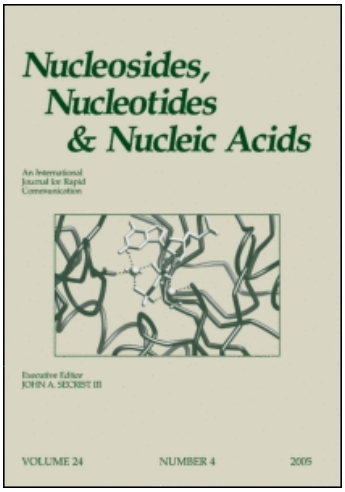

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ISOLATION AND IDENTIFICATION OF 5'-METHYLTHIOADENOSINE SULFOXIDE FROM HUMAN URINE

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Abstract - An adenine nucleoside isolated from human urine has been identified by mass spectra and other techniques as 5'-deoxy-5'-methylthioadenosine sulfoxide. Elevated levels (3-5 nmols/μmol creatinine) were noted in two children with severe combined immunodeficiency.

INTRODUCTION

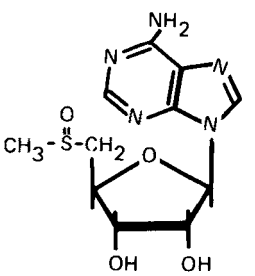

FIG. 1. 5'-Methylthioadenosine sulfoxide.

Inherited deficiency of adenosine deaminase usually results in a syndrome of severe combined immunodeficiency. Less severe immunodeficiencies result from purine nucleoside phosphorylase or purine 5'-nucleotidase deficiencies. However, most immunodeficient children have no demonstrated metabolic defect. In an attempt to establish evidence of other metabolic defects in these children, we have evaluated urinary excretion levels of many purine and pyrimidine nucleosides[1]. More recently, while extending these procedures, we have isolated an unknown adenine nucleoside. This compound was elevated in two of six patients with severe combined immunodeficiency, and appeared to have similar chromatographic properties to those found for an unknown adenine nucleoside by Hirschhorn, et al.[2].

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They noted elevated levels of their nucleoside in urine of four adenosine deaminase deficient children. Mass spectrometry studies of our isolated nucleoside provided sufficient information to suggest the identification of the compound as 5'-methylthioadenosine sulfoxide. The identity was confirmed by additional chromatographic and chemical studies with the authentic compound. These studies also showed that our adenine nucleoside was distinct from that of Hirschhorn, et al.[2].

MATERIALS AND METHODS

Chemicals and Solutions.

Analytic grade ion exchange resins (AG1-X4 and AG50-X4, 200/400 mesh) were obtained from Bio-Rad Laboratories. XAD-4 (20/50 mesh) was obtained from Mallinckrodt Chemicals Works. Chloroacetaldehyde was prepared from the dimethylacetal derivative as described previously[3]. Orcinol was recrystallized from toluene prior to use. 5'-Deoxy-5'-methylthioadenosine (MTA), thiodiglycol, 2'-O-methyladenosine, adenosine, and adenosine deaminase (Type I from calf intestinal mucosa) were obtained from Sigma Chemical Co. 5'-Deoxy-5'-methylthioadenosine sulfoxide (MTA-sulfoxide) was prepared with a yield of ca. 100% by oxidizing MTA with H_2O_2[4], followed by cation exchange chromatography (HCl elution) to purify the MTA-sulfoxide. Its identity as the sulfoxide of MTA was confirmed by the molecular weight of 313 obtained by CI mass spectra (Fig. 3). All components of buffers were reagent grade, and their pH values refer to pH at 23°C. For the sodium acetate buffer (prepared from sodium acetate and acetic acid), the listed concentration refers to the acetate concentration. The sodium borate solution (0.010 M) was prepared from $Na_2B_4O_7 \cdot 10\ H_2O$ (3.81 g/liter). The ammonium acetate + acetic acid eluent used for cation exchange chromatography was prepared from acetic acid and ammonium hydroxide with the concentration of the latter determined by titration with HCl.

