Reactivity of Aliphatic Peptides toward Hydroxyl Radicals in Aqueous Solution

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Rate constants for the hydrogen abstraction reactions of hydroxyl radicals from aliphatic peptides and N-acetyl amino acids were determined by the p-nitrosodimethylaniline method in gamma-irradiated solutions. For peptides, enhancement of reactivity toward hydroxyl radicals was expected, because of disappearance of suppression by NH₃⁺ group. The expectation was confirmed by the obtained rate constants. In order to estimate the partial rate constants for peptides, partial rate constants for the hydrogen abstraction reactions from amino acids were corrected by the factors calculated on the basis of effect of peptide bond formation. The rate constants of peptides and N-acetyl amino acids calculated from the corrected partial rate constants were in good agreement with the experimentally obtained ones.

INTRODUCTION

It is well known that reactivity of an aliphatic compound toward hydroxyl radicals increases with the increasing number of C–H bond in the molecule. In a previous paper, we reported that the rate constant for hydrogen abstraction reaction of hydroxyl radicals from an aliphatic amino acid molecule could be divided into partial rate constants which are assigned to each of C–H bonds of the amino acid molecule. It was also reported that the rate constants of aliphatic amino acids calculated from the estimated partial rate constants could be approximate enough to the observed ones.

In the argument on reactivity of aliphatic peptides toward hydroxyl radicals, effect of peptide bond formation should be taken into consideration. Deprotonation of NH₃⁺ group of an amino acid is expected to increase the reactivity toward hydroxyl radicals, since the reactivity of amino acid measured at alkaline pH was reported to be larger than that at neutral pH. The present determination of rate constants for the reactions of aliphatic dipeptides and N-acetyl amino acids with hydroxyl radicals by a p-nitrosodimethylaniline method indicates that peptide bond formation enhances the reactivity. On the basis of the experimentally obtained rate constants and the assumption of linear relationship between position of carbon atom and natural logarithm...
of the difference in activation energy, partial rate constants were estimated for aliphatic dipeptides and N-acetyl amino acids.

MATERIALS AND METHOD

p-Nitrosodimethylaniline (PNDA) obtained from the British Drug Houses Ltd. was used after recrystallization. Glycyl peptides were purchased from Nutritional Biochemicals Corp. and alanylglucose from ICN Pharmaceuticals, Inc. N-acetyl amino acids were obtained from Tokyo Kasei Kogyo Co. Ltd. and β-alanine from Wako Pure Chemical Industries, Ltd.

Rate constants for reactions of aliphatic peptides and N-acetyl amino acids with hydroxyl radicals were determined by competition kinetics using PNDA as a reference substance, as described in previous papers. Gamma rays from $^{137}$Cs source were used for irradiation of air-saturated aqueous solutions containing $5 \times 10^{-4}$M of PNDA and various concentrations of the substrates.

RESULTS AND DISCUSSION

Rate constants for the reactions of simple aliphatic peptides and N-acetyl amino acids determined by PNDA method are shown in the third column of Table 1. In the

Table 1

| Peptide                  | pH | Observed     | Calculated |
|-------------------------|----|--------------|------------|
| N-acetylglucose         | 6.6| $3.8 \times 10^8$ | $3.8 \times 10^8$ |
| N-acetylalanine         | 6.6| $4.7 \times 10^8$ | $5.4 \times 10^8$ |
| N-acetylvaline          | 6.6| $1.4 \times 10^8$ | $1.7 \times 10^8$ |
| N-acetyleucine          | 6.4| $3.1 \times 10^8$ | $3.2 \times 10^8$ |
| gIgly                 | 6.2| $2.4 \times 10^8$ | $2.4 \times 10^8$ |
| gIyala                 | 6.2| $5.1 \times 10^8$ | $4.0 \times 10^8$ |
| gIy-β-ala              | 6.2| $6.6 \times 10^8$ | $7.2 \times 10^8$ |
| gIy-α-NH$_2$-butyric a. | 7.1| $1.2 \times 10^9$ | $1.3 \times 10^9$ |
| gIval                  | 6.1| $1.6 \times 10^9$ | $1.5 \times 10^9$ |
| gIynorval              | 6.7| $2.8 \times 10^9$ | $2.8 \times 10^9$ |
| gIyle                  | 7.3| $2.5 \times 10^9$ | $3.0 \times 10^9$ |
| gIynorleu              | 6.6| $4.3 \times 10^9$ | $4.2 \times 10^9$ |
| gIyle                  | 7.2| $3.1 \times 10^9$ | $3.0 \times 10^9$ |
| alagly                 | 7.1| $3.9 \times 10^9$ | ---         |
| glyglygly              | 6.9| $5.0 \times 10^9$ | ---         |
| glyglyglygly           | 6.9| $7.0 \times 10^9$ | ---         |

