Location of Amylose in Normal Starch Granules. I. Susceptibility of Amylose and Amylopectin to Cross-Linking Reagents[1,2]

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ABSTRACT

When granular starch was cross-linked, more amylopectin than amylose molecules were found cross-linked. For example, when corn starch was treated with cross-linking reagent (0.07% epichlorohydrin) (pH 10.5 for 24 hr), 91% of its amylopectin and 45% of its amylose became insoluble. Cross-linking of pregelatinized and dispersed starch caused less difference in the proportion of soluble amylose and amylopectin than did the cross-linking of native granular starch. After the starch had been cross-linked in the granular form, gel-permeation chromatograms showed no increase in the size of amylose as a result of cross-linking between two or more amylose molecules. However, susceptibility of the amylose to sequential hydrolysis by isoamylase and β-amylase decreased. The relative blue values of amylopectin peaks indicated that amylose was cross-linked to amylopectin. This was confirmed when the amylopectin isolated from cross-linked starches was debranched with isoamylase. These results are consistent with the view that amylose is interspersed among amylopectin molecules in corn and potato starch granules.

Cereal Chem. 69(4):405-409

Amylose and amylopectin are the major molecular constituents of starch. Proportions of both depend on starch sources (Lineback 1984). For example, normal corn starch contains about 28% amylose and 72% amylopectin, and potato starch contains about 20% amylose and 80% amylopectin. When starch, heated in aqueous alcohol solutions, undergoes a thermal transition from a double to a single helical conformation, normal starch granules retain integrity, whereas waxy corn starch granules are disrupted (Jane et al 1986). It is plausible that amylose in the normal starch interacts with amylopectin, thereby preserving starch granule integrity (Jane et al 1986). Comparison of the amylose content in starch of different maturities has suggested that amylose is more concentrated at the periphery of the granule (Boyer et al 1976). An investigation of starch granule erosion using $CaCl_2$ confirmed the results of that study (Shen and Jane 1991). It is not clear, however, where amylose is located in relation to amylopectin in native starch granules and whether amylose and amylopectin intersect in the granule. Blanshard (1986) proposed that amylose was present in bundles at amorphous regions in wheat starch but partly cocrystallized with amylopectin in potato starch.

In this study, we intended to explore the location of amylose in relation to amylopectin in starch granules by introducing low to intermediate degrees of cross-linking. Epichlorohydrin (ECH) (Wurzburg 1960; Gough and Pybus 1967, 1968) and adipic anhydride (Wurzburg 1960) were selected as cross-linking reagents. Both of the reagents react with starch and produce cross-links as well as monoderivatives (Wurzburg 1960). ECH and adipic acid have respective molecular chain lengths of about 4 and 7.5 Å. Therefore, ECH and adipic anhydride can preferentially react with two −OH groups no more than 4 and 7.5 Å apart, respectively. In other words, only two adjacent molecules (within distances of 4 and 7.5 Å, compared with 3.5 Å, the width of a glucose unit) can be cross-linked by a single molecule of the reagent. If amylose molecules are interspersed among amylopectin molecules, the cross-linking reaction should covalently link amylose to amylopectin. The cross-linking sites are likely at the amorphous region (between clusters) of the amylopectin (Hood and Mercier 1978).

[1]Journal Paper J-14405 of the Iowa Agriculture and Home Economics Experiment Station, Ames, IA. Project 2863.
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Because the amylopectin molecule is about 100 times larger than the amylose molecule (Banks et al 1972, Banks and Greenwood 1975), the two can be resolved by gel-permeation chromatography (GPC). Cross-linking between amylose and amylopectin should result in an increase of blue value in the amylopectin peak in a gel-permeation profile. If amylose molecules are isolated from amylopectin molecules and are present in bundles at the amorphous region (Blanshard et al 1984, Blanshard 1986), the amylose molecules should be preferentially cross-linked among themselves at low to intermediate degrees of cross-linking. This should result in an increase in amylose molecular size and a corresponding shift of the amylose peak in a gel-permeation profile to a lower elution volume.