Analytical Methods.

A Gilford spectrophotometer (Model 222) was used for spectrophotometric measurements, while an Aminco-Bowman spectrophotofluorometer was used for fluorescence measurements. The orcinol reaction for ribose was carried out with heating in a boiling water bath for 20 minutes using the orcinol-$FeCl_3$-HCl reagent prepared as described by

Brown[5]. Color yield was measured with the spectrophotometer at 670 nm. Acid hydrolysis of adenine nucleosides to form adenine was carried out using either HCl or perchloric acid. Adenine formation was measured by determining sample absorbance at 275 and 260 nm after various time intervals, and comparing the absorbance ratios to known values (adenosine $A_{275/260} = 0.40$, adenine $A_{275/260} = 0.60$). A millimolar absorbancy of 14.4 at 260 nm was used for determining the concentration of MTA-sulfoxide, MTA and adenosine. The time required for 50% hydrolysis ($t_{\frac{1}{2}}$ value) was determined by plotting the log of the nucleoside concentration against the time. Although the $t_{\frac{1}{2}}$ value is independent of substrate concentrations, the latter were varied in different experiments from 15 to 70 μM. Different temperatures and acid concentrations for the hydrolysis were used as indicated in Table 2. Etheno derivatives of adenine nucleosides were produced by incubation of samples with chloroacetaldehyde as described previously[3]. Activity of adenine nucleosides as substrates for calf intestinal adenosine deaminase were studied at pH 7.3 in 0.034 M Tris buffer at 23°C by measuring the decrease in sample absorbance at 265 nm. Substrate concentrations varied from 12 to 60 μM. The difference in the millimolar absorbancy of adenosine and inosine at this wave length is 8.5. Desalting of solutions as required for rechromatography was carried out with a 1.3 x 30 cm XAD-4 column as described previously[6].

Mass Spectrometry.

Aqueous samples containing ca. 1 μg/μl of nucleoside were transferred to the glass insert of the mass spectrometer probe and evaporated to dryness in a vacuum desiccator. Mass spectra were obtained on a Finnigan 4000 mass spectrometer, using an ionizing voltage of 70 eV. Methane was used as the chemical ionization gas. The emission current was 1 amp for electron ionization (EI) and 0.3 amp for chemical ionization (CI). The electron multiplier voltage was 900 volts. The sample was introduced via a vacuum-lock direct insertion probe. The probe was heated rapidly to 350°C and spectra were taken when total ion production reached a maximum.

Isolation of MTA-sulfoxide from Urine.

The procedure for separation of MTA-sulfoxide from urine involved an initial separation of urinary nucleosides from uric acid and other

organic acids. This was achieved by passage of the urine (20-30 ml) through an AG1-X4 anion exchange column, formate form (1.5 x 16 cm). The column was washed with 200 ml of water, and the combined washes were concentrated to ca. 20 ml. For removal of salts, the combined washes were applied to a 1.3 x 30 cm XAD-4 column with elution of nucleosides using 19% ethanol as described previously[6]. A short anion exchange column (AG1-X4, Cl^- form, 1 x 6 cm) operated at pH 10, was utilized to separate nucleosides that were anionic at pH 10 from those that are neutral at that pH[7]. The uncharged nucleosides, including MTA-sulfoxide, were subjected to cation exchange chromatography (AG50-X4, H^+ form, 0.50 x 40 cm column) using gradient elution with HCl (0 → 1.5 N). The last peak in the A_{260} elution profile contained MTA-sulfoxide and 1-methylguanine[8]. Prior to rechromatography, HCl was removed from the appropriate pooled fractions by neutralizing with NaOH and removal of the NaCl on an XAD-4 column. After concentration by solvent evaporation, fractions containing MTA-sulfoxide were applied to an AG50-X4 cation exchange column (NH_4^+ form, 0.5 x 40 cm). Elution was carried out by gradient elution with a pH 4.4 ammonium acetate buffer. At this pH, 1-methylguanine was eluted ahead of MTA-sulfoxide, and the latter compound had consistent absorbance ratios (240/260, 250/260, 275/260, 290/260) throughout the peak. After removal of the ammonium acetate buffer on an XAD-4 column, the isolated MTA-sulfoxide was used for the additional studies described below.

