# Interaction of Amylose with Iodine. II. Kinetic Studies of the Complex Formation by the Temperature-jump Method

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Kinetic studies of the amylose–iodine complex formation for the amylose of degree of the polymerization (DP) of 32 were made using the temperature-jump method. The reciprocal relaxation time increases with increase of the concentration of iodine and decreases with that of amylose. Based on these results, the reaction, $H_0+3\ I_3^- \rightleftharpoons H_3$ was proposed as a plausible mechanism for the relaxation phenomenon, where $H_0$ denotes the free helical segment of amylose and $H_3$ the helical segment containing three $I_3^-$. The relaxation phenomena were observed for the amyloses of DP 42, 57, and 76, and analyzed by the same mechanism as that proposed in the case of DP 32. The rate constants for the forward and the backward reactions ($k_f$ and $k_b$) were determined: the $k_f$ value increases with DP of amylose and the $k_b$ value decreases. Examination of the relaxation time and the relaxation amplitude suggested that the relaxation phenomenon is not based on the whole process of the amylose–iodine complex formation but on the $H_3$ complex formation reaction which was shown to be the nucleation process.

Amylose–iodine complexes show the well-known specific blue color which is widely utilized for the detection of starch and iodine: a number of static studies have been made on the characteristics of the complex.[1–5] On the other hand, few kinetic studies of the amylose–iodine complex formation have been carried out and plausible mechanisms have been proposed.[6–9] Thompson and Hamori[6] revealed that the complex formation begins with the very rapid formation of $H_3$ followed by the slow rate-determining reaction of $H_4$ formation and that successive uptake of iodine molecule by $H_4$ is very fast. Hiromi *et al.*[7] studied the reaction with use of the amyloses of various degree of the polymerization (DP) and observed two processes: one is the very fast formation of $H_n$[††] ($n$=2—4) and the other is the subsequent slow rearrangement of $H_n$. Grätzel *et al.*[8] investigated the reaction by the pulseradiolysis with a mechanism proposed by Thompson and Hamori[6] and obtained the rate constants of $H_6$ and $H_7$ complex formation reactions, but the rapid $H_3$ complex formation process was not studied.

Although the nucleation process is significant as a source of the propagation process, and its existence has been supported by the above investigations, it is still not completely elucidated kinetically. One of the reasons it remains open is the difficulty of measuring the nucleation process independently of the propagation process; the other is the rapidity of the nucleation process. Taking these into account we used, in the present study, the amylose of low DP which has the advantage of lacking the propagation process because of its short helical part[10–13] and low facility for binding iodine.[14] Considering that the nucleation process is very fast and the amylose–iodine complex formation equilibrium is perturbed effectively with temperature, the temperature-jump method is an appropriate technique for the present investigation. The persent study was undertaken to clarify the nucleation process by the temperature-jump method using amylose of low DP.

## Experimental

*Materials.* Amyloses of various DP were prepared as described in a previous paper.[14] Iodine and potassium iodide were of guaranteed reagent and were used without further purification. Freshly prepared amylose solution is used for each measurement.

*Measurements.* Kinetic measurements were carried out by the temperature-jump method with optical detection in the range of 350—550 nm. Since the addition of electrolytes to the sample solution is required for the Joule-heating temperature-jump method, 50 mmol dm$^{-3}$ of KI was added. The concentration of iodine was determined by amperometric titration as shown in the previous paper.[14] All of the experiments were performed at 22 °C.

## Results and Discussion

For the solution of amylose of DP 32 with iodine, the relaxation phenomenon was observed at 530 nm and 350 nm, *i.e.*, the maximum wavelengths of the amylose–iodine complex and that of free $I_3^-$, respectively: in the actual experiments, the relaxation spectra were recorded at 530 nm, because of the larger relaxation amplitude. Representative relaxation curves are shown in Figs. 1a and 1b. The absorbance change observed in Fig. 1a indicates the decrease of the complex, while that in Fig. 1b indicates the increase of $I_3^-$. Both curves were characterized as single relaxation phenomena and the relaxation times were coincident. These results indicate that the temperature-jump decomposes the complex and produces free $I_3^-$ at the same time, and suggests that the present relaxation phenomenon is due to the amylose-iodine complex formation.[15]

The kinetic experiments were carried out by changing the concentration of iodine while keeping the concentration of amylose constant, or *vice versa*. The plots of the reciprocal relaxation time ($\tau^{-1}$) *vs.* the total concentration of $I_3^-$ [16] and that of amylose are shown in Figs. 2 and 3, respectively. As shown in these figures, the value of $\tau^{-1}$ increases with increase of the concentration of iodine but decreases with that of amylose.

