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The reaction of starch with iodine vapor. Determination of iodide-ion content of starch–iodine complexes

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Abstract

The molecular-iodine and iodide-ion contents of starch–iodine complexes, prepared by subjecting corn amylose (average degree of polymerization, $\overline{dp}$ 1050), low-molecular-weight amylose ($\overline{dp}$ 61–69), amylose–cyclohexanol complexes, and native corn starches to iodine vapor for 30 days under different conditions of relative humidity (RH), were determined by a differential method requiring titrations with both KIO_3 and $Na_2S_2O_3$. Iodide content generally increased with increase in relative humidity. However, the analytical method was incapable of providing reliable iodide values for complexes of very low total iodine content (<5 wt%), such as those of ordinary corn starch, waxy maize starch, and low-$\overline{dp}$ amylose. A granular hybrid corn starch of 64% amylose content yielded, at 100% RH, a complex containing 10.0 wt% of total iodine ($I/I^- = 3.7$). Low-$\overline{dp}$ amylose exhibited low reactivity toward iodine vapor, even at 100% RH; however, a cyclohexanol complex of low-$\overline{dp}$ amylose reacted readily at 100% RH, with concurrent elimination of cyclohexanol, to produce a starch–iodine complex of unusually high iodine content (33.1 wt% of total iodine; $I/I^- = 3.65$). The behavior of corn amylose toward iodine was found to be dependent upon its method of preparation from corn starch. One method of preparation yielded an amylose that, under anhydrous conditions, was very resistant to complexation with iodine, but which was very reactive at 30–100% RH, producing a complex that contained as much as 18.5 wt% of total iodine ($I/I^- = 3.49$). A different procedure for preparing amylose gave a starch that reacted readily under anhydrous conditions to produce a complex containing 21.8 wt% of molecular iodine and no detectable amount of iodide ion. A cyclohexanol complex of corn amylose reacted with iodine vapor at 100% RH, with concurrent elimination of cyclohexanol, to form a starch–iodine complex having a total iodine content of 31.6 wt% ($I/I^- = 3.91$). For most of the iodine complexes, there was a close similarity between I^- content and H^+ content, suggesting that the source of iodide ion was hydrolysis of molecular iodine by water of hydration in the starch.

Keywords: Starch; Amylose; Iodine; Iodide ion; Starch–iodine complex; Amylose–cyclohexanol complex; Glass transition

1. Introduction

Reactions of iodine with starch to form starch–iodine complexes have been studied extensively by many investigators and are used widely for determining the amylose content of starches by both potentiometric and spectrophotometric methods. The subject has been reviewed by Banks and Greenwood (1975) and given additional study by numerous workers. That the starch–iodine complex consists of a linear array of iodine atoms occupying the cavity of a helical polysaccharide molecule was first suggested by Hanes (1937) and later confirmed by X-ray diffraction analysis by Rundle and coworkers (1943). It was shown that this ordered structure exists not only in the crystalline state but also in solution.

Despite the large body of research, there continues to exist considerable uncertainty regarding the mechanism and stoichiometry of complex formation. Most of the literature has dealt with reactions between starch and iodine in aqueous solution where the presence of iodide ion was assumed by most workers to be essential in initiating complexation between amylose and iodine (Thoma & French, 1960). Although the essentiality of iodide ion in initiating the reaction has been questioned recently by Calabrese and Khan (1999), there is ample evidence that, in aqueous solution, the concentration of iodide ion is an important factor determining the extent to which iodine is bound by starch and that a significant fraction of bound iodine is iodide ion.

Mukherjee and Bhattacharyya (1946) employed NaCl to salt-out iodine complexes of potato amylose from aqueous solutions differing in KI concentration. They found that

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when the KI concentration was varied from 0.046 to 1.21 M, the ratio of I^-/I_2 rose from 0.141 to 0.470. The low value was compatible with a binding species composed of 15 iodine atoms per negative charge. The higher value approached closely the proportion required for the triiodide ion, I_3^-. Teitelbaum, Ruby, and Marks (1980) examined solid iodine complexes of potato amylose (prepared in aqueous media as well as by reaction of amylose with iodine vapor) by Raman and Mössbauer spectroscopy and concluded that I_5^- was the major chromophore in the complexes. Potentiometric measurements by Knutson, Cluskey, and Dintzis (1982) have shown that, when 100 mg of corn amylose is treated with a large excess of I_2 in KI solution, approximately 30 mg of total iodine (molecular iodine and iodide ion combined) is bound by the starch substrate. Their studies indicated that, at 0.2 M KI, the ratio of iodine atoms per negative charge was 3.3, a value that is very close to the ratio of 3.0 for the binding species I_3^-. At 5×10^{-4} M KI, the ratio was 11.84, suggesting the species I_{12}^-. Cesàro, Jerian, and Saule (1980) obtained a ratio of about 7 at KI concentrations of 10^{-4}–10^{-5} M, but did not report the plant source of their amylose.

Rundle and French (1943a,b) reported that an amylose–iodine complex can be prepared under anhydrous conditions from interaction of corn amylose with iodine vapor, provided the amylose is initially in the V-form. The amylose sorbed 26% of its own weight in iodine, which is equivalent to 21 wt% of iodine and indicates the presence of one molecule (i.e. two atoms) of iodine per 6 glucose residues (anhydroglucose units; AGUs) in each helical turn of the amylose chain. According to Murdoch (1992), such an arrangement would result in a complex in which the iodine molecules do not completely fill the space in the helical cavity. Basing his calculations on the length (6.97 Å) of an uncharged molecule of I_2 (i.e. the sum of two covalent radii and two van der Waals radii) and on the assumption that the entire amylose molecule was helical and capable of accommodating the maximum number of I_2 molecules, he concluded that the maximum iodine content should be 30 wt%. This value was close to the value of 28.8 wt% that he found in his own studies with potato amylose and which was based solely on weight increase. The average interatomic spacing for iodine atoms in this model of an amylose–iodine complex was 3.49 Å. However, early X-ray studies by West (1947) had indicated a considerably shorter interatomic spacing of 3.10 Å. Using this shorter spacing in a recalculation, Murdoch obtained a theoretical value of 33.7 wt% for maximum iodine content.

No investigators are known to have published any calculated values for maximum iodine content based upon the length of the linear triiodide ion, I_3^-. It is conceivable that such an ion is the predominant binding species in amylose–iodine complexes formed in aqueous media containing high concentrations of iodide ion. The sum of the lengths of the two covalent bonds in I_3^- is 5.92 Å (Kleinberg, Argersinger, & Griswold, 1960). If it is assumed that the van der Waals radius for each of the two terminal atoms in I_3^- is 2.15 Å (Moeller, 1963), then the length of the triiodide ion would be 10.22 Å; and the average interatomic spacing would be 3.41 Å. If an additional assumption is made that the negative charges on the linear chain of iodine atoms have no important effect on available space within the helical cavity of amylose, then a calculated value for maximum iodine content would be about 30.7 wt%, a value that is not greatly different from values calculated on the basis of a complex containing only uncharged iodine atoms.

