Biochimica et Biophysica Acta, 350 (1974) 354–357

BBA 67234

BIOSTEREOCHEMISTRY OF HISTIDINE METABOLISM*

II. THE STERIC COURSE OF AMMONIA ELIMATION FROM L-HISTIDINE

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(Received January 23rd, 1974)

SUMMARY

[2-^{2}H]Urocanic acid was obtained from the enzymatic reaction of racemic and stereoselectively labelled, DL-(±)-(3*S*):(3*R*)-[2,3-$^2H_2$]histidine with histidase prepared from *Pseudomonas striata*. The stereochemistry of deamination was assigned to be a stereospecific *trans*-elimination including *pro-R* proton liberation from the C-3 position of L-histidine.

It has been well established that the metabolism [2] of L-histidine (I) involves decarboxylation to histamine (II) or transformation via the urocanic acid pathway. The latter is the major quantitative pathway of histidine degradation in animals and in certain microorganisms, and produces several glutamate derivatives through a ring scission of the imidazole moiety (Fig. 1).

The stereochemistry of decarboxylation has been tentatively assigned [3] as proceeding with the retention of the configuration on the C-2 position of the original L-histidine (I). As to the urocanic acid pathway, there is some stereochemical interest in the ammonia elimination step and further in the subsequent hydration pathway to imidazolone propionic acid (IV) which has not been isolated yet because of its reported lability [4]. Givot et al. [5] and Retey et al. [6] have independently carried out the former non-oxidative deamination reaction of histidase with enzymatically, thus stereospecifically tritiated L-histidine and obtained unlabelled urocanic acid (III). They also have determined the chirality of the tritiated substrate by correlation with labelled succinic and aspartic acids, respectively, whose absolute configurations were known, and have asserted this enzymatic process to be a *trans*-elimination with removal of the *pro-R* proton from the C-3 position of the substrate (L-histidine).

In order to reexamine the proposed steric course and to obtain ^{2}H-labelled urocanic acid for the next study of the hydration step, the authors traced this deamination employing doubly and stereoselectively labelled histidine (I) and histidase pre-

* No. I in this series is ref. 1.

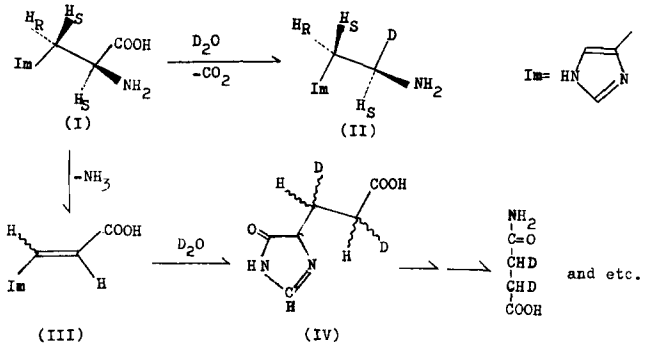

Fig. 1. The stereochemistry in L-histidine metabolism.

pared from *Pseudomonas striata*. The substrate of a racemic mixture of L-(−)-(3*R*)- and D-(+)-(3*S*)-[2,3-$^2H_2$]histidine, (75% ^{2}H-labelled), was prepared [1] stereoselectively by the azlactone synthesis. Histidase was extracted [7] from *Ps. striata* ICR 3200 grown in L-histidine medium via the methods of ultrasonification, centrifugation, heat treatment, $(NH_4)_2SO_4$ fractionation and dialysis. The enzyme activity was finally enhanced approx. 20-fold and this preparation was confirmed not to have histidine racemase and urocanase activities [7].

The incubation was carried out at 30 °C for 12 h, in a reaction mixture containing 2.0 g of racemic and labelled histidine (I), 100 mmoles of pyrophosphate buffer (pH 9.0) and 900 mg of histidase preparation. After quenching the reaction with methanol the formed acid (urocanic acid) was separated from unchanged substrate, and purified by ion-exchange column chromatography and by recrystallization, (yield of urocanic acid, 450 mg, 45% based on the L-form in the racemic substrate (DL-histidine), mp. 224–225 °C, lit. 225 °C). The product was submitted for the analyses of mass and NMR spectroscopic measurements.

