Phytochemistry, Vol. 22, No. 12, pp. 2687–2689, 1983.
Printed in Great Britain.

0031–9422/83 $3.00 + 0.00

LYSINE-KETOGLUTARATE REDUCTASE ACTIVITY IN MAIZE: ITS POSSIBLE ROLE IN LYSINE METABOLISM OF DEVELOPING ENDOSPERM

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(*Revised received* 18 *March* 1983)

Key Word Index—*Zea mays*; *Gramineae*; *maize*; lysine-ketoglutarate reductase; lysine catabolism.

Abstract—Lysine-ketoglutarate reductase has been isolated from maize (*Zea mays*) endosperm during development. The enzyme activity was dependent upon lysine, 2-oxoglutarate and NADPH as substrates. Several protein amino acids included in the assays at 10 mM concentration did not affect the enzyme activity. Lysine-ketoglutarate reductase activity increased sharply with the onset of endosperm development, reached a peak at 20 days after pollination and then declined towards seed maturity. The peak of activity coincided with the period of maximum rates of nitrogen and zein accumulation in the endosperm. Amino acid analyses of sap taken from the ear vascular system during ear development showed considerable amounts of lysine and other protein amino acids. Lysine found in the sap could account for more than twice the lysine required for protein synthesis in the endosperm. The results suggest that lysine-ketoglutarate reductase is associated with lysine breakdown in the endosperm during seed development.

INTRODUCTION

Maize seed is considered a poor source of protein for mammalian growth because of the relative low content of lysine in its endosperm [1, 2]. The low lysine content of the endosperm is generally associated with a high proportion of zein, which is known to contain only small amounts of lysine [1–4]. Although significant progress has been made in elucidating the mechanism of zein synthesis in maize endosperm, little is actually known about the lysine metabolism of this tissue. Two origins for the lysine utilized during endosperm development are available. Lysine can be synthesized through the aspartate pathway and also can be delivered, preformed from other plant parts, to the endosperm via stem sap. Lysine synthesis via the aspartate pathway was evidenced by Sodek [5] by means of tracer experiments. Additional evidence for the operation of the aspartate pathway in the endosperm was provided enzymatically [6–8]. Lysine delivery via stem sap was suggested by Arruda and Silva [9] due to the considerable amounts of lysine found in the ear peduncle vascular sap during ear development. On the other hand, there is some evidence that lysine is catabolized in developing maize endosperm. Lysine catabolism was demonstrated by Sodek and Wilson [10], who showed that [^{14}C]lysine supplied to the endosperm via the stem was converted into other amino acids such as glutamic acid and proline. Comparing the amounts of lysine found in the ear vascular sap with the lysine content in the endosperm, Silva and Arruda [11] suggested that most of the lysine translocated to the endosperm should be catabolized. Lysine catabolism was also found to occur in barley and wheat seeds [12, 13].

The importance of lysine catabolism in the lysine metabolism of maize endosperm has now been investigated. We recently characterized an enzyme that converts lysine to saccharopine, which may well be the first step in lysine catabolism in maize endosperm [14]. The enzyme named lysine-ketoglutarate reductase, showed high activity in the endosperm and was specific for lysine as substrate. We describe here the pattern of lysine-ketoglutarate reductase activity in the developing maize endosperm and its possible involvement in the lysine metabolism of this tissue.

RESULTS AND DISCUSSION

Lysine-ketoglutarate reductase was partially purified, by ammonium sulphate fractionation and gel filtration on Sephadex G-25, from maize endosperm during development. The enzyme was specific for lysine, 2-oxoglutarate and NADPH as substrates. None of the other protein amino acids replaced lysine in the assays (data not shown).

Maize endosperm is known to contain large amounts of free amino acids at the early stages of seed development [15]. In order to determine whether these amino acids affect the enzyme activity, we included in the assays several protein amino acids at a concentration of 10 mM (Table 1). Except for leucine, which produced a slight inhibition, no other amino acid affected the enzyme activity.

The pattern of lysine-ketoglutarate reductase activity and the rates of total and zein nitrogen accumulation during endosperm development of a maize single cross hybrid are shown in Fig. 1. The activity increase after the onset of endosperm development reached a peak at 20 days after pollination (DAP) and then decreased progressively towards seed maturity. The pattern of enzyme activity was well correlated with both rates of total and zein nitrogen accumulation, with the peak of activity coinciding with the period of maximum rates of nitrogen accumulation and zein synthesis in the endosperm. It is interesting to note that the enzyme activity was about

Table 1. Effect of various amino acids on lysine-ketoglutarate reductase activity of maize endosperm 20 days after pollination

Amino acid	Activity (% of control)	Amino acid	Activity (% of control)
Glu	107	Ile	100
Pro	95	Val	102
Met	96	Leu	84
Cys	100	Asp	114
Trp	104	Ser	105
Arg	93	Tre	104
Ala	104	Phe	100

All amino acids were present in the assays at 10 mM.

