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Determination of Rate Constants for Reactions of Dichloride Anion Radical with Some Dipeptides in Aqueous Solution of KCl and $K_2S_2O_8$ by Flash Photolysis

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Determination of rate constants for reactions of dichloride anion radical was conducted by conventional flash photolysis of aqueous solutions of KCl and $K_2S_2O_8$, where no participation of $HOCl^{-\cdot}$ is expected in decay kinetics of dichloride anion radical. The optimum conditions found for the determination are 0.1 mol dm^{-3} KCl and 10^{-4} mol dm^{-3} $K_2S_2O_8$. Under the present experimental conditions, $S_2O_8^{2-}$ serves to convert chloride ion to dichloride anion radical through reaction with hydrated electron produced by photolysis of chloride ion. For glycyltryptophan, the competition method was employed, because its transient absorption overlaps the absorption due to dichloride anion radical. The rate constants determined are almost the same as those obtained in N_2O-saturated aqueous solution of KCl (1 mol dm^{-3}). The reactivity of dipeptides toward dichloride anion radical is discussed.

Reaction of dichloride anion radical produced by radiolysis or photolysis of aqueous chloride with a protein molecule is an interesting subject, because physiological concentrations of the chloride ion in living cells are relatively high and because other dihalide anion radicals such as $Br_2^{-\cdot}$ were reported to react with proteins in a specific manner.[1-5] Rate constants for reactions of dichloride anion radicals with a variety of substrates have been determined in neutral aqueous solutions by radiation-chemical and photochemical method.[6-10] However, there remain some problems for the reported methods, because $HOCl^{-\cdot}$. is inevitably produced in radiolysis of neutral aqueous chloride and photolysis of N_2O-saturated aqueous chloride and because its absorption overlaps the absorption due to dichloride anion radical.[11] Furthermore, concentrations of chloride ion were inadequately high for protein, particularly in photolysis.

Peroxodisulfate ion reacts with hydrated electron to form $SO_4^{-\cdot}$, which oxidizes chloride ion to chlorine atom.[12,13] Chlorine atom is readily converted to dichloride anion radical in aqueous solution of potassium chloride:

$$e_{aq}^- + S_2O_8^{2-} \longrightarrow SO_4^{-\cdot} + SO_4^{2-}, \quad (1)$$

$$SO_4^{-\cdot} + Cl^- \longrightarrow SO_4^{2-} + Cl^{\cdot}, \quad (2)$$

$$Cl^{\cdot} + Cl^- \rightleftharpoons Cl_2^{-\cdot}. \quad (3)$$

In the present study, a determination of rate constants for reactions of dichloride anion radical with some biomolecules, especially dipeptides, was attempted in aqueous solutions of KCl including $K_2S_2O_8$, where no participation of $HOCl^{-\cdot}$ is expected. A comparison of the results obtained by the present method with those determined in N_2O-saturated solutions disclosed no essential difference between them.

Experimental

Peptides obtained from Nutritional Biochemicals Corp. were used without further purification. The other chemicals were all of reagent grade. Solutions for irradiation were prepared with triply distilled water by dissolving 0.1 mol dm^{-3} KCl, 10^{-4} mol dm^{-3} $K_2S_2O_8$, and a desired amount of substrate. The solutions were adjusted to pH 7 and subjected to bubbling of nitrogen for 20 min. Flash photolysis was carried out with a conventional apparatus under the conditions of 10 μs duration and 200 J input energy. Decay process of dichloride anion radical was monitored at 350 nm and analyzed on the basis of the following reactions:

$$Cl_2^{-\cdot} + Cl_2^{-\cdot} \longrightarrow Cl_2 + 2Cl^-, \quad (4)$$

$$Cl_2^{-\cdot} + S \longrightarrow 2Cl^- + \text{product(s)}. \quad (5)$$

The procedure of kinetic analysis was described in detail elsewhere.[9,10] Calculation was carried out with the aid of a microcomputer PC 8001 of Nippon Electric Co.

