Bull. Chem. Soc. Jpn., 58, 223—226 (1985)

Reactions of Dichloride Anion Radical with Nucleosides, Nucleotides, and Polynucleotides

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(Received January 27, 1984)

Rate constants for reactions of dichloride anion radical with nucleosides, nucleotides, and polynucleotides were determined in neutral aqueous solutions of potassium chloride by the conventional flash photolysis. The reactivities of these compounds toward the anion radical are in the order: bases>nucleosides>nucleotides>polynucleotides. This trend is similar to that observed with the corresponding reactions with hydroxyl radical. A comparison of the reactivities of nucleosides, nucleotides, and polynucleotides toward dichloride anion radical with those toward hydroxyl radical has revealed that the reactivities of these compounds are governed mainly by their base moiety. This trend in the reactivities is discussed in terms of steric effect.

Reactions of nucleic acid and related compounds with dichloride anion radical produced in photolysis or radiolysis of aqueous chloride are of interest from biological point of view, considering their possible role in light or radiation induced damage on living cells. On the other hand, kinetic study on their reactions is expected to provide information on the relation between structure and reactivity of these biologically important molecules.

In previous studies,[1–3] rate constants for reactions of hydroxyl radical with nucleic acid bases, nucleosides, nucleotides, and polynucleotides were determined by the competition method using *N*,*N*-dimethyl-*p*-nitrosoaniline as a reference compound, and some trend observed in reactivities of bases, nucleosides, and nucleotides was explained in terms of steric hindrance, that is, mutual protection between base and sugar moieties. However, the applicability of this explanation is not examined for other radical reactions. Dichloride anion radical is known to resemble hydroxyl radical in oxidative nature but differ with respect to reactions with unsaturated compounds.[4–6] For the anion radical one-electron oxidation was found,[7,8] whereas addition to a double bond was observed for hydroxyl radical.

The present study was undertaken to ascertain whether or not the same explanation may apply to some trend in reactivities of a series of compounds toward dichloride anion radical. For this purpose, rate constants for reactions of dichloride anion radical with nucleosides, nucleotides, homopolynucleotides, and nucleic acids were determined in neutral aqueous solutions of KCl saturated with dinitrogen monoxide by the conventional flash photolysis. The rate constants obtained indicate that the trend in reactivities of these compounds is similar to that observed for reactions with hydroxyl radical. On the basis of these results, reactivities of these DNA related compounds are discussed from the view point of steric hindrance of substrates.

Experimental

Purine, 1-methylcytosine, D-ribose, 2-deoxyribose 5-phosphate, nucleosides, nucleotides, polynucleotides, ribonucleic acid (baker's yeast), and deoxyribonucleic acid (calf thymus) were obtained from Sigma Chemical Co. and used without further purification. The other chemicals were of reagent grade.

Solutions for irradiation were prepared by dissolving 1 mol dm^{-3} KCl and a desired amount of a substrate in triply distilled water and adjusting pH to 7. The solutions were subjected to bubbling of N_2O for 20 min immediately before flash photolysis. In UV-photolysis of the solutions, the following reactions are expected to take place:

$$\mathrm{Cl^- + h\nu \xrightarrow{197\,nm} Cl\cdot + e_{aq}^-}, \tag{1}$$

$$\mathrm{Cl\cdot + Cl^- \rightleftharpoons Cl_2^{-}\cdot}, \tag{2}$$

$$\mathrm{e_{aq}^- + N_2O \longrightarrow N_2 + \cdot OH + OH^-}, \tag{3}$$

$$\mathrm{Cl_2^{-}\cdot + Cl_2^{-}\cdot \longrightarrow Cl_2 + 2Cl^-}, \tag{4}$$

$$\mathrm{Cl_2^{-}\cdot + S \longrightarrow 2Cl^- + product(s)}, \tag{5}$$

where S designates a substrate. By kinetic analysis of the rate of decay of dichloride anion radical due to Reactions 4 and 5, the rate constant of Reaction 5 was estimated. The procedure for the estimation was described in detail elsewhere.[6,9] Calculations in kinetic analysis and of solid angles were carried out with the aid of microcomputers PC-8001 and PC-8801 (Nippon Electric Co.).