MATERIALS AND METHODS

Normal corn and potato starches, ECH, adipic acid, acetic anhydride, anthrone, and β-amylase were purchased from Sigma Chemical Co. (St. Louis, MO). One unit of β-amylase was defined as 1.0 mg of maltose liberated from starch in 3 min at pH 4.8 and 20°C. Potato amylose was a product of Baker Chemical Co. (Phillipsburg, NJ). High-amylose corn starches (Hylon V and VII) were gifts from National Starch and Chemical Corp. (Bridgewater, NJ). *Pseudomonas amyloderamosa* isoamylase was purchased from Hayashibara Biochemical Laboratories, Inc. (Okayama, Japan). Isoamylase activity is defined by the increase in iodine blue color after the debranching of waxy rice starch (Yokobayashi et al 1970). A mixture of waxy rice starch (0.71%) and enzyme in 0.07*M* acetate buffer (pH 3.5) was incubated for 1 hr at 40°C. An aliquot (0.5 ml) of the reaction mixture mixed with 0.01*M* iodine-potassium iodide solution (0.5 ml) was made to 12.5 ml with water, and absorbance was read at 620 nm. One unit of activity increased A_{620} by 0.1. All other chemicals were reagent grade and used without further treatment.

Gel-Permeation Column Chromatography

Columns (2.6 cm i.d. × 90 cm, or as specified) of Sepharose CL-2B and Sephadex G-25 and G-100 (Pharmacia, Sweden) were used for the GPC study (Colonna and Mercier 1984). The columns were run in the ascending mode with 0.02% aqueous sodium azide or 0.02% sodium chloride solution as the eluant (0.5 ml/min, or as specified). Starch (1%) was stirred with dimethyl sulfoxide (DMSO) (90%, v/v) in a water bath for 1 hr at 96°C and then stirred for 24 hr at 25°C. The starch solution aliquot (15 mg/1.5 ml, dry-starch basis [dsb]) was mixed with absolute ethyl alcohol (4.5–6 ml) to precipitate the starch, which was recovered by centrifugation. The precipitated starch was redissolved in boiling water (5 ml) and stirred for 20 min, and the mixture was centrifuged (5,000 × *g* for 30 min) to remove the insoluble residues. The supernatant then was injected into the column, and fractions (5 ml, or as specified) were collected for total carbo-

hydrate and blue value determinations. Starch moisture content was determined by drying the starch in a forced-air oven at 115°C for 4 hr.

Determinations of Total Carbohydrate and of Blue Value

Fractions were analyzed simultaneously for total carbohydrate and amylose using a dual-channel Technicon AutoAnalyzer II (Tarrytown, NY). In some instances, the fractions were manually processed. The anthrone-sulfuric acid procedure (Wright and Gann 1966) was used for total carbohydrate analysis with the AutoAnalyzer. The resulting blue-green complex was measured at 630 nm. The phenol-sulfuric acid procedure was used in the manual analyses (Dubois et al 1956), where absorbance was read at 470 nm. In both cases, glucose was the standard reference carbohydrate. Iodine reagent (Juliano 1971) was used for the blue-value analysis in both the automatic and manual analyses, where a blue color was measured at 640 nm.

Cross-Linking of Starch and Amylose

Cross-linking with ECH. Starch (180 g, or as specified) was suspended in 300 ml of distilled water and stirred for 2 hr at 25°C for rehydration. ECH was added at predetermined concentrations (0.013, 0.07, and 0.13% [ECH/starch = w/w]), and the mixture was stirred for 2–24 hr, depending on the type of starch, to allow ECH diffusion into the starch granules. The solution pH then was adjusted to pH 10.5, or as specified, with sodium hydroxide solution (1*M*) to facilitate cross-linking. The reaction was allowed to proceed (24 hr, or as specified) and was stopped by neutralizing the mixture to pH 5.5 with glacial acetic acid. The starch was collected by filtration, washed twice with distilled water and once with alcohol, and dried at 40°C for 48 hr in a forced-air oven.

Amylose (4 ml at 15 mg per milliliter of 90% DMSO) was mixed with ECH (1.6 mg in 0.8 ml of 30% ethanol) and a subsequent addition of NaOH (1*M*) to achieve the final concentration of 0.06*M*. After the addition of NaOH, a precipitate formed, both in the presence and the absence of ECH. The mixture was stirred 24 hr at 25°C, and the reaction was stopped by neutralizing the solution to pH 5.5 with glacial acetic acid. The mixture containing ≤5.3% ECH (ECH/amylose = w/w) eventually appeared homogeneous and turned clear, but that with ≥8% ECH remained cloudy even after the neutralized mixture was stirred for several hours. GPC was performed with the amylose cross-linked with 2.67% ECH.

