Eur. J. Med. Chem. 35 (2000) 283–289

S0223523400001707/FLA

Original article

Quantum mechanical study of the intermediates formed following the reaction of the histidine decarboxylase's substrate and inhibitors with coenzyme

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Received 27 May 1999; revised 31 August 1999; accepted 7 September 1999

Abstract – Histidine decarboxylase catalyses the decarboxylation of l-histidine to histamine using pyridoxal-5'-phosphate (PLP) as coenzyme. The PM3 quantum mechanical conformation method of analysis and heat of formation calculation were carried out for intermediates which are probably formed during the interaction of histidine (substrate), (s)-α-methylhistidine, (s)-α-hydrazinohistidine, (s)-α-fluoromethylhistidine and (s)-α-difluoromethylhistidine (inhibitors) with PLP-dependent histidine decarboxylase from *Morganella morganii*. The results suggest that the structures of the intermediates before and after decarboxylation were found to exist in a conformation showing a planar arrangement of the double bonds with the pyridoxylidene ring and the bond to the carboxyl group being perpendicular to this plane. After decarboxylation, all the double bonds are in the plane of the pyridoxylidene ring which facilitates the electron displacement for the following protonation at C^{α}. The values of the enthalpy for intermediates would increase the probability of their formation in the enzyme's active site which are consistent with all available stereochemical and mechanistic data. © 2000 Éditions scientifiques et médicales Elsevier SAS

quantum chemistry / pyridoxal-5'-phosphate / histidine decarboxylase / inhibitors

1. Introduction

Pyridoxal 5'-phosphate (PLP) is a cofactor for a large number of enzymes which catalyse reactions at C^{α} of amino acid substrates including transamination, decarboxylation, racemization, deamination and elimination [1–4]. PLP-dependent histidine decarboxylase (HDC) changes l-histidine to histamine [5, 6]. HDCs from mammalian and Gram-negative bacteria require PLP as coenzyme, while those from Gram-positive organisms have proved to be pyruvoyl-dependent [7]. In HDC from *Morganella morganii* (HDC-MM) PLP is present, as in other PLP-dependent enzymes, as an internal aldimine formed with the amino group of a specific lysine residue (*figure 1*) [8]. This internal aldimine reacts with the amino acid substrate by transaldimination to form an external aldimine (ES complex) which, by virtue of the strongly electron withdrawing nature of the pyridoxylidene moiety, weakens the bond to the carboxyl group through the extended π-system of the Schiff bases formed with amino acids, resulting in loss of CO_2^-. Then a proton adds to the resulting carbanion and the product is released [8, 9].

Effects of various compounds on HDC activity have been compiled in the literature [10–13]. Imidazole derivatives such as (s)-α-methylhistidine [14, 15], (s)-α-hydrazinohistidine [16] and (s)-α-fluoromethylhistidine (α-FMH) are potent inhibitors of PLP-dependent histidine decarboxylases [11, 17–19]. α-FMH irreversibly inactivates PLP-dependent HDC from mammals [20–23] and bacteria such as HDC-MM [24–26].

Subsequent studies [25, 27] on HDC-MM confirmed the mode of its inhibition by α-FMH. Bhattacharjee et al. [28] proposed that the inactivation of HDC-MM by α-FMH is mechanism based and is initiated by decarboxylation followed by elimination of fluoride ions (F^-) (I → II → III, *figure 2*). According to their proposal, after decarboxylation of the substrate analogue and β-elimination of F^-, transaldimination releases the enamine (VI), which is free to rotate around its carbon–carbon

