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A FLOW-CALORIMETRIC STUDY OF THE BINDING OF IODINE TO AMYLODEXTRIN FRACTIONS*

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ABSTRACT

Acid hydrolyzates of waxy-maize starch were separated to give Fractions I, II, and III [T. WATANABE, AND D. FRENCH, *Carbohydr. Res.*, 84 (1980) 115–123]. Watanabe and French suggested that Fraction II, which contains approximately 25 D-glucose residues including an α-D-(1→6)-linked branch, has a double helical structure. In the present study, the thermodynamics of binding of iodine to Fractions II and III, and debranched Fraction II (Fraction II′) was measured by isothermal-flow calorimetry. If four binding sites for Fraction II and two for Fractions II′ and III are assumed, the standard free-energy changes, ΔG_b^0, for the binding of I_2 are −18.5, −18.8, and −18.4 kJ·(mol I_2)$^{-1}$, and the enthalpy changes, ΔH_b, are −28.4, −24.7, and −26.9 kJ·(mol I_2)$^{-1}$, respectively. The similarity of these values for the three fractions indicates that the conformation of Fraction II is essentially the same as those of Fractions II′ and III, and that Fraction II, therefore, does not have a double helical structure in solution. The values for ΔG_b^0 are ~15 kJ·mol^{-1} less negative, and those for ΔH_b ~40 kJ·mol^{-1} less negative than published values for the starch–I_2 complex. These differences are due to the relatively very short D-glucose chains in the amylodextrin fractions employed in the present work.

INTRODUCTION

Three fractions of amylodextrin were isolated from acid hydrolyzates of starch granules by Watanabe and French[1], who found that one of these fractions, Fraction II, has approximately 25 D-glucose residues and contains one α-D-(1→6)-

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linked branch located between two parts of the molecule having about 11 and 14 D-glucose residues, respectively. Another of these fractions, Fraction III, has 12–14 D-glucose residues with α-D-(1→4) bonds and no branch. Watanabe and French[1] proposed a single helical structure for Fraction III and an intertwined or double helical structure for Fraction II. It was proposed that these structures arise from the cluster model of amylopectin in which the outer chains are in a crystalline arrangement of double[2] helices, considered as one of the basic structures of starch granules[3].

We report herein the results of a study, by means of isothermal-flow calorimetry, of the binding of iodine to the Fractions II and III of amylodextrin, and also to a fraction (II′) formed by hydrolyzing the amylodextrin of Fraction II at its α-D-(1→6) bond. Molecules of I_2 (ref. 4), or of I_2 and I_3^- (ref. 5), or of I_5^- (ref. 6), penetrate into the helical structure of amylodextrin to form an inclusion complex, with one I_2 or I^{-3} molecule bound per turn of a single-stranded helix composed of approximately six D-glucose residues[7]. The iodine complex presumably could not be formed with the double helical structure proposed for Fraction II. We have found the thermodynamics of iodine binding to all three fractions, II, II′, and III, to be very similar. This indicates that Fraction II does hot have a double helical structure in solution.

EXPERIMENTAL

Materials. — Following the procedures of Watanabe and French[1], three fractions of amylodextrin were isolated by means of Sephadex G-50 column chromatography from a hydrolyzate of starch (waxy maize). Fractions II and III were used in the present study, as well as Fraction II′, prepared by treatment of a 0.08% solution of Fraction II with isoamylase (120 units per 10 mL of Fraction II solution) in 0.02M acetate buffer, at pH 4.5 and 25° for 70 h. The isoamylase used (*Pseudomonas* amyloderamosa) was a purified sample which hydrolyzes only α-D-(1→6) bonds, and was obtained from Hayashibara Biochemical Laboratories, Okayama, Japan. The degree of hydrolysis of Fraction II was measured by the reducing value and was found to be 96%. According to the structure of Fraction II mentioned earlier, Fraction II′ is an equimolar mixture of two amylodextrins, one made up of approximately 11 glucose residues and the other of approximately 14 residues.