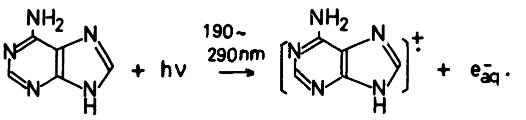
Flash photolysis was carried out under the conditions of 10 µs duration and 200 J input energy with the same conventional apparatus, that had been described in a previous paper,[10] except additional use of a transient recorder TM-1410 (Kawasaki Electronica Co.). Decay rate of dichloride anion radical was measured by using the absorption at 350 nm with 7600 dm^3 mol^{-1} cm^{-1} taken as its absorption coefficient.[11]

Results and Discussion

Rate Constants for Reaction of Dichloride Anion Radical with Nucleic Acid Components in Aqueous

Solution. Rate constants for reactions of dichloride anion radical with nucleosides, nucleotides, and some related compounds are summarized in Table 1, together with the values previously reported for nucleic acid bases.[6] These values were obtained from more than fifteen experiments and their errors are expressed in standard deviation.

We failed in determination of the rate constant for the reaction with guanine because of its low solubility in neutral solution. The rate constant for the reaction with adenine could not be determined either, because a relatively long-lived absorption of a maximum at 335 nm appeared in the region of the absorption due to dichloride anion radical so that the decay process of the anion radical could not be followed in this case. Since the transient absorption was observed even in the absence of chloride ion, it may be attributable to a cation radical of adenine produced by the photoionization in aqueous solution



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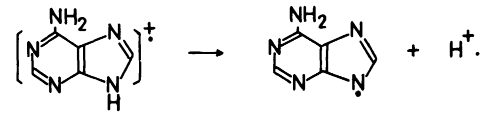
The species responsible for the transient absorption will be a neutral radical formed from the cation radical after deprotonation of N-9, because no absorption was observed for adenosine or adenosine 5′-phosphate in the time scale of flash photolysis:

TABLE 1. RATE CONSTANTS FOR REACTIONS OF DICHLORIDE ANION RADICAL WITH NUCLEIC ACID BASES, NUCLEOSIDES, NUCLEOTIDES, AND RELATED COMPOUNDS

Compound	Rate constant 10^8 dm^3 mol^{-1} s^{-1}
1. Uracil[a]	2.8±0.1
2. Uridine	1.6±0.3
3. Uridine 5′-phosphate	1.5±0.2
4. Cytosine[a]	3.5±0.1
5. Cytidine	2.5±0.8
6. Cytidine 5′-phosphate	2.2±0.6
7. Thymine[a]	3.2±0.3
8. Thymidine	2.4±0.2
9. Thymidine 5′-phosphate	1.8±0.3
10. Adenosine	3.2±0.5
11. Adenosine 5′-phosphate	3.0±0.5
12. Adenosine 5′-diphosphate	3.6±0.2
13. Adenosine 5′-triphosphate	2.0±0.6
14. Guanosine	14±7
15. Guanosine 5′-phosphate	8.8±0.8
16. D-Ribose	0.11±0.02
17. 2-Deoxyribose 5-phosphate	0.19±0.08
18. Purine	0.52±0.08
19. 1-Methylcytosine	3.3±0.5

a) Ref. 6.



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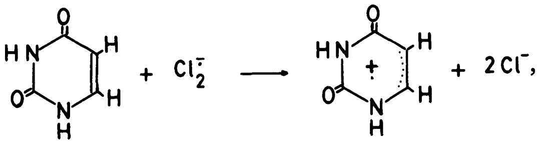
From the values given in Table 1, it is noticed that the reactivities of nucleic acid components may be arranged in the order: bases>nucleosides>nucleotides. The only exception was found for adenosine 5′-diphosphate. A duplicate determination resulted in fairly good reproducibility and this high value cannot be explained at the present stage of study. D-ribose or 2-deoxyribose 5-phosphate was chosen as a model compound of sugar or sugar phosphate moiety, respectively. Their low reactivities toward dichloride anion radical suggest that a hydrogen atom abstraction from these compounds occurs, as discussed by Hasegawa and Neta.[5]

In Table 2, rate constants for reactions with homopolynucleotides, ribonucleic acid, and deoxyribonucleic acid are summarized. They are presented as a value per nucleotide unit. Before flash photolysis, heat treatment was carried out on nucleic acids at 80 °C for 40 min in order to investigate the effect of conformational change on reactivity. An apparent increase in reactivity was observed after the treatment.

