Studies on Starches of High Amylose Content. XI. Some Physicochemical Properties of Dispersions of Amylomaize Starch, and Observations on the Nature of High-Amylose Starches[1]

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ABSTRACT

The extent to which granules of laboratory-isolated maize starches of different genotype are dispersed into aqueous solution under various conditions has been studied. Analysis by low-speed centrifugation showed that aqueous dispersion was always incomplete. Complete solution of the granules was achieved in dimethyl sulfoxide (DMSO). These DMSO-solutions have been studied by analytical ultracentrifugation. Waxy-maize starch sedimented as one component, and regular maize as two, corresponding to amylose and amylopectin, as expected. In contrast, amylomaize starches with 57 to 75% of amylose sedimented as one component, the apparent sedimentation coefficient of which decreased with increase in reputed amylose content. This decrease in molecular size was confirmed by light-scattering and viscosity measurements. These results are discussed briefly in relation to current concepts of high-amylose starches.

Maize starches of high amylose content are of much academic and industrial interest, but results in the literature are not in agreement (1). We have discussed elsewhere (1) three particularly pertinent problems: (a) Isolation and purification of amylomaize starch is more difficult than for regular maize and requires the use of rather drastic methods; (b) fractionation procedures are all cumbersome and use reagents and conditions likely to degrade the starch; and (c) the actual amylose content of an amylomaize is difficult to characterize.

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Recently, we have been able to devise laboratory methods for isolating amylomaize starch in a high yield and of high purity without the use of any reagents likely to cause modification (2), and also have characterized its iodine-binding capacity (3). We describe here, as a preliminary to further studies of fractionation procedures, investigations of the efficiency of various methods of solubilizing the granular starches and some properties of the resultant dispersions. The implications of these results with regard to the nature of high-amylose starches are discussed briefly.

MATERIALS AND METHODS

Starch Samples

Starch was isolated in the laboratory from waxy maize (WM), regular maize (RM), and five samples of high-amylose maize (HA57, HA62, HA67, HA70, and HA75, where the number represents the reputed percentage of amylose). Details of the extraction procedure and the maize samples have been given elsewhere (2). The yields (dry-weight basis) and the purity of the starches were:

Maize Type	Yield %	Protein %	Maize Type	Yield %	Protein %
WM	72	0.09	HA62	62	0.35
RM	71	0.19	HA67	60	0.46
HA57	61	0.39	HA70	60	0.38
			HA75	58	0.44

Methods of Dispersing Starch Granules

1. Water slurries of granules at pH 6.5 were heated at 98°C. in an oxygen-free atmosphere for 50 min. Stirring kept the granules in suspension; the concentration (4 to 15% depending on amylose content) was high enough to cause shear and rupture between swollen granules. The dispersion was finally diluted with water at 98°C. to a concentration of 0.5%.

2. Granules at 0.5% concentration were gently stirred with 0.2M KOH for 16 hr. at 20°C.

3. The same as method 2 except that 0.5M KOH was used.

4. Granules were treated with 0.5M KOH for 16 hr., then neutralized with HCl to pH 6.5, and allowed to stand for a further 16-hr. period at 20°C.

5. Aqueous slurries of granules were centrifuged, and the hydrated granules (about 40% of water) were stirred with dimethyl sulfoxide (DMSO) for 5 hr. at 20°C. (4). The concentration was adjusted to suit the viscosity potential of the particular starch; i.e., for waxy maize, 1 to 2%; for regular maize, 3 to 4%; and for the amylomaizes, 5 to 7%.

6. Granules were dispersed into DMSO as in method 5 and then precipitated in the nongranular form prior to re-solution. The DMSO-solution was diluted with water (2 vols.) before the addition of acetone (3.5 vols.) with vigorous stirring to give a flocculent precipitate. The precipitate was removed on the centrifuge, dehydrated by repeated washing with acetone, and dried to a friable powder in a stream of nitrogen. The product was then redispersed into DMSO.

Determination of Granule Solubility by Centrifugation at Low Force-Field

The conditions of Leach et al. (5) were used. Dispersions (0.5%) were centrifuged at a force-field of 900 X g for 20 min. The concentration of polysaccharide remaining in the supernatant liquor was then determined, and the results were expressed as a percentage of original concentration. Concentrations of polysaccharide were determined by hydrolysis of aliquots to glucose and titration of the resultant reducing sugar by alkaline ferricyanide.