Combining the above results together with the static

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[††] $H_n$: The helical segment containing $nI_3^-$.

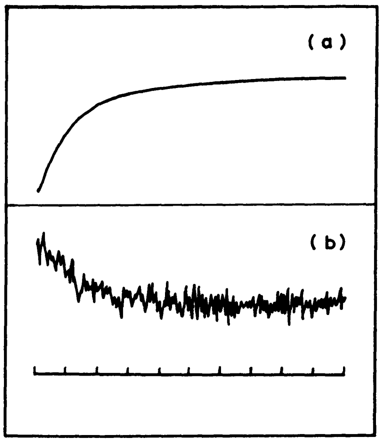

Fig. 1. Representative relaxation spectra of the amylose–iodine complex formation by the temperature-jump method at 22°C. OD increases downwards. $\Sigma$[iodine] $= 1.2\times10^{-4}$ mol dm$^{-3}$, $\Sigma$[amylose of DP 32] $=5\times10^{-4}$ mol dm$^{-3}$ in AGU, sweep: 20 μs div$^{-1}$, (a): 530 nm, (b): 350 nm.

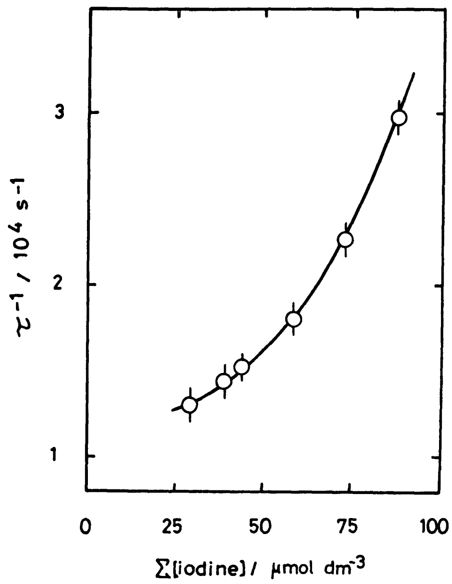

Fig. 2. Iodine concentration dependence of the reciprocal relaxation time for the amylose–iodine complex formation. The solid line drawn through the points has no theoretical significance. $\Sigma$[amylose of DP 32] $=5\times10^{-4}$ mol dm$^{-3}$.

information, the following reaction was proposed as a plausible mechanism for the present relaxation phenomenon:

$$H_0 + nI_3^- \underset{k_b}{\overset{k_f}{\rightleftharpoons}} H_n, \quad (1)$$

where $H_0$ denotes the free helical segment of amylose. The relaxation equation for the above reaction is given as

$$\tau^{-1} = k_f(\bar{C}_I^{\,n}+n^2\bar{C}_I^{\,n-1}\bar{C}_{H_0}) + k_b, \quad (2)$$

where $k_f$ and $k_b$ are the forward and the backward rate constants, respectively, $\bar{C}_I$ is the concentration of free iodine and $\bar{C}_{H_0}$ is the concentration of free helical

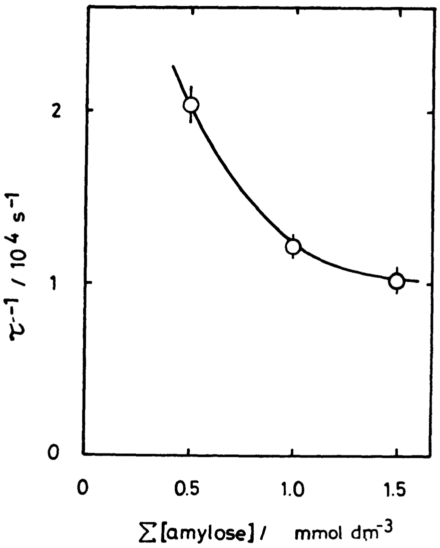

Fig. 3. Amylose concentration dependence of the reciprocal relaxation time for the amylose–iodine complex formation. $\Sigma$[iodine] $=4.5\times10^{-5}$ mol dm$^{-3}$.

