Differential Scanning Calorimetry of Raw and Annealed Starch Isolated from Normal and Mutant Maize Genotypes[1]

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ABSTRACT

Differential scanning calorimetry was used to study gelatinization of laboratory-isolated starch granules from normal and mutant maize varieties. Samples were studied in their native condition and after annealing by heating at 50° C in excess water for 48 hr. Native starches exhibited considerable variation in enthalpy and temperature range of gelatinization in a pattern consistent with the composition and degree of crystallinity of the starch, i.e., starches with the highest amylopectin content (and hence the highest degree of crystallinity) had the highest enthalpy and narrowest temperature range of gelatinization. Annealing caused an increase in enthalpy and narrowing of temperature range for all starches; the extent of these changes was inversely proportional to the degree of crystallinity of the native starch.

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Differential scanning calorimetry (DSC) was first used for measuring gelatinization of starch by Stevens and Elton (1971). As part of their study, they examined maize starches from normal, waxy, and high-amylose genotypes and found significant variation in temperature range and enthalpy of gelatinization. From those studies they concluded that the role of amylopectin was more important than that of amylose in gelatinization. DSC has since become an important tool for study of starch gelatinization, and numerous studies have been published. The work of Donovan (1979) was especially important for developing a well-founded thermodynamic approach to gelatinization based on DSC measurements.

Recently, we reported the use of DSC for studying the effects of annealing on the gelatinization characteristics of corn starch (Krueger et al 1987). Annealing, defined as the heating of starch in excess water at subgelatinization temperatures, was first shown by the birefringence studies of Gough and Pybus (1971) to narrow the temperature range and raise the peak temperature of wheat starch gelatinization; however, quantitative data such as enthalpy measurements could not be obtained by their technique. In our studies, we found that annealing increased the enthalpy of gelatinization, and that annealed (or partially annealed) starches from different maize varieties varied in gelatinization characteristics. We also demonstrated that commercially prepared starches showed characteristics of annealed starch, indicating that the temperature and moisture conditions employed in the wet milling process cause annealing of the starch. In this study, we are extending our investigation to starches from mutant genotypes in order to determine the effect of annealing on starches of varying composition and degree of crystallinity.

Several maize endosperm mutants have their primary effect on the synthesis of starch or of a particular protein. Many mutants in the group, including shrunken (*sh*), shrunken-2 (*sh2*), brittle (*bt*), brittle-2 (*bt2*), waxy (*wx*), amylose-extender (*ae*), sugary (*su*), sugary-2 (*su2*), and dull (*du*), cause variation in amylose percentage or the total amount of starch accumulation. In addition to their effects on polysaccharide composition, these mutants alone and in combination have noticeable effects on kernel development or on starch granule development and morphology (Brown et al 1971). The group of mutants including opaque-2 (*o2*), opaque-6 (*o6*), opaque-7 (*o7*), floury (*fl*), floury-2 (*fl2*), and floury-3 (*fl3*) change endosperm protein production. The prolamin (zein) fraction of the storage proteins is reduced, accompanied by an increase in albumin and globulin fractions. In the *o2* mutant, the proportion of lysine and tryptophan in the total endosperm protein is increased, making it a good source of protein and the subject of extensive nutritional study.

For this study, three mutants affecting starch synthesis (*ae*, *sh*, *wx*) and one (*o2*) that affects protein synthesis were used; all were from the same genetic background, W64A. DSC was used to study gelatinization of laboratory-isolated starch granules from these genotypes in their native condition and after annealing by heating at 50° C in excess water for 48 hr.

MATERIALS AND METHODS

Whole Corn Samples

Corn samples were grown in the Northern Regional Research Center (Peoria, IL) greenhouse. The seeds were planted in pots three weeks after germination. Plants were grown with artificial lighting 14 hr/day, with temperatures of 70° F at night and 90° F during the day. The plants were watered two times a week; once with water and once with full-strength Hoagland's solution (Hoagland and Arnon 1950), to provide a source of plant nutrients. Plants were hand-pollinated. Seed was harvested at full maturity.

