3-10-1993

Amylopectin and Intermediate Materials in Starches from Mutant Genotypes of the Oh43 Inbred Line.

Y. J. Wang
Iowa State Univeristy

Pamela J. White
Iowa State University, pjwhite@iastate.edu

Linda M. Pollak
United States Department of Agriculture

J. Jane
Iowa State University

Amylopectin and Intermediate Materials in Starches from Mutant Genotypes of the Oh43 Inbred Line.

Abstract
Amylopectin (AP) and intermediate materials (IM) from five endosperm mutant genotypes in a common Oh43 inbred line were isolated and examined by gel-permeation chromatography, iodine affinity, blue value (BV), and viscosity. The chain-length distributions of AP and IM were determined using an enzymatic-chromatographic method. The degrees of branching in AP and IM decreased when the amylose-extender (ae) gene was present. The dull-1 (du1) gene produced AP and IM with the highest degrees of branching among the samples. The ae starch had a significantly (P less than 0.01) longer peak average chain length (CL) of the long-B chains in the IM fraction (177 glucose units) than did the AP faction (73 glucose units) or the other starches (37-56 glucose units). The higher iodine affinity in ae starch of the IM (6.1) compared with that of the AP (2.8) supported the idea that the IM had a longer CL than did the AP. There were no significant differences in the peak CL of A or B chains in AP and IM fractions of brittle-1 (bt1), du1, ae bt1, and ae du1 starches. The IM of ae and ae du1 starches had higher BV than did the AP fractions; however, the IM of du1 and ae bt1 had lower BV than did the AP fractions. The limiting viscosity number and gel- permeation chromatography results indicated that the AP and IM fractions of bt1 and du1 starches possessed more branching and larger hydrodynamic volume properties than those of the ae, ae bt1, and ae du1 starches. The present study demonstrated that genetic background affects the CL of starch branches, degree of branching, and iodine binding properties of starches.

Disciplines
Food Biotechnology | Food Chemistry | Food Processing | Food Science | Human and Clinical Nutrition

Comments
This article is from *Cereal Chemistry*, March 1993, 70(5); 521-525.

CAMPBELL G. M. 1991. The aeration of bread dough during mixing. Ph.D. thesis. University of Cambridge: Cambridge.
CHAMBERLAIN N., COLLINS T. N., and ELTON G. A. H. 1967. The Chorleywood bread process: Effect of rate of dough mixing. FMBRA Rep. 1. Flour Millers and Bakers Research Association: Chorleywood, Herts, U.K.
CHAMBERLAIN N., COLLINS T. N., and REDMAN B. I. 1970. The Chorleywood bread process: Effect of reduced pressure during mixing. FMBRA Rep. 40. Flour Millers and Bakers Research Association: Chorleywood, Herts, U.K.
DUS S. J., and KOKINI J. L. 1990. Prediction of the nonlinear viscoelastic properties of a hard wheat flour dough using the Bird-Carreau constitutive model. J. Rheol. 34:1069-1084.
JUNGE R. C., HOSENEY R. C., and VARRIANO-MARSTON E. 1981. Effect of surfactants on air incorporation of dough and crumb grain of bread. Cereal Chem. 58:338-342.

[Received August 4, 1992. Accepted March 10, 1993.]

Amylopectin and Intermediate Materials in Starches from Mutant Genotypes of the Oh43 Inbred Line[1]

Y.-J. WANG,[2] P. WHITE,[2,3] L. POLLAK,[4] and J. JANE[2]

ABSTRACT

Cereal Chem. 70(5):521-525

Amylopectin (AP) and intermediate materials (IM) from five endosperm mutant genotypes in a common Oh43 inbred line were isolated and examined by gel-permeation chromatography, iodine affinity, blue value (BV), and viscosity. The chain-length distributions of AP and IM were determined using an enzymatic-chromatographic method. The degrees of branching in AP and IM decreased when the amylose-extender (*ae*) gene was present. The dull-1 (*du1*) gene produced AP and IM with the highest degrees of branching among the samples. The *ae* starch had a significantly ($P < 0.01$) longer peak average chain length (CL) of the long-B chains in the IM fraction (177 glucose units) than did the AP fraction (73 glucose units) or the other starches (37–56 glucose units). The higher iodine affinity in *ae* starch of the IM (6.1) compared with that of the AP (2.8) supported the idea that the IM had a longer CL than did the AP. There were no significant differences in the peak CL of A or B chains in AP and IM fractions of brittle-1 (*bt1*), *du1*, *ae bt1*, and *ae du1* starches. The IM of *ae* and *ae du1* starches had higher BV than did the AP fractions; however, the IM of *du1* and *ae bt1* had lower BV than did the AP fractions. The limiting viscosity number and gel-permeation chromatography results indicated that the AP and IM fractions of *bt1* and *du1* starches possessed more branching and larger hydrodynamic volume properties than those of the *ae*, *ae bt1*, and *ae du1* starches. The present study demonstrated that genetic background affects the CL of starch branches, degree of branching, and iodine binding properties of starches.

