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Note

Iodine binding to amylodextrin fractions studied by difference spectrophotometry and potentiometry*

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Kikumoto *et al.*[1] and Umeki and Kainuma[2] prepared some amylose and amylodextrin fractions from waxy-maize starch and succeeded in separating an amylodextrin, designated "Fraction II" and having d.p. 28–30, which consists of two unit-chains of glucose polymer, of d.p. 14–16, linked together by a (1→6)-α-D-glucosidic bond near the reducing end of the molecule. This amylodextrin fraction was expected to be useful for studies of the structure of starch. Watanabe and French[3,4], who also prepared Fraction II having d.p. 25–30 or 30–33 from the Nägeli amylodextrin of waxy-maize starch, proposed a model for the structure of the amylodextrin fraction; they suggested it is in the form of a double helix, which is considered to be a basic structure of starch granule.

In the present work the binding of iodine molecules to Fraction II, and to its preparation debranched at the (1→6)-α-glucosidic bond, named "Fraction II'", was studied by difference-absorption spectrophotometry and by potentiometry. The intensity of the difference absorption at 440 nm for the Fraction II–iodine system was observed to be nearly equal to that for the Fraction II'–iodine system. Moreover, four and two molecules of iodine were found to be bound by each molecule of Fraction II and Fraction II', respectively. These findings indicate that the branching (1→6)-α-glucosidic bond does not disturb the formation of the amylodextrin–iodine complex.

EXPERIMENTAL

Materials. — Amylodextrin Fraction II was prepared from waxy-maize starch granules by the method of French[3,5]. Isoamylase, a debranching amylase, was purchased from the Hayashibara Biochemical Laboratories Co. Iodine and other

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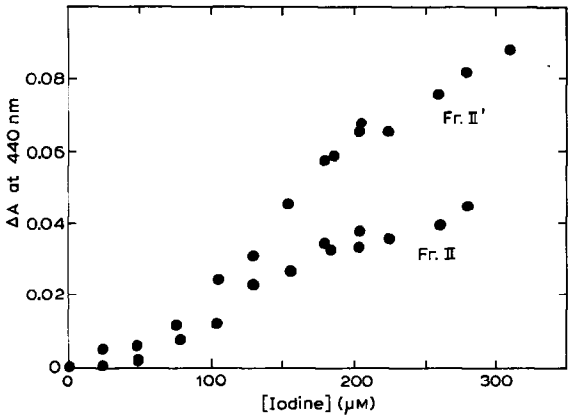

Fig. 1. Dependence of the difference absorption at 440 nm on the concentration of iodine. Amylodextrin fractions, 0.005%; pH 4.5; 25°.

chemicals, obtained from the Fisher Scientific Co., were used without further purification.

For studies on the effect of the branch, Fraction II (0.08%) was hydrolyzed for 50 h at pH 4.5, 25° by treatment with isoamylase (0.2 μg/mL), which was found not to hydrolyze Fraction III (a fraction of the Nägeli amylodextrin having d.p. 15–18) under the conditions employed. By this hydrolysis (>96%) Fraction II was converted into the linear Fraction II′. The degree of hydrolysis to Fraction II′ was shown to be 96% by measurement of the reducing value, which should be twice the starting value if Fraction II has only one branch point. Iodine stock solution was prepared by dissolving iodine in 1M KI, the final concentration of KI was kept at 0.1M in all measurements.

Methods. — Reducing power was determined with a Technicon Autoanalyzer using the method of Robyt *et al.*[6]. The binding of iodine to Fraction II and Fraction II′ was studied by measurements of difference-absorption spectra using a Cary 15 spectrophotometer[7], and by potentiometric titration with an Instrumentation Laboratories Model 135A potentiometer[8]. All the measurements were carried out in 0.02M acetate buffer, pH 4.5, at 25°.