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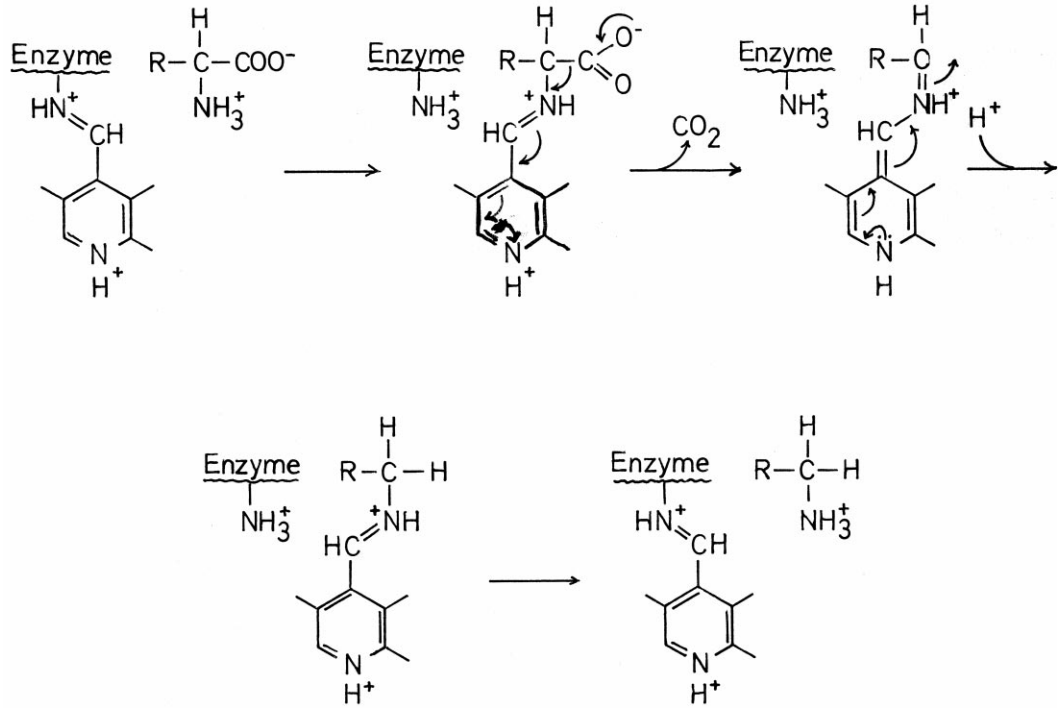


Figure 1. Present concepts of the role of PLP in decarboxylation of amino acids (from Bocker & Snell) [34].

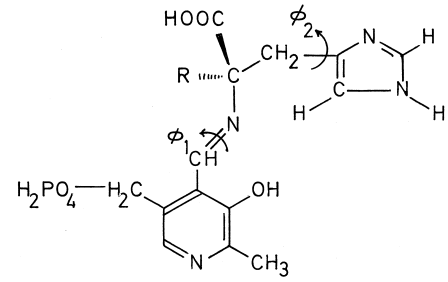
single bond. This reaction would place the nucleophilic methylene group close to the internal aldimine bond which it attacks to form VII. The adduct formed at the active site (VIII or IX) was firmly bound but could be released upon denaturation.

We intended to apply stereochemical analysis on l-histidine and some of the HDC inhibitors using experimental and computational methods [29, 30]. We report here the semi-empirical quantum chemical analysis of the predicted adducts (*figures 1* and *2*) formed during the reaction of substrate (histidine) and inhibitors with coenzyme, PLP.

2. Experimental protocols

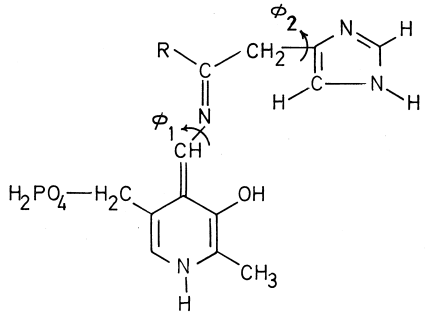
2.1. Intermediates formed following the reaction of l-histidine (substrate), (s)-α-methylhistidine and (s)-α-hydrazinohistidine (competitive inhibitors) with coenzyme

Histidine as a substrate reacts with the internal aldimine in the HDC active site to form an external aldimine with PLP (*figure 1*) [8]. Intermediates formed during the metabolism of l-histidine to histamine (compounds (**1a–3a**) (i.e. compound **1**, before decarboxylation; compound **2**, after decarboxylation and compound **3**, protonation at C^{α}), those of the inhibition of HDC with α-methylhistidine (compounds **1b–3b**) and α-hydrazinohistidine (compounds **1c–3c**) were built and preliminary energy minimisation was then performed within the PCMODEL [31] program to ensure a reasonable attracting conformation for the subsequent PM3 calculation. These initial geometries were used as input to the semi-empirical quantum chemical program 'MOPAC' [32] for full geometry optimisation.



