

Article

**Structural analysis of the human histidine decarboxylase Y334F mutant**

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**Histamine is an important chemical messenger involved in a wide variety of physiological reactions. L-histidine decarboxylase (HDC) is the primary enzyme responsible for the synthesis of histamine from histidine in a one-step reaction. So far, the crystal structure of human HDC complex with the inhibitor has been determined, and the tyrosine residue (Y334) in the catalytic loop is suggested to play an important role in the decarboxylation reaction. In this study, Y334F, a point mutant of human HDC was subjected to X-ray crystallographic analysis under the same crystallization conditions that were used for the HDC–inhibitor complex; however, despite maintaining the same conditions, different types of crystals of the Y334F mutant were obtained. Furthermore, the structure of the reaction intermediate was determined by soaking the substrate histidine into the crystal of Y334F mutant. In this study, we discuss the role of the catalytic loop in histidine decarboxylation based on the structure of the reaction intermediate.**

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**Key words: X-ray crystallography; crystal structure; histamine; PLP-dependent decarboxylase**

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**Introduction**

Histamine is a physiologically important amine playing key roles as a chemical

messenger in a variety of physiological reactions, including allergies [1], gastric acid secretion [2], and neurotransmission [3]. L-Histidine decarboxylase (HDC) is the primary enzyme responsible for histamine synthesis from L-Histidine in a one-step reaction in mammals [4]. Mammalian HDC is a pyridoxal 5'-phosphate (PLP)-dependent decarboxylase that belongs to the same family as mammalian glutamate

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decarboxylase (GAD) and aromatic L-amino-acid decarboxylase (AroDC) [4-6]. These enzymes catalyze the formation of physiologically important amines such as  $\gamma$ -aminobutyric acid, dopamine and serotonin [5-6].

So far, we have analyzed the crystal structure of the active form of the human HDC complex (amino acid residues 2-477) and one of its inhibitors, histidine methyl ester (HME) [7-8]. Human HDC forms homodimers similar to GAD and AroDC. We found that a region called the catalytic loop (amino acid residues 330-340) of human HDC was arranged to block the substrate binding site (Fig.1) [8]. Limited proteolysis was performed for human HDC in the presence or absence of the inhibitor (HME), showing that the inhibitor-free enzyme was more easily degraded than the inhibitor-bound complex [8]. The degradation probably occurred in the catalytic loop region of the inhibitor-free enzyme. We assumed that the structure of the catalytic loop was very flexible and had different conformations depending on the ligand [8]. We also predicted that tyrosine at position 334 (Y334) in the catalytic loop plays a prominent role in the enzymatic activity [8]. Histamine synthesis by HDC is thought to occur in two steps: decarboxylation and protonation. In the second step, protonation of the decarboxylated  $\alpha$ -carbon cation is thought to involve Y334. Therefore, in the Y334F mutant, the ability to synthesize histamine is completely lost [8]. In the Y334F mutant, aldehydes are synthesized via reaction with oxygen molecules and not via protonation after decarboxylation [9]. However,

the detailed mechanism of this reaction remains unclear. In this study, we analyzed the crystal structure of the Y334F mutant to understand the protonation step.

## Materials and Methods

A recombinant human HDC Y334F mutant (amino acid residues 2-477) was prepared as described in our previous study [7]. The recombinant Y334F mutant was expressed as a glutathione S-transferase (GST) fusion protein. After digestion by PreScission Protease (GE Healthcare) to cleave the GST tag, the untagged enzymes were purified using anion-exchange chromatography.