Pregelatinized starch was treated with ECH after being dissolved in 0.5*M* NaOH at 4°C for 2 hr. The starch solution (5%, dsb) was neutralized (pH 5.8), and ECH (0.5% = ECH/starch = w/w) was added. NaOH solution (1*M*) was added to adjust the solution to pH 11. The cross-linking reaction was allowed to proceed for 4 hr and was stopped by neutralizing the solution to pH 5.5 with glacial acetic acid. The cross-linked starch was isolated by precipitation with excess alcohol and washed with methanol.

Cross-linking with adipic anhydride. Mixed adipic anhydride was prepared by mixing adipic acid and acetic anhydride (1:30, or as specified; pH 9) at 25°C for 1 hr (Wurzburg 1960). The mixture was added to a starch slurry at 25°C, and the pH was maintained at 9 throughout the reaction (Wurzburg 1960).

Index of Amylose Incorporation

An index of amylose incorporation into amylopectin (I_{am}) was calculated according to the following equation: $I_{am} = [(\text{blue value}/\text{total CHO})_{\text{amylopectin}}]/[(\text{blue value}/\text{total CHO})_{\text{amylose}}]$. In this equation, the ratio of blue value to total carbohydrate in the amylopectin peak was divided by the same ratio in the amylose peak of a given GPC profile.

Pasting Properties of Native and Cross-Linked Starches

A Brabender Viscoamylograph (C. W. Brabender Inc., Hackensack, NJ) was used for starch-pasting analyses (Smith 1964). Suspensions (400 g, total weight) of both corn starch (8%, dsb) and potato starch (5%, dsb) were prepared at 25°C and heated to 97.5°C (1.5°C/min), held (97.5°C for 30 min), and finally cooled to 50°C (1.5°C/min).

Debranching Reactions of Amylopectin and Starch

Amylopectins of native and cross-linked corn starches were isolated using a Sepharose CL-2B gel-permeation column. All of the fractions except the last two in the amylopectin peak were pooled, and the combined fraction was vacuum-evaporated to remove excess water. Ethanol (four volumes) was added, and the precipitated amylopectin was isolated by centrifugation.

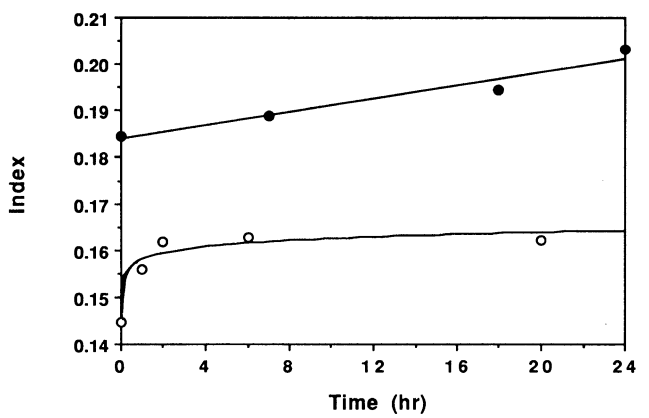

Fig. 1. Indices of amylose attachment to amylopectin in corn starch (○) cross-linked with 0.0067% epichlorohydrin (ECH/starch = w/w) at pH 12.2 in the presence of 2*M* sodium sulfate and potato starch (●) cross-linked with 0.0067% ECH at pH 9.5. The indices are plotted against the duration of the cross-linking reaction. Amylose and amylopectin were separated by a Sepharose CL-2B column (2.5 cm i.d. × 80 cm).

