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Kinetic Study of the Formation and Decomposition of Amylose-iodine-iodide Complex

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The complex formation reaction among amylose, iodine and iodide ion in aqueous solution was investigated with a stopped-flow apparatus. The forward reaction rate was found to be expressed as $v=k[\text{Amylose}]([\text{I}_3^-]-\theta)$ for $[\text{I}_3^-]>\theta$, where θ is a critical value determining the mechanism. For $[\text{I}_3^-]>\theta$, the rate was considered to be determined by the process between a free amylose unit and free I_3^-. For $[\text{I}_3^-]<\theta$, the rate-determining step is considered to be intramolecular conversion from an intermediate to a final product. The rate of the reaction between amylose units and I_3^- is close to that of a diffusion-controlled process ($7\times10^8\ \text{M}^{-1}\,\text{sec}^{-1}$). These conclusions are supported by the study of the decomposition reaction of complex, using $\text{Na}_2\text{S}_2\text{O}_3$ or $\text{Ce(SO}_4)_2$.

The blue complex of amylose and iodine has been the subject of many investigators since its discovery in the last century. Recently a few investigations proved that a kinetic study, using stopped-flow technique, is a powerful approach to look into the formation mechanism and the structure of the amylose-iodine complex.[1,2] Hiromi *et al.* pointed out in his excellent paper that there exists an intermediate form of a complex before the final stable product appears.[1] Since the intermediate complex has an absorption maximum at a shorter wavelength than that of a final product, it is thought to be a short poly-iodine-chain randomly distributed in the amylose helix. The intermediate complex rearranges intramolecularly to the stable product. The growth of the triiodide chain was undoubtedly confirmed by the shift of an absorption peak and also by the fact of the high order dependence (about five) of the formation rate on triiodide ion concentration. These observations are a definite proof to show that a kinetic approach is powerful to study this complicated phenomena. There are, however, several problems left unsettled. For example, the magnitude of the rate constant of the initial attack of triiodide ion on the amylose helix is yet to be determined. If this rate constant is obtained, it will help us to consider what is happening in the initial stage of the reaction. It is also important to discriminate the role of iodide ions from that of iodines. This will explain why iodide ion is necessary for the complex formation in aqueous solution.[3] Another problem is about the effect of temperature on the reaction, since temperature is known to have a large influence over the amount of the complex in equilibrium.[4]

We tried to settle these questions under the appropriate conditions of amylose, iodide ion and iodine amounts.

Experimental

Materials. Commercial corn amylose (Sigma) was recrystallized twice from *n*-butanol under nitrogen atmosphere. Fractional precipitation was made, using dimethylsulfoxide and ethanol as solvents according to Everett and Forster.[5] Precipitates were dried *in vacuo.* The molecular weights of five fractions were determined by viscosity measurements, following Potter and Hassid.[6] A fraction with MW 1.0×10^4 was used for kinetic studies.

All inorganic reagents were commercial reagent grade and used without further purification.

Solutions. About 5 mg of amylose was dissolved in 1 m*l* of 1 M KOH solution, and neutralized by 1 M HClO_4 solution. The solution was diluted to 100 m*l* and used as a stock solution. This solution was freshly prepared before use.

About 1 g of iodine was added into a 250 m*l* aqueous solution containing 1.4×10^{-6} M KIO_3 and 0.1 M HClO_4, where KIO_3 and HClO_4 were added in order to suppress the hydrolysis of iodine.[7] After this mixture was stirred at room temperature for about 10 hr, it was filtered through sintered glass. The concentration of iodine and triiodide ion were determined spectrophotometrically from the molar extinction coefficients given by Awtrey and Connick;[7] 746 at 460 mμ for iodine and 26400 at 352 mμ for triiodide ion. The typical value of iodine concentration in a stock solution was about 1×10^{-3} M, and the upper limit of triiodide ion concentration 2×10^{-7} M.

Number of Amylose Equivalents. The concentration of amylose equivalents present in a solution was calculated from the spectra of an amylose-iodine complex. In Fig. 1 is shown an optical density of the complex at 620 mμ under the condition of excess iodine over amylose. Seeing that the optical density increases linearly with the concentration of amylose and that it stays constant in the range from 10 to 25°C, it was concluded that amylose was complexed to its maximum capacity. Using the molar extinction coefficient for one bound-iodine in case of $(0.1—0.3)[\text{I}^-]/[\text{I}_2]$, given by Schneider *et al.*,[4] we obtained that 7.8 glucose units take up one iodine molecule. This is in good agreement with the generally accepted value.[4] Therefore, we used this relationship to calculate the number of amylose equivalents from the weight percentage of amylose.