Using the isolation procedure described above, urinary levels of MTA-sulfoxide of 5.0 and 2.9 nmols per μmol creatinine were found in two children with severe combined immunodeficiency (JRG and LLA, ref 1). Corresponding levels in six normal children were 0.34 ± 0.21 (mean ± s.d.).

pH 10 Anion Exchange Separation with Borate.

The pH 10 anion exchange separation previously used in this laboratory[7] has been modified in order to retain uncharged nucleosides containing a ribose moiety with free hydroxy groups in the 2' and 3' positions. An AG1-X4 column, 1.0 x 5.5 cm, Cl^- form is used. Pretreatment of the column is as follows: (a), 20 ml of 0.10 M NaCl + 1.5 mM NH_4OH (pH 10.0), (b), 5 ml of H_2O, and (c), 10 ml of 0.010 M sodium tetraborate. The sample, which should contain only a small amount of salts, is adjusted to pH 10.1 - 10.5 with NaOH. After the sample has passed

through the column, the resin is washed with two 25-ml portions of water. Bound nucleosides are eluted with 25 ml of a 0.092 M, pH 5.2 sodium acetate buffer followed by 25 ml of H_2O.

Nucleosides which are uncharged at pH 10 and which contain deoxyribose as the sugar moiety (e.g., deoxyadenosine), or which contain 2'-O-methyl groups on the ribose (e.g., 2'O-methyladenosine) are not retained by the resin and are present in the column wash. Adenosine and MTA-sulfoxide are eluted when the pH falls to a value where the borate complex is no longer ionized. They appear in the sodium acetate buffer eluate and in the subsequent water wash. This borate complexing procedure is useful only for compounds with no charge at pH 10. Enolic compounds with pk_a values of 10 or less (e.g., guanosine) are retained by the anion exchange column at pH 10 either with or without borate.

Separation and Analysis of Urinary MTA.

The success of this procedure depends upon the stronger retention of MTA compared to other nucleosides on an anion exchange column. This retention appears to be due to adsorption to the column, since none of the nucleosides are charged at the pH used for elution. Thiodiglycol, 0.10% (v/v) is added to the sample prior to application to the column and to the solutions used for elution. An AG1-X4 column, Cl^- form (1.25 cm x 8.7 cm) is pretreated with (a), 20 ml of 0.10 M NaCl + 1.5 mM NH_4OH (pH 10.0), and (b), 10 ml of 0.010 M sodium tetraborate. Urine (2-4 ml) is diluted to 10 ml with H_2O and the pH is adjusted to 10.2 - 10.4 with NaOH. The sample is applied to the column and washed on with a few ml of water. Elution is carried out with 20 ml of 0.092 M sodium acetate buffer (pH 5.2) in a closed mixing reservoir and 30 ml of the same buffer in the upper reservoir. Fractions of ca. 5 ml are collected with a flow rate of ca. 1.2 ml/min. When the upper reservoir has emptied, gradient elution is begun by adding 100 ml of 10% (v/v) ethanol to the upper reservoir. Most of the nucleosides are eluted rapidly from the column as soon as the pH falls, but the MTA is retained and eluted in a symmetrical peak as the ethanol concentration is gradually increased (Fig. 5). For the fluorescence assay of the MTA peak, an 0.80 ml aliquot of each fraction was added to 0.20 ml of 0.092 M, pH 5.2 sodium acetate buffer; 0.50 ml of 0.09 M chloroacetaldehyde was added and samples were heated at 80°C for 40 minutes.

Procedures for Rechromatography of MTA-sulfoxide.

