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MECHANISM OF INACTIVATION OF MAMMALIAN L-HISTIDINE DECARBOXYLASE BY (*S*)-α-FLUOROMETHYLHISTIDINE

HIROAKI KUBOTA,* HIDEYUKI HAYASHI, TAKEHIKO WATANABE,†
YOSHITAKA TAGUCHI and HIROSHI WADA
Department of Pharmacology II, Osaka University School of Medicine, Osaka 530, Japan

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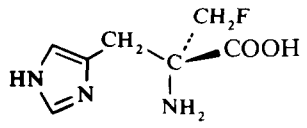
Abstract—The mechanism of inactivation by (*S*)-α-fluoromethylhistidine (FMH) of L-histidine decarboxylase (HDC, L-histidine carboxy-lyase, EC 4.1.1.22) purified from whole bodies of fetal rats was studied. FMH inhibited the activities of HDC purified from fetal HDC as well as HDCs from the brain and stomach of adult rats. The activity was not restored by extensive dialysis, indicating that the inhibition was irreversible. The inactivation of HDC was time and concentration dependent and followed pseudo first-order kinetics. L-Histidine, a substrate, protected HDC against inactivation, but D-histidine did not. Apo-HDC was not inactivated by FMH. On labeling of HDC with [^{3}H]FMH, a correlation was found between the extent of incorporation of radioactivity into the enzyme and the degree of inactivation. Two moles of the inhibitor were incorporated into one mole of HDC (108,000 daltons). Experiments with [*carboxyl*-^{14}C]FMH and [*ring* 2-^{14}C]FMH showed that decarboxylation was necessary for the inactivation and that one molecule of FMH moiety was incorporated into an HDC monomer during every three decarboxylations of FMH.

Histamine is present in a variety of mammalian tissues and is important as an autacoid or neurotransmitter [1]. L-Histidine decarboxylase (HDC‡, L-histidine carboxy-lyase, EC 4.1.1.22) catalyzes the decarboxylation of L-histidine to histamine [2–5]. Thus, a specific inhibitor of HDC activity that leads to depletion of histamine *in vivo* is useful for studies on the physiology, pharmacology and pathology of histamine. Many inhibitors have been developed and used for this purpose, but none is satisfactory, because none is so specific or strong an inhibitor *in vivo* as expected from studies *in vitro* [6–14]. Therefore, an HDC inhibitor with strict specificity and high potency is still required.

Recently, Kollonitsch *et al.* [15] tested a series of α-fluoromethyl amino acids as specific inhibitors of decarboxylases. These compounds are of the type called k_{cat} inhibitors [16], suicide substrates [17], or enzyme-activated irreversible inhibitors [18]. (*S*)-α-Fluoromethylhistidine (A), abbreviated as FMH, was found to be a potent and specific inhibitor of HDC [15]. Garbarg *et al.* [19] showed that FMH completely inactivates brain HDC and reduces the histamine level. Subsequently, Maeyama *et al.* [20, 21] studied the effects of FMH on the HDC activities and histamine contents of various tissues of mice. However, with regard to the mechanism of inhibition of HDC by this compound, so far only kinetic studies on the inactivation have been reported [15, 19].



(A)

In this work, to clarify the mechanism of inactivation of purified fetal rat HDC by FMH, we studied the inactivation kinetics and the incorporation of radioactive FMH into HDC.

MATERIALS AND METHODS

Chemicals. FMH, [*ring* 4-^{3}H]FMH, [*carboxyl*-^{14}C]FMH, [*ring* 2-^{14}C]FMH, (*R*)- and (*S*)-α-fluoromethylhistamine, (*R*, *S*)-α-fluoromethyldopa, and (*R*, *S*)-α-fluoromethylornithine were donated by Dr. J. Kollonitsch of Merck Sharp & Dohme Research Laboratories, Rahway, NJ, U.S.A. All other chemicals used were of reagent grade.

Preparation of histidine decarboxylases. HDCs were purified from whole bodies of fetal rats (Wistar, 16–20 days of gestation) and from the brains and stomachs of pregnant rats as described previously [22, 23]. The purifications achieved were 7700-, 160- and 380-fold respectively.

Assays of HDC activity. HDC activity was assayed as described previously [22]. Briefly, the reaction mixture contained 0.25 mM L-histidine, 1% (w/w)

* Present address: Suntory Institute for Biomedical Research, 1-1-1 Wakayamadai, Shimamoto-cho, Mishima-gun 618, Japan.
† Correspondence should be addressed to: Dr. Takehiko Watanabe, Department of Pharmacology II, Osaka University School of Medicine, 3-57 Nakanoshima 4-chome, Kita-ku, Osaka 530, Japan.

