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Transient Intermediate Produced from 5-Aminouracil in Reaction with Dichloride Anion Radical

Takahiro Masuda,* Ryoji Gunji, Takako Kimura, and Kotaro Yamauchi
Department of Chemistry, Faculty of Science, Tokyo Metropolitan University, Fukasawa, Setagaya-ku, Tokyo 158

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A transient absorption, which is relatively long-lived, was found in a flash photolysis of aqueous potassium chloride containing 5-aminouracil. The transient absorption with a peak at about 335 nm was assigned to an intermediate produced from 5-aminouracil through a one-electron oxidation by dichloride anion radical. For the intermedicate, a possible structure is proposed to explain its long life-time. On the basis of the transient absorption, a determination of the rate constant for the reaction of 5-aminouracil with dichloride anion radical was carried out by use of a competition kinetics. The value of $(2.9 \pm 0.2) \times 10^8$ dm^3 mol^{-1} s^{-1} was obtained as the rate constant on the basis of rate constants of several reference compounds. By comparing the values obtained by the competition method with those estimated by a direct analysis of the decay rate of dichloride anion radical, the validity of the competition method was examined.

In the course of our study on reactions of dichloride anion radical with pyrimidine derivatives,[1] a relatively long-lived transient absorption with a peak at about 335 nm was found in a flash photolysis of aqueous solutions of potassium chloride containing 5-aminouracil. It overlaps a transient absorption due to dichloride anion radical and obstracts the determination of the rate constant for the reaction of 5-aminouracil with dichloride anion radical by anaysis of the decay rate of the anion radical (a direct method).

Intermediate products of pyrimidine bases produced through reaction with dichloride anion radical have been suggested as π-cation radicals by ESR studies.[2,3] Absorption spectra of intermediate species formed from pyrimidine bases through reaction with oxidizing radical such as $SO_4^{-\cdot}$ or $Cl_2^{-\cdot}$ have been reported to have absorption maxima at 410 and 560 nm for uracil, 400 and 500 nm for thymine, and 410 nm for isoorotic acid.[2] However, no transient absorptions were observed in the time-scale of flash photolysis. It implies a fast decay of transient species responsible for the absorptions. A half-life of the transient intermediate formed from 5-aminouracil was estimated to be *ca.* 800 μs, which is considerably long compared with those for transient species from other bases and for dichloride anion radical.

In the present study, an assignment of transient absorptions was attempted by comparison of transient spectra observed under various conditions where different kinds of intermediate species are allowed to form. Furthermore, a competition method using a transient absorption at 330 nm was applied to the determination of the rate constant of 5-aminouracil with dichloride anion radical. With several referance compounds, the method was confirmed to give a reasonable value for the rate constant of 5-aminouracil with dichloride anion radical. The validity for wide application of the method was examined and discussed in terms of the reactivity of products produced from substrates and of a possible interaction between a substrate and 5-aminouracil in aqueous solutions.

Experimental

5-Aminouracil (abbreviated to 5AU) and other pyrimidine bases were obtained from Sigma Chemical Co. and peptides from Nutritional Biochemical Corp. as preparations of high purity. They were used without further purification. Potassium chloride and other chemicals were of reagent grade.

Solutions for irradiation were prepared with triply distilled water and adjusted to pH 7. The solutions were bubbled with dinitrogen monoxide, sulfur hexafluoride, or nitrogen for 20 min immediately before flash photolysis. A conventional type of apparatus was used for flash photolysis with 10 μs of 1/e duration and 200 J of input energy. Details were described in a previous paper.[4] Transient spectra were measured by a kinetic spectrophotometry. Calculation for kinetic analysis was carried out with the aid of microcomputers PC 8001 and 8801 (Nippon Electric Co.).

Results and Discussion

Transient Absorption Spectra. Transient absorption spectra of flash photolyzed aqueous solutions of 5-aminouracil were measured under several conditions (Fig. 1(a)). For the N_2O-saturated neutral solution of potassium chloride containing 5-aminouracil, absorption peaks at about 335 and 390 nm were observed at 150 μs after start of flash, while a peak at 335 nm was observed for the SF_6-saturated solution. For the acid solution (pH 3.15), a maximum was seen at 335 nm. The peak at 335 nm was also observed in the absence of potassium chloride. This suggests that the direct photoionization takes place to yield a similar intermediate.