RESULTS AND DISCUSSION

Spectrophotometric measurements of the binding of iodine to the amylodextrin fractions. — When iodine was added to the amylodextrin fraction, the observed difference-absorption spectra had a peak around 440 nm, and the shape of the peak for the Fraction II–iodine system was almost the same as that for the Fraction II′–iodine system (data not shown). We concluded that the difference-absorption spectra were produced by complex formation between the amylodextrins and iodine molecules. When the difference absorption, ΔA, at 440 nm was evaluated from the spectra and graphed against the concentration of iodine, plots such as

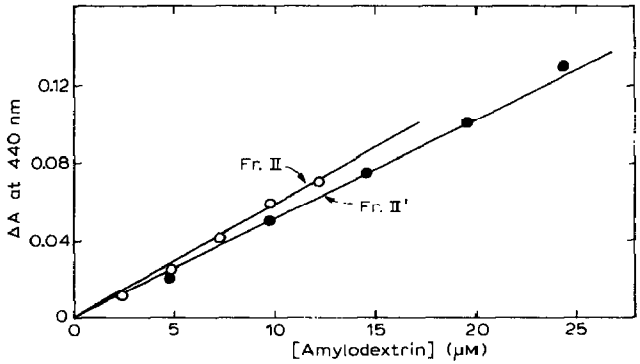

Fig. 2. Dependence of the difference absorption at 440 nm on the concentration of amylodextrin fractions. Iodine concentration, 250μM; pH 4.5; 25°.

those in Fig. 1 were typically obtained. For both the Fraction II and the Fraction II′ systems, the dependence of ΔA on iodine concentration appears sigmoidal, but this appearance results from ambiguities in the data. Accurate measurement of the difference absorption at high concentrations of iodine is difficult, because the absorbance of iodine itself becomes large under these conditions, and at low concentrations of iodine the difference absorption is small and again subject to measurement errors. Therefore, the dependence of ΔA on the concentration of Fraction II or Fraction II′ was analyzed at a fixed concentration of iodine (present in large excess), giving the results shown in Fig. 2. The plots for both Fraction II and Fraction II′ are linear, and thus ΔA is proportional to the Fraction concentration. Moreover, the plots clearly indicate that for a given molar concentration ΔA is nearly equal for the two fractions; the slope of the plot for Fraction II is 1.13 times as large as that for Fraction II′.

Rundle and French[9] confirmed from X-ray crystallographic studies that one molecule of iodine is bound to one turn of amylose helix, with each turn consisting of six glucose residues. Based on their findings, we assumed that the number of iodine molecules bound to each molecule of Fraction II′, which consists of 12–13 glucose residues on the average, is two. However, ΔA for Fraction II was found to be nearly equal to that for Fraction II′ (see Fig. 2). Therefore, the actual number of iodine molecules bound to each molecule of the amylodextrins required further evaluation.

Potentiometric measurements of iodine binding. — The binding of iodine to the amylodextrins can be described by the following equation,

$$F + nI \overset{K}{\rightleftharpoons} FI_n \qquad (1)$$

where F and I represent amylodextrin and molecular iodine, respectively, FI_n is a complex containing n molecules of iodine, and K stands for K_1, K_2, . . .K_n, the

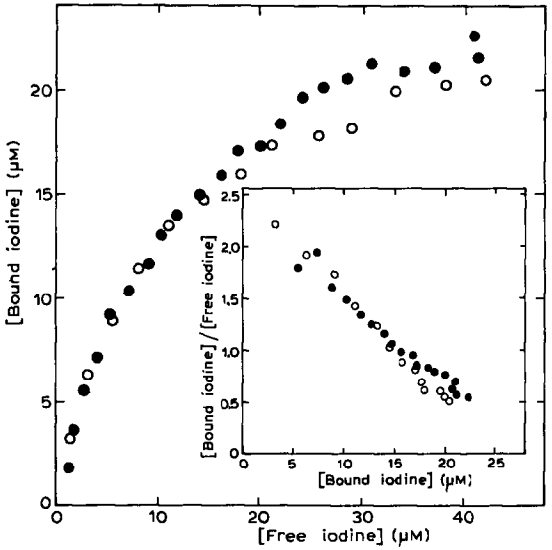

Fig. 3. Potentiometric titration of amylodextrin fractions with iodine. ○, Fraction II; ●, Fraction II'. Amylodextrin fractions, 0.00255%; pH 4.5; 25°.

successive association constants for complex formation. By measurement of the molar concentration of free iodine (C_I) using a potentiometer, the concentration of bound iodine (C_{BI}) can be evaluated, because the total concentration of iodine (C_I^0) is known. An example of the potentiometric measurements is shown in Fig. 3. In these experiments the concentration of Fraction II was 6.25μM and that of Fraction II', 12.5μM.

