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PROPERTIES OF AMYLOSE–IODINE COMPLEXES PREPARED IN THE PRESENCE OF EXCESS IODINE

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ABSTRACT

A potentiometric-titration procedure, in which samples are always exposed to an excess of I_2–KI has been developed for measuring iodine-binding capacity of starches. Binding capacity of amylose under these conditions is ~30% as opposed to 20% by conventional potentiometric titration. Spectrophotometric absorbance is essentially the same for either method, but is proportional to potentiometric values only in the excess-iodine titration procedure. Effects of variation of concentration of I_2, KI, and phosphate buffer and of temperature on the reaction have been examined. Calculations based on concentration of reactants in solution indicate that the binding species varies from I_3^- at 10^{-1}M KI to I_{11}^- at 5×10^{-4}M KI.

INTRODUCTION

The wellknown reaction of iodine with amylose to produce a blue color has been studied extensively. The reaction is used widely for the determination of the amylose content of starches by both potentiometric and spectrophotometric methods. Despite active use of the reaction and a large body of research, there continues to be considerable uncertainty regarding the mechanism and stoichiometry of the complex formation. This subject is examined in a recent book by Banks and Greenwood[1] and by several recent papers[2–5].

The differential potentiometric titration method of Banks *et al.*[6] is often used to measure iodine binding of starches and amyloses, and these workers have compared results with their technique to those obtained by spectrophotometric methods[7]. In attempting to use the method of Banks *et al.*[6] to analyze starch-granule fractions, we found that reproducibility was less than needed to detect relatively small changes in apparent amylose content. Therefore, a potentiometric excess-iodine (EI) titration method was developed, which not only improved reproducibility, but yielded an iodine-binding capacity that was ~50% greater than previously obtained values of

*The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

20–22 mg of iodine bound per 100 mg of amylose[8]. Iodine binding by amylopectin was negligible under these conditions.

In this paper, we examine properties of the differential EI-titration method, compare our results with those obtained from the well established potentiometric method of Banks *et al.*[6], and consider the implications of our findings to the properties and stoichiometry of the amylose–iodine complex. The essential difference between the foregoing two methods is that in the EI-titration method, amylose or starch is always exposed to an oversaturation of iodine–KI, whereas the usual potentiometric practice has been to approach iodine-binding saturation by adding small increments of I_2 to the system.

MATERIALS AND METHODS

All studies were conducted with an amylose sample that had been extracted at 70° from defatted corn starch and crystallized from aqueous solution saturated with 1-butanol. Intrinsic viscosity was determined by measuring flow times of aqueous solutions in Cannon viscometers. A molecular weight of 1.76×10^4, equivalent to d.p. 1100, was calculated by using the Mark–Houwink equation recommended by Burchard[9]: $\eta = 1.32 \times 10^{-2} M_v^{0.68}$. Stock solutions for iodine-binding studies were prepared by dissolving 100-mg samples in 25 mL of 9:1 (v/v) dimethyl sulfoxide–water. The stock solution was diluted 1:10 with water immediately before use, to yield a concentration of 400 μg per mL.

Standard electrolyte solution was prepared by mixing 203 mL of 0.1M KI with 20 mL of 0.2M KH_2PO_4–K_2HPO_4 buffer (pH 5.8) and diluting this to 1 L with water. Electrolyte quantities were altered by substituting KI solutions from 0.0025 to 1.0M and by varying the volume of phosphate buffer added from 10 to 40 mL. Thus, final KI concentrations were varied from 5.32×10^{-4} to 2.03×10^{-1}M, whereas phosphate buffer varied from 2×10^{-3} to 8×10^{-3}M.

Stock iodine solution was 0.025M I_2 dissolved in 0.1M KI, and was standardized with primary arsenious oxide. This iodine stock-solution was diluted 1:10 with water immediately before use.

The titration cell, which was equivalent to that used by Banks *et al.*[6], consisted of two 1-L round-bottom, three-necked flasks connected by a liquid bridge. Each flask contained a platinum electrode connected to a Keithley Model 155 Null Detector microvoltmeter, which was operated at an input resistance of 1 megohm. Flasks were placed in a refrigerated water-bath to maintain constant temperature, and contents were stirred with magnetic stirrers.

