Fluorimetric Assay of Histamine N-Methyltransferase by High-performance Liquid Chromatography

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A sensitive method for the assay of histamine N-methyltransferase in rat tissues is described. N'-Methylhistamine formed from histamine under the optimum conditions for the enzyme reaction is extracted with chloroform, derivatized to a fluorescent product with the use of o-phthalaldehyde and 2-mercaptoethanol, and determined by high-performance liquid chromatography with fluorescence detection. This method allows the simultaneous determination of histamine and N'-methylhistamine. The limit of detection for N'-methylhistamine formed is 3.5 pmol per assay. The method is simple and is suitable for the routine assay of histamine N-methyltransferase in sample preparations obtained from tissues.

Keywords—histamine N-methyltransferase; rat tissues; N'-methylhistamine; fluorimetric assay; high-performance liquid chromatography; o-phthalaldehyde; 2-mercaptoethanol

Histamine N-methyltransferase (HMT, EC 2.1.1.8), one of the enzymes in the histamine metabolic pathways in mammalian tissues, catalyzes the methylation of histamine to N'-methylhistamine in the presence of S-adenosylmethionine as a methyl donor. The activity of this enzyme has been measured exclusively by radioisotopic techniques using 14C-histamine or 14C-S-adenosylmethionine in various studies so far reported.

Recently, we presented a simple method for the simultaneous determination of histamine and N'-methylhistamine in human urine and rat brain by high-performance liquid chromatography (HPLC) with fluorescence detection, based on the pre-column derivatization of these amines with the use of o-phthalaldehyde (OPT) and 2-mercaptoethanol.

The present paper describes an HPLC method for the assay of HMT in sample solutions from tissue homogenates. N'-Methylhistamine formed from histamine under the optimum conditions for the enzyme reaction is determined by the above mentioned method after extraction with chloroform. N'-Methylhistamine was used as an internal standard for the quantitation of N'-methylhistamine. An HMT preparation from rat kidney was employed.

Experimental

Materials—All chemicals and organic solvents used were of reagent grade. Deionized and distilled water was used.

Reaction Buffer: The pH of 0.05 M sodium tetraborate solution was adjusted to 10.0 with 5 M NaOH. A reaction buffer was prepared by mixing 5.0 ml of the borate solution, 5.0 ml of methanol and 0.1 ml of 0.5% (v/v) 2-mercaptoethanol in methanol.

HMT Preparation from Rat Kidney: Supernatant from rat kidney homogenate was used as an HMT preparation. It was prepared by the method of Snyder and Axelrod with a minor modification as follows. Sprague-Dawley male rats (5–7 weeks old) were killed by cervical dislocation. The kidneys were quickly removed, placed in about five volumes of 0.01 M phosphate buffer (pH 7.4) and then homogenized in an Omega electric homogenizer. After centrifugation at 10,000×g at 4°C for 20 min, the supernatant was collected and stored at −20°C. The protein concentration was measured by the method of Lowry et al. using bovine serum albumin as a standard protein, and adjusted to approximately 2 mg/ml with water. When required for use, the supernatant was diluted with three volumes of the phosphate buffer.

Apparatus and HPLC Conditions—A Hitachi Horiba M-7 pH meter and a Vapour Mix S-10 test tube evaporator (Tokyo Rikakikai, Tokyo, Japan) were used. A Hitachi 635-A liquid chromatograph equipped
with a universal injector and a Hitachi 650-10S spectrophotometer fitted with a flow-cell unit (cell volume 18 µl) operating at an excitation wavelength of 335 nm and an emission wavelength of 445 nm were used. A stainless-steel column (150 x 4 mm I.D.) was packed with LiChrosorb RP-18 (particle size, 5 µm; Japan Merck, Tokyo, Japan) as described previously.9) The mobile phase was 0.07 M Na2HPO4-methanol (47:53, v/v) and the flow rate was 0.7 ml/min. The column temperature was ambient.

Procedure—The incubation mixture consisted of 0.5 ml of 0.1 M phosphate buffer (pH 7.4), 0.1 ml of 0.25 mm histamine, 0.1 ml of 1.0 mm S-adenosylmethionine (as its sulfate; Tokyo Kasei, Tokyo, Japan) and 0.1 ml of HMT preparation. The mixture was incubated at 37°C for 30 min, then 0.25 ml of 1 M NaOH was added. Next, 0.3 g of NaCl, 2.5 ml of chloroform and 0.1 ml of 20 nm N'-methylhistamine (internal standard) were added successively. After 5-min shaking, the mixture was centrifuged at 1200 x g for 5 min. The aqueous layer was discarded by suction, and 2.0 ml of the chloroform layer was taken into a test tube and evaporated to dryness in vacuo at room temperature. To the residue, 0.2 ml of the reaction buffer and 5 µl of 0.5% OPT in methanol were added to develop the fluorescence, and an aliquot (50 µl) of the reaction mixture was subjected to HPLC.

