[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, IOWA STATE COLLEGE]

Effect of Disaggregation of Amylose on the Properties of the Iodine Complex[1]

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Corn amylose was separated into four sub-fractions by crystallization from 15% aqueous pyridine. Light scattering studies indicated that all fractions were highly aggregated in aqueous solution. Disaggregation by aging in neutral aqueous solution resulted in a decrease in iodine binding affinity and a decrease in the wave length of maximum absorption of the iodine complex in all cases. In the case of the most highly aggregated sample the binding capacity was markedly increased. Some implications of these observations in terms of the nature of the amylose aggregates and the mechanism of iodine binding are discussed.

The authors have recently presented evidence which suggests that some or most amylose samples prepared by the conventional butanol or Pentasol crystallization procedures exist in the form of aggregates. Such aggregates are not molecularly dispersed by alkali, but break down slowly in neutral solution (*p*H 6.0–6.5).[2] In another communication[3] an empirical study has been made of some of the factors which influence the affinity of amylose for iodine in aqueous solution. It appeared probable that some of these effects might be related to the state of aggregation. The present study was initiated to attempt a clarification of these relationships.

Materials and Methods

Amylose Preparation.—A Pentasol fractionated corn amylose was employed. The method of preparation was essentially the same as that described by Schoch.[4] A 2 to 3% starch suspension was autoclaved for approximately three hours at 15 to 18 lb. pressure. The dispersed starch was crystallized with Pentasol and the precipitated amylose complex redispersed and recrystallized in similar fashion.

Purification of Amylose.—The Pentasol fractionated amylose supposedly represents essentially all of the amylose in the original starch. However, it is probably contaminated to an appreciable extent by amylopectin. The next step then was an attempt to free this material of the branched fraction. Higginbotham and Morrison[5] have employed aqueous pyridine for this purpose and control experiments on mixtures of amylose and amylopectin suggested that 15% aqueous pyridine was particularly effective. Thus amylose precipitates slowly from this solvent at 44–45° while amylopectin does not precipitate even on prolonged standing. The procedure employed was to disperse the impure amylose in 15% pyridine at 80–90° (concentration 0.6–0.7%), and to allow the solution to stand at 44–45° until precipitation was substantially complete (as evidenced by no further change in specific rotation of supernatant solution prepared by centrifugation). Approximately seven days were required. The product was redispersed and recrystallized twice, 5 days and 3 days being required for precipitation, respectively. Yields obtained were approximately 88% in each of the first and third crystallizations, 95% in the second. The progress of the purification was followed by potentiometric titration with iodine after the second and third crystallization. Figure 1 shows that the iodine binding ability was appreciably enhanced by two crystallizations but that the third accomplished little or nothing.

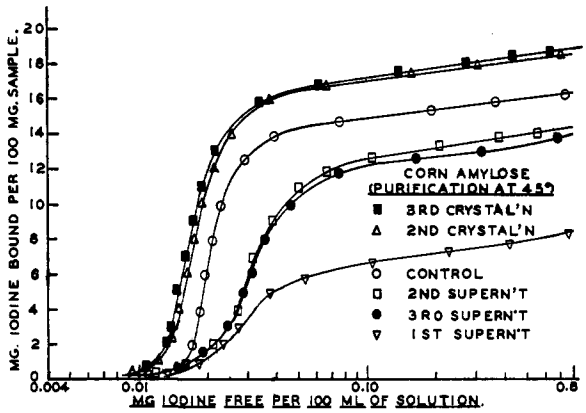

Fig. 1.—Iodine titration results on amylose before and following purification and on the removed impurity. The curve labeled control corresponds to the titration of the original amylose.

Sub-fractionation of Amylose.—The purified amylose was sub-fractionated on the basis of rate of precipitation using conditions similar to those used for the purification. The amylose was dispersed in 15% aqueous pyridine at elevated temperature, cooled to 45°, and allowed to stand at this temperature for 12 hours. A relatively small precipitate, cut 1, was removed. The temperature was then reduced to 25° and crystallization allowed to proceed for 10 hours giving a relatively large fraction, cut 2. Cut 3 came down also at this temperature after 12 hours further standing. Finally the supernatant solution was evaporated to a small volume and precipitated with ethyl alcohol to yield cut 4. All pyridine complexes were freed of pyridine by dispersing in warm water, precipitating with alcohol, washing with absolute ethanol and vacuum drying. The iodine binding properties of these fractions are compared in Fig. 2.

