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Short Communication

Lysine-Ketoglutarate Reductase Activity in Developing Maize Endosperm[1]

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PAULO ARRUDA, LADASLAV SODEK, AND WILLIAM JOSÉ DA SILVA
Departments of Genetics and Evolution (P. A., W. J. S.) and Plant Physiology (L. S.), Instituto de Biologia, Universidade Estadual de Campinas, 13100 Campinas, SP., Brazil

ABSTRACT

Lysine-ketoglutarate reductase activity was detected and characterized in the developing endosperm of maize (*Zea mays L.*). The enzyme showed specificity for its substrates: lysine, α-ketoglutarate, and NADPH. Formation of the reaction product saccharopine was demonstrated. The pH optimum of the enzyme was close to 7, and the K_m for lysine and α-ketoglutarate were 5.2 and 1.8 millimolar, respectively.

Lysine-ketoglutarate reductase catalyzes the following reaction:

lysine + α-ketoglutarate + NADPH → saccharopine + $NADP^+$

The enzyme has been characterized in human and animal tissues (3, 7, 8) where it is believed to catalyze the first step of lysine catabolism (4). A similar reaction involving NAD is catalyzed by saccharopine dehydrogenase, an enzyme found in yeast and fungi where the reverse of the above reaction is considered to be the final step of lysine biosynthesis (5, 6, 12, 16). Thus, in some organisms saccharopine is an intermediate of lysine biosynthesis while in the others it appears to be involved in lysine breakdown. Actually, several pathways for lysine catabolism are known (11), but in higher plants no general pathway has been established. Nevertheless, tracer studies with barley seedlings have provided strong evidence for the pathway involving saccharopine as intermediate (10), suggesting that this route may be important for lysine breakdown in cereals at least. However, we are unaware of any studies with enzymes in higher plants in support of this pathway.

In this report, we describe the characterization of lysine-ketoglutarate reductase in developing maize endosperm, a tissue known to degrade lysine extensively (14).

MATERIALS AND METHODS

Extracts were prepared from immature endosperm of a maize (*Zea mays* L.) hybrid synthesized from the inbreds ML-2000 × ML-2001, which were grown in the experimental field of the Universidade Estadual de Campinas. Fifty endosperm were isolated from material harvested 30 d after pollination, and ground in a chilled mortar with 25 ml of 100 mM K-phosphate (pH 7.0) containing 1 mM EDTA and 1 mM 2-mercaptoethanol. The homogenate was centrifuged at 12,000*g* for 15 min, and the supernatant was treated with $(NH_4)_2SO_4$. The precipitate obtained between 25 and 70% saturation was collected and taken up in 2.5 ml of extraction buffer. After desalting on a Sephadex G-25 column (1.5 × 16 cm), the protein fraction was assayed for lysine-ketoglutarate reductase activity using conditions based on those described by Hutzler and Dancis (7). The assay mixture contained K-phosphate (pH 7.0; 300 μmol), L-lysine (50 μmol), α-ketoglutarate (25 μmol), NADPH (300 nmol), and enzyme (0.1–0.2 mg protein) in a total volume of 3 ml. The mixture was incubated at 30°C, and oxidation of NADPH was monitored in a spectrophotometer at 340 nm.

RESULTS AND DISCUSSION

The data in Table I show that partially purified extracts of maize endosperm oxidized NADPH in the presence of lysine and α-ketoglutarate. In the absence of one or both of these substrates, the oxidation of NADPH was minimal. Specificity for the substrates is indicated by the fact that several amino acids, including closely related structures such as L-ornithine and D-lysine, would not substitute for L-lysine, nor would oxaloacetate or pyruvate substitute for α-ketoglutarate. NADH would not replace NADPH as electron donor, as was found for the enzyme from human liver (7).

To confirm that the activity being measured was truly lysine-ketoglutarate reductase, it would be essential to demonstrate the formation of saccharopine in the assay. This was attempted using [^{14}C]lysine in the reaction mixture to increase the sensitivity of product detection. For this purpose, the NADPH concentration was increased to 750 μM and 10 μCi of [U-^{14}C]L-lysine per ml included in the assay. During the incubation at 30°C, aliquots were taken at 0 and 60 min, and a portion (10 μl) was applied to a TLC plate (Merck-Kiesselgel 60) and developed with phenol:H_2O (7:3). Saccharopine, synthesized chemically essentially as described by Moller (10), was used as standard. Radioactive compounds were located by autoradiography, and amino acids were located by spraying with *o*-phthalaldehyde reagent (1). These techniques revealed that a radioactive compound was formed in the assay with an R_F of 0.16 corresponding to that of saccharopine (Fig. 1). This spot was absent when NADPH was substituted with NADH. After removal and counting of the regions corresponding to saccharopine and lysine (1), an activity of 6.86 μmol/min·endosperm was obtained (based on the specific radioactivity of lysine of [2 × 10^6 cpm/μmol). This is reasonably close to the value of 8.27 μmol/min·endosperm calculated from the oxidation of NADPH in a second assay run simultaneously. Similar information was obtained in parallel experiments with [1-^{14}C]DL-lysine. This evidence suggests that not only was saccharopine formed in the assay, but the measure of NADPH oxidation dependent on lysine and α-ketoglutarate is a reasonable estimate of the lysine-ketoglutarate reductase activity.