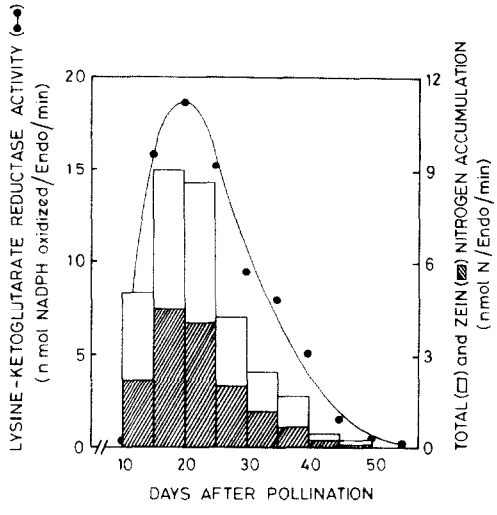

Fig. 1. Lysine-ketoglutarate reductase activity and rates of total and zein nitrogen accumulation in maize endosperm during development. The rates of total and zein nitrogen accumulation were calculated from the values of nitrogen content per endosperm at several stages of seed development. The values observed for both characteristics were: 0.40, 0.90, 1.78, 2.70, 3.08, 3.33, 3.50, 3.57, 3.55 and 3.53 mg of nitrogen per endosperm for total nitrogen; and 0.03, 0.20, 0.70, 1.20, 1.33, 1.43, 1.47, 1.52, 1.57 and 1.53 mg of nitrogen per endosperm for zein, at 10, 15, 20, 25, 30, 35, 40, 45, 50 and 55 DAP, respectively.

twice as high as the rate of nitrogen accumulation. For example, at the maximum rate of nitrogen accumulation, lysine-ketoglutarate reductase activity was 18.7 nmol of NADPH oxidized per endosperm per min, compared with the rate of nitrogen accumulation of 9 nmol per endosperm per min. Such an extremely high activity should have some physiological significance. We have shown previously [9] that the sap of the ear vascular system contains considerable amounts of lysine. The amino acid composition of the ear vascular sap taken from ears sampled in the same experiment carried out for enzyme analysis was determined (Table 2). Unfortunately it is difficult to know whether the sap represents the xylem or phloem fluids. Analysis for nitrate and sucrose has

Table 2. Amino acid composition of ear vascular sap during ear development

Amino acid	Days after pollination 14	21	28	35
Ala	5.9	8.8	9.0	6.9
Gly	2.2	4.0	3.4	3.4
Val	3.4	5.1	4.7	5.7
Thr	3.4	5.1	5.2	8.0
Ser	8.1	10.6	10.3	11.5
Leu	1.5	2.2	2.1	2.3
Ile	1.5	2.2	2.1	3.4
Pro	n.d.	n.d.	n.d.	n.d.
Met	2.0	0.4	0.4	0.8
Asx	29.7	22.7	22.3	20.7
Phe	0.2	0.7	0.9	0.6
Glx	34.6	27.1	27.9	24.1
Lys	2.5	4.8	5.6	4.6
Tyr	3.4	2.9	2.6	3.4
Arg	1.6	3.4	3.5	4.6
Trp	n.d.	n.d.	n.d.	n.d.
His	n.d.	n.d.	n.d.	n.d.
Cys	n.d.	n.d.	n.d.	n.d.

Amino acids are expressed in mol %.
Asx = Aspartic acid plus asparagine, Glx = glutamic acid plus glutamine, n.d. = not determined.

suggested that the sap should be derived from both xylem and phloem [11]. As was found previously [9], most of the protein amino acids occurred in the sap. Glx was the main nitrogen carrier for developing seeds, followed by Asx. The lysine concentration of the sap accounted for more than 4% of the total amino acid pool during the most active period of nitrogen accumulation. Assuming that the amino acids found in the sap are the only source of nitrogen for developing seeds, we estimated, by comparing the amount of lysine in the sap with the lysine content of endosperm, that the amount of lysine

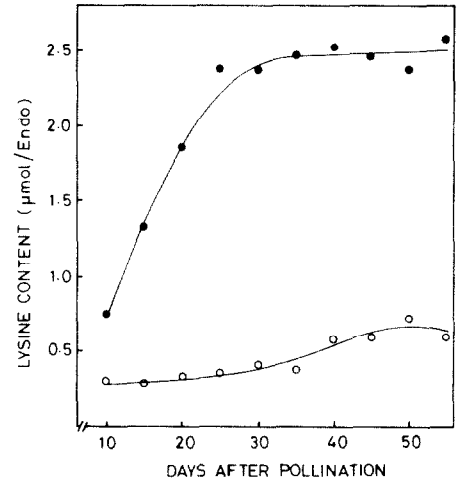

Fig. 2. Total (●) and free (○) lysine content in maize endosperm during development.

