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MULTIPLE FORMS OF STARCH BRANCHING ENZYME OF MAIZE: EVIDENCE FOR INDEPENDENT GENETIC CONTROL

Charles D. Boyer and Jack Preiss

Department of Biochemistry and Biophysics
University of California
Davis, California 95616

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SUMMARY

Purification of starch branching enzymes from kernels of two nonlinked mutants of maize, sugary and amylose-extender, showed the basis of the two mutations to be associated with branching enzymes I and IIb, respectively. Branching enzyme I from sugary kernels purified as nonmutant branching enzyme I, but had an altered pattern of activity when amylose was used as a substrate. In addition to the typical fall in absorbance at high wavelengths (550-700 nm) of the amylose-iodine complex, branching of amylose by sugary branching enzyme I caused an increase in absorbance at low wavelengths (400-550 nm). Branching enzyme IIb was undetected in extracts of amylose-extender kernels, while branching enzymes I and IIa appeared unaltered. Low umprimed starch synthase activity was also observed in DEAE-cellulose fractions of amylose-extender maize, but this activity was regenerated by the addition of any branching enzyme.

A previous study (1) showed that multiple forms of starch branching enzymes were present in 22 day old maize kernels. These enzymes were separable on DEAE-cellulose chromatography and could be distinguished by using two different assay procedures. Although both enzymes were active when measured by the stimulation of glucan formation from glucose-1-phosphate by phosphorylase a and by the decrease in absorbance of the amylose-iodine complex resulting from the branching of amylose, the relative ratios for the two assay systems for the two enzyme fractions differed. Branching enzyme I had a ratio of activity for the two assays (units of phosphorylase stimulation/units of decrease in absorbance of the amylose-iodine complex) ranging from 30 to 60, whereas branching enzyme II had a ratio of activity ranging from 300 to 500. Branching enzyme II was separated into two fractions, IIa and IIb, by chromatography on 4-aminobutyl Sepharose (1). These fractions were most readily distinguished by their relative rate of reaction on amylopectin (IIb being greater than IIa).

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Two independent nonlinked mutants of maize, amylose-extender; ae (chromosome 5) and sugary; su (chromosome 4) are known to affect the polysaccharide storage products formed in maize endosperm. Starch from ae endosperm, in addition to containing a higher proportion of linear amylose, contains amylopectin with fewer branch points than normal amylopectin (2-5). Endosperm of su contains reduced quantities of starch and, in addition, a highly branched water soluble polysaccharide termed phytoglycogen (6). As with other glycogens, phytoglycogen contains a greater proportion of branch points than amylopectin. These product characterizations of the polysaccharides from su and ae endosperms (2-6) suggested that these mutants are associated with branching enzyme activity, and we have now found that the su and ae mutants are indeed associated with different branching enzyme fractions (I and IIb).

MATERIALS AND METHODS

Genetic Material. The amylose-extender mutant was grown in the isogenic line W64A, and had been incorporated into this line by standard backcrossing procedures. The sugary genotype was the commercial sweet corn line, Improved Golden Bantam. Ears were harvested at 22 days after pollination by freezing on dry ice and stored at -15° until used.

Assays of Branching Enzyme. Assay A. The stimulation by branching enzyme of α-glucan formation catalyzed by phosphorylase a was assayed as previously described (7). The reaction mixture contained 0.1 M sodium citrate (pH 7.0), 1 mM AMP, 50 mM [^{14}C]glucose-1-P (5.0 x 10^4 cpm/μmole), 40 μg crystalline rabbit muscle phosphorylase a and branching enzyme in a volume of 0.2 ml. Assay B. The decrease in absorbance of the amylose-iodine complex was measured as previously described (1). The reaction mixture contained 350 μg amylose, 35 μmoles sodium citrate (pH 7.0) and branching enzyme in a total volume of 0.35 ml.

Assay of Starch Synthase Activity. Glucose incorporation into α-glucan from ADP-glucose was measured in the primed and unprimed conditions described by Hawker et al. (7).

Enzyme Purification. Branching enzyme fractions were purified as described for normal (nonmutant) maize (1). Protein concentrations were determined by the method of Lowry et al. (8) using bovine serum albumin as a standard. All other procedures used are described elsewhere (1).

