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HEXOKINASE FROM MAIZE ENDOSPERM*,†

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Abstract—Hexokinase was detected in the endosperm of developing and germinating maize seeds. Hexokinase activities in the endosperm of germinating dent, flint, and floury genotypes were quite similar, but were only one-fifth of the activity of shrunken-2 (sugary) genotype. A reduced enzyme level in endosperm of germinating compared to developing seeds, and a continual decline in enzyme during germination suggest that hexokinase in the endosperm of germinating maize is residual and has no major metabolic role. In the endosperm of developing seed the enzyme would be important as a first step in the metabolism of translocated sugars.

INTRODUCTION

HEXOKINASE is widely regarded as a key enzyme in the metabolism of hexoses. The mammalian enzyme is thought to regulate the flow of hexoses into the hexose phosphate pool because it is severely inhibited by glucose-6-phosphate, and the inhibition is partially relieved by orthophosphate.[1] Yeast hexokinase is activated by several metabolites including 3-phosphoglycerate, citrate and malate.[2] Measurement of appropriate metabolites in germinating barley indicates that hexokinase regulates glycolysis in that tissue.[3]

Hexokinase occurs in a wide variety of higher plant species and tissues,[4–13] but, in contrast to the mammalian and yeast enzymes, kinetic studies are reported for only a few species.[5,6,10] Hexokinase is present in starch storing organs of several plants including banana fruits,[13] potato tubers,[12] pea seeds,[10] and germinating barley endosperm.[7] In another paper, hexokinase was mentioned as one of a series of enzymes present in the endosperm of developing maize seed, but no quantitative data was presented.[14] However, one worker did not detect the enzyme in endosperm of germinating maize.[6]

A recent report indicates that ^{14}C from translocated ^{14}C-sucrose appears initially in free hexoses in the endosperm of developing maize with subsequent incorporation into

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sucrose and starch.[15] The first step in the metabolism of these hexoses would be formation of hexose phosphates via the hexokinase reaction. Hence, this enzyme could be an important factor in the efficiency of sucrose and polysaccharide synthesis by endosperm cells.

This communication is concerned with extraction and identification of hexokinase from maize endosperm. The enzyme was detected in extracts of endosperm from both maturing and germinating seeds.

RESULTS AND DISCUSSION

In the present study, hexokinase was always detected in maize endosperm using a variety of extraction procedures, different stages of seed development, and various genotypes. The relatively low activity in the endosperm of germinating seeds (about 0·006 enzyme units/mg protein vs. about 0·05 units/mg protein from scutellum) may explain why Jones[6] did not detect hexokinase in endosperm from germinating seeds of Funk's G-76.

The presence of kexokinase was demonstrated isotopically using extracts from endosperms of the maize genotypes WF9 × M14 and shrunken-2, seeds of which were germinated for 5 days. Formation of ^{14}C-glucose-6-P from ^{14}C-glucose by these extracts was confirmed by showing that the radioactive product co-chromatographed with authentic glucose-6-P in solvents A and B (see Experimental). The radioactive product was eluted from the paper chromatogram after development with solvent A and different portions were rechromatographed on cellulose thin layers with solvents A and C. In these solvents the product migrated as a single radioactive compound with the same R_f as authentic glucose-6-P. Treating the eluted material with highly purified alkaline phosphatase caused complete conversion to a new radioactive compound that co-chromatographed with authentic D-glucose in solvent A.

Subsequent studies with the endosperm of developing and germinating seeds of WF9 × M14 established optimum extraction conditions (see Experimental) and showed that less than 5% of the enzyme was precipitated from the crude homogenate by 10 min at 10,000 *g*. Most of the enzyme (91%) remained in the 40,000 *g* supernatant after 20 min, when procedures suitable for isolating intact mitochondria were followed.[16] This contrasts with hexokinase from potato tubers and wheat germ,[12] pea seed,[10] and lentil root[11] which are partially associated with the particulate fraction. The pH optimum of the maize endosperm enzyme resembles that of hexokinases from other plants. Partially purified enzyme (ammonium sulfate fractionation, DEAE cellulose chromatography) required Mg^{2+} for activity. Relative rates of fructose and glucose utilization (fructose V_{max}/glucose V_{max}) are 1·5–2·0 for endosperm hexokinase compared to a reported ratio of 0·97 for the maize scutellum enzyme.[6]

The survey of maize genotypes (Table 1) was done using the standard extraction and assay procedures found to be optimum for WF9 × M14. Each experiment was done on a different day. Enzyme activity per endosperm was surprisingly similar for all genotypes except shrunken-2 which was higher. This difference is even more pronounced when the comparison is based on specific activity. Low total enzyme activity in experiment 11 was partly due to incomplete extraction of soluble protein.

