Reversible Stepwise Mechanism Involving a Carbanion Intermediate in the Elimination of Ammonia from L-Histidine Catalyzed by Histidine Ammonia-Lyase*

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L-Histidine labeled with deuterium at the C-5' position of the imidazole ring, L-[5'-2H]histidine (His-5'-D), was used as a probe for investigating a stepwise reversible mechanism via a carbanion intermediate in the elimination of ammonia catalyzed by histidine ammonia-lyase (EC 4.3.1.3). The labeled L-histidine (His-5'-D) (2.45 mM) was incubated with histidine ammno-lyase (200 units) from Pseudomonas fluorescens at pH 7.0 or 9.0 at 25.0 °C for 24 h. The time course of the reaction was examined to determine the rates of enzyme-catalyzed hydrogen exchange at C-5' of L-histidine and urocanic acid. The finding of the enzyme-catalyzed hydrogen exchange at C-5' of both L-histidine and urocanic acid in the presence of L-histidine provided a rational explanation for a stepwise reversible mechanism via a carbanion intermediate in the elimination reaction. The rate of increase in the concentration of urocanic acid exchanged with hydrogen (UA-5'-D) did not depend on the formation rate of urocanic acid and UA-5'-H was continuously formed at a constant rate (25.6 μM/h) even after the completion of urocanic acid formation. These observations suggested the presence of the reversible reaction of urocanic acid and a carbanion intermediate. Since there was only a minor contribution for the formation of UA-5'-H from L-histidine exchanged with solvent hydrogen (His-5'-H), the main pathway in the enzymatic reaction of His-5'-D must be the formation of UA-5'-H via a carbanion intermediate (carbanion-D). Regeneration of the carbanion-D from UA-5'-D by its reverse reaction and subsequent hydrogen incorporation at C-5' would contribute to a large extent for the formation of UA-5'-H. The stability of carbanion was also demonstrated to be approximately three times higher at pH 7.0 than at pH 9.0.

Histidine ammonia-lyase (EC 4.3.1.3) catalyzes the elimination of ammonia from L-histidine to produce urocanic acid (1–7). During the past three decades, it has been suggested that the elimination proceeds by a concerted reaction mechanism (1, 3, 6, 7). Recently, we have demonstrated direct evidence for a stepwise mechanism via a carbanion intermediate in the elimination reaction (8, 9). The reaction mechanism was rationalized based upon the observation that the enzyme-catalyzed hydrogen exchange (approximately 45%) occurred at the C-5' position of urocanic acid formed by the reaction of labeled histidine (L-[3,5'-2H3,15N] histidine or DL-[2,5'-2H3]histidine) (10) with the enzyme at pH 9.0 for 24 h. The stepwise mechanism in the β elimination reaction involves two processes of the hydrogen abstraction and the subsequent cleavage of the C-N bond to release ammonia. In principle, either or both of these processes would be largely associated with the overall reversibility of the enzymatic reaction. Our interest in investigating how the carbanion intermediate participates in the forward and reverse reactions led us to examine a time-course of the extent of enzyme-catalyzed hydrogen exchange at C-5' of L-histidine and urocanic acid in the enzymatic reaction. In the present study, L-histidine selectively deuterium-labeled at C-5' of the imidazole ring (L-[5'-2H]histidine) was synthesized to investigate the reversible stepwise reaction mechanism catalyzed by histidine ammonia-lyase from Pseudomonas fluorescens. This study was designed to determine the rates of hydrogen exchange at C-5' of the imidazole ring in the course of the urocanic acid formation on incubation at pH 7.0 and 9.0.

EXPERIMENTAL PROCEDURES

1H NMR spectroscopy was performed on a Bruker AM-400 400-MHz spectrometer. Chemical shifts are reported relative to the isotope impurity peak for a given solvent (D2O, 4.75 ppm). Capillary gas chromatographic-mass spectrometric (GC-MS) analysis was done on a Shimadzu GCMS-QP2000 equipped with a data-processing system. GC-MS employed a Durabond (DB-5) fused-silica capillary column (30 m × 0.32 mm internal diameter, 0.1-μm film thickness; J & W Scientific Inc.). HPLC was done on a Hitachi 655 liquid chromatograph. The reversed-phase HPLC column was a 5-μm Inertsil-ODS-2 column (25 × 0.45 cm internal diameter, GL Sciences Inc.). The mobile phase was a solution of 1.18 mM tetra-n-butylammonium hydrogen sulfate and 20 mM potassium dihydrogen phosphate containing 1.0% (v/v) acetonitrile and 0.005% (v/v) morpholine. HPLC for the determination of optical purity was carried out on a chiral stationary-phase column (Chiralpak WH column, 25 × 0.45 cm internal diameter, Daicel Chemical) in a solution of 0.25 mM CuSO4 as the mobile phase.