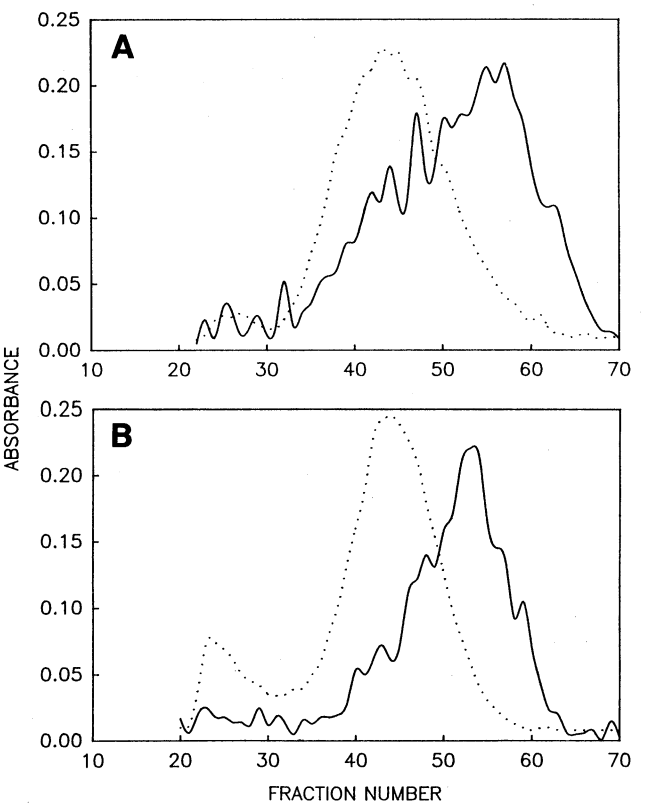

Fig. 2. Sephadex G-100 column (2.5 cm i.d. × 60 cm) chromatogram of *Pseudomonas* isoamylase hydrolysate of amylopectins isolated from native corn starch **(A)** and cross-linked corn starch **(B)**. The column was developed with 0.02% sodium azide solution, and the flow rate was 0.32 ml/min. Fractions (6 ml) were analyzed for total carbohydrate (phenol-sulfuric acid procedure) (solid line) and blue value (amylose-iodine complex) (dotted line).

The amylopectin (about 10 mg) was redissolved in 10 m*M* sodium acetate buffer (5 ml, pH 3.8) containing 20% DMSO. Excess isoamylase (300 units) was added to the amylopectin-buffer solution, and the mixture was incubated at 40°C for 24 hr (Lee et al 1968, Yokobayashi et al 1970, Mercier and Kainuma 1975). After incubation, the digest was heated in a water bath (96°C for 20 min) and centrifuged (5,000 × *g* for 30 min) to remove insoluble material. The debranched amylopectin was applied to a Sephadex G-100 column (2.5 cm i.d. × 60 cm). The column was developed at a flow rate of 0.32 ml/min, and fractions (6 ml) were collected and analyzed for total carbohydrate.

β-Amylolysis of Amyloses Isolated from Native and Cross-Linked Starches

Amyloses of both native and cross-linked normal corn starches were isolated with a Sepharose CL-2B column. The fractions in the amylose peak were pooled, and the amylose was isolated as described. The amylose (15 mg) was redissolved in 20 m*M* acetate buffer solution (pH 5, 20 m*M*, 15 ml) and incubated with 30 units of β-amylase at 30°C for 24 hr (Ring 1985). After incubation, the digest was heated in a water bath (96°C) for 20 min to inactivate the enzyme, and the hydrolysate was analyzed with a Sephadex G-25 column.

The amylose (about 15 mg) also was dissolved in 10 m*M* acetate buffer (15 ml, pH 3.8), and isoamylase (328 units) was added. After incubation at 45°C for 24 hr, the solution was adjusted to pH 5 and incubated with excess β-amylase at 30°C for 24 hr. The percentage of β-amylolysis was calculated from the total carbohydrate present in the maltose peak.

RESULTS AND DISCUSSION

GPC (Sepharose CL-2B) profiles of cross-linked starches exhibited greater blue values in the amylopectin peak than did those of native starches. This finding suggests that amylose was cross-linked to amylopectin and, thus, eluted with it. The index of amylose incorporation (I_{am}) increased with the degree of cross-linking for both corn and potato starch (Fig. 1). Isoamylase debranching experiments revealed that amylopectin isolated from native starch released only amylodextrins (Fig. 2A), whereas amylopectin isolated from cross-linked starch, which had a higher index of amylose incorporation, released amylose (as indicated by increase of blue value at the void volume of Sephadex G-100 column) as well as amylodextrins (Fig. 2B). These findings confirmed that the increased blue value in the amylopectin peak resulted from amylose-amylopectin cross-linking.

