

# Fractionation of High-Amylose Maize Starches by Differential Alcohol Precipitation and Chromatography of the Fractions

Jeffrey D. Klucinec<sup>1</sup> and Donald B. Thompson<sup>1,2</sup>

## ABSTRACT

Cereal Chem. 75(6):887–896

Three high-amylose maize starches (HAS) and a common corn starch (CCS) were subjected to differential alcohol precipitation using isoamyl alcohol and 1-butanol to obtain fractions designated as amylose (AM), amylopectin (AP), and intermediate material (IM). For each starch, IM had a blue value and an iodine binding wavelength maximum ( $\lambda_{\max}$ ) between the  $\lambda_{\max}$  of the respective AM and AP. Size-exclusion chromatography (SEC) showed similarities in the AM from CCS and HAS. HAS AP had higher blue values and iodine binding  $\lambda_{\max}$  values than CCS AP. SEC of the intact HAS AP and IM both showed large proportions of material eluting after the void volume (45–85%) when compared to CCS AP and IM. Chain length (CL) distributions of debranched AP and IM indicated that

these fractions from each starch were highly branched, and that AP had a shorter average chain length than IM. Consequently, the differential precipitation behavior of the HAS AP and IM appears dependent on general branching structure rather than size. We conclude that in both CCS and HAS, AP and IM are subsets of the branched molecules with AP as the predominant fraction. For HAS, AP and IM include molecules of a size typical for AM and contain a higher proportion of chains that are longer than those of CCS AP. Differential alcohol precipitation is a useful method of separating amylose, amylopectin, and intermediate material from HAS.

Schoch (1942) developed a method for separating starch into two fractions based on the differential ability of the fractions to precipitate with aqueous 1-butanol. In the same laboratory, Wilson et al (1943) modified the procedure of Schoch (1942) by using a combination of isoamyl alcohol and 1-butanol for the initial precipitation. This modified procedure could be used for smaller quantities of starch (Schoch 1954) and produced a precipitate that could be separated by low-speed centrifugation.

Lansky et al (1949) examined the iodine binding differences of starch fractions precipitated first by aqueous Pentasol (a mixture of amyl alcohols) and then purified by subsequent precipitations with either 1-butanol or Pentasol. When the initial precipitate was purified using aqueous 1-butanol, the iodine binding capacity increased and reached a maximum after two precipitation cycles. When Pentasol was used for purification, the iodine binding capacity of the precipitate steadily increased through seven purification cycles but was always lower than that for the 1-butanol precipitate. Lansky et al (1949) concluded that using 1-butanol for purification of the Pentasol precipitate resulted in subfractionation of the initially precipitated material.

Starch has also been fractionated into three components using a variety of other precipitation agents. Whistler and Doane (1961) fractionated corn starches into amylose, amylopectin, and intermediate material fractions by first precipitating amylose from dispersed starch using aqueous 1-butanol or 1-nitropropane and then precipitating an intermediate material fraction from the remaining supernatant material using 2-nitropropane. The material precipitated by the 2-nitropropane had a lower iodine binding capacity than the material precipitated by 1-butanol or 1-nitropropane (Whistler and Doane 1961). Adkins and Greenwood (1969) precipitated a crude amylose fraction from dispersed starch using 1-butanol and then precipitated additional material (which they called intermediate material) from the remaining supernatant material using iodine. They also obtained a fraction from the crude amylose that would not reprecipitate with 1-butanol; they considered this fraction to be intermediate material as well. Using aqueous 1-butanol, Wang and White (1994) fractionated oat starches into amylose (precipitated material), amylopectin (supernatant material), and intermediate material (a middle layer after centrifugation). Takeda et al (1986) fractionated

normal rice starch using aqueous isoamyl alcohol and 1-butanol together in an initial precipitation followed by a redispersal and precipitation with 1-butanol alone, as described by Wilson et al (1943). Takeda et al (1986) defined intermediate material as the material that precipitated with isoamyl alcohol and 1-butanol but not with 1-butanol alone. They observed that intermediate material had a higher average chain length and blue value than amylopectin, but it was not examined further. In all cases where three fractions were obtained from normal starches, the fraction (or the sum of the fractions from Adkins and Greenwood [1969]) termed intermediate material was  $\approx 5\%$  of the starch and appeared to have an iodine binding wavelength maximum ( $\lambda_{\max}$ ) and a blue value intermediate between that of amylose and amylopectin.

Whistler and Doane (1961) and Adkins and Greenwood (1969) also fractionated high amylose starches (HAS) with their methods. Whistler and Doane (1961) obtained 7–9% intermediate material from HAS; values were somewhat higher than those obtained for normal starch (4.5%). Adkins and Greenwood (1969) obtained larger (and more variable) quantities of intermediate material from HAS (25–39%) when compared to normal starch (4%).

For amylose determination in normal maize, the use of 1-butanol precipitation, size-exclusion chromatography (SEC), and potentiometric titration results in a similar estimate of amylose (Ferrone 1996). The amylose from normal maize starch obtained by 1-butanol fractionation elutes predominantly after the void volume when using Sepharose CL-2B or Toyopearl HW-75 SEC media (Yeh et al 1981; Takeda et al 1988, 1992, 1993). Unfractionated waxy maize starch and amylopectin from normal maize obtained by 1-butanol fractionation elute predominantly at the void volume with these SEC media (Yeh et al 1981, Baba and Arai 1984, Boyer and Liu 1985, Takeda et al 1988, Wang et al 1993).

When the 1-butanol supernatant material from HAS is separated by SEC using Sepharose CL-2B, polymer is observed at both the void volume and after the void volume (Baba et al 1982, Baba and Arai 1984, Wang et al 1993). Baba et al (1982) considered the molecules in the 1-butanol supernatant eluting after the void volume to be intermediate material, and they suggested that this material corresponded to intermediate material recovered by Whistler and Doane (1961). This suggestion was based on the similarity in the iodine binding characteristics of the 1-butanol supernatant molecules eluting after the void volume and of the precipitation-based intermediate material Whistler and Doane (1961) collected. The later-eluting material from the 1-butanol supernatant of Baba et al (1982) was “intermediate” in the sense that it behaved as neither normal amylose (it did not precipitate with 1-butanol) nor normal

<sup>1</sup> Department of Food Science, Penn State University, University Park, PA 16802.

<sup>2</sup> Corresponding author. Phone: 814/863-2950. Fax: 814/863-6132. E-mail: dbt1@psu.edu

amylopectin (it eluted after the void volume). Wang et al (1993) observed that 1-butanol supernatant material from HAS has a moderate iodine binding capacity that varied with SEC elution volume, showing that the materials at and after the void volume differed in a way other than size. Definition of intermediate material as low molecular weight material by SEC of starch molecules obtained from the supernatant of 1-butanol fractionation methods has resulted in identification of as much as 55% of HAS as intermediate material (Baba et al 1987), in contrast to the 5–10% determined by differential precipitation (Whistler and Doane 1961, Takeda et al 1986). For HAS, the differences between intermediate material obtained by the differential precipitation methods of starch fractionation and by the precipitation-SEC method of starch fractionation are not well understood. The purpose of this study was to separate different HAS sources into fractions designated as amylose (AM), amylopectin (AP), and intermediate material (IM) fractions using differential alcohol precipitation and then use chromatographic and iodine binding techniques to gain insight into the molecular composition and properties of the fractions and thus of the molecular composition of HAS.

## MATERIALS AND METHODS

### Granular Starches and Reagents

Two commercial *ae* genotype starches (Hylon V and Hylon VII) and a commercial normal maize starch (Melojel) were obtained from National Starch and Chemical Company (Bridgewater, NJ). A commercial *ae du* genotype starch (Amerimaize 2300) was ob-

tained from American Maize Products Company (now Cerestar USA, Inc., Hammond, IN). These HAS will be referred to as *ae V*, *ae VII*, and *ae du*. The normal maize starch will be referred to as common corn starch (CCS). All reagents were ACS reagent grade or better (Fisher Scientific; Fair Lawn, NJ).