Kinetic Measurements. The rate of formation reaction between amylose and iodine was measured by following the rise of optical density at 620 mμ in a Yanagimoto stopped-flow apparatus. Iodide ions were added in an iodine solution before mixing. The rate of decomposition reaction was

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[1] K. Hiromi, T. Shibaoka, and S. Ono, *J. Biochem.*, **68**, 205 (1970).

[2] J. Thompson and E. Hamori, *J. Phys. Chem.*, **75**, 272 (1971).

[3] J. A. Thoma and D. French, *J. Amer. Chem. Soc.*, **82**, 4144 (1960).

[4] C. L. Cronan and F. W. Schneider, *J. Phys. Chem.*, **73**, 3900 (1969).

[5] W. W. Everett and J. F. Foster, *J. Amer. Chem. Soc.*, **81**, 3460 (1959).

[6] A. L. Potter and W. Z. Hassid, *ibid.*, **73**, 593 (1951).

[7] A. D. Awtrey and R. E. Connick, *ibid.*, **73**, 1842 (1951).

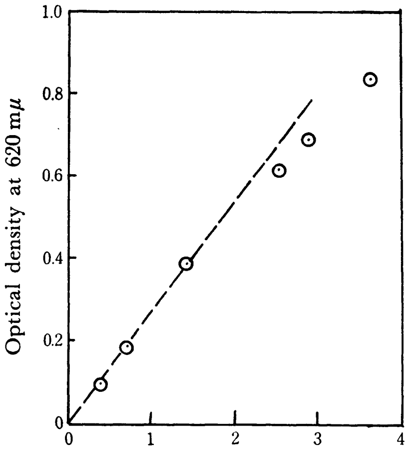

Fig. 1. The dependence of the optical density at 620 mμ on the concentration of amylose.
$[I_2] = 7.4 \times 10^{-5}$ M, $[I^-] = 1.0 \times 10^{-5}$ M. Temperature 10—25°C.

measured by mixing a solution containing amylose-iodine-iodide complexes with a solution containing decomposing reagents $(Ce(SO_4)_2$ or $Na_2S_2O_3)$. The decrease of optical density was followed at 620 mμ. In both experiments, a 10 mm observation cell was used. Temperature was maintained within ±0.1°C.

Results and Discussion

(A) Dependence of Amount of Complex on the Concentration of Iodide Ions. It is known that the complex is not produced when no iodide ion is present in aqueous solution.[3] In order to obtain the dependence of the amount of the complex on the concentration of iodide ion, we mixed the solution of amylose with that of iodide ion and iodine in a stopped-flow apparatus, varying the concentration of iodide ion, $[I^-]$. The time course of the rise of the optical density is shown in Fig. 2 for several values of $[I_2]$ and $[I^-]$. As $[I_2]$ and $[I^-]$ decrease, it changes from an exponential curve to non-exponential one. Non-exponential curves consist of at least two relaxations. We call the first and the second relaxations the relaxation (I) and (II) respectively. In Fig. 3, the amount of the complex formed in the relaxation (I) is plotted against the initial value of $[I_2]$, where we assumed that in the exponential curves the product is formed only through the relaxation (I). In Fig. 4 the same result is also plotted against the initial concentration of triiodide ion, $[I_3^-]$, which was calculated from an equilibrium constant given by Awtrey and Connick.[7] The latter plot shows that the amount of complex seems to be determined uniquely by the initial $[I_3^-]$ in spite of various values of $[I^-]$ and $[I_2]$. Thus from the figure, 1.0×10^{-5} equivalents of amylose are all complexed when 6×10^{-7} M of $[I_3^-]$ is present. In other words one molecule of I_3^- is required for about 17 equivalents of amylose to form the complex by a single relaxation. Another conclusion is that the complex contains a smaller number of iodide ions in comparison with amylose equivalents, since nearly all amylose equivalents become complexed for sufficiently high $[I_2]$ under the condition of $[I^-]/[\text{Amylose Equivalent}] = 0.125$ as shown in Fig. 3.