Protein crystallization protocols were followed previously described [7]. The recombinant Y334F mutant was crystallized under the same conditions as those used for crystallization of the HDC-HME complex. The crystals were obtained at 20 °C with a reservoir solution containing 0.1 M Tris-HCl (pH 8.5), 28% (w/v) polyethylene glycol (PEG) 3350, and 0.2 M lithium sulfate. Protein droplets were prepared by mixing 1  $\mu$ l of the protein solution containing 10 mg / ml HDC and 0.1 mM PLP with 1  $\mu$ l of reservoir solution; the droplets were equilibrated against 100  $\mu$ l of the reservoir solution. X-ray diffraction data sets were collected with a charge coupled device (CCD) X-ray detector (ADSC Q350), using a synchrotron radiation source at the BL38B1 beamline at SPring-8 (Harima, Japan). Diffraction images were processed using HKL2000 [10] and CCP4 [11] programs.

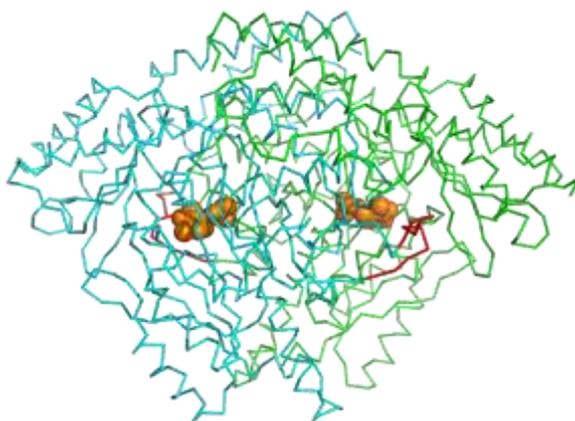
The crystal structure of the Y334F mutant was determined by the molecular replacement method implemented by the PHASER [12] using the structure of human HDC [Protein Data Bank (PDB) code: 4E1O] as the primary search model. The model was completed manually using COOT [13] and interspersed with reciprocal space refinement cycles in the program REFMAC5 [14]. The progress and validity of the refinement process were checked by monitoring the  $R_{\text{free}}$  value for 5% of the total reflections [15]. Model geometry analysis using the MolProbity server [16] showed that all residues were in the Ramachandran-favored regions. The data collection and refinement statistics are summarized in Table 1. The figures were prepared with PyMOL [17] using coordinates from the PDB file (4E1O) for the human HDC–HME complex. The atomic coordinates and experimental data have been deposited in the PDB (Table 1).

## Results and Discussion

Although human HDC could be crystallized in the form of HDC–HME complex [7], the enzyme could not be crystallized in the absence of the bound inhibitor. In the presence of HME, the catalytic loop region was fixed near the active site (Fig. 1), where Y334 interacts with the amino group of S195 at the active site. Here, we successfully crystallized the human HDC Y334F mutant without any bound ligands. It is considered that the mutation Y334F changes the structure of the catalytic loop region at the active site and affects crystal packing. Under the

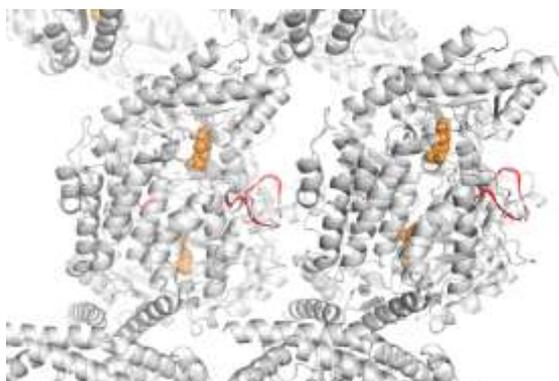
same crystallization conditions used for HDC–HME complex, different types of crystals of Y334F mutant were obtained. The space group of the crystal of the Y334F mutant was different from that of the HDC–HME complex (space group  $C2$ ,  $a = 215.2 \text{ \AA}$ ,  $b = 112.7 \text{ \AA}$ ,  $c = 171.4 \text{ \AA}$  and  $\beta = 110.3^\circ$ ) (Table 1) (Fig. 2) [7-8]. Although the overall structure of the Y334F mutant was almost identical to that of the HDC–HME complex, the catalytic loop region had a slightly different structure. Since the electron density of the catalytic loop region containing the mutation site Y334F was disordered, this part of the catalytic loop region (amino acid residues 333-343) could not be confirmed. However, structural comparisons of the HDC–HME complex and Y334F mutant revealed a significant difference in the structure of the catalytic loop region (Fig. 3). It appeared to be located a little far from the active site in the Y334F mutant, unlike in the case of the HDC–HME complex. In the active site, an internal Schiff base was formed between the PLP and  $\epsilon$ -nitrogen of the lysine residue (K305) (Fig. 4).