The molecular ion peak of the product was $M^+ = 139$ *m/e* which revealed the formation of urocanic acid mono-substituted with ^{2}H. This evidence shows that one ^{2}H is liberated from the doubly labelled substrate (histidine). By the nmr analysis (in $^2H_2O/O^2H^-$, 5% solution, 60 MHz, Fig. 2) the chemical shifts of the corresponding protons of the authentic urocanic acid and the enzymatic product, (A and B, respectively, in Fig. 2) were assigned as follows; for A: 1 H at C-2, δ (ppm) 6.20 d; 1 H at C-3, 7.32 d, ($J_{2,3,trans} = 16$ cps); 1 H at C-2′ (ring proton), 7.52 m and 1 H at C-5′, 7.25; and for B: almost the same spectral pattern as that of A, except that the amount of the integral value of the proton at C-2, is 0.3 of the normal amount and a new broad peak appeared at the centre of gravity of the doublet proton signal for the proton at C-3. The B spectrum suggests that the product is a mixture of isomers of urocanic acid unlabelled (30%) and labelled (70%) at the C-2 position, and that the specific loss of the 3*R*-^{2}H on C-3 in the labelled L-histidine has occurred in the elimination step. Since the partially deuterated histidine (75% labelled at C-2 and C-3) was used as the substrate in the present work, the unlabelled contaminated L-histidine (25%) was preferentially consumed to urocanic acid by the enzyme, for which the isotope effects might be responsible. Thus the unlabelled product was accumulated up to 30% in the metabolite. The broad peak at δ 7.32 ppm was assigned to the proton

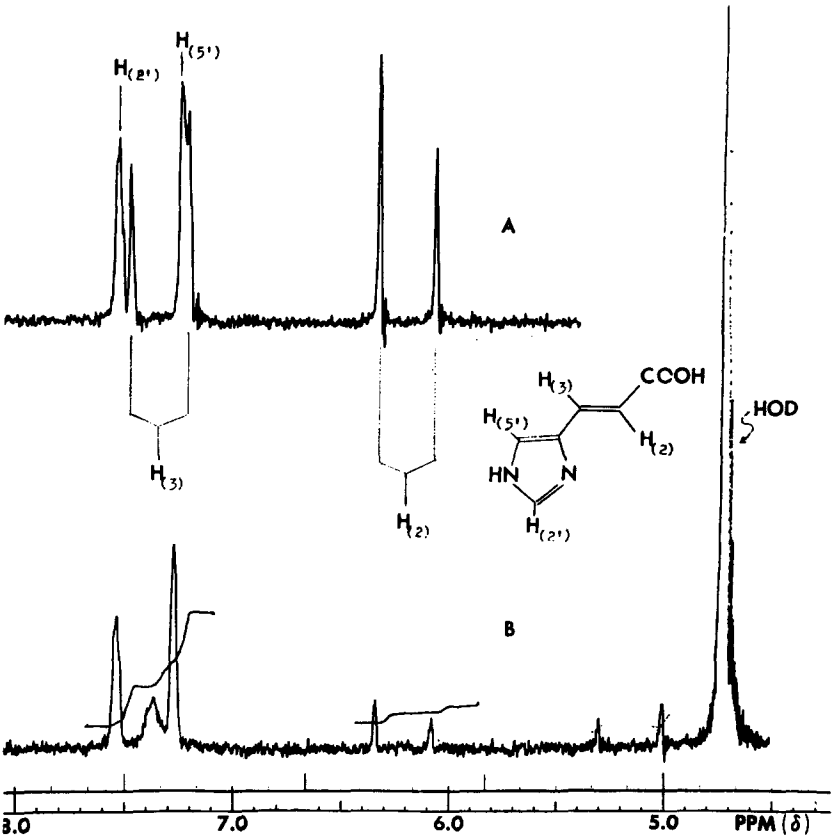

Fig. 2. NMR spectra of authentic (A) and [2-^{2}H]- (B) urocanic acids in $^2H_2O/O^2H^-$, 5% solution taken by a Varian A-60 spectrometer.

at C-3 of the urocanic acid labelled at C-2, ([2-^{2}H]uronic acid), and the broadening of the peak should be attributed to the small coupling constant between a *trans*-olefinic proton and a deuterium, ($J_{H^2H,\ trans} = 2.10$ cps [8]).

In addition, since this histidase employed was shown [7] to have no activity against the D-isomer, it was deduced that urocanic acid was derived only from the L-(−)-(3*R*)-enantiomer of histidine even though the incubation was carried out with the racemate. The contamination of the diastereomer, (L-(−)-(3*S*)-[2,3-$^2H_2$]histidine) in the substrate, which has already been neglected in the previous paper [1], and the subsequent stereospecific deamination of this counterpart with histidase should lead to the expectation of another isomer of urocanic acid deuterated at the C-3 position, ([3-^{2}H]urocanic acid). Any signal corresponding to this isomer, however, could not be detected in the B spectrum of the product.

Consequently it was ascertained by means of mass and NMR spectroscopies that non-oxidative ammonia elimination reaction of L-histidine catalyzed by histidase from *Ps. striata* proceeds in a *trans*-fashion, liberating the *pro-R* proton from C-3 in the substrate. This result compares well with those by Givot et al. [5] and Retey et al. [6], and the steric course of the α,β-elimination reaction of other amino acids [8, 9].

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