Starch Isolation

Kernels of whole corn were soaked in water for 5 min, then peeled and degermed by hand. Three kernels per sample were saturated and steeped in 0.2% aqueous SO_2 at 50° C for 24 hr. The kernels were ground with a mortar and pestle and slurried in 3 ml of water. Toluene was added (3 ml) and the mixture was mixed in a vortex mixer. The emulsion layer of denatured protein that formed at the toluene-water interface was discarded. The starch in the water layer was retained and again mixed with toluene. This series of steps was repeated until the emulsion layer became negligible. Excess water was removed by centrifugation. The starch was washed with acetone and air-dried (Watson and Sanders 1961, Adkins and Greenwood 1966).

Annealing

Starch was annealed by heating 10–20 mg of starch in excess water at 50° C for 48 hr. Samples were centrifuged to remove excess water, washed with acetone, and air-dried.

DSC Procedure

Approximately 3 mg of starch was placed into an aluminum sample pan. Water (approximately 10 μl) was added with a Hamilton microsyringe, and pans were hermetically sealed with a Perkin-Elmer volatile sample sealer. The samples were allowed to equilibrate for at least 1 hr.

DSC was performed with a Perkin-Elmer DSC-2 interfaced to a Perkin-Elmer thermal analysis data station. Samples were heated at 10° C/min from 33 to 123° C. A tristearin standard was used to

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calibrate the instrument. Enthalpy, onset temperature, and peak temperature were computed automatically. All samples were run in quadruplicate. Heat of gelatinization (ΔH, cal/g) was corrected for total carbohydrate content to compensate for moisture and small amounts of residual protein in the sample. Enthalpy values for raw starch samples all showed standard deviations of 0.2 or less; annealed samples were all less than 0.12. Standard deviation of T_0 values for raw starch were all 0.7° C or less; T_P values 0.5° C or less. Annealed samples had standard deviations for T_0 and T_P of 0.3° C or less. Because the standard deviations for the temperature parameters are so low, the differential T_0-T_P is very constant and allows quantitative evaluation of variations in peak shape by use of the peak height index (PHI), which is the ratio $\Delta H / T_0$-T_P (Krueger et al 1987). This expression varies as the height of a peak changes in relation to its width. When endotherms are symmetrical, doubling the T_0-T_P value represents the gelatinization range of the sample.

Proximate Analysis

Protein. Protein determinations were made by a micro-Kjeldahl procedure, AOAC method 47.021 (AOAC 1980), with a nitrogen conversion factor of 6.25.

Total carbohydrate. Total carbohydrate was determined by the method of Dubois et al (1956). Concentrated sulfuric acid and 5% phenol were added to a prepared sample of known concentration. Absorbances were measured at 490 nm and compared to a standard of glucose or maltose.

Amylose. Amylose was determined by a modified amylose-iodine procedure (Knutson 1986). A known quantity of sample was dissolved in $6 \times 10^{-3} M$ I_2 in 90% dimethyl sulfoxide. A blue iodine-amylose complex formed upon addition of water, and the absorbance was measured at 600 nm. Pure corn amylose was used as a standard to determine amylose content.

RESULTS AND DISCUSSION

Endosperm and Starch Composition Analysis

Compositional analysis of the endosperm and purified starch are shown in Table I. The protein levels in the starch were in the range of 1.4–2.1% after purification. The starch contents of the *sh*, *wx*, and *ae* endosperms were lower than that of the normal, whereas the *o2* endosperm starch content was greater than that of the normal. The *sh* sample contained the smallest amount of starch in the endosperm; this mutant imposes a block to starch synthesis but does not alter the ratios of amylose and amylopectin (Nelson 1980). The *o2* endosperm contained the lowest amount of protein in the sample series.

TABLE I
Compositional Analysis of Normal and Mutant Genotypes (m.f.b.)

Sample	Endosperm		% Amylose in Starch
	Protein (%)	Starch (%)	
W64A-N	15.1	82.6	29.9
W64A-*o2*	10.2	90.8	27.4
W64A-*sh*	15.0	79.7	27.9
W64A-*wx*	15.6	80.5	0
W64A-*ae*	17.4	80.1	60.4

DSC Analysis

Raw starch. A summary of DSC analyses on four mutants and the normal W64A is given in Table II. DSC thermograms of these five samples are shown in Figure 1. The unique properties of the starch from these mutants are reflected in the varying peak shapes (depth, width, and sharpness of transition), which are in turn reflected in PHI values. Previous studies (Krueger et al 1987) show that ΔH differences of 0.5 or PHI differences of 0.1 are statistically significant.