Published data on amylose–iodine interactions have shown unequivocally that iodide ions can be, depending upon the experimental conditions, important constituents of the chain of iodine atoms in amylose–iodine complexes. In all instances where samples of amylose were exposed to iodine vapor, the reactions were conducted under strictly anhydrous conditions. Extent of iodine binding was determined only by measuring the increase in weight of the amylose substrates; and no attempts were made to determine the iodide-ion content of the products. The present paper describes studies designed to provide information on the contribution of factors that influence not only the extent to which starches complex with iodine vapor but also the ratio of molecular iodine to iodide ion (I/I^-) in starch–iodine complexes. Among the variables investigated were degree of polymerization of amylose, use of amylose–alcohol complexes as substrates, and relative humidity. All of the low-$\overline{dp}$ amylose used in these investigations were prepared synthetically from α-cyclodextrin.

2. Experimental

2.1. Materials

Cyclodextrin glucanotransferase (CGTase; EC 2.4.1.19) from *Bacillus macerans* was obtained as an aqueous solution (600 units/ml, according to the method of Tilden and Hudson (1942) from Amano International Enzyme Co. (Troy, VA, USA). Corn amylopectin (11.5% H_2O) was from Sigma Chemical Co. (St. Louis, MO, USA); waxy maize starch (10.7% H_2O) and ordinary corn starch (10.1% H_2O) were from Cerestar USA (Hammond, IN, USA); Hylon VII (64% amylose; 11.1% H_2O), a high-amylose hybrid corn starch, was from National Starch and Chemical Corp. (Bridgewater, NJ, USA); and α-cyclodextrin (α-CD; 8.8% H_2O) was from Anspec/Ohio (Columbus, OH, USA). Corn amylose (100%, $\overline{dp} \sim 1050$; 6.3% H_2O) was prepared at this laboratory from its 1-butanol complex by means of the Schoch method (Lansky, Kooi, & Schoch, 1949; Schoch, 1942). Absence of 1-butanol in the isolated amylose was ascertained by NMR analysis. Low-molecular-weight

amyloses of $\overline{dp}$ 56 (12.1% H_2O), $\overline{dp}$ 69 (14.3% H_2O), and $\overline{dp}$ 72 (9.1% H_2O) were prepared by the action of CGTase on α-cyclodextrin in aqueous solution at 25, 50, and 70 °C, respectively, according to procedures reported by Rendleman and Knutson (1998). Amylose of $\overline{dp}$ 61 (11.6% H_2O) was prepared in 54% yield by subjecting α-cyclodextrin (22.4 g, anhydrous basis) to the action of CGTase (300 units) at 2 °C in aqueous solution (215 ml) at pH 7 for 46 days. Streptomycin sulfate (7 mg) was added to prevent microbial growth. At completion of reaction, the mixture was heated for 1 h at 100 °C to inactivate the enzyme. After standing overnight at room temperature (24 °C), the mixture was filtered and the amylose washed thoroughly with water, dried in open air, and finally equilibrated at 31% RH. A similar yield could be obtained in only 3 days when a much larger amount of CGTase (1800 units) was employed.

The amylose was evaluated for its $\overline{dp}$ by a modified version (Rendleman & Knutson, 1998) of the spectrophotometric method of Knutson (1986). All starches were stored in a chamber maintained at a constant 31% RH (24 °C). Moisture contents of starches were determined by heating samples at 120 °C under vacuum for 2 h.

Dextran standards for gel-filtration chromatography were from Fluka (Milwaukee, WI, USA); 1-Butanol (99.7%), cyclohexanol (99%), methyl sulfoxide (DMSO; 99 + %), chloroform (99.9%), carbon tetrachloride (99.9%), methanol (HPLC grade), and sodium thiosulfate pentahydrate (99.5%) were from Aldrich Chemical Co. (Milwaukee, WI, USA); potassium iodate (ACS grade) and streptomycin sulfate were from Sigma; and crystalline iodine (99.9%) was from J.T. Baker Chemical Co. (Phillipsburg, NJ, USA). Water was distilled and deionized. Columns for gel-filtration HPLC chromatography were supplied by Supelco (Bellefonte, PA, USA).

2.2. Analytical methods

The weight-average molecular weight of native corn amylose (170,000) was determined by gel-filtration chromatography with a combination of two TSK-GEL G5000 PW_{XL} HPLC columns (7.8 mm × 30 cm) in series. Elution was with 0.1 M $NaNO_3$ at 50 °C and at a flow rate of 0.5 ml/min. The injection-loop volume was 50 μl and each reference solution contained 1 mg of dextran standard and 0.5 mg of NaN_3 (as preservative) per ml of aqueous 0.1 M $NaNO_3$. The general method for preparing an amylose solution for injection was as follows: amylose (14.0 mg; hydrate form) and DMSO (0.5 ml) were heated together at 100 °C in a screw-cap culture tube until solution was complete; 0.1 M $NaNO_3$ (0.5 ml) was added and the resulting solution filtered by syringe through a Millex HV filter unit of 0.45-μm pore size (Millipore Corp., Bedford, MA, USA) before injection. The filtered solution was stable for at least two days at 25 °C before onset of precipitation.

X-ray powder diffraction patterns were obtained by means of a Philips PW1830 X-ray diffractomer (Philips Electronic Instruments Co., Mahwah, NJ, USA). Data were taken from 4 to 30° 2Θ in increments of 0.05, 2Θ/step at 4 s/step. Elemental analysis for sulfur was by means of emission spectroscopy. Analysis for possible presence of butanol in samples of amylose was by NMR. Differential scanning spectroscopic (DSC) analyses were performed on a TA Instruments Model 2920 by ramping at 10 °C/min from −25 to 150 °C. DSC data was analyzed for peaks and glass transitions by means of the Universal Analysis software provided by the same company.

A combination of two titrimetric analyses was employed to determine molecular iodine (I) and iodide ion (I^-) in starch–iodine complexes: (1) titration with standard sodium thiosulfate to obtain molecular-iodine content and (2) titration with standard potassium iodate in ice-cold concentrated HCl solution to obtain information that, when combined with knowledge of molecular-iodine content, permitted a calculation of iodide-ion content. The final product of the reaction of KIO_3 with either molecular iodine or iodide ion is iodine chloride, as shown in the following reaction scheme:

$$KIO_3 + 5KI + 6HCl = 3I_2 + 6KCl + 3H_2O$$

$$2I_2 + KIO_3 + 6HCl = KCl + 5ICl + 3H_2O$$

The titration end-point was indicated by the disappearance of the pink color imparted by molecular iodine to a chloroform layer in the mixture being titrated. Use of these reactions to determine iodide ion quantitatively was reported first by Andrews (1903) and then later by Conant and Hussey (1924) who recognized the value of the reactions in determining iodide ion in organic solvents. However, there have been no reports of these reactions being used to determine iodide ion in mixtures of iodide ion and molecular iodine.

