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A study of pyrophosphorylase activities in maize endosperm

The pyrophosphorylase class of enzymes (EC 2.7.7.9) may be particularly important in the biological regulation of carbohydrate polymer synthesis[1-3]. The relative levels of activity of the UDPG and ADPG pyrophosphorylases (UTP:α-D-glucose-1-phosphate uridylyltransferase and ATP:α-D-glucose-1-phosphate adenylyltransferase, respectively) may determine which of the nucleotide sugar products is available in greatest abundance for the synthesis of starch. In addition, other pyrophosphorylases which may be present could compete for glucose 1-phosphate thereby influencing the availability of the entire range of nucleotide sugars for further reactions. Alternatively these enzymes, whose reactions are readily reversible, may control the availability of the nucleotide sugars by reducing their concentrations as they are formed directly from sucrose or similar donors through other pathways. This communication describes a study of the endosperm pyrophosphorylases of a number of maize genotypes especially bred to have as common a genetic background as possible. Evidence is presented for the previously unreported existence in these tissues of an enzyme having CDPG-pyrophosphorylase activity which appears to be distinct from the UDPG and ADPG pyrophosphorylases commonly studied. The enzyme had been primarily observed in animal and bacterial organisms and only recently discovered in trace amounts in pea and parsley[4]. These latter activities were 4–5 times lower than the ADPG activity in these same organisms and no attempt was made to distinguish between individual enzymes. In contrast, the CDPG activity in maize endosperm is comparable to or somewhat higher than the ADPG activity in the same tissue, suggesting that it may have a similarly important role in the regulation of carbohydrate polymer synthesis.

Normal, sh_2, su_2 and $su_1^{66}su_2$ maize genotypes were selected for these studies because of the wide variations evident in their starch–sucrose percentages[5] and the known absence of ADPG pyrophosphorylase in the sh_2 mutant[6]. Ears of these four genotypes were harvested 20 days after hand pollination, a time when starch synthesis is proceeding actively. They were immediately placed in liquid nitrogen and kept frozen until used. Preliminary studies with the ADPG and CDPG enzymes showed a linear increase in product formation with time to 1.5 h under the conditions of the assay. The more active UDPG-enzyme preparations were incubated for shorter periods with similar results. In each case the quantity of product formed reached a stable plateau following extended incubation. This observation and the lack of accumulation of degradative products such as glucose on the radioautograms indicate the probable absence of hydrolytic enzymes in the preparations. Such products did appear when the 0–25% ammonium sulfate fractions were studied.

The results of quantitative determinations of ADPG-, UDPG-, CDPG-, and GDPG-pyrophosphorylase activities in each of the four maize genotypes are illustrated in Fig. 1. In all cases the UDPG-synthesizing enzyme was extremely active, the CDPG and ADPG enzymes were less active than the UDPG enzyme but comparable to each other in activity (except in sh_2) and GDPG pyrophosphorylase was not detected in any preparation.

