

CHARACTERIZATION OF INTERMEDIARY FRACTIONS OF HIGH-AMYLOSE CORN STARCHES¹

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ABSTRACT

Starches from high-amylose corn of *du su*₂, *ae su*₁, *ae ae* types, and from commercial corn have been shown to possess a fraction intermediate between amylose and amylopectin, which has been isolated in yields of 8.7, 7.5, 6.6, and 4.5%, respectively. Separation is effected by fractionating out the amylose as either the 1-butanol or 1-nitropropane complex and then precipitating the intermediate fraction from the supernatant with 2-nitropropane. Intermediate fractions from these different starches are similar in iodine color, iodine blue value, iodine sorptive capacity, optical rotation, and rate of retrogradation. Those from the high-amylose starches have much lower viscosities and have possibly lower molecular weights than the fraction from commercial corn. All properties suggest that the intermediate fractions contain less highly branched molecules than does amylopectin and that the molecules are intermediate in shape between amylose and amylopectin.

It is well known from the work of Schoch (10,15) and others (6,11,20) that starches cannot be divided sharply into amylose and amylopectin but that these two major fractions shade into each other through an intermediate fraction. Fractions which are not intermediate but rather are more highly branched than amylopectin are also well known (2,4,5,14). Because of mounting interest in high-amylose corn starch, the characteristics of the intermediate fraction are here investigated with the expectation that increased information concerning this component will be of use in further understanding the properties of these starches and, perhaps, the manner of starch formation.

While various means are available for isolation of the intermediate fraction, the method chosen here is sequential precipitation of a starch dispersion, first with 1-butanol or 1-nitropropane and then with 2-nitropropane. This choice was based on the observation that 1-butanol or 1-nitropropane, on addition to a dispersion of normal corn starch, produces a precipitated fraction consisting of 26 to 28% of the starch with a high iodine sorption capacity, whereas 2-nitropropane produces a fraction of 28 to 31% with lower iodine sorption capacity (20). Differences in yield and iodine sorption capacities persist even when the isolated fractions are purified by recrystallization of the respective complexes. It is deduced that 2-nitropropane is a

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broad-spectrum complexing agent capable not only of complexing the linear amylose molecules but also some slightly branched molecules. Therefore, addition of 2-nitropropane to a starch dispersion from which amylose has been removed by means of 1-butanol or 1-nitropropane should lead to precipitation of the slightly branched molecular fraction.

Materials and Methods

Starches were isolated by conventional laboratory procedures from corn produced in the 1956 crop year. Starches of high amylose content were from corn of known genetic background (9) and were designated *du su*₂, *ae su*₁, and *ae ae*, containing 53, 64, and 66% amylose, respectively. The total starch content of the kernels approached that of normal corn. Before fractionation, the starch was extracted with methanol (8,16).

Each starch was fractionated by two procedures. In one, the amylose fraction was first separated with 1-butanol (17) and, after distillation of 1-butanol from the supernatant, the intermediate fraction was precipitated by addition of 2-nitropropane. The second fractionation procedure was conducted similarly, except that the amylose fraction was first separated with 1-nitropropane (20). High-amylose starches were not completely dispersed in hot water saturated with 1-butanol or 1-nitropropane. Therefore, these starches were pretreated by soaking in liquid ammonia (7) before being subjected to aqueous dispersion. The supernatant solution remaining after removal of the 1-butanol- or 1-nitropropane-amylose complex was concentrated slightly under reduced pressure to remove 1-butanol or 1-nitropropane. The solutions at pH 5.6–6.2 were adjusted to about 2% concentration (volume, 6 l.) and heated to 85°C.; 135 g. of 2-nitropropane were added. The vessel holding the dispersion was insulated by cloth wrappings, and the solution was allowed to cool slowly with stirring. After 48 hours, the mixture was centrifuged in a Spinco ultracentrifuge. The precipitate was redispersed to 2% concentration in water at 85°C., an excess of 2-nitropropane added, and the mixture allowed to cool slowly with stirring.

Iodine sorption capacity was measured potentiometrically by the procedure of Bates, French, and Rundle (3) as modified by Wilson, Schoch, and Hudson (22) and colorimetrically by the procedure of McCready and Hassid (12).

Osmotic molecular weights were determined on the acetylated fractions dissolved in chloroform using the Stabin-Immergut modification (18) of the Zimm-Myerson osmometer (23).

Intrinsic viscosities were measured in 1N potassium hydroxide solution in an Ostwald-Cannon-Fenske viscometer following the procedure of Lansky, Kooi, and Schoch (10).

