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CONVERSION OF SACCHAROPINE TO LYSINE IN BARLEY

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Abstract—Labelled saccharopine was synthesized and showed a low conversion to lysine in barley seedlings. The results indicate a role of saccharopine in either lysine biosynthesis or catabolism.

INTRODUCTION

Recent studies indicate that lysine biosynthesis proceeds via the 2,6-diaminopimelic acid pathway in higher plants. Thus the labelling pattern observed in lysine when different specifically labelled precursors were administered to seedlings of barley was in agreement with this pathway [1]. Furthermore, the presence of dihydrodipicolinate synthase (EC 4.2.1.52) has been demonstrated in maize [2]. However, due to scrambling of the radioactivity administered, the operation of the other known lysine pathway via 2-aminoadipic acid at a much lower rate could not be totally excluded. This possibility was increased by the fact that in the same studies a small conversion of labelled 2-aminoadipic acid to lysine occurred [1]. Chemical degradation of the labelled lysine formed showed that the incorporation occurred directly and not after breakdown of 2-aminoadipic acid to pyruvic or acetic acids [1,3]. If this conversion occurs according to the 2-aminoadipic acid pathway known from fungi, saccharopine should be an intermediate [4]. It has been claimed that saccharopine is present in the seed kernels of *Fagopyrum esculentum* (buckwheat), but the amount isolated was so low that fungal origin could not be excluded [5]. However, large quantities of saccharopine have recently been isolated from the inflorescence of *Reseda odorata* [6]. Saccharopine is therefore established as a constituent of higher plants. Consequently a study of the capabilities of barley seedlings to synthesize lysine from saccharopine was undertaken.

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RESULTS AND DISCUSSION

The results of the biosynthetic experiments are presented in Table 1. The saccharopine administered to the endosperm was transported to both the primary leaf and to the root, although this transport proceeded slowly compared to the precursors previously tested under the same experimental conditions [1]. Autoradiography showed the formation of several labelled compounds. Besides untransformed saccharopine, the major activity was found in aspartic acid, glutamic acid, 2-aminoadipic acid and lysine. This shows that saccharopine is not metabolically inert in plants. The labelled saccharopine administered presumably was a mixture of equal parts of the two diastereoisomers L-saccharopine, ((2S, 2S')-[1,2,3,4,5,6-^{14}C]-N^6-(2'-glutaryl)-lysine), and D-*allo*saccharopine ((2S, 2R')-[1,2,3,4,5,6-^{14}C]-N^6-(2'-glutaryl)-lysine) [7] of which only L-saccharopine (in the absence of evidence to the contrary) is metabolically active.

The incorporation percentages in Table 1 show that saccharopine can be converted directly to lysine, but the incorporation percentages are lower than those percentages obtained with 2-aminoadipic acid and most of the precursors of the 2,6-diaminopimelic acid pathway tested under the same experimental conditions [1]. Saccharopine and 2,6-diaminopimelic acid are the ultimate lysine precursors in the 2-aminoadipic acid and 2,6-diaminopimelic acid pathways, respectively. However, the percentage incorporation of saccharopine is only 3% of that of 2,6-diaminopimelic acid, even though the percentage incorporation of saccharopine was doubled in order to correct for the presumably inactive D-*allo*saccharopine present in the administered precursor. If the 2-aminoadi-

Table 1. Distribution of radioactivity in basic amino acids in root, endosperm and primary leaf of barley seedlings

	Root		Endosperm		Primary leaf		Whole seedling	
	(dpm)	(%*)	(dpm)	(%)	(dpm)	(%)	(dpm)	(%)
Total activity	21700	2.0	645000	60.6	48100	4.5	714700	67.2
Histidine	0	0	209	0.02	0	0	209	0.02
Lysine	62	0.006	2480	0.23	769	0.07	3310	0.31
Arginine	0	0	304	0.03	172	0.02	476	0.04

* Incorporation percentages are based on the total amount of saccharopine injected into the endosperm.

