β-ASPARTOKINASE FROM DEVELOPING ENDOSPERM OF MAIZE

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SUMMARY: β-aspartokinase (EC 2.7.2.4.) has been isolated from the developing endosperm (30 days post-pollination) of *Zea mays* (cv. Pioneer 3145). Enzyme activity was dependent upon ATP, Mg^{++} or Mn^{++}, aspartate, and protein concentration. Double reciprocal plots of velocity vs. aspartate concentrations deviated from a straight line at low aspartate concentration indicating two apparent Km's of 0.5 and 6.6 mM. Enzyme activity was inhibited by lysine but not by methionine or threonine. The endosperm-derived β-aspartokinase behaved similarly to enzyme isolated from 6-day-old etiolated shoot tissue. The presence of β-aspartokinase in developing endosperm provides new insight into the source of the aspartate-derived amino acids in maize endosperm.

INTRODUCTION

Recent attempts to improve protein quality in the cereals have resulted in naturally occurring and induced-mutant high lysine varieties (1-4). In high lysine maize, barley, and sorghum, shifts in the relative proportions of the lysine-rich and lysine-poor storage proteins provide the bulk of the lysine increase. Much effort has been given to elucidating the underlying biochemical and genetic events conferring the "high lysine" trait in an attempt to improve the nutritional value of the amino acid complement in the cereal grains (5). Fundamental to an understanding of the biological constraints on storage protein quality and quantity is information concerning the input of nitrogen and its transformations into the other required nitrogenous substances in the developing grain. The limited data on the composition and rates of nitrogen input suggest that most of this nitrogen is transported from the vegetative portions of the plant into the developing grain via the phloem. The predominant forms of this nitrogen are asparagine and glutamine (for example, in *Spartium junceum* they amount to about 75% of the total amino nitrogen) with the remaining protein amino acids either absent

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or present in low amounts (6, 7). It is likely that the amounts of most amino acids provided to the developing seed via the phloem are insufficient for the formation of storage proteins (8). This suggests that the developing grains themselves synthesize large amounts of specific amino acids from sucrose and the amino acids supplied by the phloem sap.

We present here the first characterization of the enzyme β-aspartokinase (EC 2.7.2.4.) from the developing endosperm of maize. This enzyme catalyzes the conversion of aspartate to β-aspartate phosphate, the first step in the biosynthesis of the family of amino acids which includes lysine, methionine, and threonine. Evidence is presented regarding feedback regulation of the enzyme, and the properties of the enzyme from developing maize endosperm are compared to those of the enzyme found in vegetative tissue of the same organism.

MATERIALS AND METHODS

Maize (*Zea mays* cv. Pioneer 3145) seeds were surface sterilized with 0.26% sodium hypochlorite, planted on filter paper in a pyrex storage dish, and allowed to grow in the dark at 26°C. Shoots of 6-day-old seedlings were used as the source of β-aspartokinase from vegetative tissue. The endosperm-derived β-aspartokinase was obtained from the kernels of greenhouse-grown plants 30 days post-pollination. Endosperm in the early dough state was isolated essentially free of associated tissue by peeling away the seed coat and aleurone layer and excising the embryo axis and scutellum. The enzyme was extracted and partially purified from both shoot and endosperm according to the methods of Cheshire and Miflin (9).

The activity of β-aspartokinase was assayed by a modification of the method of Black and Wright (10). The standard assay mixture contained in 1 ml total volume: 50 mM L-aspartate, 25 mM ATP, 25 mM MgCl, 400 mM hydroxylamine, 100 mM Tris HCl pH 8.0, and enzyme. The standard reaction was initiated with the enzyme, carried out for 60 min at 30°C, and terminated with 1.5 ml of an acid/ferric chloride reagent (11). The protein was pelleted by centrifugation and the absorbance of the supernatant measured with a Beckman Model 25 double beam spectrophotometer at 505 nm 15 to 20 min after the termination of the reaction.