In the N_2O-saturated neutral solution of potassium chloride containing a sufficient amount of 5-aminouracil, the following reactions are expected to proceed in flash photolysis:

$$Cl^- + h\nu \longrightarrow Cl\cdot + e^-, \tag{1}$$

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

$$e^- + aq \longrightarrow e_{aq}^-, \tag{3}$$

$$e_{aq}^- + N_2O \longrightarrow N_2 + HO\cdot + OH^-, \tag{4}$$

$$HO\cdot + Cl^- \longrightarrow ClOH^{-\cdot}, \tag{5}$$

$$HO\cdot + 5AU \longrightarrow 5AUOH\cdot, \tag{6}$$

$$ClOH^{-\cdot} + 5AU \longrightarrow 5AUOH\cdot + Cl^-, \tag{7}$$

$$Cl_2^{-\cdot} + 5AU \longrightarrow 5AU^{+\cdot} + 2Cl^-. \tag{8}$$

In flash photolysis of the N_2O-saturated neutral solution of 5-aminouracil, Reaction 9, a direct photoionization, will occur, followed by Reactions 3, 4, and

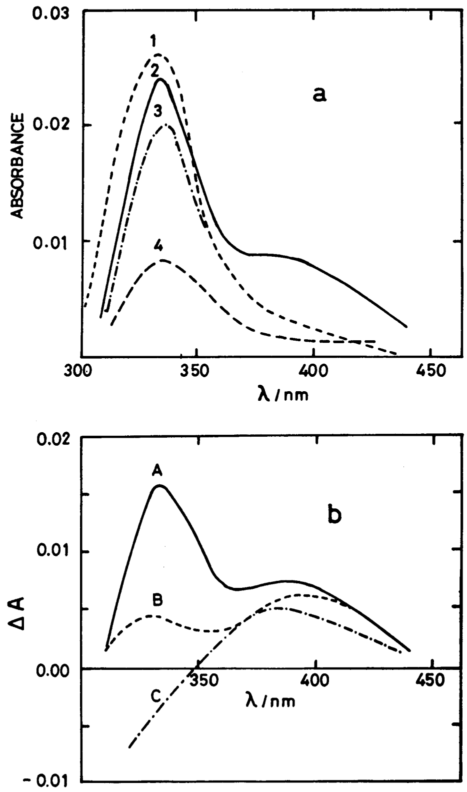


Fig. 1. Absorption spectra of the intermediates produced from 5-aminouracil in flash photolysis.
(a) Transient spectra measured at 150 μs after start of flash. [5AU] = 5×10^{-5} mol dm^{-3}. 1: 1 mol dm^{-3} KCl, N_2O saturated, pH 3.15. 2: 1 mol dm^{-3} KCl, N_2O saturated, neutral pH. 3: 1 mol dm^{-3} KCl, SF_6 saturated, neutral pH. 4: N_2O saturated, neutral pH.
(b) Difference spectra. A: 2-4. B: 2-3. C: 2-1.

6; then the formation of a cation radical ($5AU^{+}\cdot$) and an OH-adduct ($5AUOH\cdot$) is expected:

$$5AU + h\nu \longrightarrow 5AU^{+\cdot} + e^{-}. \tag{9}$$

When an acid solution of potassium chloride containing 5-aminouracil is photolyzed with N_2O-saturation, Reactions 10 and 11 will be added to Reactions 1—5 and 8:

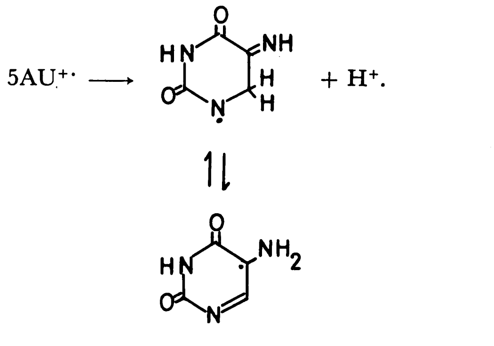
$$e_{aq}^{-} + H^{+} \longrightarrow H\cdot, \tag{10}$$