Starch is composed of two primary components: amylose (AM), an essentially linear molecule; and amylopectin (AP), a highly branched molecule. The existence of a third component in normal maize starch, an intermediate material (IM) with properties different from those of AM and AP, was first postulated by Lansky et al (1949). Banks and Greenwood (1975) suggested that type and amount of IM depended primarily on the AM percentage of the starch, although it varied considerably among starches.

The AM content in normal maize (*Zea mays* L.) starch ranges from 25 to 30%; however, the AM content can be altered with mutant genes. Amylomaize, which contains the recessive mutant gene amylose-extender (*ae*), has an apparent AM content of up to 80% (Banks and Greenwood 1975) and is associated with the presence of abnormal AP (Mercier 1973, Ikawa et al 1978, Boyer et al 1980, Ikawa et al 1981). Wolff et al (1955) demonstrated that the AP fraction in a 50% amylomaize starch had longer inner and outer chains than those of normal AP, where the inner and the outer chains were divided at the point of α-1,6-glucoside bonds. Montgomery et al (1964) supported the proposal of Wolff et al (1955) that the AP in amylomaize starch was less highly branched than was normal AP. Some investigators (Banks and Greenwood 1968, Adkins and Greenwood 1969), however, proposed that the resulting abnormal AP came from contaminating short-chain AM. The discrepancy might result from the fact that investigators seldom used exactly the same techniques for the dispersion and fractionation of starch (Banks and Greenwood 1975). Although the fine structure of abnormal AP from amylomaize has not been completely clarified, there are many reports showing the presence of abnormal AP in amylomaize starch (Mercier 1973, Ikawa et al 1978, Boyer et al 1980, Ikawa et al 1981).

More recently, Baba and Arai (1984) examined the fine structures of AP and IM in a 50% amylomaize starch. They found that the IM and the AP of amylomaize possessed an average chain length (CL) that was 20 and 10 glucose units longer than that of the AP of waxy (*wx*) maize, respectively. The IM of amylomaize had an average degree of polymerization of 250–300 glucose units per molecule, with four or five branches having a CL of approximately 50 glucose units.

In addition to the *ae* gene, the combinations of a dull-1 (*du1*) or sugary-1 (*su1*) gene with other recessive mutant genes (except the *wx* gene) also produced starches with increased AM contents (Ikawa et al 1981; Yeh et al 1981; Inouchi et al 1983, 1987; Boyer and Liu 1985; Wang et al 1993). In a previous report (Wang et al 1993), several mutant genotypes (*ae, du1, ae* brittle-1 [*bt1*], and *ae du1*) evidenced increased AM as well as increased IM content. This study was undertaken to examine the structures and the physicochemical properties of AP and IM from several mutant genotypes in an Oh43 inbred background to illustrate the influences of recessive mutant genes on the starches.

[1]Journal Paper J-15147 of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA. Projects 3128 and 3052, a joint contribution with the Field Crops Research Unit, USDA-ARS.
[2]Graduate student, professor, and associate professor, respectively, Department of Food Science and Human Nutrition, Iowa State University, Ames.
[3]Author to whom correspondence should be addressed at 2312 Food Sciences Building, Iowa State University, Ames.
[4]Research geneticist, USDA-ARS, Department of Agronomy, Iowa State University, Ames.

MATERIALS AND METHODS

Materials

Mature kernels of single mutants (*ae, bt1,* and *du1*) and double mutant combinations (*ae bt1* and *ae du1*) from a common Oh43 inbred line were harvested from a summer nursery near Ames, Iowa, in 1991. Development of the genotypes and sampling and storing of the kernels were described previously (Wang et al 1993).

Isolation of Starch

Starch was isolated by a wet-milling procedure (Steinke and Johnson 1991). The isolated starches were purified by treating with five volumes of 0.2*M* sodium chloride-toluene (5:1, v/v) at least five times, until no protein residue was present in the sodium chloride-toluene interface. After each treatment, the starch granules were sedimented by centrifugation (8,700 × *g*), and the final sediment was washed three times with distilled water and dried at 45°C for 24 hr.