To analyze the detailed structure of the active site trapping the reaction intermediate state, Y334F mutant crystals were flash-cooled in a stream of gaseous nitrogen at 100 K after a few minutes of soaking in histidine solution (6 mM). Histidine was immersed in the crystals of the Y334F mutant and its crystal structure was determined by X-ray crystallographic analysis. In the crystal of Y334F mutant soaking in histidine solution, PLP formed an external Schiff base with Histidine (Fig. 5). Since the catalytic residue of Y334 was mutated, it was assumed



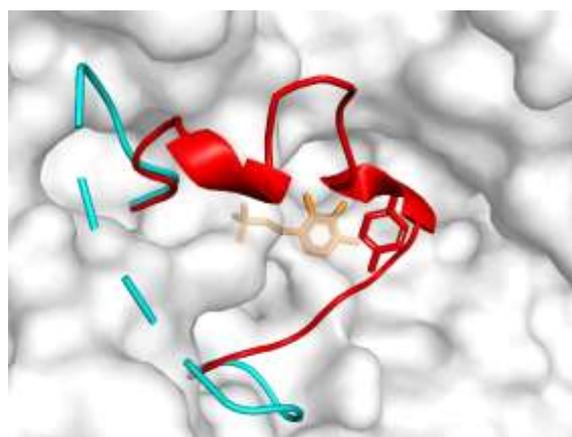
**Fig. 1. Overall structure of the HDC dimer in complex with HME (PDB ID: 4E1O)**

Molecules A and B are shown in green and cyan, respectively. The catalytic loop regions are shown in red. PLP molecules in the active sites are shown in orange.



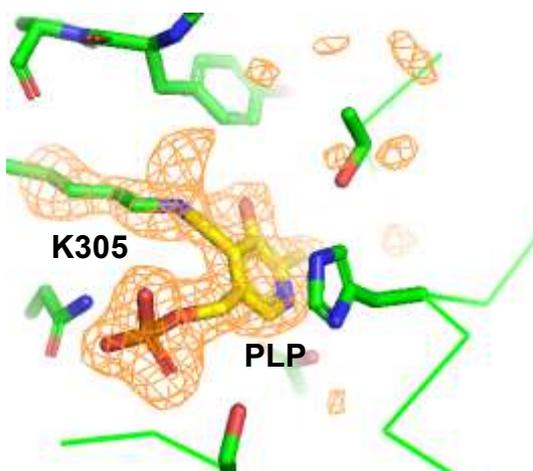
**Fig. 2. Crystal packing of Y334F mutant**

Y334F mutants are shown in gray. The loop areas are shown in red. PLP molecules are shown in orange.



**Fig. 3. A comparison of the loop region of HDC-HME complex and Y334F mutant ligand free form**

The structures of HDC-HME complex and Y334F mutant are superimposed. The loop regions of HDC in complex with HME and Y334F mutant are shown in red and cyan, respectively. PLP molecules are shown in orange. The disordered region in Y334F mutant is shown as dash line. The other parts of the molecule are shown in a transparent gray surface model.