The EI-titration procedure was as follows: 830 mL of electrolyte solution was added to each flask and allowed to equilibrate to a temperature of either 2 or 20°. Between 0.1 and 2.0 mL of 25mM I_2 in KI was added to the reaction flask. An equal volume of I_2, allowing enough variation (±2%) to exactly null the microvoltmeter, was added to the reference flask. Either 0.5 or 1.0 mL of amylose solution was then added to the reaction flask and an equal volume of diluted Me_2SO solution without

amylose was added to the reference flask to compensate for possible potentiometric variations caused by the presence of Me_2SO. The system was allowed to equilibrate for 5 min. The microvoltmeter was then balanced by adding iodine to the reaction flask, thus restoring the free iodine activity in the reaction flask to its original level. A portion of the solution from the reaction flask was placed in a 10-cm spectrophotometer cell, and the absorbance spectrum was measured between 650 and 250 nm in a Cary Model 14 recording spectrophotometer. The sample from the spectrophotometer cell was returned to the reaction flask, and another quantity (0.5 or 1.0 mL) of amylose solution was introduced. This sequence was repeated 5–10 times per determination. Data, expressed as either absorbance at λ_{max} (~615 nm) or mg iodine bound *vs.* mg amylose, was analyzed by least-square regression to determine linear correlation.

We define standard reaction conditions as follows: 20°, with reagent concentrations of 3.0×10^{-6}M I_2, 2.03×10^{-2}M KI, and 4×10^{-3}M phosphate buffer. Initial iodine content is 630 μg I_2; initial amylose addition is 200 μg. These values are achieved by starting with 1 mL of 0.0025M I_2 solution and adding 0.5 mL of amylose solution. The effects of variations in reagent concentrations were compared to this standard condition. Reagents as used in the standard condition were also used in running comparison studies of the conventional titration-procedure as described by Banks *et al.*[6].

Effects of temperature on the formation and disruption of the complex were studied by using samples prepared by either the EI-titration or the conventional titration-procedure to an amylose volume of 8 mL (3.2 mg) at 2°. A portion of the sample was placed in a jacketed spectrophotometer cell connected to a variable-temperature water bath. Temperature was raised in 5° increments and absorbance was monitored at 650 nm in the spectrophotometer. About 5–15 min were required for absorbance to stabilize at each new temperature: The spectrum from 650 to 250 nm was then recorded. This operation required an additional 20 min, so that solutions were maintained at each temperature for ~30 min. In this manner, solutions were heated sequentially to 80° and then cooled sequentially back to 2°.

RESULTS

Potentiometric EI-titration measurements of iodine binding for 10 aliquots of amylose are displayed in Fig. 1 for the standard condition (X) and for an iodine concentration of one-half the standard condition (o). The lines drawn through the points denote the least-squares fit; from the slopes of the lines, iodine-binding values are calculated. These values are calculated to be 31.6 mg of I_2 bound per 100 mg of amylose for the standard condition and 24 mg for one-half the initial iodine concentration. We note that the values shown for the half-iodine concentration do not extrapolate through the origin. These values compare with 22.6 mg amylose bound per 100 mg amylose obtained by the procedure of Banks *et al.*[6] on the same amylose stock solution.

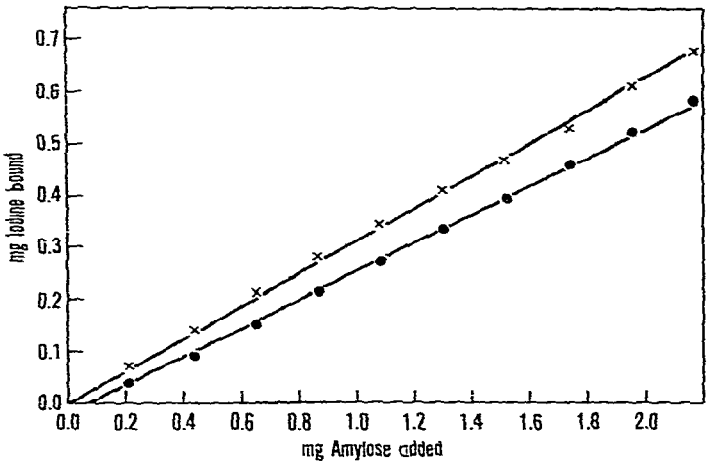

Fig. 1. Iodine binding by differential potentiometric back-titration, 20°, 2.03 × 10^{-2}M KI. x–x Standard condition, 3.02μM iodine; ●—● one-half standard condition, 1.51μM iodine.