### Preparation of Nongranular Starch

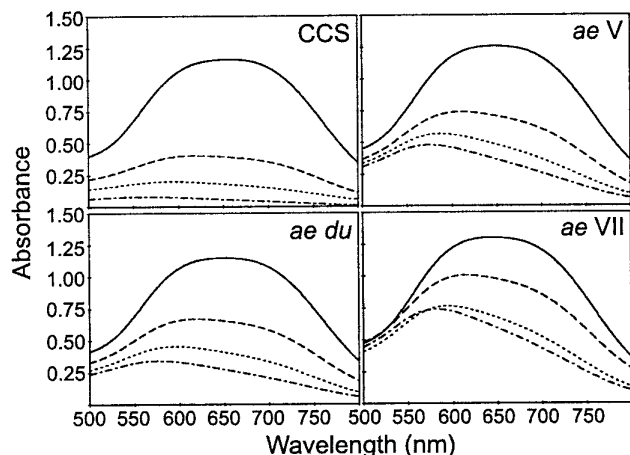
Granular starches (10 g, dry weight) were dispersed in 200 mL of 90% (v/v) dimethyl sulfoxide (DMSO) (in H<sub>2</sub>O) by heating the mixtures in a boiling water bath with constant stirring for 3 hr. Following dispersion, 4 volumes of ethanol were added and the mixture was then centrifuged at 6,500 × *g* for 15 min at 4°C. The supernatants were discarded and the pellets were washed by suspending them in 50 mL of ethanol followed by recentrifugation (6,500 × *g*). The washing procedure was repeated once with ethanol and once with acetone. The acetone-washed precipitate was then dried in a forced-air oven at 50°C for 24 hr. The nongranular starches (NG by the terminology of Banks and Greenwood [1975]) were stored under desiccation (CaSO<sub>4</sub>). For use in a particular experiment, NG samples from all starches were prepared simultaneously.

### Preparation of Starch Fractions

The NG starch was fractionated based on the procedure of Schoch (1954) with modifications. NG starch (2.5 g) from each starch was redispersed in 70 mL of 90% DMSO. Seven volumes (490 mL) of an aqueous mixture of 6% 1-butanol and 6% isoamyl alcohol were added to the DMSO-redispersed starches in 1-L Erlenmeyer flasks. The mixtures were stirred and then placed in a 95°C water bath for 1 hr, after which the entire system (≈25 L of water and the flasks) was allowed to cool to 28°C (≈18 hr).

After cooling, the mixtures were gently agitated to resuspend any precipitated starch and then centrifuged at 10,000 × *g* for 15 min at 4°C. The supernatants were saved and the precipitates were resuspended in water to bring the starch concentrations to 1–2% (estimated based on preliminary experiments). Isoamyl alcohol and 1-butanol were then added to bring both concentrations to 5.2% (v/v), the same concentration as in the first precipitation. This mixture was heated, cooled, and centrifuged (10,000 × *g*) as described above. The resultant supernatant was combined with the first supernatant. The precipitate was redispersed in the mixture of isoamyl alcohol and 1-butanol, and the reprecipitation procedure was then repeated; the supernatant from the final step was combined with the first two supernatants. The combined supernatants containing isoamyl alcohol and 1-butanol are referred to as the AP fraction.

The precipitate was redispersed by stirring in 70 mL of 90% DMSO and then mixed with 490 mL of 6% 1-butanol. This mixture was heated and allowed to cool in the water bath and then centrifuged (10,000 × *g*), as described above. The supernatant from this step in the fractionation procedure was decanted and saved separately from the previously collected supernatants. This secondary supernatant is referred to as the IM fraction.



**Fig. 1.** Iodine binding by nongranular starches and fractions obtained by differential alcohol precipitation. Nongranular starches (---), amylose (—), amylopectin (- · - · -), and intermediate material (- - - -). CCS = common corn starch; *ae du*, *ae V*, *ae VII* represent high amylose starches. Blue values and  $\lambda_{\max}$  for nongranular starches and fractions are presented in Table II.

**TABLE I**  
Recovery of Starch Fractions (% w/w) by Differential Alcohol Precipitation<sup>a</sup>

Starch <sup>b</sup>	Total Recovery <sup>c</sup> (%, w/w)	Amylopectin <sup>d</sup> (AP)	Intermediate Material <sup>d</sup> (IM)	Amylose <sup>d</sup> (AM)
CCS	84.6 ± 3.5	70.4 ± 2.0a <sup>e</sup>	6.9 ± 0.4a	23.1 ± 1.4a
<i>ae du</i>	85.2 ± 0.7	51.5 ± 4.2b	7.9 ± 1.7a	40.5 ± 4.1b
<i>ae V</i>	84.3 ± 1.2	54.4 ± 2.0b	7.5 ± 0.3a	38.3 ± 1.9b
<i>ae VII</i>	85.7 ± 1.4	42.5 ± 1.0c	6.8 ± 3.1a	48.9 ± 1.8c

<sup>a</sup> Values are mean ± standard deviation based on five replicates of the fractionation procedure.

<sup>b</sup> Common corn starch (CCS) and high amylose starches *ae du*, *ae V*, and *ae VII*.

<sup>c</sup> Based on the sum of weights of recovered fractions divided by the starting weight of nongranular starch. Moisture content assumed to be the same for all materials.

<sup>d</sup> Based on the weight of recovered fraction divided by the sum of the weights of the three recovered fractions.

<sup>e</sup> Values followed by the same letter in the same column are not significantly different ( $P < 0.05$ ).

The final precipitate (AM fraction) was dispersed in 50 mL of 90% DMSO, precipitated with 95% ethanol, washed, and then dried as described for the preparation of NG starch. The AP and IM in aqueous alcohol mixtures were both concentrated fivefold using a rotary evaporator (model RE111 Rotovapor with a model B-169 vacuum system, Büchi, Switzerland) with the water bath set at 50°C and a condenser connected to a circulating bath set at 5°C. The concentrated fractions were then precipitated and dried as described for the preparation of NG starch. All dried fractions were stored under desiccation (CaSO<sub>4</sub>). The same NG starch preparations were fractionated on five separate occasions. On each occasion, a sample of NG starch prepared from each starch type was fractionated.

### High-Performance SEC of Debranched Molecules

High-performance SEC analysis of debranched NG starch and debranched starch fractions was done using a system consisting of a pump (6000A solvent delivery system; Waters, Millipore Corp.; Milford, MA) connected in series to an injector (U6K universal chromatograph injector, Waters) and a differential refractometer (model 410 differential refractometer, Waters). Two 30-cm columns packed with 6- $\mu$ m porous silica microspheres (Zorbax PSM 60S, DuPont, Wilmington, DE) connected in series were used for the separations. The columns were maintained at 35°C using a column heater (Waters); the detector was also maintained at 35°C. The mobile phase in the system was 100% DMSO, which was degassed using sonication and vacuum before use. The flow rate of the system was 0.5 mL/min.

Detector responses and the resultant chromatograms were analyzed using Millennium Chromatography Manager 2010 software

**TABLE II**  
Iodine Binding Properties of Nongranular Starch (NG) and Starch Fractions Recovered by Differential Alcohol Precipitation