Determination of Granule Solubility by Centrifugation at High Force-Field

DMSO-dispersions of starch granules were examined in the Beckman Spinco analytical ultracentrifuge at 20°C. with 30-mm. cells in conjunction with schlieren optics and speeds of 33,540 and 50,740 r.p.m. Particles sedimenting before attainment of a force-field of about 29,000 X g (20,000 r.p.m.) were considered to be undispersed.

Physical Measurements

Limiting viscosity numbers, $[\eta]$, of DMSO-dispersions of the starches were measured with a modified Ubbelohde viscometer at 20°C. (6).

Weight-average molecular weights, M_w, were determined from measurements with a Brice-Phoenix light-scattering photometer. This technique has been described elsewhere (7); large cylindrical cells were used, and dust was removed from the DMSO solvent and solution by preparative ultracentrifugation at 20,410 r.p.m. and 12,590 r.p.m. for 1.5 and 1 hr., respectively.

Values of sedimentation coefficient, at 20°C., S_{20}, were evaluated in the usual manner from the runs in the analytical ultracentrifuge.

RESULTS AND DISCUSSION

Dispersion of Maize Starches

Table I shows the extent to which the freshly isolated maize starches are solubilized by dispersion methods 1 to 6, as measured by low-speed centrifugation

TABLE I. APPARENT SOLUBILITY[a] OF STARCH IN AQUEOUS DISPERSION AS MEASURED BY LOW-SPEED CENTRIFUGATION (900 X g)

Dispersion Method[a]	Potential Solvent	Starch				
		WM	RM	HA57	HA70	HA75
1	Water	99	85	38	30	25
2	0.2M KOH	90	72	49	n.d.[b]	n.d.
3	0.5M KOH	94	96	97	95	n.d.
4	KOH-HCl	94	87	27	20	29
5	DMSO	95	98	99	99	99
5[c]	DMSO	99	99	99	99	99
6	DMSO	99	99	99	99	99

[a]See text.

[b]n.d. = not determined.

[c]After standing for 10 days at 20°C.

TABLE II. ULTRACENTRIFUGATION OF "DMSO" DISPERSIONS[a] OF AMYLOMAIZE

Starch	Concentration		Ratio: Calculated / Experimental
	Experimental[b]	Calculated[c]	
HA57	5.68	5.34	0.94
HA62	4.38	4.27	0.98
HA67	7.34	7.25	0.99
HA70	4.65	4.06	0.88
HA75	8.95	9.08	1.02

[a]After four treatments with DMSO.

[b]Determined by hydrolysis and alkaline ferricyanide titration.

[c]Calculated from area under schlieren pattern obtained at 50,740 r.p.m.

(5). Water at 98°C. gave apparent solution of only the waxy-maize starch, and 0.2M KOH was ineffective KOH (0.5M) gave visually clear dispersions and high apparent solubilities, but with the regular and high-amylose maizes this solubility was reduced drastically by neutralization and aging at 20°C. High apparent solubility of all the starches was achieved when the *hydrated* granules were treated with DMSO, and no insolubility effects occurred on aging in this solvent. Nongranular starches which had been precipitated and freed from DMSO redispersed into DMSO very readily.

When the dispersions in 0.5M KOH were subjected to analytical ultracentrifugation, they were found to consist essentially of gel, and it became apparent that centrifugation at 900 X g (5) was not an effective method of assessing complete dispersion of starch granules.

In contrast, the DMSO-dispersions of the starches indicated the presence of only small amounts of gel, and retreatment with DMSO gave solutions which showed no gel and whose peak areas on the sedimentation patterns were consistent within experimental error with the actual concentration of starch (Table II).

Sedimentation Behavior of Starches

Although the analysis of the sedimentation pattern of a multicomponent system, such as starch, can be complicated (8), we have found that a mixture of amylose (30%) and amylopectin (70%), both from regular maize, behaves as expected; i.e., there are two peaks in the pattern which move independently (S_{20} = 10.8 and 1.9, respectively; 0.3% concentration), and the area of each is proportional to the appropriate concentration. It would therefore be expected that maize starches of differing amylose content would give two-component patterns in which the peak areas are directly proportional to the amount of amylose present. However, this is not found. The sedimentation patterns (compare Fig. 1) showed that (a) waxy-maize starch was homogeneous and had the largest sedimentation coefficient, (b) regular maize was a two-component system with peaks corresponding to amylose and amylopectin, and (c) for all the amylomaize starches there was only one apparent component, except for HA57 where there was an incipient trace of a fast-moving component, and HA62 where such a fast-moving component was present in even smaller amount.