1

a R = H ; b R = CH_3 ; c R = $NHNH_2$



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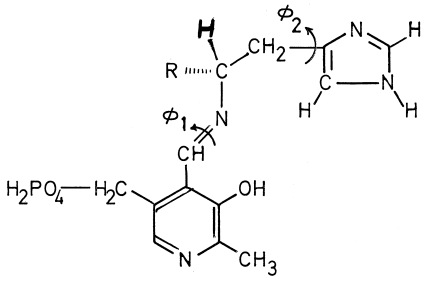
a R = H ; b R = CH_3 ; c R = $NHNH_2$

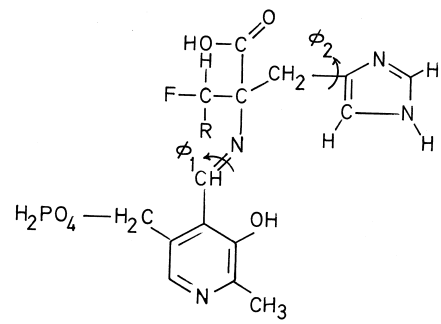


3

a R = H ; b R = CH_3 ; c R = $NHNH_2$

*2.2. Intermediates formed following the reaction of (s)-α-fluoromethylhistidine and (s)-α-difluoromethylhistidine (irreversible inhibitors) with coenzyme (s)-α-fluoromethylhistidine inactivates HDC-MM as a mechanism-based inhibitor, (*figure 2*).*

Intermediate compounds formed during the inactivation of PLP-dependent histidine decarboxylase by (s)-α-fluoromethylhistidine [28] (compounds **4a–6a**) (i.e. compound **4**, before decarboxylation, compound **5**, after decarboxylation and compound **6**, which is equivalent to adduct VIII in *figure 2*) were studied using the quantum chemical calculations.



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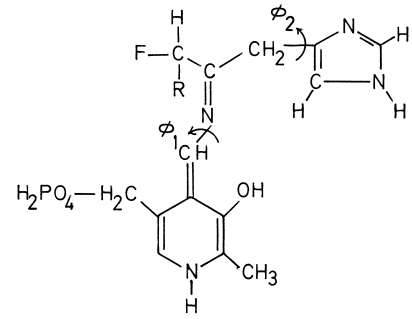
a R = H ; b R = F



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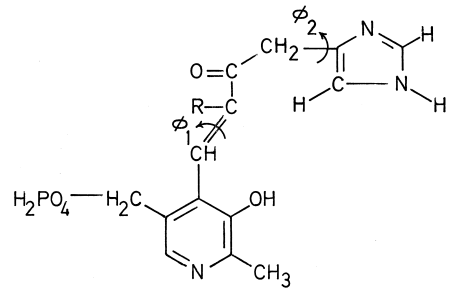

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a R = H ; b R = F

An attempt has also been made to consider (s)-α-difluoromethylhistidine (α-DFMH) as a probable HDC inhibitor and intermediates resulting from the reaction of

