STEREOCHEMICAL STUDY OF FORMATION OF HISTAMINE FROM (2S)-HISTIDINE BY MAMMALIAN HISTIDINE DECARBOXYLASE

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1. Introduction

The physiological activity of histamine (as (2)) is well known; it is produced by decarboxylation of (2*S*)-histidine (as (1)) but to our knowledge, no specific inhibitor of histidine decarboxylases is available. As a contribution to knowledge of these enzymes, stereochemical studies have been carried out and histidine decarboxylase (EC 4.1.1.22) from bacterial sources (e.g., from *Lactobacillus* 30a [1]) was found to decarboxylate (2*S*)-histidine (1) with retention of configuration [2]. This bacterial enzyme is unique in using a pyruvate residue as cofactor [1] rather than pyridoxal phosphate which is usual for mammalian systems. This difference in cofactors made it important to compare the stereochemistry of action of a mammalian histidine decarboxylase; the level of this enzyme is usually low but is highest for tissues in fast growth, e.g., embryos or tumours [3].

2. Materials and methods

2.1. *Enzyme and substrate*

Mammalian histidine decarboxylase was isolated from fetal rats (19–20 days after mating; placenta rejected) by the method in [4] with some modifications: a 0.1 M acetate buffer was used (pH 6.0), the pH being adjusted throughout to 6.0; after the first dialysis, the solution was not centrifuged but was added to the above acetate buffer before the first $(NH_4)_2SO_4$ precipitation (28% saturation). From 50 g fetus, the enzyme solution showed spec. act. $\geqslant$ 60 nmol histidine decarboxylated .mg protein^{-1} .h^{-1} at 30°C (pH 6.8). The assay was run using (2*S*)-[2′-^{14}C]histidine (as (1)), the [2′-^{14}C]histamine (as (2)) was extracted, converted into its *N*-phenylurea derivative which was purified and counted as in [2]. Since the initial tissue contains aromatic amino acid decarboxylase of relatively low specificity (EC 4.1.1.28), a repeat assay was carried out at pH 8.5, the optimum for the general decarboxylase [5]; little decarboxylation occurred in support of the isolated enzyme being the specific histidine decarboxylase.

(2*S*)-[2-^{3}H,2′-^{14}C]Histidine (1) was synthesised as in [2]. Liver alcohol dehydrogenase, catalase and NAD^+ were obtained from Sigma. Diamine oxidase from pea seedlings was isolated as in [6]; its specific activity [6] was 0.064 units .mg protein^{-1}.

2.2. *Decarboxylation of histidine and stereochemical assay on histamine*

The incubation mixture comprised the foregoing mammalian enzyme preparation (2 ml) (pre-incubated at 30°C under N_2 with pyridoxal-5-phosphate (21.2 μg)), reduced glutathione (245 μg), (2*S*)-[2-^{2}H,2′-^{14}C]-histidine (1.55 mg) and sufficient phosphate buffer (pH 6.8) to make the incubate 0.1 M. After the mixture had been held at 30°C under N_2 for 36 h, the labelled histamine (2) was diluted with unlabelled histamine hydrochloride (53.4 mg) and the filtered solution was evaporated. Half the residue was extracted with ethanol and the product was converted as in [2] into histamine *N*-phenyl urea (5), m.p. 182–184°C, for multiple crystallisation to constant specific activity and ^{3}H:^{14}C ratio (table 1).

The remaining half of the histamine salt was incubated in 0.1 M phosphate buffer (pH 7) for 36 h

Table 1

Substance	^3H:^{14}C ratio	^3H retention (%)
(2*S*)-[2-^{3}H,2'-^{14}C]Histidine (1)	14.4 ± 0.4	100
(1*S*)-[1-$^3H_1$,2'-^{14}C]Histamine (2), as urea (5)	14.8 ± 0.4	103 ± 3
[2'-^{14}C]Histaminol (4), as urethane (6)	0.26 ± 0.02	1.8 ± 0.2

at 30°C with pea seedling diamine oxidase (4 ml, 0.75 mg protein .ml^{-1}), liver alcohol dehydrogenase (5 mg, 9 U), catalase (1 mg), NAD^+ (10 mg) and ethanol (1 ml); mixture total vol. 10 ml. The histaminol (4) produced was converted into its *N*-phenylurethane (6), m.p. 132–134°C, as in [2] and was crystallised from aqueous ethanol to constant ^{3}H:^{14}C ratio (table 1).

3. Results and discussion

Pea seedling diamine oxidase specifically removes only the *Si*-hydrogen from histamine (2) to produce the aldehyde (3) which can be trapped reductively by liver alcohol dehydrogenase and NADH [2]. The results (table 1) show that this sequence removes essentially all the ^{3}H from histamine (2) produced by decarboxylation of (2*S*)-[2-^{3}H]histidine (1). It follows that the histamine has the (1*S*)-configuration (2) and thus has been produced with retention of configuration as found earlier [2] for bacterial enzymes. Thus the reactions catalysed by histidine decarboxylases using either pyridoxal phosphate or a pyruvate residue as cofactor occur in the same stereochemical sense.

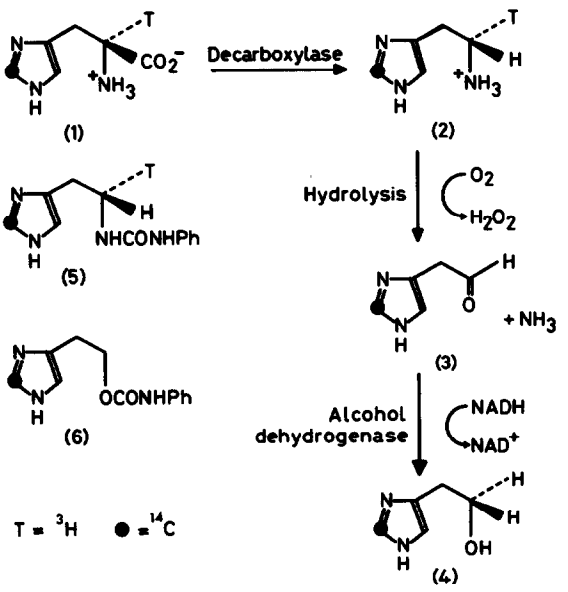

Fig.1

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