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Amylose-Iodine Complex. V. An Estimation of Number of Anhydroglucose Units of Helical Segments in Amylose-Iodine Complex*1

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The amylose molecules in neutral or acidic aqueous solution would take a conformation of deformed helix;[1-5] the individual helical segments are connected by random segments. The dissolved amylose-iodine complex has a similar structure as stated above, *i. e.* a single amylose molecule contains more than one independent polyiodine chains. According to Szejtli *et al.*[5] the number of anhydroglucose units composing the helical segment was about 120 which was obtained from the study of the correlation between the degree of polymerization of amylose molecule and the iodine-binding capacity or the wavelength of the maximum absorption peak of amylose-iodine complex.

In the present paper, the number of anhydroglucose units per helical segment was estimated from spectrophotometric measurement of the change in the absorbance at 288, 352 and 630—660 mμ with addition of amylose to iodide-iodine solution.

Experimental

The amylose subfraction used is the one described in the previous report[4], having viscosity-average molecular weight ($\overline{M}_v$) 1×10^6 calculated from limiting viscosity number, $[\eta]$ (m*l*/g) in dimethyl sulfoxide at 25°C, using the following equation proposed by Everett and Foster[6], $[\eta]=3.06\times10^{-2}\ M_v^{0.64}$.

The spectrophotometric measurements were carried out by using Hitachi Recording Spectrophotometer Model EPS-3 with silica cells 1.00 cm thick.

Results and Discussion

The iodide-iodine solution possesses two absorption peaks (at wavelength, 288 and 352 mμ), which have been attributed by Awtrey and Connick[7] to the I_3^- ion. On the addition of amylose to the solution, blue colored complex is formed and the complex solution possesses three absorption peaks at 288, 352 and 630—660 mμ. Absorbance of iodide-iodine solution with and without amylose

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1) J. Holló and J. Szejtli, *Period. Polytech., Chem. Eng.* (Budapest) **1**, 223 (1957); *Stärke*, **10**, 49 (1958).
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3) V. S. R. Rao and J. F. Foster, *Biopolymers*, **1**, 527 (1963).
4) S. Ono, T. Watanabe, K. Ogawa and N. Okazaki, This Bulletin **38**, 643 (1965).
5) J. Szejtli, S. Augustat and M. Richter, *Stärke*, **18**, 38 (1966); *Biopolymers*, **5**, 5 and 17 (1967); **6**, 27 (1968).
6) W. W. Everett and J. F. Foster, *J. Amer. Chem., Soc.*, **81**, 3464 (1959).
7) A. D. Awtrey and R. E. Connick, *ibid.*, **73**, 1842 (1951).

changes with the concentration of iodide as shown in Figs. 1 and 2. In the solution of iodide-iodine-amylose, the following equilibria will hold

$$\mathrm{Am(h)} + n\mathrm{I_2} + m\mathrm{I^-} \rightleftharpoons \mathrm{Am(h)(I_2)}_n\mathrm{(I^-)}_m \quad (1)$$

$$\mathrm{I_2} + \mathrm{I^-} \rightleftharpoons \mathrm{I_3^-} \quad (2)$$

where Am(h) is the helical segment of amylose molecule and $\mathrm{Am(h)(I_2)}_n\mathrm{(I^-)}_m$ is the iodine complex. The dissociation constant of I_3^- in Eq. (2), K, was given by Davies and Gwynne[8] as follows.

$$K = 1.22 \times 10^{-3} \quad \text{(at 20°C)} \quad (3)$$

The concentration of I_3^- ion in iodide-iodine solution can be calculated from the molar extinction coefficients given by Awtrey and Connick;[7] 40000 at 288 mμ and 26400 at 352 mμ.

As shown in Figs. 1 and 2, absorbance at 352 mμ of iodide-iodine solution increases with the addition of amylose in the range of low iodide concentration, therefore the complex is assumed to have appreciable contribution to this absorption peak as well as the I_3^- ion in solution. On the other hand, absorbance at 288 mμ of the iodide-iodine solution is always suppressed by the addition of amylose. However, the amount of iodine in the complex calculated by the following equation, was not in agreement with that predicted from the experimental results shown in Figs. 1 and 2.

