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Kinetic Studies of Amylose-iodine-iodide Reaction by Stopped-flow Method*

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1. The kinetics of the complex formation between iodine-iodide and amyloses of various number-average degrees of polymerization (DP_n) at large excess of amylose was studied by stopped-flow method, over the wavelength range of 280–800 mμ, at pH 4.9 and 24.7°C.

2. With amyloses of $\overline{DP}_n$=15 and 28, the reaction was found to be completed within about 1 msec, the dead-time of the apparatus. However, with amyloses of $\overline{DP}_n$ above about 40, at least two stages with considerably different velocities were observed.

3. A typical example with amylose of $\overline{DP}_n$=8,700 and at low iodine concentration showed that the spectral change which was developed within the dead-time had an absorption peak around 570 mμ, while that developed in the first stage which was observable by the stopped-flow method (stage C) had a peak at longer wavelength around 660 mμ. Most of the free triiodide ions have been taken up within the dead-time.

4. The time course of stage C followed apparent first-order kinetics, and the first-order rate constant, k, of this stage was roughly proportional to the fifth power of added iodine concentration, $(I_2)_0$, at lower $(I_2)_0$ range, and tended to saturate at higher $(I_2)_0$. k was almost independent of amylose concentration over the range studied (0.0025–0.1%). At fixed concentration of added iodine, k increased with about the fifth power of DP_n up to $\overline{DP}_n \fallingdotseq 140$, above which it was independent of $\overline{DP}_n$ of amylose.

5. These results are essentially consistent with a simplified mechanism, in which a complex with shorter poly-iodine chain is formed rapidly and reversibly within the dead-time (about 1 msec), and this complex subsequently transforms into a complex with longer poly-iodine chain without appreciable participation of free triiodide ion in solution. The latter change is considered to correspond to stage C.

6. Evidence has been obtained which indicated that stages following stage C may involve the rearrangement of poly-iodine chain once formed in stage C into a thermodynamically more stable state, which could have shorter poly-iodine chain than that involved in stage C.

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Starch-iodine reaction is one of the most familiar color reactions, which has been the subject of a number of investigations (*1–9*). It has been established that the color (red to blue depending on the chain length of amylose) is due to the formation of complex (a kind of inclusion compounds), in which iodine atoms are linearly arranged to form poly-iodine chain (*10–13*) in the helical part of amylose chain, and that the wavelength and the extinction of maximum absorption are dependent on the length of the poly-iodine chain in the helix (*14–20*). The dependence of the wavelength of maximum absorption, λ_{max}, on the chain length of amylose has been accounted for on the basis of a free electron model in a one-dimensional potential box (*15, 21, 22*). Evidence has been accumulated for believing that the species present in solution contributing to the formation of poly-iodine chain is mainly tri-iodide ion (I_3^-) rather than iodine molecule (I_2) (*5, 7, 23–26*). For this reason, the term "amylose-triiodide complex" will be used throughout this paper.

Studies on the formation of amylose-tri-iodide complex have so far been confined to static or thermodynamic ones, dealing with the equilibrium properties. Kinetic studies of the reaction seem not to have been published, probably because it is too rapid to be studied with usual techniques. Flow methods (*27*) and relaxation methods (*28*) should be useful for this purpose. Because of the reversibility of the reaction and high temperature dependency of the equilibrium (*6, 24, 29, 30*), temperature-jump method (*28*) would be applicable. However, the addition of neutral salt to the system which is necessary for the conventional temperature-jump method very often brings about the precipitation of amylose-triiodide complex and sometimes alters the absorption spectrum (*23*). Moreover, repeated electrode discharges could result in decrease in iodine concentration in the system. For this reason, we first attempted the kinetic studies of the reaction by stopped-flow method, utilizing amyloses of various number-average degrees of polymerization ($\overline{DP}_n$), with the hope for obtaining information on the mechanism of reaction which cannot be gained with static methods so far employed.

EXPERIMENTAL

Meterials–Amyloses used in this study were prepared by the fractionation with ethanol-dimethly sulfoxide according to the method of Everett and Foster (*31*) from the native potato amylose, or the partially degraded potato amylose which was prepared with Taka-amylase A [EC 3.2.1.1] as described elsewhere (*32*). Maltodextrin ($\overline{DP}_n = 15$) was prepared from starch digest according to Hizukuri's method (*33*). Some of the samples are the gifts from Drs. T. Watanabe and M. Takagi and their coworkers in our laboratory. The number-average molecular weights of the amyloses were determined by several methods including polarographic method (*34*), exo-enzyme kinetic method (*32*), osmotic pressure and reducing-end measurements, or combination of these.

The amyloses were dissolved in a small amount of 1 N NaOH and neutralized with acetic acid after dilution, pH being adjusted to 4.9. The final concentrations of sodium ion and acetate ion were both 0.04 M.