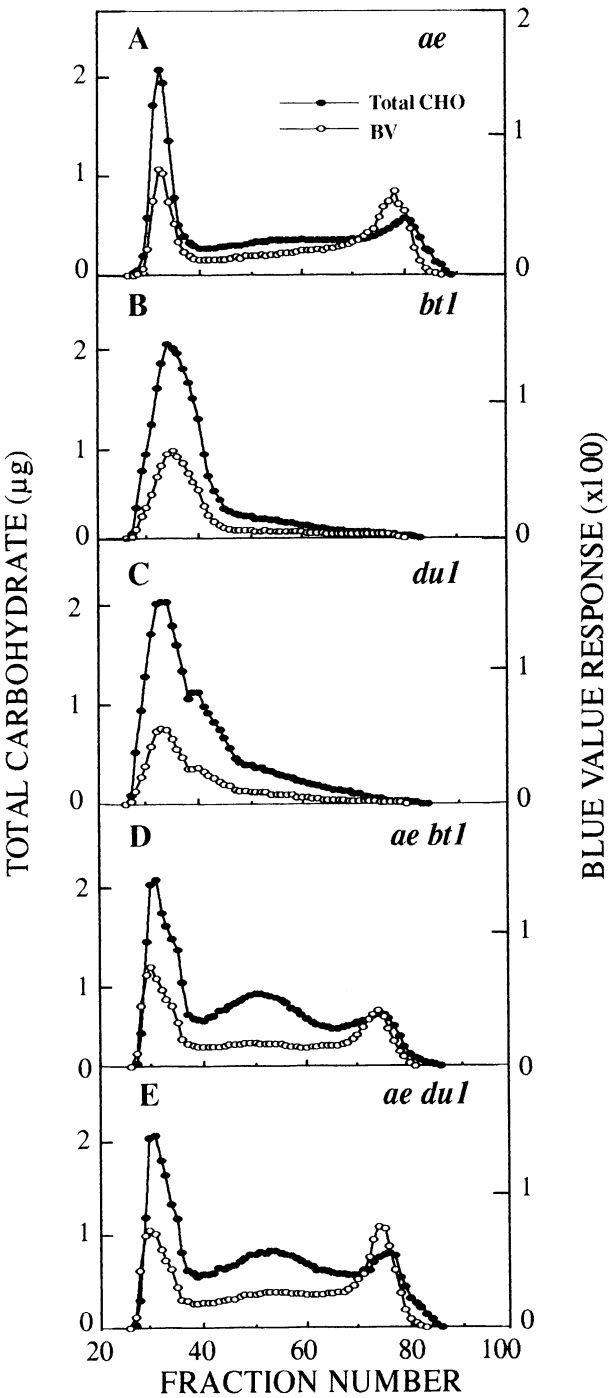

Fig. 1. Elution profile on Sepharose CL-2B column of butanol non-complexing fractions from five mutant genotypes. BV = blue value response. Total CHO = total carbohydrate value.

Separation of AP and IM

Starch was fractionated according to the general procedure of Schoch (1942) and Jane and Chen (1992) with the modifications of Wang et al (1993). The fraction not complexing with 1-butanol (butanol noncomplexing fraction, BNF) during fractionation was considered to include AP and IM. It was purified by three further treatments with 1-butanol to remove contaminating AM. The supernatant precipitated with methanol was collected and redissolved in 90:10 (v/v) dimethyl sulfoxide-deionized water (Wang et al 1993) for further study. The amount of BNF collected from different starch genotypes ranged from 0.5 to 4.0 g, depending upon the availability of starch, and it represented about a 50% yield.