a This was calculated on the basis of the partial rate constants which were estimated for β-alanine and γ-amino-n-butyric acid.
The rate constants of peptides are not understood by the simple summation of those of the constituent amino acids. Enhancement of reactivity is expected as an effect of peptide bond formation. Suppressive effect of NH$_3^+$ group on partial reactivity was observed in case of basic amino acid, as reported in the previous paper.$^{3}$ Carboxyl group reduces the reactivity toward hydroxyl radicals as well.$^{3}$ Formation of peptide bond is equivalent to disappearance of NH$_3^+$ and COO$^-$ groups and hence an increase in reactivity toward hydroxyl radicals is expected. The rate constants determined in the present study indicate that there is an enhancement of reactivity of peptides toward the radicals as compared with those of the constituent amino acids.

The partial rate constants for aliphatic dipeptides were estimated from the partial reactivity of amino acids by making corrections for activation energy, as in case of basic amino acid.$^{3}$ The estimated values are shown in Table 2, where $f$ represents a correction factor for each position of carbon atom. The $f$-values were calculated by drawing a best-fitted straight line on diagram of natural logarithm of the difference in activation energy plotted against the position of carbon atom.

| Type of C-H bond | $f$ | Partial rate constant M$^{-1}$ sec$^{-1}$ |
|------------------|-----|----------------------------------------|
| $\alpha$-sec     | 12.0| $1.02 \times 10^8$                    |
| $\alpha$-tert    |    | $2.04 \times 10^8$                    |
| $\beta$-prim     | 2.49| $5.15 \times 10^7$                    |
| $\beta$-sec      |    | $4.41 \times 10^8$                    |
| $\beta$-tert     |    | $8.82 \times 10^8$                    |
| $\gamma$-prim    | 1.40| $6.75 \times 10^7$                    |
| $\gamma$-sec     |    | $7.17 \times 10^8$                    |
| $\gamma$-tert    |    | $1.43 \times 10^9$                    |
| $\delta$-prim    | 1.13| $7.63 \times 10^5$                    |

The rate constants of dipeptides calculated from the estimated partial rate constants are shown in the fourth column of Table 1. With a series of N-acetyl amino acids, the partial rate constant for acetyl group was assumed to be $1.8 \times 10^8 $M$^{-1}$sec$^{-1}$, which is in accordance with that calculated from the value for $\beta$-primary C-H bond of carboxylic acid reported by Anbar and his coworkers.$^5$ In case of glycyl dipeptide, the partial rate constant for glycyl moiety was taken to be $4 \times 10^7$M$^{-1}$sec$^{-1}$, assuming that the observed rate constant for glycylglycine is correct. There is a good agreement between the calculated rate constants and the observed ones, except for N-acetylanaline, glycylalanine, glycyl-$\beta$-alanine and glycyleucine, although the differences are within the range possibly allowable for determination of rate constants.
Rate constant of β-alanine was determined to be \(1.4 \times 10^8 \text{M}^{-1}\text{sec}^{-1}\) and \(4.4 \times 10^8 \text{M}^{-1}\text{sec}^{-1}\) was reported previously for γ-amino-n-butyric acid. In the molecules of these amino acids, amino and carboxyl groups are separated to both ends of an aliphatic chain. Partial rate constants for these amino acids were estimated from those of carboxylic acid by multiplying a factor of \(1/28.6, 1/5.59,\) or \(1/2.49\) for α', β' or γ' position, respectively. The correction factors were estimated by a linear relationship between natural logarithm of the difference in activation energy and position of carbon atom.