Materials—L-Histidine free base, urocanic acid, glutathione, histidine ammonia-lyase (EC 4.3.1.3) from P. fluorescens, and acylase I (EC 3.5.1.14) from porcine kidney were purchased from Sigma. Tri- fluoracetic anhydride (Kanto Chemicals) and diethyl pyrocarbonate (Aldrich) were obtained commercially. Deuterium oxide (D2O; 99.75 atom%) and 35% deuterium chloride (DCl; 99 atom%) in D2O were obtained from Merck Sharp & Dohme. All other chemicals and enzyme-catalyzed hydrogen exchange (approximately 45%) occurred at the C-5' position of urocanic acid formed by the reaction of labeled histidine (L-[3,5'-2H3,15N] histidine or DL-[2,5'-2H3]histidine) (10) with the enzyme at pH 9.0 for 24 h. The stepwise mechanism in the β elimination reaction involves two processes of the hydrogen abstraction and the subsequent cleavage of the C-N bond to release ammonia. In principle, either or both of these processes would be largely associated with the overall reversibility of the enzymatic reaction. Our interest in investigating how the carbanion intermediate participates in the forward and reverse reactions led us to examine a time-course of the extent of enzyme-catalyzed hydrogen exchange at C-5' of L-histidine and urocanic acid in the enzymatic reaction. In the present study, L-histidine selectively deuterium-labeled at C-5' of the imidazole ring (L-[5'-2H]histidine) was synthesized to investigate the reversible stepwise reaction mechanism catalyzed by histidine ammonia-lyase from Pseudomonas fluorescens. This study was designed to determine the rates of hydrogen exchange at C-5' of the imidazole ring in the course of the urocanic acid formation on incubation at pH 7.0 and 9.0.

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Reagents were of analytical-reagent grade and were used without further purification.

**Synthesis of L-[5'-2H]Histidine** — A solution of L-histidine free base (2.0 g, 12.9 mmol) in D_2O (100 ml) was adjusted to pH 5.0 with 35% HCl and heated at 100 °C for 15 h in a nitrogen-filled sealed tube. After cooling, the residue was dissolved in CH_2Cl_2 (100 ml) and heated at 180 °C for 15 h again. After evaporating the solvent to 50–60 °C under reduced pressure, the product (di-[2'-2',5'-2H]histidine) was dissolved in H_2O (100 ml). The solution was neutralized to pH 7.2 with 1 M LiOH and then heated at 80 °C for 12 h for the selective back-exchange of deuterium at the C-2' position of the imidazole ring. After evaporating the solvent below 60 °C under reduced pressure, the residue was washed with absolute EtOH and recrystallized from aqueous EtOH to give di-[2',5'-2H]histidine (0.95, 99%). A solution of di-[2',5'-2H]histidine as a free base (1.57 g, 10.0 mmol) in CH_2COOH-CH_2COOH (22 ml) was then heated at 80 °C under a stream of nitrogen, providing N-acetylation with concomitant racemization at C-2. After removal of the solvent below 50 °C under reduced pressure, the residue was taken up twice in H_2O (20 ml) and then evaporated to dryness each time to give N-acetyl-di-[5'-2H]histidine. The almost pure material was dissolved in H_2O (50 ml), and the solution was neutralized to pH 7.2 with 0.5 M LiOH. Acetylsine I (EC 3.5.1.14) in three portions (50 mg, 0.050 mmol units each) was added at 24-h intervals on incubation at 37 °C for 72 h. The reaction was terminated by acidification with CH_3COOH (pH 5.0) and the precipitated protein was filtered with aid of charcoal. The filtrate was treated with 30% CH_2Cl_2 (10 ml) to precipitate the histidine. The solution was purged with EtOH and then evaporated to dryness in a stream of nitrogen to give di-[2',5'-2H]histidine (1.66 g, 85%) as a free base. 1 H NMR (D_2O) δ 3.15 (2H, d, 3-H), 2.97 (1H, t, 2-H) and 7.75 (1H, s, 2'-H). A proton at C-5' (δ 7.68) completely disappeared. The isotopic purity was estimated to be 97.4 atom %, based upon the ion intensities in the region of the molecular ion of GC derivative, i.e. N'-trifluoroacetylated N'-ethoxycarbonyl)histidine n-butyl ester (11–13). Optical purity (δ 99.9%) was determined by HPLC with a chiral stationary-phase column.