‡ Abbreviations: HDC, L-histidine decarboxylase, L-histidine carboxy-lyase, EC 4.1.1.22; FMH, (*S*)-α-fluoromethylhistidine; dopa, 3-(3,4-dihydroxyphenyl)-alanine; PLP, pyridoxal 5′-phosphate; and U, units of HDC activity, as pmoles of histamine formed per min.

polyethylene glycol (average molecular weight 300), 0.2 mM dithiothreitol, 0.01 mM pyridoxal 5'-phosphate and 0.1 M potassium phosphate buffer, pH 6.8, in a total volume of 1.0 ml. After incubation at 37°, histamine was separated by Amberlite CG-50 column chromatography and determined by the *o*-phthalaldehyde method [24] in a Technicon autoanalyzer by the method of Martin and Harrison [25] as described previously [22]. One unit of enzyme catalyzes the production of 1 pmole of histamine per min under these conditions.

Measurement of protein. Protein was measured by the method of Lowry *et al.* [26] with bovine serum albumin as a standard.

Preparation of apo-HDC. Apo-HDC* was prepared by extensive dialysis of holo-HDC against a reaction buffer without PLP.

Production of rabbit anti-HDC serum. An antibody was raised in New Zealand rabbits (3.0 to 3.5 kg) by injecting about 250 μg of fetal HDC (0.5 ml) emulsified with 0.5 ml of complete Freund's adjuvant into the foot pads of animals under pentobarbital anesthesia. Four booster injections into the abdominal skin were given at 10-day intervals and, 10 days after the last injection, blood was taken from the marginal ear vein. The antiserum was freeze-dried and stored at 0°.

Inactivation of HDC by FMH and other α-fluoromethyl compounds. Fetal HDC was preincubated with various concentrations of FMH for various periods at 37° in the reaction mixture and dialyzed at 4° against two 100-volumes of the same solution. The dialyzates were assayed for remaining activity. Inactivations of fetal HDC by other α-fluoromethyl compounds and of brain and stomach HDCs by FMH were examined in the same way.

Kinetic studies on the inhibition of HDC by FMH. For studies on inhibition kinetics, the fetal enzyme was preincubated with various concentrations of FMH for the indicated periods; then samples were transferred to a reaction mixture to lower the FMH concentration to below that inhibiting HDC under the assay conditions (less than 10^{-9} M) and were assayed for activity as described above.

*Labeling of HDC with [*ring 4-^{3}*H]FMH.* Purified fetal HDC (500 U, equivalent to 1.67 μg because a pure enzyme has the sp. act. of 300,000 U/mg protein [22]) was incubated with 0.01 mM [*ring* 4-^{3}H]FMH (sp. act. 11.5 mCi/mmole) in 2.0 ml of reaction mixture for 30 min at 37°. Under these conditions, the enzyme was 80% inactivated. The mixture was applied to a Sephadex G-25 column (0.9 × 10 cm) equilibrated with the reaction mixture minus histidine, and 1.0-ml fractions were collected. Samples of 0.2 and 0.4 ml of each fraction were used for HDC assay and for measurement of radioactivity, respectively. Radioactivity was measured in 5 ml of ACS-II (Amersham) in an Aloka liquid scintillation counter.

Alternatively, after treatment of HDC with [*ring* 4-^{3}H]FMH as described above, 0.1 ml was used for HDC assay, and 0.4 ml was mixed with 0.05 ml of 60% perchloric acid and kept at 4° for 10 min. The precipitate was then collected by filtration on a glass microfiber filter (GF/C, Whatman) and washed with 5 ml of 10% perchloric acid. The precipitate was dried, dissolved in 1 ml of Protosol (New England Nuclear) in a glass vial, and heated at 60° for 30 min. Then, 0.5 ml of acetic acid was added, and the radioactivity was counted in 5 ml of ACS-II as described above.

*Precipitation of [*ring 4-^{3}*H]FMH-treated HDC with anti-HDC antibody.* Samples of 0.6 ml of [*ring* 4-^{3}H] FMH-treated HDC were incubated with 0.2 ml of either anti-HDC antiserum or control serum for 12 hr at 4°. The antigen–antibody complex was co-sedimented by incubation with 0.2 ml of goat anti-rabbit IgG for 1 hr at 4°. Then the mixture was centrifuged, and the radioactivity of 0.4 ml of the supernatant fraction was measured as described above.

