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Note

A simple and sensitive liquid chromatographic method for the determination of 5′-methylthioadenosine in tissues

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Methylthioadenosine (MTA) is formed from S-adenosyl-L-methionine by several pathways, including those leading to biosynthesis of polyamines. This product is a potent inhibitor of enzymes involved in the synthesis of spermidine and spermine. The enzyme responsible for the metabolic degradation of MTA, namely MTA phosphorylase, is a potential chemotherapeutic target enzyme [1], and this enzyme is lacking in some malignant tumors and cells [2—4].

The growing interest in MTA (for review, see refs. 1, 5 and 6) has demanded the development of methods for its measurement in tissues. The concentration of this nucleoside is low in comparison with those of related compounds, and early methods for measurement of MTA suffered from low sensitivity [1]. Recent methods, based on high-performance liquid chromatography (HPLC), allow the determination of MTA in several tissues. Purification of the tissue samples on borate affinity gel [7, 8] or Dowex 50 [9] was required before HPLC.

The present communication describes a liquid chromatographic method for the determination of MTA, involving filtration of neutralized tissue extract through DEAE-Sephadex at pH 7.5. MTA appeared in the effluent while interfering substances were retained on the column. The effluent was lyophilized and subjected to reversed-phase chromatography on a short 3-μm ODS (C_{18}) column eluted at high flow-rate. 2-Chloroadenosine served as a convenient internal standard.

EXPERIMENTAL

Materials

MTA, 2-chloroadenosine, adenine, S-adenosyl-L-homocysteine, S-adenosyl-

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L-methionine and adenosine were obtained from Sigma (St. Louis, MO, U.S.A.). ODS Hypersil, 3-μm packing material for HPLC, empty stainless-steel columns (10 × 0.5 cm) and guard columns (2.5 × 0.5 cm) were purchased from Shandon (Cheshire, U.K.). Packing material for guard columns, Pelliguard LC 18, 40 μm, was from Supelco (Houston TX, U.S.A.). DEAE-Sephadex A-50 was obtained from Pharmacia (Uppsala, Sweden).

The 3-μm ODS columns were slurry packed using a Shandon column packer. The guard column was dry-packed and subjected to mechanical compression and refilling.

Preparation and purification of tissue extracts

Tissue was homogenized in 0.8 *M* perchloric acid (1:4, w/v) containing 5 μM 2-chloroadenosine (internal standard). Precipitated protein was removed by centrifugation, using a Beckman type B microfuge. The supernatant was immediately neutralized to pH 7.5 by adding 1.44 *M* KOH—1.2 *M* $KHCO_3$. Then 1.5 ml of the solution were applied to DEAE-Sephadex columns (0.6 × 5 cm) equilibrated with 10 m*M* Tris—HCl, pH 7.5, and eluted with the same buffer. The first 2 ml were discarded, and the following 5 ml were collected, lyophilized and resuspended in 100 μl of distilled water.

Reversed-phase liquid chromatography

Samples of 5—25 μl were analyzed on a 3-μm ODS Hypersil column (0.5 × 10 cm) equipped with a guard column (0.5 × 2.5 cm). The column was eluted isocratically with 8 m*M* phosphate buffer, pH 6.0, containing 6% acetonitrile. The liquid chromatographic system consisted of a Spectra-Physics SP 8700 solvent delivery system, a Perkin-Elmer ISS 100 autosampler for HPLC, a Beckman ultraviolet (UV) detector, Model 160, and a Hewlett-Packard HP 3390 A integrator. For the determination of UV spectra of MTA in tissue extracts, a variable-wavelength detector from Kratos, Model Spectroflow 773, was used.

RESULTS AND DISCUSSION

MTA and 2-chloroadenosine were not retained on DEAE-Sephadex columns equilibrated with 10 m*M* Tris—HCl, pH 7.5. More than 95% of MTA and 2-chloroadenosine applied to the column was eluted with this buffer. The recovery of exogenous MTA and 2-chloroadenosine added to the tissue extract was 20—40%, depending on the elution volume collected from the DEAE-Sephadex column. Tissue samples were prepurified by filtration through DEAE-Sephadex columns, followed by lyophilization of the effluent. This step removed UV-absorbing material, which interfered with the determination of MTA by HPLC, and greatly reduced the solvent front of the chromatogram (Fig. 1). The concentration of MTA was increased about 5-fold relative to that in the crude tissue extract.

Some methods for determining MTA include purification of the tissue extract on borate affinity columns prior to HPLC [7—9]. The high cost of this material and the variable quality of some commercial preparations led us to use DEAE-Sephadex to purify the tissue extract. These columns could easily be regenerated by elution with a high salt concentration.