**Enzymatic Reaction and HPLC and GC-MS Analyses** — Histidine ammonia-lyase (200 units, P. fluorescens) was incubated in 13.15 ml of 200 mM Tris buffer at 25 °C for 30 min at pH 7.0 or at pH 9.0 in the presence of 10 mM MgCl_2 and 100 mM glutathione (reduced form). To the incubation mixture was added L-[5'-2H]histidine (0.5 mg, 12.9 μmol) and then incubated at 25 °C for 24 h. The reaction mixture (100 μl each) was collected every 2 h for HPLC and GC-MS analyses, respectively. The enzymatic reaction was terminated by adding 2 ml of absolute EtOH, and the solution was evaporated at 50 °C under a stream of nitrogen. The residue was reconstituted with 1.0 ml of distilled water containing 0.998 mM L-tryptophan as an internal standard. The solution (10 μl) was analyzed by HPLC and the peak height ratios were measured.

The extents of hydrogen exchange at C-5' of L-histidine and urocanic acid in the reaction were measured by capillary GC-MS. L-Histidine and urocanic acid were simultaneously derivatized to N'-trifluoroacetylated N'-ethoxycarbonyl)histidine n-butyl ester for GC and N'-ethoxycarbonyl)urocanic acid n-butyl ester for GC-MS. A portion of the reaction mixture (100 μl) was collected, deproteinized and extracted with 2 ml of EtOH. The solution was transferred to a Vial (2 ml) and evaporated to dryness at 50 °C under a stream of nitrogen. To the residue containing L-histidine and urocanic acid were added 200 μl of 5 M HCl in n-butyl alcohol. The reaction mixture was sealed under a nitrogen atmosphere and heated at 100 °C for 15 min. After removal of the solvent at 70 °C under a stream of nitrogen, 200 μl of CH_3Cl_2 were added and the solution was evaporated to dryness again. The residue was dissolved in 2 ml of CH_3Cl_2 and then applied to a Sep-Pak silica cartridge (Waters). The cartridge was washed with 5 ml of CH_3Cl_2 and then eluted with 5 ml of MeOH. After evaporation of the eluate in a conical centrifuge tube (10 ml) under a stream of nitrogen, 1.5 ml of MeOH was added to the residue. The solution was transferred to a Vial (2 ml), and then the solution was evaporated to dryness. The residue was reconstituted in 1 ml of 20% trifluoroacetic anhydride in CH_3Cl_2 and then heated at 150 °C for 5 min under a nitrogen atmosphere. After the reaction, the excess reagent and solvent were evaporated at room temperature under a gentle stream of nitrogen. The residue was dissolved in 200 μl of 1.5% dietary fiber complete in CH_3Cl_2, heated at 150 °C for 20 min, then evaporated to dryness. The residue was reconstituted with 100 μl of CH_3Cl_2. The solution (0.5 μl) was analyzed by GC-MS, and the peak height ratios were measured. The GC-MS multiple ion detector was focused on the molecular ions of the respective GC derivatives of L-histidine and urocanic acid, m/z 379 for L-histidine exchanged with hydrogen at C-5' (His-5'-H), m/z 380 for L-histidine retaining deuterium at C-5' (His-5'-D), m/z 266 for urocanic acid exchanged with hydrogen at C-5' (UA-5'-H) and m/z 267 for urocanic acid retaining deuterium at C-5' (UA-5'-D). In calculating the molar ratios of His-5'-H to His-5'-D and of UA-5'-H to UA-5'-D, corrections were made for the mutual contribution of the multiplicity of the molecular ions due to the isotope peaks from the natural abundance.

