VARIATIONS IN β-ASPARTATE KINASE ACTIVITY DURING THE DEVELOPMENT OF MAIZE ENDOSPERM

Randolph R. HENKE and Rosemarie WAHNBAECK-SPENCER
Comparative Animal Research Laboratory, The University of Tennessee, Oak Ridge, TN 37830, USA

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1. Introduction

Information concerning amino nitrogen input and its conversion into other nitrogenous compounds necessary for grain development is fundamental to an understanding of the biological constraints affecting cereal grain protein quantity and quality. Two origins for the amino acids utilized in grain development are generally acknowledged:

(1) Amino acids are 'delivered' to the developing grain by translocation through the vascular connections;
(2) Amino acids are synthesized de novo within the developing grain from precursors supplied by the translocation stream.

Evidence supporting the delivery of amino acids has been provided primarily by the demonstration of the presence of most amino acids in the translocation stream which supplies the developing seeds of legumes and the subsequent incorporation of those amino acids into reserve protein [1–4]. Although most of the protein amino acids are present in this translocation stream, the bulk of the nitrogen is translocated as amide (asparagine and glutamine [4]).

Amino acids synthesized from precursors provide a means by which the seed may overcome the imbalance in the amino acids supplied, thus meeting the requirements of the developing seed. Several laboratories have followed the distribution of ^{14}C in the developing storage tissues of cereals and legumes from ^{14}C photosynthate supplied by the translocation stream, and by ^{14}C-labeled precursors injected into the translocation stream or directly into the developing seed [3,5–8]. These studies demonstrate the involvement of the known plant amino acid biosynthetic pathways in the conversion of these labeled precursors into various amino acids.

Recent evidence indicating how the amide nitrogen of glutamine is made available for amino acid biosynthesis has been provided by the detection of glutamate synthase in developing maize endosperm [9,10] and pea cotyledons [11,12]. An important role for amino acid biosynthesis is also suggested in maize endosperm by the presence of the nitrogen assimilatory enzymes (glutamate dehydrogenase, glutamine synthetase and asparagine synthetase [10]) and enzymes of the aspartate family of amino acids (aspartate kinase (AK) [13,14], homoserine dehydrogenase [14] and diaminopimelate decarboxylase [14,15]).

This paper presents evidence that relates changes in AK activity to other physiological events throughout maize endosperm development.

2. Materials and methods

2.1. *Plant material*

Maize (*Zea mays* c.v. Pioneer 3145) was field grown. The first two emerging ears on each plant were self-pollinated and ears were harvested at intervals from 5–55 days after pollination. After harvesting, kernels were removed from the ear, quickly frozen, lyophilized and stored at –20°C. Endosperm was isolated essentially free of associated tissue by peeling away the outer integuments and excising the embryo axis and scutellum. This endosperm tissue was used for subsequent analyses.

2.2. *AK isolation and assay*

AK (ATP: L-aspartate 4-phosphotransferase, EC 2.7.2.4) was extracted and partially purified from endosperm using the methods in [16] as in [13].

The activity of AK was assayed by the colorimetric β-aspartylhydroxymate assay [17] as in [13]. Identification of the activity as AK was performed according to [18].

2.3. *Dry weight, protein, nitrogen and α-amino nitrogen determination*

Endosperm dry weight was determined directly from the lyophilized material. Soluble protein was determined by the method in [19] on the 10% trichloroacetic acid-precipitable material in the crude enzyme extract. Bovine serum albumin was used as the protein standard. Total nitrogen was determined by a micro-Kjeldahl method on the lyophilized endosperm [20]. Total protein was calculated by multiplying total nitrogen values by 6.25. Soluble amino nitrogen was determined on a 20% ethanol endosperm extract by a ninhydrin method [21].

3. Results

Endosperm-derived AK activity was dependent upon aspartate, ATP, Mg^{2+} and enzyme concentration throughout development. Reaction rates were linear with both time (for 75 min) and enzyme concentration (from 0.5–4.0 mg protein) under standard assay conditions. Double reciprocal plots of velocity versus aspartate concentration deviated from a straight line at low aspartate concentrations. Two app. K_m values for aspartate were estimated to be 0.5 and 7.0 mM for AK obtained from endosperm 15, 25 and 35 days after pollination. This was similar to K_m values reported for endosperm obtained from greenhouse-grown plants 30 days after pollination [13].

Asparagine synthetase and aspartyl tRNA ligase may also contribute to the apparent total activity of AK observed with the aspartylhydroxymate assay used [18,22,23]. However, these enzyme activities could not be demonstrated in the enzyme preparations for the following reasons.

(1) The level of activity was not affected by 10 mM $NaK_4P_2O_7$, a strong inhibitor of asparagine synthetase [24] and aspartyl tRNA ligase [25].

(2) About 85% of the activity was lost if hydroxylamine was added after the enzyme reaction (at 30°C for 60 min) was terminated. This is consistent with the lack of stability of β-aspartylphosphate, the product of the AK reaction [17,18,26].

(3) No asparagine synthetase activity could be detected in a radiolabeled assay [23].