The equivalent weight of KIO_3 for converting one equivalent weight of iodide ion (i.e. I^-/1 or 126.91) to ICl is MW/2 or 107.0. The equivalent weight of KIO_3 for converting one equivalent weight of molecular iodine (i.e. I_2/2 or 126.91) to ICl is MW/1 or 214.0. The reliability of the KIO_3 titration for determining combinations of I and I^- was tested by introducing an artificial mixture of I (10.54 mg; 0.08305 meq) and KI (1.00 ml of 0.01602 M KI; 0.01602 meq) into 40 ml of ice-cold concentrated HCl and titrating with standard KIO_3 (0.00796N with respect to I; 0.00398N with respect to I^-). The theoretical volumes required for these amounts of I and I^- were 10.43 and 4.03 ml, respectively. The sum of these two volumes (14.46 ml) was in satisfactory agreement with the experimentally determined volume of 14.54 ml.

2.3. Reaction procedure

Samples of starch (generally about 0.3 g) were accurately weighed out into weighed glass vials that were then placed for 30 days or longer in glass desiccators containing iodine

crystals. Three types of such reaction chambers were employed. One type contained a bed of Drierite desiccant to create an anhydrous atmosphere (RH 0%). Another type contained water to provide a water-saturated atmosphere (RH 100%). A third type had no special humidity control; its relative humidity was maintained close to that of the laboratory (about 30%) by occasionally removing the lid of the desiccator for brief periods of time. All reactions were conducted at 24 °C. Because water of hydration in starch–iodine complexes formed at 100% RH is much larger than that in complexes formed at 30% RH, excessive water content of complexes formed at 100% RH was reduced to a lower, more stable level by placing vials containing the complex into a 30% RH iodine chamber for 5 days. Knowledge of both the initial weight of starch substrate and the final weight of iodine complex was necessary in order to calculate the starch content of a complex.

The following description of a typical analysis will illustrate the general procedure for determining molecular iodine, iodide ion, and hydrogen ion in a starch–iodine complex prepared from corn amylose at 100% RH. The complex was produced by exposing amylose hydrate (0.5000; 0.4685 g, anhydrous) to iodine vapor at 100% RH for 30 days. After the weight of the product (0.7227 g) was reduced to 0.6329 g in a 30% RH iodine chamber, the vial containing the product was tightly capped to prevent any further weight change. Portions of this material were subsequently taken for analysis.

Titration with $Na_2S_2O_3$. To a sample of amylose–iodine complex (0.1351 g; anhydrous amylose content, 0.1000 g) in a 250-ml Erlenmeyer flask (equipped with ground-glass stopper) was added 40 ml of water, 1 ml of glacial acetic acid, 5 ml of $CC1_4$, and 0.2 g of NaI. Standard 0.00767N $Na_2S_2O_3$ (eq. wt. = mol. wt.) was then added dropwise with rotary agitation until the last trace of purple or lavender color was gone. Volume of $Na_2S_2O_3$ was 18.17 ml, which is equivalent to 0.1392 meq or 0.01767 g of I. Error in determining the volume of titrant required for complete reaction was no greater than 0.10 ml (equivalent to 0.0008 meq of I). Patience was required in conducting these titrations, because of the slowness with which thiosulfate reacted with molecular iodine tightly bound within its amylose host. Many hours were generally required. Large particles of complex were frequently broken up by mashing with a glass rod in order to facilitate contact of thiosulfate with the imbedded iodine. With some complexes, the observed purplish color in the mixture being titrated was caused by numerous, very fine, microscopic, purplish particles adhering to the surface of CCl_4 globules.

Titration with KIO_3. A measured amount of iodine complex identical in weight to that used for the thiosulfate titration (0.1351 g) was placed in a 250-ml Erlenmeyer flask. Concentrated HCl (40 ml) was added and the resulting mixture swirled manually at room temperature for 5 min. During this time most, if not all, of the amylose dissolved, releasing molecular iodine and iodide ion. The mixture was then cooled to approximately 0 °C by placing the flask in an ice-water bath and manually agitating the contents by means of a rapid rotary movement. Chloroform (5 ml) was added and the mixture titrated with standard KIO_3 (0.00792N with respect to I and 0.00396N with respect to I^-) to an end-point indicated by the disappearance of pink color in the $CHCl_3$ layer. Throughout the titration, the flask was frequently returned to the ice-water bath in order to keep the temperature of the contents close to 0 °C; and each addition of titrant was followed by vigorous, prolonged agitation. Error in determining the required volume of KIO_3 was no greater than 0.05 ml. Volume of KIO_3 required for combined I and I^- was 27.65 ml.

Determination of hydrogen-ion content. In a closed screw-cap vial a sample of amylose–iodine complex (0.09442 g) was stirred rapidly and magnetically in 15.00 ml of 0.00766N $Na_2S_2O_3$ at room temperature (24 °C). The mixture became colorless in approximately 1 h, at which time the pH was measured and found to be 2.748, which corresponded to a hydrogen-ion concentration of 0.00179 M (equivalent to 0.0269 mmol of H^+ in 15 ml of solution. A sample of iodine complex weighing 0.1351 g (and containing 100 mg of anhydrous amylose) would, therefore, have generated 0.0385 mmol of H^+. Some of the iodine complexes prepared in these studies required considerably less time for complete reaction with thiosulfate, In general, complexes with a high iodine content required the most time. Many complexes, particularly those of low iodine content, required only 10 min. For reasons not presently understood, pH measurements on thiosulfate solutions of iodine complexes of very low or no iodide-ion content required the presence of added iodide ion (as NaI). Without the added iodide, pH readings were too low and gave erroneously high values for H^+ content.