Iodine was purified several times by sublimation. Potassium iodide was a reagent of guaranteed grade. Iodine-iodide solution was prepared in a usual manner, the concentration of iodide added being fixed at 0.01 M before mixing (5 mM after mixing) throughout the experiments.

Methods–Equal volumes of amylose and iodine-iodide solutions were mixed in a stopped-flow apparatus (Yanagimoto SPU-1) which was described elsewhere (*35*). The apparatus has a minimum dead-time of 0.5 msec, and the observation cell of the optical path of 2 mm or 10 mm was used depending on the absorbance of the mixed solution. Temperature was controlled within ±0.1°C by circulating water from a thermostat to the fluid-handling system of the apparatus. The light transmittance change with time, at various wavelengths ranging from 280 mμ to 800 mμ, was recorded on a storage oscilloscope (Iwasaki Electronics, MS-5019 Memoriscope) screen and on a pen recorder (Toa Electronics, EPR-2T) at the same time. The magnitude of the transmittance

change was calibrated with calibration signal (5% transmittance change) generated by the apparatus (*35*).

The absorption spectrum of the complex was taken at 25°C with a recording spectrophotometer (Hitachi EPS-3) at appropriate time (1 min or longer) after mixing, when the color development was virtually complete.

In order to simplify the kinetic treatment, amylose concentration was usually chosen to be in large excess over the iodine-equivalent (about 2×10^{-3} g atom iodine per 1 g amylose).

RESULTS AND DISCUSSION

Amyloses with $\overline{DP}_n$ of 15 and 28, whose triiodide complex have absorption maxima at 460 mμ and 520 mμ, respectively, showed no detectable change in absorption, when they were mixed with iodine-iodide solution in the stopped-flow apparatus, although the mixed solution was colored. This means that the reaction is completed within the dead-time of the stopped-flow apparatus, slightly less than 1 msec under the operating conditions. According to the X-ray analysis of amylose-iodine complex of Rundle *et al.* one helical turn consisting of 6 glucose residues accomodates two iodine atoms (*11*). Thus amyloses with $\overline{DP}_n$ of 15 and 28 are considered to include 2 and 3 triiodide ion units, respectively, since several glucose residues at both ends of amylose helix may be unable to form rigid helix (*9*). The result, therefore, indicates that the formation of amylose-triiodide complex which involves 2 or 3 triiodide units (6 or 9 iodine atoms, respectively) is essentially finished within the dead-time (about 1 msec), being too rapid to be observed by the stopped-flow apparatus.

However, with amyloses with $\overline{DP}_n$ larger than 40, time course of the transmittance change was actually observed. A typical example with amylose of $\overline{DP}_n$=8,700 at 660 mμ is shown in Fig. 1, in which two stages of considerably different velocities are clearly discernible. The magnitudes and velocities of the two stages were dependent on the concentration of iodine-iodide and $\overline{DP}_n$ of amylose used. In addition to these stages, other slower stages were observed depending on the conditions.

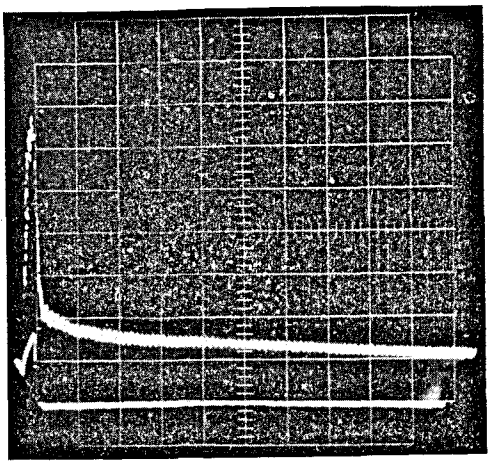

Fig. 1. The time course of amylose-iodine-iodide reaction observed by stopped-flow method. Amylose of $\overline{DP}_n$=8,700, 0.025%. $(I_2)_0$=15 μM, (KI)=5 mM, pH 4.9, 24.7°C. Optical path 2 mm, 660 mμ. Horizontal scale, 50 msec per major division. Upper curve, reaction signal. 0.007 ΔO.D. per major division. O.D. increases downwards. The rapid increase in O.D. after flow-stop corresponds to stage C (see text). Lower curve, flow velocity trace. 18 ml/sec per major division. Dead-time 0.54 msec.

In this paper, we shall confine our attention mainly to the first stage observed by the stopped-flow method. This stage will be termed "stage C". Stage which precedes stage C and has been finished within the dead-time of the apparatus cannot be observed directly. This unobservable stage will be termed "stage B". The absorbance change which had occurred within stage B, though not obtained directly, can be calculated by combining the stopped-flow data with usual static spectrophotometric measurement in the following way.