The calculated rate constants were \(1.4 \times 10^8\) and \(4.5 \times 10^8 \text{M}^{-1}\text{sec}^{-1}\) for β-alanine and γ-amino-n-butyric acid, respectively. They are in accordance with the experimentally obtained rate constants. The calculated rate constant for glycyglycylglycine was obtained by correcting the partial rate constants of β-alanine for the effect of peptide bond formation.

With alanylglycine, \(8.9 \times 10^8 \text{M}^{-1}\text{sec}^{-1}\) was determined as the rate constant. If \(1.0 \times 10^8 \text{M}^{-1}\text{sec}^{-1}\) is taken as the partial rate constant for α-secondary C-H bond of glycine moiety, \(1.9 \times 10^8 \text{M}^{-1}\text{sec}^{-1}\) is calculated for an alanyl moiety. In case of glycyglycine, \(2 \times 10^8 \text{M}^{-1}\text{sec}^{-1}\) was estimated as the partial rate constant for α-secondary C-H bond of glycyl moiety. Assuming that the partial rate constant for α-tertiary C-H bond of alanyl moiety is two times greater than that for α-secondary C-H bond of a glycyl moiety, the partial rate constant for β-primary C-H bond of the alanyl moiety is obtained to be \(5 \times 10^7 \text{M}^{-1}\text{sec}^{-1}\). It is approximately 2.4 times greater than the value for free amino acid.

The partial rate constant for the middle glycyl moiety of glycyglycylglycine can be obtained to be \(2.6 \times 10^7 \text{M}^{-1}\text{sec}^{-1}\) by comparing the observed rate constant of glycyglycylglycine with that of glycyglycine, while comparison of glycyglycylglycine with glycylglycylglycine gives \(2.0 \times 10^8 \text{M}^{-1}\text{sec}^{-1}\). The value for α-secondary C-H bond of the middle glycyl moiety is \(1.3 \times 10^8 \text{M}^{-1}\text{sec}^{-1}\) in the former estimation and \(1.0 \times 10^8 \text{M}^{-1}\text{sec}^{-1}\) in the latter. It is not known at present whether the difference is due to experimental errors or to a conformational effect of the tetrapeptide.

In previous papers, the rate constants of free amino acids were used to estimate the rate constants for reactions of some enzyme proteins with hydroxyl radicals. In general, reactivity of amino acid residue toward hydroxyl radicals is larger than that of free amino acid. Fairly good agreement between the observed rate constants and the estimated ones might be attributed to overestimation of steric factors which were obtained for the direction of attack of hydroxyl radical by a cubic model. In case of long molecules such as leucine, isoleucine, lysine or arginine, application of the cubic model is apparently inadequate. If a hydroxyl radical attacks an amino acid residue along the direction of molecular axis, the position of C-H bond may affect the reaction rate. In order to obtain the steric factor for amino acid residue located on the surface of globular protein accurately, a solid angle admitted for the radical attack should be calculated for each C-H bond of the residue. However, it is probably better to discuss the problem after the rate constants of aromatic peptides are determined, because aliphatic amino acid residues generally share about 30% of...
reactivity of enzymes toward hydroxyl radicals and aromatic amino acid residues are responsible for a greater part of the reactivity.

Asmus and his coworkers\textsuperscript{7} reported the relative probabilities for hydrogen atom abstraction from the $\alpha$ position, from the OH group, and from other positions of alcohols by hydroxyl radicals, determined by pulse radiolysis. The probabilities accord substantially with the calculated values from the partial rate constants for aliphatic alcohols reported by Anbar and his coworkers.\textsuperscript{3} This is evidence which supports the hypothesis that in hydrogen abstraction reaction by hydroxyl radicals the whole reactivity of a molecule can be divided into the partial reactivities assigned to each structural unit of the molecule.

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