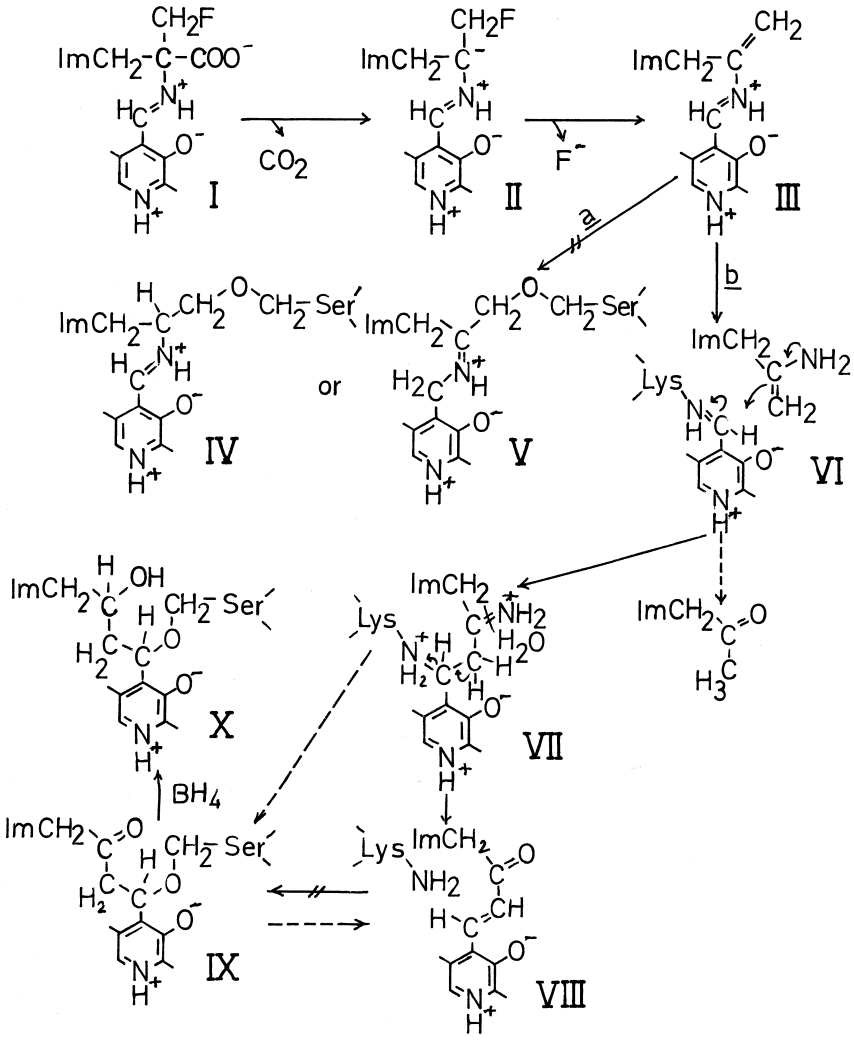

Figure 2. Proposed mechanism for inactivation of HDC by α-FMH. Arrows (→) indicate the mechanism proposed by Hayashi et al [25]; dotted arrows (----->) indicate modifications proposed by Bhattacharjee et al. [28].

α-DFMH with PLP (compounds **4b–6b**) were taken into conformational calculations.

3. Results and discussion

PM3 calculations show that all of the intermediates formed following the reaction of l-histidine (substrate), (s)-α-methylhistidine, (s)-α-hydrazinohistidine, (s)-α-fluoromethylhistidine and (s)-α-difluoromethylhistidine (inhibitors) with PLP (compounds **1–6**) are stable forms (*table I*).

Histidine decarboxylase catalyses the decarboxylation of l-histidine to histamine (*figure 1*). Its competitive inhibitor, (s)-α-methylhistidine, could make an external aldimine with PLP in a similar way to l-histidine (*figure 3*).

Previous work with both enzymatic and non-enzymatic models has confirmed that direct labilization of the C^{α}–COOH bond occurs in external aldimine formation (compound **1b**) and C^{α}–methyl amino acids are decarboxylated [8].

Our results point out that α-substituted inhibitors could also be decarboxylated in the same manner, but with different speed.

Poulin's group [33] demonstrated inactivation of the PLP-dependent ornitine decarboxylase, the enzyme re-

Table I. Heat of formation in kcal/mol calculated by the PM3 method for the preferred conformation of the compounds **1–6**.

Compound	Heat of formation (kcal/mol)
1a	–315.59035
1b	–319.8892
1c	–284.22
2a	–216.97554
2b	–224.9585
2c	–193.13179
3a	–231.13245
3b	–235.27287
3c	–199.6786
4a	–360.6356
4b	–408.6019
5a	–263.9269
5b	–315.0562
6a	–263.6140
6b	–304.025

sponsible for producing putrescine from ornitine, by the α-difluoromethyl derivative of ornitine.

Based on our data, (s)-α-difluoromethylhistidine might be able to inhibit histidine decarboxylase in a similar manner as its monofluoro derivative (α-FMH) (*figures 3–5*).