RESULTS

β-aspartokinase activities derived from both etiolated shoots and endosperm were dependent upon aspartate, ATP, Mg^{++} or Mn^{++}, and enzyme concentration. Fe^{++} was unable to replace the requirement for Mg^{++} while Ca^{++} and Zn^{++} did allow low levels of activity to be detected (Table 1). The reaction

Table 1. Divalent cation effects on β-aspartokinase activity.*

Cation	nmoles BAP/minute 12.5 mM	25 mM
Mg^{++}	2.50	2.90
Mn^{++}	2.00	2.10
Zn^{++}	1.00	2.00
Ca^{++}	0.60	1.00
Fe^{++}	0.35	0.20
-Mg^{++}	0.30	

*Activity represented as nmoles of β-aspartate phosphate (BAP) formed/minute under standard assay conditions, using 25 mM ATP, 1.5 mg enzyme, 60 minutes, 30°C.

rates were linear with both time (for 60 min) and enzyme concentration under standard assay conditions. Specific activities ranged from 2.5 to 4.0 and 1.5 to 2.5 nm of product per mg protein per min for shoot- and endosperm-derived enzyme, respectively, at 30°C and pH 8.0 (Table 2). Both enzyme preparations were stable for approximately 2 months when maintained frozen, with a gradual loss of activity thereafter.

In the shoot-derived enzyme, double reciprocal plots of velocity vs. aspartate concentration deviated from a straight line at low aspartate concentrations (Fig. 1). Two apparent Km's for aspartate were estimated to be 0.5 and 6.6 mM, which are similar to the two Km's reported by Bryan (12) in enzyme derived from maize shoots. The endosperm-derived β-aspartokinase also exhibited this complex kinetic relationship. Two Km's of 1.5 and 8.0 were estimated from a Lineweaver-Burk plot of assays where aspartate concentration was varied over the same range as the shoot-derived enzyme (Fig. 1). Total extractable activities from both tissues were similar (Table 2).

The effects of the potential endproducts lysine, methionine, and thre-

Table 2. Characteristics of shoot- and endosperm-derived β-aspartokinase of maize.

		Shoot (6 days old)	Endosperm (30 days post-pollination)
Specific activity* (nmoles of product/mg protein/min)		3.5	1.5
Total activity* (nmoles of product/min/g fresh weight)		7.0	5.9
Km (mM) for aspartate		0.5 and 6.6	1.5 and 8.0
V_{max}		3.6-7.4	1.7-2.1
Endproduct inhibition		(% of control)	
Lysine	0.5 mM	98	96
	1.0 mM	78	71
	5.0 mM	40	46
Methionine	1.0 mM	116	-
	5.0 mM	104	107
	10.0 mM	95	111
Threonine	1.0 mM	108	-
	5.0 mM	106	96
	10.0 mM	93	106

*Values reported represent the average obtained from three or more repetitions run under standard assay conditions, as discussed in the text.

onine were studied to determine the nature of feedback inhibition that may exist. In the endosperm-derived enzyme, lysine inhibited activity by 54% at 5 mM, while threonine and methionine showed little effect (Table 2). The shoot-derived enzyme was also inhibited by lysine and not by threonine or methionine. Lysine, but not methionine or threonine, also partially protected the enzyme from heat inactivation at 45°C.

