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BIOSYNTHESIS OF LYSINE AND OTHER AMINO ACIDS IN THE DEVELOPING MAIZE ENDOSPERM

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Abstract—Tracer studies with aspartic acid-[4-^{14}C], alanine-[1-^{14}C] acetate-[2-^{14}C] and diaminopimelic acid-[1,(7)-^{14}C] injected into the developing endosperm of maize revealed that the biosynthesis of lysine and other amino acids occurs in this organ. The data suggest that lysine is synthesized via the diaminopimelic acid pathway.

INTRODUCTION

The low lysine content of maize endosperm is chiefly reponsible for the nutritional inadequacy of maize as a source of protein. Following the discovery of the high-lysine maize mutant, opaque-2 [1] with its high nutritive value [2], there has been considerable interest in the biochemical changes underlying the increased lysine content. The opaque-2 gene appears to alter the balance of the reserve proteins, but it mainly reduces the synthesis of zein, a major storage protein of the maize endosperm that contains virtually no lysine [1,3–6].

The higher utilization of lysine by the opaque-2 endosperm presumably involves some change in the metabolism of lysine in the devloping grain. There is some evidence that lysine may be catabolized to a lesser extent in the high-lysine mutant than in the normal version in the case of maize [7] and barley [8]. The possible involvement of the biosynthetic pathway on the other hand, cannot be evaluated since it is not known whether lysine is actually synthesized in the developing endosperm or supplied pre-formed from other parts of the plant. For this reason a study was made of the fate of radioactive precursors of lysine biosynthesis following their injection directly into the endosperm. Evidence is presented that the synthesis of lysine and other protein amino acids occurs in the developing endosperm of maize.

RESULTS

Labeling of amino acids. After aspartate-[4-^{14}C], alanine-[1-^{14}C] and acetate-[2-^{14}C] were administered to developing maize endosperm, all protein amino acids became labeled (Table 1). A non-amino acid fraction, which includes sugars and organic acids, was also highly labeled, especially when alanine-[1-^{14}C] was precursor. Alanine-[1-^{14}C] labeled amino acids derived from glycolytic intermediates more strongly than those derived from the Krebs cycle acids, in contrast to acetate-[2-^{14}C] and aspartate-[4-^{14}C]. Acetate-[2-^{14}C] was the most efficient precursor of amino acids, which is probably related to the greater metabolic stability of its labeled carbon. Estimates of the recovery of injected label indicate that a major portion of the label was lost, presumably in the form of CO_2.

When diaminopimelic acid (DAP)-[1,(7)-^{14}C] was administered, lysine was the only protein amino acid labeled (Table 2). Some label was also found in several unidentified compounds, two of which co-elute with 2-amino-adipic acid and pipecolic acid. Many of these compounds are possibly products or intermediates of lysine catabolism. A large part of the administered DAP

Table 1. Incorporation of ^{14}C into total amino acids in the developing endosperm of maize following injection of labeled compounds

	dpm/100 mg lyophilyzed endosperm		
	Asp-[4-^{14}C]	Ala-[1-^{14}C]	Ac-[2-^{14}C]
	(%)*	(%)*	(%)*
Lys	660 (6.4)	750 (1.7)	3300 (1.4)
His	285 (2.7)	2730 (6.3)	630 (0.3)
Arg	750 (7.2)	2130 (4.9)	21000 (9.1)
Asp	192000 (–)	9530 (21.8)	76300 (33.0)
Thr	540 (5.2)	390 (0.9)	3150 (1.4)
Ser	345 (3.3)	4500 (10.3)	5300 (2.3)
Glu	4935 (47.6)	4980 (11.4)	83650 (36.1)
Pro	825 (8.0)	1650 (3.8)	7200 (3.1)
Gly	375 (3.6)	2900 (6.6)	7680 (3.3)
Ala	570 (5.5)	50100 (–)	4700 (2.0)
Val	345 (3.3)	7080 (16.2)	3480 (1.5)
Ile	150 (1.4)	210 (0.5)	1200 (0.5)
Leu	90 (0.9)	430 (1.0)	11700 (5.1)
Tyr	150 (1.4)	2010 (4.6)	580 (0.2)
Phe	350 (3.4)	4310 (9.9)	1530 (0.7)
Total A.A.	202370	93700	231400
Non amino acid	24800	291000	38400
Total recovered	227170	384700	269800
Total hydrolysate	226000	394000	283000

* % Of total ^{14}C in amino acids, discounting precursor amino acids.

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Table 2. Incorporation of ^{14}C from injected DAP-[1,(7)-^{14}C] into amino acids and other compounds in the developing endosperm of maize

	dpm/100 mg Lyophilyzed endosperm
Lysine	10400
Other protein amino acids	0
DAP	42200
2-amino adipic acid	2330
Pipecolic acid	2370
Others (not identified)	8350
Total	65650
Organic acids/sugars	300
Total recovered	65950
Total hydrolysate	67900

was not metabolized which may be due, in part at least, to the presence of the unnatural DD isomer in the racemic mixture injected. The amount of DAP metabolized is greater than indicated by straightforward comparison of counts since the loss of half of the label as CO_2 upon conversion to lysine should be taken into account.