BNF (75 mg) was fractionated on a Sepharose CL-2B column following the procedure of Wang et al (1993), except that 75 mg of starch was loaded onto the column. Fractions (4.9 ml of effluent) were collected every 9.5 min and subjected to total carbohydrate and BV response analyses using the anthrone-sulfuric acid method (Wright and Gann 1966) and the iodine staining test (Juliano 1971), respectively. The minimum value from iodine staining after elution of the first major fraction was used to identify the end of the eluted AP. The rest of the effluent was considered to be IM. Only one major fraction was observed for *bt1* and *du1* starches; thus, the AP was separated from the IM at the inflection point of the BV response. The corresponding fractions from AP and IM were pooled, respectively, condensed by vacuum evaporation, and precipitated with five volumes of methanol. The precipitates were separated by centrifugation at 8,700 × *g* for 20 min at 4°C and redissolved in 90:10 (v/v) dimethyl sulfoxide-deionized water. The separation procedure was repeated several times to get sufficient AP and IM for all BNF samples. Previously, the separation of IM from AP was achieved by ultracentrifugation (Adkins and Greenwood 1966), complexing with iodine (Adkins and Greenwood 1969), or leaching (Banks et al 1971). It has been suggested that, by using gel-permeation chromatography (GPC), AP can be cleanly separated from IM (Baba and Arai 1984).

GPC of Isoamylase-Debranched AP and IM

The starch was prepared, debranched by isoamylase, fractionated on a Bio-Gel P-6 column (Wang et al 1993), and assayed for total carbohydrate using the anthrone-sulfuric acid method (Wright and Gann 1966). Crystalline *Pseudomonas* isoamylase was used (Hayashibara Shoji, Inc., Olayama, Japan). The eluted materials were separated into two fractions. The division was made at minimum points between the two major peaks according to the response of the polysaccharide. The CL of debranched starch was also calculated (Hizukuri et al 1981, Jane and Chen 1992).

Physicochemical Properties of AP and IM

The isolated AP and IM in dimethyl sulfoxide of different mutant genotypes were characterized by selected physicochemical properties. BV was determined according to Gilbert and Spragg (1964). Two separate determinations were done on each sample.

An Ostwald viscometer at 22.5°C was used to determine the limiting viscosity number [η] of starch sample (0.5%) in 1*N* KOH. The flow time was 124 sec for 1*N* KOH. Each sample was measured three times.

Iodine affinity (IA), expressed as milligrams of iodine bound to 100 mg of starch, was determined by amperometric titration (Schoch 1964) at 30°C. The AP and IM of the *ae* and *du1* starches were measured twice. Only one determination of *bt1* and *ae bt1* starches was done because of a limited sample size. There was insufficient AP and IM of *ae du1* to determine IA.

Statistical Analyses

Correlations were computed between structural characteristics and physicochemical properties, and also among structural characteristics and physicochemical properties using the SAS program (SAS Institute 1990). Least significant differences were computed at a significance level of $P < 0.05$.

RESULTS AND DISCUSSION

Separation of AP and IM

The BNF of starches from five maize mutant genotypes of the Oh43 inbred line was eluted on the Sepharose CL-2B column. Profiles are presented in Figure 1. The *ae* starch (Fig. 1A) showed two major fractions. The *bt1* and *du1* starches (Figs. 1B and C, respectively) showed only one major fraction. The *ae bt1* and *ae du1* starches (Figs. 1D and F, respectively) exhibited three fractions. The first, and also the major, fraction in the elution profile was composed of high molecular weight molecules, considered to be AP. The rest of the material was regarded as IM, with mainly low molecular weight molecules (Baba and Arai 1984). For *ae bt1* and *ae du1* starches, the third fraction exhibited a greater BV response than did the second fraction, although the second fraction had a greater polysaccharide content than did the third. These data suggest that the third fraction possessed longer branch CL than did the second (Bailey and Whelan 1961).

Individual Characterization of AP and IM

The chromatograms of isoamylase-debranched AP and IM from the five maize mutant genotypes are presented in Figure 2. To calculate the amount of materials in low and high molecular weight fractions, each elution profile was divided into two fractions (I and II) at the point of minimum value of the polysaccharide response between the two major peaks. The CL at the apices of peaks from fraction I and II and the percentage of fraction I and II were determined (Table I). Materials eluting at the void volume (V_o) of elution, except for those of AP from *ae* and *bt1* starches, were suspected to be contaminating AM after