Procedure A. The sample was applied to an AG50-X4 column, H^+ form, 0.50 cm x 40 cm. The MTA-sulfoxide was eluted by gradient elution with 75 ml of H_2O initially in the closed mixing reservoir and 1.5 N HCl in the upper reservoir. Fraction volumes were 3.5 - 4.0 ml with a flow rate of ca. 0.25 ml/min.

Procedure B. The sample was applied to an AG50-X4 column, NH_4^+ form, 0.50 cm x 40 cm. The column was equilibrated with the initial eluting buffer prior to sample application. Elution was carried out with 75 ml of 0.105 N acetic acid + 0.045 M ammonium acetate in the closed mixing reservoir and 0.70 N acetic acid + 0.30 M ammonium acetate in the upper reservoir. Fraction volumes were 3.0 - 3.5 ml with a flow rate of 0.20 ml/min.

Procedure C. Samples (10-20 ml) were applied to an XAD-4 column, 1.3 cm x 30 cm. After washing the column with 50 ml of water, elution was begun with 19% (v/v) ethanol. Fraction volumes ranged from 10 to 5 ml with flow rates ranging from 1.0 to 0.50 ml/min.

RESULTS

Identification of Isolated Adenine Nucleoside as MTA-sulfoxide.

The EI and CI mass spectrometry studies on the isolated and authentic MTA-sulfoxides are shown in Figs. 2 and 3. The EI mass spectrum for adenosine is included for comparison. Both the 314 m/z (M + 1) CI ion and the major 298 m/z ion in EI mass spectrometry contain isotopic distributions characteristic of a compound containing sulfur. The spectra show peaks at m/z 296 (CI spectra) and 298 (EI spectra) resulting from loss of H_2O and CH_3 as evidence that 314 is the M + 1 molecular ion. The EI spectra of MTA-sulfoxide shows the pattern of adenine base at m/z 135 and 136, which agrees with the ultraviolet absorption spectra (Table 1) showing that the adenine moiety is not modified. The EI spectra also contains a peak at m/z 164 indicative of $(adenine\text{-}CHO)^+$ ion as well as the peak at m/z 178 indicating that the C_2OH group of the ribose ring is unsubstituted. The presence of the m/z 178 peak is consistent with the observed formation of borate complexes by both adenosine and MTA-sulfoxide (Table 1).