**Fig. 4. Active site of Y334F mutant**

Carbon atoms of HDC and PLP are shown in green and yellow, respectively.  $F_o - F_c$  omit map of PLP-K305 contoured at  $2.5 \sigma$  is shown in orange.

that a normal reaction could not occur. In addition, considering the crystal packing of the Y334F mutant (Fig. 2), it was not possible to form a suitable conformation of the catalytic loop region around the active site, and as a result, it was expected that the decarboxylation reaction would not proceed easily in the crystal. The orientation of the imine linkage was similar to the general orientation of PLP-dependent enzymes, in which the NH group pointed toward the ketone group of PLP interacting via a hydrogen bond (Fig. 5). On the contrary, the NH group points to different sides of the imine linkage in the HDC–HME complex (4E1O) [8] (Fig. 6). The chemical structure of HME is similar to that of histidine; however, the slightly bulky methyl group of HME could distort the original substrate–PLP binding into a different conformation, which presumably prevents keto–enol tautomerism between the NH group of the amino acid substrate and the ketone group of the PLP that is essential for the decarboxylation reaction [8, 18].

## Conclusion

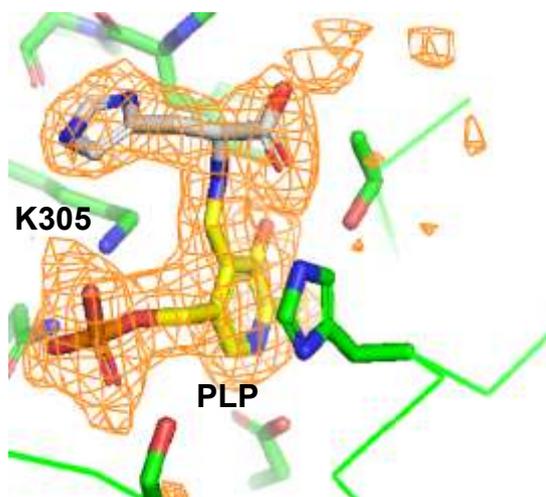
In this study, a point mutant of human HDC, Y334F, was subjected to X-ray crystallographic analysis. Under the same crystallization conditions as those used for the HDC–HME complex, crystals with different packing modes were obtained because of the structure of the catalytic loop region caused by slight differences in amino acid residues (Y334F). Furthermore, by soaking the substrate histidine in the crystal of the Y334F mutant, the decarboxylation

reaction was suppressed and the reaction intermediate was captured. The substrate-binding mode was consistent with the previously deduced structure. Thus, this study showed the importance of the catalytic loop region, including Y334, in human HDC.

**Table 1. Crystallographic data**

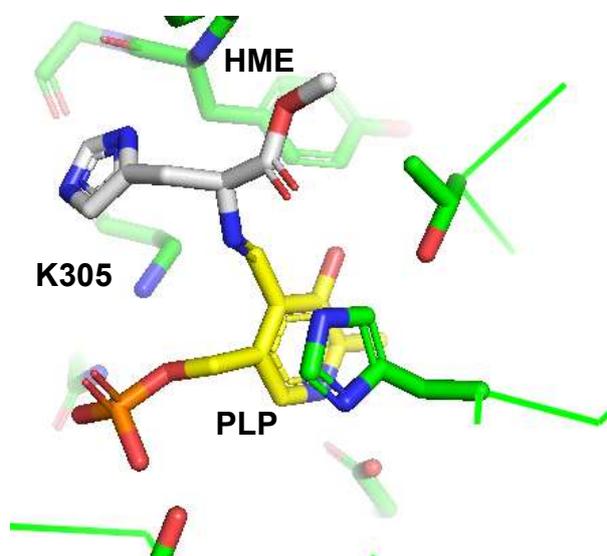
Y334F	Ligand free	Soaking with histidine
PDB ID	7EIX	7E1Y
Space group	<i>P</i> 2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>	<i>P</i> 2 <sub>1</sub> 2 <sub>1</sub> 2 <sub>1</sub>
Unit-cell <i>a</i> , <i>b</i> , <i>c</i> (Å), $\gamma$ (°)	57.98, 85.09, 188.30	57.93, 85.00, 187.42
Resolution (Å)	50.0 – 1.90 (1.90-1.97)	50.0 - 2.20 (2.28-2.20)
No. of unique reflections	73859 (7309)	47992 (4720)
Multiplicity	6.5 (6.5)	7.3 (7.4)
Completeness (%)	99.2 (100.0)	100.0 (100.0)
<i>I</i> / $\sigma$ ( <i>I</i> )	22.5 (2.2)	19.9 (3.2)
<i>R</i> <sub>merge</sub> (%)	8.6 (75.9)	10.7 (61.0)
<i>R</i> -value (%)	16.7 (23.2)	16.7(22.2)
<i>R</i> -free (%)	20.6 (25.7)	22.4 (28.0)
No. of non-hydrogen atoms	7898	7731
No. of HDC molecules	1 dimer	1 dimer
No. of solvent molecules	399	233
Average B-factor (Å <sup>2</sup> )	27.5	34.3
RMS deviations Bond lengths(Å) Bond angles (°)	0.011 1.723	0.017 2.098
Ramachandran Favored (%) Allowed (%)	96.7 100.0	96.1 100.0