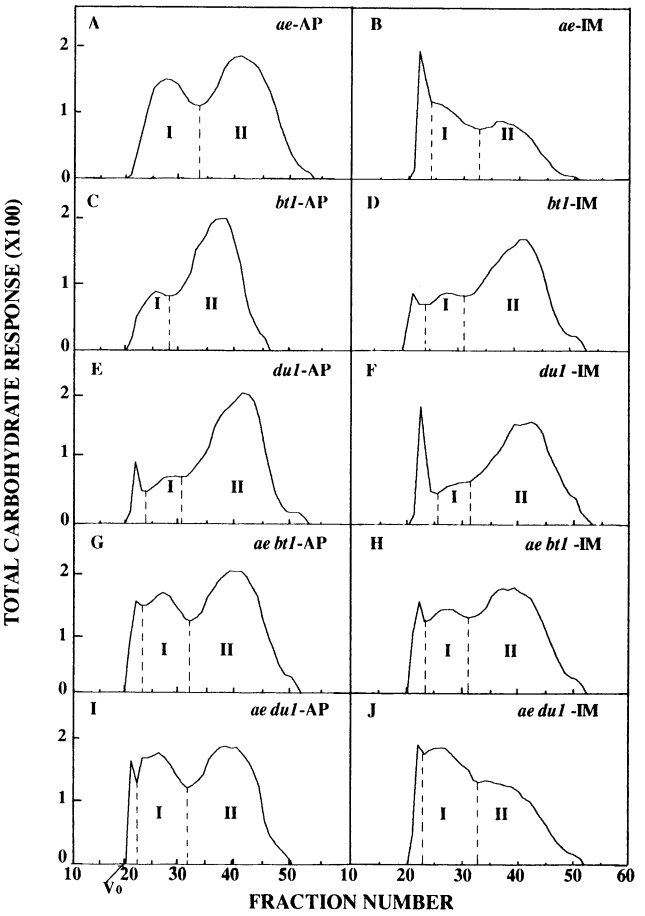

Fig. 2. Elution profile of isoamylase-debranched starch from amylopectin (AP) and intermediate materials (IM), divided into fractions I and II, for five mutant genoytpes on Bio-Gel P-6 column. Void volume = V_o.

TABLE I
Percentage Compositions of Polysaccharides and Chain Length Distribution of Debranched Amylopectin (AP) and Intermediate Materials (IM) from Five Mutant Genotypes of the Oh43 Inbred Line[a]

Starch Component	Intermediate Materials (%)[b]	AP or IM, %		Ratio of Fraction II to Fraction I	Peak Average Chain Length of Fraction I[c]	Peak Average Chain Length of Fraction II
		Fraction I	Fraction II			
ae-AP		39.2	60.8	1.6	73	26
		(0.3)	(0.3)	(0)	(3)	(4)
ae-IM	15.3	60.1	39.9	0.6	177	26
		(0.2)	(0.2)	(0)	(8)	(7)
bt1-AP		21.2	78.8	3.7	55	16
		(0.5)	(0.5)	(0.1)	(10)	(0)
bt1-IM	1.8	22.3	77.7	3.5	38	20
		(0.2)	(0.2)	(0)	(8)	(0)
du1-AP		17.8	82.2	4.6	37	17
		(0.2)	(0.2)	(0.1)	(2)	(5)
du1-IM	15.2	16.0	84.0	5.2	51	14
		(0.2)	(0.2)	(0.1)	(2)	(3)
ae bt1-AP		35.9	64.1	1.8	50	18
		(0.1)	(0.1)	(0)	(4)	(0)
ae bt1-IM	22.5	37.6	62.4	1.7	52	21
		(0.3)	(0.3)	(0)	(19)	(5)
ae du1-AP		39.9	60.1	1.5	56	23
		(1.2)	(1.2)	(0.1)	(3)	(3)
ae du1-IM	18.9	56.9	43.1	0.8	55	23
		(2.0)	(2.0)	(0.1)	(5)	(4)
$LSD_{0.05}$				0.1	18	8

[a]Values are the average of two separate determinations. Standard deviations are listed immediately below. *ae* = amylose extender, *bt1* = brittle 1, *du1* = dull 1.
[b]Percentage of composition of intermediate materials in total starch. Data are summarized from Table I of Wang et al (1993).
[c]Glucose units.

fractionation and were not included in either fraction I or fraction II. In the chromatograms for AP, fraction I was assumed to include long B chains, and fraction II was assumed to be composed of A and short-B chains (Hizukuri 1986). In the present study, the same designation of fraction I and II was adopted for the IM. The ratio of fraction II to fraction I may be used as an index of the extent of branching, in that the larger the ratio, the greater the degree of branching (Biliaderis et al 1981).