With a low level of cross-linking agent (ECH, 0.0067%) and with identical rehydration (2 hr) and premixing (2 hr) steps, the I_{am} for corn starch (pH 12.2) plateaued after 2 hr, whereas the I_{am} for potato starch (pH 9.5) increased linearly up to 24 hr. This difference may be attributable to different cross-linking rates at pH 12.2 and 9 and perhaps to granular size and porous structure in the granules. Nevertheless, both results showed that amylose was cross-linked to amylopectin by ECH.

The viscosity consistencies of corn and potato starch pastes increased with the degree of cross-linking (data not shown), whereas the solubility of the starches decreased for cross-linked corn starch (data not shown). Both results confirmed that cross-linking occurred. Gel-permeation profiles of cross-linked starches indicated that amylopectin was preferentially cross-linked and insolubilized (Fig. 3A–D). Proportions of the soluble amylopectin and amylose, calculated from the sum of carbohydrate in the two peaks, are listed in Tables I and II for corn and potato starches, respectively. The results indicated that the amylopectin peak decreased with the amount of the cross-linking agent applied. The profile (Fig. 3B) of cross-linked corn starch (0.07% ECH, pH 10.5 for 24 hr) exhibited a small amylopectin peak equivalent to 9.1% of the peak area observed in native corn amylopectin, whereas the amylose peak of the cross-linked starch was reduced to 55% of the peak area of amylose in native corn starch (Fig. 3A and B).

The solution of the cross-linked corn starch in 90% DMSO remained turbid after 2 hr of heating and stirring in a water bath (96°C) and after 24 hr of stirring (25°C). The cooked and swollen granules, when viewed under a light microscope, showed sharp boundaries. These results suggest that about half of the amylose originally in the granule was not connected to the cross-linked polymer network. Elution profiles and solubilities of cross-linked starch prepared with adipic anhydride were similar to those prepared with ECH (data not shown).