Results and Discussion

Optimum Conditions. In the presence of $S_2O_8^{2-}$, formation of dichloride anion radical was markedly enhanced even at low concentrations of chloride ion. Peroxodisulfate ions are photolyzed to give $SO_4^{-\cdot}$ as follows:[14,15]

$$S_2O_8^{2-} + h\nu \longrightarrow 2SO_4^{-\cdot}. \quad (6)$$

In addition to Reaction 1, therefore, Reaction 6 produces $SO_4^{-\cdot}$ and in turn increases the yield of dichloride anion radical through Reactions 2 and 3. However, formation of a large amount of the anion radical was found to be inadequate for analysis of the decay process, because it gave rise to a practical difficulty in monitoring absorption in the initial stage. Consequently, nitrogen-saturated solution of 0.1 mol dm^{-3} KCl and 10^{-4} mol dm^{-3} $K_2S_2O_8$ was chosen as an optimum condition for experimental reasons.

In this system, reaction of $SO_4^{-}\cdot$ with a substrate of dichloride anion radical was neglected because Reaction 2 takes place predominantly ($k_2=3.1\times10^8$ dm^3 mol^{-1} s^{-1}).[13] Similarly, bimolecular decay of $SO_4^{-}\cdot$ was neglected since the rate constant of Reaction 7 was reported to be 1.0×10^9 dm^3 mol^{-1} s^{-1} [16] and since $k_7[SO_4^{-}\cdot]$ was much less than $k_2[Cl_2^{-}\cdot]$:

$$SO_4^{-}\cdot + SO_4^{-}\cdot \longrightarrow S_2O_8^{2-}. \quad (7)$$

In fact, no absorption at 460 nm due to sulfate radical was observed in flash photolysis of the above solution. This implies that a decay of sulfate radical is almost complete within flash duration. The lifetime of sulfate radical was estimated to be 3.2×10^{-8} s from rate data for Reaction 2. In the present system, a complete consumption of hydrated electrons by Reaction 1 cannot be expected, because rate data of Reaction 1 suggest a possibility that at least several percent of hydrated electrons may react with dichloride anion radicals; this reaction causes a decrease in the yield of dichloride anion radicals. In any cases, the greater part of hydrated electrons will be consumed within flash duration, because the life time is estimated to be 1 μs. At 40 μs after start of flash, when analysis of the decay process starts, there may remain dichloride anion radicals as an overwhelming majority of reactive transients in the system.

Comparison of Rate Constants. Table 1 shows the rate constants determined by the present method, together with those obtained in N_2O-saturated solutions. Two kinds of substrates were taken for comparison, because dichloride anion radical abstracts a hydrogen atom from an aliphatic compound and oxidizes an unsaturated compound.[6,16,17] The values determined in the present study are in good agreement with those previously obtained in N_2O-saturated solutions. This implies that the contribution of $HOCl^{-}\cdot$ may be neglected in kinetic analysis based on the decay of the absorption at 350 nm. Since an OH-adduct of 5-aminouracil was observed in neutral N_2O-saturated solution,[10] the formation of hydroxyl radicals is confirmed. In neutral N_2O-saturated solutions, reactions

$$e_{aq}^{-} + N_2O \longrightarrow N_2 + \cdot OH + OH^{-}, \quad (8)$$

$$\cdot OH + Cl^{-} \rightleftharpoons HOCl^{-}\cdot \quad (9)$$

take place. The equilibrium constant of Reaction 9 was reported to be (0.70±0.13) dm^3 mol^{-1} by Jayson *et al.*[11] and hence the initial concentration of $HOCl^{-}\cdot$ is estimated as 70 percent of dichloride anion radicals on the assumption that Reaction 8 proceeds with 100 percent efficiency. Since the absorption coefficient at 350 nm is approximately half that of the dichloride anion radical,[11] the contribution of $HOCl^{-}\cdot$ amounts to about 26 percent of the absorbance at 350 nm. However, the decay constant of dichloride anion radical ($2k$ for Reaction 4) in N_2O-saturated solution was found to be essentially the same as that determined in the present system. Studying pulse radiolysis, Anbar and Thomas[8] pointed out that hydroxyl radicals formed in the bulk of a solution containing 10^{-3} mol dm^{-3} H_2O_2 do not produce any dichloride anion radicals (no change in absorbance at 365 nm). However, Jayson *et al.*[11] reported the transient spectrum of $HOCl^{-}\cdot$ and kinetic data for this species. The discrepancy cannot be interpreted at present, although the present results agree with the former.