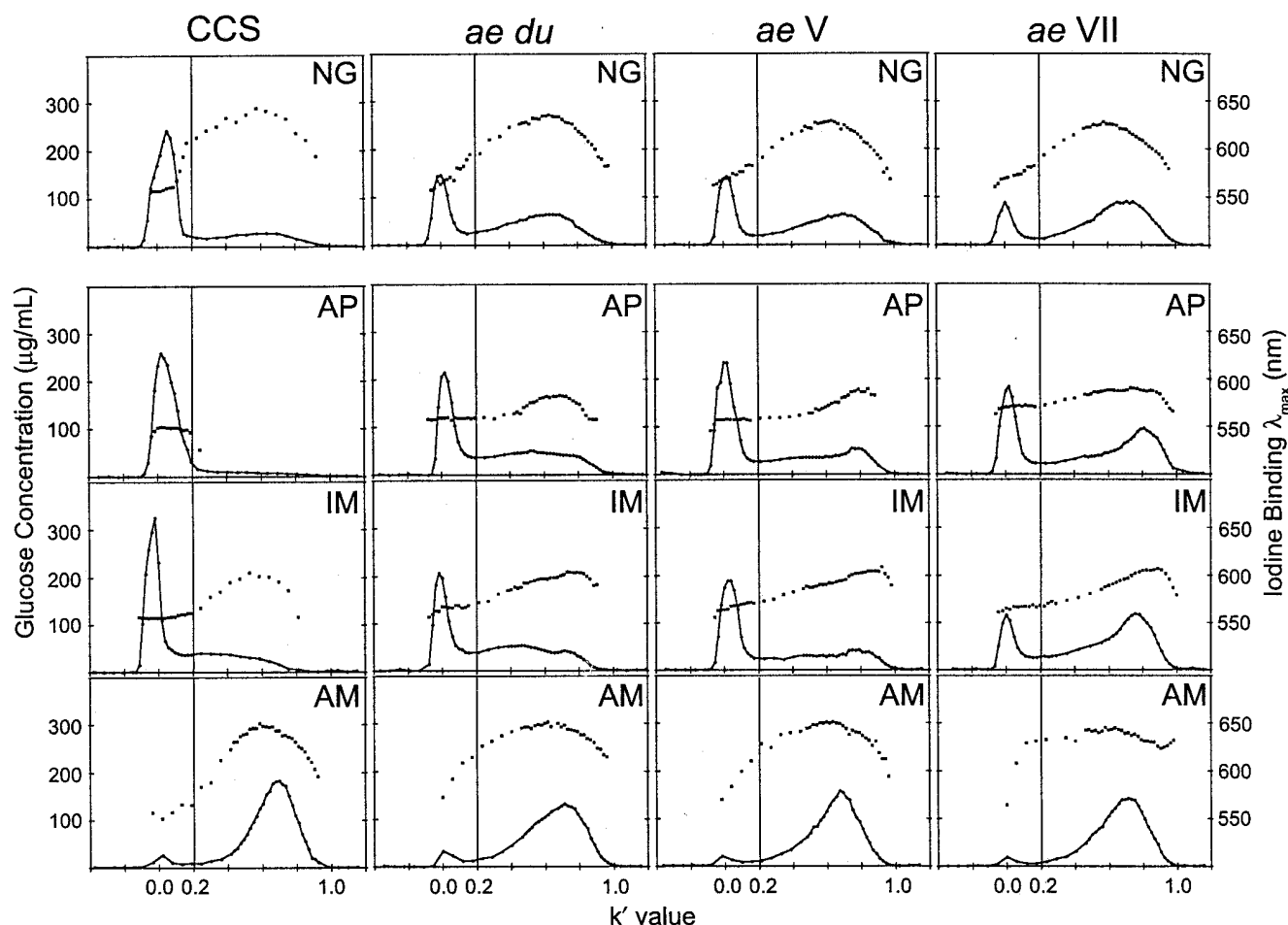
Starch Sample <sup>a</sup>	Blue Value <sup>b</sup>	$\lambda_{\max}$ <sup>c</sup>
CCS		
NG	0.41 ± 0.021	619 ± 0.6
AP	0.12 ± 0.071a <sup>d</sup>	559 ± 1.9
IM	0.21 ± 0.036b	594 ± 1.1
AM	1.22 ± 0.091c	655 ± 1.7
<i>ae du</i>		
NG	0.67 ± 0.018	618 ± 0.6
AP	0.29 ± 0.005a	577 ± 1.1
IM	0.42 ± 0.002b	597 ± 1.7
AM	1.17 ± 0.042c	648 ± 2.8
<i>ae V</i>		
NG	0.72 ± 0.004	611 ± 1.6
AP	0.42 ± 0.032a	575 ± 0.5
IM	0.49 ± 0.012b	588 ± 0.5
AM	1.27 ± 0.027c	647 ± 1.5
<i>ae VII</i>		
NG	0.98 ± 0.032	615 ± 0.5
AP	0.49 ± 0.003a	583 ± 0.3
IM	0.63 ± 0.012b	593 ± 0.5
AM	1.30 ± 0.005c	643 ± 1.9

<sup>a</sup> Common corn starch (CCS) and high amylose starches *ae du*, *ae V*, and *ae VII*. AM = amylose; AP = amylopectin; IM = intermediate material.

<sup>b</sup> From duplicate determinations. Blue value is the absorbance of starch-iodine mixture at 635 nm (Morrison and Laingelet 1983). See Fig. 1.

<sup>c</sup> Iodine binding wavelength maximum (Morrison and Laingelet 1983). From duplicate determinations. See Fig. 1.

<sup>d</sup> Values followed by the same letter within each starch are not significantly different ( $P < 0.05$ ).



**Fig. 2.** Size-exclusion chromatograms of nongranular starches (NG) and starch fractions. AM = amylose; AP = amylopectin; IM = intermediate material. CCS = common corn starch; *ae du*, *ae V*, *ae VII* represent high amylose starches. Total carbohydrate (solid line) and iodine binding  $\lambda_{\max}$  (broken line).

(Version 2.15; Waters). The system was calibrated using separate 50- $\mu$ L injections of five separate solutions: maltotriose (Sigma Chemical Co., St. Louis, MO), maltoheptaose (Sigma), and three pullulan standards (P-5, P-10, and P-20, molecular weights of  $5.8 \times 10^3$ ,  $1.22 \times 10^4$ , and  $2.37 \times 10^4$ , respectively) (Showa Denko, obtained through Waters).

**TABLE III**  
**Partial Chromatogram Areas of Nongranular Starch (NG)**  
**and Respective Starch Fractions from Size-Exclusion**  
**Chromatography**

Starch Sample <sup>b</sup>	Starch Eluting (%) <sup>a</sup>	
	Below $k' = 0.2$	Above $k' = 0.2$
CCS		
NG	70	30
AP	88	12
IM	68	32
AM	7	93
<i>ae du</i>		
NG	38	62
AP	47	53
IM	49	51
AM	9	91
<i>ae V</i>		
NG	32	68
AP	54	46
IM	51	49
AM	2	98
<i>ae VII</i>		
NG	19	81
AP	31	69
IM	16	84
AM	1	99

<sup>a</sup> Values are based on single determinations.

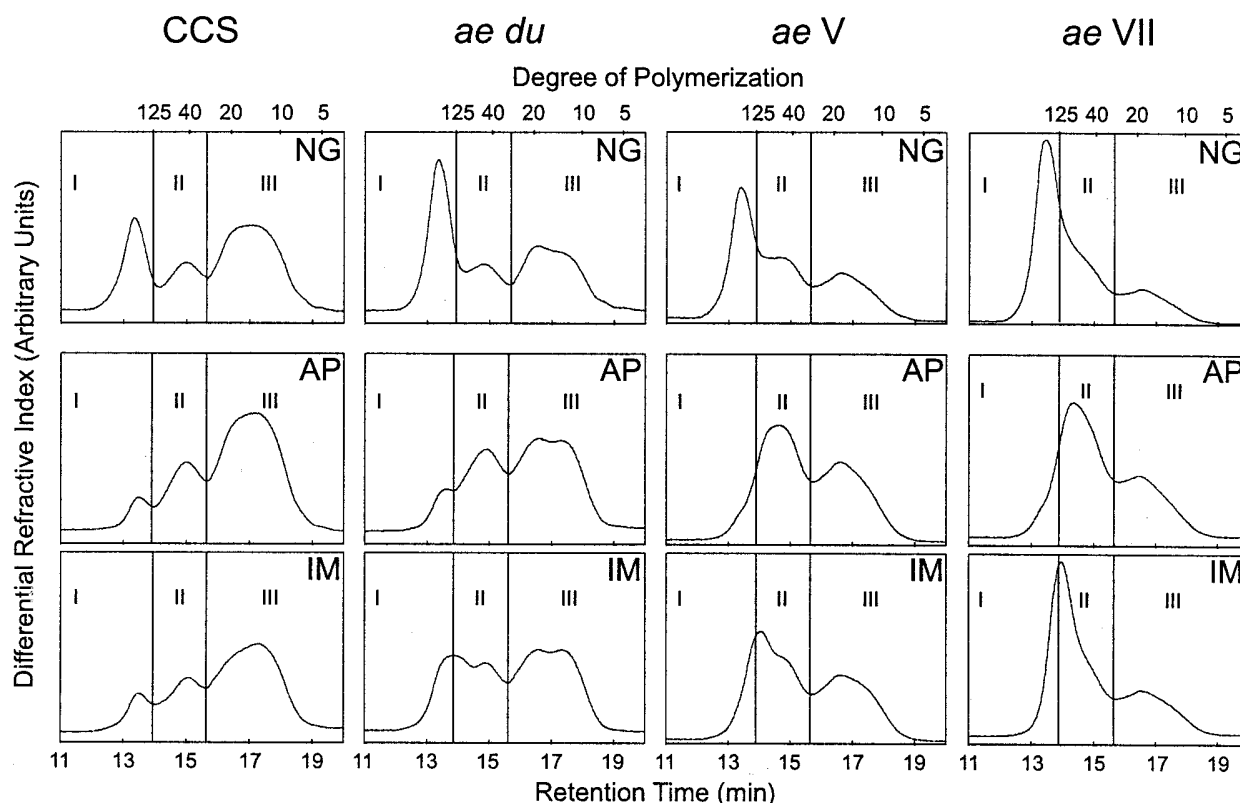
<sup>b</sup> Common corn starch (CCS) and high amylose starches *ae du*, *ae V*, and *ae VII*. AM = amylose; AP = amylopectin; IM = intermediate material.