Calculations. Volume of KIO_3 reacting with

$$\mathrm{I} = \frac{0.1392 \text{ meq I}}{0.00792\text{N } KIO_3} = 17.58 \text{ ml}$$

Volume of KIO_3 reacting with

$$\mathrm{I}^- = 27.65 \text{ ml} - 17.58 \text{ ml} = 10.07 \text{ ml} = 0.0399 \text{ meq } \mathrm{I}^-$$

$$= 0.00506 \text{ g}$$

Total iodine

$$(\mathrm{I} + \mathrm{I}^-) = 0.01767 \text{ g} + 0.00506 \text{ g} = 0.02273 \text{ g}$$

$$= 0.1791 \text{ meq}$$

Wt% of total iodine in anhydrous complex

$$= \frac{0.02273 \text{ g}}{0.12273 \text{ g}} \times 100 = 18.5\%$$

$$\frac{\mathrm{I}}{\mathrm{I}^-} = \frac{0.1392 \text{ meq}}{0.0399 \text{ meq}} = 3.49$$

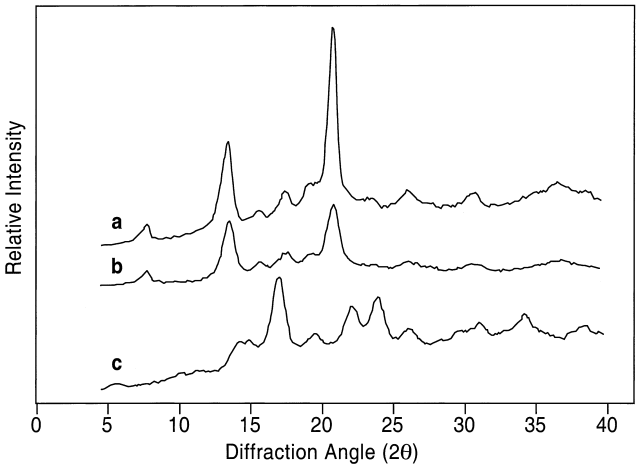

Fig. 1. X-ray diffraction patterns of amylose. Pattern **a** is of corn amylose ($\overline{dp}$ 1050) isolated from corn starch by the method of Schoch (1942). Pattern **b** is of corn amylose reprecipitated from DMSO–water–butanol and freed of solvent according to the method of Murdoch (1992). Pattern **c** is of granular, low-molecular-weight amylose ($\overline{dp}$ 69) produced by an enzymatic conversion of α-CD.

$$\frac{\text{mmol of glucose residues}}{\text{meq of total iodine}} = \frac{0.617}{0.1791} = 3.45$$

2.4. Preparation of amylose–cyclohexanol complexes

Amylose–cyclohexanol complexes were prepared by heating aqueous mixtures of cyclohexanol and either low-$\overline{dp}$ or high-$\overline{dp}$ amylose in sealed tubes at a temperature high enough to effect either total or nearly total dissolution of the starch. Into a heavy-wall glass pressure tube equipped with a Teflon screw cap were placed amylose (0.5 g; hydrate form), water (5 ml), and cyclohexanol (0.3 ml). The tube was capped and then heated in an oven at 145–150 °C for approximately 0.5 h with occasional agitation on a Vortex mixer. Where low-$\overline{dp}$ amylose was used, there was complete dissolution. However, with high-$\overline{dp}$ corn amylose, dissolution was not complete. The hot contents were allowed to cool very slowly to room temperature over a period of several hours and remain thus for 24 h. The mixture containing high-$\overline{dp}$ amylose was filtered with suction through a sintered-glass filter of medium porosity; the product was dried over Drierite under partial vacuum (50 mm Hg) for 3 days; yield, 0.57 g. The reaction mixture containing low-$\overline{dp}$ amylose was pastey and could not be filtered. Excess water and cyclohexanol were eliminated by placing the mixture in a desiccator over Drierite for several days at ambient temperature and atmospheric pressure; yield, 0.48 g. X-ray diffraction patterns for cyclohexanol complexes of corn amylose and low-$\overline{dp}$ amylose were very similar and are shown in Fig. 1.

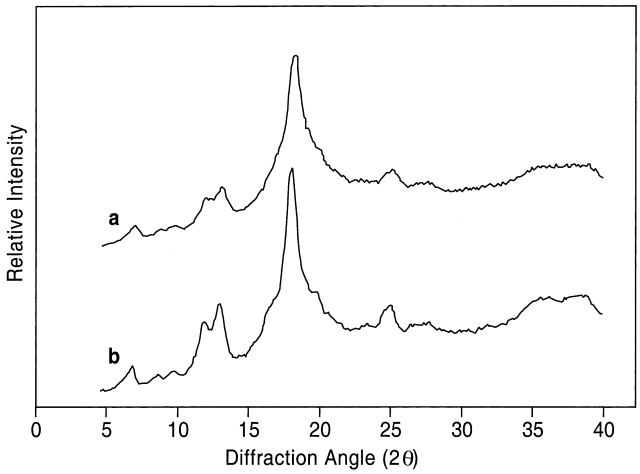

Fig. 2. X-ray diffraction patterns of cyclohexanol complexes of (a) corn amylose of $\overline{dp}$ 1050 and (b) low-molecular-weight amylose of $\overline{dp}$ 69.

3. Results and discussion

3.1. Reactions of amylose

Foremost among factors found to influence reactivity of amylose toward iodine vapor was starch macrostructure. Its influence was evident from the observed difference in behavior of iodine toward low-$\overline{dp}$ amylose and high-$\overline{dp}$ amylose, and also from differences in the behavior of iodine toward high-$\overline{dp}$ amyloses prepared from corn starch by different methodologies. The two low-molecular-weight amyloses ($\overline{dp}$ 61 and $\overline{dp}$ 69) used in these studies were in the form of spherical granules whose X-ray diffraction patterns were sharp and typical of the B type (see Fig. 2, Pattern **c**). The sharpness suggests a high degree of organization ('crystallinity'); and this high degree might explain the resistance of these granules not only toward iodine vapor (see Table 1) but also toward the digestive enzyme α-amylase (Rendleman, 2000). For the purpose of convenience, high-molecular-weight amylose of $\overline{dp}$ 1050, isolated from corn starch by complexation with 1-butanol in a purely aqueous medium according to the method of Schoch (1942), is labeled *corn amylose A*. This amylose did not appear granular, even under a scanning electron microscope at 10,000 × magnification (Fig. 3). However, it did possess a relatively sharp X-ray diffraction pattern of the V-type (see Fig. 2, Pattern **a**). When a solution of this amylose in DMSO was diluted with water and the amylose precipitated as the butanol complex according to the method of Murdoch (1992), the recovered amylose (freed of 1-butanol and any DMSO by multiple treatment with boiling methanol, followed by drying over P_2O_5 at 30 °C), also had a V-pattern (see Fig. 2, Pattern **b**), but differed from corn amylose A in its behavior toward iodine vapor. Amylose that was precipitated according to the method of Murdoch is labeled *corn amylose B*. Both amyloses were shown by NMR analysis to be free of 1-butanol. Amylose B contained only a trace of DMSO (0.0095 wt%, as determined by

Table 1
Reaction of iodine vapor with amylose and amylose–cyclohexanol complexes under different conditions of relative humidity at 24 °C. Corn amylose A and B were prepared according to the methods of Murdoch (1992) and Schoch (1942), respectively. Low-$\overline{dp}$ amylose was prepared by enzymatic conversion of α-CD. AGU symbolizes anhydroglucose unit (glucose residue)