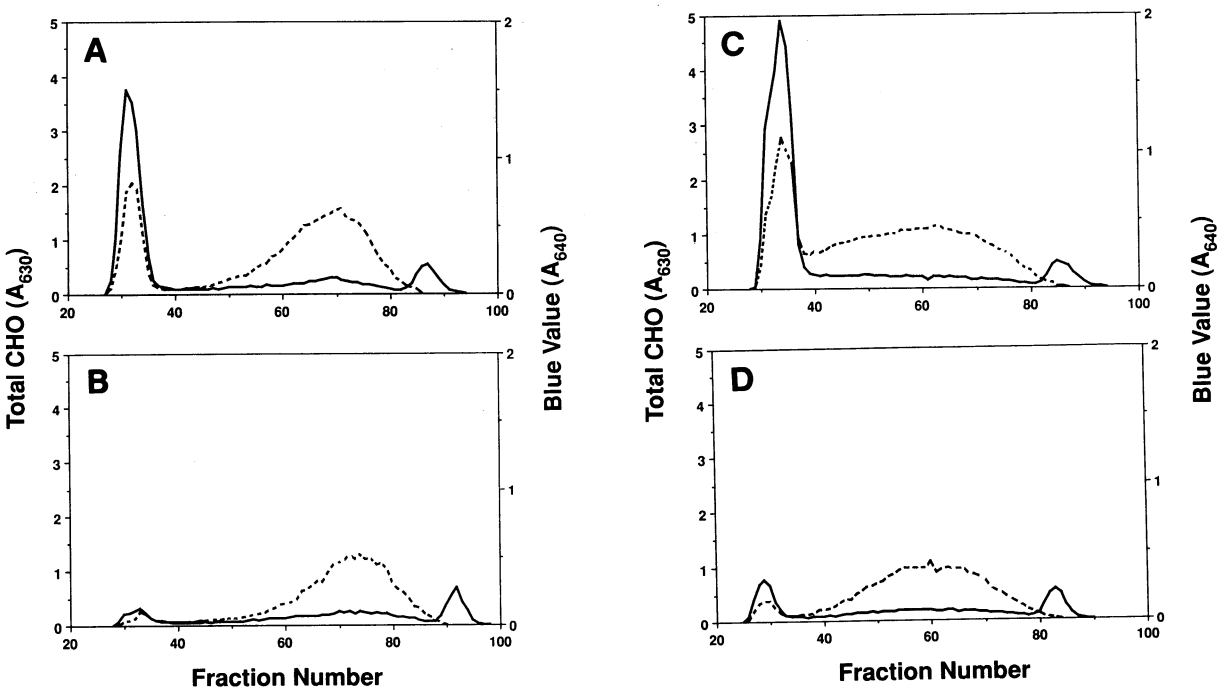

Fig. 3. Sepharose CL-2B column (2.6 cm i.d. × 90 cm) profiles of native corn starch **(A)**, corn starch cross-linked with 0.07% epichlorohydrin (ECH) at pH 10.5 for 24 hr **(B)**, native potato starch **(C)**, and potato starch cross-linked with 0.07% ECH at pH 10.5 for 24 hr **(D)**. The column was eluted with 0.02% sodium chloride aqueous solution, and the flow rate was 0.5 ml/min. Fractions (5 ml) were analyzed for total carbohydrate (anthrone/sulfuric acid procedure) (solid line) and blue value (amylose-iodine complex) (dotted line). Glucose was used as a marker.

Cross-linking of pregelatinized normal corn and potato starches, however, produced GPC profiles that had proportions of amylose and amylopectin similar to those of native starches (Table III). Differences in the proportions of amylose and amylopectin between cross-linked granular and cross-linked pregelatinized starches could be attributed to the closely packed, highly crystalline structure of amylopectin in the starch granule. In the granule, closely packed amylopectin was more susceptible to cross-linking than was amylose. In the pregelatinized form, however, amylopectin crystallites were destroyed, and the amylose and amylopectin in the solution were equally susceptible to cross-linking. Thus, the proportions of soluble amylose and amylopectin were similar to those of the native starches.

Cross-linking of amylose in DMSO solution with ECH showed that after the reaction was initiated with aqueous NaOH, amylose precipitated rapidly from the solution. The precipitated amylose, which was in close proximity, resembled the model wherein amylose is postulated to be separated from amylopectin and located in bundles. A Sepharose CL-2B column profile showed that the peak of the cross-linked amylose (2.67% ECH for 24 hr) broadened and shifted to higher molecular weights than did the peak of native amylose (Fig. 4A and B). At this high level of ECH (one ECH per 21 glucose units), some amylose was highly cross-linked and was eluted at the void volume. In granular form, however, amylose from neither normal starches (Fig. 3B and D) nor high-amylose corn starch (data not shown) would cross-link, as indicated by no observable increase in molecular size, even to a high level of ECH that insolubilized the starches. Additionally, normal corn starch treated with adipic anhydride (7.5-Å chain length) did not exhibit any increase in amylose molecular size either (data not shown). All of these results suggested that in the granule, amylose molecules were not located in bundles but rather were interspersed among amylopectin molecules.

Amylose isolated from cross-linked corn starch showed more resistance to β-amylolysis than amylose isolated from native corn starch (Table IV). Because there was no evidence of increase in molecular size by cross-linking, we attributed the resistance to monosubstitutes of ECH along the amylose chains instead of

TABLE I
Proportion of Amylopectin (AP) and Amylose (AM) Present in Soluble Fraction of Native and Cross-Linked Granular Corn Starches

Corn Starch	AP[a] (%)	AM[a] (%)
Native corn	72.7 ± 0.5	27.3 ± 0.5
Cross-linked corn[b]		
0.013% ECH,[c] 6 hr	71.1 ± 0.2	28.9 ± 0.2
0.013% ECH, 17 hr	68.0	32.0
0.07% ECH, 24 hr	26.9 ± 3.4	73.1 ± 3.4
0.13% ECH, 24 hr	8.2 ± 1.2	91.8 ± 1.2

[a]Calculated on the basis of total carbohydrate present in amylose and amylopectin peaks separated by the Sepharose CL-2B column.
[b]pH 10.5.
[c]Epichlorohydrin.