Iodine binding throughout the range of iodine concentrations studied was measured both potentiometrically and spectrophotometrically. Values obtained, relative to the standard condition, are given in Table I. Iodine binding increased with increasing iodine concentration until a constant value was reached, within the limits of reproducibility, at iodine concentration of 1.5μM I_2 at 2° and 2.26μM I_2 at 20°. Iodine concentrations above these values are hereafter referred to as "excess iodine", whereas lower iodine concentrations are considered to cause the system to be in a state of iodine undersaturation or "deficiency". Under standard conditions or at higher iodine concentrations, an absorbance of 0.668 units per 280 mg amylose was measured

TABLE I

EFFECTS OF INITIAL IODINE CONCENTRATION AND TEMPERATURE

Temperature	*Iodine concentration (μM)*						
	6.02	*3.01*	*2.26*	*1.50*	*1.05*	*0.752*	*0.301*
A. Potentiometric EI titration: relative values of I_2 bound[a]							
2°	0.997	0.998	0.990	1.020		0.870	0.734
20°	0.982	1.000	0.981	0.798	0.418		
B. Relative absorbance values: A_{max}[a]							
2°	1.081	1.054	1.090	1.042		0.928	0.589
20°	1.036	1.000	0.964	0.839	0.236		

[a]All values given are relative to measurements made at standard conditions: KI = 2.03 × 10^{-2}M, I_2 3.01 × 10^{-6}M, 20°, and λ_{max} 615 nm.

TABLE II

EFFECT OF KI CONCENTRATION ON IODINE-BINDING CAPACITY OF AMYLOSE IN THE EI TITRATION

$[KI] \times 10^3$	*Potentiometric measurement* *mg of I_2 bound/100 mg of amylose*	*Spectrophotometric measurement*	
		λ_{max}	*Absorbance*[a]
203	26.8	570	0.260
20.3	30.2	615	0.320
2.03	31.2	630	0.362
0.53	32.4	630	0.318

[a]Absorbance of 1 mg of amylose per 830 mL of total solution, measured in a cell of 10.0-cm pathlength against phosphate-buffered KI solution in the reference cell.

in the 10-cm cell; wavelength of maximum absorbance (λ_{max}) under standard conditions was 615 nm. During the course of carrying out potentiometric EI-titrations, it was observed that absorbance at 353 and 288 nm, which are the absorbance maxima of the I_3^- ion, increased after each sequence of amylose addition and replenishment of iodine.

Variation in concentration of phosphate in the supporting electrolyte did not, within experimental error, affect the results of either the potentiometric or the spectrophotometric measurements. The effect of variation of KI concentration is shown in Table II. As seen here, potentiometric iodine-binding values do change with KI concentration; nevertheless, all values are well in excess of the 19–22% binding values obtained in the conventional potentiometric titration. Both λ_{max} and absorbance values also vary somewhat with KI concentration.

Throughout our potentiometric studies, it was observed that there was always a slightly higher binding value obtained when smaller quantities of amylose were introduced. Namely, the slope of the binding was slightly less (~5%) with 1 mL of amylose than with 0.5 mL. The scatter of points was too great, relative to the difference involved, to allow any significant calculation to be made, but the tendency generally was present.

The effect of exposure of amylose to variable concentrations of iodine, as occurs in the conventional titration-procedure, was examined by carrying out the EI-titration in two steps as follows: a potentiometric EI-titration was performed with a deficient initial concentration of iodine (1.26μM), to which 0.5-mL aliquots of amylose solution were added to a total volume of 4 mL. The slope of the line through these points indicated an iodine-binding value of 11.7 mg per 100 mg of amylose. Additional iodine was then introduced to the reference cell to give a concentration of 6.02μM. Sufficient iodine was then added to the reaction flask to null the voltmeter. The excess of iodine required to balance the cells indicated that the iodine now bound by the amylose in the reaction flask totaled 22 mg per 100 mg of amylose, equivalent to the amount that would be obtained by the conventional procedure. With the