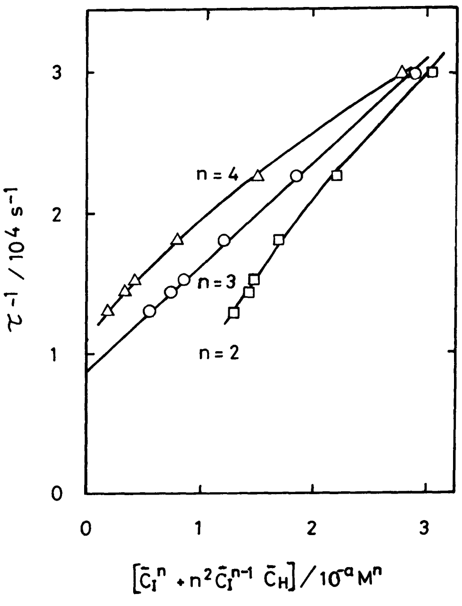

Fig. 4. Plots of the reciprocal relaxation time *vs.* the concentration term in Eq. 2 calculated for each $n$ value. □: $a=9$; ○: $a=13$, △: $a=17$. 1 M $=1$ mol dm$^{-3}$.

segment of amylose.19) An increase of $\Sigma$[amylose] increases $\bar{C}_{H_0}$ but decreases $\bar{C}_I$ due to the shift of the equilibrium (1) to the right under the constant initial concentration of iodine. Since $\bar{C}_I$ contributes to $\tau^{-1}$ by the $n$ and $(n-1)$ powers in Eq. 2, the variation of $\bar{C}_I$ predominantly effects $\tau^{-1}$. Therefore, the experimental results in Figs. 2 and 3 seem to be well described by Reaction 1. The plots of $\tau^{-1}$ *vs.* the concentration term in Eq. 2 with various $n$ values are shown in Fig. 4. As shown in the figure, the plot with $n\leqq2$ gives a negative intercept and in the cases of $n\geqq4$, it does not give a straight line. Only the plot with $n=3$ gives a best straight line, *i.e.*, Reaction 1 is shown to be

$$H_0 + 3I_3^- \underset{k_b}{\overset{K_f}{\rightleftharpoons}} H_3. \tag{3}$$

Reaction 3 is considered to be composed of the following three elementary reactions:

$$H_0 + I_3^- \overset{K_1}{\rightleftharpoons} H_1, \tag{4}$$

$$H_1 + I_3^- \overset{K_2}{\rightleftharpoons} H_2, \tag{5}$$

$$H_2 + I_3^- \underset{k_{-3}}{\overset{\overset{K_3}{k_3}}{\rightleftharpoons}} H_3, \tag{6}$$

Assuming that the concentrations of $H_1$ and $H_2$ are very low and Reaction 6 is the rate-determining step of Reaction 3, the relaxation time for Reaction 3 is given by

$$\tau^{-1} = K_1K_2k_3(\bar{C}_I{}^3 + 9\bar{C}_I{}^2\bar{C}_{H_0}) + k_{-3}. \tag{7}$$

In comparison to Eq. 2 with $n=3$, $K_1K_2k_3$ corresponds to $k_f$, and $k_{-3}$ to $k_b$. From the slope and intercept of the straight line of the plot with $n=3$ in Fig. 4, we can evaluate the values of $k_f$ and $k_b$ to be $(7.7\pm0.2)\times10^{16}$ $(\text{mol dm}^{-3})^{-3}\,\text{s}^{-1}$ and $(8.4\pm0.9)\times10^3\,\text{s}^{-1}$, respectively, and the equilibrium constant $K$ was obtained to be $(9.2\pm0.8)\times10^{12}$ $(\text{mol dm}^{-3})^{-3}$ from the ratio of $k_f$ and $k_b$.