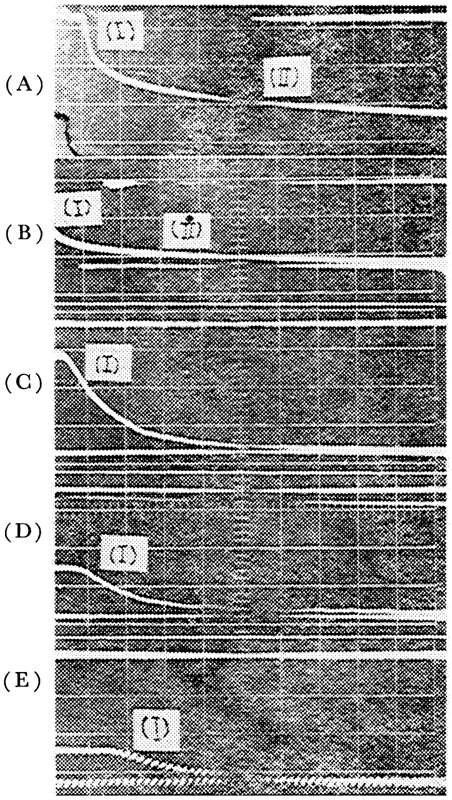

Fig. 2. The increase of the optical density at 620 mμ with time for various concentration of iodine and iodide ion at 25°C.
The initial values of $[I_2]$ and $[I^-]$ and the time scale are: (A) 5.5×10^{-5} M, 1.25×10^{-6} M, 5 sec/div., (B) 1.1×10^{-4} M, 1.25×10^{-6} M, 2 sec/div., (C) 1.6×10^{-4} M, 2.0×10^{-6} M, 50 msec/div., (D) 2.6×10^{-4} M, 3.0×10^{-6} M, 2 msec/div., (E) 2.6×10^{-4} M, 4.5×10^{-6} M, 1 msec/div. The vertical scale 0.1 ΔO.D./div. Amylose concentration 1.0×10^{-5} eq. The horizontal line above each signal shows the initial position of the optical density.

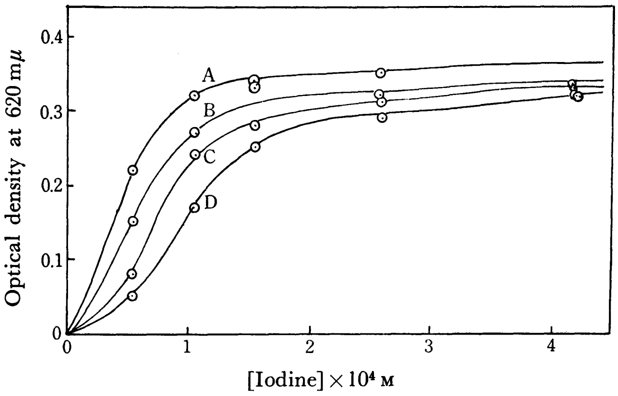

Fig. 3. The dependence of the optical density at 620 mμ on the iodine concentration for various values of iodide ion concentrations at 25°C.
$[I^-]$ is (A) 4.50×10^{-6} M, (B) 3.0×10^{-6} M, (C) 2.0×10^{-6} M, (D) 1.25×10^{-6} M. Amylose concentration 1.0×10^{-5} eq.

(B) Dependence of the Rate of Complex Formation on the Initial Concentration of Amylose. The rate of formation was measured, varying the initial number of amylose equivalents, [Am.]. The result is shown in

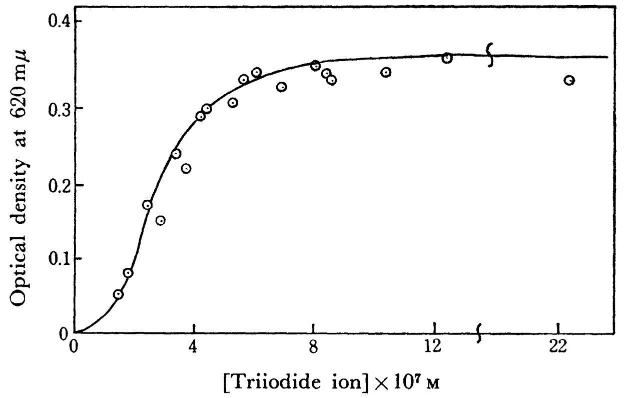

Fig. 4. The dependence of the optical density at 620 mμ on the triiodide ion concentration at 25°C.