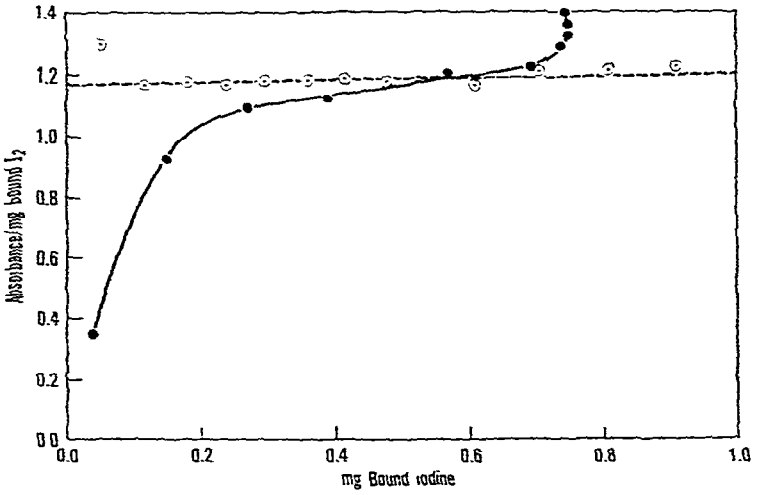

Fig. 2. Absorbance (in 10-cm cell at 615 nm) per mg of bound iodine (measured potentiometrically) plotted *vs.* mg of bound iodine. —●—●— conventional titration, —○—○— EI titration.

system now in a condition of excess iodine, additional 0.5-mL volumes of amylose solution were introduced as usual in the EI-titration procedure to a total volume of 8 mL of amylose solution. The second 4 mL of amylose bound iodine at the normal rate for the EI-titration procedure (30 mg/100 mg amylose).

Although potentiometric measurements indicate that iodine binding by the EI titration is 50% higher than by the conventional method, spectrophotometric data reveal that, for equivalent amounts of amylose, absorbance at λ_{max} is essentially the same for both methods. If the relationship between absorbance and potentiometrically measured binding is linear, as reported by Banks *et al.*[6,7], the increased binding should be reflected in higher absorbance values. Examination of data throughout the course of each titration reveals, however, that whereas a linear relationship does exist in the EI titration, the conventional titration deviates from linearity when a condition of excess iodine is reached. There is an increase of ~8% in the absorbance after no more binding can be measured potentiometrically. Comparison between absorbance values and potentiometric-binding values for both systems is given in Fig. 2. The ratio of absorbance to bound iodine is plotted *vs.* bound iodine to emphasize deviation from linearity. A linear relationship in this plot would be represented by a straight line of zero slope. This relationship is found for the EI titration, but is true only for the middle regions of the conventional titration.

Effects of temperature variation on the amylose–iodine complex prepared at constant iodine excess are illustrated in Fig. 3. These experiments were conducted under the four conditions listed in Table III, and hysteresis was observed in all cases. As samples were heated, the wavelength of maximum absorbance decreased from ~620 to ~600 nm before complete disappearance of color. Upon cooling and reformation of the colored complex, the first measurable absorbance appeared at

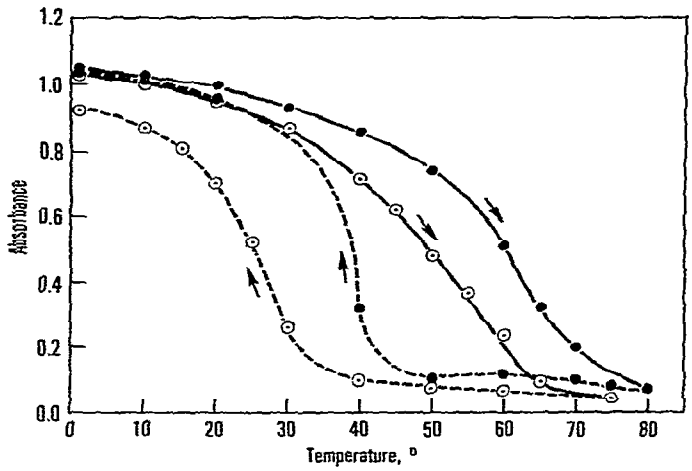

Fig. 3. Change in absorbance of amylose–iodine complex with temperature: λ_{max} 590–615 nm; ●—● 6.02μM iodine; and ⊙—⊙ 1.51μM iodine.