Mechanism of Reaction of Dichloride Anion Radical with Nucleoside. It has already been reported that dichloride anion radical reacts with a nucleic acid base to form 5 yl radical through one-electron oxidation and deprotonation[7,8]



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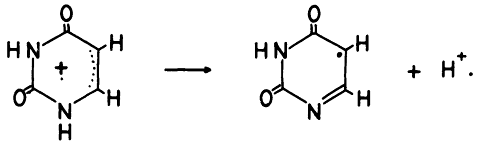

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TABLE 2. RATE CONSTANTS FOR REACTIONS OF DICHLORIDE ANION RADICAL WITH HOMOPOLYNUCLEOTIDES AND NUCLEIC ACIDS

Compound	Rate constant 10^8 dm^3 mol^{-1} s^{-1}
20. Polyuridylic acid	0.98±0.22
21. Polycytidylic acid	1.1±0.3
22. Polyadenylic acid	1.5±0.1
23. Ribonucleic acid	1.1±0.2
24. Ribonucleic acid pretreated at 80 °C, 40 min	2.2±0.1
25. Deoxyribonucleic acid	0.74±0.11
26. Deoxyribonucleic acid pretreated at 80 °C, 40 min	1.4±0.3

On the other hand, it is well known that anion radical $Cl_2^- \cdot$ abstracts a hydrogen atom from an aliphatic compound as follows:[12,13]

$$RH + Cl_2^- \cdot \longrightarrow R\cdot + H^+ + 2Cl^-. \qquad (10)$$

Therefore, two types of reactions are expected for nucleoside; one-electron oxidation of base ring (Reaction 8) and hydrogen abstraction from sugar moiety. Thus the apparent rate constant for the reaction of nucleoside, k, may be expressed by

$$k = f_1 k_b + f_2 k_s, \qquad (11)$$

where k_b and k_s designate the rate constants independently determined for base and sugar, respectively, and f_1 and f_2 are correction factors for k_b and k_s, respectively, with respect to the bonding between base and sugar.

The correction factors will be evaluated by considering the following causes for the decrease in reactivity toward dichloride anion radical: 1) change in energy state due to formation of glycoside bond; 2) decrease in diffusion rate owing to increase in molecular size; 3) steric hindrance, that is, mutual protection of two moieties.

The effect of energy state on reactivity is evaluated from the rate constant determined for 1-methylcytosine. As shown in Table 1, the value for 1-methylcytosine, $(3.3\pm0.5)\times10^8$ dm^3 mol^{-1} s^{-1}, is closer to the value of cytosine than to that of cytidine or cytidine 5′-phosphate. This suggests that the change in energy state due to formation of glycoside bond does not significantly affect the reactivity toward dichloride anion radical at least in neutral aqueous solution.

Furthermore, the first ionization potentials determined by photoelectron spectroscopy for the nucleic acid components and related compounds are in the following orders for each series: uracil (9.6—9.45 eV) >uridine (9.0 eV)>1-methyluracil (8.74 eV); thymine (9.20—9.02 eV)>thymidine (8.7 eV); cytosine (9.1 eV)> 1-methylcytosine (8.655 eV)>cytidine (8.6 eV); adenine (8.48 eV)>adenosine (8.4 eV)>9-methyladenine (8.39 eV); guanine (8.28 eV)>9-methylguanine (8.02 eV)> guanosine (8.0 eV).[14—20] If the order of magnitude in gas phase does not change in the liquid phase, the order of magnitude suggests that a nucleoside or an N-methyl derivative is more reactive than a base in one-electron oxidation reaction.

In relation to diffusion rate, the reactivity would be affected by the encounter number of reactants in solution. The degree of this contribution will be evaluated by Smoluchowski's equation. Bowen and Martin[21] reported diffusion constants of some nucleosides, nucleotides, and purine bases in the presence and absence of salt such as KCl, $MgCl_2$, or $CaCl_2$. The values for adenine, adenosine, and adenosine 5′-phosphate are 7.7×10^{-6}, 5.2×10^{-6}, and 4.3×10^{-6} cm^2 s^{-1} in the presence of 5×10^{-3} mol dm^{-3} $MgCl_2$, respectively. From these values and the diffusion constant of the anion radical, 1.67×10^{-5} cm s^{-1}, which has been estimated from the rate constant of Reaction 4 by Debye's equation for diffusion-controlled reactions between charged species, 0.90 and 0.86 can be obtained as the relative reactivities of adenosine and adenosine 5′-phosphate, respectively, with unity taken for adenine. Since the observed values given in Table 1 are much less than the values estimated above from diffusion rate, the second cause will be eliminated.

The third possible cause is steric hindrance, namely mutual protection between base and sugar moieties. In Fig. 1, rate constants of nucleic acids and related compounds for hydroxyl radical are plotted against those for dichloride anion radical. In a previous paper,[6] we reported that a linear correlation with slope 11 was found between reactivities of pyrimidine bases toward hydroxyl radical and those toward dichloride anion radical.