pic acid pathway is operating, this percentage may be taken as an estimate of the relative quantitative importance of the two different pathways in the seedlings. It must, however, be stressed that neither the ability of the seedlings to biosynthesize 2-aminoadipic acid from simple precursors, nor the ability of plants to synthesize lysine via aldol condensation of 2-oxoglutaric acid and acetyl-CoA according to the 2-aminoadipic acid pathway, have been demonstrated. On the other hand, this type of reaction is well-known from plants, e.g. in the initial steps of the TCA cycle and in the biosynthesis of leucine. Similar aldol condensations also occur in the biosynthesis of secondary plant products such as the cyanogenic glucoside acacipetalin [8], the non-protein amino acid γ-phenylbutyrin and the glucosinolate gluconasturtiin [9]. Glucosinolates are often derived from the keto analogues of protein amino acids either directly or following aldol condensations with acetyl-CoA as described above [10]. Thus, according to this scheme 3-methoxycarbonyl glucosinolate, which occurs in *Erysimum* spp. can be derived by condensation of 2-oxoglutaric acid and acetyl-CoA. In fact 2-aminoadipic acid has been shown to be a good precursor, but unfortunately the glutamic acid which was also tested was uniformly labelled and gave low incorporation so that it is difficult to say whether direct incorporation occurred [11]. Similar studies with specifically labelled glutamic acids followed by chemical degradation might show whether plants are able to synthesize 2-aminoadipic acid by use of the steps known from the 2-aminoadipic acid pathway in most fungi. In animals, it has been shown recently that lysine is catabolized by a reversal of the steps in this pathway [12–14]. If a similar degradative pathway operating in higher plants is slightly reversible, this may also account for the low production of lysine from the two compounds. Preliminary studies on the catabolic fate of lysine and saccharopine in barley seedlings indicate such a degradative pathway.

In metabolic studies where different pathways are employed by different organisms, care must be taken to avoid contamination by other organisms. Thus the plant material was sterilized before the biosynthetic experiments were conducted, and bacterial and fungal infection during the biosynthetic experiments was monitored throughout by tests of tissues and homogenates on meat peptone and malt extract agars and of tissues on moist filter paper [15,16]. In none of the experiments described here could fungal infection be detected.

EXPERIMENTAL

Radioisotopic chemicals. L-lysine-U-^{14}C was obtained from Calatomic, Los Angeles, California. Synthesis of labelled saccharopine (N^6-(2′-glutaryl)-L-lysine-[L-lysine-U-^{14}C] was performed by a Strecker synthesis [17]. The reaction was carried out in a 500 μl ampoule and the reaction mixture consisted of 0.73 mg 2-oxoglutaric acid, 0.82 mg L-lysine, HCl, 2.21 mg KCN, 20 μl L-lysine-U-^{14}C (8.11 μCi, 10 mCi/mmol) and 50 μl of N NaOH. The clear soln was kept at 50–52° for 24 hr. Conc HCl (250 μl) was added and the soln was refluxed for 24 hr. The reaction mixture was lyophilized, dissolved in H_2O and applied to Dowex 50W resin (×8, 200–400 mesh, H^+, 0.2 × 1.5 cm). After washing with 8 ml H_2O, labelled saccharopine was eluted with 8 ml N Py. After lyophilization the residue was dissolved in H_2O. PC of the Py eluate on Whatman No 3MM [1] followed by autoradiography showed two strongly labelled spots corresponding to saccharopine and pyrosaccharopine and several minor spots. Co-chromatography of authentic saccharopine and pyrosaccharopine with aliquots of the eluted two major compounds proved the identity. Radiochemical yield: 0.44 μCi of saccharopine and 0.35 μCi of pyrosaccharopine. To increase the yield of saccharopine, untransformed L-lysine was recovered from the ion exchange resin by elution with 8 ml of N NH_3. After evaporation to dryness *in vacuo*, the Strecker synthesis was repeated. This was done twice, and the total radiochemical yields were 1·04 μCi of saccharopine (13%) and 0.80 μCi of pyrosaccharopine (10%).

Biosynthetic experiments. Labelled saccharopine was dissolved in sterile H_2O to give a soln of 6 nCi/μl. A Terumo micro syringe was used to inject 1 μl of this soln into the endosperm of 4-day-old seedlings of *Hordeum vulgare* cv. Emir grown as earlier described [1]. 80 seedlings were employed for the expt. 2 days after injection, the seedlings were harvested and separated into primary leaf, endosperm and root. Each part was dried and hydrolyzed in 6 N HCl. Ion exchange resins were used to obtain chemically pure histidine, lysine, and arginine as earlier described [1].

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