Debranching of the NG starch, AP, and IM was based on the method of Hizukuri et al (1981) with modifications. NG starch, AP, and IM (5 mg) were redispersed in 120  $\mu$ L of 90% DMSO and heated for 10 min in a boiling water bath. Warm (50°C) sodium acetate buffer (880  $\mu$ L, pH 3.75, 0.05*N*) was added to the warm samples, which were then mixed gently, reheated for 10 min in a boiling water bath, and then cooled to 37°C.

A solution of isoamylase (Megazyme USA, Inc., Boseman, MT) (100  $\mu$ L; 0.5 units/mL, 0.05*N* sodium acetate, pH 3.75) was added to the redispersed starches. Samples were digested for 24 hr at 37°C with constant agitation in a water bath. After digestion, a 100- $\mu$ L aliquot of the mixture was diluted with 900  $\mu$ L of DMSO. The DMSO mixtures were heated in a boiling water bath for 10 min, allowed to cool to room temperature, then centrifuged at  $6,000 \times g$  for 10 min. After centrifugation, 50  $\mu$ L of the supernatant was injected into the high-performance SEC system. Two independent debranching experiments were conducted on all NG starch, AP, and IM samples. Preliminary experiments showed no change in the chromatographic profile of the debranched starch samples after an additional 24 hr of incubation with an additional 100  $\mu$ L of isoamylase solution.

#### Sepharose CL-2B Chromatography of Intact Molecules

NG starches and all starch fractions were chromatographed on a Sepharose CL-2B (Supelco Chromatography Products, Bellefonte, PA) SEC column (74 cm  $\times$  2.5 cm, Pharmacia Fine Chemicals, Sweden) using gravity flow. The mobile phase in the system was 0.01*M* sodium hydroxide containing 0.02% (w/v) sodium azide. Starch samples (15 mg) were dispersed in 1 mL of concentrated (10 $\times$ ) mobile phase for one to two days. The dispersed starches were diluted with 5 mL of deionized water, and then the entire 6 mL was loaded onto the column using a sample applicator (model SA-5; Pharmacia). For each sample, 500 mL of eluent was collected as 5-mL fractions using a fraction collector. Flow rate ranges were 20–30 mL/hr. The void volume and salt volume of the column were determined for a mixture of 1 mg of waxy starch and 1 mg of



**Fig. 3.** High-performance size-exclusion chromatography of isoamylase-debranched nongranular starches (NG) and starch fractions. AM = amylose; AP = amylopectin; IM = intermediate material. CCS = common corn starch; *ae du*, *ae V*, *ae VII* represent high amylose starches.

## RESULTS

### Properties of NG Starches and Starch Fractions

For all starches, the total recovery for the fractionation procedure was  $\approx 85\%$  (Table I). The highest proportion of AP was recovered from CCS, and the smallest proportion of AP was recovered from *ae VII* starch. For all starches, the proportion recovered as IM (6.8–7.9%) was not significantly different.

For each starch, the NG starting material and the three fractions had different iodine binding behaviors (Fig. 1, Table II). The AM from each starch had the highest absorbance at all wavelengths, the highest  $\lambda_{\max}$ , and the highest blue value. The AP from each starch had the lowest absorbance at all wavelengths (except for *ae VII* at low wavelengths), the lowest  $\lambda_{\max}$ , and the lowest blue value. The IM from each starch had a  $\lambda_{\max}$  and blue value between those of the respective AM and AP fractions. It was more similar to AP than to AM. The blue values of AP and of IM both decreased in the order: *ae VII* > *ae V* > *ae du* > CCS. For each starch, the wavelength scans from IM appeared to have an inflection between 700 and 720 nm not present in the AP wavelength scans (Fig. 1).

### Size Distribution of Intact Molecules

The Sepharose CL-2B chromatograms were divided into two regions: a region preceding  $k' = 0.2$  (considered to represent material eluting in the void volume) and a region after  $k' = 0.2$  (the region after the void volume) (Fig. 2). The void volume peak from all NG starch materials had an iodine binding  $\lambda_{\max}$  between 560 and 570 nm. The CCS NG starch  $\lambda_{\max}$  increased rapidly just before  $k' = 0.2$  to values >600 nm, with later fractions as high as 640 nm. For the HAS NG materials, the  $\lambda_{\max}$  gradually increased from the values of the void volume region, unlike the rapid increase observed for CCS. The highest  $\lambda_{\max}$  values of the HAS eluting after the void volume (620–630 nm) were somewhat lower than those observed with CCS. A gradual decrease in the  $\lambda_{\max}$  approaching  $k' = 1.0$  was observed for both CCS and the NG of HAS (Fig. 2).

Approximately 88% of AP from CCS was within the void volume region of the chromatogram and had a  $\lambda_{\max}$  near 550 nm (Fig. 2, Table III). Only 7% of AM from CCS was within the void volume region of the column. These molecules had an iodine binding  $\lambda_{\max}$  of

glucose by total carbohydrate analysis. Elution volumes for chromatograms of samples were converted to capacity factors ( $k'$ ) based on the elution volume of waxy starch ( $k' = 0$ ) and glucose ( $k' = 1$ ) (Yau 1969).

Every third SEC fraction was examined for total carbohydrate and iodine binding  $\lambda_{\max}$ , as was every fraction within 10 fractions of the total carbohydrate peak. Total carbohydrate of the fractions was determined using the phenol-sulfuric acid assay of Dubois et al (1956). The iodine binding  $\lambda_{\max}$  of all fractions examined for total carbohydrate was determined using the procedure of Krisman (1962) as modified by Baba et al (1982) and Boyer and Liu (1985). Immediately before the analysis, 0.5 mL of the stock iodine solution (0.026 g of  $I_2$  and 0.26 g of KI/mL of water) was diluted in 130 mL of deionized water to make the iodine solution used in the assay. A 0.8-mL aliquot of an SEC fraction was neutralized with 1.0N HCl (25  $\mu$ L) and mixed with 5.0 mL of iodine solution. The absorbance of the solutions was examined between 450 and 800 nm (DU650, Beckman Instruments, Inc.; Columbia, MD). Blanks for both total carbohydrate and iodine binding  $\lambda_{\max}$  analyses were prepared from fraction number 99 of the chromatography run. Areas of the chromatograms were determined by summation of the carbohydrate content of each fraction. The carbohydrate content of fractions that were not analyzed were obtained by interpolation.

### Blue Value and $\lambda_{\max}$

The blue value and  $\lambda_{\max}$  of NG starch and starch fractions were determined by the method of Morrison and Laignelet (1983) with slight modifications. Starches (40 mg) were redispersed in 10 mL of DMSO containing 10% 6M urea (UDMSO). A 1.0-mL aliquot of each sample was placed in a 100-mL volumetric flask, to which  $\approx 95$  mL of deionized water and 2 mL of an aqueous  $I_2$ -KI solution (2 mg of  $I_2$ /mL and 20 mg of KI/mL) were added. The mixture was brought to 100 mL with deionized water and mixed immediately. Blank solutions were made identically but without starch. All samples were examined from 500 to 800 nm. The blue value of the starch was defined as the absorbance at 635 nm. The  $\lambda_{\max}$  was the peak absorbance value over the range of wavelengths examined. Duplicate determinations were conducted on each NG starch sample and starch fraction.