The equilibrium constant is also obtained amperometrically by the following equation:

$$K = [H_3]/[H_0][I_3^-]^3 = \bar{C}_B/(3\Sigma\bar{C}_H - \bar{C}_B)(\bar{C}_I)^3, \tag{8}$$

where $\bar{C}_B$ denotes the concentration of the bound iodine and $\Sigma\bar{C}_H$ is the total concentration of the helical segment of amylose. Introducing the same assumptions as those used in the derivation of Eq. 7, *i.e.*, $[H_1]=[H_2]\simeq0$ and $H_3$ is the only species of the complex, the value of $K$ was determined to be $(1.3\pm0.6)\times10^{13}$ $(\text{mol dm}^{-3})^{-3}$, which is in good agreement with the above value.

The same experiments as those described above were performed for the amyloses of DP 42, 57, and 76 and the effect of DP of amylose was investigated. The iodine concentration dependences of $\tau^{-1}$ for amyloses of various DP are shown in Fig. 5, where the positive and negative dependences were observed below DP 42 and above DP 57, respectively. In the case of lower DP amyloses, the binding of iodine is so weak that the added iodine increases the concentration term of Eq. 7 and induces the increase of $\tau^{-1}$ value. On the other hand, in the case of higher DP amyloses, most of the added iodine binds to the amylose and makes a negative contribution to the increase of the concentration term of Eq. 7. Consequently, the apparently opposing behavior of the concentration dependences of $\tau^{-1}$ can be well understood.

It is well known that the amylose–iodine complex becomes longer with increase of the DP of the amylose. In the case of these higher DP amyloses, the value of $n$ for the final species of the complex ($H_n$) will exceed 3, *e.g.*, $n=4$ or more.[10–14] Therefore, if we are observing the whole process of the amylose–iodine complex formation in the present temperature-jump experiment

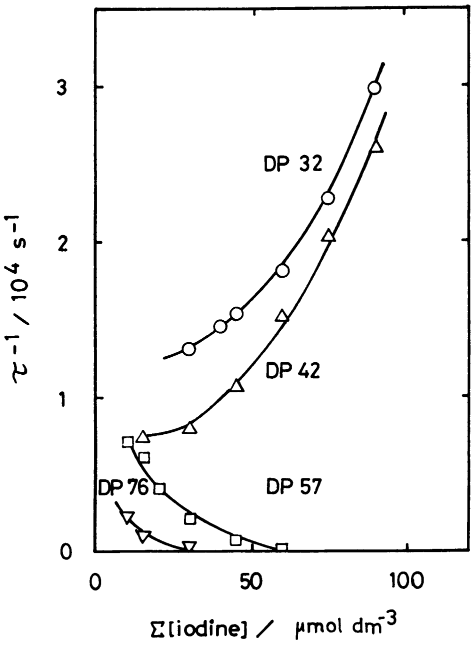

Fig. 5. Iodine concentration dependences of the reciprocal relaxation time for the amyloses of various DP and iodine system.
$\Sigma$[amylose]$=5\times10^{-4}$ mol dm$^{-3}$.

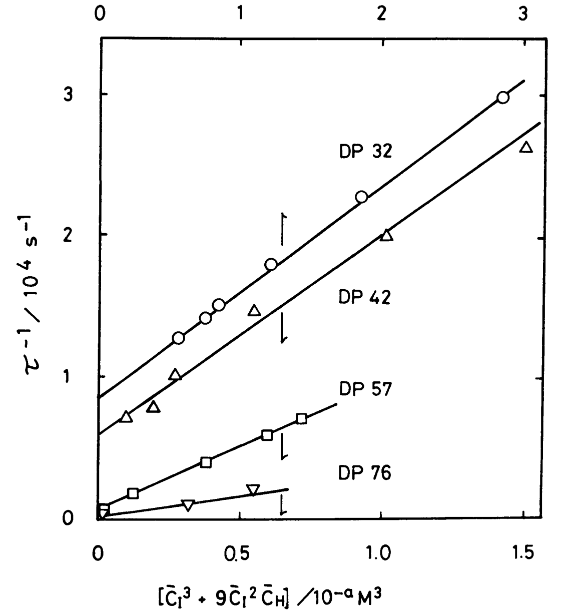

Fig. 6. Plots of the reciprocal relaxation time *vs.* the concentration term in Eq. 7 with $n=3$ for the amyloses of various DP and iodine system.
○: $a=13$, △: $a=13$, □: $a=15$, ▽: $a=16$.