The elution profiles of debranched AP from *ae, ae btl,* and *ae dul* starches were similar (Figs. 2A, G, and I) with similar percentage compositions of fraction I and II (Table I). The *btl* and *dul* starches had similar elution profiles of debranched AP (Fig. 2C and E) in which the percentage of fraction II was much greater than that of fraction I (Table I). The elution patterns of debranched IM from the five samples (Figs. 2B, D, F, H, and J) suggest a structural difference among IM of the different starch genotypes. The elution patterns of AP and IM from the same sample were similar to each other for *btl, dul,* and *ae btl* samples (Figs. 2C–H, respectively), but were different for *ae* and *ae dul* samples (Figs. 2A, B, I, and J, respectively). The *ae* gene decreased the proportion of fraction II in the IM more than in the AP for *ae* and *ae dul* starches, but this effect was not seen in *ae btl* starch.

The ratios of fraction II to fraction I of debranched AP ranged from 1.5 for *ae dul* starch to 4.6 for *dul* starch. The ratios of fraction II to fraction I of debranched IM ranged from 0.6 for *ae* starch to 5.2 for *dul* starch (Table I). These small ratios for AP and IM from *ae, ae btl* and *ae dul* starches indicated small degrees of branching in these polysaccharides resulting from the effect of the *ae* gene (Wang et al 1993). Moreover, the *ae* gene decreased the degree of branching more on the IM than on the AP. In contrast, the large ratios (fraction II to fraction I) of AP and IM of *dul* starch suggested the presence of more highly branched molecules. This finding agrees with a previous report (Wang et al 1993). The ratio of fraction II to fraction I in the AP was slightly larger than that in the IM for the same starch, except for *dul* starch. Thus, the IM was generally more lightly branched than was the AP for the same starch. These findings agree with those of previous reports (Banks and Greenwood 1975, Baba and Arai 1984).

The peak CL of fraction I for the AP ranged from 37 for *dul* starch to 73 glucose units for *ae* starch. The peak CL of fraction I for the IM ranged from 38 for *btl* starch to 177 for *ae* starch (Table I). The peak CL of fraction II for AP and IM were more similar for all starches and ranged from 14 to 26 glucose units. For the *ae* starch, the peak CL at fraction I of the IM was significantly ($P < 0.01$) longer than that of the AP. No significant difference ($P < 0.05$) was noted between the peak CL (fraction I) of the IM and that of the AP for *btl, dul, ae btl,* and *ae dul* starches. For fraction II, no significant difference ($P < 0.05$) was noted between the peak CL of the AP and the IM for all starches. The ae starch had the longest peak CL at fraction I for both AP and IM of all starches.

The influence of the *ae* gene on the fine structure of AP has been extensively studied. Evidently, the AP from amylomaize has a unique structure, with a longer CL than that of the AP of normal and waxy maize (Wolff et al 1955, Montgomery et al 1964, Mercier 1973, Baba and Arai 1984). When combined with other genes (in *ae btl* and *ae dul*), however, the *ae* gene affected the fine structure of the starches less. These data suggested that the combination of two mutant genes affected the starch structure differently than the respective single mutant genes.

TABLE II
Physicochemical Properties of Amylopectin (AP) and Intermediate Materials (IM) from Five Mutant Genotypes of the Oh43 Inbred Line Background

Sample[a]	Blue Value[b]	Limiting Viscosity Number (ml/g)[c]	Iodine Affinity
ae-AP	0.277 ± 0.006	124 ± 3	2.8[d]
ae-IM	0.318 ± 0.007	70 ± 4	6.1[d]
btl-AP	0.164 ± 0.002	137 ± 2	1.6[e]
btl-IM	0.162 ± 0.001	82 ± 0	2.0[e]
dul-AP	0.186 ± 0.005	142 ± 2	1.7[d]
dul-IM	0.155 ± 0.010	80 ± 1	2.2[d]
ae btl-AP	0.189 ± 0.004	...[e]	1.0[e]
ae btl-IM	0.159 ± 0.001	...[e]	1.1[e]
ae dul-AP	0.252 ± 0.008	127 ± 1	...[f]
ae dul-IM	0.304 ± 0.001	50 ± 1	...[f]
$LSD_{0.05}$	0.011	6	...[g]

[a] *ae* = amylose extender; *btl* = brittle 1; *dul* = dull 1.
[b] Values are the average ± standard deviation of two separate determinations.
[c] Average of three determinations.
[d] Two measurements.
[e] One measurement.
[f] Not determined.
[g] Value unavailable due to lack of replication.

