Stereochemistry of the Enzymic Decarboxylation of L-Lysine

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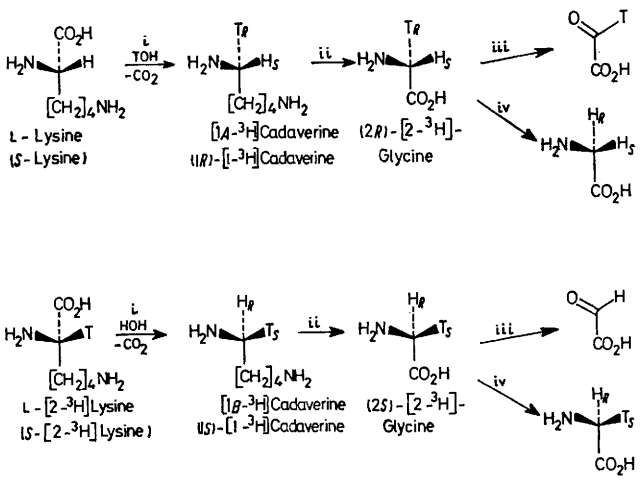
Summary The decarboxylation of L-lysine to yield cadaverine, catalysed by L-lysine decarboxylase (EC 4.1.1.18, L-lysine carboxylyase), takes place with retention of configuration.

EVEN though it is known that in the enzymic decarboxylation of L-lysine replacement of the carboxy-group by a proton takes place stereospecifically,[1] it is not known whether this process leads to retention or inversion of configuration. We now present evidence to show that the reaction takes place with retention of configuration.

Samples of the two enantiomers of [1-^{3}H]cadaverine (1,5-diaminopentane) were prepared[2] by the action of L-lysine decarboxylase (from *Bacillus cadaveris*) on L-lysine in the presence of tritiated water (yielding [1*A*-^{3}H]cadaverine) and on L-[2-^{3}H]lysine (yielding [1*B*-^{3}H]cadaverine). If decarboxylation of L-lysine occurred with retention, the absolute configuration of [1*A*-^{3}H]- would be (1*R*)-[1-^{3}H]-, and that of [1*B*-^{3}H]- would be (1*S*)-[1-^{3}H]cadaverine. If decarboxylation of L-lysine took place with inversion, [1*A*-^{3}H]- would be (1*S*)-[1-^{3}H]-, and [1*B*-^{3}H]- would be (1*R*)-[1-^{3}H]cadaverine.

The key step in the assignment of configuration to the enantiomers of [1-^{3}H]cadaverine was their conversion into the corresponding enantiomers of [2-^{3}H]glycine, by oxidation with CrO_3–conc. H_2SO_4 (2% yield). It was shown in separate experiments that proton exchange in the course of this oxidation was not extensive.

The absolute configuration of the chirally tritiated samples of glycine was determined by established methods. When D-amino-acid oxidase (EC 1.4.3.3), which catalyses the oxidative deamination of D-amino-acids (but not of L-amino-acids) to the corresponding α-oxo-acids, acts upon glycine to yield glyoxylic acid, it is the *pro-S* hydrogen at



SCHEME. i, L-Lysine decarboxylase; ii, [O]; iii, D-amino-acid oxidase, loss of H_S; iv, L-alanine aminotransferase, exchange of H_R.

C-2 of glycine, corresponding to the α-hydrogen of a D-amino acid such as D-alanine, which is removed.[3] When L-alanine aminotransferase (EC 2.6.1.2), which normally

TABLE

Substrate	Incubation of [2-^{3}H,1-^{14}C]glycine With D-amino-acid oxidase: ^{3}H/^{14}C ratio of glycine	^{3}H/^{14}C ratio of glyoxylic acid	Retention of tritium (%)	With L-alanine aminotransferase: ^{3}H/^{14}C ratio of initial glycine	^{3}H/^{14}C ratio of reisolated glycine	Retention of tritium (%)
Glycine from [1*A*-^{3}H]cadaverine	14·1 ± 0·6	12·4 ± 1·5	88	18·5 ± 2·3	4·8 ± 0·2	26
Glycine from [1*B*-^{3}H]cadaverine	11·1 ± 0·6	2·7 ± 0·4	24	17·9 ± 2·9	15·9 ± 0·8	89

catalyses the reversible transfer of an amino-group from L-alanine to α-oxoglutarate, acts upon glycine in the absence of an amino-group acceptor, it is the *pro-R* hydrogen at C-2 of glycine, corresponding to the α-hydrogen of L-alanine, which is labilized.[4]

The tritium-labelled samples of glycine, obtained by chromic acid oxidation of [1*A*-^{3}H]- and [1*B*-^{3}H]cadaverine, were incubated with each of the two enzymes, in the presence of [1-^{14}C]glycine as an internal standard. The ^{3}H : ^{14}C ratios of the samples of glycine, prior to incubation with enzyme, were compared with those of the products of the enzymic reactions, glyoxylic acid and reisolated glycine, respectively, and served as a measure of the retention of tritium.

The results (Table) indicate that (2*R*)-[2-^{3}H]glycine is formed from [1*A*-^{3}H]cadaverine (88% retention of tritium, relative to ^{14}C, with D-amino-acid oxidase, 74% loss of tritium with L-alanine aminotransferase), whereas (2*S*)-[2-^{3}H]glycine is obtained from [1*B*-^{3}H]cadaverine (76% loss of tritium with D-amino-acid oxidase, 89% retention of tritium with L-alanine aminotransferase). It follows that [1*A*-^{3}H]cadaverine is (1*R*)-[1-^{3}H]cadaverine and that [1*B*-^{3}H]cadaverine is the (1*S*)-compound, and, further, that the decarboxylation of L-lysine takes place with retention of configuration. These stereochemical relationships are summarized in the Scheme.

Belleau predicted that the enzyme-catalysed decarboxylation of α-amino-acids should take place with retention of configuration, and established such a steric course for the decarboxylation of L-tyrosine by a kinetic method.[5] A similar steric course is now established for L-lysine. Circumstantial evidence indicates that decarboxylation of L-histidine also takes place with retention.[6]

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