Stereochemical Course of the Transmethylation Catalyzed by Histamine N-Methyltransferase

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The stereochemical course of the methyl group transfer catalyzed by histamine N-methyltransferase was studied using S-adenosylmethionine (AdoMet), which carried a chiral methyl group. The incubation of these AdoMet samples and histamine with a partially purified enzyme obtained from guinea pig brains gave the corresponding methylated histamine. The N-methylhistamine samples were degraded to convert the N-methyl group into the methyl group of acetate using a reaction sequence of known stereochemistry. The results of the configurational analysis of these acetate samples indicated that the enzymatic transfer of the methyl group from the sulfur of AdoMet to the nitrogen of histamine occurs with inversion of configuration.

Recently, our laboratory (1-4) and that of Arigoni (5) have examined the stereochemical course of several reactions catalyzed by methyltransferases using AdoMet as substrate. The studies were made possible by employing S-adenosylmethionine which was stereospecifically labeled at the S-methyl group with 2H and 3H. The enzymatic transfer of the methyl group to acceptors such as carbon, nitrogen, sulfur, or oxygen in all the examples studied so far has been shown to proceed with inversion of configuration.

Histamine N-methyltransferase (EC 2.1.1.8) catalyzes the N-methylation of the imidazole ring of histamine to form 1-methyl-4-(2-aminoethyl)imidazole (N-methylhistamine). This methyltransferase plays an important role in the metabolism of histamine, functioning in neuronal and neuroendocrine mechanisms (6, 7).

In this paper, we report on the stereochemical fate of the methyl group of AdoMet in the histamine N-methyltransferase-catalyzed formation of N-methylhistamine.

EXPERIMENTAL PROCEDURES

Materials. Organic, inorganic, and biological chemicals were purchased from Aldrich Chemical Company and from Sigma Chemical Company. All the chemicals were reagent grade or better and were used without further purification.

Histamine N-methyltransferase was isolated from guinea pig brains by the method of Brown et al. (8). The (methyl-S)- and (methyl-R)-[methyl-14C,2H3,3H0]-AdoMet samples were synthesized from R- and S-[2-14C,2H3,3H0]acetate, respectively, as previously described (2).

Enzyme incubation. The reaction mixture contained 4 μCi of (methyl R)AdoMet (1 μCi/μmol), 200 μmol of...
phosphate buffer (pH 7.25), 4 mmol of histamine, and 0.012 units of histamine N-methyltransferase in a total volume of 10 ml. The reaction mixture using 
(methyl-S)AdoMet was 2 μCi of AdoMet (1 μCi/μmol), 200 μmol of phosphate buffer, 2 mmol of histamine, and 0.012 units of enzyme in a total volume of 5 ml. Incubations were carried out at 37°C for 4 h. An additional 0.012 units each of the enzyme were added to the reaction mixtures at 0.75, 1.5, 2.25, 3, and 3.75 h. The reaction was terminated by addition of 10 N NaOH. After saturating the reaction mixture with sodium sulfate the methylated histamine was isolated by extraction with chloroform (3 × 4 ml).

Degradation (cf. (9)). To the methylated histamine samples was added 10 ml of 2.5 N H₂SO₄, the reaction was heated with stirring at 80°C, and then 0.6 ml of 0.24 M NaNO₂ solution was added dropwise. After 30 min, the mixture was heated to 110°C and 0.06 M KMnO₄ solution was added dropwise so that the color of the mixture remained violet. After 2.5 h, 1 ml of 0.1 M oxalic acid solution was added to reduce excess KMnO₄. While cooling with ice, pellets of NaOH (about 1 g) were carefully added to neutralize the solution. The mixture was refluxed for 24 h and then transferred to a three-neck, round-bottom flask equipped with a gas inlet and reflux condenser. Two traps, each containing 10 ml of 1 N HCl, were connected to the system through the condenser outlet. Five milliliters of 20% NaOH solution were added to the flask and the mixture was heated at 110°C for 24 h while a stream of N₂ was passed through the apparatus. An additional 5 ml of 20% NaOH solution were added after 6 h. The contents of the first trap, containing radioactive methylamine hydrochloride, were evaporated to dryness. One hundred micromoles of nonlabeled methylamine hydrochloride in 1 N HCl was mixed with the radioactive material and the solution was evaporated to dryness. The methylamine hydrochloride was then converted to acetic acid via the N,N-ditosylate as described earlier (2). The overall radiochemical yield was 5 to 6%.

The configuration of the methyl group of acetate was determined by the method of Cornforth et al. (10, 11) and Arigoni and co-workers (12) as described previously (13).

The radioactivity of compounds was determined in a Beckman LS 7500 liquid scintillation counter using Bray's solution or Aquasol-2. [¹⁴C]Toluene and [³H]toluene were used as internal standards.