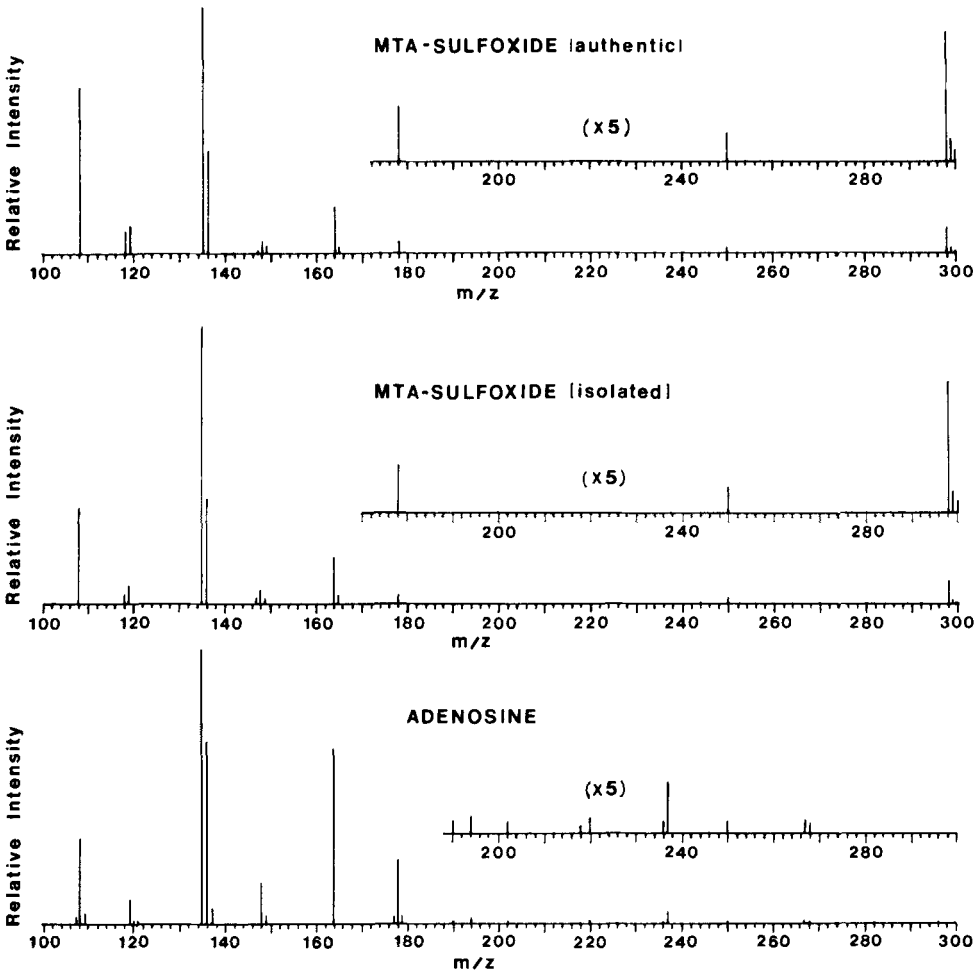

FIG. 2. EI mass spectra of adenosine and MTA-sulfoxides.

Both the m/z 164 and 178 peaks also are prominent in the adenosine EI spectra, indicating that MTA-sulfoxide and adenosine are identical in regard to the adenine and C_1 and C_2 carbons of the ribose. MTA-sulfoxide is the only compound that has the proper molecular weight of 313, would be expected to undergo the facile loss of a methyl group, contains adenine and ribose moieties, and contains a sulfur atom. Moreover, the isolated compound has EI and CI spectra that are identical with those of the authentic MTA-sulfoxide. The slight discrepancies in peak heights are within the usual experimental variation.

As further proof of their identity, the isolated and authentic MTA-sulfoxides were subjected to rechromatography in three different