Thermodynamics measurements. — The enthalpy of binding of I_2 to amylodextrin was determined as a function of the I_2 concentration in a flow modification of the Beckman Model 190 batch calorimeter[8]. A flow rate of 43.5 $\mu L \cdot min^{-1}$ was used for both the amylodextrin and I_2–0.2M KI solutions. The amylodextrin was also dissolved in 0.2M KI in order to avoid large heats of dilution, and the pH of both solutions was adjusted to 4.5. In the case of Fraction II, it was shown that experiments using 0.01 and 0.1M KI in 0.02M acetate buffer (pH 4.5) gave the same results as obtained with 0.2M KI. All measurements were made at

25°. The calorimeter was calibrated[9] by use of the reaction of HCl with a small excess of NaOH.

On the assumption of equivalent and independent binding sites, the binding equilibrium should follow Eq. (*1*)

$$2\frac{y-y_0}{y_\infty-u_0}=1+\frac{B_0}{nA_0}+\frac{K}{nA_0}-\sqrt{\left(1+\frac{B_0}{nA_0}+\frac{K}{nA_0}\right)^2-4\frac{B_0}{nA_0}} \qquad (1)$$

where y is any property which varies linearly with the degree of saturation by the ligand, with $y = y_0$ at zero I_2 concentration and $y = y_0$ at a very large I_2 concentration, A_0 is the total amylodextrin concentration (mM), B_0 is the total I_2 concentration (mM), K is the dissociation constant (mM), and n is the number of binding sites per amylodextrin molecule. The data for the calorimetric titration of each amylodextrin fraction (y = enthalpy per mol of amylodextrin, $y_0 = 0$) were fit to Eq. (*1*) according to the least squares criterion, taking as adjustable parameters (*a*) y_∞, K and, or (*b*) y_∞ and K, with n = 4 for Fraction II and n = 2 for Fractions II′ and III.

RESULTS AND DISCUSSION

Figure 1 shows the thermal titration curve observed with Fraction II, with the observed heat of binding in kJ/mol of amylodextrin plotted as a function of the total concentration of I_2. The parameters giving the best fit of the experimental data with those of Eq. (*1*) are given in Table I for the fitting procedures (*a*) and (*b*) outlined above. The solid curve in Fig. 1 was drawn using the procedure (*a*) parameters. It is clear that the assumption of identical and non-interacting binding sites is supported by the titration data.

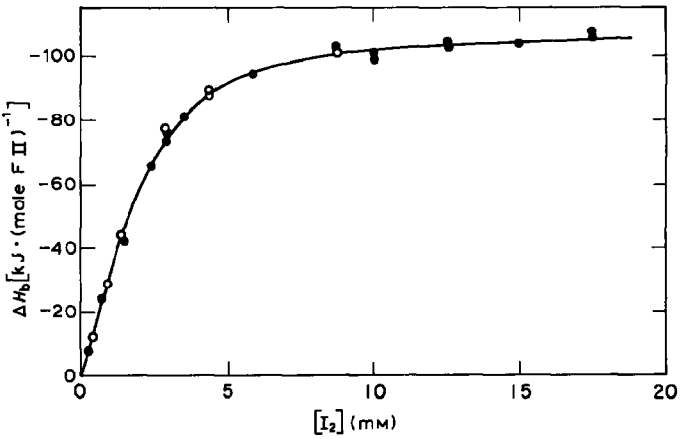

Fig. 1. The enthalpy of binding I_2 to amylodextrin Fraction II as a function of the total I_2 concentration. Fraction II concentration, 2.56 mg·mL^{-1}, pH 4.5, 25°: (●) 0.2M KI; (○) 0.01M and 0.1M KI; (——) calculated with parameters (*a*) given in Table I.

TABLE I

PARAMETERS FOR FITTING THE EXPERIMENTAL THERMAL DATA TO EQ. (*1*)

Fraction	*Fitting procedure*	y_∞ *(kJ·mol^{-1})*	K *(mM)*	*n (Sites/molecule)*	*Stand. dev. (% of y_∞)*
II	(*a*)	−113	0.484	4.39	1.6
II	(*b*)	−114	0.580	4.00[a]	1.7
II′	(*a*)	−49.8	0.637	1.75	1.1
II′	(*b*)	−49.4	0.500	2.00[a]	1.5
III	(*a*)	−55.2	0.905	1.45	1.3
III	(*b*)	−53.5	0.590	2.00[a]	2.1

[a]Assumed value.

