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Short Communication

High-performance liquid chromatographic determination of histamine N-methyltransferase activity

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ABSTRACT

A method for the determination of histamine N-methyltransferase (HMT) activity by high-performance liquid chromatography based on post-column derivatization with *o*-phthalaldehyde is described. The determination involves the separation of the substrate, histamine, from its product, N^{τ}-methylhistamine, using a weak cation exchanger, followed by on-line derivatization of these imidazoleamines with *o*-phthalaldehyde and their detection and quantitation with a fluorimetric detector. This assay method is suitable for the measurement of HMT activity during enzyme purification.

INTRODUCTION

Histamine N-methyltransferase (S-adenosylmethionine:histamine N-methyltransferase, HMT, EC 2.1.1.8), one of the enzymes involved in the metabolism of histamine in mammalian tissues, catalyses methyl transfer from S-adenosyl-L-methionine (SAM) to histamine (HA) to form N^{τ}-methylhistamine (N^{τ}-MeHA) [1,2]. The reported methods for determining HMT activity may be divided into two types: a radioisotopic procedure using [^{3}H]- or [^{14}C]SAM [3–6] and a spectrofluorimetric method using *o*-phthalaldehyde (OPA) as a reagent [7,8]. The high-performance liquid chromatographic (HPLC) separation combined with fluorimetric detection using OPA derivatization of HA and N^{τ}-MeHA is also

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used for the measurement of HMT activity [9]. As this method is based on pre-column derivatization of these amines with OPA in the presence of 2-mercaptoethanol, the procedure is tedious and not suitable for the assay of HMT activity in multiple samples such as those obtained during enzyme purification.

In this paper, a new method for the determination of HMT activity is described which allows the separation of the substrate (HA) and its N^{τ}-methylated product (N^{τ}-MeHA) using a post-labelling OPA–thiol fluorimetric method. This method was useful for the analysis of samples during the purification of rat kidney HMT [10].

EXPERIMENTAL

Chemicals

Citric acid, acetonitrile and OPA of HPLC grade were purchased from Wako Pure Chemicals (Osaka, Japan). Histamine diphosphate was from Wako; N^{τ}-methylhistamine dihydrochloride and N^{π}-methylhistamine dihydrochloride (N^{π}-MeHA) were from Calbiochem (La Jolla, CA, U.S.A.) and were dissolved in 0.05 *M* hydrochloric acid and stored at 4°C for daily use. The stock solutions were diluted daily to 100 μM concentrations of each amine in 0.05 *M* hydrochloric acid and used as standards for calibration. Pargyline and aminoguanidine were obtained from Sigma (St. Louis, MO, U.S.A.) and the other chemicals were of analytical-reagent grade and were used without further purification. Water purified through a Milli RO and Milli Q system (Japan Millipore, Tokyo, Japan) was used.

High-performance liquid chromatographic procedure

The mobile phase was prepared by dissolving 5.25 g of citric acid and 10 g of imidazole in 800 ml of water, and mixing the resultant solution with 200 ml of acetonitrile. The OPA reagent was prepared as follows: 2.47 g of boric acid and 0.12 g of Brij 35 were dissolved in 100 ml of water, and the solution was adjusted to pH 10.5 ± 0.02 with 5 *M* potassium hydroxide solution. OPA (60 mg) was dissolved in 5 ml of methanol and added to the borate solution together with 100 μl of 2-mercaptoethanol. The mobile phase and reagents were freshly prepared, filtered and degassed by passage through a 0.20-μm PTFE membrane filter (Toyo Roshi, Tokyo, Japan) just before use.

The HPLC system consisted of a Hitachi 635 liquid chromatograph (Hitachi Seisakusho, Tokyo, Japan) equipped with an autosampler (Model 638-08, Hitachi), a spectrofluorimeter (JLC-FL detector, JEOL, Tokyo, Japan) and a recorder.

The mobile phase was delivered at a flow-rate of 1.0 ml/min to a stainless-steel column (150 mm × 4.0 mm I.D.) packed with a weak cation exchanger (TSKgel CM2SW, 5-μm particles, Tosoh, Tokyo, Japan). The OPA reagent was added to the column eluate at a flow-rate of 1.0 ml/min with mixing in a reaction coil made

of PTFE tubing (1 m × 0.5 mm I.D.) at room temperature. The fluorescence intensity of the reaction mixture was then measured at the emission wavelength of 410–800 nm and excitation wavelength of 230–400 nm in a 15-μl flow cell.