entering the endosperm was enough to supply more than twice the requirement of lysine for protein synthesis. These results are in good agreement with those found previously [11] suggesting lysine catabolism in the endosperm. Thus, lysine-ketoglutarate reductase may well be associated with the breakdown of an excess of lysine supplied to the endosperm during seed development. If this is so, the lysine supplied to the developing endosperm from both synthesis through the aspartate pathway [5] or delivered through sap, should have two destinations; utilization for lysine-rich protein synthesis or degradation through the reaction catalysed by lysine-ketoglutarate reductase. The pattern of total lysine accumulation and the levels of free lysine during endosperm development are shown in Fig. 2. Total lysine increased early at the onset of endosperm development, reached its maximum at 30–35 DAP and remained apparently constant up to seed maturity. Free lysine, which represented *ca* 15–20% of total lysine, remained apparently constant from 10 to 35 DAP and increased slightly from 35 DAP to seed maturity. These results show that lysine is no longer accumulated in a free form. Based on the water content of the endosperm, the concentration of free lysine was estimated to be *ca* 5.8 mM at 10 DAP, 2–3 mM at the intermediate stages and 7–11 mM from 40 to 55 DAP. These concentrations of free lysine in the endosperm are high enough to support enzyme activity, since the K_m for lysine was shown to be *ca* 5 mM [14]. However, further evidence for '*in vivo*' activity of lysine-ketoglutarate reductase is necessary since the localization of the enzyme within the endosperm is unknown.

The data presented here, taken together with the reported results on transport and catabolism of lysine in maize endosperm [9–11], lead us to draw some conclusions. First, preformed lysine should be an important source of lysine for developing maize endosperm and may explain the relationship between lysine-ketoglutarate reductase activity and the rates of nitrogen accumulation in the endosperm. Second, due to the high rates of zein synthesis (Fig. 1) and since this protein is devoid of lysine [1–4], little lysine should be utilized for protein synthesis. Third, accumulation of free lysine in the endosperm is prevented by the activity of lysine-ketoglutarate reductase.

EXPERIMENTAL

Plant material. A single cross hybrid of maize obtained by crossing inbred lines ML649 and ML674 was used in this study. Sixty field-grown plants were self-pollinated on the same day and used for subsequent sampling.

Vascular sap collection. Ears sampled at 14, 21, 28 and 35 DAP were used for sap collection. The ears with attached 3 cm long peduncles were husked, cut in half and placed in the 500 ml chamber of a conventional Scholander pressure bomb. The peduncles were fixed to the top cover of the chamber with rubber rings so that only the peduncle emerged outside the chamber. The sap fluid was forced out under N_2 at a pres. up to 0.5 atm. The fluid that emerged on the peduncle surface was collected with capillary glass tubes. Saps of 5 ears at each stage of development were pooled and stored at $-20°$

Amino acid composition of sap was determined by GLC according to the method of ref. [10]. Samples (0.2–0.8 ml) were applied to a short column (1 × 6 cm) of Dowex 50—1 × 8 (100–200 mesh) H^+ and washed with 20 ml H_2O. Amino acids were eluted with 4 × 5 ml 7 M NH_4OH. The eluate was dried, redissolved in a small vol. of 0.1 M HCl, transferred to a 100 × 16 cm test-tube and dried at 50° under a stream of dry N_2. Amino acid derivatization was performed in the standard way. Amino acids as their heptafluorobutyryl *N*-propyl esters were separated in a 3.5 m × 2 mm i.d. glass column packed with 3% SE-30 on 80–100 mesh Chromosorb WHP. Norleucine was used as an internal standard to calculate the retention time of each amino acid.

Enzyme extraction and assay. For enzyme studies, ears were sampled at 5 day intervals from 10 to 55 DAP and stored at $-20°$. A portion of the seeds was used for enzyme extraction and the remaining seeds were freeze-dried for chemical analysis. Enzyme extraction, purification and assay was carried out as described in ref. [14]. The protein content of enzyme extracts was determined by the method of ref. [17].

Nitrogen, zein and lysine determinations in the endosperm. Freeze-dried seeds were dehusked, separated from the embryos and ground to a powder. All analyses were carried out on duplicate 100 mg samples. Total nitrogen was determined by the method of ref. [18]. Zein was extracted by the method of ref. [19]. Zein nitrogen was determined as for total nitrogen. Lysine was determined by the method of ref. [20]. Free lysine was determined by the same method excluding the enzymatic digestion. For this purpose, endosperm powder was extracted with borate buffer, pH 8 (1 ml/100 mg endosperm powder), for 2 hr at 5°. After centrifugation at 12 000 *g*, 0.5 ml supernatant was used for free lysine determination.

Acknowledgement—Part of this work was supported by Grant PIG-CNPq 40523/80.

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