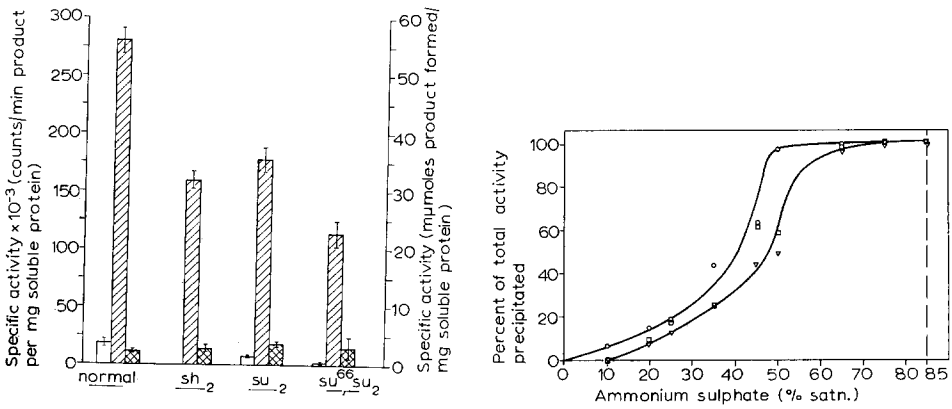

Fig. 1. Comparison of the specific activities of ADPG (open columns), UDPG (hatched columns) and CDPG pyrophosphorylase (cross-hatched columns) in the 25–65% ammonium sulfate-insoluble fractions of normal, sh_2, su_2, and $su_1{}^{66}su_2$ maize endosperm homogenates. A modified procedure of TSAI AND NELSON[6] was used in the isolation and assay of the enzymes. 5 g of maize endosperm from each of the four genotypes was homogenized in 5 ml of 0.05 M phosphate buffer (pH 7.0), and centrifuged at 22 000 × *g* for 20 min. Solid ammonium sulfate was added to the supernatants to obtain the 25–65% of saturation fractions. The pellets obtained by centrifugation at 18 000 × *g* for 20 min were dissolved in 5 ml of distilled water and dialyzed against distilled water for 14 h at 0°. Protein was determined by the method of LOWRY *et al.*[7]. Each enzyme preparation was assayed for ADPG-, UDPG-, CDPG- and GDPG-pyrophosphorylase activity. 40 μl of the enzyme solution were incubated at 37° for 1 h with 0.5 μmole of a nucleotide triphosphate (ATP, UTP, CTP or GTP depending on the particular assay), 10 μmoles Tris–HCl buffer (pH 7.4), 0.6 μmole $MgCl_2$, and 0.05 μmole α-D-[$^{14}C_6$]Glc-1-*P*, 1 μC, to produce a total reaction volume of 85 μl. The reaction was halted by placing the tubes into boiling water for 1 min. Water was then added to a final volume of 150 μl. 10 μl of the reaction mixtures were spotted on acid-washed Whatman No. 1 chromatographic paper and run in a solvent system of 95% ethanol–1 M ammonium acetate (7.5:3, v/v), pH 3.8 (ref. 8), for 12 h. The nucleotide sugar products were eluted with distilled water and co-chromatographed with standards in a solvent system of 95% ethanol–1 M ammonium acetate (7.5:3, v/v), pH 7.6 (ref. 8), using similarly prepared paper. A third chromatographic system of 1 M acetic acid–3 M LiCl (9:1, v/v) on a polyethylene imine cellulose paper[9] further verified the identities of the radioactive nucleotide sugar products. The radioactive spots were counted with a Baird Atomic GM Scaler operated at 850 V. The efficiency of elution for re-chromatography was at least 98% in all cases. The ratios of UDPG- to CDPG-pyrophosphorylase activity shown for the above four mutants are 24.0, 11.4, 9.8 and 7.5 ,respectively.

Fig. 2. Precipitation curves for ADPG- (○—○), UDPG- (▽—▽) and CDPG-pyrophosphorylase (□—□) activity in normal maize endosperm with ammonium sulfate fractionation. The data are plotted as the cumulative percentage of total activity obtained from successive salt fractions and were determined by the procedures given in the legend of the preceding figure. No GDPG pyrophosphorylase was detected.

The discovery in these experiments of the relatively high CDPG-pyrophosphorylase activity in each of the four genotypes led to the question of the specificity of the enzyme responsible for the reaction. A study of the ammonium sulfate fractionation of the normal pyrophosphorylases showed that the UDPG and CDPG activities were similar in their precipitation characteristics suggesting that these could be caused by a single enzyme. This was in contrast to the ADPG enzyme which precipitated at distinctly lower salt concentrations as shown in Fig. 2.

On the other hand, a comparison of the ratios of specific activities of the UDPG and CDPG pyrophosphorylase, assayed on single preparations, shows a variation from

TABLE I

EFFECTS ON THE UDPG PYROPHOSPHORYLASE OF NORMAL MAIZE ENDOSPERM OF THE EQUIMOLAR ADDITION OF CTP OR ATP TO THE UTP SUBSTRATE IN THE PYROPHOSPHORYLASE ASSAY

The complete reaction mixture for the (UTP only) experiment contained 1.0 μmole UTP, 20 μmoles Tris–HCl buffer (pH 7.4), 1.2 μmoles $MgCl_2$, 0.10 μmole α-D-[$^{14}C_6$]Glc-1-*P*, 2 μC, and 40 μl enzyme preparation obtained as described in Fig. 1 legend. The blank (*Minus* enzyme) contained the above components *minus* the enzyme. The (UTP + CTP) experiment contained the complete reaction mixture except that 0.5 μmole CTP was substituted for half the UTP. The same applied to the (UTP + ATP) experiment except that the 0.5 μmole ATP was substituted for half the UTP. These three reaction mixtures and the blank were incubated at 37° for 1 h. The products were isolated by paper chromatography from 10 μl of each mixture and counted. The data are expressed as counts/min.

Experimental conditions	*Spotted at origin*	*UDP[$^{14}C_6$]G obtained*	*UDP[$^{14}C_6$]G formed (normalized to 10 000 counts/min applied to origin)*
Minus enzyme	11 929	140	120
UTP only	13 131	3800	2900
UTP + CTP	11 813	3700	3000
UTP + ATP	10 055	2600	2600

24.0 for normal to 7.5 for $su_1^{66}su_2$ (Fig. 1). This variation appears quite significant, indicating the activities are probably caused by variable concentrations of two distinct enzymes.

A competitive inhibition study was also directed toward clarifying the question of whether the UDPG and CDPG activities are associated with different enzymes. The results, presented in Table I, show that there is no observable inhibition of the UDPG pyrophosphorylase by the addition of an equimolar concentration of CTP, thus supporting the conclusion that the two activities are caused by separate enzymes in normal maize endosperm. The effect of adding ATP to the reaction mixture is also reported.

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Department of Biochemistry,
Pennsylvania State University,
University Park, Pa. (U.S.A.)

J. D. VIDRA
J. D. LOERCH

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