Expt.	Substrate	Time (days)	Weight		Analysis of iodine complex based upon 100 mg of amylose content							
			Initial (g)	Final (g)	H^+ (mmol)	I (mg)	I (meq)	I^- (mg)	I^- (meq)	AGU (mmol)/Total iodine (meq)	I (meq)/I^- (meq)	Wt% of total iodine in complex
RH 0%												
1	Corn amylose A (6.3% H_2O)	30	0.2500	0.2442	0.0016	2.45	0.0193	0.31	0.0024	28	8.0	1.3
2	Corn amylose A, anhydrous, vacuum dried at 120 °C	30	0.2360	0.2402	0.00026	1.35	0.0107	0.12	0.0010	52	11.0	1.5
3	Corn amylose A-cyclohexanol complex, 13.7% solvent	30	0.1786	0.1734	0.0010	1.38	0.0109	0.000	0.000	57	∞	1.4
4	Corn amylose B, anhydrous, dried over P_2O_5 at 30 °C	30	0.2469	0.3158	0.00010	28.17	0.2220	0.000	0.000	2.78	∞	21.8
5	Corn amylose A, (6.3% H_2O)	30	0.2000	0.2308	0.0215	17.12	0.1349	3.31	0.0261	3.83	5.76	16.9
RH 30%												
6	Corn amylose A, (6.3% H_2O)	60	1.0001	1.1777	0.0336	19.41	0.1530	4.25	0.0335	3.31	4.57	19.2
7	Low-$\overline{dp}$ amylose ($\overline{dp}$ 61, 11.6% H_2O)	30	0.2500	0.2454	n.d.	1.55	0.0122	0.000	0.000	50.6	∞	1.5
8	Low-$\overline{dp}$ amylose ($\overline{dp}$ 69, 14.3% H_2O)	30	0.2500	0.2554	0.0023	4.76	0.0376	0.000	0.000	14.3	∞	5.2
9	Low-$\overline{dp}$ amylose ($\overline{dp}$ 69, cyclohexanol complex 11.7% solvent)	30	0.3019	0.3096	0.000	0.05	0.0004	0.000	0.000	2000	∞	0.4
RH 100%												
10	Corn amylose A (6.3% H_2O)	30	0.5000	0.6329	0.0385	17.67	0.1392	5.06	0.0399	3.45	3.49	18.5
11	Corn amylose A (6.3% H_2O)	60	0.2000	0.2481	0.0327	25.3	0.1994	3.10	0.0244	2.76	8.2	22.1
12	Corn amylose B (7.8% H_2O)	30	0.3002	0.3700	0.0019	24.19	0.1906	0.00025	0.0020	3.2	90	19.6
13	Corn amylose A-cyclohexanol complex (13.7% solvent)	30	0.1602	0.2191	0.0803	36.92	0.2909	0.00944	0.0744	1.69	3.91	31.6
14	Low-$\overline{dp}$ amylose ($\overline{dp}$ 69, 14.3% H_2O)	30	0.2000	0.2152	0.0009	1.57	0.0124	0.31	0.0024	42	5.1	1.9
15	Low-$\overline{dp}$ amylose ($\overline{dp}$-69, cyclohexanol complex 11.7% solvent)	30	0.3006	0.4378	0.0847	38.78	0.3056	10.62	0.0837	1.58	3.65	33.1

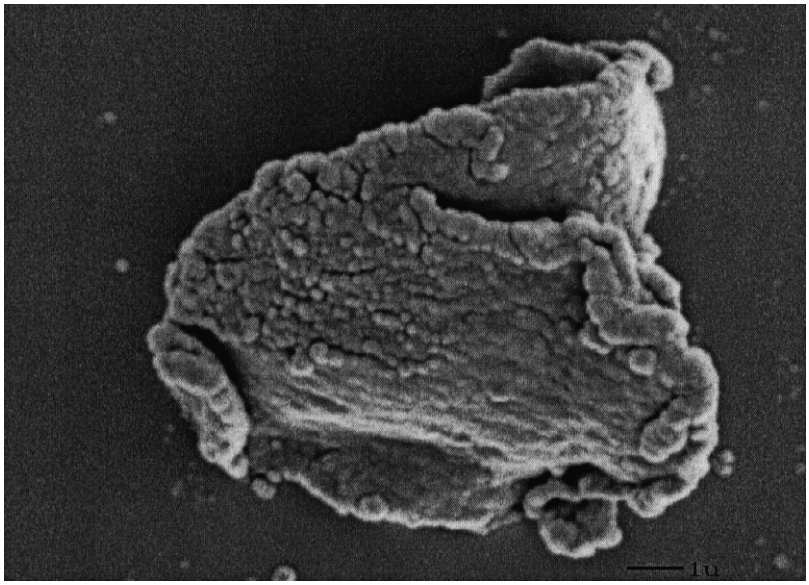

Fig. 3. Scanning electron microphotograph (SEM) of corn amylose A at 10,000 × magnification.

emission spectroscopic analysis for sulfur). The similarity in the V-patterns of the two corn amylose preparations suggests that the polymer chains in both are predominantly helical and are probably not associated as double helices to any large extent. Such helical chains would be free to accommodate guest molecules within their empty cavities.

Scanning electron microphotographs of corn amylose A (see Figs. 3–5) were virtually indistinguishable from those of corn amylose B (see Figs. 6–8). Both starches were isolated as microscopically small flakes which, under high magnification, appeared to be tightly bound clusters of spherules of very small diameter (≤ 0.5 μm). It is perhaps significant that low-molecular-weight amyloses ($\overline{dp}$ 61–71), whose shapes are in the form of globular granules with diameters as large as 5 μm, likewise consist of agglomerates of small spherules (Rendleman, 2000).

Data in Table 1 show that when samples of corn amylose A were exposed to iodine vapor in a humid atmosphere, considerable binding occurred and that the resulting amylose–iodine complexes contained both molecular iodine and iodide ion. Presumably, the primary source of iodide ion was a reaction in which molecular iodine was hydrolyzed by water of hydration in the starch substrate (see Table 1 for moisture contents of various starches at different levels of relative humidity). Both H^+ and I^- are produced according to the following reversible reactions:

$$I_2 + H_2O = I^- + H^+ + HOI$$

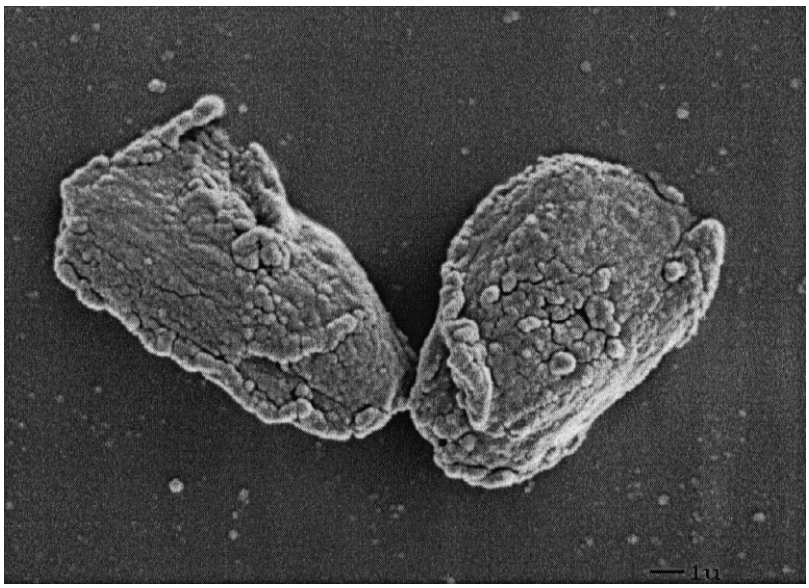

Fig. 4. SEM of corn amylose A at 6000 × magnification.