For convenience, the total absorbance change at a given wavelength, which was denoted by A, may be divided into three parts, B, C and F, as is schematically shown in Fig. 2.

A is simply obtained by usual static spectrophotometry by

$$A = A_{comp.} - A_i/2 \qquad (1)$$

where $A_{comp.}$ is the absorbance of the complex at a given wavelength in 1 cm optical path measured at a definite time (60 or 90 sec) after

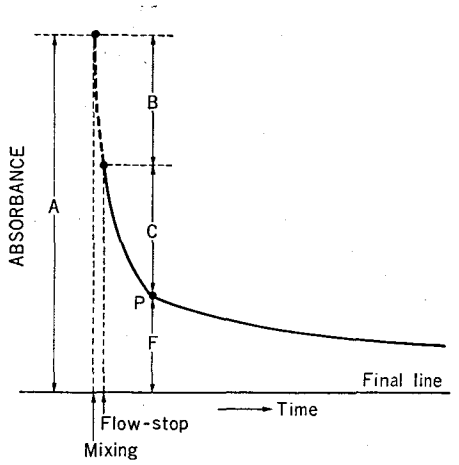

Fig. 2. Schematic illustration for the determination of absorbance changes in various stages of reaction. *A*, total O.D. change due to reaction (statically observable by usual spectrophotometry). *B*, O.D. change in the dead-time of the stopped-flow apparatus, stage B (not directly observable). *C*, O.D. change in stage C (from the flow-stop to the break-point P). *F*, O.D. change in stage F (from the break-point P to the final line which was superimposed at a definite time after mixing where no further change was observed).

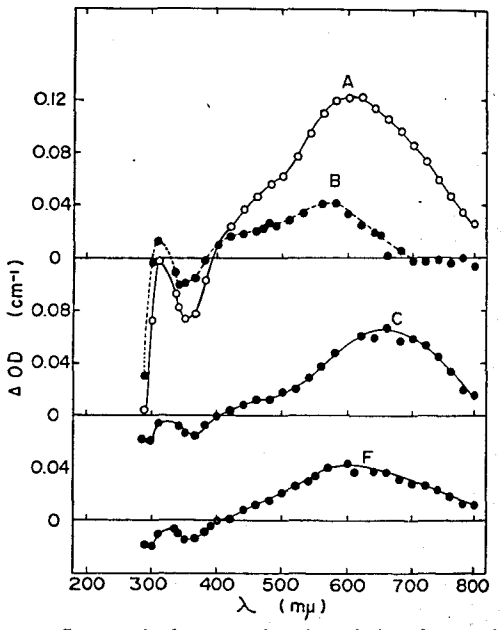

Fig. 3. Spectral changes developed in the various stages of reaction. Amylose of $\overline{DP}_n$=8,700, 0.025%. $(I_2)_0$=5 μM, (KI)=5 mM, pH 4.9, 24.7°C. A, B, C and F refer to the O.D. changes developed in stages A, B, C and F, respectively. The time ranges are: A, 0–90 sec; B, 0–1 msec; C, 1–200 msec; F, 0.2–90 sec; after mixing.

mixing (the color development was complete at that time), and A_I is the absorbance of iodine-iodide solution before mixing at the same wavelength. *C* and *F* in Fig. 2 are the absorbance changes (converted into 1 cm optical path) developed in stages C and F, respectively, both of which can be directly observed by stopped-flow method. The point P in Fig. 2 which separates stages C and F was conveniently chosen at the break-point of the reaction signal trace shown in Fig. 1. Thus "stage F" covers the time range from point P to that of the final line (*cf.* Fig. 1) which was superimposed at a definite time (60 or 90 sec) after mixing. The absorbance change which had occurred within the dead-time (stage B), denoted by *B*, can be obtained from the relationship;

$$B=A-(C+F) \qquad (2)$$

Spectral Changes Developed in Various Stages of Reaction—The absorbance changes for various stages of reaction, B, C and F obtained as stated above were calculated at various wavelengths from 280 mμ to 800 mμ. Figure 3 shows a typical example of the spectral changes developed in stages B, C and F, obtained in this manner, together with the overall (total) change of absorption spectrum of complex formation denoted by A obtained by usual spectrophotometry.

Two things are noticeable in Fig. 3: First, the spectral change developed within the dead-time (stage B) has an absorption peak around 570 mμ, whereas that developed in stage C has a peak at much longer wavelength around 660 mμ. Second, most part of the decrease in free triiodide concentration, as judged from the change at 288 mμ, is finished within stage B, while the decrease at 288 mμ is much less in stage C in spite of considerable increase in absorption at longer wavelength region. This trend is more clearly shown by comparing the ratios of ΔO.D. at 288 mμ to that at wavelength of maximum absorption at visible region. Thus $\Delta O.D._{570}/\Delta O.D._{288}=-0.67$ in stage B is in contrast with $\Delta O.D._{660}/\Delta O.D._{288}=-3.8$ in stage C.