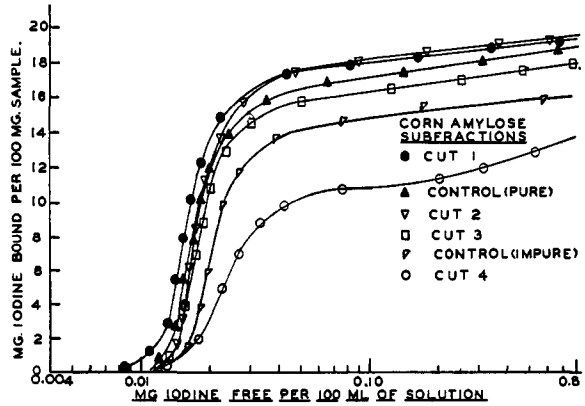

Fig. 2.—Iodine titration results on the various subfractions of corn amylose. The curve labeled control corresponds to the purified amylose used for sub-fractionation.

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(2) E. F. Paschall and J. F. Foster, *J. Polymer Science*, **9**, 73, 85 (1952).

(3) E. F. Paschall and J. F. Foster, THIS JOURNAL, **75**, 1177 (1953).

(4) T. J. Schoch, *Adv. Carbohydrate Chem.*, **1**, 247 (1945).

(5) R. S. Higginbotham and G. A. Morrison, *J. Text. Inst.*, **40**, T208 (1949).

Titration Procedure.—The titration procedure was the same as previously described.[5] Briefly, the amylose samples were dissolved in 1 *N* alkali, neutralized to *p*H 6–6.5 with HCl, and adjusted to 0.05 *N* in both KCl and KI. Titrations were carried out with 0.00157 *N* iodine in 0.05 *N* KI plus 0.05 *N* KCl. A bright platinum electrode and commercial calomel reference electrode were used in conjunction with a Leeds and Northrup Type K potentiometer. All titrations were carried out at 25°.

Light Scattering Procedure.—The light scattering instrument was a Zeiss photometer, modified for angular measurements, which has been described previously.[2,6] All measurements were made in six-sided cells permitting measurements at 45, 90 and 135° with respect to the direction of the incident beam.

Prior to scattering measurements solutions were clarified by centrifuging for one hour at 20,000*g* in a Sorvall SS-1 high-speed centrifuge.

Disaggregation Procedure.—Disaggregation of aggregated amylose samples was effected by dissolving in 1 *N* alkali, neutralizing to *p*H 6–6.5 and allowing to stand at room temperature until a turbidity drop and gross retrogradation had taken place.[2] The retrograded material was then dissolved by making the retrograded suspensions 0.5 *N* in alkali and physical measurements carried out on these solutions.

Absorption Spectra of the Iodine Complexes.—Absorption spectra were determined on dilute (approximately 0.002%) amylose solutions containing 1.6×10^{-4} *N* iodine in KI using a Coleman Universal spectrophotometer.

Experimental Results

Iodine titration curves for the four sub-fractions before and after disaggregation are summarized in Figs. 3, 4, 5 and 6. In addition the values determined for the wave length of maximum absorption of the iodine complexes and for the particle weights (or molecular weights) before and after disaggregation are included. To facilitate discussion Table I summarizes these data plus data on the relative yield of fractions and the iodine affinities before and after disaggregation.

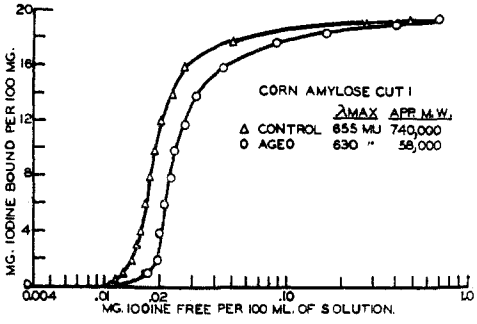

Fig. 3.—Iodine titration curves on cut 1 before (control) and after disaggregation.