Figures 2 and 3 are the thermal titration curves for Fractions II′ and III, respectively, and the corresponding least-squared parameters are listed in Table I. The solid curves in Figs. 2 and 3 were drawn with the procedure (*a*) parameters, and the dashed curve in Fig. 3 with the procedure (*b*) parameters for Fraction III. This is the only case in which the curves for (*a*) and (*b*) are detectably different.

It is evident that the overall enthalpy of binding, y_∞, is the best determined of the various parameters, as is to be expected in calorimetric titrations. In the case of Fraction II, for example, 10% changes in y_∞, K, and n cause, respectively, 500, 12, and 38% increases in the standard deviation. However, as indicated in Table I and Fig. 3, large compensating changes in the K value permit large changes in n without undue deviation of the calculated curve from the experimental points. The

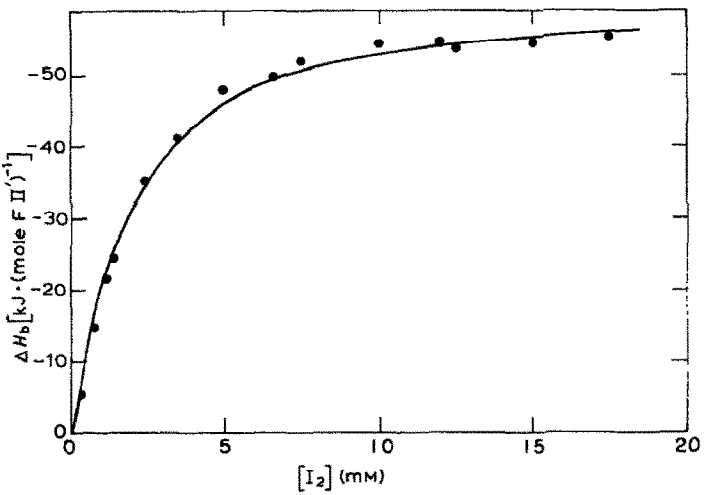

Fig. 2. Calorimetric titration of Fraction II′ with I_2 in 0.2M KI, pH 4.5, 25°. Fraction II′ concentration, 2.80 mg·mL^{-1}: (●) Experimental data; (——) calculated with parameters (*a*) listed in Table I.

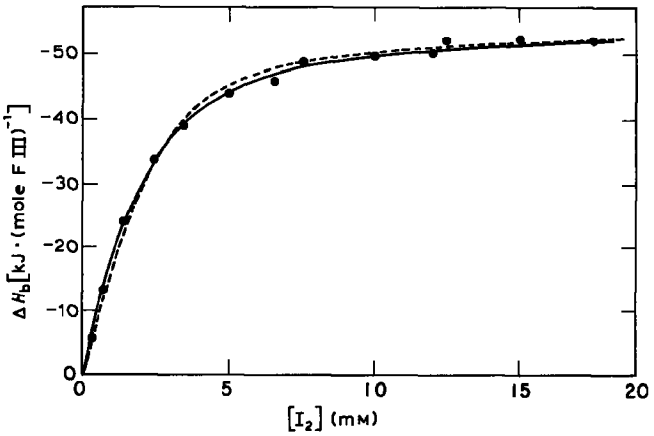

Fig. 3. Calorimetric titration of Fraction III with I_2 in 0.2M KI, pH 4.5, 25°. Fraction III concentration, 2.58 mg·mL^{-1}: (●) Experimental data; (——) calculated with parameters (*a*) listed in Table I; (----) calculated with parameters (*b*) in Table I.

resulting large uncertainty in assigning values to K and n is a manifestation of the fact that, as pointed out by Weber and Anderson[10], curves such as those in Figs. 1–3 are actually of severely limited information content.