Further work showed a progressive decrease in hexokinase activity of WF9 × M14 endosperms during germination (Fig. 1). In endosperm of developing seeds (F_2 kernels from WF9 × M14 plants), hexokinase activity increased 8-fold between 14 and 27 days

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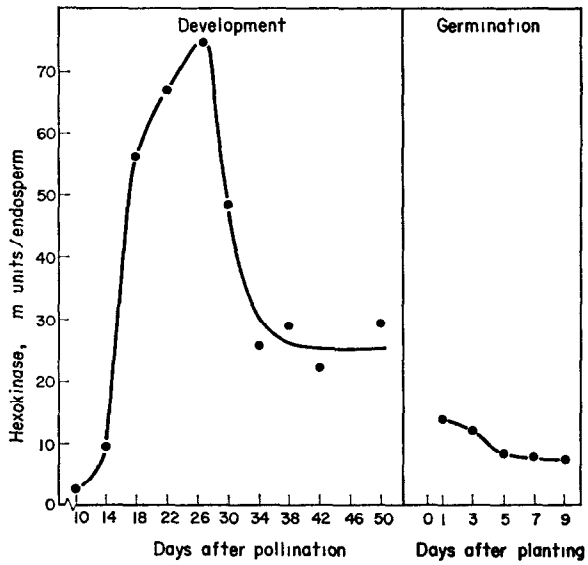

FIG. 1. HEXOKINASE IN ENDOSPERM FROM DEVELOPING AND GERMINATING MAIZE SEEDS (WF9 × M14).

TABLE 1. HEXOKINASE ACTIVITY IN DIFFERENT ENDOSPERMS 5 days AFTER PLANTING

Endosperm* type	Experiment	Enzyme Activity†		Soluble Protein	Dry Weight	%
		mUnits/endosp.	mUnits/mg protein	mg/endosp.	mg/endosp.	moisture
Dent						
WF9 × M14	1	9·1	10·0	0·91	126	50
WF9 × M14	2	6·9	7·5	0·92	134	42
WF9 × M14	3	8·6	8·8	0·97	130	40
WF9	4	9·0	6·1	1·48	177	48
M14	5	5·0	6·2	0·80	109	42
G76	6	8·1	4·6	1·76	255	37
G76	7	8·5	6·6	1·30	248	41
Flint	8	12·3	47·3	0·26	50	51
Flour	9	13·4	22·7	0·59	80	55
Shrunken	10	63·1	204	0·31	49	60
Shrunken	11	11·6	145	0·08	44	64
Shrunken	12	50·3	314	0·16	36	61

* The seed stocks used in this survey are described in Experimental.

† One unit of enzyme activity is defined as one μmole of glucose phosphorylated/min at pH 8·0 and 25°. The spectrophotometric assay was used as described in Experimental.

after pollination, then decreased to 35% of peak activity by 34 days after pollination (Fig. 1). The rise in enzyme activity coincided with the period of maximum increase in starch content of the endosperm. These results suggest that hexokinase in endosperm of germinating maize is a residual enzyme and that its function was to phosphorylate hexoses during seed development and maturation. Hence, the enzyme would appear to have no major catalytic function during germination.

It remains to be established that hexokinase from endosperm of developing seeds is identical to the enzyme from endosperms of germinating seeds. Comparison of the properties of endosperm and scutellum hexokinase will also be of interest, and regulatory properties of endosperm hexokinase remain to be established. Preliminary studies indicate that the maize enzyme resembles wheat germ hexokinase[12] in being only slightly inhibited by glucose-6-P.

The relatively high levels of hexokinase in the endosperms of developing dent seeds and of germinating shrunken-2 seeds indicate that these are good sources of enzyme for further experiments dealing with purification and characterization. It is not known whether hexokinase activity in shrunken-2 endosperm is higher than in dent endosperm during development. These two tissues may have similar enzyme levels initially. The difference in germinating seeds may be due to a decline in enzyme level with maturation in dent seed while the enzyme level remains high in shrunken-2 seed.