$$ClOH^{-\cdot} + H^{+} \longrightarrow Cl\cdot + H_2O. \tag{11}$$

Although an addition reaction of a hydrogen atom to 5-aminouracil can be expected in acid solutions, data on rate constants and concentrations indicate that Reaction 4 overwhelms Reaction 10, since the rate constants for Reactions 4 and 10 are reported to be 8×10^{9} [5] and (2.2—2.4)$\times10^{10}$ dm^{3} mol^{-1} s^{-1},[6] respectively, and the concentrations of N_2O and H^{+} are 2.5×10^{-2} and 7×10^{-4} mol dm^{-3}, respectively. Therefore, $5AU^{+\cdot}$ is considered as the main intermediate product formed from 5-aminouracil in the acid solution. In the SF_6-saturated solution of potassium chloride containing 5-aminouracil, Reaction 12 may occur in addition to Reactions 1—3 and 8:

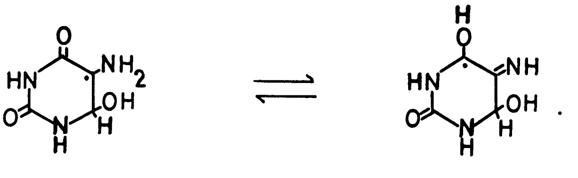
$$e_{aq}^{-} + SF_6 \longrightarrow SF_5\cdot + F^{-}. \tag{12}$$

The difference spectra shown in Fig. 1(b) can be well understood by combination of the above reactions. The absorption peaks at 335 and 390 nm are attributable to $5AU^{+\cdot}$ and $5AUOH\cdot$, respectively. Danziger *et al.*[7] reported that the OH-adduct of uracil shows an absorption maximum at 390 nm. Similarly Mayers *et al.*[8] indicated that the OH-adducts of uracil and thymine have absorption spectra with a maximum at 375 nm and that the second-order decay constants of the OH-adducts are 2.1×10^{9} dm^{3} mol^{-1} s^{-1} for uracil and 2.2×10^{9} dm^{3} mol^{-1} s^{-1} for thymine. The half-life was also estimated by the authors to be *ca.* 100 μs for 5×10^{-6} mol dm^{-3} of initial concentration. For the OH-adduct of 5-aminouracil studied in the present work, however, about 600 μs of half-life was obtained at the initial concentration of 3.5×10^{-6} mol dm^{-3}. Apparently the OH-adduct of 5-aminouracil is more stable than those of other bases. Bansal and Fessenden[2] and Sevilla *et al.*[3] have proposed structure of π-cation radicals produced from pyrimidine bases through reaction with oxidizing radicals on the basis of ESR studies. The structures correspond to neutral radicals with a high electron density at C-5, which are produced from cation radicals after deprotonation respectively. For the intermediate species of 5-aminouracil responsible for the absorption at 335 nm, therefore, deprotonated forms of the cation radical ($5AU^{+\cdot}$) can be considered. The possible structure is as follows:



(13)

The N-radical will be an explanation for the long life-time. An analogous argument with the OH-adduct leads to the following structure:



(14)

Competition Kinetics. Assuming a competition between Reactions 8 and 15, Eq. I can be derived:

$$Cl_2^{-\cdot} + S \longrightarrow 2Cl^{-} + \text{product(s)} \quad k_s, \tag{15}$$

$$\frac{A_0}{A} = 1 + \frac{k_s[S]_0}{k_8[5AU]_0}, \tag{I}$$

where A_0 and A represent the absorbances at 330 nm in the absence and presence of substrate, respectively, and $[S]_0$ and $[5AU]_0$ designate the initial concentrations of the substrate and 5-aminouracil, respectively. By plotting A_0/A against $[S]_0/[5AU]_0$, k_s/k_8 is obtained from the slope of a straight line. Equation I is valid when the dichloride anion radical converts to the intermediate

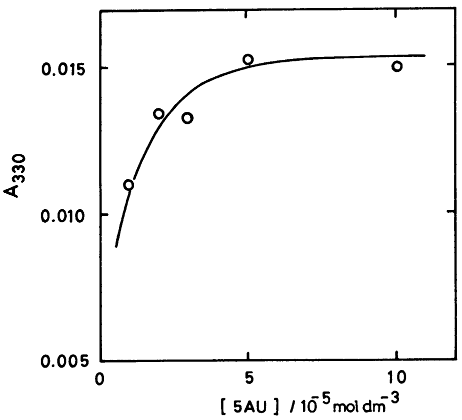


Fig. 2. Dependence of the absorbance at 330 nm of the intermediate of 5-aminouracil upon the concentration of 5-aminouracil.
1 mol dm^{-3} KCl, N_2O saturated, neutral pH.