Detection of the reaction products of FMH by thin-layer chromatography. A sample of 1.0 ml of a mixture consisting of 100 U of HDC and 0.1 mM FMH was mixed with 50 μl of 60% perchloric acid and centrifuged 10 min later. The supernatant fraction was evaporated to dryness *in vacuo*, and the residue was dissolved in a small volume of H_2O and chromatographed on TLC plates (silica gel 60 F254, Merck) in solvent systems (a) *n*-butanol–ethylacetate–ethanol–water (6:2:3:1, by vol.), and (b) chloroform–methanol–ethylacetate–28% ammonia (4:3:2:1, by vol.). The products were located by spraying the plates with iodine or 1% ninhydrin solution.

Measurement of α-fluoromethylhistamine by HPLC. For determination of whether α-fluoromethylhistamine (FMHA) is produced by reaction of HDC with FMH, 0.3 ml of reaction mixture consisting of 1500 U HDC and 0.1 mM FMH was incubated at 37° for 1 hr, and then all the reaction mixture was subjected to high performance liquid chromatography by the method of Yamatodani *et al.* [27]. FMH and FMHA were separated on a column of strong cation exchange resin and determined with *o*-phthalaldehyde [24]. The minimum amount of FMHA detectable by this method was 1 pmole injected into the instrument.

Labeling of HDC with ^{14}*C-labelled FMH.* Purified HDC (100 U) was incubated with a 0.25 mM concentration of either [*carboxyl*-^{14}C]FMH (sp. act. 3.91 mCi/mmole) or [*ring* 2-^{14}C]FMH (sp. act. 3.46 mCi/mmole) in the reaction buffer described above. In some experiments, the enzyme was pretreated with 0.1 mM "cold" FMH for 60 min at 37° and dialyzed against the above buffer without FMH before labeling with [*carboxyl*-^{14}C]FMH or [*ring* 2-^{14}C]FMH. These reaction mixtures were incubated at 37° for 30 min. After incubation, each mixture was dialyzed three times against 1000 ml of the same buffer for 12 hr. Then the radioactivities of the mixtures were counted in 5 ml of ACS-II as described above.

Determination of the partition ratio of FMH. The following experiments were performed to determine the partition ratio of FMH, i.e. the number of turnovers per inactivation. Purified HDC was incubated with 0.25 mM [*carboxyl*-^{14}C]FMH (sp. act. 3.91 mCi/mmole) in the reaction mixture described

* Strictly speaking, we should use the terms "holo-HDC" and "apo-HDC" to distinguish them. But for simplicity, we used "HDC" in the sense of "holo-HDC".

Table 1. Effect of dialysis on the enzyme activity of HDC treated with FMH*

FMH (M)	Activity (%) Before dialysis†	After dialysis‡
1×10^{-3}	5.2	5.6
5×10^{-7}	52.3	46.8

* HDC was incubated with FMH for 30 min at 37°, and the activity was assayed before and after dialysis against the reaction buffer without FMH for 24 hr, as described in Materials and Methods.

† Activity of enzyme solution incubated without FMH was taken as 100%.

‡ Activity of enzyme solution incubated without FMH and then dialyzed was taken as 100%.

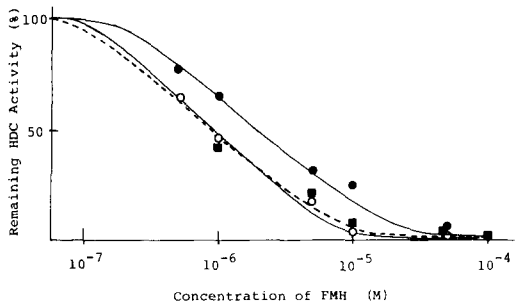


Fig. 1. Inhibition of fetal, brain and stomach HDCs by FMH. Purified fetal (■), brain (●) and stomach (○) HDCs were preincubated with various concentrations of FMH at 37° for 30 min. The remaining activities were assayed as described in Materials and Methods.

above for 30 min at 37°. The amount of CO_2 generated was calculated from the radioactivity of $^{14}CO_2$ trapped on filter paper soaked in 10% NaOH by the method of Hayashi *et al.* [28]. These enzyme solutions were also incubated with 0.25 mM [*ring* 2-^{14}C]FMH (sp. act. 3.46 mCi/mmole) for 30 min at 37° with or without pretreatment by cold FMH as described above, and the amount of FMH specifically bound to HDC was calculated from the radioactivity incorporated into the protein.

RESULTS

Inactivation of HDC by fluoromethyl compounds. HDC was preincubated with 1×10^{-3} and 5×10^{-7} M FMH at 37° for 30 min, and remaining activities were compared before and after dialysis. As shown in Table 1, FMH reduced the HDC activity, and no activity was restored by extensive dialysis, indicating that the inhibition was irreversible. The effects of other fluoromethyl compounds on purified fetal rat HDC were also tested (Table 2). α-Fluoromethylornithine or α-fluoromethyldopa at 1×10^{-5} M, a concentration at which FMH almost completely inactivated the enzyme, did not inactivate HDC. Both (*R*)- and (*S*)-α-fluoromethylhistamine inactivated HDC about 50%. Apo-HDC was not inactivated by 1×10^{-3} M FMH, which inactivated HDC 100% (data not shown).