TABLE IV  
Chain Length Distributions of Isoamylase-Debranched Nongranular Starch (NG), Amylopectin (AP), and Intermediate Material (IM) from Common Corn Starch<sup>a</sup>

Sample <sup>b</sup>	Total Area	Chromatographic Region		
		I	II	III
NG				
Peak DP		310 $\pm$ 19	45 $\pm$ 4	15 $\pm$ 0
DP <sub>n</sub>	23 $\pm$ 0	251 $\pm$ 6	50 $\pm$ 1	15 $\pm$ 0
DP <sub>w</sub>	70 $\pm$ 1	276 $\pm$ 6	60 $\pm$ 1	16 $\pm$ 0
Wt%		23.0 $\pm$ 0.96	20.0 $\pm$ 1.29	57.0 $\pm$ 0.33
Adj wt% <sup>c</sup>			26.0 $\pm$ 1.36	74.0 $\pm$ 0.55
Mol%		2.2 $\pm$ 0.03	9.7 $\pm$ 0.89	88.1 $\pm$ 0.87
AP				
Peak DP		245 $\pm$ 14	45 $\pm$ 2	15 $\pm$ 0
DP <sub>n</sub>	23 $\pm$ 0	230 $\pm$ 7	48 $\pm$ 1	15 $\pm$ 0
DP <sub>w</sub>	50 $\pm$ 0	253 $\pm$ 8	56 $\pm$ 2	16 $\pm$ 0
Wt%		4.9 $\pm$ 1.69	24.3 $\pm$ 1.36	70.8 $\pm$ 0.33
Adj wt% <sup>c</sup>			25.6 $\pm$ 0.97	74.4 $\pm$ 0.24
Mol%		0.4 $\pm$ 0.13	10.1 $\pm$ 0.83	89.5 $\pm$ 0.19
IM				
Peak DP		245 $\pm$ 14	43 $\pm$ 0	15 $\pm$ 0
DP <sub>n</sub>	25 $\pm$ 1	234 $\pm$ 2	49 $\pm$ 0	15 $\pm$ 0
DP <sub>w</sub>	63 $\pm$ 3	258 $\pm$ 2	59 $\pm$ 0	16 $\pm$ 0
Wt%		10.3 $\pm$ 0.63	24.8 $\pm$ 0.51	65.0 $\pm$ 0.12
Adj wt% <sup>c</sup>			27.6 $\pm$ 0.97	72.4 $\pm$ 0.64
Mol%		0.9 $\pm$ 0.06	10.8 $\pm$ 0.21	88.3 $\pm$ 0.55

<sup>a</sup> Values are means from two independent analyses.

<sup>b</sup> DP = degree of polymerization.

<sup>c</sup> Adjusted weight percentage does not include the area from region I of the chromatogram.

550 nm. The  $\lambda_{\max}$  of AM molecules eluting after  $k' = 0.2$  gradually increased, reached a maximum of  $\approx 660$  nm, then decreased as the  $k'$  value approached 1.0. For the IM of CCS, the  $\lambda_{\max}$  changed with the  $k'$  value in a manner similar to AM, but the highest  $\lambda_{\max}$  was only 600 nm. The IM within the void volume region appeared to have a slightly higher  $\lambda_{\max}$  (560 nm) than did AP.

The proportions of the HAS AP eluting after the void volume were all considerably higher than the 12% observed within the separation region for CCS AP (Fig. 2, Table III). The chromatograms (as observed by total carbohydrate and iodine binding  $\lambda_{\max}$ ) of the three HAS AP fractions were different from each other. For the three HAS, the proportions of IM eluting after the void volume were higher than the 32% observed for IM from CCS (Table III). The elution profiles of IM from the different HAS resembled the total carbohydrate profiles of the respective AP. However, the changes in the iodine binding  $\lambda_{\max}$  of IM and respective AP were different: for IM the  $\lambda_{\max}$  increased steadily through  $k'$  values of 0.7–0.8 to a maximum value of  $\approx 600$  nm. AM for the three HAS were similar to AM from CCS in the total carbohydrate elution profiles. The proportion of AM in the void volume was between 1% (for *ae* VII) and 9% (for *ae* *du*). The iodine binding behavior of AM from *ae* *du* and *ae* V was also very similar to AM from CCS; a  $\lambda_{\max}$  of 650 nm and similar  $\lambda_{\max}$  changes with  $k'$  value. The iodine binding behavior of AM from *ae* VII was different from the other three starches, as the  $\lambda_{\max}$  remained between 630 and 640 nm for nearly the entire chromatogram.

#### Chain Length Distribution of Debranched Molecules

For purposes of comparison, high-performance SEC chromatograms of debranched NG, AP, and IM for each of the four starches were divided into three regions (Fig. 3) based on the retention times of the two response minima for debranched AP from CCS. For NG from CCS, regions I, II, and III corresponded to 23, 20, and 57% of the total area (Table IV). The proportion of starch in region III of the NG starches decreased in the order: CCS > *ae* *du* > *ae* V > *ae* VII (Fig. 3, Tables IV–VII).

The proportion of starch in region I from the debranched AP and IM was less than that in region I from the respective debranched NG starch samples (Fig. 3, Tables IV–VII). In addition, for each

starch the peak degree of polymerization (DP) (when a peak was observed),  $DP_n$ , and  $DP_w$  of the region I material in AP and IM appeared to be different from that for the NG starch. For CCS, region I in AP and IM had a smaller peak DP,  $DP_n$ , and  $DP_w$  than did the NG starch. For the HAS, the relationships between AP and IM were different than for CCS, as region I in the HAS IM had a much lower  $DP_w$  and  $DP_n$  than did region I in AP.

The chromatograms of the debranched AP and IM of each HAS illustrate the major differences between regions I and II of each fraction (Fig. 3). In the debranched IM of HAS, the peaks in regions I and II overlapped considerably. For *ae* V and *ae* VII starches, IM did not exhibit a shoulder or peak in region I; instead these IM had a peak in the high molecular weight area of region II that was not observed in AP.

## DISCUSSION

### Molecular Composition of Starches

For NG CCS, the results from differential alcohol precipitation, from Sepharose CL-2B chromatography, and from chromatography of the debranched NG, indicated that normal amylose and amylopectin have been reasonably well fractionated from NG starch by the precipitation procedure. The NG CCS eluting after the void volume may be considered to be largely classical amylose because most of the molecules in the AM eluted after the void volume (93%) (Fig. 2). Moreover, only a small proportion of the molecules in the AP from CCS eluted after the void volume (12%). In NG CCS, the rapid shift in iodine binding  $\lambda_{\max}$  values to higher wavelengths also indicates a sharp transition from amylopectin to amylose. However, the proportion of amylose from CCS obtained by differential alcohol precipitation was only 23% of the total recovered material (Table I), smaller than the 30% amylose estimate obtained from chromatography of unfractionated starch (Fig. 2, Table III). Chromatograms of debranched NG CCS also indicated that 23% of the material eluted in region I (Fig. 3, Table IV), which likely originated from amylose (perhaps initially slightly branched) in CCS (Ferrone 1996). Although 32% of the CCS IM (Fig. 2, Table III) eluted after the void volume of the Sepharose CL-2B column (adding to a putative amylose content as determined by CL-2B

TABLE V  
Chain Length Distributions of Isoamylase-Debranched Nongranular Starch (NG), Amylopectin (AP), and Intermediate Material (IM) from *ae* *du* Starch<sup>a</sup>

Sample <sup>b</sup>	Total Area	Chromatographic Region		
		I	II	III
NG				
Peak DP		304 ± 30	51 ± 1	18 ± 1
$DP_n$	30 ± 0	250 ± 5	56 ± 1	15 ± 0
$DP_w$	108 ± 2	276 ± 5	69 ± 1	17 ± 0
Wt%		37.5 ± 1.21	22.4 ± 1.54	40.1 ± 0.33
Adj wt% <sup>c</sup>			35.8 ± 1.77	64.2 ± 1.59
Mol%		5.0 ± 0.12	13.4 ± 1.08	81.6 ± 1.54
AP				
Peak DP		215 ± 4	48 ± 1	19 ± 0
$DP_n$	23 ± 0	250 ± 5	56 ± 1	16 ± 0
$DP_w$	50 ± 0	252 ± 1	62 ± 1	17 ± 0
Wt%		9.0 ± 0.14	32.5 ± 0.26	58.5 ± 0.12
Adj wt% <sup>c</sup>			35.7 ± 0.23	64.3 ± 0.11
Mol%		0.9 ± 0.01	15.0 ± 0.25	84.1 ± 0.01
IM				
Peak DP		143 ± 7	48 ± 1	18 ± 1
$DP_n$	25 ± 1	222 ± 3	56 ± 0	15 ± 0
$DP_w$	63 ± 3	245 ± 4	69 ± 0	17 ± 0
Wt%		15.1 ± 1.41	31.7 ± 0.49	53.2 ± 0.91
Adj wt% <sup>c</sup>			37.4 ± 0.04	62.6 ± 0.73
Mol%		1.7 ± 0.18	14.6 ± 0.04	83.7 ± 0.92

<sup>a</sup> Values are means from two independent analyses.