Gelatinization is a semicooperative, or cooperative process

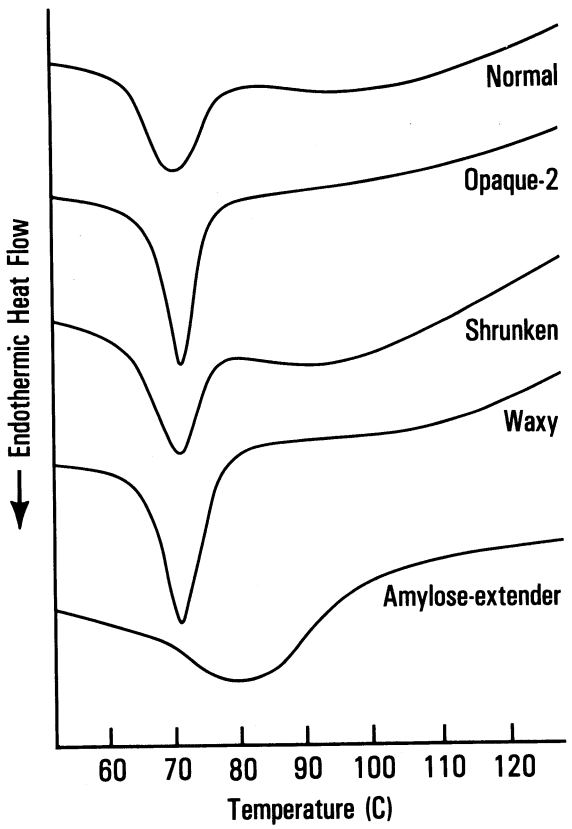

Fig. 1. Differential scanning calorimetry thermograms of raw starch from normal and mutant genotypes (variety W64A).

TABLE II
Differential Scanning Colorimetry Thermogram Values of Raw and Annealed Starches

Sample	T_0 (° C)	T_P (° C)	ΔH (cal/g)		ΔH Ratio (annealed/raw)	PHI[a]	PHI[a] Ratio (annealed/raw)
			Starch	Amylopectin			
W64A-N							
Raw	63.9	70.3	2.61	3.72		0.41	
Annealed	68.9	72.6	3.10	4.42	1.19	0.85	2.07
W64A-*o2*							
Raw	67.1	71.5	2.78	3.83		0.65	
Annealed	70.8	73.7	3.25	4.48	1.17	1.1	1.69
W64A-*sh*							
Raw	64.7	71.1	2.69	3.73		0.42	
Annealed	68.4	73.4	3.07	4.26	1.14	0.61	1.45
W64A-*wx*							
Raw	66.7	71.5	3.48	3.48		0.73	
Annealed	69.0	73.3	3.40	3.40	0.98	0.79	1.08
W64A-*ae*							
Raw	70.6	79.8	1.78	4.49		0.19	
Annealed	72.3	79.2	2.27	5.73	1.28	0.33	1.74

[a] PHI = Peak height index.

(Marchant and Blanshard 1978, Donovan 1979, Evans and Haisman 1982, French 1983) in which the amorphous region hydrates and swells to a gel phase, straining the crystalline regions and tearing away molecular chains from the crystallites. This action stresses the crystallites so that they cooperatively melt at a lower temperature than if they were not associated with the gel phase. Structural relationships between the amorphous regions and crystallites within the starch granule are responsible for the sharpness of the transition and the temperature at which it occurs. Size and perfection of crystallites do not have a significant effect on these characteristics during thermal analysis (Donovan and Mapes 1980).

The *o2* starch has a sharper transition than the normal starch, and the gelatinization range is narrowed and at a higher temperature; therefore, the PHI value is also higher. These observations indicate differences in the granule structures of *o2* and normal starches.