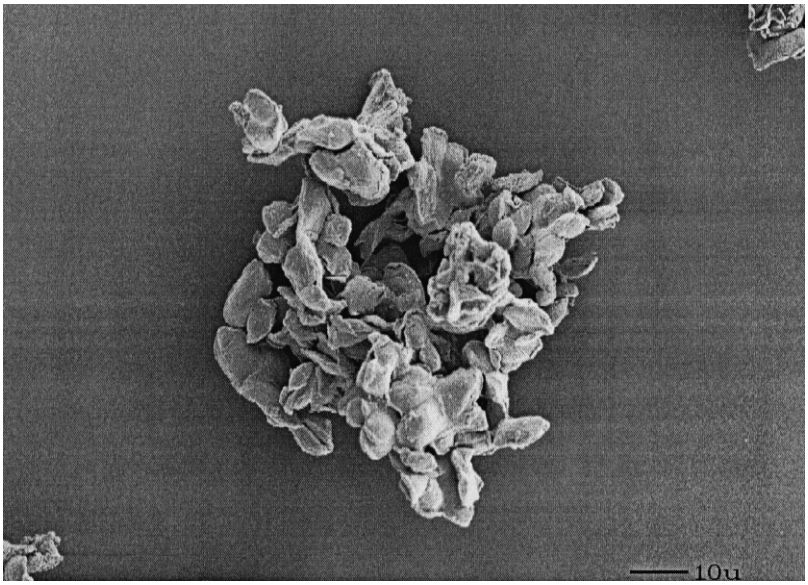

Fig. 5. SEM of corn amylose A at 1000× magnification.

$$3I_2 + 3H_2O = IO_3^- + 5I^- + 6H^+$$

The hypoiodous acid does not contribute a significant amount of free H^+ ions, because of its extremely low dissociation constant. Iodide ions produced in the reactions above would be expected to combine with molecular iodine to form triiodide ions (I_3^-) and possibly other polyiodide ions that would become constituents of the linear array of iodine atoms within the starch–iodine complex. The negative charges on these anions would be counterbalanced by H^+ ions. Because of the differential method of analysis employed by the author to determine iodide ion, there probably was considerable error in the calculated values for iodide in complexes containing low levels of iodine (<5 wt%). In such cases, calculated values for I/I^- have relatively little significance. Nevertheless, with few exceptions, iodide contents of starch–iodine complexes (expressed in meq) were very similar to H^+ contents. Severe deviation from a 1:1 relationship occurred only in Expt. 11 where meq of I^- was much lower than meq of H^+. This anomaly might have occurred because of unavoidable side reactions that were pronounced because of a high relative humidity and an extremely long reaction period. An abnormally low iodide content would explain the curiously high value of 8.2 for I/I^-. In general, an increase in relative humidity resulted in an increase in iodide content and a decrease in I/I^-.

Moisture was found to be essential for iodine binding by only one of the two corn amyloses used in the present studies. In Expts. 1 and 2 of Table 1, corn amylose A

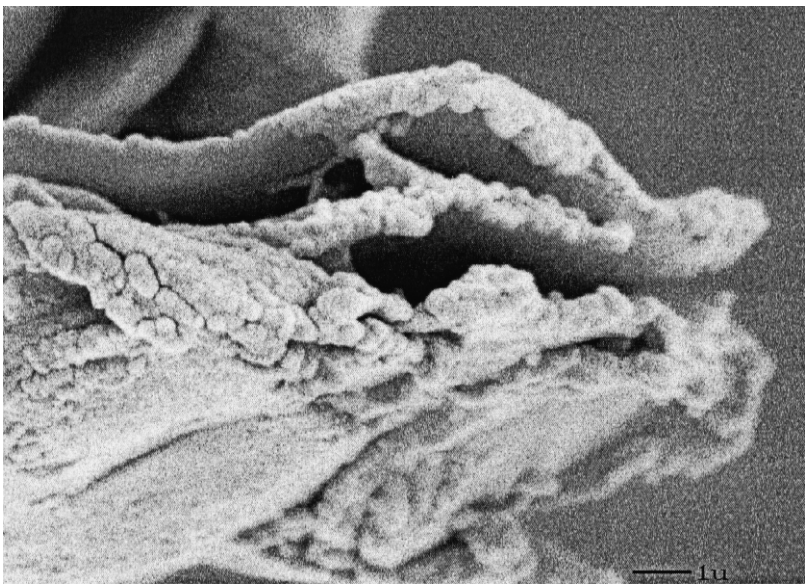

Fig. 6. SEM of corn amylose B at 10,000× magnification.

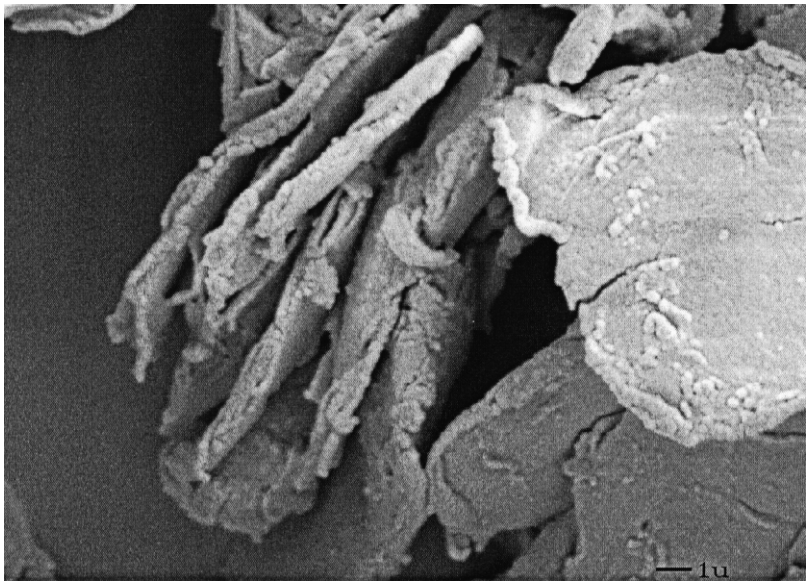

Fig. 7. SEM of corn amylose B at 6000× magnification.

exhibited almost no ability to bind iodine at 0% RH, even when the initial substrate was in its hydrate form. In an anhydrous atmosphere over Drierite, the hydrate form of corn amylose A probably lost most of its water of hydration within the first 24 h and, before any appreciable reaction of iodine with the hydrate could occur, acquired the inert characteristic of the anhydrous form (Expt.2). At 30–100% RH, where the moisture content of corn amylose ranged from moderate (6.3 wt%) to very high (27.6 wt%), moisture probably loosened the macrostructure of corn amylose A in a manner that facilitated sorption of iodine (Table 1, Expts. 5, 6, 10, and 11). At 100% RH, iodine bound by corn amylose A in 60 days was 22.1 wt%, a value only slightly greater than the 19.2 wt% for binding at 31% RH in 60 days; and iodine binding for a 60-day reaction period was only slightly greater than that for a 30-day period under the same RH conditions (Fig. 9).