<sup>b</sup> DP = degree of polymerization.

<sup>c</sup> Adjusted weight percentage does not include the area from region I of the chromatogram.

chromatography of NG CCS), 90% of IM was degraded to short chains by isoamylase. Thus, much of IM appeared to have a branched fine structure which resulted in a smaller region I peak by high-performance SEC than expected from the chromatogram.

Aside from the differences in the SEC elution profiles of the CCS AP and IM, the two branched fractions appeared to have some structural differences based on iodine binding  $\lambda_{\max}$  values (Table II). The CCS IM eluting in the void volume and after the void volume of the column had intermediate iodine binding behavior. The  $\lambda_{\max}$  values of the void volume material from IM were slightly higher (560 nm) than those of AP (545 nm) (Fig. 2), suggesting the void volume material in IM appeared to contain longer iodine-complexible chain segments than did AP. The IM eluting after the void volume appeared to behave similarly to AM, as indicated by the similarity in the changes in  $\lambda_{\max}$  with  $k'$  value for AM and IM on Sepharose CL-2B. Chromatograms of debranched AP and IM were very similar; the only major difference between the two fractions was in the proportion of debranched starch in region I (AP 5%, IM 10%). This difference would be consistent with the idea that IM is AP with AM contamination. However, chromatograms of CCS IM and AM at  $k' > 0.2$  suggest the situation is more complex (Fig. 2).

For HAS, if all of the material eluting after the void volume in the chromatograms of the NG starch were considered to be amylose, they would contain at least twice the amylose of CCS (Fig. 2, Table III). However, the proportion of AM in HAS determined by the differential alcohol precipitation (Table I) was smaller than the proportion of NG eluting after the void volume of the column (Table III) by  $\approx 20\%$  for *ae du* and *ae V*, and by  $\approx 30\%$  for *ae VII*. Chromatography of the fractions obtained by differential alcohol precipitation indicated that a large proportion of the total material from the NG HAS eluting after the void volume did not precipitate as AM (Fig. 2, Table III). As IM was only 6–8% of the total recovered starch (Table I), these discrepancies are due primarily to AP fractions from HAS (Fig. 2). When Baba et al (1982) and Wang et al (1993) chromatographed 1-butanol supernatant fractions of HAS, low molecular weight material was observed and defined as intermediate material. The present work would be consistent with

the interpretation that much of the HAS defined as intermediate material by Baba et al (1982) is a low molecular weight material which we would consider as amylopectin. We suggest that amylopectin from HAS may have a broad distribution of molecular weight, containing a subfraction of molecules smaller than amylopectin from CCS. SEC would separate this subfraction only for HAS.

Chain profiles of the debranched AP from HAS showed that only 9% of the debranched *ae du* AP was in region I of the chromatogram (Fig. 3, Tables V–VII). Similarly, 7.7% of *ae V* AP and 12.1% of *ae VII* AP were found in region I of the debranched chromatograms. Although this region I material might be interpreted as AM contamination, a larger proportion of the material eluted in region II of AP from HAS when compared to AP from CCS, and the peak DP, DP<sub>n</sub>, and DP<sub>w</sub> were higher for region II of AP from HAS than AP from CCS (Fig. 3, Tables IV–VII). For AP of HAS, much of the area of region I may be related to the major peak in region II; it is likely that the partial area of region I is not simple AM contamination for these samples. The  $\lambda_{\max}$  values for AP from HAS were higher than those of AP from CCS both for the materials in the void volume and for the materials eluting after the void volume in the chromatograms (Fig. 2). These higher values are consistent with the differences in the chain profiles of the debranched AP. All AP from HAS had larger proportions of starch chains in regions I and II than did AP from CCS.

For all starches, the debranched IM contained a larger proportion of chains in regions I and II (as a sum) when compared to the respective AP (Tables IV–VII). For the debranched HAS IM chromatograms (Fig. 3), a peak (at a DP  $\approx 125$ ) was observed in the high molecular weight portion of region II. It is not clear whether these long chains from the debranched molecules are from linear small molecules or originated as part of a branched molecule. Takeda et al (1993) suspected that the 1-butanol supernatant fractions contained low molecular weight linear materials (DP < 100) before debranching. Contrary to the hypothesis of Takeda et al (1993), our data (Figs. 2 and 3) suggest that the peak in regions I and II for IM may not represent short-chain linear materials at all; instead, the chains observed in regions I and II of the debranched IM (even with CCS) could be integral to the structure of the branched

TABLE VI  
Chain Length Distributions of Isoamylase-Debranched Nongranular Starch (NG), Amylopectin (AP), and Intermediate Material (IM) from *ae V* Starch<sup>a</sup>

Sample <sup>b</sup>	Total Area	Chromatographic Region		
		I	II	III
NG				
Peak DP		291 ± 11	—	19 ± 0
DP <sub>n</sub>	37 ± 0	243 ± 3	58 ± 0	16 ± 0
DP <sub>w</sub>	113 ± 1	269 ± 3	71 ± 0	18 ± 0
Wt%		36.9 ± 0.44	32.8 ± 0.49	30.3 ± 0.04
Adj wt% <sup>c</sup>			52.0 ± 0.42	48.0 ± 0.80
Mol%		6.1 ± 0.06	22.9 ± 0.10	71.0 ± 1.03
AP				
Peak DP		na <sup>d</sup>	61 ± 1	19 ± 0
DP <sub>n</sub>	29 ± 0	205 ± 1	56 ± 0	17 ± 0
DP <sub>w</sub>	56 ± 3	223 ± 1	73 ± 1	18 ± 0
Wt%		7.7 ± 1.68	48.2 ± 1.15	44.1 ± 0.53
Adj wt% <sup>c</sup>			52.2 ± 0.29	47.8 ± 0.00
Mol%		1.1 ± 0.21	25.3 ± 0.44	73.6 ± 0.19
IM				
Peak DP		na	118 ± 9	18 ± 0
DP <sub>n</sub>	30 ± 0	205 ± 1	60 ± 0	16 ± 0
DP <sub>w</sub>	69 ± 1	225 ± 1	73 ± 0	17 ± 0
Wt%		14.7 ± 0.57	43.4 ± 0.32	41.8 ± 0.25
Adj wt% <sup>c</sup>			50.6 ± 0.53	49.4 ± 1.10
Mol%		2.2 ± 0.07	22.2 ± 0.31	75.6 ± 1.49

<sup>a</sup> Values are means from two independent analyses.

<sup>b</sup> DP = degree of polymerization.

<sup>c</sup> Adjusted weight percentage does not include the area from region I of the chromatogram.

<sup>d</sup> Not applicable.

molecules. Banks et al (1970) examined the iodine binding abilities of acid-hydrolyzed waxy starches whose external chains were then extended using glucose-1-phosphate and phosphorylase. They found that the modified amylopectin had higher iodine binding capacities and increased  $\lambda_{\text{max}}$  values approaching 600 nm with increasing degrees of chain extension. If the structure of IM is similar to the modified amylopectin from Banks et al (1970), the long chains observed in the debranched IM could be integral to a branched, larger molecule and thus would be less likely to precipitate in the presence of 1-butanol. Long chains present in the intact IM fraction could also explain the presence of a shoulder between 650 and 750 nm observed in the spectrophotometric analysis of starch-iodine complexes of the IM fraction (Fig. 1).