Endosperm from *o2* kernels, which is very floury with little evidence of horny protein matrix, was used to examine the effect of growth environment of gelatinization behavior. Portions of endosperm were scraped directly from the kernel and analyzed by DSC directly, without any steeping or protein separation. Results are seen in Figure 2. The endotherm of the unsteeped starch has a ΔH value of 2.59 and a PHI of 0.54, not very different from the values of 2.78 and 0.65, respectively, for the *o2* starch obtained by conventional isolation procedures (Table II). The major difference between the steeped and unsteeped starch is the lack of symmetry of the unsteeped endotherm. The fact that gelatinization of the unsteeped starch is possible in this genotype is in sharp contrast to our findings with normal corn starch, in which any evidence of a gelatinization endotherm on unsteeped starch was barely discernible (Krueger et al 1987).

DSC behavior of the *sh* starch is similar to that of the normal, but with a slightly higher T_P. The PHI is almost identical to that of the normal; visual comparison of the curves in Figure 1 shows them to be quite similar.

The curve of the waxy mutant starch exhibits a sharp transition and is similar to that of *o2*. PHI and ΔH are significantly higher than those of normal starch (Table II), in agreement with Donovan and Mapes (1980), who report values of peak maxima for maize and waxy maize at 71 and 73° C, respectively. Starch granules from normal and waxy maize exhibit many similarities in terms of birefringence, A-type diffraction patterns, and higher birefringence end-point temperature (Shannon and Garwood 1983). Because waxy starch is primarily amylopectin, the granules possess a different crystalline-amorphous structural relationship than that of the normal starch granule. Both Stevens and Elton (1971) and Inouchi et al (1984) report higher ΔH values for amylopectin starch than normal starch. Inouchi and co-workers (1984) conclude that the results indicate a more important contribution from amylopectin than from amylose in gelatinization. Amylopectin is considered the primary participant in the crystalline regions (Robin et al 1974). The narrowed transition range for gelatinization of waxy starch granules indicates that melting is highly cooperative, and more energy is needed for initiation in the absence of the amylose-rich amorphous regions.

The transition of the *ae* starch is quite wide in comparison with the other samples, and the ΔH is significantly smaller. Amorphous regions dominate the starch granule because of the higher amylose content. Consequently, a smaller amount of amylopectin crystallites are present in these granules. The crystallites may be so far removed from each other that cooperative melting is not possible.

Annealed starch. Annealing in this study is defined as heating starch in the presence of excess water at a temperature of 50° C. These conditions have been shown to cause changes in the thermal properties of starches, as measured by DSC (Krueger et al 1986).

The DSC curves of the annealed starches are shown in Figure 3, and the PHI, ΔH, T_0, and T_P values are presented in Table II. In each case, it can be seen that the PHI of the annealed sample is greater than that of the raw sample. All ΔH values are increased except for the waxy mutant, and all T_P values are increased about 2° C except for the *ae* starch. Increases in T_0 values vary widely.

The *wx* and *ae* starches show atypical DSC behavior. The waxy mutant starch exhibits a sharp endotherm transition prior to annealing. After annealing the thermogram closely resembles the raw waxy starch thermogram, but with a higher T_P. The amylose-

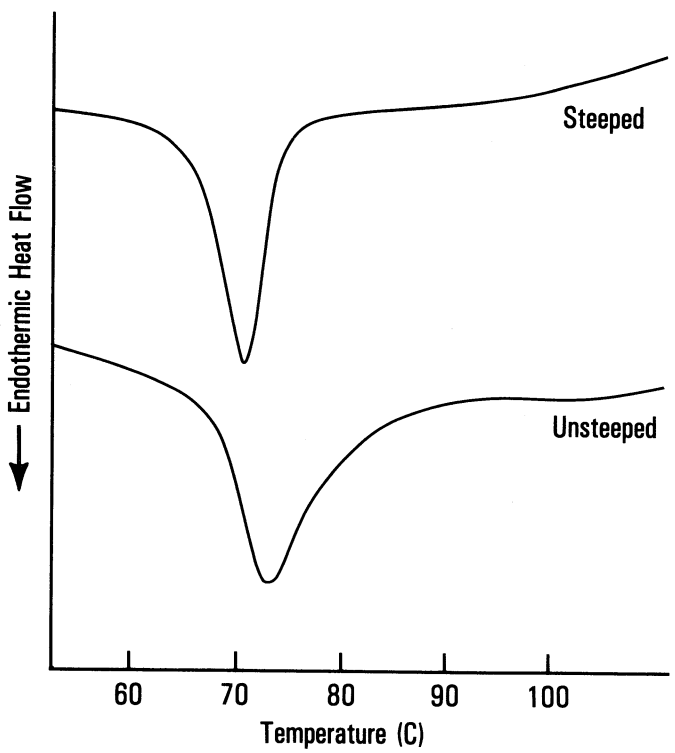

Fig. 2. Differential scanning calorimetry thermograms of raw starch from *opaque-2* corn.