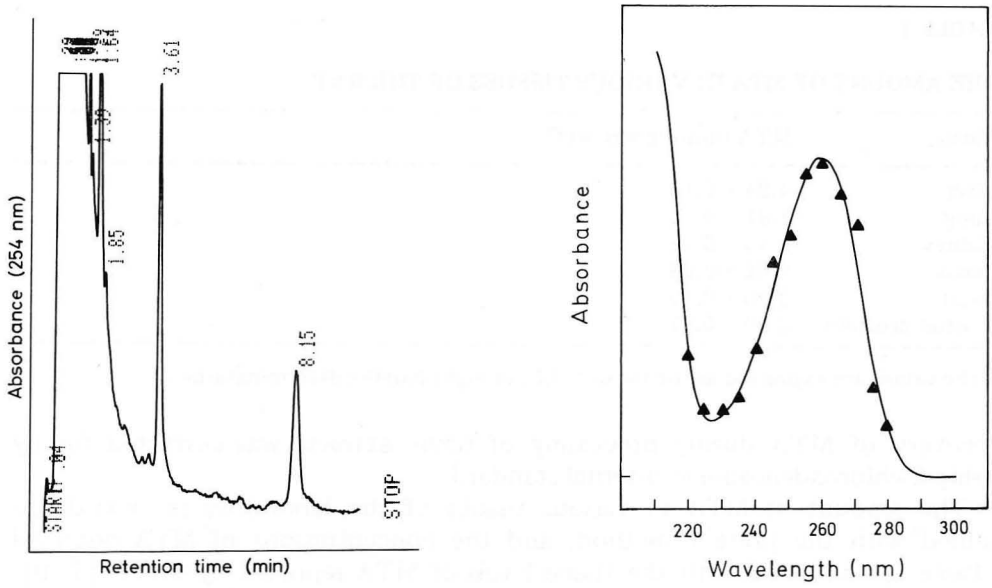

Fig. 1. Chromatographic analysis of rat liver extract. Rat liver was homogenized in perchloric acid containing 2-chloroadenosine, and the neutralized extract was purified on a DEAE-Sephadex column as described in the text. The purified extract was analysed on an ODS Hypersil column eluted isocratically with 6% acetonitrile in 8m*M* phosphate buffer, pH 6.0, at a flow-rate of 3 ml/min. The retention times of 2-chloroadenosine and MTA were 3.61 and 8.15 min, respectively.

Fig. 2. Ultraviolet absorption spectrum of authentic MTA and the MTA peak in liver extract. The solid line shows the absorption spectrum of MTA dissolved in phosphate buffer, pH 6.0. The absorption spectrum of the MTA peak (▲) was obtained by repetitive analysis of purified rat liver extract. The absorption of the effluent was monitored at the wavelengths indicated.

The lyophilized samples from the DEAE-Sephadex columns were analyzed by reversed-phase liquid chromatography on a short (10 cm) highly efficient (about 9000 theoretical plates) 3-μm ODS column eluted at high flow-rate (3 ml/min). Baseline separation of MTA from interfering substances was obtained, and the internal standard, 2-chloroadenosine, eluted just after the solvent front (Fig. 1). Adenosine, S-adenosylhomocysteine, S-adenosylmethionine, inosine, adenine, and various oxypurines eluted at lower retention times than MTA (data not shown). MTA in the tissue extract was identified by its retention time relative to an authentic standard in various reversed-phase systems (8 m*M* potassium dihydrogen phosphate pH 6.0 containing 6% acetonitrile or 10 m*M* potassium dihydrogen phosphate pH 6.0 containing 20—25% methanol), and in a cation-exchange system as described elsewhere [10], by the increase in the MTA peak upon addition of MTA to the tissue extract, and by the UV spectrum of MTA in the tissue extract (Fig. 2). (Fig. 2).

The determination of MTA in tissue extracts was performed by comparison of the area under the peak with that of known MTA standards. The standard curve for MTA was linear in the range 0.2—30 μM, and the amount of MTA was calculated by calibrating the reporting integrator. The variable

TABLE I

THE AMOUNT OF MTA IN VARIOUS TISSUES OF THE RAT

Tissue	MTA (nmol/g wet wt)*
Liver	4.24 ± 0.13
Lung	1.31 ± 0.06
Kidney	1.43 ± 0.06
Testis	0.62 ± 0.03
Heart	3.90 ± 0.15
Ventral prostate	2.00 ± 0.07

*The values are expressed as the mean ± S.E. of eight parallel determinations.

recovery of MTA during processing of tissue extracts was corrected for by using 2-chloroadenosine as internal standard.

The amount of MTA in various tissues of the laboratory rat was determined with the present method, and the concentrations of MTA obtained (Table I) compared with the tissue levels of MTA reported by others [7, 9]. Using a low-noise, fixed-wavelength detector, equipped with a mercury lamp (254 nm) and a relatively large flow-cell (18 μl), 2 pmol of MTA in tissue extracts could be detected.

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