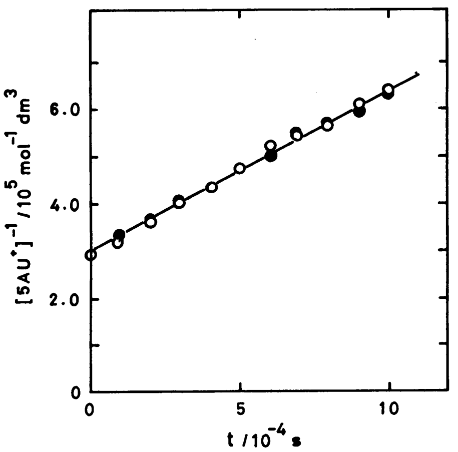


Fig. 3. Second-order decay of the intermediate of 5-aminouracil. ○: 5×10^{-5} mol dm^{-3} 5-aminouracil. ●: 10^{-4} mol dm^{-3} 5-aminouracil.

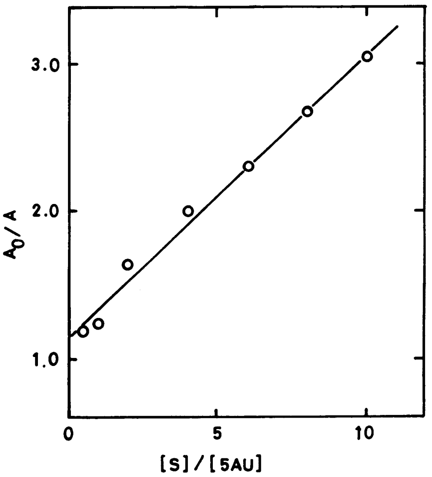


Fig. 4. Competition between 5-aminouracil and glycylnorvaline against the dichloride anion radicals.

Table 1. Rate constants for the reaction of dichloride anion radical with 5-aminouracil estimated by the competition method

Reference compound	Slope	$\dfrac{k_S}{10^8\ dm^3\ mol^{-1}\ s^{-1}}$
Cytosine	1.09	3.21
Thymine	1.06	3.02
Glycyl-α-amino-*n*-butyric acid	0.114	2.63
Glycylnorvaline	0.182	2.80
Glycylvaline	0.150	2.73
Glycylleucine	0.170	3.18
	Average	2.93 ±0.24

of 5-aminouracil completely. The dependence of the absorbance at 330 nm on the concentration of 5-aminouracil (Fig. 2) and the decay rate of the intermediate of 5-aminouracil (Fig. 3) seem to confirm this condition. The absorbance at 330 nm was observed to reach the upper limit at *ca.* 5×10^{-5} mol dm^{-3}, as shown in Fig. 2. For 5×10^{-5} and 10^{-4} mol dm^{-3} solutions of 5-aminouracil, an identical decay rate of the intermediate was found (Fig. 3). The decay process obeys a second-order kinetics and the value of 5.8×10^4 was obtained as $2k/\varepsilon$. The absorption coefficient of the intermediate of 5-aminouracil is estimated to be 6200 $dm^3\ mol^{-1}\ cm^{-1}$, on the assumption that a complete conversion of dichloride anion radical to the intermediate at 10 μs after start of flash. Consequently the value of $3.6\times10^8\ dm^3\ mol^{-1}\ s^{-1}$ was obtained as $2k$. The result obtained by plotting A_0/A *vs.* $[S]_0/[5AU]_0$ supports the reliability of Eq. I. Figure 4 shows an example of competition plot obtained for glycylnorvaline.