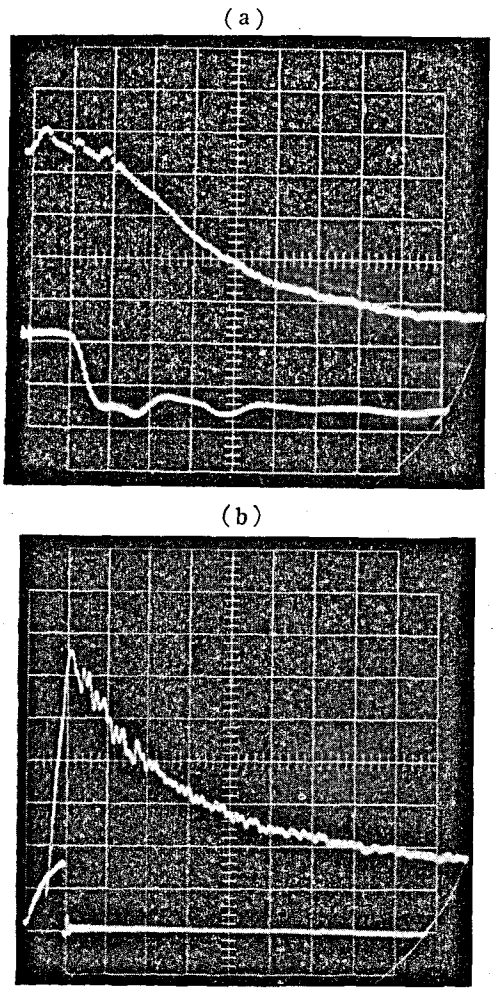

Fig. 4. Typical examples of the time course of stage C. Amylose of $\overline{DP}_n$=8,700, 0.025%. (KI)=5 mM, pH 4.9, 24.7°C. Optical path 2 mm, 600 mμ. Upper curve, reaction signal. Lower curve, flow velocity trace (18 ml/sec per major vertical division) (dead-time 0.54 msec). (a), $(I_2)_0$=12.5 μM. Vertical scale, 0.007 ΔO.D. per major division for upper curve. Horizontal scale, 1 msec per major division. (b), $(I_2)_0$=4.4 μM. Vertical scale, 0.0014 ΔO.D. per major division for upper curve. Horizontal scale, 20 msec per major division.

These results suggest that a complex with rather short poly-iodine chain (including 3–4 triiodide units as judged from the absorption maximum, λ_{max}=570 mμ) is formed within the dead-time of the apparatus (stage B), and that the length of the poly-iodine chain is increased in stage C mainly by the rearrangement of triiodide ion already complexed with the amylose helix without appreciable participation of free triiodide ion in solution. The failure of observing the time course of reaction with amyloses of $\overline{DP}_n$ less than 30, as stated above, is in accordance with this supposition.

Essentially similar results were obtained at higher iodine concentration (25 μM) and also with amylose of lower $\overline{DP}_n$ ($\overline{DP}_n$=83).

Kinetics of Stage C—Two typical examples of the time course of stage C at different added iodine concentrations $(I_2)_0$ are reproduced in Fig. 4, and their Guggenheim plots (*36*) are shown in Fig. 5.

The straight lines of Guggenheim plots show that stage C obeys apparent first-order kinetics, and the apparent first-order rate constant k can be evaluated from the slope of the plot.

At a fixed added iodide concentration of 5 mM, the effect of added iodine concentration upon the rate constant k was examined, and the results are shown in logarithmic plot in Fig. 6. In consequence of the equilibrium;