Evidence for the direct conversion of DAP into lysine was obtained after decarboxylation of lysine with ninhydrin. 93% of the ^{14}C in lysine was recovered as CO_2 on decarboxylation, demonstrating that virtually all the label was present in C-1 of lysine. This would be expected if DAP, being labeled only in the carboxyl carbons, were transformed directly into lysine.

Incorporation into storage proteins. The distribution of label in the various protein and non-protein fractions is shown in Table 3. Label derived from all the precursors studied was incorporated into each of the protein fractions. In general the albumin and globulin fractions incorporated higher amounts of labeled amino acids relative to their size than the major storage proteins (the zeins and glutelin).

Although varying amounts of label were incorporated into the endosperm proteins, most of the label was found in either of the non-protein fractions. In the aspartate-[4-^{14}C] experiment, 95% of the label was in the TCA-soluble fraction. Only aspartic acid (see Table 1) has enough label to account for the total radioactivity in this fraction, so it appears that a sizeable proportion of the labeled aspartate administered was not metabolized. In the alanine-[1-^{14}C] experiment 77% of the label was recovered in the residual (starch) fraction. This is in agreement with the data in Table 1 where a very high proportion of label was in the nonamino acid fraction. Acetate-[2-^{14}C] also contributed significantly to the starch fraction, but in contrast to alanine-[1-^{14}C] this was much smaller when considered in relation to the labeling of amino acids.

DISCUSSION

The data presented in this paper show that the biosynthesis of lysine and other protein amino acids occurs in the developing maize endosperm. This is in agreement with the work of McConnell's group [9,10] who demonstrated the labeling of many amino acids in the developing wheat kernel following the injection of precursors into the stem. Any doubt as to the site of amino acid formation is removed in the present experiments since the precursors were administered at the location under study. However, since preformed amino acids could be the main source of amino acids for protein synthesis in the endosperm, the importance of *de novo* amino acid synthesis cannot be evaluated without a better knowledge of the transport form of nitrogen to the ear. In other plants, it has been shown that all protein amino acids are present in the translocation stream, but glutamine and/or asparagine are the principle forms of nitrogen transported to the fruits [11,12]. Since this nitrogen is readily transferred to the amino group of other amino acids, this implies a considerable contribution of *de novo* amino acid syntheis to meet the needs of storage protein synthesis.

The widespread distribution of label in the protein amino acids might not perhaps be expected with the precursors used. The formation of oxaloacetate, via transamination and β-carboxylation reactions, is the probable key to the general distribution of label in the alanine-[1-^{14}C] experiment. Aspartate-[4-^{14}C] and acetate-[2-^{14}C] are also readily transformed into oxaloacetate which, as precursor of the gluconeogenic pathway, can explain the labeling of sugars and amino acids derived from the glycolytic intermediates. However, to fully explain the results, it must be assumed that label entering either C-1 or C-4 of oxaloacetate is subsequently randomized in the carboxyls after equilibration with fumarate in the Krebs cycle [13]. It might be added that the labeling of starch by the precursors used may not signify a net movement of carbon towards sugar synthesis unless

Table 3. Distribution of ^{14}C in the protein fractions of maize endosperm following the injection of labeled compounds

	Asp-4-^{14}C dpm*	Ala-1-^{14}C dpm*	Ac-2-^{14}C dpm*	DAP-1,(7)-^{14}C dpm*	(Nitrogen content)*
TCA soluble	95.2	13.4	35.7	88.8	
Albumin	0.3	1.2	3.5	1.2	(6.0)
Globulin	0.8	2.3	8.0	2.3	(6.3)
Zein I	0.3	1.8	15.3	0.3	(13.6)
Zein II	0.2	0.9	2.4	0.3	(12.0)
Glutelin	1.7	3.4	9.9	5.4	(39.8)
Residue	1.5	77.0†	25.0	1.9	
Total dpm/100 mg	256200	490000	355000	74400	

* % Total. † About 85% of the ^{14}C in the residue fraction was recovered as glucose after hydrolysis in 0.5 M HCl at 100° for 3 hr, and chromatography with butanol–acetic–H_2O (3:1:1)

an external supply of some precursor of the gluconeogenic pathway were to exist.