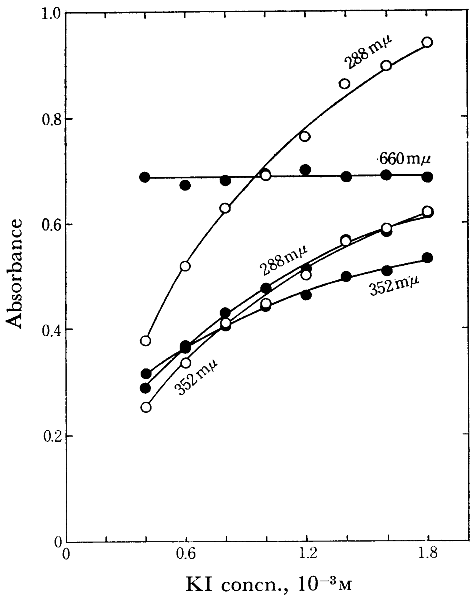

Fig. 1. Relation between the absorbance (at 288, 352 and 660 mμ) and KI concentration of iodide-iodine solution with (—●—) and without (—○—) amylose: concn. of I_2, 4×10^{-5}M; concn. of amylose, 0.02 g/l; at 20°C.

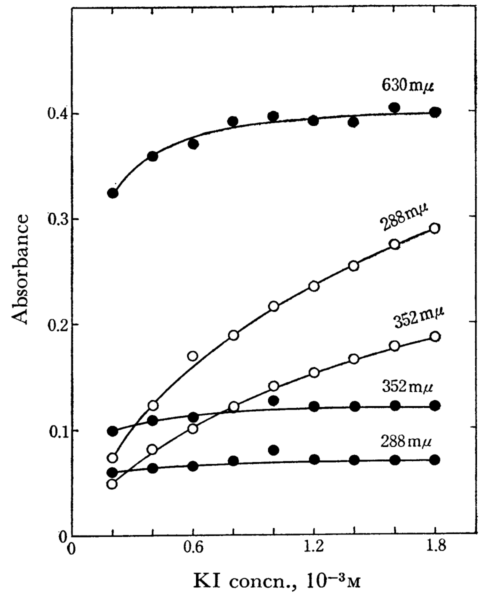

Fig. 2. Relation between the absorbance (at 288, 352 and 630 mμ) and KI concentration of iodide-iodine solution with (—●—) and without (—○—) amylose: concn. of I_2, 1.2×10^{-5}M; concn. of amylose, 0.02 g/l; at 20°C.

(amount of iodine in the complex)

= (amount of iodine dissolved)

$$-\left[\begin{pmatrix}\text{amount of iodine present as } I_3^- \text{ ion calculated}\\ \text{from the relation, \{absorbance at 288 m}\mu/40000\}\end{pmatrix} + \begin{pmatrix}\text{amount of iodine in equilibrium with}\\ \text{the } I_3^- \text{ ion calculated by Eq. (3)}\end{pmatrix}\right] \quad (4)$$

Therefore, polyiodine chain in the complex may have some contribution to the peak at 288 mμ. Thereupon, the molar extinction coefficient of the iodine complex was evaluated by trial-and-error solution and the most probable values per mol iodine obtained were as follows; 0.7×10^4 at 288 mμ, 1.2×10^4 at 352 mμ and 4×10^4 at 630—660 mμ. The latest value at 630—660 mμ is in good agreement with the proposed one by Rundle *et al.*[9] The above values of extinction coefficient were obtained on the basis of the following assumption; both ends of the polyiodine chain are in the form of I_3^- and these I_3^- as well as the free I_3^- ion in solution, take part in the equilibrium, existing in solution according to Eq. (2), with free iodine. The most probable values of n and m were obtained as 11 and 2, respectively. Therefore, the polyiodine chain in the complex is assumed to be composed of linearly linked 11 iodine molecules and the both ends of the chain are in the form I_3^- such as shown as follows; $^-I_3(I_2)_9I_3^-$. When one helical turn

8) M. Davies and E. Gwynne, *ibid.*, **74**, 2748 (1952).

9) R. E. Rundle, J. F. Foster and R. R. Baldwin, *ibid.*, **66**, 2116 (1944).

consists of 6 anhydroglucose units, the helical segment will be expected to be composed of $6\times 11=66$ anhydroglucose units provided the condition for the complex formation *i. e.* one iodine molecule per one turn of the helix is maintained. This amount 66 is in good agreement with the maximum number of anhydroglucose units composing a stable single helix obtained from the correlation between the wavelength of the maximum absorption peak of iodine-complex and the degree of polymerization of maltodextrin.[10] The amount of iodine combined in the complex in the presence of excess amount of iodine in solution (Fig. 1), was about 83% of the saturation (2.06×10^{-5} mol iodine per 0.02 g amylose), therefore a random segment between two helical segments in the helical segments may be assumed to be composed of about 14 anhydroglucose units.

10) K. Sakon, T. Watanabe and S. Ono, This Bulletin, **43**, in press.