$$I_2 + I^- \rightleftharpoons I_3^- \qquad (3)$$

$$K=(I_2)(I^-)/(I_3^-)=1.40\times10^{-3}\ \text{M at } 25°C\ (37) \qquad (4)$$

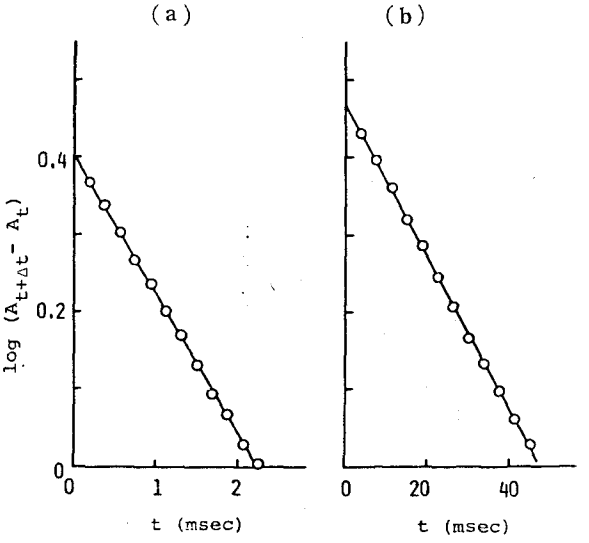

Fig. 5. Guggenheim plots of the reaction curves of Fig. 4 (a) and (b). A_t, absorbance change (in arbitrary units) at time t after flow-stop. $A_{t+\Delta t}$, absorbance change at time $t+\Delta t$. Δt was chosen equal to the half-time of the reaction. The apparent first-order rate constant k was evaluated from the slope to be 460 sec^{-1} and 23 sec^{-1} for (a) and (b), respectively.

the concentration of triiodide ion initially present in the system (before complex formation) is nearly proportional to the added iodine concentration $(I_2)_0$ under the experimental conditions. Hence, for convenience, $(I_2)_0$ will be used to represent the concentration of the reactant iodine species.

Figure 6 shows that at lower $(I_2)_0$, k is crucially dependent on $(I_2)_0$, the limiting slope of the log-log plot being about 5, and that k tends to saturate with increasing $(I_2)_0$, approaching a finite value. The dependency of k on $(I_2)_0$ was exactly the same for amylose of $\overline{DP}_n$=8,700 and 620, as seen from Fig. 6. With amylose of $\overline{DP}_n$=83, a similar dependency, but slightly lower value of the limiting slope and appreciably lower saturation level, were obtained.

The rate constant k was found to be quite insensitive to the amylose concentration (Am), as is shown in Fig. 7, over the (Am) range studied (0.0025–0.1%). Obviously these results cannot be reconciled with a single step equilibrium such as:

$$\mathrm{Am}+n\mathrm{I} \rightleftharpoons \mathrm{Complex}$$

where Am and I denote the amylose and triiodide ion, respectively, n the number of triiodide units necessary for complex formation.

The concentration dependency of k (Figs. 6 and 7) and the spectral change in the various stages (Fig. 3) strongly suggest that the following reaction mechanism in a simplified form as a reasonable one:

$$\mathrm{Am}+n\mathrm{I} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \text{Complex I} \underset{k_4}{\overset{k_3}{\rightleftharpoons}} \text{Complex II} \quad (5)$$

where Am and I denote amylose helix (or binding site for triiodide) and triiodide ion, respectively, n the number of triiodide units involved in the complex formation. Complex I represents a complex in which n triiodide units are rather randomly distributed in the amylose helix and some fraction of bound triiodide units forms relatively short poly-iodine chain (probably corresponding to 2–4 triiodide units). This complex I which absorbs at shorter wavelengths is formed rapidly and reversibly, and is considered to correspond to the complex formed within the dead-time (stage B). Complex II,

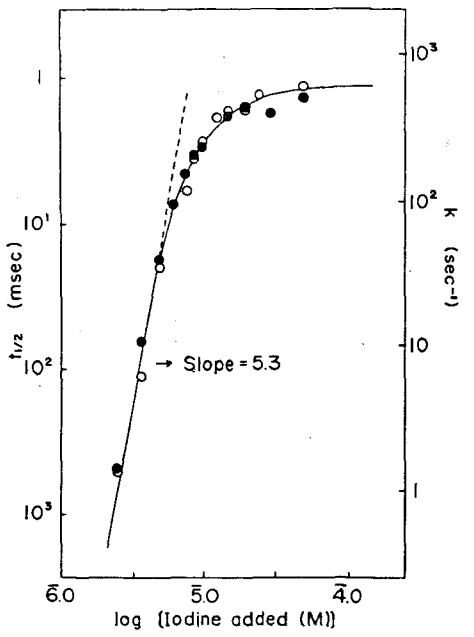

Fig. 6. The effect of the added iodine concentration $(I_2)_0$ upon the apparent first-order rate constant k and the half-time $t_{1/2}$ of stage C. Amylose concentration 0.025%, (KI)=5 mM. pH 4.9, 24.7°C. 660 mμ. Open circle, amylose of $\overline{DP}_n$=8,700. Closed circle, amylose of $\overline{DP}_n$=620.