The sequence of events during catalysis is not yet known (*figure 2*); however, the lysine residue could function catalytically as a proton donor to the carbanion formed as CO_2 is lost, thus facilitating the decarboxylation of l-histidine. This role is supported by the position of lysine in the active site.

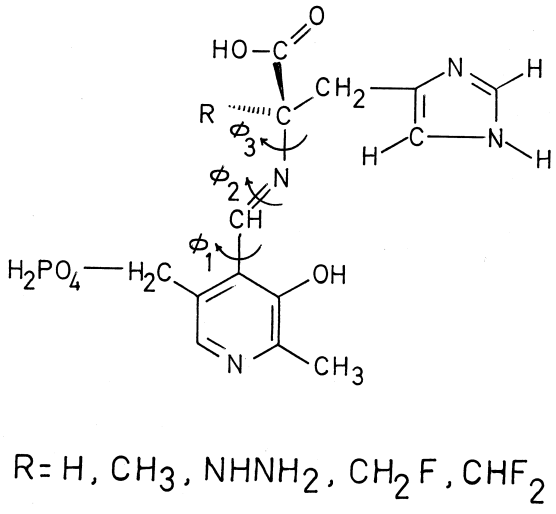

Figure 3. Conformational angles φ_1, φ_2 and φ_3 in the intermediates formed during the reaction of l-histidine and inhibitors with PLP.

In the scheme of *figure 1*, the protein obviously provides, in addition to the specific lysine residue that interacts with PLP, other specific binding sites for PLP and for the substrate that properly orients the two for reaction, as well as catalytic residues which greatly enhance the reaction rate. In HDC-MM, Lys-232 provides the amino group that forms the internal aldimine with coenzyme. The sequence around PLP-binding lysine, -Ser-X-His-Lys-, contains two completely conserved residues, a serine which forms a side chain H-bond with the 5'-phosphate ester group of PLP, and the active site lysine, which makes Schiff's base with PLP and which also serves to shuttle protons between C^{α} and C_4' of aldimine [34, 35].

By comparison with aminotransferases, the amount of stereochemical, mechanistic and structural information available for the decarboxylase group is not much. Transaminases are the best-understood PLP-dependent enzymes and much of the early work in the area was concerned with assessing the stereochemical course of aldimine formation in transaminases [36]. In both groups, the residues forming aldimine with PLP is lysine, and the major difference between transaminases and decarboxylases appear to be the conformation of the substrate aldimine C^{α}–N bond which is controlled by side chain binding, and in decarboxylases by the presence of the positively charged imidazolium side chain of a residue which interacts electrostatically with the α-carboxylate group [1].

Previous studies with the carbon and nitrogen isotope effects showed that the overall decarboxylation rate is jointly limited by the rate of transamination and by the intrinsic rate of decarboxylation [37].

Table II summarises the results of the PM3 calculation of conformation for the intermediates (compounds **1–6**) formed during the reaction of substrate and inhibitors with coenzyme. The preferred conformational angle, φ_1, for the torsion of the C_4'–N is verified to be close to 180° in all of the intermediates. In addition, it is found that the preferred conformational angle, φ_2, for the torsion of C–C varies from 53°–127° in most of the compounds considered.

In 1966 Dunathan proposed [38], on the basis of stereoelectronic arguments that all double bonds in the intermediates before decarboxylation are thought to lie in a plane which facilitates the electron displacements, and for efficient labilization in such a system, the bonds to be broken at C^{α} of PLP-amino acid Schiff's bases should be held at 90° to the plane of the extended conjugated system [39].

Table II. Preferred conformational angles φ_1 and φ_2 (compounds **1–6**) in the intermediates formed during the reaction of histidine and inhibitors with PLP calculated by the PM3 approximation.

Compound	φ_1	φ_2
1a	–177.94	–63.9
1b	–178.56	–57.64
1c	178.54	61.32
2a	–176.64	126.93
2b	–179.48	91.91
2c	175.79	107.75
3a	–179.92	70.03
3b	–178.35	59.68
3c	–177.85	70.07
4a	–178.42	–62.58
4b	178.67	–60.04
5a	–178.52	76.23
5b	176.44	104.17
6a	178.12	53.83
6b	178.46	63.53

*The values of φ_1 and φ_2 are in degrees

According to our results, all double bonds in the intermediates before decarboxylation are in a plane (φ_1 and φ_2 in *figure 4, table III)*, and the bond to the carboxyl group lies perpendicular to this plane (φ_3 in *figure 4, table III*).