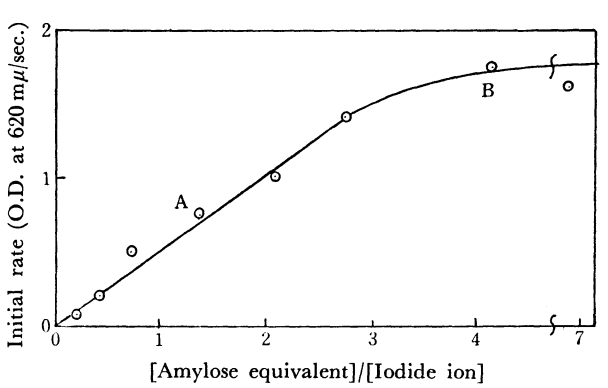

Fig. 5. The dependence of the initial rate of the complex formation on the ratio of the number of amylose equivalents to the iodide ion concentration at 10°C. $[I_2]=2.6\times10^{-5}$ M, $[I^-]=5.0\times10^{-6}$ M. The region A denotes a linear portion, and the region B a staturated portion.

Fig. 5. As expected from the assumption that a certain unit of amylose polymer behaves independently in the course of formation reaction, the initial rate increases in proportion to [Am.] for small [Am.]. However, the rate becomes saturated to a constant value as [Am.] becomes comparable to $[I^-]$. In this region the time dependence of the rise of optical density becomes non-exponential, coinciding with the results in section (A). The result means that, although we called the initial stage of complex formation the relaxation (I) over all amylose range, the mechanism itself changes as the amylose amount passes through a critical value. Taking the results reported by Hiromi into account,1) we concluded that, for small [Am.] (region A), the formation rate is determined by the reaction between a free amylose unit and free I^- or I_2 or its variants. On the other hand, for large [Am.] (region B), the rate is determined by the intramolecular process of an intermediate. The rise of optical density was followed in the wavelength range from 700 mμ to 300 mμ in order to find the spectra of a transient compound. However, no shift of the absorption peak was observed in both region A and B. Therefore, it is assumed that the process following the rate-determining step is too rapid to be observed, and that the transient compound has no absorption in the visible region,

(C) Dependence of the Rate of Complex Formation on the Concentration of Iodine and Iodide Ion. The rate of formation was measured for various values of $[I^-]$ and $[I_2]$. The results are shown in Fig. 6, in which the reciprocal of the half-life of the relaxation (I), $t_{1/2}$, is plotted against the initial $[I_3^-]$. The reason to choose $[I_3^-]$ as a parameter is that this plot is found to give a single curve in the wide range of $[I^-]$ and $[I_2]$. For small $[I_3^-]$, corresponding to region B in section (B), the rate is extremely slow and depends crucially on $[I_3^-]$. For large $[I_3^-]$, corresponding to region A, the rate increases linearly with $[I_3^-]$. Therefore, $t_{1/2}$ is expressed approximately as

$$t_{1/2}^{-1} = k([I_3^-]-\theta) \qquad (1)$$

where θ is a value of the $[I_3^-]$ at the intercept obtained by extrapolating the linear portion of the curve. In section (B) it was suggested that in the region of $[I_3^-]<\theta$, the rate of complex formation is determined by the intramolecular process and that in the region of $[I_3^-]>\theta$, the rate is determined by the reaction between free amylose units and I^- or I_2 or its variants. From the relationship of Eq. (1), one of the possibilities for the rate-determining step is a reaction between an amylose unit and I_3^-,

$$\mathrm{Am} + I_3^- \rightarrow \mathrm{Am}\cdot I_3^-. \qquad (2)$$

Since the complex $\mathrm{Am}\cdot I_3^-$ is thought to have no absorption in the wavelength longer than 600 mμ,8) reaction (2) may be followed by chain growth reaction. This later step which is too fast to be observed as stated in section (B) involves a reaction between an intermediate complex and I_2, *e.g.*

$$\mathrm{Am}\cdot I_3^- + I_2 \rightarrow \mathrm{Am}\cdot I_3^-\cdot I_2. \qquad (3)$$