Inactivation of brain and stomach HDCs by FMH. Figure 1 shows the inactivation of fetal, brain and stomach HDCs by FMH. Like fetal HDC, stomach HDC was inactivated by FMH. The brain enzyme was inactivated slightly less than the other HDCs.

Kinetics of inactivation of HDC by FMH. Figure 2 shows the time- and concentration-dependences of inactivation of fetal rat HDC by FMH. The inactivation followed pseudo first-order kinetics (Fig. 2A).

Table 2. Inhibition of fetal rat HDC by α-fluoromethyl compounds*

Addition	Activity (%)
Control	100
(*R*,*S*)-α-Fluoromethyldopa	91.3
(*R*,*S*)-α-Fluoromethylornithine	92.0
(*R*)-α-Fluoromethylhistamine	56.3
(*S*)-α-Fluoromethylhistamine	50.6
(*S*)-α-Fluoromethylhistidine	4.4

* HDC was incubated with 0.01 mM α-fluoromethyl compounds for 30 min at 37° and dialyzed against the reaction buffer. The remaining activity was assayed as described in Materials and Methods.

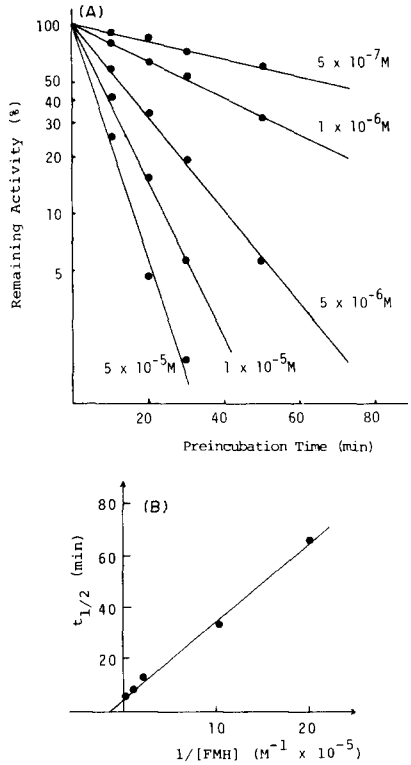


Fig. 2. Kinetics of inactivation of HDC by FMH. (A) Time-dependent inactivation of fetal rat HDC by FMH. Fetal rat HDC was preincubated with various concentrations of FMH in reaction buffer of pH 6.8 at 37°. Remaining activity was expressed as a percentage of the control activity (HDC activity incubated without FMH). (B) Plots of $t_{1/2}$ against 1/[FMH]. The half-time of inactivation ($t_{1/2}$) at each concentration of inhibitor was determined from (A) and plotted against 1/[FMH].

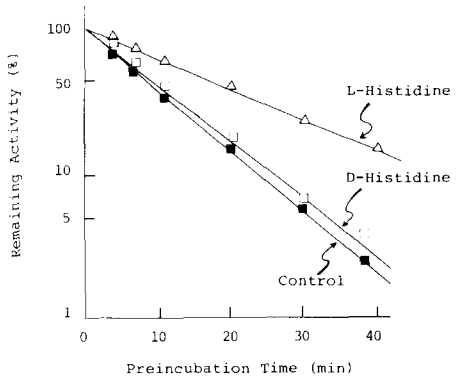


Fig. 3. Effects of L- and D-histidine on inhibition of HDC by FMH. HDC was preincubated with 0.01 mM FMH plus L- or D-histidine (0.5 mM), and the remaining activity was measured as described in Materials and Methods. The control sample was preincubated with 0.01 mM FMH.

The half-times ($t_{1/2}$) of inactivation were determined for each FMH concentration and were plotted versus 1/[FMH] as shown in Fig. 2B by the method of Kitz and Wilson [29]. From this plot, the K_I value (see Appendix) was calculated to be 0.83×10^{-5} M.

Effects of substrate analogs on inactivation by FMH. If FMH is directed to an active site of HDC, a substrate analog should influence the inactivation by FMH. As shown in Fig. 3, L-histidine, a substrate, protected HDC against inactivation, whereas D-histidine, a non-substrate, did not.