The behavior of anhydrous corn amylose B toward iodine vapor at 0% RH was totally different from that of anhydrous corn amylose A. Under anhydrous conditions, corn amylose B was very reactive and bound 21.8 wt% of iodine in 30 days (Expt. 4), whereas binding by corn amylose A (Expt. 2) was very low (1.5 wt%). Furthermore, the iodide-ion content of the corn amylose B complex was too low to be measured. At 100% RH, corn amylose B bound almost as much iodine (19.6 wt%) as it did under anhydrous conditions (compare Expts. 4 and 12); yet, it contained only a trace of iodide ion.

No satisfactory explanation in terms of differences in macrostructure can be offered at this time for the differences

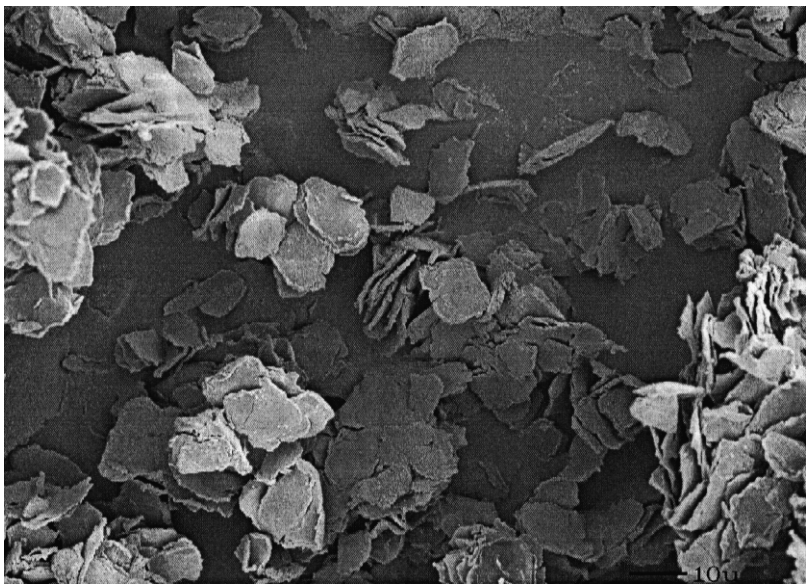

Fig. 8. SEM of corn amylose B at 1000× magnification.

Table 2
Reaction of iodine vapor with starches other than amylose under different conditions of relative humidity at 24 °C. AGU symbolizes anhydroglucose unit (glucose residue)

Expt.	Substrate	Time (days)	Weight		Analysis of iodine complex based upon 100 mg of amylose content							
			Initial (g)	Final (g)	H^+ (mmol)	I (mg)	I (meq)	I^- (mg)	I^- (meq)	AGU (mmol)/Total iodine (meq)	I (meq)/I^- (meq)	Wt% of total iodine in complex
RH 30%												
1	Corn starch, ordinary (10.1% H_2O, ~25% amylose)	30	0.2500	0.2484	n.d.	1.19	0.0094	0.015	0.00012	65	78	1.2
2	Waxy maize starch (10.7% H_2O, ≤1% amylose)	30	0.2500	0.2492	n.d.	1.21	0.0095	0.126	0.00099	59	9.6	1.3
3	Hylon VII (11.1% H_2O, 64% amylose)	30	0.2500	0.2537	n.d.	3.33	0.0262	0.69	0.0054	23	4.9	3.9
RH 100%												
4	Corn starch, ordinary (10.1% H_2O, ~25% amylose)	30	0.2000	0.2108	n.d.	1.74	0.0137	0.268	0.00211	39.1	6.5	2.0
5	Waxy maize starch (10.7% H_2O, ≤1% amylose)	30	0.2500	0.2630	0.0002	3.71	0.0292	0.071	0.00056	21	52	3.7
6	Hylon VII (11.1% H_2O, 64% amylose)	30	0.2000	0.2288	n.d.	8.72	0.0687	2.34	0.0184	7.1	3.7	10.0

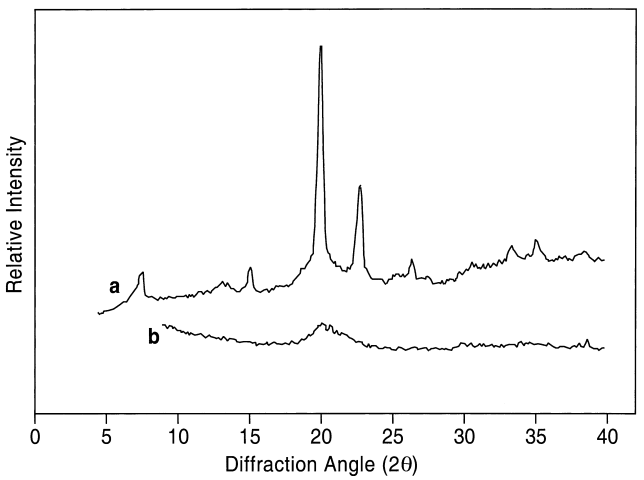

Fig. 9. X-ray diffraction patterns of corn amylose–iodine complexes produced by (a) treating corn amylose A with iodine vapor for 30 days at 30% RH and (b) treating the cyclohexanol complex of corn amylose A with iodine vapor for 30 days at 100% RH.

in behavior between the two corn amyloses. Both amyloses bound similar amounts of iodine; yet, amylose B was able to do so with very little or no concomitant formation of iodide ion. Although the results of Expt. 4 imply strongly that the presence of I^- is not necessary for reaction of corn amylose B with iodine vapor, they must not be taken as conclusive evidence that I^- is inessential. It is quite possible that the iodide content was much too low for either detection or determination by the analytical means employed in these studies.

