Interaction of Amylose with Iodine. I. Characterization of Cooperative Binding Isotherms for Amyloses

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The interaction of amylose with iodine was investigated as a function of degree of polymerization (DP) of amylose by amperometric titration, and the binding isotherms were obtained. The intrinsic equilibrium constant and cooperative parameter were determined with a one-dimensional Ising model for amyloses of various DPs. The inflection of the plot of the intrinsic equilibrium constant *vs.* DP observed at about DP 60 suggests that a helical segment of amylose consists of anhydroglucose residues less than 60. The discontinuity of the cooperativity at about DP 60 was interpreted by two kinds of cooperative effects, intra- and interhelical effects. These results suggest that the interrupted helix is a good model for the amylose structure in aqueous solution. The temperature dependencies of the cooperative parameters show that the cooperativity of amylose-iodine complexation is entropy-controlled.

The structure of amylose in aqueous solution has not yet been established, in spite of its important role for food and industry. Figure 1 shows three representative structural models proposed for amylose in aqueous solution. Model A is a random coil structure,[1] model B is a deformed helix structure,[2,3] and model C is an interrupted helix structure composed of the helix regions with intervals of random coils between them.[4,5] These studies clarified that the iodine staining characteristics, *e.g.*, maximum wavelength λ_{max}, or iodine binding capacity I_b, increases with DP of amylose.[6,7] These results were interpreted by the increase of the affinity for iodine, which implies the existence of a cooperative effect for the complexation, *i.e.*, neighboring iodine forms a more stable complex.[8] A quantitative analysis of the cooperative effect was introduced by Schneider and Cronan.[9] They examined precisely the binding isotherms of iodine to high DP amylose from the standpoint of the cooperative phenomenon and determined the intrinsic equilibrium constant and cooperative parameters. However, no quantitative investigation of the effect of DP on these parameters has been performed.

In the present study, the effect of DP of amylose on the intrinsic equilibrium constant and the cooperative parameter was studied by the amperometric titration, with special emphasis on the change in cooperativity. These results are discussed from the point of view of the structure of amylose in aqueous solution.

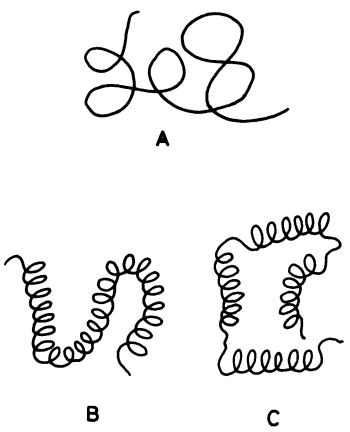

Fig. 1. Proposed models for amylose structure in aqueous solution. A) Random coil structure,[1] B) deformed helix structure,[2,3] and C) interrupted helix structure.[4,5]

Experimental

Materials. Potato amylose was purchased from Pierce Chemical and purified by precipitation three times from 1-butanol. The DP was evaluated to be 1100 by gel-filtration chromatography on Sepharose 4B gel. Degraded amyloses were obtained by hydrolysis of the above amylose with α-amylase[10] and fractionated in a dimethyl sulfoxide–ethanol solvent system. Further purification was done by gel chromatography[11] on Sephadex G-25 and G-75. The DP of each fraction was determined by the relationship between λ_{max} and DP reported by Banks *et al.*[12] The results are listed in Table 1 together with I_b. All solutions containing amylose were freshly prepared immediately before measurements.

Table 1. Properties of the amylose samples

DP	K_{av} [a]	λ_{max} / nm	I_b [b] / mg
27±2	0.24	510	1.5
32±1	0.31	530	4.7
42±3	0.41	545	8.6
57±5	0.51	570	12.6
76±3	0.65	588	15.0
142±20	0.88	610	18.1
1100±50	1.0	620	19.6

a) Elution constant[15] for a Sephadex G-75 gel. b) The amount of iodine binding to 100 mg of amylose.

Measurements. In the amperometric titration, an apparatus similar to that described by Kainuma *et al.*[13] was used in conjunction with two bright platinum electrodes. A solution of 50 cm³ of amylose (0.5 mM** anhydroglucose) was titrated by adding 30 mm³ portions of 10 mM iodine solution at constant concentrations of KI (50 mM) and KCl (50 mM) at 75 s intervals. The temperature was controlled at 20.0 °C by circulating thermostatically controlled water around the cell.

** In this paper 1 M = 1 mol dm^{-3}.

Results and Discussion

The binding isotherms of the amylose–iodine system were obtained for a series of amyloses, from DP 27 to 1100 by amperometric titration; these are shown in Fig. 2. As can be seen, the maximum amount of bound iodine increases with increase of DP of amylose.

