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STEREOCHEMISTRY OF THE REACTION CATALYSED BY GLUTAMIC ACID DECARBOXYLASE FROM A HIGHER PLANT (*HORDEUM VULGARE*)

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Key Word Index—*Hordeum vulgare*; Gramineae; barley; L-glutamic acid decarboxylase; pyridoxal phosphate; γ-aminobutyric acid.

Abstract—The decarboxylation of (2*S*)-glutamic acid to yield γ-aminobutyric acid catalysed by L-glutamic acid decarboxylase (EC 4.1.1.15) from *Hordeum vulgare* proceeds with net retention. The result is interpreted in terms of a single progenitor hypothesis of the pyridoxal phosphate enzymes and confirms that not only bacteria and animals but also plant decarboxylases catalyse the biosynthesis of biogenic amines from amino acids with net retention.

INTRODUCTION

The stereochemistry of the enzymic decarboxylation of amino acids may proceed with net retention or net inversion of configuration. Net inversion has only been observed in the case of meso-α,ε-diaminopimelic acid decarboxylase (EC 4.1.1.20) [1] (Fig. 1, path B), an enzyme that generates (2*S*)-lysine by decarboxylation of the 2*R* chiral centre of meso-α,ε-diaminopimelate. In all other cases [2–26] the decarboxylation of the chiral centre of (2*S*)-amino acids was shown to occur with net retention regardless of whether the enzymic reaction depends on pyridoxal phosphate [2–12, 17–25]* or pyruvate [13–16, 26] as a cofactor. The enzymes that have been investigated were mainly bacterial [2–20, 26], but some were mammalian [3, 21–24]. Plant decarboxylases have been investigated in only two cases [3, 25] where chirally labelled precursors were administered to intact alkaloid containing plants. Thus, the mode of incorporation of (2*S*)-[2-^{3}H, U-^{14}C]tyrosine into papaverine and morphine [3] and of labelled (2*S*)-ornithine and chirally labelled putrescine into nicotine [25] led to the conclusion that retention of configuration has taken place.

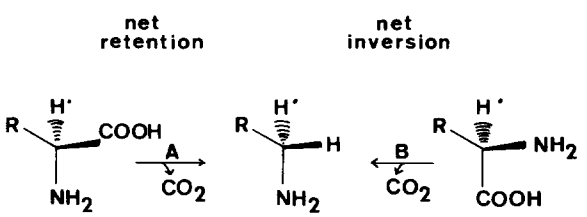

Fig. 1. Mode of enzymic decarboxylation of (2*S*)-amino acids (path A) and of the 2*R* chiral centre of meso-α,ε-diaminopimelic acid (path B)

*The participation of pyridoxal phosphate has not been rigorously tested in every single case

RESULTS AND DISCUSSION

The results on plant decarboxylases [3, 25] are particularly important because the stereochemistry of pyridoxal phosphate catalysed reactions have been interpreted in evolutionary terms [27–29]. It is assumed that all pyridoxal phosphate dependent enzymes in all organisms (including plants) are derived from a common progenitor as indicated by the consistency of the stereochemical course of pyridoxal phosphate catalysed reactions [27–29].

In an attempt to corroborate previous findings [3, 25] in the light of this concept [27–29] we have isolated L-glutamic acid decarboxylase (EC 4.1.1.15) from *Hordeum vulgare* L. (barley) and investigated *in vitro* the stereochemistry of the reaction catalysed by this plant enzyme.

The reaction of the bacterial decarboxylase has been previously shown to proceed with retention [9–11]. We have reinvestigated this enzyme using methods which had also been applied in our study of the glutamic acid decarboxylase from a plant source (Tables 1 and 2). L-Glutamic acid decarboxylase was either purchased (*Escherichia coli*) or isolated from *Hordeum vulgare* L. The plant enzyme was used after extraction and ammonium sulphate precipitation [30]. Enzymes from both sources were incubated with either (2*S*)-[2-^{3}H]glutamic acid (Table 1) or (2*S*)-glutamic acid in deuterated water (Table 2). The chirality of the isotopically labelled γ-aminobutyric acid samples so obtained was analysed using diamine oxidase from *Pisum sativum*, an enzyme that removes the xi-proton during deamination [5, 31, 32]. The resulting succinic semialdehyde was isolated as the 2,4-dinitrophenylhydrazone. During this derivatization, the aldehyde proton was stable [33]. Analysis of the isotope content of the hydrazone was carried out using either liquid scintillation counting (experiments 1 and 2) or mass spectrometry (experiments 3 and 4).