RESULTS

Purification and Characterization of Branching Enzymes from Amylose-Extender Kernels. Unlike extracts from normal maize, crude extracts from ae could not be assayed for branching activity (Table 1) and little (less than

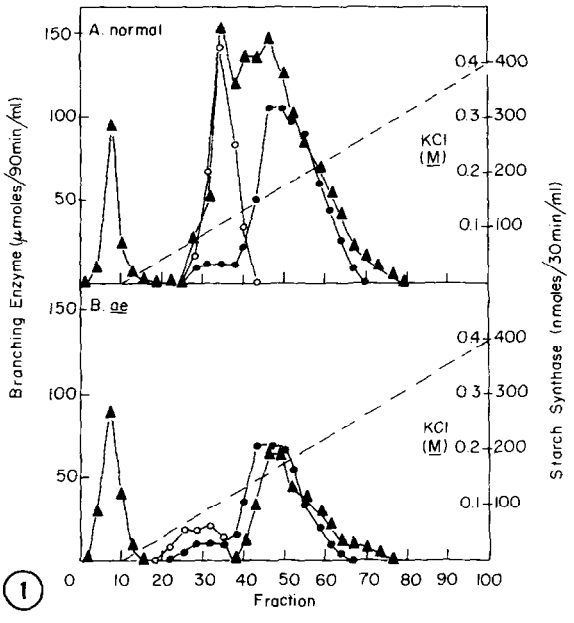
Table 1. Purification of maize branching enzymes I and II from amylose-extender maize.

Fraction	Volume (ml)	Protein (mg)	Total Activity[a] (units)	Specific Activity[a] (units/mg)
1. Crude	365	4106	0	0
2. 45,000 x g supernatant	305	1865	940	0.5
3. $(NH_4)_2SO_4$ (0-40%)	52	681	885	1.3
4. DEAE-cellulose				
Fraction I	42	201	393	2.0
Fraction II	38	81	319	4.0

[a]One unit of activity is defined as 1 μmole of glucose transferred per minute under the conditions of Assay A.

10 total units) or no activity was detected. However, after removal of the starch granules (presumably most of the primer) by centrifugation, branching enzyme activity was detected (Table 1), but the recovered total activity was only 20% the amount recovered from equivalent quantities of normal tissue (1). Both the primed and unprimed starch synthase activities were observed at levels similar to normal extracts in fractions up to DEAE-cellulose chromatography.

Chromatography of ae extracts on DEAE-cellulose (Fig. 1B) resulted in two distinct differences from the elution profiles observed for normal (Fig. 1A). First, no branching enzyme was observed to coelute with the unprimed starch synthase activity (Fractions 20-35) and second, the overall unprimed starch synthase activity was reduced. However addition of any of the three branching enzymes from normal (I, IIa or IIb) at ratios of activity (unprimed synthase units/branching enzyme units) observed for these fractions in preparations from normal maize stimulated the unprimed activity 3 to 4 fold, bringing the total unprimed activity up to the level observed for normal preparations The unprimed and primed starch synthases had K_m's for ADP-glucose of 0.10 mM in the primed conditions. The unprimed synthase had greater activity with



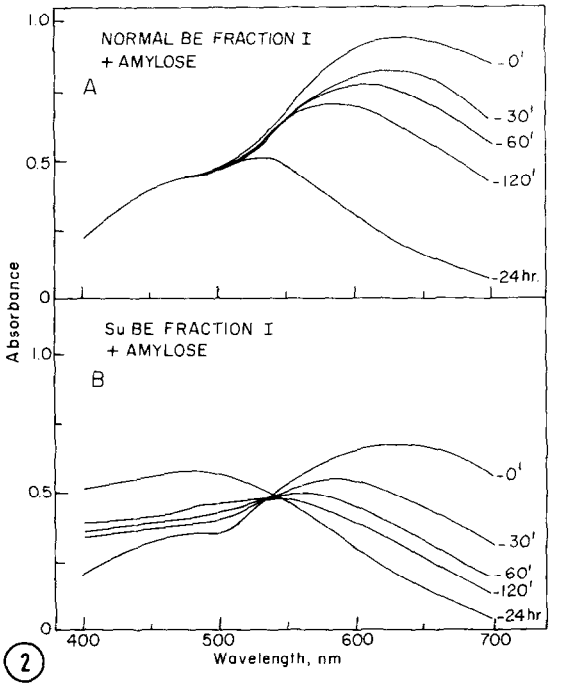


Figure 1. Chromatography of maize branching enzymes on DEAE-cellulose; branching enzyme activity (▲), unprimed starch synthase activity (O), and primed starch synthase activity (●). A. Chromatography of normal maize extracts. B. Chromatography of amylose-extender maize extracts.

Figure 2. Absorption spectra of the iodine complexes in saturated $CaCl_2$ of the α-glucans formed by the branching of amylose by branching enzyme I. A. Branching enzyme I from normal maize. B. Branching enzyme I from sugary maize.

different glycogens as substrates than amylopectin, while the primed synthase showed greater activity with amylopectin than the glycogens. Both of these observations for the unprimed and primed synthases agree with those values previously reported for these fractions from waxy maize (9).