The radioactive precursors used in this study were chosen especially to help decide which of the two pathways of lysine biosynthesis [14] operates in maize endosperm. Thus aspartate-[4-^{14}C] and alanine-[1-^{14}C] should label lysine only via the DAP pathway whereas acetate-[2-^{14}C] is a direct precursor of lysine in the α-aminoadipic acid pathway, although it can follow the DAP pathway via aspartate after metabolism in the Krebs cycle [13, 15]. The fact that aspartate-[4-^{14}C] and alanine-[1-^{14}C] labeled all amino acids, of which many were more effectively labelled than lysine itself, considerably diminishes their usefulness to distinguish between the two pathways simply on the basis of incorporation. Possibly, aspartate must be converted to one of the dicarboxylic acids of the Krebs cycle before being transformed into lysine [13]. Nevertheless, information on the biosynthetic pathway followed may be obtained by comparing the ratio of counts in lysine and threonine for each of the precursors studied, since the initial steps of the DAP pathway of lysine biosynthesis are the same as those for threonine. It may be calculated from Table 1 that this ratio is similar when aspartate-[4-^{14}C] (= 1.2) and acetate-[2-^{14}C] (= 1.05) are precursors. This would be expected if the DAP pathway were operating since acetate would label lysine and threonine via aspartate and therefore give a similar ratio as aspartate itself. The higher ratio obtained in the alanine-[1-^{14}C] experiment (= 1.92 from Table 1) would also be expected if the DAP pathway were operating. Alanine-[1-^{14}C] may label pyruvate which is a direct precursor of lysine by the DAP pathway, but not of threonine. Aspartate, which was highly labeled in the alanine-[1-^{14}C] experiment, must account for the labeling of threonine and some of the lysine, but direct labeling of lysine via pyruvate would result in a higher lysine to threonine ratio than that obtained with labeled aspartate. Moller [13] obtained strong evidence that alanine-[1-^{14}C] and pyruvate-[1-^{14}C] label lysine not only directly but in part via aspartate in barley seedlings.

Additional evidence for the DAP pathway is provided in the present experiments where it was shown that DAP-[1,(7)-^{14}C] was converted directly to lysine, in agreement with similar work with wheat kernels [16] and previous work with maize where DAP-[1,(7)-^{14}C] was administered by shank injection (L. Sodek and C. M. Wilson, unpublished). The possibility that *meso*-DAP-[1,(7)-^{14}C] was converted to L-lysine-[1-^{14}C] by a nonspecific D-amino acid decarboxylase is unlikely since a DAP decarboxylase has been partially purified from maize endosperm and found to have no activity towards any of the many D-(and L-) amino acids tested (L. Sodek, unpublished).

There is now strong evidence, both tracer [13] and enzymic [17], that the biosynthesis of lysine in higher plants follows the DAP pathway. Our data, which demonstrates the synthesis of lysine in the developing endosperm of maize is consistent with this view.

EXPERIMENTAL

Maize (*Zea mays*, L. cv. Maya opaque-2) growing in the field was used in this study. The isotopes were obtained with the following specifications: Na acetate-[2-^{14}C] (23.5 mC/mmol), L-alanine-[1-^{14}C] (23.8 mC/mmol), L-aspartic acid-[4-^{14}C] (12.4 mC/mmol), and diaminopimelic acid-[1,(7)-^{14}C] (48 mC/mmol).

Tracer expts. Isotopes were dissolved in H_2O to give a concn of 2 μC/μl for acetate and alanine, 0.5 μC/μl for aspartate, and 0.1 μC/μl for diaminopimelic acid. Each isotope (1 μl) was injected by microsyringe directly into the endosperm 30 days after pollination (early dough stage). At least 25 kernels were injected for each isotope studied, and to assure similar experimental conditions different regions of the same ear were employed for each isotope. One week after treatment, the ears were harvested and stored frozen. The kernels were dehulled, the endosperms separated from the embryos and lyophilized.

Analysis for labeled amino acids. Powdered endosperm (100 mg) was hydrolyzed in 10 ml 6 N HCl at 110° for 24 hr in a screw-cap culture tube. After drying by rotary evaporator the hydrolysate was taken up in a small vol of Na citrate buffer, pH 2.2 [18] and run through a small column of Dowex-1 × 8 Cl-(about 1 ml of resin) to remove humin [19]. The sample was eluted with more buffer such that the hydrolysate of 200 mg of material was in a total vol of 3 ml. This clean-up procedure was necessary to avoid problems during the liquid scintillation counting of the whole hydrolysates and also to avoid contamination of the resin of the amino acid analyser used subsequently to separate the amino acids. The separation of the amino acids together with identification and counting of the fractions was performed as described in ref. 7.

Fractionation and counting of endosperm proteins. This was carried out as described in refs. 5 and 7.

Chemical decarboxylation of lysine. The lysine fraction obtained after separation on the ion exchange column was decarboxylated with ninhydrin following the method of ref. 20 with minor modifications. The CO_2 liberated was collected in KOH, and counted after transformation into $BaCO_3$ [21]. DL-lysine-[1-^{14}C] was used to standardize the procedure.

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