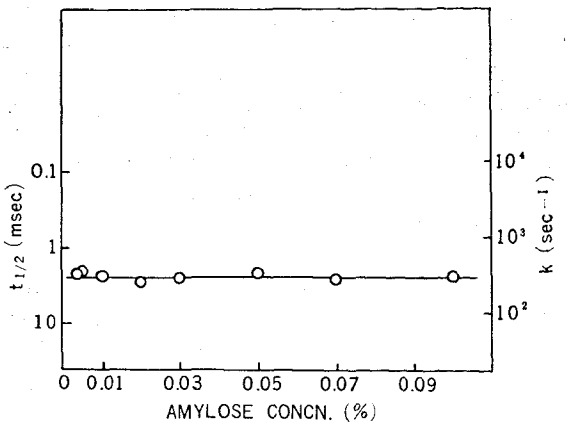

Fig. 7. The effect of amylose concentration upon the apparent first-order rate constant k and the half-time $t_{1/2}$ of stage C. Amylose of $\overline{DP}_n$=8,700, $(I_2)_0$ =12.5 μM, (KI)=5 mM. pH 4.9, 24.7°C.

on the other hand, represents a blue complex which absorbs at longer wavelengths. It is formed from Complex I and is assumed to involve the same number of triiodide units n (without appreciable participation of free triiodide ion), but the average length of the polyiodine chain is increased than that of Complex I through the rearrangement of triiodide units already involved in Complex I. Stage C which is observed by stopped-flow method is considered to be this transformation process from Complex I to Complex II.

If we assume that the pre-equilibrium, Am $+nI \rightleftharpoons$ Complex I, is rapidly established within the dead-time of the apparatus, and the rate of formation of blue Complex II is limited by the apparent unimolecular process, Complex I $\rightleftharpoons$ Complex II, a simplified treatment leads to the following approximated rate equation for the formation of Complex II (see Appendix):

$$\frac{d\,(\text{Comp. II})}{dt} = k\left\{\frac{(\text{I})_0}{n^2} - (\text{Comp. II})\right\} \qquad (6)$$

where $(\text{I})_0$ denotes the initial triiodide concentration which is almost proportional to the added iodine concentration $(\text{I}_2)_0$.

Equation 6 indicates that a linear Guggenheim plot will be obtained with the apparent first-order rate constant k, which is given by

$$k = \frac{n^2 k_3 K_1 (\text{Am})\,(\text{I})_0{}^{n-1}}{1 + n^2 K_1 (\text{Am})\,(\text{I})_0{}^{n-1}} \qquad (7)$$

where k_1, k_2, k_3 and k_4 are the rate constants specified in the reaction scheme, Eq. 5, and $K_1 = k_1/k_2$.

This equation predicts that k is proportional to the $(n-1)$th power of $(\text{I}_2)_0$ (since $(\text{I}_2)_0 \propto (\text{I})_0$), at lower $(\text{I})_0$ range where $1 \gg n^2 K_1 (\text{Am})\,(\text{I})_0{}^{n-1}$ holds, and that k asymptotically approaches k_3 as $(\text{I})_0$ increases. Experimental results shown in Fig. 6 are consistent with the prediction of Eq. 7, according to which the limiting slope in Fig. 6 represents $(n-1)$. Thus we have $(n-1) \fallingdotseq 5$, hence $n \fallingdotseq 6$. This means that about six triiodide units are necessarily involved in the formation of the blue complex (Complex II). The value of n is reasonable in view of the relationship between the length of the poly-iodine chain and the wavelength of maximum absorption.*

The rate constant k at the saturation level represents the rate constant k_3 of rearrangement of triiodide ion, Complex I→Complex II. This process could be either intramolecular or intermolecular depending on the reaction conditions ($\overline{\text{DP}}_\text{n}$ of amylose and $(\text{I}_2)_0$ *etc.*). Important is that this process does not necessarily involve the further participation of free triiodide ion.

Effect of $\overline{\text{DP}}_\text{n}$ of Amylose on the Rate Constant k—The effect of the $\overline{\text{DP}}_\text{n}$ of amylose on the rate constant k of stage C was studied at the fixed concentrations of added iodine and iodide, $(\text{I}_2)_0 = 25\ \mu\text{M}$ and $(\text{KI}) = 5\ \text{mM}$, which is near the saturation level of k (see Fig. 6). The results are shown in log k *versus* log $(\overline{\text{DP}}_\text{n})$ plot in Fig. 8.

A striking feature is that there is a sharp break in the plot around $\overline{\text{DP}}_\text{n} \fallingdotseq 140$, above which k is perfectly independent on $\overline{\text{DP}}_\text{n}$ of amylose. For $\overline{\text{DP}}_\text{n}$ below 140, k is crucially dependent on $\overline{\text{DP}}_\text{n}$, the slope of the plot being about 5. The value of $\overline{\text{DP}}_\text{n}$ where the sharp break oc-