Branching enzyme I (Fractions 1-10, Fig. 2B) from ae had all the characteristics of normal branching enzyme I. Ratios of activity (Assay A/Assay B) of 30 to 60 were observed throughout the purification. In addition, as with normal, a K_m of 160 μg/ml for amylose in Assay B was observed. Chromatography on 5-aminopentyl Sepharose showed elution in a 0-1.0 M KCl gradient at the same salt concentration as normal enzyme and gel filtration gave a similar

native molecular weight, 70-90,000. Separate or co-gel electrophoresis of normal and ae branching enzymes I yielded a single band when gels were stained for protein or activity. The second peak of branching activity (Fractions 40-70, Fig. 2B) had similar properties to branching enzyme IIa observed in normal. As with both normal branching enzymes IIa and IIb, the fraction II enzyme of ae had an activity ratio (Assay A/Assay B) ranging from 300 to 500 and a K_m for amylose between 500 and 600 µg/ml. Likewise, ae branching enzyme II had a molecular weight around 80,000 based on gel filtration and sodium dodecyl sulfate gel electrophoresis and was primarily monomeric. Branching enzyme II of ae was identified as normal branching enzyme IIa by early elution from 4-aminobutyl Sepharose resin in a 0-1.0 M KCl gradient, and low activity on amylopectin (1). The elution order of branching enzymes IIa and IIb is reversed on DEAE-cellulose and 4-aminobutyl Sepharose.

Purification of Branching Enzymes from Sugary Kernels. The purification of the branching enzymes from su kernels (Table 2) did not differ significantly from the purification of normal branching enzymes (1). The total activities on a per gram basis, as well as specific activities in crude extracts were similar in both genotypes. In addition, chromatography of su extracts on DEAE-cellulose gave similar enzyme profiles to normal for branching enzyme and primed and unprimed synthases (not shown). As observed in normal preparations branching enzyme I from su contained only 30% of the total recovered activity when assayed by phosphorylase a stimulation (Assay A) but contained 70% of the recovered activity when assayed by the branching of amylose (Assay B). However, branching enzyme I of su was observed to differ from normal branching enzyme I in the pattern of amylose branching. The branching of amylose characteristically can be followed by the decrease in absorbance at the higher wavelengths and the shift in the wavelength maxima to lower wavelengths of the amylose-iodine complex (Fig. 2A). This pattern has been observed for all maize branching enzymes studied including normal I, IIa and IIb and ae I and IIa using iodine reagents prepared in either water

Table 2. Purification of maize branching enzymes I and II from sugary maize.

Fraction	Volume (ml)	Protein (mg)	Total Activity[a] (units)	Specific Activity[a] (units/mg)
1. Crude	74	1462	1431	1.0
2. 45,000 x g supernatant	58	383	1251	3.3
3. $(NH_4)_2SO_4$ (0-40%)	30	257	1098	4.3
4. DEAE-cellulose				
Fraction I	8	30	260	8.8
Fraction II	10	14	666	47.4

[a]One unit of activity is defined as 1 μmole of glucose transferred per minute under the conditions of Assay A.

or saturated $CaCl_2$ (10). Branching enzyme I of su, however, showed an additional increase in the absorbance at the lower wavelengths of the amylose-iodine complex when iodine reagents made with saturated $CaCl_2$ were used (Fig. 2B).

DISCUSSION

The observed differences of the branching enzymes for the mutants ae and su correspond well with the reported characterizations of the polysaccharides found in these genotypes (2-6). The fewer branch points in the ae amylopectin agrees with the absence of branching enzyme IIa. Likewise, the altered activity of branching enzyme I in the branching of amylose (Fig. 2B) correlates with the presence of phytoglycogen in the su genotype. The phytoglycogen branching enzyme has been studied previously (11) and the observations made here confirm their observations. However, the same group (12) was unable to relate the su enzyme to an enzyme found in normal maize. This report bridges that gap and provides evidence for the relationship of normal maize branching enzymes and the enzymatic affects of the mutants ae and su.

The detection of multiple branching enzymes is interesting in light of recent studies of the structure of amylopectins and glycogen (13,14). The unit chains from enzymatic debranching of amylopectin have been shown to differ from the unit chains of glycogen by having a greater average length as well as a bimodal versus a unimodal distribution when fractionated by gel filtration (13). The ratio of A to B chains in amylopectins generated by enzymatic techniques was observed to be considerably higher than in glycogens (2:1 compared to 1:1; reference 14). These two observations considered together suggest an assymmetry in the structure of the amylopectin molecule. Investigations are now being initiated into the relationship of the multiple forms of branching enzyme and the possible assymmetry of the amylopectin molecule.

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