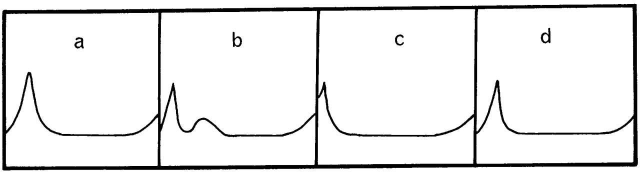

Fig. 1. Tracings of typical schlieren patterns obtained on the ultracentrifugation of maize starches in DMSO solution. Concentration = 0.3%; speed = 33,410 r.p.m.; temperature = 20°C. (a) waxy maize starch after 11.5 min.; (b) regular maize after 16 min.; (c) HA57 after 53 min.; (d) HA75 after 111 min.

The apparent homogeneity of these amylomaize starches is significant, although the presence of a few percent of normal, high-molecular-weight amylopectin would not necessarily be detected. McGuire and Erlander (9) have also found one peak on the ultracentrifugation of a comparable HA70 starch, which had, however, been isolated by a procedure involving enzymatic digestion of the kernel.

Physical Characteristics of DMSO Solutions of Maize Starches

Table III shows that the sedimentation coefficient for the amylomaize starches essentially decreases with increase in reputed amylose content, indicating a decrease in average molecular size of the glucan mixture.

In agreement with the results of Leach and Schoch (4), viscosity experiments showed that the DMSO solutions of the starches were stable. When the limiting viscosity numbers of the starch solutions were determined, it was found that $[\eta]$ also decreased with increase in reputed amylose content (see Table III). This effect is again unexpected and may indicate that the amylose, and indeed the starch material, present in the amylomaize is of smaller molecular size. This was confirmed by the results of light-scattering experiments (Table III) on the DMSO-starch solutions; a profound decrease in average molecular size occurred between the regular maize and starches HA57 and HA75.

It should be noted that a direct correlation between the sedimentation and light-scattering experiments in Table III is not to be expected, for different average values are being measured. The sedimentation coefficient is that of the most abundant molecular species, so that in the heterogeneous amylostarches it will reflect essentially the size of the amylose-type material and will be unaffected by any high-molecular-weight amylopectin. In contrast, the weight-average value from

TABLE III. PHYSICAL CHARACTERISTICS OF MAIZE STARCH SOLUTIONS IN "DMSO"

Starch	WM	RM	HA57	HA62	HA67	HA70	HA75
S_{20} [a]	17.2	9.9,2.4 [b]	1.12	1.21	1.01	1.25	0.80
$[\eta]$ [c], ml. g.$^{-1}$	170	173	117	112	101	99	90
$\overline{M}_w$, $\times 10^{-6}$	92	n.d.	13.5	n.d.	n.d.	n.d.	5.0

a Apparent sedimentation coefficient for 0.3% solution (values not corrected to water).

b Values corresponding to fast and slow components, respectively.

c Range of concentration = $(0.5^{-7}) \times 10^{-4}$ g. ml.$^{-1}$.

the light-scattering measurements will depend essentially on the amount of amylopectin present (10).

Concept of Amylomaize Starch

The nature and structure of amylomaize starch is a matter of some controversy (compare ref. 1). The above results show that, whatever the exact nature of the starch components, an increase in apparent amylose content is accompanied by an over-all reduction in average molecular size. Elsewhere (3,11,12) we have proposed that many features of the behavior of an amylomaize starch are not inconsistent with the concept that the starches contain degraded, linear material. Furthermore, amylostarches of increasing apparent amylose content also contain decreasing amounts of normal, high-molecular-weight amylopectin (10).

We would suggest that these three facts indicate that alpha-amylolytic activity may be causing fairly large-scale degradation of the starch material in an amylomaize starch. Our current results would indicate that such degradation actually increases with increase in apparent amylose content, but the whole position is complicated and further work is required.

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