### Differential Precipitation Behavior of AM, AP, and IM

In the simple case of an  $\alpha$ -(1 $\rightarrow$ 4) glucose chain of a particular DP interacting with either iodine, 1-butanol, or isoamyl alcohol, binding behavior would be affected by both the length of the glucose chain as well as a particular complexing agent. Extending this concept to starch (either fractionated or not) becomes complicated because of the distribution of chain lengths within the starch molecules (or more precisely, the distribution of lengths of uninterrupted chains) as well as the spatial relationship among complexed chains (if more than one complex per molecule).

Long, largely unbranched chains of amylose will all have a high iodine binding capacity and  $\lambda_{\text{max}}$ . Precipitation of amylose with 1-butanol involves a step after complexation: the aggregation of complexed chains into a separate phase sufficiently more dense than the aqueous phase (Liu et al 1997). One might envision that orientation could be accommodated by the light branching of amylose. Iodine complexation and 1-butanol precipitation are related because both require the formation of a complex. However, the set of molecules that will complex is not necessarily identical to the set that will precipitate, even for the same complexing agent. Some material not precipitated by 1-butanol could also have a high iodine affinity as well as a strong tendency to form a 1-butanol complex. In the specific case of AM, iodine complexation and 1-butanol precipitation could be closely related due to the lightly branched

structure and long chains, both of which would accommodate iodine binding and alcohol precipitation events.

The relatively short chains of amylopectin have relatively low affinities for iodine. In the specific case of AP, separation was based on a failure to precipitate with the combination of 1-butanol and isoamyl alcohol. Although the inability to precipitate does not imply that the alcohols cannot complex with AP, prior work (Rundle and French 1943) indicated that amylopectin from CCS does not complex with 1-butanol. Amylose and amylopectin from CCS are relatively well separated by SEC; consequently, precipitation with alcohols might appear to be dependent on the size of starch molecules. However, the chromatograms of AP from HAS, which contain considerable proportions of starch with a size similar to that of AM, indicate otherwise. The highly branched structure of AP appears to have the strongest influence on the alcohol precipitation ability of AP of all of the starches. Although the AP molecules of each HAS were contained within the same alcohol precipitation fraction, differences were observed in the iodine binding behavior of AP from different HAS. Consequently, differences in AP structure (based on iodine binding) can be accommodated among molecules that fail to precipitate with the combination of the two alcohols. A schematic diagram to explain the nature of the materials isolated as AM, AP, and IM from CCS and HAS is presented in Fig. 4.

The behavior of the IM fractions is the most difficult to account for. Relative to AM and AP, the respective IM of each starch has an intermediate ability to complex iodine. Because IM precipitates in the presence of 1-butanol and isoamyl alcohol but not in the presence of 1-butanol alone, IM has an intermediate capacity to precipitate. High-performance SEC of the debranched IM from HAS indicates that IM is a fraction of branched molecules that contain long chains not present in the debranched AP. SEC of intact molecules indicates that IM and AP have similar size distributions. Similar proportions of IM are recovered from both CCS and HAS. It is not clear why recovery of IM should be similar among all of the starches.

The properties and recovery of IM might be explained by: 1) amylose with amylopectin contamination due to insufficient reprecipitation, 2) interaction between amylose and amylopectin during precipitation leading to coprecipitation, or 3) the existence of an

TABLE VII  
Chain Length Distributions of Isoamylase-Debranched NG (non-granular starch), Amylopectin, and Intermediate Material from *ae* VII Starch<sup>a</sup>

Sample <sup>b</sup>	Total Area	Chromatographic Region		
		I	II	III
NG				
Peak DP		258 ± 24	—	19 ± 1
DP <sub>n</sub>	50 ± 1	239 ± 3	63 ± 0	18 ± 1
DP <sub>w</sub>	140 ± 3	264 ± 4	78 ± 0	18 ± 1
Wt%		46.4 ± 1.22	34.2 ± 0.86	19.4 ± 0.35
Adj wt% <sup>c</sup>			63.8 ± 0.16	36.2 ± 0.68
Mol%		10.7 ± 0.41	29.8 ± 0.04	59.5 ± 1.07
AP				
Peak DP		na <sup>d</sup>	82 ± 1	20 ± 0
DP <sub>n</sub>	35 ± 0	207 ± 1	58 ± 0	17 ± 0
DP <sub>w</sub>	69 ± 0	229 ± 1	70 ± 0	19 ± 0
Wt%		12.1 ± 0.23	54.1 ± 0.19	33.8 ± 0.04
Adj wt% <sup>c</sup>			61.5 ± 0.05	38.5 ± 0.23
Mol%		2.0 ± 0.03	32.5 ± 0.22	65.5 ± 0.47
IM				
Peak DP		na	127 ± 8	19 ± 0
DP <sub>n</sub>	40 ± 0	197 ± 2	68 ± 0	16 ± 0
DP <sub>w</sub>	89 ± 2	212 ± 3	82 ± 0	18 ± 0
Wt%		20.7 ± 1.56	51.3 ± 1.83	28.0 ± 0.28
Adj wt% <sup>c</sup>			64.7 ± 1.04	35.3 ± 0.97
Mol%		4.2 ± 0.30	31.0 ± 0.85	69.0 ± 1.01

<sup>a</sup> Regions were distinguished as described in the text. Values are means from two independent analyses.

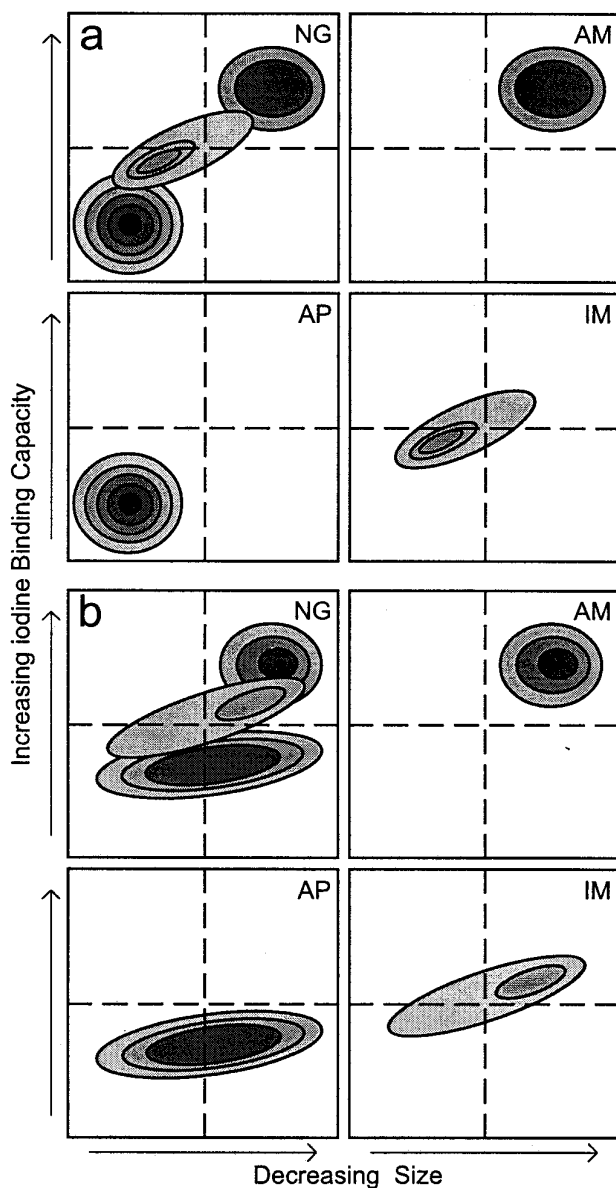
<sup>b</sup> DP = degree of polymerization.

<sup>c</sup> Adjusted weight percentage does not include the area from region I of the chromatogram.

<sup>d</sup> Not applicable.

intermediate molecular fraction with a size distribution and branching pattern related more closely to amylopectin than to amylose. The three precipitation steps with the combination of isoamyl alcohol and 1-butanol in the precipitation procedure would be expected to remove contaminating nonprecipitated material (amylopectin), leaving mostly amylose. However, most of the material in IM appears to be amylopectin-like. Thus, it is unlikely that the IM is amylose with amylopectin contamination.