Labeling of fetal HDC with [ring 4-^3H] *FMH.* The inactivation of HDC by FMH was investigated further by treating HDC with [*ring* 4-^{3}H]FMH. Figure 4 shows the elution profile on a Sephadex G-25 column of HDC treated with [^{3}H]FMH. The protein fraction containing slight HDC activity due to incomplete inactivation under these conditions

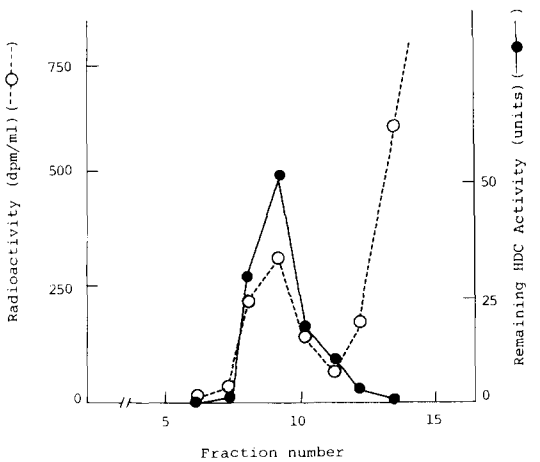


Fig. 4. Labeling of HDC with [*ring* 4-^{3}H]FMH. Fetal HDC (500 U) was treated with 0.01 mM [*ring* 4-^{3}H]FMH (sp. act. 11.5 mCi/mmole) for 30 min at 37°. The mixture was passed through a Sephadex G-25 column, and fractions of 1.0 ml were collected. Aliquots (0.2 and 0.4 ml) of the eluate fractions were used for measuring HDC activity and radioactivity, respectively, as described in Materials and Methods.

Table 3. Immunoprecipitation with anti-HDC antibody of [*ring* 4-^{3}H]FMH-treated HDC*

Addition	Radioactivity in supernatant (dpm)
None	2740
Control serum	2448
Anti-HDC serum	202

* HDC treated with [*ring* 4-^{3}H]FMH was incubated with anti-HDC serum or control serum and then goat anti-rabbit IgG serum as described in Materials and Methods, and the radioactivity of the supernatant fraction was measured.

gave a peak of radioactivity well-separated from the remaining [*ring* 4-^{3}H]FMH. To confirm that the radioactivity was associated with HDC, we precipitated [*ring* 4-^{3}H]FMH-labeled protein with anti-HDC antiserum. As shown in Table 3, protein-bound radioactivity was precipitated with anti-HDC serum but not with control serum. Figure 5 shows that the incorporation of radioactivity into protein was a mirror image of the degree of HDC inactivation, indicating that FMH is incorporated into the HDC molecule during inactivation. At complete inactivation, the protein-bound radioactivity was 162 dpm, indicating the incorporation of 6.35×10^{-12} moles of FMH into 100 U of HGC, equivalent of 0.333 μg of pure enzyme. From this result the stoichiometry of the inactivation reaction was calculated as 1.03 mole of FMH per mole of HDC subunit, when the molecular weight of the HDC subunit is assumed to be 54,000 daltons (Taguchi *et al.*, *J. biol. Chem.* in press).

Analysis of reaction products of HDC with FMH. The reaction product(s) of HDC with FMH was analyzed by thin-layer chromatography as described in Materials and Methods. Four spots were detected with iodine at R_f values of 0.15, 0.27, 0.33 and 0.53 (R_f 0.15 for FMH) in solvent (a) (data not shown). Only the spot of R_f 0.15 corresponding to FMH stained with ninhydrin. No spot corresponding to FMHA was detected: it should be at R_f 0.02 as a ninhydrin positive spot. Thin-layer chromatography with solvent (b) gave similar results to those with solvent (a). When the reaction product was analyzed

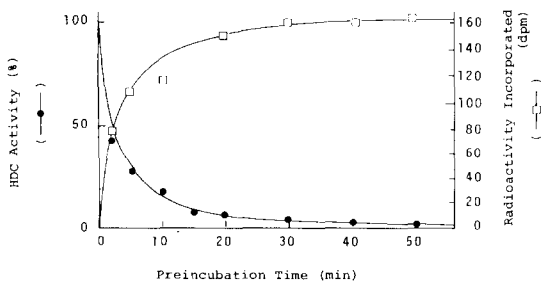


Fig. 5. Titration of fetal HDC with [*ring* 4-^{3}H]FMH. Purified fetal HDC (500 U) was preincubated with 0.01 mM [*ring* 4-^{3}H]FMH (sp. act. 11.5 mCi/mmole). Samples of 0.1 and 0.4 ml of the reaction mixture (total volume 2 ml) were used for assay of HDC activity and measurement of radioactivity, respectively, as described in Materials and Methods.