TABLE III

VARIATION OF THE DISRUPTION–REFORMATION TEMPERATURE OF AMYLOSE–IODINE COMPLEX WITH CHANGING IODINE AND KI CONCENTRATION

KI (mM)	I_x (μM)	*Temperature of maximum inflection-absorbance at* A_{max}		
		Heating	*Cooling*	*Differential*
20.3	1.50	50	26	24
20.3	3.01	56	33	23
20.3	6.02	60	39	21
2.03	6.02	50	22	28

~580 nm, and then stabilized at 590 nm. The amount of hysteresis between heating and cooling curves, measured as the difference in temperature between the maximum slopes of the curves, remained fairly constant, although the temperature required for elimination of color increased with increasing concentration of iodine. Some degree of hysteresis was observed also when samples were heated only to 50° and then cooled. The hysteresis corroborates results reported by Banks and Greenwood[1] and by Moulik and Gupta[4].

We investigated whether amylose–iodine complexes prepared under conditions of constant excess iodine might exhibit different thermal behavior than those prepared by conventional titration. Thus, two amylose–iodine systems were prepared with identical total contents, one by the EI-titration method and one by the titration method of Banks *et al.*[6] Each system was formed at 0–2° and heated in 10° increments from 0–50° as previously described, and absorbance was measured at each tempera-

ture interval. The decrease in absorbance was, within experimental error, the same for both systems.

DISCUSSION

Our experimental results may be summarized as follows: (*a*) potentiometric measurements show that ~30 mg of iodine is bound per 100 mg of amylose when amylose is constantly exposed to a sufficient excess of I_2 in KI; (*b*) when amylose first is exposed slowly to iodine under conditions of iodine deficiency and then iodine is added rapidly to a sufficient final excess, potentiometric measurements indicate the iodine-binding value to be the same as if the iodine were slowly added, namely, 19–22 mg of iodine per 100 mg of amylose [when additional amylose is added to the foregoing system (*b*) at sufficient iodine excess, potentiometric measurements indicate the additional amylose binds ~30 mg iodine per mg amylose]; (*c*) within the region of KI and phosphate-buffer concentration examined herein, the absorbance intensity of the blue complex at final excess iodine concentration is independent of the rate at which amylose is exposed to I_2. However, absorbance of the I_3^- peak at 353 nm is greater when amylose is slowly exposed to the iodine; (*d*) at identical final conditions of excess iodine, the decrease in absorbance in the 600-nm range upon heating from 0 to 50° is the same for complexes prepared either under conditions of excess or deficient iodine. The temperature required to disrupt the amylose–iodine complex increases with increased iodine concentration. Provided that final solution conditions are identical, the behavior of blue-color intensity of the amylose–iodine complex upon heating and cooling appears to be independent of the rate at which amylose was exposed to iodine.

The mass ratio of iodine to amylose in the complex prepared by EI titration as described in (*a*) is one I_2 per five glucose residues, and in complexes prepared by conventional titration is one I_2 per seven glucose residues. If these ratios are compared on a constant amylose basis, the masses of the complexes vary by only 7%. Thus, if the final blue color intensity of the complex is controlled by the mass ratio of amylose to bound iodine, absorbance values at 615 nm would be expected to vary by 7%. Failure to observe this variation is attributed to the previously mentioned observation that absorbance of the conventionally prepared complex increases by ~8% after no further binding can be measured potentiometrically (see Fig. 2).

The following calculations use the potentiometric and spectrophotometric data from these experiments to enable us to further consider the nature and stoichiometry of the amylose–iodine complex. In these calculations, we assume that extinction coefficients reported in the literature[5,10–14] for I_3^- and for the amylose–iodine complex are valid for our experimental conditions, and that the contribution of the amylose–iodine complex absorbance at 353 nm is proportional to the absorbance at λ_{max} under all conditions used. The following extinction coefficients are used:

E_{353,I_3^-} = 26,400 (molar extinction coefficient of I_3^- at 353 nm),

TABLE IV

FREE I_3^- ABSORBANCE CALCULATIONS

KI (mM)	*Amylose (μg)*	A_{max}	A_{353}		
			Total (measured)	*Complex (0.27 × A_{max})*	*I_3^- free*
A. Calculation of free I_3^- absorbance during back titration					
20.3	0	0	0.628	0	0.628
20.3	434	0.157	0.674	0.042	0.632
20.3	867	0.307	0.705	0.083	0.622
20.3	1300	0.473	0.762	0.128	0.634
20.3	1730	0.608	0.795	0.164	0.631
20.3	2170	0.752	0.812	0.203	0.609
20.3	2600	0.908	0.881	0.245	0.630
2.03	0	0	0.400	0	0.400
2.03	406	0.139	0.433	0.037	0.395
2.03	813	0.274	0.450	0.074	0.375
2.03	1220	0.383	0.482	0.104	0.378
2.03	1630	0.496	0.530	0.134	0.402
2.03	2030	0.686	0.579	0.185	0.395
2.03	2440	0.840	0.621	0.227	0.394
0.532	0	0	0.160	0	0.160
0.532	405	0.124	0.203	0.034	0.169
0.532	810	0.258	0.238	0.069	0.168
0.532	1210	0.396	0.286	0.107	0.179
0.532	1620	0.522	0.312	0.141	0.171
0.532	2030	0.651	0.360	0.176	0.184
0.532	2430	0.777	0.383	0.210	0.173
B. Calculation of free I_3^- absorbance at final conditions in conventional titration, compared to EI titration					
2.03[a]	3250	1.030	1.030	0.278	0.752
2.03[b]	3250	1.044	0.900	0.302	0.598

[a]Conventional potentiometric titration. [b]Potentiometric EI titration.

$E_{353,AI}$ = 10,000 (molar extinction coefficient of amylose–iodine complex at 353 nm),

$E_{max,AI}$ = 37,000 (molar extinction coefficient of amylose–iodine complex at λ_{max}, 590–630 nm).

The ratio $E_{353,AI}/E_{max,AI}$ is 0.27. This ratio is used to calculate the contribution of the amylose–iodine complex to absorbance at 353 nm which is then subtracted from total absorbance at 353 nm, leaving the absorbance at 353 nm due to I_3^-. Absorbance of I_2 at 353 is negligible. Thus:

$$A_{353} - 0.27 \times A_{max} = A_{353,I_3^-} \qquad (1),$$

and from A_{353,I_3^-} and E_{353,I_3^-}, the concentration of free I_3^- in solution may be calculated at any stage of the EI titration.

Concentration of free I_3^- for EI titrations conducted at various KI concentrations are given in Table IV. We note that I_3^- concentrations vary with KI concentration, but remain constant throughout the course of the titration at any given concentration of KI. Free I_3^- concentrations in EI titration and conventional titration at final conditions are compared in part B of Table IV, where total concentrations of amylose and iodine added are equivalent. We note that the free I_3^- concentration in the conventional titration is higher.

To determine the concentration of bound I_3^- and I_2 in these titrations, the following relationships are used:

$$[I_3^-]_B = [I_3^-]_T - [I_3^-]_F \quad (2),$$

and

$$[I_2]_B = [I_x]_B - [I_3^-]_B \quad (3)$$

where $[I_x]$, $[I_3^-]$, and $[I_2^-]$ are concentrations of the unknown species x, involved in the binding reaction, and of I_3^- and I_2, respectively. Subscripts T, B, and F indicate total, bound, and free, (namely, not bound to amylose) in solution, respectively. From the extinction coefficient of I_3^- at 353 nm and the known amount of iodine added to the solution, $[I_3^-]_T$ is determined. From spectrophotometric measurements, $[I_3^-]_F$ is known as shown in Table IV, and $[I_x]_B$ is the molar concentration of bound iodine measured potentiometrically.

If the validity of equation (*3*) is assumed, the proportions of bound I_2 and I_3^- are then known, and the ratio of the number of iodine atoms per charge, N, may be calculated. From this value and potentiometric measurement of the mass of iodine bound per unit mass of amylose, one may also calculate a ratio of glucose residues per bound iodine atom, Q. These values plus the amounts of I_2 and I_3^- bound per glucose residue are given in Table V for experiments conducted with various concentrations of KI. We note in part A of Table V that for the EI titration, the charge of

TABLE V

RELATIVE PROPORTIONS OF BOUND I_2 AND I_3^- IN THE AMYLOSE–IODINE COMPLEX

[*KI*] (mM)	$[I_3^-]_B$/[*Glucose*]	$[I_2]_B$/[*Glucose*]	*N*[a]	*Q*[b]
A. EI titration				
203	0.148	0.024	3.30	2.04
20.3	0.138	0.048	3.72	1.90
2.03	0.108	0.097	4.72	1.98
0.53	0.038	0.169	11.84	2.21
B. Conventional titration				
20.3	0.118	0.029	3.48	2.44

[a]Number of iodine atoms per charge in bound species. [b]Ratio of D-glucose molecules per bound iodine atom.

the bound species decreases as KI decreases and that the ratio of glucose residues per iodine atom is constant, except at the very lowest concentration of KI.