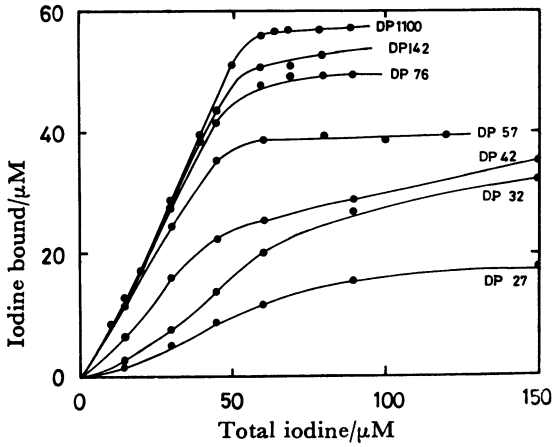

Fig. 2. Binding isotherms of iodine for a series of amyloses obtained by the amperometric titration at 20 °C. Concentration: amylose; 0.5 mM, and KI; 50 mM.

The binding equilibrium of amylose–iodine system is written as

$$A + (I_3^-)_f \rightleftarrows (I_3^-)_b, \quad (1)$$

where A is the binding site, and $(I_3^-)_f$ and $(I_3^-)_b$ are the free and bound triiodide, respectively.[14] The Scatchard equation for reaction (1) is given by

$$r/C_f = K_{app}(n-r), \quad (2)$$

where r and n are the amount of bound iodine and the maximum amount of bound iodine per glucose residue, respectively, C_f is the concentration of free iodine,[16] and K_{app} is the apparent equilibrium constant. Representative Scatchard plots are shown in Fig. 3. All of the amylose–iodine systems are indicative of the cooperativity in the complex formation.[18] If the complexation is independent of cooperativity, the plot

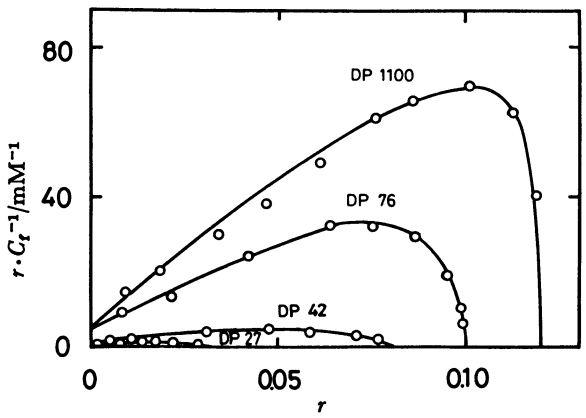

Fig. 3. Representative Scatchard plots of binding isotherms of iodine to amyloses. Solid lines show the theoretical curves obtained by Eq. 3.

Table 2. Binding parameters of the complex formation

DP	n / 10^{-2}	K_0 / 10^3 M^{-1}	K_{st}
27	3±1	4.7±0.2	5±1
32	7±1	4.9±0.1	6±1
42	8±2	7.9±0.1	7±1
57	9±1	15±5	9±2
76	10±2	14±1	40±5
142	10±1	14±1	50±10
1100	12±1	14±2	85±10

should show a straight line with negative slope, as can be seen in Eq. 2. The values of n were obtained from the intercept at $r/C_f=0$ in Fig. 3, and are listed in Table 2. The continuous elevation of n with DP will be due to the decrease of the "terminal effect" which means that each end of amylose does not make helix structure and so iodine can not bind there. According to the one-dimensional Ising model of McGhee and von Hippel,[19] the Scatchard equation (2) can be rewritten as

$$\frac{r}{C_f} = K_0(n-r)\left\{\frac{n-2r+R}{2(n-r)}\right\}^2, \quad (3)$$

with $R = \{(n-2r)^2+4K_{st}r(n-r)\}^{1/2}$,

where K_0 is the equilibrium constant for the intrinsic binding of iodine to amylose site (intrinsic equilibrium constant) and K_{st} is the cooperative parameter expressing the first nearest neighboring interaction between bound iodines.

The values of K_0 and K_{st} were determined so as to give the best fit of the data to the Scatchard equation, these are also listed in Table 2. The values of K_0 and K_{st} for amylose of DP 1100 were in good agreement with the corresponding values obtained by Schneider and Cronan.[9]

The value of K_0 increases with DP up to DP 60 and then remains constant, as shown in Fig. 4. With increase of a helical region, the iodine included in the center of a helix will be stabilized gradually, due to the interruption

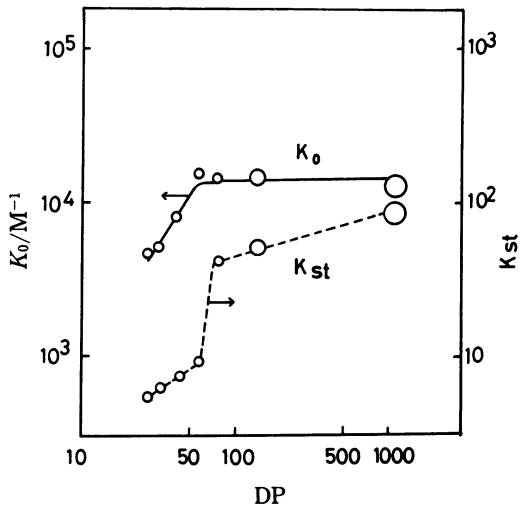

Fig. 4. The DP dependencies of the binding parameters of the amylose–iodine complex.

