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## **HYDROGEN-DEUTERIUM EXCHANGE OF HISTIDINE AND HISTAMINE WITH DEUTERATED TRIFLUOROMETHANESULFONIC ACID**

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This paper is dedicated to Prof. Dr. Kaoru Fuji on the occasion of his 80th birthday.

**Abstract** – Histidine and its decarboxylated metabolite, histamine, containing imidazole ring, play an important role for biological activity. Deuterated trifluoromethanesulfonic acid (TfOD) is one of the acid hydrogen-deuterium exchange reagents for aromatic compounds. Hydrogen-deuterium exchanges of histidine and histamine with TfOD were examined in this report.

Based on the progress of the mass analysis, stable isotope-labeled bioactive ligands are attractive tools in the field.<sup>1</sup> Deuterium is one of the simplest stable isotopes and can be utilized for post-introductions to bioactive compounds. Hydrogen-deuterium exchange (H-D exchange)<sup>2-4</sup> for  $\alpha$ -amino acids at a specific position is one of the most important methods to reveal biological interactions between protein-protein, protein-DNA and protein-small molecules etc. Histidine is one of the essential amino acids and contained imidazole moiety. It has been reported a few examples for hydrogen-deuterium or tritium exchange of histidine derivatives. Acidic conditions with deuterium or tritium chloride at high temperature preferred H-D(T) exchange at 5-position of imidazole or  $\alpha$ -proton.<sup>5-8</sup> Alkaline conditions with deuterated or tritium labeled sodium hydroxide preferred H-D(T) exchange at 2- and 5-positions of imidazole ring at 130 °C.<sup>7,8</sup> *N*-Acetylhistidine derivatives are easily racemized and tritium was incorporated at  $\alpha$ -position with acetic acid and acetic anhydride.<sup>9</sup> Enantiospecific deuterium incorporation at 2, 5 and  $\alpha$ -positions was achieved using ruthenium nanoparticles dispersed in a polyvinylpyrrolidone matrix very recently.<sup>10</sup> Iodide-deuterium exchange was also applied to synthesize deuterated histidine.<sup>11</sup> Histamine was

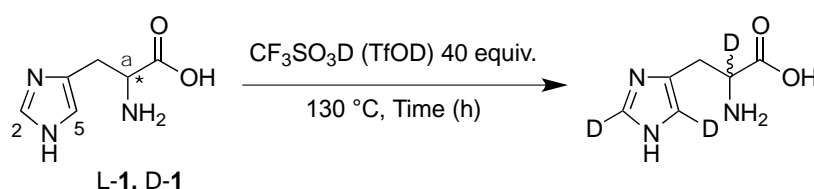
metabolized from histidine via decarboxylation. Deuterated or tritiated histamines were prepared from corresponding labeled histidine by enzymatic decarboxylation<sup>7,12</sup> or iodine-deuterium exchange.<sup>13</sup> To the best of our knowledge, there are no reports for H-D(T) exchange from histamine directly.

**Table 1.** Hydrogen-deuterium exchange of L-histidine with deuterated trifluoromethanesulfonic acid (TfOD) at various temperature

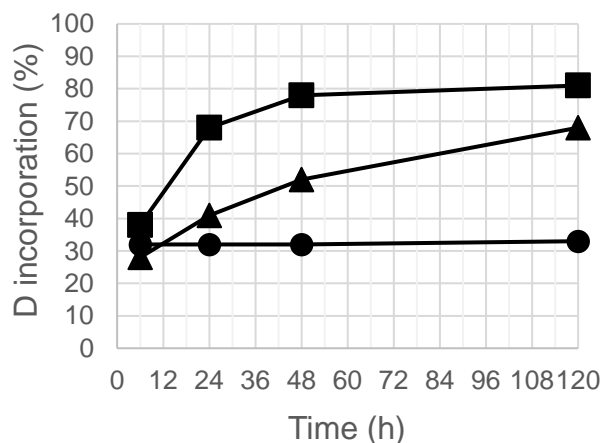
L-1

	Position	D incorporation (%)		
		2-	5-	α-
Temp.	4 °C	0	0	0
	rt	0	0	0
	60 °C	0	0	0
	130 °C	32	28	35