Rate Constants of Dipeptides. In our previous paper,[8] rate constants for several aliphatic dipeptides reacting with dichloride anion radical were reported. The present method was applied to other dipeptides whose corresponding rate constants for the anion radical had not been determined. Values determined by the present method are listed in Table 2. The present method could not be applied to glycyltryptophan because of a transient absorption appearing in the region of the absorption due to dichloride anion radical. Tryptophan is known to yield an indolyl radical on one-electron oxidation and deprotonation.[19-22] The indolyl radical has another absorption with a maximum at 510 nm and is relatively long-lived. The decay constant in 0.1 mol dm^{-3} KCl was determined to be $(6.9\pm0.6)\times10^8$ dm^3 mol^{-1} s^{-1}. Since the dichloride anion radical has no absorption at 510 nm, a com-

Table 1. Comparison of rate constants determined in N_2O system with those in N_2-$K_2S_2O_8$ system

Compound	Rate constant/10^7 dm^3 mol^{-1} s^{-1}	
	N_2O	N_2-$K_2S_2O_8$
Thymine	32±3[a]	36±2
Cytosine	35±1[a]	35±2
Uracil	28±1[a]	28±3
Glycylvaline	4.1±0.5[b]	4.3±0.3
Glycylleucine	5.4±0.1[b]	5.1±0.1
Glycylisoleucine	5.8±0.7[b]	5.2±0.3

a) Ref. 9. b) Ref. 8.

Table 2. Rate constants of dichloride anion radical with several dipeptides

Peptide	Rate constant/10^7 dm^3 mol^{-1} s^{-1}
Glycylserine	4.0±0.2
Glycylthreonine	2.7±0.5
Glycylaspartic acid	3.9±0.2
Glycylasparagine	5.2±0.1
Glycylglutamic acid	2.7±0.4
Glycyllysine	3.4±0.7
Glycylproline	4.2±0.2
Glycylhistidine	21±3
Glycylphenylalanine	19±2
Glycyltyrosine	59±2
Glycyltryptophan	280±30[a]

a) Competition method.

petition kinetics was attempted to determine the rate constant for glycyltryptophan, in which use was made of the absorption at 510 nm due to the indolyl radical produced from glycyltryptophan through oxidation by dichloride anion radical:

$$Cl_2^{-\cdot} + TH \longrightarrow 2Cl^- + TH^{+\cdot}, \quad (10)$$

$$TH^{+\cdot} \longrightarrow T^{\cdot} + H^+, \quad (11)$$

where TH represents glycyltryptophan and TH^+ is the cation radical of glycyltryptophan produced by one-electron oxidation. $T^{\cdot}$ designates a neutral radical formed from the cation radical after deprotonation. Since glycyltryptophan yields also indolyl radical by photoionization according to

$$TH + h\nu \longrightarrow TH^+ + e_{aq}^-, \quad (12)$$

Reactions 5, 10, 11, and 12 should be considered in the competition kinetics. For the decrease in the absorbance at 510 nm caused by the presence of a substrate, the following equation is derived:

$$\frac{1}{A_0 - A} = \frac{1}{\varepsilon l[T^{\cdot}]}\left(1 + \frac{k_{10}[TH]}{k_5[S]}\right), \quad (13)$$

where A_0 and A designate the absorbances at 510 nm in the absence and presence of substrate, respectively at 150 μs after start of flash. A reciprocal plot of $(A_0 - A)$ *vs.* [TH]/[S] provides a straight line and k_{10}/k_5 is obtained from the slope and intercept as shown in Fig. 1. As reference substrates, were used glycylvaline, glycylleucine, glycylnorleucine, and glycyl-α-aminobutyric acid, because their reaction products have no absorption at 510 nm.

As shown in Table 2, dipeptides having C=C double bonds are more reactive than saturated ones toward the anion radical. For aliphatic dipeptides, the ratio of the rate constant with hydroxyl radical to that with the anion radical has been found to be about 66.[9] The reactivity of glycylproline can be explained in terms of this correlation. However, a large deviation from the correlation was observed for aliphatic dipeptides having such a functional group as hydroxyl, carboxyl, or carbamoyl group. Since the ratio of the rate constant with hydroxyl radical to that with dichloride anion radical is much less than 66 for the aliphatic dipeptide having some functional group, these groups may affect the reaction rate differently from hydroxyl radical working in hydrogen atom abstraction reaction.