Table 4. Incorporation of ^{14}C-labeled FMH into HDC*

Pretreatment	Incorporation of radioactivity when incubated with	
	[*Ring* 2-^{14}C]FMH	[*Carboxyl*-^{14}C]FMH
	(cpm)	
None	64	18
Cold FMH	21	19

* Purified HDC (about 100 U) was incubated with 0.25 mM [*ring* 2-^{14}C]- or [*carboxyl*-^{14}C]FMH for 30 min. The same amount of HDC which had been inactivated by pretreatment with cold FMH (0.1 mM for 60 min) was incubated similarly. The radioactivity was counted as described in Materials and Methods. The background radioactivity of 5 ml of ACS-II containing 1 ml of buffer was 19.2 ± 2.0 cpm (mean ± S.D., N = 10).

by HPLC as described in Materials and Methods, no peak of FMHA, which is eluted in the same position as histamine, was detected. The lower limit of sensitivity of this system for FMHA was 1 pmole.

*Labeling of HDC with [*ring 2-^{14}*C]- and* [carboxyl-^{14}*C]FMH.* We tested whether the decarboxylation of FMH is necessary for inactivation to occur, using [*ring* 2-^{14}C]- and [*carboxyl*-^{14}C]FMH. If decarboxylation is necessary before inactivation, the radioactivity of [*carboxyl*-^{14}C]FMH should not be incorporated into HDC but that of [*ring* 2-^{14}C]FMH should be incorporated; if decarboxylation is not necessary, the radioactivities of both compounds should be incorporated similarly. The incorporation of radioactivity into protein treated with [*ring* 2-^{14}C]FMH was 64 dpm. When the enzyme had been pretreated with "cold" FMH for 60 min at 37°, the radioactivity was within the background range. On the other hand, when [*carboxy*-^{14}C]FMH was used for labeling, the radioactivities of pretreated and untreated HDCs were both within the background range (Table 4).

Partition ratio of FMH. Finally, we studied the efficiency of labeling of HDC by FMH, i.e. how many decarboxylations of FMH are coupled to the covalent binding of one molecule of FMH to HDC, as follows. HDC was incubated with 0.25 mM FMH for 30 min, and the amounts of CO_2 evolved and that of FMH incorporated into HDC were measured as described in Materials and Methods. The ratio of the amount of CO_2 to that of FMH incorporated into the protein (partition ratio) under these conditions was 2.82, as shown in Table 5.

DISCUSSION

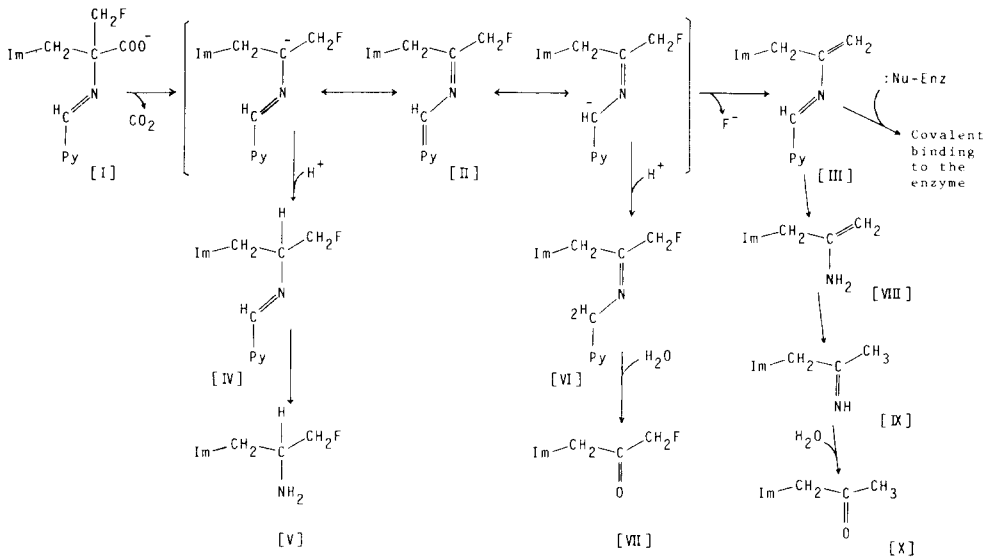
Enzyme-activated irreversible inhibitors [18], suicide substrates [17], or k_{cat} inhibitors [16] have been investigated extensively and used widely in *in vitro* studies [30–34]. Of these inhibitors, α-fluoromethyl amino acids, which were developed by Kollonitsch *et al.* [15], are potent and specific inhibitors of decarboxylases and are useful in investigations on biogenic amines. However, their mechanism of inhibition was only speculative until recently when Maycock *et al.* [33] made extensive studies on the process of inactivation of dopa decarboxylase by α-fluoromethyldopa.