Preparation of tissue extracts

Fresh kidneys were isolated from male Wistar rats (freely fed, body weight 150–300 g) and homogenized with four volumes of ice-cold 0.05 *M* sodium phosphate buffer solution (pH 7.4) containing 1 m*M* dithiothreitol and 1% polyethyleneglycol (average molecular mass 300) by a Polytron homogenizer (Kinematica, Lucerne, Switzerland) operated at the maximum setting for 60 s in an ice-bath. The homogenate was centrifuged at 180 000 *g* for 1 h at 4°C, and the supernatant was dialysed three times for 12 h each against 100 volumes of the buffer.

The reaction of HMT was carried out at 37°C in 1 ml of a mixture of 0.7 ml of 0.1 *M* phosphate buffer solution containing 0.1 m*M* pargyline and 0.1 m*M* aminoguanidine, pH 7.4, 0.1 ml of 1.0 m*M* histamine (0.1 m*M*), 0.1 ml of 2.5 m*M* SAM (0.25 m*M*) and 0.1 ml of enzyme preparation [10]. The blank and standard for calibration were incubated without enzyme, and the latter was then supplemented with 100 μ*M* N^{τ}-MeHA. After incubation, the reaction was terminated by adding 50 μl of 60% perchloric acid (PCA). The mixtures were then centrifuged at 1000 *g* for 3 min and the supernatant was adjusted to pH 6.5 ± 0.2 by the dropwise addition of 5 *M* potassium hydroxide solution after adding 20 μl of 5% bromothymol blue solution as a pH indicator. The precipitated potassium perchlorate was removed by brief centrifugation and the supernatant was applied to a short column of Amberlite CG-50 (Type II, 200–400 mesh, Na^+ form, 20 mm × 4.0 mm I.D.) which had been equilibrated with 0.2 *M* sodium phosphate buffer solution, pH 6.5. After the sample had passed through the column, the column was washed twice with 3 ml of 5 m*M* Na_2EDTA (adjusted to pH 6.5 with 4 *M* sodium hydroxide solution) and with 3 ml of 0.1 *M* hydrochloric acid. Histamine and N^{τ}-MeHA were eluted from the column with 1.0 ml of 0.5 *M* hydrochloric acid [11]. This eluate was applied to the HPLC system.

RESULTS AND DISCUSSION

The principle of this HMT assay is the separation of N^{τ}-MeHA from the substrate, HA, on a cation exchanger, and the detection and quantitation of N^{τ}-MeHA by a post-column OPA–thiol reaction and fluorimetry. Fig. 1 shows a typical chromatogram of the standard mixture of N^{τ}-MeHA, HA and N^{π}-MeHA. These amines were completely separated from each other with retention times of 12, 15 and 17 min, respectively.

Of various heterocyclic compounds such as imidazole, pyridine, piperidine, N-methylpiperidine and piperazine tested, it was found that imidazole was the most suitable for use in the mobile phase, because pyridine quenched the fluo-

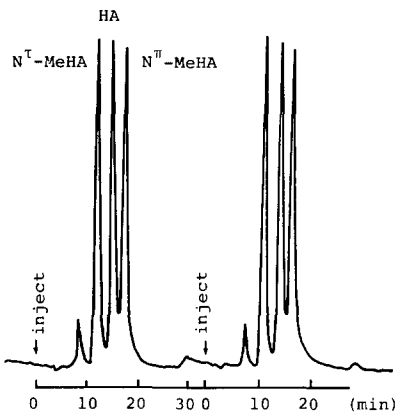

Fig. 1. Chromatograms obtained on successive application of a standard mixture of HA, N$^{\tau}$-MeHA and N$^{\pi}$-MeHA to a TSKgel CM2SW cation-exchange column. Sample: 10 μl, 1.0 nmol.

rescence, and piperidine, N-methylpiperidine and piperazine reacted with OPA resulting in an increase in the baseline fluorescence. The effect of the concentration of imidazole in the mobile phase on the k' values (capacity factor) for the N$^{\tau}$-MeHA and HA was studied at pH 6.85 (Fig. 2). The k' values of N$^{\tau}$-MeHA and HA decreased with an increase in the imidazole concentration. The two peaks overlapped at imidazole concentrations lower than 50 mM. The absence of imidazole caused a delay and broadening of the peak of N$^{\tau}$-MeHA, and the elution order of the two amines was reversed. When the imidazole concentration was higher than 100 mM, the N$^{\tau}$-MeHA peak was completely separated from that of HA. The concentration of 1% (w/w) imidazole (145.3 mM) was therefore chosen for the mobile phase.