RESULTS AND DISCUSSION

This paper reports studies in which we determined the stereochemical course of the methyl group transfer catalyzed by histamine N-methyltransferase using 
(methyl-R)- and (methyl-S)-[methyl-¹⁴C, ²H, ³H]AdoMet as a substrate. Each of the two diastereomers of AdoMet was incubated with histamine and histamine N-methyltransferase partially purified from guinea pig brains. The methyl group of the resulting N-methylhistamine was chemically converted into the methyl group of acetic acid by the reaction sequence of known stereochemistry shown in Scheme 1. The N-methylhistamine was oxidized with KMnO₄.

\[\text{AdoHcy} \rightarrow \text{KCN} \rightarrow \text{HMPA} \rightarrow \text{H₂O₂, NaOH} \rightarrow \text{NaNO₂} \rightarrow \text{CONH₂, H₂SO₄} \rightarrow \text{COOH} \]

\[\text{KMnO₄, H₂SO₄} \rightarrow \text{NH₂} \]

\[\text{1/NaOH / TsCl} \rightarrow \text{2/NaH / TsCl} \]

\[\text{SCHEME 1} \]
TABLE I

CHIRALITY ANALYSIS OF STARTING MATERIALS AND PRODUCTS IN THE ENZYMATIC METHYLATION OF HISTAMINE

<table>
<thead>
<tr>
<th>Compound</th>
<th>$^3$H/$^14$C ratio</th>
<th>F value</th>
<th>Methyl group configuration</th>
<th>$^3$H/$^14$C ratio</th>
<th>F value</th>
<th>Methyl group configuration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Starting $[1^{-14}$C, $^3$H,$^3$H] acetate$^a$</td>
<td>2.6</td>
<td>28</td>
<td>S (76% e.e.)</td>
<td>3.1</td>
<td>68</td>
<td>R (62% e.e)</td>
</tr>
<tr>
<td>$[methyl-^14$C, $^3$H,$^3$H] methionine</td>
<td>2.3</td>
<td></td>
<td>R</td>
<td>3.2</td>
<td></td>
<td>S</td>
</tr>
<tr>
<td>$[methyl-^14$C, $^3$H,$^3$H] AdoMet</td>
<td>2.4</td>
<td></td>
<td>R</td>
<td>3.1</td>
<td></td>
<td>S</td>
</tr>
<tr>
<td>N-methylhistamine</td>
<td>2.7</td>
<td></td>
<td>S</td>
<td>3.3</td>
<td></td>
<td>R</td>
</tr>
<tr>
<td>Acetate from N-methylhistamine degradation</td>
<td>3.0</td>
<td>64</td>
<td>R (48% e.e)</td>
<td>4.3</td>
<td>41</td>
<td>S (31% e.e)</td>
</tr>
<tr>
<td>Malate</td>
<td>2.70</td>
<td></td>
<td></td>
<td>3.70</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fumarate</td>
<td>1.73</td>
<td></td>
<td></td>
<td>1.51</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$These are the samples described in ref. (2) and (3).

in sulfuric acid to yield methylamine (9). The methylamine was converted into its N,N-ditosyl derivative, which was then subjected to cyanoanalysis to give acetonitrile with inversion of the methyl group configuration. The acetonitrile was hydrated with alkaline hydrogen peroxide to give acetamide, followed by diazotation with sodium nitrite and sulfuric acid (2). The overall yield of acetate was about 5–6%.

The configuration of the acetic acid thus obtained was assayed enzymatically by the method of Cornforth et al. (10, 11) and Arigoni and co-workers (12). The method is based on a kinetic deuterium isotope effect in the malate synthase reaction. The acetate sample is converted into acetyl-CoA by the action of acetate kinase and phosphoacyltransferase. The resulting acetyl-CoA is then incubated with glyoxylic acid and malate synthase. A significant primary kinetic isotope effect in the abstraction of a hydrogen from the methyl group of acetyl-CoA results in an unequal distribution of tritium between the diastereotopic positions at C-3 of malate. The tritium distribution at that position is analyzed by incubation with fumarase, which stereospecifically equilibrates the pro-$3R$ hydrogen of 2S-malate with water. The percentage of retention of tritium in the resulting mixture of fumarate and malate relative to that of the starting malate is defined as the F value. Configurationally pure R-acetate gives an F value of 79 and pure S-acetate gives an F value of 21 (cf. (13)).

The results of the experiments are summarized in Table I. When AdoMet prepared from S-acetate was used as a substrate, R-acetate was obtained. Conversely, AdoMet prepared from R-acetate gave S-acetate. The starting acetate samples used for the synthesis of AdoMet had chiral purities of about 60–75% enantiomeric excess (e.e.).

The acetate samples obtained after the degradation of N-methylhistamine had somewhat lower chiral purity (31 to 48% e.c.). The data indicate that some racemization has occurred in the reaction sequence, most likely during the synthesis of AdoMet. Nevertheless, the stereochemical outcome is clear. Since one inversion of configuration takes place in the synthesis of AdoMet and another inversion in the degradation of N-methylhistamine, it is concluded that the enzymatic transfer of the methyl group from AdoMet to hista-

$^6$ Enantiomeric excess = $(F-50)/29 \times 100 \%$.
mine catalyzed by histamine N-methyltransferase proceeds with inversion of configuration.

The observed reaction stereochemistry conforms to that observed for all the other methyltransferases studied so far. It indicates that the methyl group must, in the process, undergo an odd number of individual, presumably S_{N2}-like, transfers. The most reasonable interpretation is that the reaction involves a single direct transfer of the methyl group from the sulfur of AdoMet to the imidazole nitrogen via an S_{N2} transition state as demonstrated for catechol-O-methyltransferase (3, 14). Specifically, the results exclude a ping-pong mechanism in which AdoMet first alkylates the enzyme, followed by a second transfer of the methyl group to the imidazole ring of histamine. Such a mechanism should lead to net retention of configuration, unless one makes the extremely unlikely assumption that the process involves methylation of two groups in the protein in a concerted, sequential fashion.

REFERENCES