TABLE I

RESULTS OF SUB-FRACTIONATION OF AMYLOSE WITH 15% PYRIDINE. PROPERTIES OF FRACTIONS BEFORE AND AFTER DISAGGREGATION

Fract.	Weight % of total	Apparent mol. wt. Before	Apparent mol. wt. After	Iodine affinity[a] Before	Iodine affinity[a] After	Wave length, mμ[b] Before	Wave length, mμ[b] After
1	10	740,000	58,000	0.018	0.023	655	630
2	62	900,000	53,000	.018	.024	655	625
3	15	1,000,000	120,000	.018	.023	...	645
4	13	1,500,000	57,000	.024	.041	630	605

[a] Iodine affinity is given as the free iodine concentration at the midpoint of the titration curve in mg. per 100 ml. of solution. [b] Wave length of maximum absorption of the iodine complex.

Discussion

The sub-fractionation procedure was originally developed in a hope of attaining molecular fractionation. It was felt that pyridine might serve the dual role of solvent at high temperature and precipitant at low. The results indicate that pyridine, under the conditions used, does not yield molecular dispersions since all of the sub-fractions appear to be highly aggregated.

It seems clear from these results that aggregation is not restricted to a small fraction of the amylose but is rather general. Any fractionation that has been attained evidently involves a separation on the basis of particle weight of the aggregates, not molecular weight. The average degree of aggrega-

(6) R. C. Rhees and J. F. Foster, *Iowa State College J. Sci.*, **27**, 1(1952).

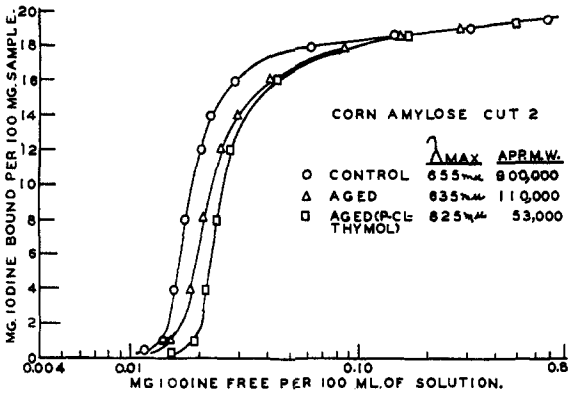

Fig. 4.—Iodine titration curves on cut 2 before (control) and after disaggregation.

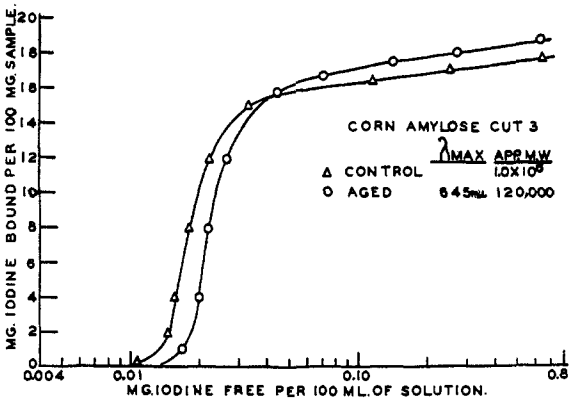

Fig. 5.—Iodine titration curves on cut 3 before (control) and after disaggregation.

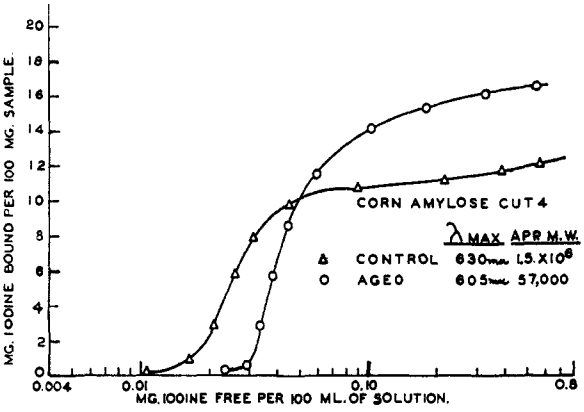

Fig. 6.—Iodine titration curves on cut 4 before (control) and after disaggregation.

tion varies from perhaps 15 in the first cut to around 30 in the last. It seems probable that cut 3 was not fully disaggregated by the treatment, and indeed no claim can be made that any of the fractions have been reduced completely to the molecular state. However, the weight-average molecular weights found by light scattering following the disaggregation treatment are of the same order of magnitude found by osmotic pressure on amylose acetates in organic solvents and by assay for terminal groups through periodate oxidation.[7]

In all cases the iodine affinity decreases upon disaggregation and there is a downward shift in the wave length of maximum absorption.[8] Indeed it is found that there is a rather good linear relation between the wave length of the maximum and the logarithm of the iodine affinity indicating that the two properties are in some manner intimately related.