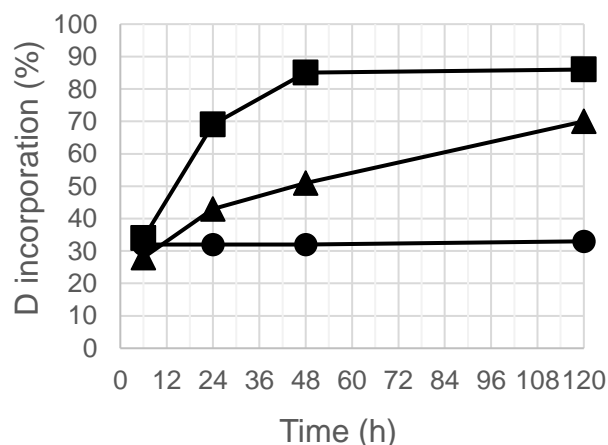
We developed a method for effective H-D(T) exchange for natural aromatic  $\alpha$ -amino acids and their containing peptides with trifluoromethanesulfonic acid-d (TfOD).<sup>14-16</sup> We report in this paper that effective H-D exchange for histidine and histamine derivatives with TfOD.



(a) L-histidine

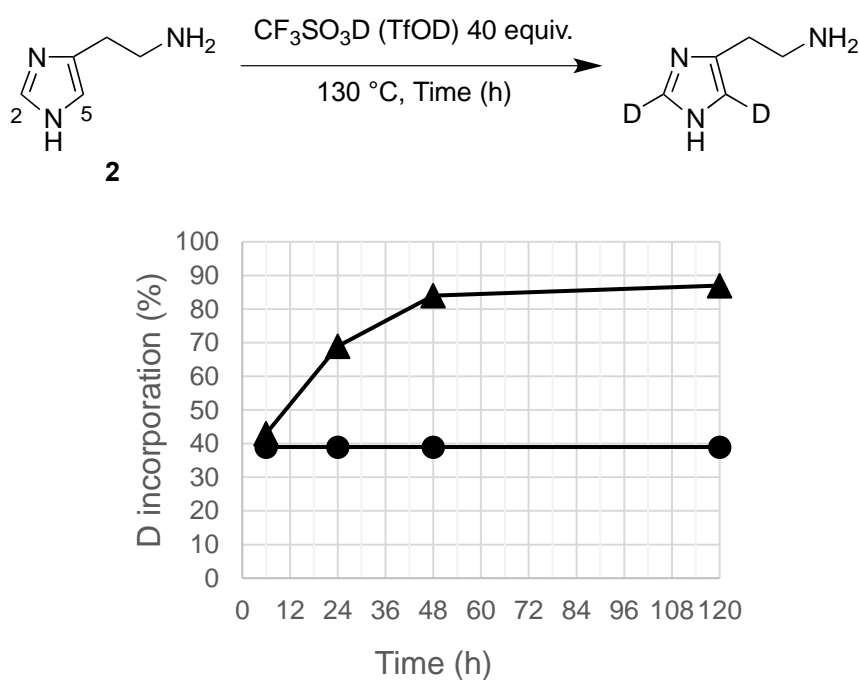


(b) D-histidine



**Figure 1.** Hydrogen/deuterium exchange of (a) L-histidine and (b) D-histidine (L- and D-1) in TfOD at 130 °C. Deuterium incorporation at 2-, 5- and  $\alpha$ -positions were presented as circle, triangle and square, respectively.