From Eq. *1* one can arrive at the relationship

$$C_{BI}/C_I = K(C_B^0 - C_{BI}), \tag{2}$$

where C_I is the molar concentration of free iodine, C_{BI} is that of bound iodine, and C_B^0 is the concentration of binding sites for iodine molecules. When the values of K_1, K_2, ...K_n are constant the binding sites are identical and have no interaction with each other, and a plot of C_{BI}/C_I *vs.* C_{BI} is a straight line. Figure 3 (inset) shows that plots of C_{BI}/C_I *vs.* C_{BI} for Fraction II and also for Fraction II' are straight lines in the range of concentrations examined, indicating that the K values for Fraction II and for Fraction II' are very similar; both slopes are nearly equal. Moreover, the intercept at the C_{BI} axis (abscissa) gives the concentration of binding sites, C_B^0, and, when an amylodextrin fraction has n sites per molecule for iodine binding

$$nC_F^0 = C_B^0, \tag{3}$$

where C_F^0 is the total concentration of amylodextrin fraction. Thus, from the experimental results the number of binding sites per molecule (n) can be estimated. In

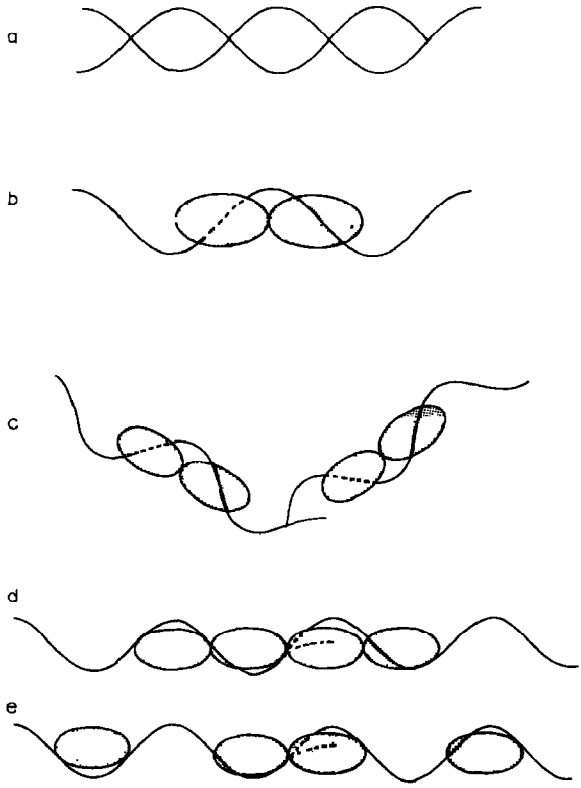

Fig. 4. Schematic illustration of complex formation between amylodextrin fractions and iodine molecules. (a) Double helical structure for Fraction II, as suggested by Watanabe and French[3]; (b) complex of Fraction II′ with two molecules of iodine; (c), (d), (e) possible structures for Fraction II complexed with four molecules of iodine.

the present case the values were found to be 4.10 ±0.07 for Fraction II and 2.16 ±0.09 for Fraction II′, that is, four and two molecules of iodine are bound to each molecule of amylodextrin Fraction II and Fraction II′, respectively. These findings, which are consistent with an evaluation carried out by the calorimetric method[10], indicate that the branch point (1→6)-α-glucosidic bond does not interfere with complex formation between iodine molecules and Fraction II.

The finding that the iodine-binding capacity of Fraction II is twice that of Fraction II′ is in good agreement with the results of Rundle and French[9]. However, the difference absorption (ΔA) of the Fraction II–iodine complex is not twice that of the Fraction II′–iodine complex, as might be expected, but rather the two values are nearly equal (II:II′ 1.13). Given these contradictory observation, a firm hypothesis regarding the three-dimensional structure of Fraction II cannot be formulated at present.

Watanabe and French[3] proposed that Fraction II has the double helical form that is a basic feature of starch structure. However, the results reported here argue against this hypothesis, because it is unlikely that iodine molecules can be accom-

modated in the double-helical arrangement. For the iodine complexes it is necessary to invoke structures such as those shown in Fig. 4. Among these possibilities, the best explanation for the low molar difference absorption of Fraction II (as compared to Fraction II') is perhaps offered by Fig. 4e, showing a structure that has two "isolated" iodine molecules and two in close contact.

It would obviously be of interest to measure iodine-complex formation at low temperature (−1–2°), where Fraction II might have a double helical structure.

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