Results and Discussion

The conditions of fluorescence reaction and HPLC were the same as described in the previous paper.7)

Figure 1 shows a typical chromatogram obtained according to the above procedure. N'-Methylhistamine is completely separated from N'-methylhistamine and histamine. Unreacted histamine, if present in a large amount in the fluorescence reaction mixture, gave a large peak which partially overlapped the peak of N'-methylhistamine. Therefore, histamine should be removed from the incubated assay mixture as far as possible. The extraction of N'- and N''-methylhistamines with chloroform served to minimize the amount of histamine. The recoveries of N'- and N''-methylhistamines (200 pmol each) in the chloroform extraction were 80±2% (mean ± standard deviation, n=10 each). The lower limit of detection for N'-methylhistamine formed enzymatically is 3.5 pmol per assay.

A linear relationship was obtained between the peak height ratio of N'-methylhistamine to the internal standard and the amount of N'-methylhistamine added in the range of 5 to at least 200 pmol in the assay mixture without incubation.

HMT was most active at pH 6.5—8.0 in 30—125 mm phosphate buffer; 62 mm phosphate buffer of pH 7.4 was used. A maximum and constant activity was obtained in the presence of 20—125 µM histamine with a Michaelis constant (Km) of 7.1 µM; 31 µM histamine was employed in the assay mixture as a saturating concentration for the enzyme reaction.

A maximum and constant activity was obtained in the presence of 93.8—312.5 µM S-adenosylmethionine with an observed K_m value of 14.3 µM; 125 µM S-adenosylmethionine was employed in the assay mixture. The activity was inhibited at a concentration of S-adenosylmethionine of 312 µM or greater.

The enzyme activity was linear with time up to at least 45 min, when the mixture containing 0.2 mg or less protein was incubated at 37°C. The precision was established as regards repeatability. The standard deviation was 11.3 (n=10) for a mean activity of 145 µmol per min per mg protein.
HMT activities in preparations obtained from the kidney, brain and stomach of a Sprague-Dawley male rat (6 weeks old) were 560, 83 and 2 μmol per min per mg protein, respectively, but no activity was detected in preparations from the lung, heart, liver, spleen and blood of the rat. The preparations from tissues other than kidney were obtained by a procedure similar to that used for the kidney.

This study provides the first method for the HPLC assay of HMT. The proposed method is sensitive, the entire procedure can be performed in about 2 h, and more than 20 samples can be assayed simultaneously. This method should be useful routinely in place of the radiochemical method.

References and Notes


High Performance Liquid Chromatographic Separation and Detection of Methoxy Derivatives of 3,4-Dihydroxyphenylalanine

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A voltammetric detector and a fluorometric detector combined with a high-performance liquid chromatograph were applied to the analysis of methoxy derivatives of 3,4-dihydroxyphenylalanine, and the results obtained were compared with those obtained with a 254 nm ultraviolet detector. Response was linear over a wide range of concentrations and detection was sensitive and selective.

The contents of 3-methoxy-4-hydroxyphenylalanine in the plasma of normal persons were measured.

**Keywords**—3,4-dihydroxyphenylalanine; 3,4-dihydroxyphenylalanine derivatives; high performance liquid chromatography; voltammetry; fluorimetry; metabolism

Catecholamines and their metabolites have been associated with a number of disease states.1,2 Recently, various techniques have been developed to measure methoxy derivatives of catecholamines.3–7 These methods have in general been proved to be applicable for the determination of only a few methoxy derivatives of 3,4-dihydroxyphenylalanine (DOPA).

Previously, we made a brief report on the detection of 3-methoxy-4-hydroxyphenylalanine(3-O-methyl-DOPA) and 3-hydroxy-4-methoxyphenylalanine(4-O-methyl-DOPA) by the use of rat liver homogenate.8 In this paper, we describe chromatographic conditions for the analysis of methoxy derivatives of DOPA, including 3-O-methyl-DOPA, in plasma