STERIC COURSE OF THE HISTIDASE REACTION

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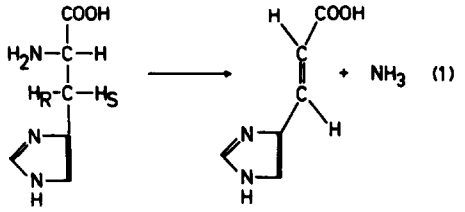
1. Introduction

Histidase (histidine ammonia lyase, E.C. 4.3.1.3) catalyses the practically irreversible* conversion of L-histidine to urocanic acid and ammonia according to equ. (1). In the course of the reaction one of the heterotopic [1] hydrogen atoms at C-3 of L-histidine is



slowly exchanged with the protons of water and this hydrogen is lost in the elimination reaction [2]. It will subsequently be shown that the H_R atom [3] of C-3 is involved both in the exchange and in the overall reaction and hence the elimination of ammonia from L-histidine occurs in a "trans" manner.

2. Methods

Enzymically labelled L-^{3}H-histidine was prepared essentially as described in [2]. A few μmoles of the tritiated histidine were freeze-dried and diluted with 1.5 g of unlabelled L-histidine hydrochloride. After chromatography on Dowex-50 (eluent 4 N HBr) 2 g crystalline L-histidine dihydrobromide were obtained (2280 cpm/mmole), the radioactivity of which remained unchanged on repeated chromatography.

2.1. *Degradation of L-^{3}H-histidine to succinic acid*

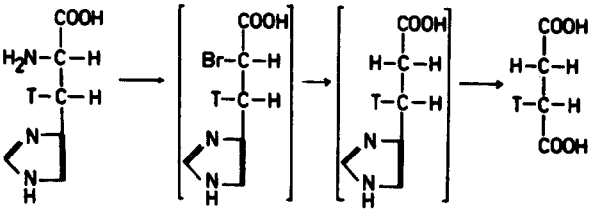
The following procedure was used: 580 mg L-^{3}H-histidine dihydrobromide were dissolved in 4 ml water and 0.87 ml HBr (48%). A solution of 125 mg $NaNO_2$ in 1.5 ml water was slowly added at 0°C and the reaction mixture stirred at 20°C for 2 hr. After addition of 100 mg PtO_2, the mixture was hydrogenated at room temperature until the uptake of H_2 ceased. A total of 43 ml H_2 were consumed. The catalyst was subsequently removed and the solution adjusted to pH 7 with 1 N KOH. A saturated aqueous solution of 1 g $KMnO_4$ was added dropwise to the mixture at 60°C. After 1½ hr stirring another portion of 1 g $KMnO_4$ was added and the reaction allowed to proceed for a further 1½ hr. In order to reduce Mn^{VII} and Mn^{IV} to Mn^{II}, 15 ml of 38% $NaHSO_3$ were introduced to the solution. After acidification with 2 N H_2SO_4, the succinic acid was extracted continuosly with ether and purified by chromatography on Dowex-1 and several recrystallisations from water as described in [4]. The yield after 3 recrystallisations was 31 mg. The same degradation procedure with unlabelled L-histidine dihydrobromide, and using ^3H-H_2O as solvent, resulted in practically no incorporation of tritium into the succinic acid.

2.2. *Determination of chirality of ^{3}H-succinic acid*

The chirality determination was carried out as described in [5]. Reference (R)-, (S)- and (RS)-^{3}H-succinate samples were oxidised to 70% conversion with the same preparation of soluble succinate dehydrogen-

* Williams and Hiroms [11] recently showed that on very long incubation histidase catalyses the conversion of urocanate and ammonia to L-histidine.

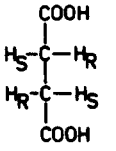
ase. Succinic and fumaric acid samples were separated and purified as described in [4]. The results of the radioactivity measurements are summarised in the table.



(2)

3. Results and discussion

A detailed study of the mechanism of the histidase reaction has revealed [2] that in addition to the overall conversion of L-histidine to urocanic acid, the following two partial reactions are catalysed by the enzyme; i) dismutation bettween L-histidine and ^{14}C-urocanic acid and ii) exchange of hydrogen atoms between L-histidine and water. The tritium atom introduced to L-histidine by the latter process was localised in the methylene group by appropriate degradation procedures [2]. Furthermore, enzymically tritiated L-histidine was converted by the enzyme into tritium free urocanic acid. This implies that only one of the diastereotopic H atoms at C-3 of L-histidine is exchangeable and that the same hydrogen is lost in the elimination reaction. In the present work, it is shown that only the H_R atom of L-histidine (equ. 1) is reactive both in the exchange and in the overall reaction. This is achieved by reductive elimination of the amino group of enzymically tritiated L-histidine and subsequent degradation of the imidazolyl propionic acid to ^{3}H-succinate (equ. 2). The latter is shown to possess the (R)-configuration by a method [5] based on the difference in isotope effects for the enzymic removal of tritium from positions corresponding to H_R and H_S in succinate (see formula 1). Thus on partial oxida-



tion of the chiral ^{3}H-succinic acids by succinate dehydrogenase the enrichment of tritium in the starting material is faster when the substrate has the (R)-configuration than when it has the (S)-configuration (cf. table). In spite of recent work [2, 6] the precise mechanism of the histidase reaction is still unknown. The present results throw light on its stereochemistry by showing that it is a trans elimination process. Other enzyme-catalysed reactions involving the elimination of ammonia, e.g. those catalysed by aspartase [7] and

Table

Substrate	Radioactivity				
	Starting succinate	Succinate recovered		Fumarate produced	
	cpm/mmole	cpm/mmole	ratio compared with substrate (%)	cpm/mmole	ratio compared with substrate (%)
^{3}H-succinate from L-^{3}H-histidine	945 ± 30	1630 ± 50	173 ± 10	574 ± 15	58 ± 5
(R)-^{3}H-succinate (reference)	1506 ± 15	2535 ± 15	168 ± 8	785 ± 15	52 ± 5
(S)-^{3}H-succinate (reference)	1663 ± 15	2087 ± 15	125 ± 5	932 ± 10	56 ± 5
(RS)-^{3}H-succinate (reference)	1581 ± 15	2321 ± 15	147 ± 7	922 ± 10	58 ± 5

β-methylaspartase [8–10], follow the same steric course although they differ from the histidase reaction in other respects.

Acknowledgement

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Addendum

After the preparation of this manuscript we received a preprint of a paper by I.L.Givot, T.A.Smith and R.H. Abeles in which they also show that the histidase reaction involves a "trans" elimination.

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