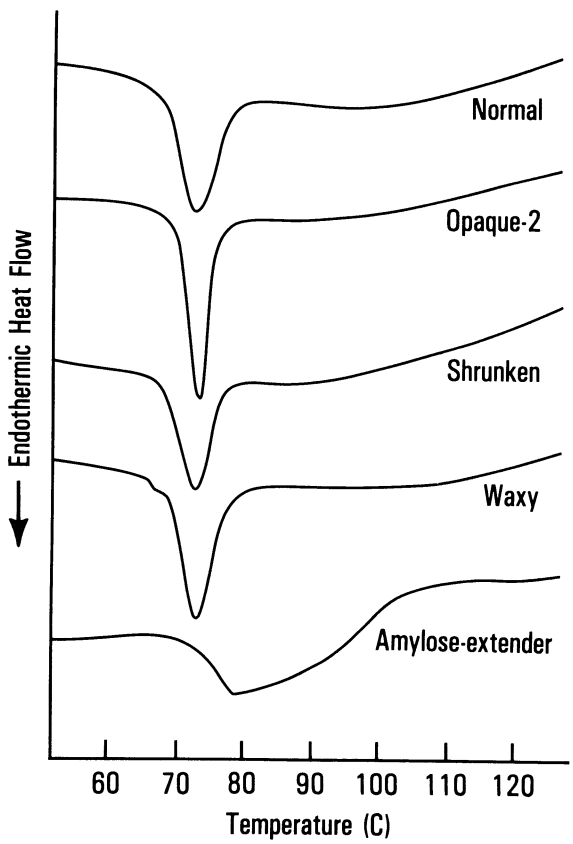

Fig. 3. Differential scanning calorimetry thermograms of annealed starch from normal and mutant genotypes (variety W64A).

extender starch does not exhibit a definite peak prior to annealing, but begins to show a peak after annealing. This suggests that annealing primarily involves amylose and that little reorganization occurs in the waxy starch granules during annealing because of the extensive crystalline structure already present. The amylose-extender does not possess as much of this type of structure, so reorganization or alignment of starch chains can occur. The data in column 5 of Table II, in which enthalpy values are calculated on the basis of calories per gram of amylopectin rather than total starch, further demonstrate the role of amylose in the annealing process The data for raw starches with "normal" amylose content are very similar to those of the waxy maize starch. However, for the annealed samples, the enthalpy values in relation to amylopectin content are significantly higher than the values for waxy maize starch, which contains no amylose. Thus, the increase in ordering that occurs during annealing depends upon the presence of amylose in the granule and suggests the development of crystallinity in the amorphous regions of the granule.

CONCLUSIONS

Mutant genotypes of maize vary in their gelatinization characteristics. The varying responses to DSC analysis suggest that structural differences are present in the starch granules. .

Annealing changes the thermal properties of starches by reorganization of the starch granule structure. The effects of annealing are a narrowing of the gelatinization range, an increase in peak temperature, and an increase in enthalpy.

Variation in response to annealing reflects either the degree of restraint imposed during growth and maturation of the kernel, as in the case of *o2* starch, or compositional differences within the starches, as in the case of the *wx* and *ae* samples. In the absence of amylose in the starch granule, near-maximum crystallinity, as measured by DSC data, develops in the native state. As amylose content of the granule increases, there is increased volume in the amorphous phase, and the degree of ordering in the native state is diminished. Annealing increases this internal ordering: "normal" starches (70–75% amylopectin) after annealing have endotherms equivalent to *wx* starch. The degree of ordering achieved in high-amylose starch is lower, but in the same direction. Further increases may be possible if higher annealing temperatures are used.

It should be noted that the DSC values obtained in this study, on normal dent corn starch obtained from plants grown in our greenhouse, differ slightly from values reported previously for starch isolated from maize inbred variety W64A from a different source (Krueger et al 1987). This variation illustrates the need to examine the effect of varying growth and storage conditions on thermal properties of starches.

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