The fluorescence intensity, expressed as peak height, was proportional to the amount of N$^{\tau}$-MeHA in the range 0.05–25 nmol. The lower limit of the determi-

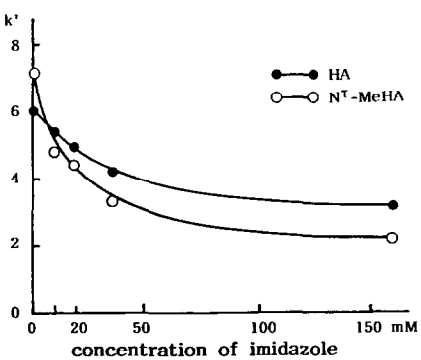

Fig. 2. Effect of imidazole on k' values (capacity factor) for HA and N$^{\tau}$-MeHA. The amines were eluted with mixtures of 27.3 mM citric acid, 20% acetonitrile and various concentrations of imidazoles, pH 6.85, at a flow-rate of 1.0 ml/min.

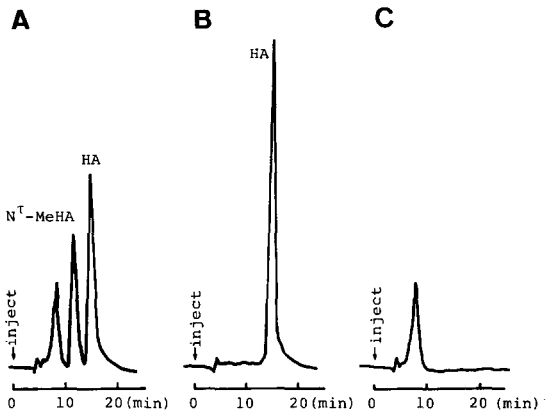

Fig. 3. Chromatograms of the HMT reaction mixture after incubation for 1 h at 37°C. Elution profiles were obtained (A) with and (B) without the enzyme, and (C) with the enzyme but no histamine in the reaction mixture.

nation at a signal-to-noise ratio of 10 was 50 pmol of N$^{\tau}$-MeHA per injection. The intra- and inter-assay coefficients of variation were less than 3 and 5%, respectively.

In this method, previous purification of N$^{\tau}$-MeHA on Amberlite CG-50 was not always necessary, as the direct injection of the incubation mixture treated with PCA to the HPLC–OPA system gave the same results. However, the application of solutions containing high concentrations of protein resulted in an earlier decrease in the separation efficiency in the HPLC system. In such cases, the samples of reaction mixtures were first purified on an Amberlite CG-50 column.

On direct application, the reaction mixture of rat kidney HMT (Fig. 3) showed a significant formation of N$^{\tau}$-MeHA during incubation at 37°C for 1 h, and the enzyme activity was estimated as 0.71 nmol/min/mg protein (Fig. 3A), whereas N$^{\tau}$-MeHA was not detected in the control mixture incubated without enzyme (Fig. 3B). Incubation of the enzyme preparation in the reaction mixture without histamine also did not produce N$^{\tau}$-MeHA (Fig. 3C), showing that appreciable endogenous histamine had been removed by the dialysis procedure in the preparation of the enzyme solution.

A maximum and constant HMT activity was observed in the presence of 100 μM histamine and 250 μM SAM. The Michaelis constant (K_M) for HA was estimated as 19.9 μM. This value is in good agreement with previously reported values [12]. The activity was inhibited by histamine at a concentration of 125 μM or more as reported by Barth *et al.* [13]. The K_M values for SAM were reported to be 8.7–43 μM [12].

This method is both simple and rapid, and it does not require extraction or pre-column derivatization. It should be useful for the assay of HMT during purification and for measuring its activity in various tissues.

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