Thus the final product contains many I_2 molecule as concluded in section (A). In the region $[I_3^-]>\theta$, since $[I_2]$ and $[I^-]$ are in excess over amylose equivalents, they are assumed to be kept constant during the reaction. Supposing the equilibration $I^-+I_2 \rightleftharpoons I_3^-$ is much faster than the formation rate,9) $[I_3^-]$ is also kept constants. In reaction (2), therefore, the decrease of amylose units is expressed as below, neglecting a reverse reaction

$$d[\mathrm{Am.}]/dt = -k[\mathrm{Am.}][I_3^-],$$

where [Am.] represents the number of free amylose units, and k a second-order rate constant. From the slope of a linear portion in Fig. 6, k can be calculated as

$$k = 7\times10^8\ \mathrm{M}^{-1}\,\mathrm{sec}^{-1} \quad \text{at } 25^\circ\mathrm{C}.$$

This value is so large that it is close to the rate of a diffusion-controlled reaction.10) Ono *et al.* concluded that amylose should be in helical form so as to form a complex with iodine.11) Thus the above result means

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8) H. Murakami, *J. Chem. Phys.*, **22**, 367 (1954).

9) O. E. Myers, *ibid.*, **28**, 1027 (1958).

10) Here we assume that a free amylose unit moves in liquid just as a free particle. However another extreme assumption that the unit is restricted to one position in medium will not affect the result very much. (*cf.* A. A. Frost and R. G. Pearson, "Kinetics and Mechanism," John Wiley and Sons, Inc., N. Y., 1961, p. 221).

11) S. Ono, T. Watanabe, K. Ogawa, and N. Okazaki, This Bulletin, **38**, 643 (1965).

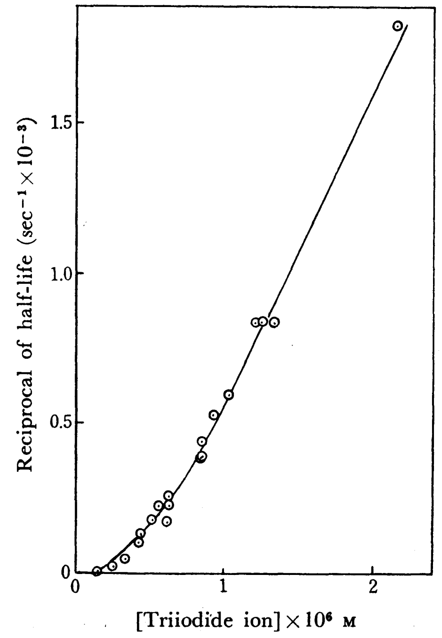

Fig. 6. The dependence of the rate of the complex formation on the triiodide ion concentration at 25°C.
This results are from the same experiments for Fig. 3.
$[I_2]=(1-4)\times10^{-4}$ M, $[I^-]=(1-5)\times10^{-6}$ M, and the amylose concentration 1.0×10^{-5} eq.

that the reaction between a triiodide ion and helical amylose is nearly a diffusion-controlled process.

The origin of the θ term in equation (2) is not clear. Apparently owing to this term, the dependence of the rate on $[I_3^-]$ is very crucial in the region of $[I_3^-]<\theta$. Also θ depends greatly upon temperature. Figure 7 shows the plot of the initial rate against $[I_3^-]$ at various temperatures. As temperature decreases, the linear portion becomes larger. Therefore in the region of $[I_3^-]<\theta$, the formation rate is greatly affected by $[I_3^-]$ and temperature. Under the present assumption of intramolecular process, it is suggested that the stability and the concentration of an intermediate depends very much on $[I_3^-]$ and temperature.

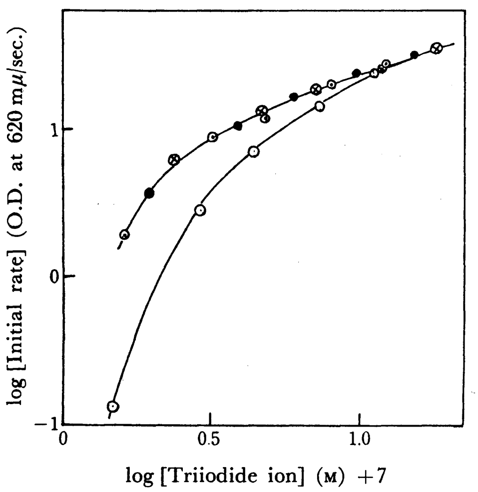

Fig. 7. The dependence of the initial rate of the complex formation on the triiodide ion concentration at various temperatures.
$[I_2]=5.4\times10^{-5}$ M, and amylose concentration 1.0×10^{-5} eq. $[I^-]=(1.5-11)\times10^{-7}$ M. Temperature ○ 20°C, ⊙ 15°C, ● 10°C, ⊗ 5°C respectively.