**RESULTS AND DISCUSSION**

L-[5'-2H]Histidine (His-5'-D) was used as a substrate for the enzymatic reaction of L-histidine exchanged with hydrogen by selective deuterium of unlabeled L-histidine using the hydrogen-deuteron exchange reaction. L-Histidine was heated twice in D_2O/DCl (pD 5.0) at 180 °C for 15 h to give di-[2',5'-2H]histidine. The selective back-exchange at C-2' of the labeled di-[2',5'-2H]histidine on heating in H_2O (pH 7.2) at 80 °C for 12 h gave di-[2',5'-2H]histidine. By treating with CH_2COOH-CH_2COOH (10:1) at 80 °C for 15 min, di-[2',5'-2H]histidine free base underwent N-acetylation with concomitant racemization at C-2 to give N-acetyl-di-[5'-2H]histidine, and the subsequent enzymatic resolution with acylase I gave L-[5'-2H]histidine. Isotopic purity was estimated to be 97.4 atom %, based upon the ion intensities in the region of the molecular ion of GC derivative, i.e. N'-trifluoroacetylated N'-ethoxycarbonyl)histidine n-butyl ester (11–13), measured by GC-MS. Optical purity of the labeled L-histidine was determined to be >99.9% by HPLC with a chiral stationary-phase column.

The labeled L-histidine (His-5'-D) (2.45 mM) was incubated with histidine ammonia-lyase (200 units) from P. fluorescens at 25 °C at pH 7.0 or 9.0 in the presence of 10 mM MgCl_2 and 100 mM glutathione (reduced form). Samples were collected every 2 h for 24 h. The extents of hydrogen exchange at C-5' of L-histidine and urocanic acid were measured by capillary GC-MS. The concentrations of L-histidine were quantitated by HPLC with a reversed-phase column.

Fig. 1 shows semilogarithmic plots of the concentration of L-histidine versus reaction time. The concentration of L-histidine at pH 7.0 declined linearly over the incubation period of 24 h, while at pH 9.0 a break was observed at about 18 h. The rate of decline in the early phase was approximately three times larger at pH 9.3 than at pH 7.0 with the half-lives being 5.4 h at pH 9.0 and 17.0 h at pH 7.0. As shown in Fig. 2, the concentration of L-histidine exchanged with hydrogen (His-5'-H) was low but increased linearly with reaction time. The rates were 4.96 μM/h at pH 7.0 and 1.43 μM/h at pH 9.0. Fig. 3 shows the time course of concentration of urocanic acid formed in the reaction. The formation of total urocanic acid (total U = UA-5'-H + UA-5'-D) began to decrease at about 16 h.

Incubation of either unlabeled urocanic acid in D_2O or labeled urocanic acid (UA-5'-D) in H_2O with the enzyme under the same conditions as described above showed no incorporation of deuterium or hydrogen into the C-5' position. In addition, deuterium of His-5'-D was completely retained under the enzyme-free conditions. The observed losses of deuterium at C-5' of labeled L-histidine and urocanic acid during the enzymatic reaction can be interpreted as the consequence of the enzyme-catalyzed hydrogen exchange. The present results of hydrogen exchange can be rationally explained by a reversible stepwise mechanism via a carbanion intermediate, as proposed in Scheme I.
Reversible Mechanism Catalyzed by Histidine Ammonia-Lyase

FIG. 1. Semilogarithmic plots of L-histidine (total His) concentration (mM) versus reaction time (h) in the enzymatic reaction of L-[5'-2H]histidine with histidine ammonia-lyase. The reaction was carried out at pH 7.0 (A) or at pH 9.0 (B) as described under "Experimental Procedures." The concentration (mM) of total His (His-5'-D and His-5'-H) was measured by HPLC. His-5'-D (mM): L-histidine retaining deuterium at C-5' (L-[5'-2H]histidine) and His-5'-H (mM): L-histidine exchanged with hydrogen at C-5'.

FIG. 2. Time course of the concentration of recovered L-histidine (total His) in the enzymatic reaction of L-[5'-2H]histidine with histidine ammonia-lyase. The reaction was carried out at pH 7.0 (A) or at pH 9.0 (B) as described under "Experimental Procedures." The concentration of total L-histidine (total His) was measured by HPLC. The extents of hydrogen exchange were determined by capillary GC-MS selected ion monitoring, measuring the ion intensities in the molecular ion of the GC derivative, N''-(trifluoroacetyl)-N'-(ethoxycarbonyl)histidine n-butyl ester, m/z 380 for His-5'-D, and m/z 379 for His-5'-H. His-5'-D (mM): L-histidine retaining deuterium at C-5' (L-[5'-2H]histidine), His-5'-H (mM): L-histidine exchanged with hydrogen at C-5' and total His: total concentration (mM) of His-5'-D and His-5'-H.