The rate constant of 5-aminouracil with dichloride anion radical can be estimated by using some reference compounds whose rate constants have been determined by the direct method.[1,9)] The direct determination of rate constant for reaction with dichloride anion radical is based on analysis of the decay rate of dichloride anion radical.[1)] Equation II is derived from Reactions 1, 2, 15, and 16:

$$Cl_2^{-\cdot} + Cl_2^{-\cdot} \longrightarrow Cl_2 + 2Cl^- , \qquad (16)$$

$$\ln\frac{[Cl_2^{-\cdot}]_0(2k[Cl_2^{-\cdot}]+k_{ob})}{[Cl_2^{-\cdot}](2k[Cl_2^{-\cdot}]_0+k_{ob})} - k_{ob}t = 0, \qquad (II)$$

where k_{ob} is equal to $k_S[S]$; $[Cl_2^{-\cdot}\ t]_0$ and $[Cl_2^{-\cdot}]$ are the concentrations of dichloride anion radical at time zero and t, respectively. $2k$ is the rate constant for Reaction 16. The concentration of the anion radical at time zero was assumed to be equal to the value obtained by applying an extrapolation of $1/[Cl_2^{-\cdot}]$ and t. Values of k_{ob} at given times were estimated by Eq. II. From the values estimated at every 10 μs from 50 to 100 μs after start of flash, an average value of k_{ob} was calculated for a given concentration of substrate. The average values of k_{ob} were plotted against the concentration of substrate and k_S was obtained from the slope. The result is summarized in Table 1. With six reference compounds, the value of $(2.9\pm0.2)\times10^8$ $dm^3\ mol^{-1}\ s^{-1}$ was obtained as k_S. The value shows a close agreement with the value of $(3.1\pm0.5)\times10^8\ dm^3$ $mol^{-1}\ s^{-1}$, reported in a previous paper,[1)] as estimated from data with cytosine, thymine, glycylleucine, and

TABLE 2. COMPARISON OF RATE CONSTANTS ESTIMATED BY THE COMPETITION METHOD WITH THOSE DETERMINED BY OTHER METHODS

Compound	Rate constant/dm^3 mol^{-1} s^{-1}		
	Competition method	Direct method	Reported value
Formate	2.0×10^6	1.3×10^7	1.9×10^6 a)
Succinate	6.5×10^6	9.2×10^6	8×10^2 a) (pH 1)
Acrylate	9.7×10^7	9.0×10^7	1.9×10^7 a)
Fumarate	3.7×10^8	3.3×10^8	4×10^6 a)
Maleate	1.7×10^8	1.7×10^8 b)	3.5×10^7 c) 3.7×10^7 d) 4.1×10^7 e)
6-Aminouracil	1.5×10^8	2.7×10^9	

a) Ref. 10. b) Ref. 1. c) Ref. 12. d) Ref. 13. e) Ref. 14.

formate.

The rate constant for a given substrate with dichloride anion radical can be estimated by the competition method making use of k_8 as the reference. Results are shown in Table 2. Agreement of the values with those obtained by the direct method seems to be fairly good with some exceptions. The values reported by other authors are in general smaller than the present values. The values cited in the fourth column of Table 2 were determined by a pseudo-first-order kinetics with pulse radiolysis. Despite the low doses applied, neglect of Reaction 16 may cause an incorrect estimation, especially for relatively low concentrations of substrate. Furthermore, the analysis of the decay rate of dichloride anion radical would be disturbed by a new absorption due to an intermediate species from the substrate, if the absorption appears in the same region of wavelength with dichloride anion radical, because the time-scale of pulse radiolysis is much shorter than that of flash photolysis.

For formate ion, the value determined by the competition method differs from that by the direct method, but is in fairly good agreement with the value determined by pulse radiolysis. The reaction of dichloride anion radical with formate ion produces a carbon dioxide anion radical ($CO_2^{-\cdot}$):

$$Cl_2^{-\cdot} + HCOO^- \longrightarrow 2Cl^- + H^+ + CO_2^{-\cdot}. \quad (17)$$

$CO_2^{-\cdot}$ will react with dichloride anion radical to form carbon dioxide:

$$Cl_2^{-\cdot} + CO_2^{-\cdot} \longrightarrow 2Cl^- + CO_2. \quad (18)$$

Therefore, the reaction of formate ion with dichloride anion radical is complicated and the direct method is not appropriate to the substrate.