(D) Decomposition of the Complex by Thiosulfate Ion and Cerium(IV). The amylose-iodine-iodide complex was found to decompose by adding sodium thiosulfate solution or cerium(IV) sulfate solution.

First the rate of decomposition was measured, using a $Na_2S_2O_3$ solution. At the sufficiently high concentration of $Na_2S_2O_3$, the decomposition occurs so rapidly within a dead time (about 1.5 msec). This is a surprising result, since this means that the decomposition rate is larger than that of formation. This is very unbelievable seeing that the formation constant is rather high.[4] One of the possible explanation is that the reversible equilibrium exists between the transient complex $Am\cdot I_3$ in Eq. (2) and the final products. They may be

$$Am\cdot I_3 + I_2 \rightleftharpoons Am\cdot I_3\cdot I_2. \qquad (4)$$

By adding $Na_2S_2O_3$, I_2 is reduced to I^- and the above equilibrium moves toward the decomposition. The fact that the equilibrium (3) is very rapid coincides well with the direct appearance of a final product as stated in section (B).

Next the rate of decomposition was measured, using a $Ce(SO_4)_2$ solution. The rate was measurable this time even at 0.05 M Ce(IV). The example is shown in

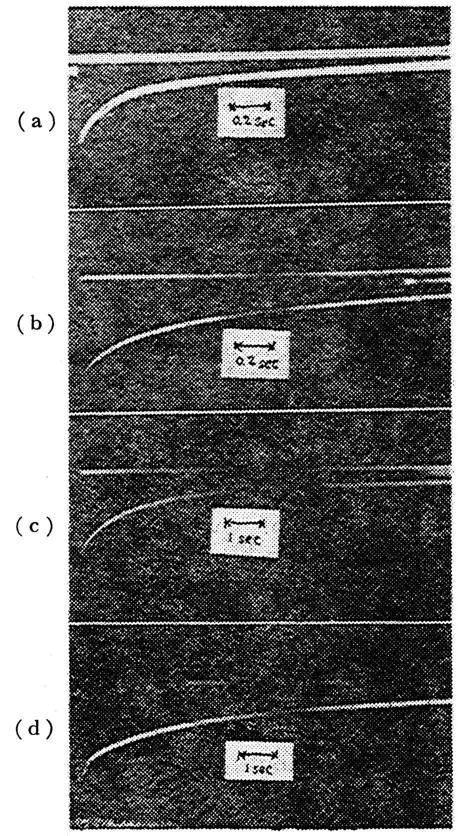

Fig. 8. The decrease of the optical density at 620 mμ with time at various temperatures, induced by the addition of $Ce(SO_4)_2$.
$[Ce(SO_4)_2]=2.8\times10^{-2}$ M, and [Complex]$=3.5\times10^{-6}$ eq. Temperature and time scale: (a) 25°C, 0.2 sec/div., (b) 20°C, 0.2 sec/div., (c) 15°C, 1 sec/div., (d) 10°C, 1 sec/div., The vertical scale 0.05 ΔO.D./div.

Fig. 8. Since the decrease of the complex was found independent of [$Ce(SO_4)_2$], we concluded that the decomposition occurs by the oxidation of free I^- by Ce(IV). The rate is considered to be determined by the reverse reaction of Eq. (2). The time dependence of decomposition is not exponential in spite that the formation is exponential in an appropriate condition. This means that triiodide ion is bound to amylose helix with various degrees of strength. In this sense, it is impossible to obtain the unique value for the equilibrium constant in this complexation reaction. Interestingly the reverse reaction is greatly temperature dependent as shown in Fig. 8. Therefore, the large temperature dependence of the stability of the amylose-iodine-iodide complex arises from the reverse reaction not from the forward reaction.

Summarizing these results, the forward, and the reverse reaction rate constants for the reaction,

$$\mathrm{Am} + \mathrm{I_3}^- \rightleftharpoons \mathrm{Am}\cdot\mathrm{I_3}^-,$$

are given as below.

Temperature	Forward	Reverse[a]
25°C	$7\times10^8\,\mathrm{M}^{-1}\,\mathrm{sec}^{-1}$	$4\,\mathrm{sec}^{-1}$
20	same	2
15	same	0.5
10	same	0.3

a) The approximate value of a unimolecular reaction rate for the initial stage of decomposition.