The endproduct L-lysine inhibited AK activity ~50% at 1.0 mM lysine. This inhibition decreased with higher aspartate concentrations. At 5 mM lysine increased inhibition of AK activity by only 5–10%. L-Threonine and L-methionine at 5 mM had little or no effect on AK activity alone or in combination

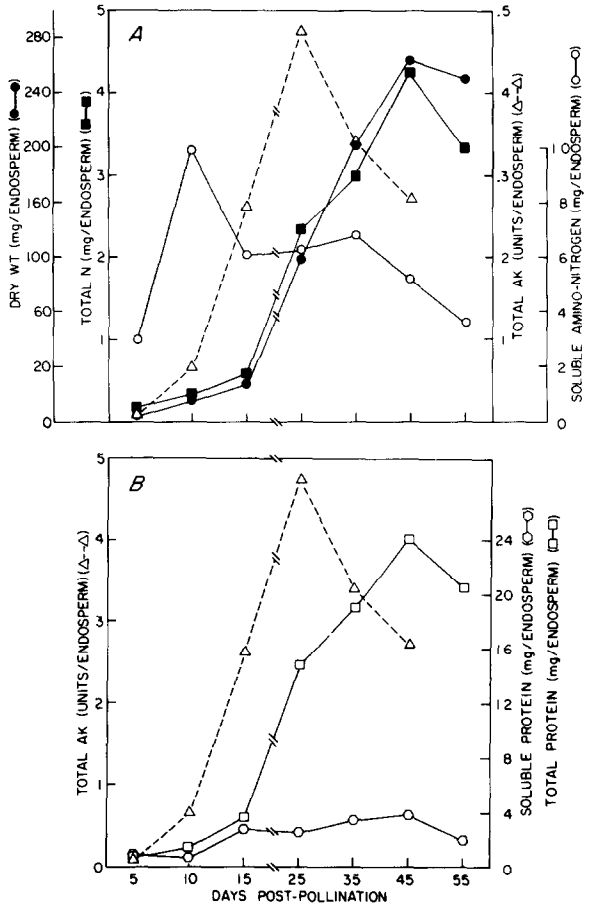

Fig.1A,B. β-aspartate kinase (AK) levels in developing maize endosperm related to changes in: (A) dry weight, total nitrogen and soluble amino nitrogen; (B) soluble protein and total protein levels per endosperm.

with lysine. AK sensitivity to lysine remained constant throughout endosperm development.

The total AK activity (units per endosperm; 1 unit = 1 nmol β-aspartylhydroxymate/min) increased sharply during early endosperm development, reached a peak at ~25 days after pollination, and then declined at kernel maturity (fig.1A,B). This increase in AK activity preceded slightly the accumulation of total nitrogen (total protein) and dry weight in the developing endosperm (fig.1A,B). Soluble α-amino nitrogen increased from 5–10 days after pollination and then declined gradually throughout endosperm development to its original level at maturity. Soluble endosperm protein remained relatively constant throughout endosperm development.

4. Discussion

A sharp increase in maize endosperm dry weight and total protein begins ~10 days after pollination and continues for ~4 weeks (fig.1) [9,27]. During this active period of storage protein deposition and protein synthesis the greatest demand for amino acids would presumably exist. As discussed, the source of amino acids for endosperm storage protein synthesis is likely to include de novo biosynthesis of many amino acids as well as a direct supply via the translocation stream. To meet the greater demand for amino acids, the developing endosperm might be expected to exhibit an increased capacity to synthesize these amino acids from the amide nitrogen and various carbon sources. A sharp increase in glutamate synthase activity was observed [9] in developing maize endosperm from 10–30 days after pollination, followed by a decline in activity to low levels at endosperm maturity. The peak they observed in glutamate synthase activity coincided with the period of most active nitrogen accumulation in the endosperm. The authors felt that the flow of nitrogen via the glutamate synthase system could play an important role in providing amino nitrogen needed to sustain the biosynthesis of other amino acids for storage protein synthesis. Similar increases in glutamate synthase activity and in other nitrogen assimilatory enzymes (glutamate dehydrogenase, glutamine synthetase and asparagine synthetase) have been observed [10] early in maize endosperm development.

β-Aspartate kinase, the first enzyme unique to the biosynthesis of the aspartate-derived amino acids (lysine, methionine, threonine and isoleucine), shows the same pattern of increasing activity in developing maize endosperm as does glutamate synthase (fig.1A,B). AK total activity increases about 25-fold from 5–25 days after pollination and declines during the remainder of endosperm development. Since soluble protein increases only 3-fold from 5–25 days after pollination, the increase in AK activity appears to have some physiological significance and is not simply associated with changes in the soluble protein levels (fig.1B). Also, the decline in AK activity after 25 days was not associated with a similar decline in soluble protein levels up to 45 days after pollination. 55 days after pollination the hardness of the endosperm and the high starch content may have interfered with the efficiency of extraction and did seem to interfere with AK assay since the level of total activity varied from 0–0.2 units/endosperm.

Since the levels of AK activity correspond to those of glutamate synthase and coincide with the period of most active protein accumulation, it is reasonable to conclude that AK is active in providing greater amounts of the aspartate-derived amino acids during the period of greatest demand in endosperm development. Similar changes in another enzyme of the pathway for the aspartate family of amino acids (homoserine dehydrogenase) has been observed [28]. These data suggest that the biosynthetic pathway for the aspartate-derived amino acids may be an important source for lysine, methionine, threonine and isoleucine in the synthesis of the endosperm storage proteins in maize.

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