It has been assumed that the conserved histidine in the decarboxylase active site could serve as a base in the deporotonation of the ammonium group of the substrate before transaldimination [39]. Following decarboxylation and generation of the quinoid intermediate, the imidazolium side chain then protonates the quinoid at C^{α} to give the product aldimine [40, 41].

We also determined the preferred conformation of the intermediates (*table IV*) in which the double bonds are in the plane of the extended conjugated π-system, thereby facilitating the protonation at C^{α} (the values of φ_1, φ_2 and φ_3 in *figure 5*).

Table III. Preferred conformational angles φ_1, φ_2 and φ_3 in the intermediates before decarboxylation (*figure 3*) calculated by the PM3 approximation.

Compound	φ_1	φ_2	φ_3
1a	177.04	–177.94	89.1
1b	175.29	–178.56	79.6
1c	–177.09	178.54	–95.8
4a	176.32	–178.42	90.93
4b	–179.85	178.67	94.93

*The values of the φ_1, φ_2 and φ_3 are in degrees

Table IV. Preferred conformational angles φ_1, φ_2 and φ_3 in the intermediates after decarboxylation (*figure 5*) calculated by the PM3 approximation.

Compound	φ_1	φ_2	φ_3
2a	–179.39	–176.64	–178.99
2b	–179.37	–179.48	–179.92
2c	–179.21	175.79	–179.08
5a	179.79	–178.52	–179.28
5b	–178.95	176.44	–178.80

*The values of the φ_1, φ_2 and φ_3 are in degrees.

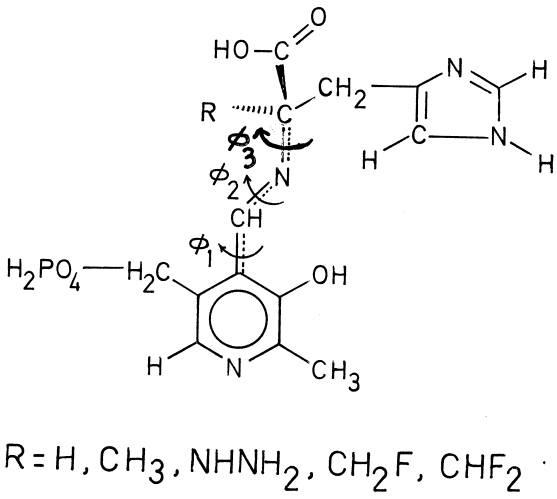

Figure 4. Conformational angles φ_1, φ_2 and φ_3 in the intermediates before decarboxylation.

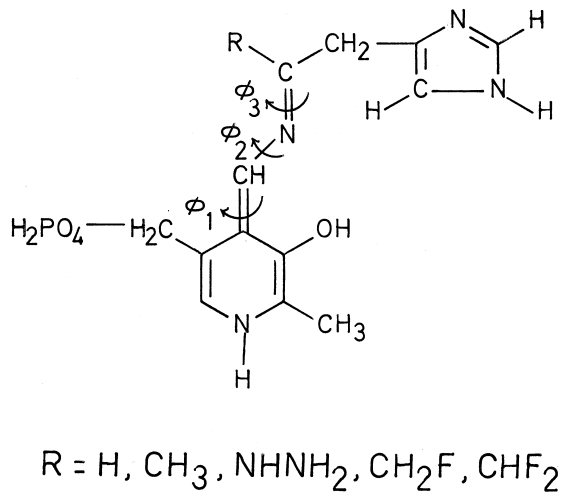

Figure 5. Conformational angles φ_1, φ_2 and φ_3 in the intermediates after decarboxylation.

In conclusion, our computational data would confirm the catalytic mechanisms used by PLP enzymes, which has been proposed by others, and further could be helpful in designing therapeutic agents targeting these proteins.

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