of the dissociation. According to this idea, the behavior of K_0 in Fig. 4 indicates that the length of a helix increases with DP up to a definite DP and then becomes constant, and suggests the model C in Fig. 1 as the amylose structure in aqueous solution. Furthermore, the length of the helical segment in the unit of DP (hereafter denoted by LHS) was estimated to be less than 60. Compared with the literature values of LHS, ranging from 66 to 140,[20-23] our value is smallest. Inconsistencies in the literature values have been ascribed mainly to the distribution of the DP of amylose.[23] However, some of them will be induced by the differences of the physical quantities used for the determination of the LHS.[24] Since the physical quantities which have been used so far were the values under the saturated condition of iodine, they should be affected significantly by the cooperativity. The value of K_0, on the contrary, does not contain any contribution from the cooperativity and is influenced only by the DP of amylose. Therefore, we believe that K_0 is the most preferable parameter for the determination of the LHS.

The value of K_{st} increases with DP of amylose up to DP 60, at this point it steeply jumps and then gradually increases, as shown in Fig. 4. Such discontinuous behavior of K_{st} can be interpreted by introducing two kinds of cooperative effects. One is the effect within a helical segment (intrahelical effect) which comes out in the region of DP up to 60, where amylose has only one helical segment. The other is the effect between different helical segments (interhelical effect) which comes out only above DP 60 where the amylose consists of more than two helical segments. This idea about cooperativity also supports the model C in Fig. 1 as the structure of amylose in aqueous solution.

Thermodynamic parameters for the intrinsic equilibrium constant and cooperativity were obtained for two kinds of amyloses (DP 42 and DP 1100). Figure 5 shows the Scatchard plots for the amylose of DP 1100

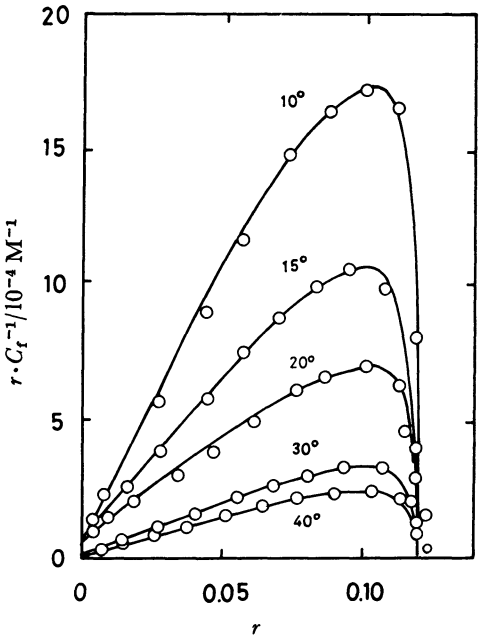

Fig. 5. The Scatchard plots of amylose (DP 1100)–iodine complex at different temperatures.

TABLE 3. EFFECT OF THE TEMPERATURE ON THE BINDING PARAMETERS OF THE COMPLEX FORMATION

Temp/°C	DP 42		DP 1100	
	K_0 / $10^3\ M^{-1}$	K_{st}	K_0 / $10^3\ M^{-1}$	K_{st}
10±0.1	16±3	7±1	24±1	85±10
15±0.1	10±1	7±1	15±1	85±10
20±0.1	7.9±0.1	7±1	14±1	85±10
30±0.1	3.9±0.7	7±1	4.7±0.3	85±10
40±0.1	—	—	3.5±0.3	85±10

TABLE 4. THERMODYNAMIC PARAMETERS OF THE COMPLEX FORMATION

Parameter	DP 42	DP 1100
ΔH_0/kJ mol^{-1}	−50	−50
ΔG_0/kJ mol^{-1}	−22	−37
ΔS_0/J K^{-1} mol^{-1}	−94	−42
ΔH_{st}/kJ mol^{-1}	0	0
ΔG_{st}/kJ mol^{-1}	−5.4	−11
ΔS_{st}/J K^{-1} mol^{-1}	19	38

measured at different temperatures. The magnitude of the plots decreases with increase of temperature, while the shape does not change. The values of K_0 and K_{st} for the two amyloses are listed in Table 3. From the temperature dependencies of K_0 and K_{st}, thermodynamic parameters were estimated, they are summarized in Table 4. It is noteworthy that the cooperative enthalpy change ΔH_{st} is zero for both amyloses and, therefore, the cooperativity is controlled not by enthalpy but by entropy.

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14) The bound species is I_3^- under the present experimental conditions, according to Cronan and Schneider.[15] Hence, in the present manuscript, iodine represents I_3^-.
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16) The concentration of I_3^- is determined by considering

the reaction $I_2+I^- \rightleftharpoons I_3^-$, using $K'=[I_3^-]/[I_2][I^-]=866\ M^{-1}$ at 20 °C.[17]

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24) In fact, in the present study, if we use λ_{max} or I_b in Table 1, the value of LHS will be about 110, which is almost two times larger than that determined from K_0.