If amylose and amylopectin coprecipitate during the precipitation procedure (perhaps due to alignment of single-helical complexing regions of the molecules), more amylopectin could remain in the IM fraction than would be expected if amylose and amylopectin did not interact. Because IM appears more similar to AP than AM, coprecipitation would have to lead to a higher proportion of AP than AM. This seems unlikely if amylose is the material most readily complexible.



**Fig. 4.** Hypothesized relationships between molecular size and iodine binding capacity for common corn starch (CCS) (a) and *ae* VII high amylose starch (b) and for fractions obtained by differential alcohol precipitation. Vertical and horizontal dotted lines are included for ease of comparison. Shaded areas indicate the relative concentrations for nongranular starches (NG) and the three fractions of each starch: AM = amylose; AP = amylopectin; IM = intermediate material.

Alternatively, IM might be considered a fraction of the branched molecules with a size distribution and gross branching structure similar to that of AP, but with some structural features different from AP resulting in altered physical behavior. The  $\lambda_{\max}$  value of the small ( $k' < 0.2$ ) IM molecules was larger than the  $\lambda_{\max}$  value of larger IM molecules, and increasingly larger differences between the  $\lambda_{\max}$  values of IM and AP molecules of similar size were observed for the small molecules. Consequently, the length of linear chains (or the extent of the branching) of IM appeared to become increasingly different from AP with decreasing molecular size (Fig. 2). The iodine binding relationships within IM and between IM and AP are consistent with the interpretation that the long chains observed in debranched IM and not present in the debranched AP (Fig. 3) were in a relatively high molar proportion in the molecules that eluted after the void volume as compared to those molecules that eluted at the void volume. In addition, the ratio of IM to AP was higher in HAS than in CCS (though the proportion of IM recovered from each starch was the same on a total recovery basis). As a result of reduced starch branching enzyme activity in *ae*-type endosperm (Boyer and Preiss 1978), it is reasonable to expect that a higher proportion of the branched molecules would behave as IM in HAS than in CCS. The similarity between the size distributions of IM and AP may indicate that IM molecules are biosynthetically related to AP molecules.

For CCS, the two major fractions are distinct in both size and iodine binding. Consequently, general agreement has been observed among chromatographic amylose determinations, iodine-binding-based amylose determinations, and 1-butanol precipitations. For the HAS, the size distributions of AP fractions may overlap considerably with AM. In addition, the relationship between alcohol precipitation behavior and iodine binding behavior of the branched molecules (AP and IM) is more complex for HAS when compared to CCS. The differences in size as well as complexation and precipitation behavior result in profound differences in the estimates of amylose in HAS by chromatography, iodine binding, and 1-butanol precipitation (Fig. 4).

## CONCLUSIONS

We conclude that HAS differ from CCS in three main respects: 1) HAS contain a higher proportion of a largely linear material similar to normal amylose, 2) both AP and IM from HAS contain molecules of a size typical for normal amylose, and 3) both AP and IM from HAS tend to have longer chains than AP from CCS. In each starch, AP and IM are subsets of the branched molecules, with AP responsible for the highest proportion. Differential alcohol precipitation is a useful method of fractionating HAS according to the nature of branching.

## ACKNOWLEDGMENTS

This research was supported in part through a United States Department of Agriculture National Research Initiative Grant 93-37500-9246.

## LITERATURE CITED

- Adkins, G. K., and Greenwood, C. T. 1969. Studies on starches of high amylose-content. X. An improved method for the fractionation of maize and amylo maize starches by complex formation from aqueous dispersion after pretreatment with methyl sulphoxide. *Carbohydr. Res.* 11:217-224.
- Baba, T., and Arai, Y. 1984. Structural characterization of amylopectin and intermediate material in amylo maize starch granules. *Agric. Biol. Chem.* 48:1763-1775.
- Baba, T., Arai, Y., Yamamoto, T., and Itoh, T. 1982. Some structural features of amylo maize starch. *Phytochemistry* 21:2291-2296.
- Baba, T., Uemura, R., Hiroto, M., and Arai, Y. 1987. Structural features of amylo maize starch: Components of Amylon 70 starch. *J. Jpn. Soc. Starch Sci.* 34:213-217.

- Banks, W., and Greenwood, C. T. 1975. *Starch and Its Components*. John Wiley and Sons: New York.
- Banks, W., Greenwood, C. T., and Kahn, K. M. 1970. The properties of synthetic amylopectin with long external-chains. *Stärke* 22:292-296.
- Boyer, C. D., and Preiss, J. 1978. Multiple forms of starch branching enzyme of maize: Evidence for independent genetic control. *Biochem. Biophys. Res. Comm.* 80:169-175.
- Boyer, C. D., and Liu, K.-C. 1985. The interaction of endosperm genotype and genetic background. I. Differences in chromatographic profiles of starches from nonmutant and mutant endosperms. *Starch/Stärke* 37:73-79.
- Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., and Smith, F. 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* 28:350-356.
- Ferrone, J. T. 1996. Glucan composition and physical behavior of three high-amylose starches. MS thesis. The Pennsylvania State University: University Park, PA.
- Hizukuri, S., Takeda, Y., Yasuda, M., and Suzuki, A. 1981. Multi-branched nature of amylose and the action of debranching enzymes. *Carbohydr. Res.* 94:205-213.
- Krisman, C. R. 1962. A method for the colorimetric estimation of glycogen with iodine. *Anal. Biochem.* 4:17-23.
- Lansky, S., Kooi, M., and Schoch, T. J. 1949. Properties of the fractions and linear subfractions from various starches. *J. Am. Chem. Soc.* 71:4066-4075.
- Liu, H., Arntfield, S. D., Holley, R. A., and Aime, D. B. 1997. Amylose-lipid complex formation in acetylated pea starch-lipid systems. *Cereal Chem.* 74:159-162.
- Morrison, W. R., and Laignelet, B. 1983. An improved colorimetric procedure for determining apparent and total amylose in cereal and other starches. *J. Cereal Sci.* 1:9-20.
- Rundle, R. E., and French, D. 1943. The configuration of starch in the starch-iodine complex. III. X-ray diffraction studies of the starch-iodine complex. *J. Am. Chem. Soc.* 65:1707-1710.
- Schoch, T. J. 1942. Fractionation of starch by selective precipitation with butanol. *J. Am. Chem. Soc.* 64:2957-2961.
- Schoch, T. J. 1954. Preparation of starch and the starch fractions. Pages 5-17 in: *Methods in Enzymology*. III. S. P. Colowick and N. K. Kaplan, eds. Academic Press: New York.
- Takeda, Y., Hizukuri, S., and Juliano, B. O. 1986. Purification and structure of amylose from starch. *Carbohydr. Res.* 148:299-308.
- Takeda, Y., Shitaozono, T., and Hizukuri, S. 1988. Molecular structure of corn starch. *Starch/Stärke* 40:51-54.
- Takeda, Y., Maruta, N., and Hizukuri, S. 1992. Structures of amylose subfractions with different molecular sizes. *Carbohydr. Res.* 226:279-285.
- Takeda, C., Takeda, Y., and Hizukuri, S. 1993. Structure of the amylopectin fraction of amylo maize. *Carbohydr. Res.* 246:273-281.
- Wang, L. Z., and White, P. J. 1994. Structure and properties of amylose, amylopectin, and intermediate materials of oat starches. *Cereal Chem.* 71:263-268.
- Wang, Y.-J., White, P., Pollak, L., and Jane, J. 1993. Amylopectin and intermediate materials in starches from mutant genotypes of the Oh43 inbred line. *Cereal Chem.* 70:521-525.
- Whistler, R. L., and Doane, W. M. 1961. Characterization of intermediary fractions of high-amylose corn starches. *Cereal Chem.* 38:251-255.
- Wilson, E. J., Schoch, T. J., and Hudson, C. S. 1943. The action of *macerans* amylase on the fractions from starch. *J. Am. Chem. Soc.* 65:1380-1383.
- Yau, W. W. 1969. Steric exclusion and lateral diffusion in gel-permeation chromatography. *J. Polym. Sci., Part A-2* 7:483-495.
- Yeh, J. Y., Garwood, D. L., and Shannon, J. C. 1981. Characterization of starch from maize endosperm mutants. *Starch/Stärke* 33:222-230.

[Received March 5, 1998. Accepted August 11, 1998.]