Physicochemical Properties of AP and IM

Physicochemical properties of isolated AP and IM, including BV, [η], and IA, are summarized in Table II. Both AP and IM components of the *ae* and *ae dul* starches exhibited higher BV than did other starches, reflecting the strong binding of AP and IM with iodine, and suggesting their long branch chains. The *ae btl* starch did not have a high BV, although it previously exhibited high BV response during fractionation on GPC (Fig. 1D). The AP and IM of *ae btl* starch may have retrograded during the BV analysis. The IM gave a higher BV than did the AP in *ae* and *ae* dul starches. However, there was no difference in the BV of AP and IM for *btl* starch, and the AP had a higher BV than did the IM for *dul* and *ae btl* starches ($P < 0.05$). Data indicate that structural differences, such as CL of A chains and B chains, causing different iodine-binding abilities, exist among and between the AP and IM.

All AP fractions exhibited significantly ($P < 0.01$) greater [η] than did the IM fractions, as expected. The AP and the IM fractions of *btl* and *dul* starches had greater [η] values than did the respective fractions of *ae* and *ae dul* starches, which reflected the more branched nature of *btl* and *dul* starch.

Because of the limited sample size, replicate IA analyses of all starches were not possible; therefore, statistical comparisons

TABLE III
Correlations Between Structural Characteristics and Physicochemical Properties of Amylopectin (AP) and Intermediate Materials (IM) from Five Mutant Genotypes of the Oh43 Inbred Line

Property	Peak CL of Fraction I[a]	Peak CL of Fraction II[a]	Blue Value	Limiting Viscosity Number	Iodine Affinity
Ratio of fraction II to fraction I	−0.35*[b]	−0.70**	−0.69**	0.38**	−0.26
Peak CL of fraction I	...	0.26*	0.45**	−0.21	0.89**
Peak CL of fraction II	...	...	0.62**	−0.21	0.55**
Blue value	...	...	...	−0.45**	0.87**
Limiting viscosity number	...	...	...	...	−0.58**

[a] Average chain length (CL).
[b] * and ** = significant at $P < 0.05$ and $P < 0.01$ levels of probability, respectively.

could not be made. Nonetheless, from the limited data, it seems that the IM of *ae* starch exhibited a greater IA than did the AP. Some contaminating molecules present at the void volume of debranched starch chromatograms were suspected to be AM because of their long branch chain (CL > 200 glucose units, data not shown) and were not included in either fraction I or fraction II. Therefore, these contaminating materials did not affect the IA determination. The long CL of fraction I (177 glucose units) in the IM may have resulted in the great discrepancy in IA between the AP and IM fractions of *ae* starch.

Correlation analyses were determined among structural characteristics, including ratios of fraction II to fraction I, peak CL at fractions I and II, and physicochemical properties (Table III). Although some correlations were expected (e.g., between IA and peak CL of fraction I and between IA and BV), the correlations did not account for all variations. Low correlations among other parameters may be important. More work needs to be done to clarify the effects of mutant genes on starch structures and on the physicochemical properties of maize.

LITERATURE CITED

ADKINS, G. K., and GREENWOOD, C. T. 1966. Studies on starches of high amylose-content. Part V. The ultracentrifugal fractionation of aqueous dispersions of amylomaize starch. Starch/Staerke 18:237.

ADKINS, G. K., and GREENWOOD, C. T. 1969. Studies on starches of high amylose-content. Part X. An improved method for the fractionation of maize and amylomaize starches by complex formation from aqueous dispersion after pretreatment with methyl sulphoxide. Carbohydr. Res. 11:217.

BABA, T., and ARAI, Y. 1984. Structural characterization of amylopectin and intermediate material in amylomaize starch granules. Agric. Biol. Chem. 48:1763.

BAILEY, J. M., and WHELAN, W. J. 1961. Physical properties of starch. I. Relationship between iodine stain and chain length. J. Biol. Chem. 236:969.

BANKS, W., and GREENWOOD, C. T. 1968. Studies on starches of high amylose-content. Part IX. The detection of linear material in the anomalous amylopectin from amylomaize starch. Carbohydr. Res. 6:241.

BANKS, W., and GREENWOOD, C. T. 1975. Fractionation of the starch granule, and the fine structures of its components. Pages 5-66 in: Starch and Its Component. Edinburgh University Press: Edinburgh.

BANKS, W., GREENWOOD, C. T., and MUIR, D. D. 1971. Studies on starches of high amylose-content. 14. The fractionation of amylomaize starch by aqueous leaching. Starch/Staerke 23:199.

BILIADERIS, C. G., GRANT, D. R., and VOSE, J. R. 1981. Structural characterization of legume starches. I. Studies on amylose, amylopectin, and beta-limit dextrins. Cereal Chem. 58:496.