TABLE 1. RATE CONSTANTS AND EQUILIBRIUM CONSTANTS FOR THE COMPLEX FORMATION OF AMYLOSES OF VARIOUS DP WITH IODINE

| DP | $k_f$ / $(\text{mol dm}^{-3})^{-3}\,\text{s}^{-1}$ | $k_b$ / $\text{s}^{-1}$ | $K$ / $(\text{mol dm}^{-3})^{-3}$ |
|---|---|---|---|
| 32 | $(7.7\pm0.2)\times10^{16}$ | $(8.4\pm0.9)\times10^3$ | $9.2\times10^{12}$ |
| 42 | $(1.4\pm0.3)\times10^{17}$ | $(5.8\pm1.0)\times10^3$ | $2.4\times10^{13}$ |
| 57 | $(8.7\pm0.9)\times10^{18}$ | $(7.6\pm0.9)\times10^2$ | $1.1\times10^{16}$ |
| 76 | $(3.0\pm0.5)\times10^{19}$ | $(1.1\pm0.4)\times10^2$ | $3.7\times10^{17}$ |

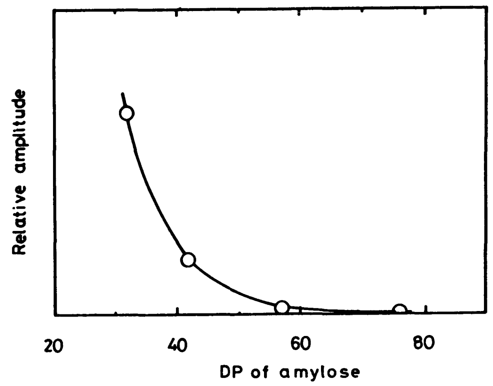

Fig. 7. The effect of DP of amylose on the relaxation amplitude.
$\Sigma$[amylose]$=5\times10^{-4}$ mol dm$^{-3}$, $\Sigma$[iodine]$=4.5\times10^{-5}$ mol dm$^{-3}$. Amplitude is plotted in arbitrary unit.

the value of $n$ should be 4 or more in the case of higher DP amyloses. For all amyloses of various DP, however, only the plot with $n=3$ gave a good straight line with positive slope and the positive intercept as shown in Fig. 6. These results suggest that only Reaction 3 is responsible for the present relaxation and the reactions having order higher than 3 are not observed in these experiments. The values of $k_f$ and $k_b$ for the amyloses of various DP were obtained from each straight line in Fig. 6; they are summarized in Table 1 along with the value of $K$. With increasing DP of the amylose, the $k_f$ value increases but the $k_b$ value decreases.

The relaxation amplitude was found to be greatly affected by the DP of the amylose as shown in Fig. 7; the relaxation amplitude decreases steeply with increase of the DP of the amylose. This behavior of the relaxation amplitude is well understood by considering the shift of the equilibrium of Reaction 3 to the right[20] with DP of amylose, as indicated by the increase of $K$ in Table 1. Another reason for the decrease of the relaxation amplitude will be in the decrease of the concentration of $H_3$ due to the propagation of $H_3$ to $H_n$ ($n\geqq4$) in the case of higher DP amyloses.

The results on the relaxation time and the relaxation amplitude suggest that the relaxation phenomenon is not based on the whole process of the amylose–iodine complex formation but on the $H_3$ complex formation reaction. This consideration implies that the $H_3$ complex is relatively unstable and is easily perturbed by temperature but the higher order complex, *e.g.*, $H_4$, very stable and not perturbed. These are consistent with the explanation proposed by Thompson and Hamori[6] where the existence of a stable nuclei, $H_4$, is postulated for the high DP amylose.