We studied the mechanism of inactivation of HDC by FMH and concluded that this compound is a suicide substrate [17] from the following evidence: (1) FMH inactivates HDC irreversibly, because extensive dialysis did not restore the activity (Table 1), as reported by other investigators [15, 19]; (2) covalent binding of [*ring* 4-^{3}H]FMH or its moiety to HDC was demonstrated by measurement of incorporation of radioactivity into the protein (Figs. 4 and 5, Table 3); 2 moles of FMH moiety were bound to one mole of HDC, assuming that the molecular weight of HDC is 108,000 daltons; (3) apo-HDC was not inactivated by FMH; (4) L-histidine protected the enzyme from inactivation, but D-histidine did not (Fig. 3); and (5) kinetic studies showed that the dose-dependent inactivation could be saturated, i.e. a plot of the half-time of inactivation ($t_{1/2}$) versus 1/[FMH] gave a straight line (Fig. 2). The K_I value of FMH

Table 5. Partition ratio of FMH*

Amount of enzyme (U)	CO_2 formation (pmoles)	FMH bound to HDC (pmoles)	Partition ratio (CO_2 formation/FMH bound to HDC)
120	20.9	7.50	2.79
240	42.0	13.2	3.18
360	61.4	21.6	2.84
480	78.0	31.4	2.48
			(Average: 2.82)

* HDC was incubated with 0.25 mM [*carboxyl*-^{14}C]FMH or 0.25 mM [*ring* 2-^{14}C] FMH for 30 min in the reaction buffer, and CO_2 formation and FMH bound to HDC were calculated from the radioactivity of $^{14}CO_2$ trapped on filter paper, and that of [^{14}C]FMH incorporated into the protein, respectively, as described in Materials and Methods.



Scheme 1. Proposed mechanism of inactivation of HDC by FMH.

for the inactivation of HDC obtained from a Kitz and Wilson plot (0.83×10^{-5} M, Fig. 2A) is smaller than that of rat brain HDC (3.2×10^{-5} M) reported by Garbarg *et al.* [19]. This difference may depend on differences between our conditions and theirs, such as in the enzyme preparation or temperature of incubation (20° in their studies). As to enzyme preparation, the difference is consistent with the finding that brain HDC was inhibited by FMH a little more weakly than fetal HDC (Fig. 1).

For confirmation that decarboxylation of FMH occurs before inactivation, which is the most important criterion for a "suicide substrate", binding studies using ^{14}C-labeled FMH were performed (Table 4). As with [*ring* 4-^{3}H]FMH, the radioactivity of [*ring* 2-^{14}C]FMH was associated with HDC. Radioactive FMH did not bind to enzyme that had already been inactivated by cold FMH. These data indicate that 5.60×10^{-12} moles of FMH were incorporated into enzyme that had an activity of 100 U. As one molecule of FMH is considered to bind to each subunit of HDC from the above result (Fig. 5), the specific activity of the enzyme was calculated as 3.31×10^{5} U/mg protein, which is consistent with the value expected for pure HDC [22]. In contrast, the radioactivity of [*carboxyl*-^{14}C]FMH did not associate with HDC, indicating that the moiety of FMH attached to the enzyme had lost the carboxyl group.

The efficiency of inactivation of HDC by FMH was examined, and the results in Table 5 show that the partition ratio (catalysis/covalent binding) was 2.82. This indicates that not all decarboxylated FMH molecules were bound to HDC. Some of them were released from the enzyme, and HDC escaped being inactivated. Then, some reaction products which do not bind to HDC may have been formed from FMH. We separated three reaction products by thin-layer chromatography. None of them corresponded to α-fluoromethylhistamine (FMHA), indicating that reaction products other than FMHA were formed. This was confirmed in experiments in which no FMHA was detected in an HPLC system. Under these conditions, the amount of the reaction products which do not bind to HDC is 169 pmoles, calculated from the amount of enzyme present (92.6 pmoles) and the partition ratio (2.82). Because the lower limit of detection of FMHA is 1 pmole, it is expected that the FMHA produced is, at most, only 0.6% of the reaction products.