For bases, nucleosides, nucleotides, polynucleotides, and nucleic acids, a similar correlation was observed, except for 2-amino derivatives of pyrimidine and purine. The slope 15 in the correlation is close to that found for pyrimidine bases. On the other hand, a slope of the correlation found for D-ribose and 2-deoxyribose 5-phosphate, 87.5, is close to that found for aliphatic dipeptides, 65.8.[6] These results suggest that the overall reactivity of a nucleoside, nucleotide,

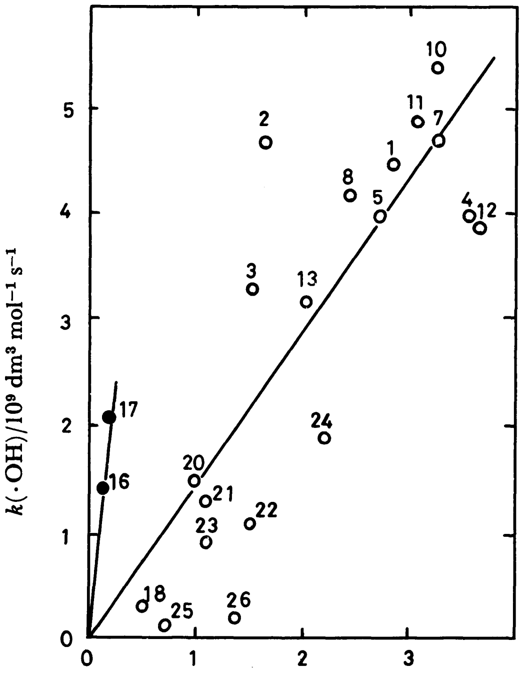

Fig. 1. $k(\cdot OH)$ *vs.* $k(Cl_2^-)$.

Figure associated with each plot corresponds to the number used in Tables 1 and 2.

polynucleotide, or nucleic acid is governed mainly by base moiety, the contribution of sugar or sugar phosphate moiety being negligible. Therefore, Eq. 11 is rewritten to $k=f_1k_b$.

The rate constant of a nucleoside will be predicted by the above equation, if f_1, the degree of protection of base moiety by sugar moiety, is estimated from the solid angle open to the attack of dichloride anion radical, as discussed in the case of hydroxyl radical.2) Although for the addition reaction of hydroxyl radical C-5 has been proposed as the reaction site by ESR studies,22,23) the reaction site on a pyrimidine base is not clear for one-electron oxidation. However, π-electron has been suggested to react with the anion radical (Reaction 8) by ESR studies.7,8)

Therefore, by assuming that the reaction site on nucleoside for the anion radical is C-5 of pyrimidine ring, a calculation of f_1 was attempted for reactions of the anion radical with nucleosides. With each atom of sugar moiety projecting on the surface of unit sphere centered at C-5 of a base, the area of projection was determined as described in a previous paper.2) Coordinates of nucleoside reported by Lakshiminarayanan and Sasisekȟaran24) were used for the calculation of solid angles. Area was calculated for stepwise change of the torsional angle about the bond between N-1 and C-1′. Contact distances were taken as the sum of van der Waals radii. For the anion radical, the van der Waals radius was assumed as the average of the long and short axes, 0.25 nm. The estimated rate constants for uridine, cytidine and thymidine are given in Table 3.

They are presented as the values estimated for the upper and lower limits of protection. Compared with the case of hydroxyl radical, the agreement with the observed values is not good except uridine. This may be due to the assumption of the reaction site, because the effective size of π-orbital was neglected in the calculation. However, steric hindrance could provide values closer to observed ones.

The results obtained for homopolynucleotides and nucleic acids indicate that the reactivity toward dichloride anion radical depends on their conformation. The reactivity of a polynucleotide is 0.5—0.65 times as high as that of the corresponding nucleotide. This may be due to a protection by the sugar phosphate backbone. The reactivity of a nucleic acid is relatively high, in spite of its tight conformation. This will be explained in terms of the presence of guanosine 5′-phosphate, which is included in the molecules and very reactive to the anion radical. The increase in reactivity induced by heat treatment may also be understood in terms of conformational degradation.

The results obtained for the reaction of dichloride anion radical can be understood in the same way with the reaction of hydroxyl radical. The explanation based on steric effect is probably applicable to reactions of these compounds with some other radicals in aqueous solutions.

TABLE 3. ESTIMATED RATE CONSTANTS OF REACTIONS OF NUCLEOSIDES WITH DICHLORIDE ANION RADICAL

Compound	Rate constant 10^8 dm^3 mol^{-1} s^{-1}
Uridine	1.3—1.8
Cytidine	1.7—2.3
Thymidine	1.5—2.1

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