As preliminary experiments, hydrogen-deuterium (H-D) exchange of L-histidine in 40 equiv. of TfOD, which was established for aromatic  $\alpha$ -amino acid, was carried out for 6 h at various temperatures. No deuterium incorporation at any position of histidine was observed until 60 °C. Moderate deuterium incorporations at 2-, 5- and  $\alpha$ - positions were observed at 130 °C (Table 1). L- and D-histidine (L-1 and D-1) were subjected to H-D exchange in TfOD at 130 °C by time dependent manner. Deuterium incorporation at  $\alpha$ -position most preferred around 80% D within 48 h, that at 5-position increased around 70% D up to 120 h, on the other hand, that at 2-position was constant (~30% D) during this period. The deuterium incorporation at  $\alpha$ -position indicated that the histidine might be loss of the optical purity during the reaction. L- and D-histidine in TfOH (c 4.4) afforded optical rotation of +17 and -17, respectively and the values did not change after store at -20 °C for 12 h. On the other hand, the optical rotation value was reached zero after heating at 130 °C for 12 h (Figure 1, SI-1 and SI-2 in Supporting Information).



**Figure 2.** Hydrogen/deuterium exchange of histamine (2) in TfOD at 130 °C. Deuterium incorporation at 2- and 5- positions was presented as closed circle and triangle, respectively.

Since the synthesis of deuterated derivatives of histamine (2) only has been reported from enzymatic decarboxylation of deuterated histidine or iodide/deuterium exchange 2,5-diiodide histamine derivative and there is no report for its direct hydrogen-deuterium exchange for histamine skeleton, then histamine was also subjected to deuterium exchange in TfOD at 130 °C. Deuterium incorporations for histamine showed similar tendency to one for histidine. Deuterium is higher incorporated at 5-position (~87% D) than 2-position (~40% D) (Figure 2, and SI-3 in Supporting Information). Deuterium incorporation at

2-position of imidazole in histidine in DCl acidic condition was observed at 180 °C,<sup>6</sup> on the other hand was not observed at 130 °C<sup>8</sup> in previous reports. The reaction might be conducted at high temperature to proceed hydrogen-deuterium exchange at 2-position in imidazole ring of histidine and histamine in TfOD. We recently found that aromatic  $\alpha$ -amino acids, phenylalanine and tyrosine, act as substrates for H-D exchange reaction with TfOD at room temperature.<sup>14-16</sup> The selective H-D exchange for aromatic  $\alpha$ -amino acid in the presence of histidine was examined. The mixture of histidine and phenylalanine or tyrosine was dissolved in TfOD at room temperature for 1 h. The aromatic proton of phenylalanine and *o*-protons against phenol moiety of tyrosine were exchanged to D atoms as determined by <sup>1</sup>H-NMR analysis (SI-4~6 in Supporting Information). These selective deuteration for aromatic  $\alpha$ -amino acids were identical with our previous results.<sup>14-16</sup> ESI-MS analysis also indicated that 5- and 2-sites were deuterated in the aromatic moiety of phenylalanine and tyrosine, respectively. On the other hand, no deuterium incorporation was observed on histidine residue (Table 2).

**Table 2.** ESI-MS analysis of mixture of L-histidine and L-phenylalanine or L-tyrosine in undeuterated (TfOH) or deuterated (TfOD) trifluoromethanesulfonic acid at rt for 1 h

A) L-histidine and L-phenylalanine

Source	Condition	<i>m/z</i>	
		His	Phe
TfOH	rt, 1h	156	166
TfOD	rt, 1h	156	171

B) L-histidine and L-tyrosine

Source	Condition	<i>m/z</i>	
		His	Tyr
TfOH	rt, 1h	156	182
TfOD	rt, 1h	156	184

In summary, hydrogen-deuterium exchange of histidine was proceeded in deuterated trifluoromethanesulfonic acid (TfOD) at high temperature. The condition was also exchange  $\alpha$ -proton to deuterium to afford racemate. Histamine, decarboxylated derivative of histidine, was also exchanged at only imidazole ring under the identical condition. These properties of hydrogen-deuterium exchange with TfOD promoted selective deuteration of aromatic  $\alpha$ -amino acids (Phe and Tyr) in the presence of histidine. Deuterium was introduced Phe or Tyr only at room temperature treatment. These results

indicated that site specific deuterium exchange for aromatic  $\alpha$ -amino acids or their containing peptides over histidine residue may be proceeded with TfOD treatment by temperature dependent manner.