EXPERIMENTAL

Plant Material

The seed stocks *Zea mays* used were: dent endosperm—WF9 and M14 inbreds, WF9 × M14 and Funk's G-76 hybrids; flint endosperm—Faribo Hulless white popcorn; floury endosperm—plant breeding stocks of $fl_1fl_1fl_1$ endosperm genotype; shrunken endosperm—Illini Chief Super Sweet sweet corn ($sh_2sh_2sh_2$ endosperm genotype).

Seeds were surface sterilized with 2% NaOCl and germinated with 10^{-4} M $CaCl_2$ in the dark at 27° ± 1° as described by Ingle *et al.*[17]

Ears of field grown WF9 × M14 were self-pollinated by hand, harvested at various times after pollination, frozen in liquid N_2 and stored at −20° until assayed.

Extraction Procedures

Endosperms were dissected from the germinating seedlings after 1–9 days, weighed, and ground in a mortar with liquid N_2. Hexokinase was extracted from the frozen powder by stirring 30 min at 1° with a medium (2 ml/g powder) containing 50 mM potassium phosphate, 5 mM $MgCl_2$, 10 mM KCl, 1 mM EDTA and 1 mM dithiothrietol, adjusted to pH 7·5. The brei was centrifuged at 40,000 *g* for 20 min and the supernatant was the source of enzyme. The same procedure was used to extract hexokinase from developing endosperms of field grown WF9 × M14 plants. Soluble protein was determined by the method of Lowry *et al.*[18] on the material precipitated by 10% TCA; bovine serum albumin was used as the standard.

Assay Procedures

Hexokinase was assayed spectrophotometrically by measuring NADP reduction at 340 nm.[19] Optimal concentrations of substrates, cofactors and indicator enzyme were determined for hexokinase isolated from endosperm of 5-day-old WF9 × M14 seedlings. The reaction mixture (total vol. 0·2 ml) contained the following in μmoles: *N*-2-hydroxyethyl-piperazine-*N*-2-ethanesulfonic acid (Hepes buffer), pH 8·0, 10; $MgCl_2$, 1; glucose, 1; ATP, 0·5; NADP, 0·2; and 245 munits of glucose-6-P dehydrogenase. Phosphorylation of fructose was measured by substituting 3 μmoles of fructose and 200 munits of phosphohexoisomerase for glucose in the standard reaction mixture. The reaction was initiated with 5–30 μl of undiluted endosperm extract and run at 25° in a recording spectrophotometer. Reaction rates, corrected for rates observed without ATP, were proportional to the quantity of endosperm extract used. One unit of enzyme activity is equal to 1 μmole hexose-P formed/min at 25° in the standard reaction mixture.

In the experiments to identify reaction products, 0·5 mM ^{14}C-D-glucose (uniformly labeled, 1·82 μc/μmole) was added to the standard reaction mixture while NADP and glucose-6-P dehydrogenase were omitted. Endosperm extract was incubated with this mixture for 10 min at 30° and the reaction was terminated by 30 sec immersion in boiling H_2O.

Chromatography of Reaction Products

TLC and paper chromatography of the isotopic reaction mixtures were carried out in three solvent systems.[20] (A), EtOA–HOAC–H_2O; 3·3:1 (to separate glucose, fructose, fructose-6-P, glucose-1-P plus

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glucose-6-P). (B), MeOH–28% NH_4OH–H_2O; 6:1:3, (for glucose and fructose from each other and from the hexose phosphates). (C), methyl cellosolve–methyl ethyl ketone–3 N NH_4OH; 7:2:3, (for glucose-1-P from glucose-6-P). Hexose standards were detected with Tollens reagent and hexose phosphates were detected by formation of the phosphomolybdate blue complex.[21] Bacterial alkaline phosphatase (Worthington, Type BAPSF) was used for enzymatic hydrolysis of the phosphate esters.

Note added in proof. After submission of this manuscript Tsai, Salamini and Nelson[22] reported the time course of development of hexokinase activity in endosperm of developing $B_{37} \times B_{14}$ maize kernels. Their data agreed well with those presented in Fig. 1.

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