When (2*S*)-[2-^{3}H]glutamic acid was incubated with glutamic acid decarboxylase (EC 4.1.1.15) from both sources, chirally labelled γ-aminobutyric acid was obtained which upon removal of the xi-proton with diamine oxidase, lost all tritium activity (Table 1). Thus, the

Table 1 Treatment of chirally labelled samples of γ-aminobutyric acid with diamine oxidase

Experiment No	Source of glutamic acid decarboxylase	$^3H/^{14}C$ ratio of chirally labelled γ-[4-^{3}H]-[U-^{14}C]-aminobutyric acid	$^3H/^{14}C$ ratio of succinic semialdehyde hydrazone	Loss of ^3H (%)
1	*Escherichia coli*	10.0	0 3	97
2	*Hordeum vulgare*	10.0	0 8	92

The chirally labelled samples of γ-[4-^{3}H]-[U-^{14}C]aminobutyric acid were obtained by decarboxylation of (2*S*)-[2-^{3}H]glutamic acid using glutamic acid decarboxylase (EC 4 1 1 15) from two sources The γ-[4-^{3}H]labelled γ-aminobutyric acid so obtained was mixed with commercially available [U-^{14}C]γ-aminobutyric acid

Table 2 Treatment of chirally labelled samples of γ-[^{2}H]aminobutyric acid with diamine oxidase

Experiment No	Source of glutamic acid decarboxylase	Atom % excess of γ-aminobutyric acid	Atom % excess of succinic semialdehyde hydrazone	Loss of ^2H (%)
3	*Escherichia coli*	89 7	87.8	3
4	*Hordeum vulgare*	86 3	81 5	5 6

The chirally labelled samples of γ-[4-^{2}H]-[U-^{14}C]aminobutyric acid were obtained by decarboxylation of (2*S*)-glutamic acid in deuterated water using glutamic acid decarboxylase (EC 4 1 1 15) from two sources The γ-[4-^{2}H]labelled γ-aminobutyric acid so obtained was mixed with commercially available [U-^{14}C]γ-aminobutyric acid

labelled proton was in the xi-position in γ-aminobutyric acid and decarboxylation had taken place with retention (path A, Fig 1) This applies to both the *E. coli* and the *Hordeum* enzymes (Table 1). In the complementary experiment (Table 2) the label was retained indicating again that net retention had taken place. This is in agreement with previous findings obtained with enzymes from various organisms [2–26] and supports the assumption that a common progenitor for all pyridoxal phosphate enzymes might exist On the other hand, it shows that, as opposed to one of our previous assumptions [5], decarboxylases from plants catalyse the formation of biogenic amines from amino acids also with net retention The previously discussed problem [5] related to the incorporation of chirally labelled substrates into *Sedum* alkaloids is, thus, unresolved

EXPERIMENTAL

Hordeum vulgare L seeds were purchased from Saatgutprufstelle der Landwirtschaftskammer Westfalen-Lippe, D-4400 Munster, West Germany and plants were propagated in a green house as described in ref [30]

Enzymes Bacterial L-glutamic acid decarboxylase was purchased from Sigma, whereas plant decarboxylase was extracted from freshly grown plant material [30]

Chirally labelled γ-aminobutyric acid Labelled samples of γ-aminobutyric acid were prepared as described in refs [9–11]

Deamination with diamine oxidase This was carried out as described [5] except that acetoacetic acid was omitted

Isolation of the 2,4-dinitrophenylhydrazone of succinic semialdehyde After termination of the diamine oxidase reaction, an acidic soln of 2,4-dinitrophenylhydrazine was added to the incubation mixture and extracted with EtOAc The washed and dried extract was evaporated and the hydrazone purified by TLC on silica ($CHCl_3$–MeOH, 9 1 and EtOAc–$CHCl_3$–MeOH, 15 80 5)

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