The average number of D-glucose units per terminal nonreducing end group was determined by the method of Anderson, Greenwood, and Hirst (1) using cold potassium metaperiodate solutions.

Extent of retrogradation was measured in 0.5% aqueous solutions. One-half g. of sample was dissolved in 50 ml. of 1N potassium hydroxide solution at 25°C.; the solution was carefully neutralized with 1N hydrochloric acid solution and centrifuged. After 15 days, precipitated material removed by centrifugation was dissolved in 1N potassium hydroxide solution, and the concentration was determined by measurement of optical rotation. Optical rotations of intermediate fractions from high-amylose starches averaged $[\alpha]_D^{25} + 150^\circ$.

Acetylation of fractions was accomplished in acetic anhydride-pyridine mixture (3.2:3.7 v/v) at 100°C. (21). Acetyl determinations done according to the method of Murray, Staud, and Gray (13) showed that the fractions were fully acetylated.

Results and Discussion

The amounts of intermediate fractions obtained from the various high-amylose starches and from starch of normal commercial hybrid corn are in the range of 8.7 to 4.5%. Thus, the yields from starches of *du su*₂, *ae su*₁, and *ae ae* lines (Purdue high-amylose genotypes) and normal corn varieties are 8.7, 7.5, 6.6, and 4.5%, respectively. The fractions are obtained in quite pure condition, as evidenced by the very slight loss observed on recrystallization and the finding that the acetates, when dissolved to 1% concentration in chloroform, precipitate within a narrow range of hexane concentration when this nonsolvent is slowly added with stirring. Intermediate fractions precipitated by 2-nitropropane following removal of amylose with either 1-butanol or 1-nitropropane are almost identical in all properties. Therefore, only the fractions obtained with the use of 1-butanol and 2-nitropropane are described here.

As might possibly be expected, the properties of the fractions from the different starch types are similar and are intermediate between those of amylose and amylopectin (Table I). All intermediate fractions give a deep-blue color with iodine, visually similar to the amylose-iodine color. However, depth of color is less, as shown by the lower blue values (12) and the lower amount of iodine taken up on potentiometric titration. In addition, the maximum absorbance for the iodine solution of the intermediate fraction is at 588–600 $m\mu$,

TABLE I
PROPERTIES OF STARCH FRACTIONS

STARCH FRACTION	COLORATION BY IODINE	BLUE VALUES (ABSORBANCE)	IODINE ABSORBED PER G. STARCH FRACTION	AVERAGE NUMBER OF D-GLUCOSE UNITS PER NONREDUCING END GROUP	LIMITING VISCOSITY IN NORMAL POTASSIUM HYDROXIDE SOLUTION	RETROGRADATION WITHIN 15 DAYS	YIELD OF FRACTION	$[\alpha]_{25}^D$
			mg.			%	%	
Intermediate from normal starch	blue	0.26	58	41	1.26	26	4.5	+153
Intermediate from $du\ su_2$ starch	blue	0.25	51	30	0.375	19	8.7	+149
Intermediate from $ae\ su_1$ starch	blue	0.26	57	40	0.308	28	7.5	+151
Intermediate from $ae\ ae$ starch	blue	0.31	61	47	0.195	34	6.6	+150
Amylose from normal starch	blue	0.45	196	409	1.04	98	..	+159
Amylopectin from normal starch	reddish-purple	0.07	3	25	1.48	3	..	+155

which is between the values for amylopectin (540 $m\mu$) and amylose (650 $m\mu$) (19).

Periodate end-group determinations show that all intermediate fractions have roughly the same degree of branching with approximately one nonreducing end group for each 30–47 d-glucose units. The intermediate fraction is much less branched, therefore, than amylopectin, having, on the average, only 64% of the branching frequencies of amylopectin.

Osmotic molecular-weight determinations on the triacetates of the amylose and intermediate fractions from commercial corn starch gave values of 303,000 and 93,000, respectively.

According to viscosity measurements, the intermediate fractions of high-amylose starches have molecular weights which are lower than those of the intermediate fraction of commercial corn starch. The bearing of this on possible schemes for starch biosynthesis is intriguing.

Intermediate fractions, even though possessing frequent branches and, in most instances, low molecular weights, are capable of retrogradation, though at a slow rate.

In view of the similarity of optical rotations of amylose and amylopectin in alkaline solution, the equivalent rotary power of these intermediate fractions is expected.

The iodine sorption and periodate values indicated the intermediate fractions to be close to amylopectin in structure but with longer branch length and perhaps smaller molecular size.

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