BOYER, C. D., and LIU, K.-C. 1985. The interaction of endosperm genotype and genetic background. 1. Differences in chromatographic profiles of starches from nonmutant and mutant endosperms. Starch/Staerke 37:73.

BOYER, C. D., DAMEWOOD, P. A., and MATTERS, G. L. 1980. Effect of gene dosage at high amylose loci on the properties of the amylopectin fractions of the starches. Starch/Staerke 32:217.

GILBERT, G. A., and SPRAGG, S. P. 1964. Iodimetric determination of amylose. Iodine sorption: "Blue value". Pages 168-169 in: Methods in Carbohydrate Chemistry, Vol. 4. R. L. Whistler, ed. Academic Press: Orlando, FL.

HIZUKURI, S. 1986. Polymodal distribution of the chain lengths of amylopectin, and its significance. Carbohydr. Res. 147:342.

HIZUKURI, S., TAKEDA, Y., YASUDA, M., and SUZUKI, A. 1981. Multi-branched nature of amylose and the action of debranching enzymes. Carbohydr. Res. 94:205.

IKAWA, Y., GLOVER, D. V., SUGIMOTO, Y., and FUWA, H. 1978. Amylose percentage and distribution of unit chain-length of maize starches having a specific background. Carbohydr. Res. 61:211.

IKAWA, Y., GLOVER, D. V., SUGIMOTO, Y., and FUWA, H. 1981. Some structural characteristics of starches of maize having a specific genetic background. Starch/Staerke 33:9.

INOUCHI, N., GLOVER, D. V., TAKAYA, T., and FUWA, H. 1983. Development changes in the fine structure of starches of several endosperm mutants of maize. Starch/Staerke 35:371.

INOUCHI, N., GLOVER, D. V., and FUWA, H. 1987. Chain length distribution of amylopectins of several single mutants and the normal counterpart, and sugary-1 phytoglycogen in maize (*Zea mays* L.). Starch/Staerke 39:259.

JANE, J.-L., and CHEN, J.-F. 1992. Effect of amylose molecular size and amylopectin branch chain length on paste properties of starch. Cereal Chem. 69:60.

JULIANO, B. O. 1971. A simplified assay for milled-rice amylose. Cereal Sci. Today 16:334.

LANSKY, S., KOOI, M., and SCHOCH, T. J. 1949. Properties of the fractions and linear subfractions from various starch. J. Am. Chem. Soc. 71:4066.

MERCIER, C. 1973. The fine structure of corn starches of various amylose-percentage: waxy, normal and amylomaize. Starch/Staerke 25:78.

MONTGOMERY, E. M., SEXSON, K. R., DIMLER, R. J., and SENTI, F. R. 1964. Physical properties and chemical structure of high-amylose corn starch fractions. Starch/Staerke 16:314.

SAS INSTITUTE, INC. 1990. The GLM Procedure. Pages 891-996 in: SAS Users Guide: Statistics. SAS Institute, Inc., Cary, NC.

SCHOCH, T. J. 1942. Fractionation of starch by selective precipitation with butanol. J. Am. Chem. Soc. 64:2957.

SCHOCH, T. J. 1964. Iodimetric determination of amylose. Potentiometric titration: Standard method. Pages 157-160 in: Methods in Carbohydrate Chemistry, Vol. IV. R. L. Whistler, ed. Academic Press Inc.: Orlando, FL.

STEINKE, J. D., and JOHNSON, L. A. 1991. Steeping maize in the presence of multiple enzymes. I. Static batchwise steeping. Starch/Staerke 68:7.

WANG, Y.-J., WHITE, P., POLLAK, L., and JANE, J. 1993. Characterization of starch structures of 17 maize endosperm mutant genotypes with Oh43 inbred line background. Cereal Chem. 70:171.

WOLFF, I. A., HOFREITER, B. T., WATSON, P. R., DEATHERAGE, W. L., and MACMASTERS, M. M. 1955. The structure of a new starch of high amylose content. J. Am. Chem. Soc. 77:1654.

WRIGHT, H. K., and GANN, D. S. 1966. An automatic anthrone method for the determination of inulin in plasma and urine. J. Lab. Clin. Med. 67:689.

YEH, J. Y., GARWOOD, D. L., and SHANNON, J. C. 1981. Characterization of starch from maize endosperm mutants. Starch/Staerke 33:222.

[Received November 16, 1992. Accepted March 23, 1993.]