3.2. Reactions of amylose–cyclohexanol complexes

When cyclohexanol complexes of corn amylose A and low-molecular-weight amylose ($\overline{dp}$ 69) were subjected to iodine vapor at 100% RH for 30 days (see Table 1, Expt. 13 and 15), unexpectedly high sorption of iodine occurred with concurrent elimination of cyclohexanol. The iodine contents of the resulting amylose–iodine complexes were 31.6 and 33.1 wt%, respectively, and both complexes contained moderately large amounts of iodide ion. Their respective I/I^- ratios were 3.91 and 3.65, values that were very similar to the ratio of 3.49 for corn amylose A under the same reaction conditions. High humidity was necessary for the displacement of cyclohexanol from its helical host and the subsequent reaction of host with iodine. At and below 30% RH, the concentration of water in the atmosphere of the reaction chambers was insufficient to remove cyclohexanol from its amylose complex (compare Expts. 3 and 9 with Expts. 13 and 15); consequently, under these conditions, very little iodine was sorbed. The exceptionally high iodine contents of 31.6 and 33.1 wt%, obtained at 100% RH, were not only appreciably higher than maximum iodine contents reported by other investigators for complexes prepared from V-amylose and iodine vapor, they were also close to the theoretical maximum content of 33.7 wt% calculated by

Table 3
Relationship between moisture content and glass transitions in amylose. The symbol T_g represents glass-transition temperature; ΔC_p represents the change in specific heat capacity (joules/gram/°C) that occurs at T_g

Amylose	Water content (wt%)	T_g (°C)	ΔC_p (J/g/°C)	Transition range (°C)
Corn amylose A[a]	0[b]	None	–	–
	6.3[c]	70.6	0.063	67.0–75.6
		89.4	0.026	87.3–91.8
	27.6[d]	66.0	0.12	57.0–78.0
Corn amylose B[e]	0[b]	63.1	0.063	58.8–68.3
		81.5	0.023	76.7–86.6
	7.8[c]	57.3	0.21	45.8–65.2
	31.0[d]	24.4	0.23	19.5–30.9
		72.8	0.11	68.1–75.5
Low-$\overline{dp}$ amylose ($\overline{dp}$ 69)[f]	0[b]	none	–	–
	14.3[c]	46.8	0.036	39.5–49.8
		60.6	0.020	58.0–63.2
	30.8[d]	18.1	0.28	13.4–25.0
		69.5	0.21	64.7–73.2

[a] Prepared from corn starch by precipitation with 1-butanol from aqueous solution (Schoch method).
[b] Amylose was dried under vacuum over Drierite at room temperature.
[c] Amylose was equilibrated at 31% RH and 24 °C.
[d] Amylose was equilibrated at 100% RH and 24 °C.
[e] Prepared by treating corn amylose A in DMSO–H_2O solution with 1-butanol (Murdoch method).
[f] Prepared by conversion of α-CD with CGTase in aqueous solution at 50 °C.

Murdoch (1992). Rundle and French (1943a) reported an iodine content of 21 wt% (equivalent to 26% of the weight of the amylose component) for a corn amylose complex prepared under anhydrous conditions. The potato amylose complex of Murdoch (1992), also prepared under anhydrous conditions, contained 28.8 wt% of iodine.

X-ray diffraction patterns of starch–iodine complexes prepared from corn amylose A directly and from the cyclohexanol complex of corn amylose A are shown in Fig. 3. The pattern of the former was well defined and suggested considerable 'crystallinity'. However, the pattern of the latter was ill defined, with no sharp peaks, and suggested a large amount of amorphous character. An iodine complex formed by the interaction of iodine vapor with the cyclohexanol complex of low-$\overline{dp}$ amylose ($\overline{dp}$ 69) also had an ill-defined pattern.

Differences in starch macrostructure that can cause differences in the behavior of amylose toward iodine vapor probably arise largely from variations in protocols chosen to separate amylose from corn starch (Table 2). Any retrogradation that occurs during isolation or purification could produce an amylose that is less reactive than one that has not suffered retrogradation. Corn amylose A may be an example of an amylose that has undergone partial retrogradation during isolation. With some starches, such as corn amylose A, but not corn amylose B, the presence of moisture is essential in order to loosen the macrostructure and, thereby, facilitate reaction with iodine. Amylose–cyclohexanol complexes serve as excellent examples of starch in a form whose macrostructure is open to facile and extensive reaction with iodine vapor. All or nearly all

Table 4
Effect of relative humidity on moisture content of starches at 24 °C

Starch	Moisture (wt%)				
	RH 2%	RH 31%	RH 50%	RH 70%	RH 100%
Native					
Corn, ordinary	8.8	10.1	11.8	14.2	25.7
Amylopectin, corn	9.1	10.1	11.9	15.0	28.7
Hylon VII (64% amylose)	9.9	11.1	13.5	16.7	26.7
Amylose, corn (non-granular; isolated by the butanol method of Schoch)	5.3	6.3	7.3	12.3	27.6
CD-derived					
Amylose, $\overline{dp}$ 56	11.2	12.1	13.9	17.0	29.8
Amylose, $\overline{dp}$ 61	10.1	11.6	13.7	16.4	30.3
Amylose, $\overline{dp}$ 69	12.8	14.3	16.0	18.6	29.0
Amylose, $\overline{dp}$ 72	8.2	9.1	10.7	12.8	25.4

of each starch chain is helical and arranged in a manner that permits a nearly theoretical amount of iodine to be bound.

Glass transition (T_g) data from DSC analyses performed on amyloses used in these studies provided evidence that chain mobility was not an important factor influencing amylose reactivity at or below 31% RH at 24 °C. At this temperature, moisture contents of the amyloses at 31% RH were not sufficient to promote any translational or rotational movement of starch molecules or movement of water molecules that would result in heat capacity increases and, consequently, the appearance of glass transitions. Table 3 contains DSC data for corn amylose A, corn amylose B, and low-$\overline{dp}$ amylose ($\overline{dp}$ 69) at different levels of moisture content. At high levels of moisture content (attained by equilibrating the amyloses at 100% RH), all three amyloses exhibited glass transitions. However, the fact that iodine-binding behavior at these high levels (~30 wt% H_2O) did not differ greatly from binding behavior at moderate levels (6–14 wt% H_2O) provided further evidence that glass transitions have little or no influence on iodine binding at 24 °C.

3.3. Reactions of native corn starches

Of the several native corn starches subjected to the action of iodine vapor (see Table 4), only Hylon VII, a hybrid containing 64% of amylose, had any substantial ability to react with iodine vapor at moderate levels of humidity. After 30 days at 100% RH, the iodine content of the Hylon VII complex was approximately one half that of iodine complexes of corn amylose A or B prepared under the same reaction conditions. The moderately high iodine content of the Hylon VII complex (10.0 wt%) reflected not only the moderately high amylose content of the starch, but also a granular structure that is easily penetrated. The I/I^- ratio for this complex was 3.7, a value very similar to the ratio of 3.45 found for the iodine complex of corn amylose A.

Acknowledgments

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