TABLE II
Proportion of Amylopectin (AP) and Amylose (AM) Present in Soluble Fraction of Native and Cross-Linked Granular Potato Starches

Potato Starch	AP[a] (%)	AM[a] (%)
Native potato	75.3 ± 1.2	24.7 ± 1.2
Cross-linked potato[b]		
0.013% ECH,[c] 6 hr	64.5 ± 3.7	34.6 ± 3.7
0.07% ECH, 24 hr	38.8 ± 1.9	61.2 ± 1.9
0.130% ECH, 24 hr	26.7	73.3

[a]Calculated on the basis of total carbohydrate present in amylose and amylopectin peaks separated by the Sepharose CL-2B column.
[b]pH 10.5.
[c]Epichlorohydrin.

TABLE III
Proportion of Amylopectin (AP) and Amylose (AM) Present in Soluble Portions of Cross-Linked Pregelatinized (PG) Corn and Potato Starches

Starch	AP[a] (%)	AM[a] (%)
Cross-linked[b] PG corn starch		
0.5% ECH,[c] 4 hr	65 ± 8	35 ± 8
Cross-linked[b] PG potato starch		
0.5% ECH, 4 hr	70 ± 2	30 ± 2

[a]Calculated on the basis of total carbohydrate present in amylose and amylopectin peaks separated by the Sepharose CL-2B column.
[b]Pregelatinized starches were prepared by dissolving the starches in KOH solution (0.5*M*) at 4°C for 2 hr. Cross-linking reactions were conducted at pH 11.
[c]Epichlorohydrin.

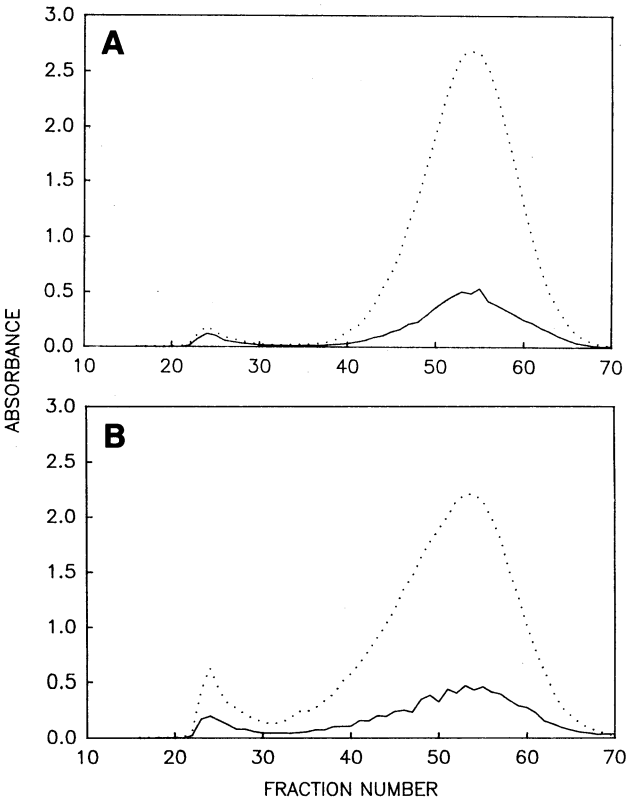

Fig. 4. Sepharose CL-2B column (2.5 cm i.d. × 80 cm) profiles of native potato amylose **(A)** and potato amylose cross-linked with 2.67% epichlorohydrin (ECH) based on amylose **(B)**. Fractions were analyzed for total carbohydrate (phenol/sulfuric acid procedure) (solid line) and blue value (amylose-iodine complex) (dotted line).

TABLE IV
Percentage of β-Amylolysis of Amyloses Isolated from Native and Cross-Linked Granular Corn Starch With and Without Prior Isoamylase Treatment[a-c]

Treatment	Native Starch (%)	Cross-Linked[d] Starch (%)
β-amylase (30 units)	64.6	53.7
β-amylase (90 units)	62.8	54.8
Isoamylase (328 units) + β-amylase (30 units)	85.1	56.7

[a]Calculated on the basis of total carbohydrate present in maltose peak separated by the Sephadex G-25 column.
[b]Amylose was isolated with the Sepharose CL-2B column.
[c]Amylose (15 mg) in 15 ml of acetate buffer solution (pH 5, 20 m*M*).
[d]Cross-linked with 0.25% epichlorohydrin (ECH) (ECH/starch = w/w).

to cross-links. Further studies are being conducted to verify this.