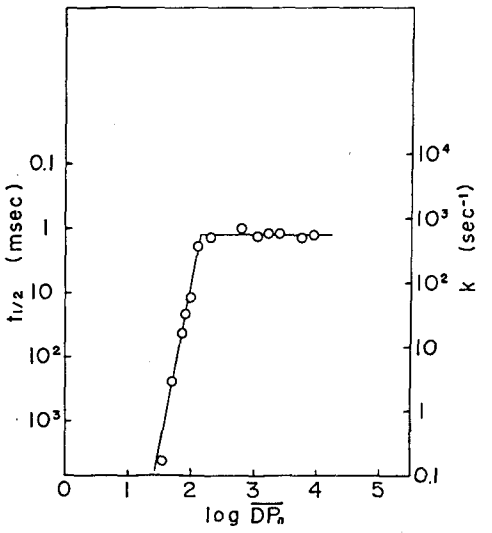

Fig. 8. The effect of $\overline{\text{DP}}_\text{n}$ of amylose upon the apparent first-order rate constant k and the half-time $t_{1/2}$ of stage C. Amylose concentration 0.025%, $(\text{I}_2)_0 = 25\ \mu\text{M}$, $(\text{KI}) = 5\ \text{mM}$, pH 4.9, 24.7°C. 660 mμ.

* K. Hiromi, T. Shibaoka and S. Ono, to be published. (Paper read at the Annual Meeting of Agricultural Chemical Society of Japan, Apr. 1969).

curs is of the same order of magnitude as the average DP_n of helical segment of amylose chain as reported by some authors (*2, 9*). The independency of k on DP_n above $DP_n = 140$, together with entirely the same dependency of k on $(I_2)_0$ of amyloses whith $DP_n = 8{,}700$ and 620 (Fig. 6), suggests that each helical segment of amylose molecule behaves independently in the amylose-triiodide reaction.

The reason why k is nearly proportional to the fifth power of DP_n below the critical value ($DP_n \fallingdotseq 140$) has not fully been accounted for. However, one possible interpretation may be as follows: The probability, f, of finding n triiodide units in the same helical segment will be proportional to the n-th power of average DP_n of helical segment of amylose, when $\overline{DP}_n$ is less than the critical value. If, in the pre-equilibrium (Complex I in Eq. 5), a helical segment contains less than n triiodide units, the development of n-unit poly-iodine chain must involve inter-segment rearrangement. This will be certainly less frequent than the intra-segment rearrangement (within the same segment) which contains originally n triiodide units. Therefore, k may be expected to be proportional to the probability f, which in turn is roughly proportional to $(\overline{DP}_n)^n$.

In parallel with the decrease in k with decreasing DP_n, the fraction of absorbance change in stage C to the total change A, C/A, also diminishes with decreasing $\overline{DP}_n$ below the critical value of DP_n (Fig. 9). It may be expected from this trend that short-chain amyloses with DP_n of 15 and 28 showed no observable change which corresponds to stage C.

Stage Which Follows Stage C–Although our present interest is focussed upon the stages

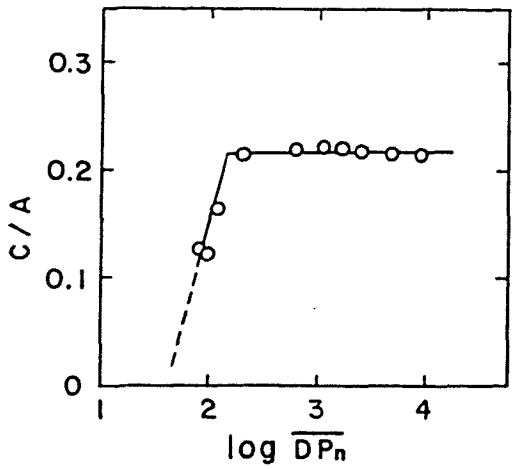

Fig. 9. The effect of $\overline{DP}_n$ of amylose upon the fraction of absorbance change in stage C to the total change A. Amylose concentration 0.025%, $(I_2)_0 = 25\ \mu M$, (KI)=5 mM, pH 4.9, 24.7°C. 660 mμ.