FIG. 3. Time course of the concentration of urocanic acid (total UA) formed by the enzymatic reaction of L-[5'-2H]histidine with histidine ammonia-lyase. The reaction was carried out at pH 7.0 (A) or at pH 9.0 (B) as described under "Experimental Procedures." The concentration of total urocanic acid (total UA) (mM) in each sample was determined by subtracting the amount of L-histidine measured by HPLC from the initial amount (2.45 mM) of L-histidine incubated. The extents of hydrogen exchange were determined by capillary GC-MS selected ion monitoring, measuring the ion intensities in the molecular ion of the GC derivative, N''-(ethoxycarbonyl)urocanic acid n-butyl ester, m/z 267 for UA-5'-D, and m/z 266 for UA-5'-H. UA-5'-D (mM): urocanic acid retaining deuterium at C-5', UA-5'-H (mM): urocanic acid exchanged with hydrogen at C-5' and total UA: total concentration (mM) of UA-5'-D and UA-5'-H.

process, the enzyme-catalyzed abstraction of C-3 hydrogen has been demonstrated to proceed stereospecifically (1, 2, 15, 16). The stereospecific abstraction of hydrogen at C-3 produces 3-carbanion (carbanion-D) that is delocalized to C-5' by conjugation. The electrophilic incorporation of solvent hydrogen into C-5' of the carbanion intermediate (carbanion-
D) gives a 5'-protonated intermediate. This 5'-protonated intermediate produces a 3-carbanion intermediate (carbanion-H) by the loss of deuterium at C-5' (route a), leading to His-5'-H or UA-5'-H. The 5'-protonated intermediate also produces a 3-carbanion intermediate (carbanion-D) retaining deuterium resulting from the loss of hydrogen at C-5' (route b), leading to His-5'-D or UA-5'-D. The reverse reaction of UA-5'-D gives rise to a 3-carbanion intermediate (carbanion-H) through the loss of deuterium at the C-5' position of the 5'-protonated intermediate, which also produces His-5'-H or UA-5'-H.

Fig. 4 shows the plots of the ratio of UA-5'-H to total UA (UA-5'-H/total UA) as a function of total concentration of urocanic acid (total UA). The ratios at pH 7.0 and 9.0 were proportional to the concentrations of urocanic acid formed. A rapid rise in the ratio at pH 9.0 was observed at concentrations of >2 mM urocanic acid (>16 h of reaction), indicating that UA-5'-H continued to be formed at a constant rate (25.6 μM/h, see Fig. 3) even after the completion of urocanic acid formation. This clearly suggested that UA-5'-H was reversibly produced from UA-5'-D via a carbanion intermediate.

Fig. 5 shows the plots of the ratio of UA-5'-H to total UA (UA-5'-H/total UA) at pH 7.0 and 9.0 as a function of reaction time. The ratio increased linearly with reaction time. In spite of the rapid rise in the ratio of UA-5'-H to total UA observed for pH 9.0 at the reaction of >16 h (Fig. 4), the rate of increase at pH 9.0 shown in Fig. 5 remained constant throughout the reaction period. This implicates that the rate of formation of UA-5'-H does not depend on that of urocanic acid from L-histidine. The slope of the curve (the rate of increase) would therefore correspond to the formation of UA-5'-H from UA-5'-D; i.e., the reverse reaction from UA-5'-D (route b) and subsequent forward reaction to UA-5'-H via a carbanion intermediate (carbanion-H) (route a). The rates of formation of UA-5'-H by this route were estimated from the slopes to be 1.03%/h at pH 7.0 and 0.99%/h at pH 9.0. If this reversible reaction (UA-5'-D → carbanion → UA-5'-H) had not occurred in the enzymatic reaction, UA-5'-H should have been
produced, depending on the rate of formation of urocanic acid from L-histidine. In this case, the ratio (UA-5'-H/total UA) would be expected to be constant throughout the reaction, as indicated by the shadow in Fig. 5. The value of the y-intercept in Fig. 5 can therefore be interpreted as the formation of UA-5'-H from His-5'-D, not from UA-5'-D by the reverse reaction. That is, the value at time zero (14.2% at pH 7.0 and 4.7% at pH 9.0) represents the extent of hydrogen incorporation into C-5' of the carbanion intermediate (carbanion-D) which has been initially formed from His-5'-D.

Since the formation of His-5'-H was not significant as observed in Fig. 2, His-5'-H would contribute little for the formation of UA-5'-H. The main pathway in the enzymatic reaction of His-5'-D must be the formation of UA-5'-D via a carbanion intermediate (carbanion-D). Regeneration of carbanion-D from UA-5'-D by its reverse reaction and subsequent hydrogen incorporation at C-5' would contribute to a large extent for the formation of urocanic acid exchanged with hydrogen (UA-5'-H).