Values of N in Table V are similar to those reported in recent literature. Cronan and Schneider[13] estimated the binding species to vary from I_3^- at a KI concentration of 10^{-3}M to I_2 at 10^{-5}M KI. Cesaro *et al.*[14] calculated R values, ratio of bound I_3^- to total bound iodine, between 0.5 and 0.1, which corresponds to a range of 5–18 iodine atoms per charge. Their average R value, 0.32, predicts 7.25 iodine atoms per charge at KI concentrations of 10^{-4} to 10^{-5}M. Teitelbaum *et al.*[15] used Raman and Mossbauer spectroscopy to examine precipitates of amylose–iodine complexes and concluded that I_5^- was the species involved.

The shift in the wavelength of maximum absorbance of the complex from 630–570 nm as KI concentration increases (Table II), in view of our calculated values for the composition of the binding species, may be of some significance. The circular-dichroism studies of Handa and Yajima[2] have shown that the primary absorbance of the complex may be resolved into two overlapping peaks with maxima at 570 and 630 nm. As these maxima coincide with the shifts we observe with increasing KI concentration, it may be that they correspond to the absorbance of two different forms of bound iodine, namely, predominantly I_3^- at 570 nm and predominantly I_2 at 630 nm.

From comparison of N values in parts A and B of Table V, and from our observations of essentially identical thermal behavior of complexes prepared by conventional and by EI titration, we infer that the I_x^- species bound within the helix at our standard conditions is probably the same for both methods of preparation. Until additional data are available, we limit this inference to regions of standard reactant concentrations. An implied corollary to this concept is that differences in iodine binding between the methods reflect steric and/or packing effects related to chain-segment behavior. These effects appear to be largely irreversible, providing the system is not heated sufficiently to permit major loss of helical character. Once the amylose–iodine complex is formed under conditions of iodine deficiency and is maintained at constant temperature, addition of an excess of iodine does not increase binding beyond the usual 19–22% level. The rate at which amylose is exposed to iodine is the crucial distinction between the EI titration and conventional titration. The importance of mixing-rate effects on spectral properties of the complex has recently been noted by other researchers[3,14,16].

The location of the "extra" iodine in the complex formed by EI titration is a matter of speculation. The maximum helix-filled binding capacity of amylose in solid state has been determined by X-ray analysis to be 26%, which is equivalent to one I_2 molecule per six glucose residues[17]. To accommodate more iodine within the helical cavity would require that the helix in solution be expanded. It is known that behavior of the amylose–iodine complex in aqueous solution is dependent upon such factors as ionic strength, reagent concentration, temperature, molecular weight of amylose, and rate of mixing. The wide range of experimental conditions employed

TABLE VI

COMPARISON OF REAGENT CONCENTRATIONS IN AMYLOSE–IODINE COMPLEX SYSTEMS

Investigator	*Solution concentrations*		
	[*KI*] (μM)	[I_2] (μM)	[*Amylose*] *mg/L*
This work			
Range	532–203,000	0.301–6.02	0.48–4.8
Standard	20,300	3.01	
Banks *et al.*[5]	10,000	6.02	0–10
Cesaro *et al.*[14]	10,000	1,000	50–100
Cronan and Schneider[13]	10–25,000	500	45
Handa and Yajima[3]	12,000	16–95	50
Pfannemüller[16]	630	412	400

by various investigators limits both direct comparison and interpretation of results. Experimental conditions used by recent workers are compared in Table VI.

Kuge and Ono[10] reported differences in enthalpy changes in potentiometric and spectrophotometric measurements, and ascribed the difference to surface adsorption of iodine, which caused errors in the photometry. Handa and Yajima[3] concluded that blue-color development must involve some action of iodine outside the helical cavity. Our data in Fig. 2 show an increase in absorbance without a corresponding increase in binding for the conventional titration, under conditions equivalent to our standard condition. It had been shown previously that aggregation of amylose–iodine complexes occurs in solutions at this concentration of amylose[18]. Thus, if one accepts the concept that significant amounts of iodine may be associated with the complex outside the helical cavity, distinctions between inter- and intrachain associations need to be considered. The evidence in this study proves that much remains to be understood about the nature of amylose–iodine systems in solution.

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