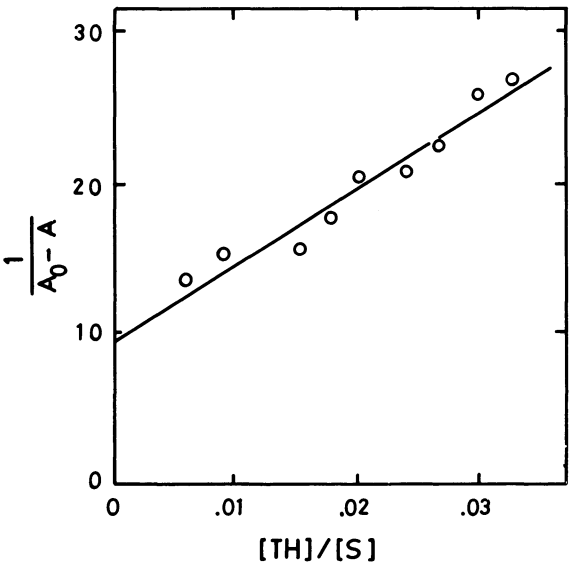

Fig. 1. Kinetic plots according to Equation 13. [TH] = 10^{-4} mol dm^{-3}. S: Clycylleucine.

Aromatic and heterocyclic dipeptides seem to undergo one-electron oxidation, because they are very reactive toward dichloride anion radicals. Glycyltryptophan is most reactive toward the anion radical in neutral solution as well as in acid solution.[10] Since tryptophan is often found in active sites of enzyme proteins and the physiological concentration of chloride ion is considerably high, the role of dichloride anion radical must be taken into consideration in radiation- or light-induced damage on biological systems.

References

1) G. E. Adams, J. E. Aldrich, R. H. Bisby, R. B. Cundall, J. L. Redpath, and R. L. Willson, *Radiat. Res.*, **49**, 278 (1972).
2) G. E. Adams, R. S. Bisby, R. B. Cundall, J. L. Redpath, and R. L. Willson, *Radiat. Res.*, **49**, 290 (1972).
3) J. L. Redpath, *Int. J. Radiat. Biol.*, **22**, 99 (1972).
4) P. B. Roberts, *Int. J. Radiat. Biol.*, **24**, 143 (1973).
5) G. E. Adams, J. L. Redpath, R. H. Bisby, and R. B. Cundall, *J. Chem. Soc., Faraday Trans. 1*, **69**, 1608 (1973).
6) K. Hasegawa and P. Neta, *J. Phys. Chem.*, **82**, 854 (1978).
7) T. Masuda, S. Shimada, and M. Kondo, *J. Radiat. Res.*, **20**, 209 (1979).
8) T. Masuda, T. Hayashi, and M. Kondo, *J. Radiat. Res.*, **23**, 298 (1982).
9) T. Masuda, T. Kimura, E. Kadoi, T. Takahashi, K. Yamauchi, and M. Kondo, *J. Radiat. Res.*, **24**, 165 (1983).
10) T. Masuda, R. Gunji, T. Kimura, and K. Yamauchi, *Bull. Chem. Soc. Jpn.*, **56**, 3595 (1983).
11) G. G. Jayson, B. J. Parsons, and A. J. Swallow, *J. Chem. Soc., Faraday Trans. 1*, **69**, 1597 (1973).
12) W. Roebke, M. Renz, and A. Henglein, *Int. J. Radiat. Phys. Chem.*, **1**, 39 (1969).
13) O. P. Chawla, and R. W. Fessenden, *J. Phys. Chem.*, **79**, 2693 (1975).
14) M.-S. Tsao and W. K. Wilmarth, *J. Phys. Chem.*, **63**, 346 (1959).
15) L. Dogliotti and E. Hayon, *J. Phys. Chem.*, **71**, 2511 (1967).
16) K. Bobrowski, *J. Phys. Chem.*, **84**, 3524 (1980).
17) M. D. Sevilla, D. Suryanarayana, and K. M. Morehouse, *J. Phys. Chem.*, **85**, 1027 (1981).
18) M. Anbar and J. K. Thomas, *J. Phys. Chem.*, **68**, 3829 (1964).
19) J. L. Redpath, R. Suntus, J. Ovadia, and L. I. Grossweiner, *Int. J. Radiat. Biol.*, **27**, 201 (1975).
20) D. V. Bent and E. Hayon, *J. Am. Chem. Soc.*, **97**, 2612 (1975).
21) E. J. Land and W. A. Prütz, *Int. J. Radiat. Biol.*, **32**, 203 (1977).
22) W. A. Prütz and E. J. Land, *Int. J. Radiat. Biol.*, **36**, 513 (1979).