Values in parentheses are for the outer resolution shell.



**Fig. 5. Active site of Y334F mutant soaking with substrate histidine**

Carbon atoms of HDC, PLP and histidine are shown in green, yellow and gray, respectively. *Fo-Fc* omit map of PLP-histidine contoured at 1.8  $\sigma$  is shown in orange.



**Fig. 6. Active site of the HDC in complex with HME (PDB ID: 4E1O)**

Carbon atoms of HDC, PLP and HME are shown in green, yellow and gray, respectively.

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### References

- Galli, S. J., Tsai, M. & Piliponsky, A. M., *Nature*, (2008) 454, 445–454.
- Andersson, K. *et al.*, *Yale J. Biol. Med.*, (1998) 71, 183–193.
- Nuutinen, S. & Panula, P., *Adv. Exp. Med. Biol.*, (2010) 709, 95–107.
- Nitta, Y., Komori, H., & Ueno, H., *Seikagaku*, (2015) 87, 321–325.
- Burkhard, P. *et al.*, *Nat. Struct. Biol.*, (2001) 8, 963–967.
- Fenalti, G. *et al.*, *Nat. Struct. Mol. Biol.*, (2007) 14, 280–286.
- Komori, H., Nitta, Y., Ueno, H., & Higuchi, Y. *Acta Cryst. F*, (2012) 68, 675–677.
- Komori, H., Nitta, Y., Ueno, H., & Higuchi, Y., *J. Biol. Chem.*, (2012) 287, 29175–29183.
- Takeshima, D., Mori, A., Ito, H., Komori, H., Ueno, H., & Nitta, Y., *Arch Biochem Biophys.*, (2020) 693, 108551.
- Otwinowski, Z., & Minor, W., *Methods Enzymol.*, (1997) 276, 307–326.
- Collaborative Computational Project, Number 4, *Acta Cryst. D*, (1994) 50, 760–763.
- McCoy, A. J. *et al.*, *Acta Cryst. D*, (2005) 61, 458–464.
- Emsley, P., & Cowtan, K., *Acta Cryst. D*, (2004) 60, 2126–2132.
- Murshudov, G. N., Vagin, A. A., and Dodson, E. J., *Acta Cryst. D*, (1997) 53, 240–255.
- Brünger, A.T., *Nature*, (1992) 355, 472–475.
- Davis, I. W. *et al.*, *Nucleic Acids Res.*, (2004) 32, W615–W619.
- DeLano, W. L., *The PyMOL Molecular Graphics System*, version 1.3r1, Schrodinger, LLC, New York, (2010)
- Fernandes H. S., Ramos M. J., Cerqueira N.M.F.S.A., *Chemistry*, (2017) 23, 9162–9173.

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