals. This fact suggests that a steric effect due to folding exists in the reaction of a peptide with a long side chain such as glycynorleucine.

The rate constant for the reaction of dichloride anion radicals with glycine was determined as $(7.1 \pm 1.3) \times 10^5$ M$^{-1}$ s$^{-1}$, which is much smaller than a half of the rate constant for glycylglycine, $(8.0 \pm 0.9) \times 10^6$ M$^{-1}$ s$^{-1}$. Apparently, the formation of a peptide bond or disappearance of the protonated amino group enhances the reactivity of C-H bond belonging to a carboxyl moiety of the dipeptide, as observed and discussed for hydroxyl radicals. The enhancement can be interpreted as a decrease in the suppressive effect of a protonated amino group. For the reaction of dichloride anion radicals, the difference in activation energy between glycine and glycylglycine can be estimated from the rate constants toward the anion radicals, assuming an identical preexponential term; at 300 K, the difference is approximately 1.5 kcal mol$^{-1}$. A similar estimation for the reactions of hydroxyl radicals gives approximately 1.5 kcal for the difference in activation energy, using $1.7 \times 10^7$ M$^{-1}$ s$^{-1}$ and $2.0 \times 10^8$ M$^{-1}$ s$^{-1}$ as the rate
constants for glycine and the carboxyl moiety of glycyglycine, respectively.

The rate constant for the middle moiety of triglycine can be estimated by comparison of the rate constants of glycyglycine and triglycine, if assumed that the rate constant for the amino and the carboxyl moieties is equal to that for glycyglycine. The calculated value is $1.0 \times 10^7$ M$^{-1}$ s$^{-1}$, which agrees with the value, $1.1 \times 10^7$ M$^{-1}$ s$^{-1}$, calculated from the rate constants for triglycine and tetraglycine.

Recently, Bobrowski has revealed that a reaction type, hydrogen abstraction or electron transfer, can be appraised from a trend in order of reactivities of several oxidizing radicals toward a common substrate. He concluded that the dichloride anion radical abstracts a hydrogen atom from aliphatic compounds. According to his study, an abstraction of hydrogen atom from tetraalkylammonium cations is three or four orders of magnitude higher for hydroxyl radicals than for dichloride anion radicals. In contrast, the present study shows only two orders of magnitude of difference. The discrepancy may be explained in part in terms of pH values of the solutions used in the present study. In acidic solution, a relatively small rate constant was obtained for the reaction of dichloride anion radicals with an amino acid. The present determination was carried out at neutral pH, and the obtained values are expected to be at least one order of magnitude larger than those in acidic solutions.

The trend of partial reactivity and the suppressive effect of a protonated amino group observed in the present study indicate that dichloride anion radicals react with aliphatic compounds in the same manner with hydroxyl radicals. Consequently, it is concluded that the dichloride anion radical abstracts a hydrogen atom from an aliphatic peptide molecule.

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REFERENCES