In conclusion, we found when granular starch was subjected to cross-linking reaction, amylose was cross-linked to amylopectin and eluted with the amylopectin. No increase in amylose molecular size was found as a result of cross-linking between amylose molecules. In contrast, if the amylose was precipitated (molecules were in close proximity) before cross-linking reaction, there was an increase in the molecular size of amylose. These results suggested that amylose was interspersed among amylopectin instead of being in bundles in the granule.

ACKNOWLEDGMENTS

We thank the Corn Refiners' Association for financial support of this study and D. J. Burden for help in preparing the manuscript. We also thank R. C. Hoseney for his interest and counsel.

LITERATURE CITED

BANKS, W., and GREENWOOD, C. T. 1975. Starch Structure and Its Components. University Press: Edinburgh.

BANKS, W., GEDDES, R., GREENWOOD, C. T., and JONES, I. G. 1972. Physicochemical studies on starches, Part 63, The molecular size and shape of amylopectin. Starch/Staerke 24:245.

BLANSHARD, J. M. V. 1986. The significance of the structure and function of the starch granule in baked products. Page 1 in: Chemistry and Physics of Baking. J. M. V. Blanshard, ed. Royal Society of Chemistry: London.

BLANSHARD, J. M. V., BATES, D. R., MUHR, A. H., WORCESTER, D. L., and HIGGINS, J. S. 1984. Small-angle neutron scattering studies of starch granule structure. Carbohydr. Polym. 4:427.

BOYER, C. D., SHANNON, J. C., GARWOOD, D. L., and CREECH, R. G. 1976. Changes in starch granule size and amylose percentage during kernel development in several *Zea mays* L. genotypes. Cereal Chem. 53:327.

COLONNA, P., and MERCIER, C. 1984. Macromolecular structure of wrinkled- and smooth-pea starch components. Carbohydr. Res. 126:233.

DUBOIS, M., GILLES, K. K., HAMILTON, J. K., REBERS, P. A., and SMITH, F. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28:350.

GOUGH, B. M., and PYBUS, J. N. 1967. Interaction of wheat starch and epichlorohydrin. Starch/Staerke 19:240.

GOUGH, B. M., and PYBUS, J. N. 1968. Interaction of wheat starch and epichlorohydrin, Part 2. Starch/Staerke 20:108.

HOOD, L. F., and MERCIER, C. 1978. Molecular structure of unmodified and chemically modified manioc starch. Carbohydr. Res. 61:53.

JANE, J., CRAIG, S. A. S., SEIB, P. A., and HOSENEY, R. C. 1986. Characterization of granular cold water-soluble starch. Starch/Staerke 38:258.

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

LEE, E. Y. C., MERCIER, C., and WHELAN, W. J. 1968. A method for the investigation of the fine structure of amylopectin. Arch. Biochem. Biophys. 125:1028.

LINEBACK, D. R. 1984. The starch granule organization and properties. Baker's Dig. 17.

MERCIER, C., and KAINUMA, K. 1975. Enzymatic debranching of starches from maize of various genotypes in high concentration of dimethylsulfoxide. Starch/Staerke 27:289-292.

RING, S. G., I'ANSON, K. J., and MORRIS, V. J. 1985. Static and dynamic light scattering studies of amylose solutions. Macromolecules 18:182-188.

SHEN, J., and JANE, J. 1991. A chemical erosion method for starch granule structure studies. Am. Chem. Soc. Natl. Meet. 201st. Abstr. 46.

SMITH, R. J. 1964. Viscosity of starch pastes. Page 114 in: Methods in Carbohydrate Chemistry. Vol. 4. R. L. Whistler, ed. Academic Press: New York.

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

WURZBURG, O. B. 1960. Preparation of starch derivatives. U.S. patent 2,935,510.

YOKOBAYASHI, K., MISAKI, A., and HARADA, T. 1970. Purification and properties of pseudomonas isoamylase. Biochim. Biophys. Acta 21:458.

[Received April 4, 1991. Revision received December 16, 1991. Accepted December 20, 1991.]