From these results and the general mechanism of decarboxylation [35], we propose the mechanism for inactivation of HDC by FMH shown in Scheme 1. First, FMH binds to PLP of the enzyme via a Schiff's base. As in the normal catalytic reaction, decarboxylation of FMH occurs, resulting in formation of an intermediate [II]. If transamination (pathway [II] $\rightarrow$ [VI] $\rightarrow$ [VII]) occurs, compound VII should be formed, and HDC is not inactivated. On the other hand, if fluoride ion is released from compound II, compound III is formed. This compound is highly reactive as judged from its structure, for its methylene carbon atom may be easily attacked by a nucleophilic residue of HDC resulting in an enzyme–inhibitor complex. However, a nucleophile such as the ε-amino group of lysine can also attack C4', and compound VIII may be formed. This compound does not inactivate HDC unless it remains at the active site of the enzyme. But as Likos *et al.* [36] reported recently, such an enamine may attack internal Schiff's base and inactivate the enzyme. Our data do not exclude this possibility.

Both (*R*)- and (*S*)-FMHA (V) at a concentration

of 0.01 mM can also inactivate HDC about 50% (Table 2). This compound can form a Schiff's base with PLP, compound IV, and then deprotonate to form compound II, the decarboxylated intermediate of the FMH–enzyme complex. Then by the same pathway as for FMH, FMHA binds covalently to the enzyme and inactivates it [15]. However, it is unlikely that FMH inactivates HDC via its decarboxylated metabolite FMHA, because the amount of FMHA formed enzymatically from FMH is too small to account for the inactivation.

Though FMH is not as good as L-histidine as a substrate (see Appendix), it can inactivate one subunit of HDC within only three decarboxylations (Table 5). FMH cannot bind to HDC unless it is decarboxylated (Table 4). Therefore, other enzymes for which L-histidine is a substrate are less likely to be inactivated.

Since FMH is a potent and specific inactivator of HDC, it will be very useful in studies on the pharmacology of histamine. It may also be useful in the therapy of some diseases, such as gastric ulcer and urticaria.

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APPENDIX

We propose the following model for inactivation of HDC by FMH:

$$E + S \underset{K_I}{\rightleftharpoons} ES \underset{CO_2}{\overset{k_1}{\rightleftharpoons}} EI \xrightarrow[k_3]{k_2} EI^* \qquad EI \xrightarrow{k_3} E + I' \tag{1}$$

where k_1, k_2 and k_3 are rate constants for the reactions as indicated, E is the enzyme, and S is the inhibitor. K_I is the dissociation constant for the ES complex. In condition for rapid equilibrium, $K_I = [E][S]/[ES]$. I is the decarboxylated product(s) of FMH. I^* is a compound that is formed from I and attaches covalently to HDC, and I' is a chemically inactive compound which is also formed from I and cannot bind to HDC.

Assuming that k_1 is much smaller than k_2 or k_3, the rate of inactivation is expressed as

$$\frac{k_2}{k_2 + k_3} k_1 [ES] \tag{2}$$

The concentration of the active form of enzyme is

$$E_a = [E] + [ES] = (1 + K_I/[S])[ES] \tag{3}$$

Therefore,

$$[ES] = \frac{[S]}{K_I + [S]} E_a \tag{4}$$

when the relation $K_I = [E][S]/[ES]$ is used.

Thus, the following differential equation is obtained:

$$-\frac{dE_a}{dt} = \frac{k_1 k_2}{k_2 + k_3} \frac{[S]}{K_I + [S]} E_a \tag{5}$$

Solving this equation using the initial value $E_a(t = 0) = E_0$

$$E_a = E_0 \exp\left(-\frac{k_1 k_2}{k_2 + k_3} \frac{[S]}{K_I + [S]} t\right) \tag{6}$$

Therefore, the half-time of inactivation ($T_{\frac{1}{2}}$) is expressed as

$$T_{\frac{1}{2}} = 0.693 \frac{k_2 + k_3}{k_1 k_2} \frac{K_I + [S]}{[S]}$$

$$= 0.693 \frac{k_2 + k_3}{k_1 k_2} \left(1 + \frac{K_I}{[S]}\right) \tag{7}$$

As a plot of $T_{\frac{1}{2}}$ versus 1/[FMH] was linear (Fig. 2B), the mechanism discussed here explains the inactivation of HDC by FMH well.

The intercept on the abscissa was $1.2 \times 10^5\ M^{-1}$. This value is equal to $-1/K_I$, as is easily obtained from equation 7. Then $K_I = 0.83 \times 10^{-5}$ M.

The intercept on the ordinate was 4 min. Therefore,

$$4 = 0.693 \frac{k_2 + k_3}{k_1 k_2} \tag{8}$$

$(k_2 + k_3)/k_2$ is equal to the partition ratio, and this value is taken from Table 5 as 2.82. So k_1 is calculated as $0.489\ min^{-1}$. The rate constant of decarboxylation of L-histidine by HDC is $17.6\ min^{-1}$, calculated from the specific activity. Thus, the rate of decarboxylation of FMH by HDC is 1/36 of that of L-histidine.

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