For uracil and 6-aminouracil, significant differences were observed between the values obtained by the competition and direct methods. An electron transfer from 5-aminouracil to a substrate cation (S^+) will be an explanation for the discrepancy. The electron transfer causes a decrease in reactivity of the substrate in appearance, when the competition kinetics is applied. However, a simulation by a computer indicated that the mechanism gives no linear correlation between A_0/A and $[S]_0/[5AU]_0$. Another explanation is the formation of a complex of 5-aminouracil with the substrate:

$$5AU + S \overset{K}{\rightleftharpoons} 5AU\cdot S, \quad (19)$$

$$5AU\cdot S + Cl_2^{-\cdot} \longrightarrow 5AU^{+\cdot} + S + 2Cl^- \quad k_c. \quad (20)$$

Assuming that the complex reacts with dichloride anion radical to give $5AU^{+\cdot}$ the following relation is derived:

$$A_0/A = 1 + \frac{k_s[S]}{k_8[5AU]+k_c[5AU\cdot S]}, \quad (III)$$

where [S], [5AU], and [5AU·S] designate the concentrations of a substrate, 5-aminouracil, and the complex at equilibrium, respectively; k_c is the rate constant for Reaction 20. When 5-aminouracil is converted to the complex completely, Eq. III is transformed to

$$A_0/A = 1 + \frac{k_s[S]}{k_c[5AU\cdot S]}, \quad (IV)$$

where [5AU·S] is equal to $[5AU]_0$ and [S] is the difference between $[S]_0$ and $[5AU]_0$, hence

$$A_0/A = \left(1-\frac{k_s}{k_c}\right) + \frac{k_s[S]_0}{k_c[5AU]_0}. \quad (V)$$

When the ratio of the initial concentrations, $[S]_0/[5AU]_0$, is sufficiently large, the slope of the straight line obtained by plotting A_0/A *vs.* $[S]_0/[5AU]_0$ provides the value of k_c. For 6-aminouracil and uracil, 5.2×10^9 and 5.8×10^8 dm^3 mol^{-1} s^{-1} were obtained as k_c, respectively. Using the equilibrium constant, K, as a parameter and the value of k_c, a simulation of Eq. III was carried out and 1.5×10^4 of K was found to give the best fitted curve in the case of 6-aminouracil, as shown in Fig. 5. For uracil, the best fitted curve was obtained for 10^4 of K. Although there is no confirmation for the formation of the complex, the resemblance of their structures suggests a possible interaction between them.

For the dicarboxylic acids, the agreement is fairly good between the two methods. Since the values of

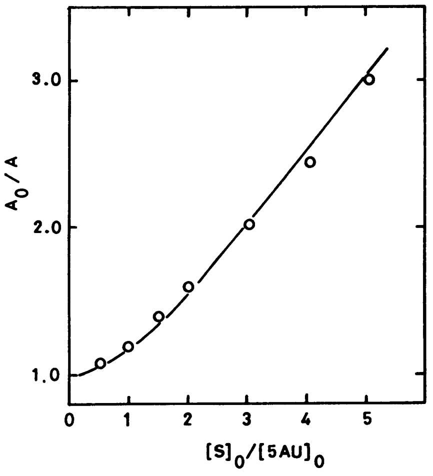


Fig. 5. Simulation of A_0/A *vs.* $[S]_0/[5AU]_0$ based on the mechanism of complex formation. Substrate: 6-aminouracil.

1.4×10^6 and 2.5×10^6 $dm^3\,mol^{-1}\,s^{-1}$ were obtained as the rate constants for acetate and propionate,[11] the value for succinate seems reliable. In a previous study,[1] the partial rate constant for the 5, 6 double bond of uracil for the reaction with dichloride anion radical was estimated to be 2.2×10^8 $dm^3\,mol^{-1}\,s^{-1}$. For a compound containing a double bond, therefore, a value of the order of 10^8 can be expected as the rate constant of reaction with dichloride anion radical if the compound has no other reactive group than the double bond or no repressive one. The observed rate constants for the unsaturated carboxylic acids appear to be consistent with this argument. Maleate was found to be less reactive than fumarate toward dichloride anion radical. This implies that a stabilization due to the interaction between two carboxyl groups in the *cis*-form may cause a decrease in the reactivity of one electron oxidation.

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