DISCUSSION

The *in vivo* labeling of amino acids from ^{14}C-precursors in developing

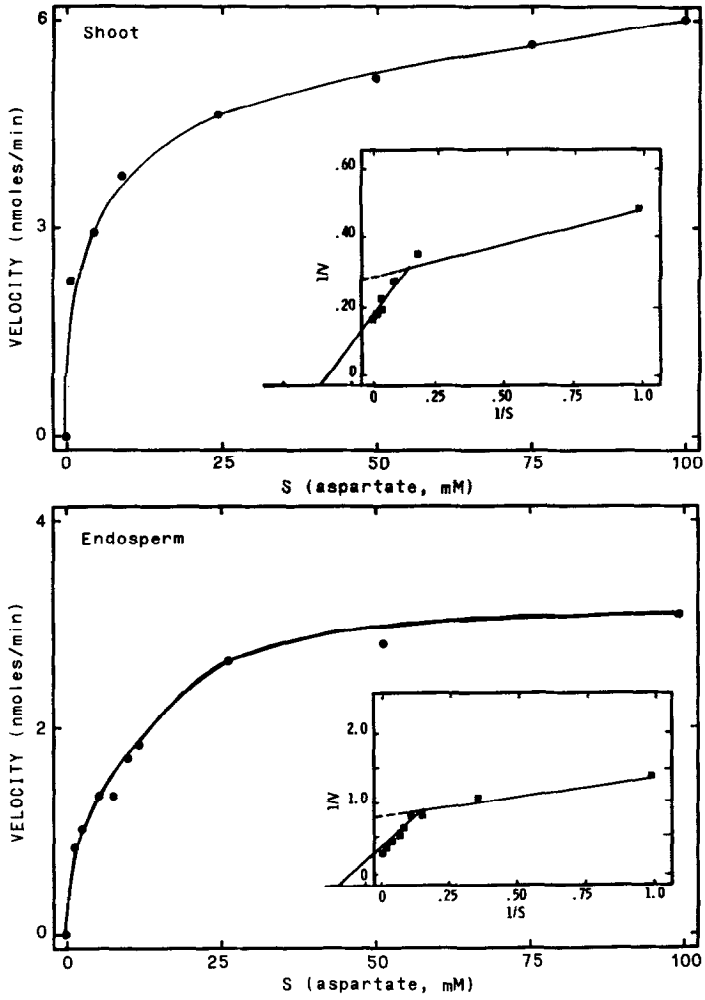

Fig. 1. Relationship of aspartate concentration to the velocity of shoot- and endosperm-derived β-aspartokinase activity under the standard assay conditions in the text. Inset: Double reciprocal plot of the same data, indicating the presence of two apparent Km's.

cereal endosperm has suggested that substantial amino acid synthesis may be occurring *in situ* (13, 14). McConnell (13) reported the appearance of ^{14}C in many amino acids of the developing wheat endosperm following injection of the ^{14}C labeled precursors (^{14}C-glutamic acid, -glutamine, -arginine, -proline, -aspartic acid, -succinic acid, and -glyoxylate) into the stem. Sodek (14) has recently provided similar supporting evidence for the developing

endosperm of maize. However, Sodek injected the precursors directly into immature endosperm tissue, removing doubt as to the site of the *de novo* amino acid synthesis. He was also able to provide evidence, based on the distribution of the ^{14}C in lysine and threonine from the injected precursors {aspartic acid-[4-^{14}C]; alanine-[1-^{14}C]; acetate-[2-^{14}C]; and diaminopimelic acid[1, (7)-^{14}C]}, indicating that the diaminopimelic acid, and not the α-aminoadipic acid lysine biosynthetic pathway, was functioning in the developing maize endosperm (15). The presence of β-aspartokinase in the developing endosperm of maize strongly suggests the existence of the biosynthetic pathway for the aspartate-derived family of amino acids (lysine, methionine, and threonine) in the endosperm. A second enzyme in this pathway, homoserine dehydrogenase (EC 1.1.1.3.), has recently been detected in developing maize endosperm as well (16).

The presence of this endproduct-controlled pathway in developing maize endosperm could offer new potentials for selecting for an improved nutritional balance of these amino acids in the kernel. Amino acid analogs or specific combinations of naturally occurring amino acids have been employed to select for mutants which are defective in their endproduct control and subsequently overproduce the amino acids which had been subject to that control. Plant cells in culture have been selected for resistance to different amino acid analogs including the lysine analog, S-2-aminoethyl-L-cysteine, and have been shown to accumulate the antagonistic, naturally occurring amino acid at levels commonly 10-fold greater than in the wild type (17-22). Similar mutation screens have been designed to be carried out on mutagenized populations of germinating seedlings (23-25).

It may be feasible to develop screens that would select for mutants with defective endproduct control of β-aspartokinase or other key control points in the biosynthesis of lysine, methionine, and threonine in the developing endosperm. Such mutants may then accumulate these essential amino acids in the free pool at levels that may significantly improve the nutri-

tional balance of the total amino acid complement of the endosperm. Mutants that overproduce lysine in yeast (26) and in rice cells (22) have been reported to exhibit an increase in protein-bound lysine as well. The biochemical behavior of such mutations and an evaluation of their agronomic advantages and/or disadvantages remain to be determined.

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