The y-intercept values at pH 7.0 (14.2%) and at pH 9.0 (4.7%) estimated from Fig. 5 may reflect the stability or the lifetime of a carbanion intermediate into which hydrogen can be incorporated. Conversion of L-histidine to urocanic acid involves the transformation of an sp3 to an sp2 geometry at C-3/C-2 carbon atoms. The delocalized carbanion to the imidazole ring may be restrained in the optimum conformation of determining whether the exchange rate is indicative of rate-limiting (6) in the reaction of L-histidine to urocanic acid, the incorporation rate of 14C-labeled urocanic acid into regenerated L-histidine substrates were essentially identical. The incorporation rate of tritium into L-histidine represents the C-H bond formation (cleavage) at C-3. The regeneration rate of radioactive L-histidine from 14C-labeled urocanic acid was assumed to correspond to the C-N bond formation (cleavage) by its reverse reaction. If the reaction is actually concerted, these exchange rates should be exactly identical. On the basis of the identical value for both rates, these authors postulated the simultaneous (concerted) occurrence of C-H/C-N bond breaking or formation in the reversible elimination reaction. However, the equivalent value does not necessarily lead to a concerted elimination mechanism. The incorporation rate of 14C-labeled urocanic acid into L-histidine would rather reflect the rate of C-N bond cleavage when the rate of C-H bond cleavage is much faster than that of C-N bond cleavage. In a stepwise mechanism via a carbanion intermediate, the tritium incorporation into L-histidine corresponds to the C6-H bond formation (step 1). The incorporation of 14C-labeled urocanic acid into the L-histidine molecule corresponds to the formation of both C5- and C6-H bonds (step 2). If the C6-H bond cleavage (step 1) is at least partially rate-limiting (6) in the reaction of L-histidine to urocanic acid, the incorporation rate of 14C-labeled urocanic acid into L-histidine may depend on the rate of the C6-H bond formation (= the rate of tritium incorporation). However, it should be noted that all studies that attempt to determine the sequence of bond cleavage by use of isotope exchange are generally faced with the difficulty of determining whether the exchange rate is indicative of covalent bond cleavage or dissociation of the group from the enzyme. In addition, solvent isotope effect on the reaction always has to be taken into account in assessing the net rate of C-H bond cleavage as long as D2O or T2O is used as solvent.

Peterkofsky (3) postulated the existence of an amino-enzyme complex in the histidine ammonia-lyase reaction. This was deduced from the observation that 14C-labeled urocanic acid was reversibly incorporated into reisolated L-histidine when L-histidine was incubated with the enzyme, while 15NH3 was not incorporated. The result implies that L-histidine cannot be formed starting from urocanic acid in the presence of NH3. The reverse reaction does occur only in the presence of a substrate, L-histidine, in the enzymatic reaction system. In the present study, selectively deuterium-labeled L-histidine
at C-5' of the imidazole ring was uniquely used as a probe for elucidating the reversible stepwise mechanism. This is the first example demonstrating how an unstable intermediate participates in the forward and reverse reactions catalyzed by an enzyme.

In recent years, mechanistic studies of the enzymatic reaction catalyzed by the ammonia-lyases such as aspartase (19, 20), methylaspartase (21-24), and phenylalanine ammonia-lyase (25, 26) have attracted much interest. The elimination mechanism of ammonia is still a subject of controversy. The fact that hydrogen exchange occurred at the conjugated carbon atoms demonstrated in the present study offers a significant value with regard to the mechanistic elucidation of elimination reactions catalyzed by these ammonia-lyases. Furthermore, the reaction mechanisms catalyzed by mammalian histidine ammonia-lyases, both in vitro and in vivo, are poorly understood. Detailed studies of L-histidine metabolism have recently been the subject of biochemical and clinical investigations concerned for patients with typical and atypical histidinemia caused by histidine ammonia-lyase deficiency (27-30). The finding of hydrogen exchange at C-5' of the imidazole ring will shed light on the elucidation of the enzymatic reaction mechanism of L-histidine metabolism in vivo. Synthesis of multilabeled L-histidine containing deuterium at the C-5' position (18) as a mechanistic probe for the in vivo metabolism in humans and the development of GC-MS assay (13) for the simultaneous determination of labeled L-histidine and uronic acid in biological fluids will be reported in due course.

REFERENCES