Hiromi *et al.*[7] have proposed the following mechanism for the amylose–iodine complex formation:

$$H + nI_3^- \underset{}{\overset{\text{fast}}{\rightleftharpoons}} \text{complex } \mathbf{1} \overset{\text{slow}}{\rightleftharpoons} \text{complex } \mathbf{2}.$$

The slow reaction with a time constant of 1—200 ms was investigated in detail but the fast reaction was just noted. Its existence in the time region is less than 1 ms. The existence of the process where three iodines bind to amylose has been suggested by other investigators.[6,8] The relaxation phenomenon observed in the present study was in the time region of less than 1 ms and ascribed to the reaction $H_0+3\,I_3^-\rightleftharpoons H_3$: these results were in complete agreement with the features predicted for the nucleation process. Finally, the fact that three iodines bind successively to the helical segment of amylose is indicative of the cooperative binding, which is compatible with the result we obtained by amperometric titration.[14]

## Appendix

For a reaction
$$H_0 + nI_3^- \underset{k_b}{\overset{k_f}{\rightleftharpoons}} H_n, \tag{A-1}$$
the rate equation is given by
$$\begin{aligned} -\frac{d}{dt}C_{H_0} &= k_f C_{H_0} C_I^n - k_b C_{H_n} \\ &= k_f(\bar{C}_{H_0}+\Delta C_{H_0})(\bar{C}_I + \Delta C_I)^n \\ &\quad - k_b(\bar{C}_{H_n}+\Delta C_{H_n}), \end{aligned} \tag{A-2}$$
where a bar over the concentration symbols indicates the equilibrium value and $\Delta$ indicates the small deviation from equilibrium. The stoichiometry gives the following relation,
$$\Delta C_{H_0} = \frac{1}{n}\Delta C_I = -\Delta C_{H_n} \tag{A-3}$$
At an equilibrium state,
$$K = \frac{k_f}{k_b} = \frac{\bar{C}_{H_n}}{\bar{C}_{H_0}\bar{C}_I^n}. \tag{A-4}$$
Neglecting the quadratic term in Eq. A-2, the rate equation is developed as follows,
$$\begin{aligned} -\frac{d}{dt}C_{H_0} &= -\frac{d}{dt}C_{H_0}(\equiv\tau^{-1}\Delta C_{H_0}) \\ &= k_f\{\Delta C_{H_0}\bar{C}_I^n + n\Delta C_I\bar{C}_I^{n-1}\bar{C}_{H_0}\} - k_b\Delta C_{H_0} \\ &= \{k_f(\bar{C}_I^n+n^2\bar{C}_I^{n-1}\bar{C}_{H_0}) + k_b\}\Delta C_{H_0}, \end{aligned} \tag{A-5}$$
then the reciprocal relaxation time $\tau^{-1}$ is given by,
$$\tau^{-1} = k_f(\bar{C}_I^n + n^2\bar{C}_I^{n-1}\bar{C}_{H_0}) + k_b. \tag{A-6}$$

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15) The relaxation phenomenon may not be directly related to the helix-coil transition of amylose by considering the present experimental conditions, 22 °C, together with the facts that (1) the helix-coil trasition occurs at 55—65 °C in general, and (2) the amount of bound iodine has been shown to be constant in the range of 10—40 °C.[14]

16) The concentration of $I_3^-$ is determined by the reaction; $I_2+I^- \rightleftharpoons I_3^-$, using $K'=[I_3^-]/[I^-][I_2]=864$ $(\mathrm{mol\ dm^{-3}})^{-1}$ at 20 °C;[17] the bound species is assumed to be $I_3^-$ according to Cronan and Schneider[18] and in the present manuscript, iodine represents the species of $I_3^-$.

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19) Since the helical segment is composed of less than about 60 anhydroglucose units (AGU), as described in the previous paper,[14] the concentration of the helical segment ($C_H$) is determined as $C_H=[\text{amylose in AGU}]/\text{DP}$ at $\text{DP}\leqq 60$ while $C_H=[\text{amylose in AGU}]/60$ at $\text{DP}\geqq 60$.

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