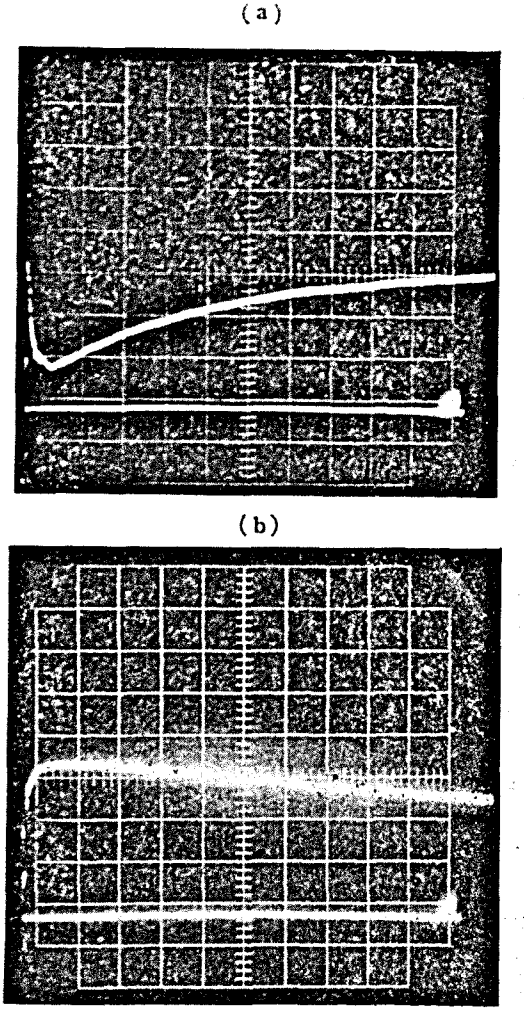

Fig. 10. Time course showing the change after stage C at two different wavelengths. Amylose of $\overline{DP}_n = 620$, 0.025%, $(I_2)_0 = 100\ \mu M$, (KI)=5 mM, pH 4.9, 24.7°C. Optical path 2 mm. (a), at 750 mμ; (b), at 560 mμ. Upper curve, reaction signal (O.D. increases downwards). Vertical scale, 0.007 ΔO.D. per major division. Lower curve, flow velocity trace. Vertical scale, 18 ml/sec per major division (dead-time 0.82 msec). Horizontal scale, 500 msec per major division.

B and C, some noticeable feature concerning the stage which follows stage C will be mentioned briefly. When higher iodine concentrations were used (at $(I_2)_0 = 50$ or 100 μM), a slow decrease in absorbance at longer wavelengths (>660 mμ) (half-time of *ca.* 1 sec) was observed after the initial rapid increase in stage C, as shown in Fig. 10a. Correspondingly, a slow increase in absorbance at 560 mμ was observed, as shown in Fig. 10b.

This result indicates that the blue complex with long poly-iodine chain which had been developed in stage C rearranges itself into complex with somewhat shorter poly-iodine chain. The long poly-iodine chain complex formed in stage C may not be thermodynamically most stable, probably energetically favorable but entropically unfavorable under such a condition that large excess amylose is present, and will transform itself into a state having minimum free energy, which is entropically more favorable, with larger number of shorter poly-iodine chains.

APPENDIX

Derivation of Eq. 6–From the assumed scheme Eq. 5, the conservation of triiodide units requires that

$$(I)_0 = (I) + n[(\text{Comp. I}) + (\text{Comp. II})] \qquad \text{(A-1)}$$

where $(I)_0$ and (I) are the concentrations of the initial (total) and free triiodide ion, respectively, and (Comp. I) and (Comp. II) are the concentrations of Complex I and Complex II, respectively, both of which contain n triiodide units.

If we assume the rapid pre-equilibrium:

$$\text{Am} + n\text{I} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \text{Comp. I}$$

then we have

$$K_1 = \frac{k_1}{k_2} = \frac{(\text{Comp. I})}{(\text{Am})(\text{I})^n} = \frac{(\text{Comp. I})}{(\text{Am})[(I)_0 - n\{(\text{Comp. I}) + (\text{Comp. II})\}]^n} \qquad \text{(A-2)}$$

where (Am) represents the concentration of amylose site for iodine-binding, which is in large excess over $(I)_0$ and may be regarded to be approximately constant during the reaction.

Expanding the denominator of Eq. A-2 and taking the first two terms, we have, as an approximation:

$$(\text{Comp. I}) = \frac{K_1(\text{Am})(I)_0^{n-1}\{(I)_0 - n^2(\text{Comp. II})\}}{1 + n^2K_1(\text{Am})(I)_0^{n-1}} \qquad \text{(A-3)}$$

The rate of formation of Comp. II is given by

$$\frac{d(\text{Comp. II})}{dt} = k_3(\text{Comp. I}) - k_4(\text{Comp. II}) \qquad \text{(A-4)}$$

The overall equilibrium Eq. 5 is much towards the right hand side, then we may drop the k_4 term in Eq. A-4. Thus we have:

$$\frac{d(\text{Comp. II})}{dt} \fallingdotseq k_3(\text{Comp. I}) \fallingdotseq \frac{k_3K_1(\text{Am})(I)_0^{n-1}\{(I)_0 - n^2(\text{Comp. II})\}}{1 + n^2K_1(\text{Am})(I)_0^{n-1}} \equiv k\left\{\frac{(I)_0}{n^2} - (\text{Comp. II})\right\} \qquad \text{(A-5)}$$

Note added in proof: After submitting this paper to publication, the authors noticed the paper of J.C. Thompson and E. Hamori on the same subject, "Kinetic study of the rapid reaction between amylose and iodine", which appeared in *Biopolymers*, 8, 689 (1969).

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