The thermodynamic parameters for the binding of iodine to amylodextrins are listed in Table II. In deriving these parameters, we have retained the values resulting from both fitting procedures (*a*) and (*b*), the latter being included since it is generally accepted that there is one binding site per six or seven glucose residues. The uncertainties given in Table II for K_b and $\Delta H_b = y_\infty/n$, the enthalpy of binding per mole of binding sites, are arbitrarily based on the variations which lead to an increase of 20% in the standard deviation, plus an additional uncertainty corresponding to one residue in the degree of polymerization of the amylodextrin fractions. The uncertainties in ΔG_b^0 and ΔS_b^0 follow from those for K and ΔH_b.

TABLE II

THERMODYNAMICS OF THE BINDING OF IODINE TO AMYLODEXTRINS AT pH 4.5 AND 25°

Fraction	*Fitting procedure*	K_b/M^{-1} ($\times 10^{-3}$)	ΔG_b^0 [a] (*kJ·mol^{-1}*)	ΔH_b [a] (*kJ·mol^{-1}*)	ΔS_b^0 [a] (*J·K^{-1}·mol^{-1}*)
II	(*a*)	2.07 ±0.35	−18.9 ±0.40	−25.6 ±1.9	−22.6 ±1.7
II	(*b*)	1.72 ±0.30	−18.5 ±0.40	−28.4 ±1.3	−33.0 ±1.7
II′	(*a*)	1.57 ±0.12	−18.2 ±0.20	−28.4 ±2.9	−34.2 ±3.6
II′	(*b*)	2.00 ±0.20	−18.8 ±0.30	−24.7 ±2.1	−19.7 ±1.9
III	(*a*)	1.10 ±0.05	−17.4 ±0.20	−40.9 ±3.8	−79.1 ±7.1
III	(*b*)	1.69 ±0.40	−18.4 ±0.50	−26.9 ±2.5	−28.3 ±2.7

[a] The values of ΔG_b^0, ΔH_b and ΔS_b^0 are per mol of binding sites.

As mentioned earlier, the various thermodynamic parameters are quite similar for all three fractions. We take this as an indication that the structure of Fraction II is similar to that of Fractions II′ and III, and that it does not involve intertwined helices.

The favorable free energy is the result, at least at 25°, of a favorable enthalpy opposed by a fairly large unfavorable entropy change. It is difficult, as is usually the case, to rationalize fully thermodynamic data in terms of the molecular process taking place. Possible contributions to the decrease in entropy are loss of translational and rotational entropy of I_2 and I_3^- in the binding process, and decreased conformational flexibility of the amylodextrin when binding takes place.

The thermodynamic parameters reported herein differ markedly from those which have been previously reported for complexes formed with starch preparations of much higher degrees of polymerization than our amylodextrin fractions. (For a summary of previous values of ΔH_b, obtained from equilibrium and from calorimetric measurements, see Cesaro *et al.*[5]). Our enthalpies are roughly 40 $kJ \cdot mol^-$ less negative and our standard free energies almost 16 $kJ \cdot mol^{-1}$ less negative than the previously published values.

The structure of the starch–I_2 complex is made up of a helical chain of glucose residues within which I_2 molecules or I_3^- ions (or both) are arranged in linear array[4,11]. Stabilization of this structure has been attributed, at least in part, to resonance involving the *p*-electrons on the I atoms similar to the resonance in polyenes[12]. A major contribution to the differences noted in the preceding paragraph may be the fact that effective resonance stabilization is not possible in very short arrays of I atoms. Support for this view is given by the observation that little or no color change in the iodine solutions resulted from mixing with amylodextrin solutions in our experiments (*cf.* Ono *et al.*[13]).

The entropy of binding derived from our data is much less unfavorable than that for the binding of I_2 to starch, which according to Takahashi and Ono[14] is $-120\ J \cdot K^{-1} \cdot (mol\ I_2)^{-1}$. Both contributions to the entropy of binding listed above may be expected to be smaller for a short system than for a very long one.

It would obviously be of interest to determine ΔH_b for a series of amylose samples covering a wide range of degrees of polymerization, and also the absorption maxima of the I_2 complexes formed with these samples[13].

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