Rundle and co-workers[10] have proposed a helical, molecular, structure for the amylose–iodine complex which is rather well supported by crystallographic evidence. To account for the "all-or-none" character of the iodine reaction a coöperative effect has been proposed. Thus the entering iodine molecules are presumed to be polarized by the permanent dipole moment of the amylose molecule, and they in turn interact through dipolar forces. There is evidence to suggest that the longer the amylose molecule the greater the iodine affinity and the longer the wave length of absorption; these facts also are accounted for by the coöperative effect. It appears highly desirable to reinvestigate this point, since the present results show that aggregation can influence both of these properties. Indeed the differences between any of the aggregated and disaggregated fractions are greater than has been found between fractions thought previously to differ widely in molecular weight.

Meyer and Bernfeld[11] have rejected the helical theory and have presented some evidence that amylose micelles are essential for complex formation. It can now be seen that aggregates do enhance the reaction, but the true situation is probably somewhere between the two extreme points of view of the molecular and micellar schools.

The authors have observed[2] that the stability of amylose toward disaggregation and retrogradation is increased by pretreatment with iodine. In addition this pretreatment markedly enhances the iodine binding affinity. For this reason we are presently inclined toward the view that these aggregates are not entirely haphazard but are, at least in part, ordered structures. It is tempting to suggest that they exist in large part in the helical configuration and are thus predisposed toward iodine binding. The retrogradation process might thus be pictured as a transition from the helical configuration, stabilized by intermolecular and possibly also intramolecular forces, to an extended configuration.

If the aggregates do indeed contain helical amylose molecules in close packing it might be suggested that the dipolar field in a given helix would be enhanced by surrounding amylose–iodine chains. Admittedly the distance between centers of close packed helices appears to be rather large to permit an appreciable dipolar interaction because of the high inverse power effect. However, in a helix surrounded by six neighboring helices the effect might conceivably be appreciable. Furthermore, a slight effect might be sufficient to reduce the distance between adjacent iodine molecules to a point where resonance stabilization could set in.

According to this view, then, the iodine binding affinity and wave length of maximum absorption would be governed by two factors: (1) the average length of uninterrupted helical amylose segments and (2) the degree of crystalline order. Interruptions in helical segments would be introduced by chain termini, by amorphous regions in the micelles, and probably also by branching sites if any branching were present.

Cut 4, although it was the most aggregated of the four, showed the lowest iodine affinity and wave length of absorption. To the extent to which the preceding reasoning is sound this could imply that this fraction was either (1) composed of shorter molecules than the others (2) contained an appreciable quantity of branched material or (3) was less perfectly ordered than the other cuts. The molecular weight after disaggregation does not lend any support to the first possibility. On the other hand, the fact that the affinity is still lower following disaggregation indicates that crystal imperfection is not entirely responsible. Possibly one is here dealing with moderately branched material of a character intermediate between amylose and amylopectin.

Perhaps the most significant observation is the pronounced increase in binding capacity of this fourth cut following disaggregation. This suggests that aggregates, if large enough, have a reduced binding capacity because of buried material which is not available for reaction. On this basis it seems probable that the difficulty encountered in preparing corn amylose fractions of high binding capacity is a result of the higher degree of aggregation present in corn amylose preparations as compared to, for example, potato.[2] It also seems entirely possible that some of the material removed in the preliminary pyridine fractionation was highly aggregated amylose rather than amylopectin.

AMES, IOWA

(7) A. L. Potter and W. Z. Hassid, THIS JOURNAL, **70**, 3774 (1948); **73**, 593 (1951).

(8) The effect of aggregation on the wave length of maximum absorption has been reported previously, on the basis of other evidence, by the authors.[9]

(9) J. F. Foster and E. F. Paschall, THIS JOURNAL, **74**, 2105 (1952).

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

(11) K. H. Meyer and P. Bernfeld, *Helv. Chim. Acta*, **24**, 389 (1941).