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Table I. *Substrate Specificity of Lysine-Ketoglutarate Reductase*

The complete system was as described in the text. Other compounds were present at 15 mM (amino acids) and 8 mM (organic acids) or as indicated in the table.

Assay Substrates	Activity, NADPH Oxidation
	μmol/min·endosperm
Complete system	9.4
− Lysine	1.2
− α-Ketoglutarate	1.9
− Lysine − α-ketoglutarate	0.9
− Lysine + L-ornithine	1.0
− Lysine + L-glutamine	2.3
− Lysine + L-asparagine	1.6
− Lysine + D-lysine	1.6
− Lysine + diaminopimelate	2.7
− α-Ketoglutarate + pyruvate	1.2
− α-Ketoglutarate + oxaloacetate	19.7*
− α-Ketoglutarate + oxaloacetate, − lysine	21.9*
− NADPH, + NADH (0.1 mM)	0.5
Complete, + NH_4Cl (50 mM)	11.1
Complete, + Amino oxyacetate (1 mM)	8.9
Lysine-ketoglutarate reductase†	8.5

* Malate dehydrogenase-type activity.

† Calculated by substracting − lysine, − α-ketoglutarate from complete.

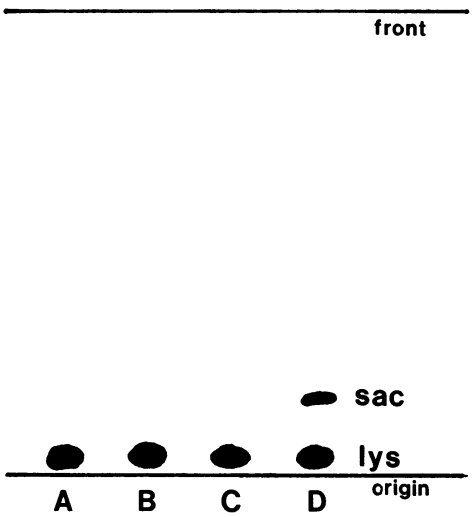

FIG. 1. TLC of amino acids in the reaction mixture of lysine-ketoglutarate reductase assay using [^{14}C]lysine. A, with NADH at zero time; B, with NADH after 60 min incubation; C, with NADPH at zero time; D, with NADPH after 60 min incubation.

Further evidence for the identity of saccharpine as a product of the reaction was obtained by TLC using silica-cellulose mixed bed plates (15) with authentic saccharopine as marker. In all three solvent systems tested (Table II), the ninhydrin-positive reaction product ran with saccharopine. Moreover, analysis of the reaction mixture for amino acids by GLC (9) revealed the formation of a substance whose *N*-heptafluorobutyryl, *n*-propyl ester emerged from the column at 256°C (lysine = 186°C). This peak corresponded to that of the authentic saccharopine derivative and was quantitatively related to NADPH oxidized in the assay.

Table II. *R_f Values for Saccharopine and Lysine by TLC on Silica-Cellulose Plates with Three Solvents*

Solvent 1, phenol:H_2O (80/20, w/v); solvent 2, 2-propanol:H_2O:HCl (65/18.4/16.6, v/v); solvent 3, methyl alcohol:H_2O:10 N HCl:pyridine (80/17.5/2.5/10, v/v).

	Solvent 1	Solvent 2	Solvent 3
Lysine	0.20	0.37	0.49
Saccharopine	0.27	0.50	0.44

Other characteristics of the enzyme determined were a pH optimum close to 7 and a temperature optimum of 30°C. NADPH oxidation was linear with time, and activity was proportional to the amount of extract added to the assay. The apparent K_m for lysine and α-ketoglutarate, as determined by direct linear plots (2), were 5.2 and 1.8 mM, respectively. The enzyme was not inhibited by 50 mM NH_4Cl, in contrast to the lysine-ketoglutarate reductase from human liver (7). The transaminase inhibitor, amino-oxyacetate, was also without effect in our assay, suggesting that no such activity underlies that being measured. Storage of material at −20°C for 2 months or more did not diminish the extractable activity.

Work is now in progress to investigate the possible importance of this enzyme in controlling lysine levels in developing maize endosperm, where different levels of lysine utilization in high-lysine mutants such as opaque-2 may involve different rates of lysine breakdown (13, 14).

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