## EXPERIMENTAL

*General Remarks.* All reagents used were of analytical grade. FT-IR spectra were recorded on a FT-IR 4100 spectrometer (JASCO, Tokyo, Japan). NMR spectra were measured by an EX 270 spectrometer (JEOL, Tokyo, Japan). Optical rotations were measured at 23 °C on a JASCO DIP370 polarimeter (JASCO, Tokyo, Japan). HRMS-ESI spectra were obtained with a Waters UPLC ESI-TOF mass spectrometer (Waters, Milford, CT, USA).

*General Procedure for the hydrogen-deuterium exchange of histidine and histamine.* Histidine and histamine were dissolved in ice cooled TfOD (40 equiv.) and the solution was heated at 130 °C for 5 d. A part of the reaction mixture (40  $\mu$ L) was diluted with 400  $\mu$ L D<sub>2</sub>O to check the exchange ratio by NMR. The NMR sample was diluted with 500  $\mu$ L H<sub>2</sub>O for ESI-MS analysis.

Histidine-D<sub>1</sub>: HRMS-ESI ( $m/z$ ) [M+H]<sup>+</sup> calcd for C<sub>6</sub>H<sub>9</sub>DN<sub>3</sub>O<sub>2</sub> 157.0836, found 157.0823.

Histidine-D<sub>2</sub>: HRMS-ESI ( $m/z$ ) [M+H]<sup>+</sup> calcd for C<sub>6</sub>H<sub>8</sub>D<sub>2</sub>N<sub>3</sub>O<sub>2</sub> 158.0899, found 158.0889.

Histamine-D<sub>1</sub>: HRMS-ESI ( $m/z$ ) [M+H]<sup>+</sup> calcd for C<sub>5</sub>H<sub>9</sub>DN<sub>3</sub> 113.0937, found 113.0960.

Histamine-D<sub>2</sub>: HRMS-ESI ( $m/z$ ) [M+H]<sup>+</sup> calcd for C<sub>5</sub>H<sub>8</sub>D<sub>2</sub>N<sub>3</sub> 114.1000, found 114.1000.

The optical rotations for L- and D-histidine in TfOH were +17.0 and -17.0, respectively (c 4.4). The solutions were heated at 130 °C for 12 h. No optical rotations were measured for the heated samples.

*General Procedure for the hydrogen-deuterium exchange of the mixture of histidine and phenylalanine or tyrosine.* L-Histidine and L-phenylalanine or L-tyrosine (each 0.14 mmol) were dissolved in ice cooled TfOD (80 equiv.) and treated at rt for 1 h. A part of the reaction mixture (40  $\mu$ L) was diluted with 400  $\mu$ L D<sub>2</sub>O to check the exchange ratio with NMR. The NMR sample was diluted with 500  $\mu$ L H<sub>2</sub>O for ESI-MS analysis.

Histidine: HRMS-ESI ( $m/z$ ) [M+H]<sup>+</sup> calcd for C<sub>6</sub>H<sub>10</sub>N<sub>3</sub>O<sub>2</sub> 156.0773, found 156.0767.

Phenylalanine-D<sub>5</sub>: HRMS-ESI ( $m/z$ ) [M+H]<sup>+</sup> calcd for C<sub>9</sub>H<sub>7</sub>D<sub>5</sub>NO<sub>2</sub> 171.1182, found 171.1177.

Tyrosine-D<sub>2</sub>: HRMS-ESI ( $m/z$ ) [M+H]<sup>+</sup> calcd for C<sub>9</sub>H<sub>10</sub>D<sub>2</sub>NO<sub>3</sub> 184.0943, found 184.0927.

## ACKNOWLEDGEMENTS

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