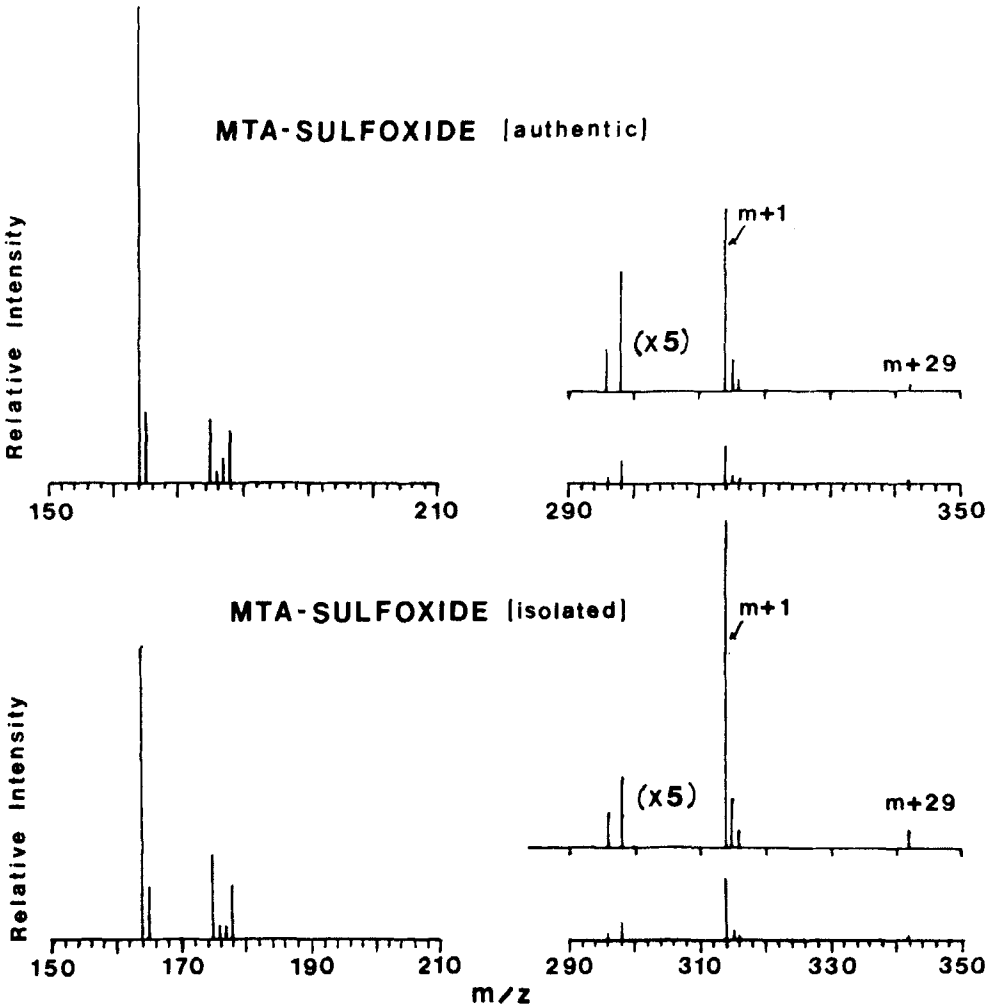

FIG. 3. CI mass spectra of isolated and authentic MTA-sulfoxides.

systems as shown in Fig. 4. In each case, the isolated and authentic compounds were eluted in the same position.

Additional Properties of MTA-sulfoxide.

Additional properties of isolated and authentic samples of MTA-sulfoxide are summarized in Tables 1 and 2. Spectral ratios of the isolated and authentic compounds are typical of adenine nucleosides and clearly show that there are no substitutions on the adenine moiety. Both the authentic and isolated compounds are retained on the pH 10

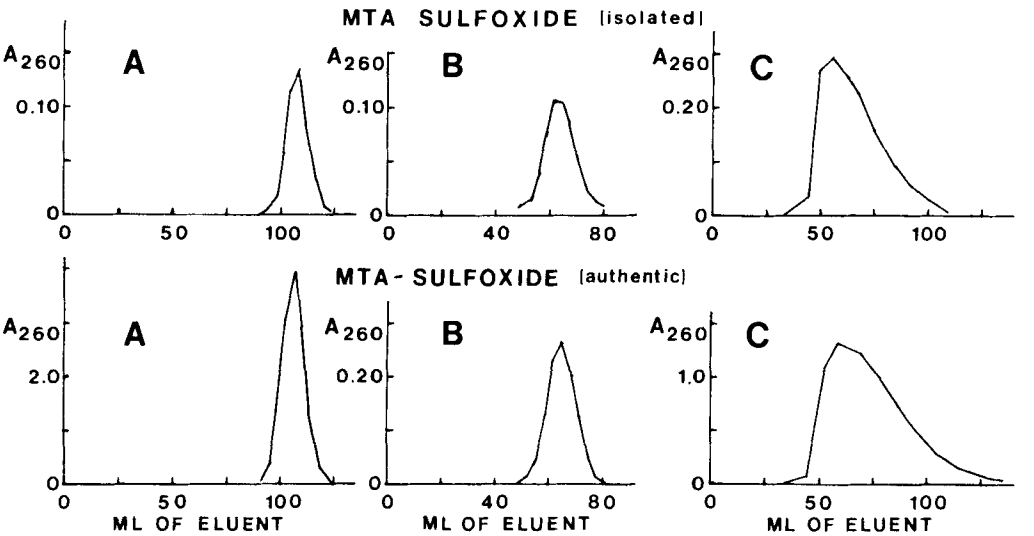

FIG. 4. Rechromatography of isolated and authentic MTA-sulfoxides. A. Cation exchange, HCl elution. B. Cation exchange, ammonium acetate + acetic acid elution. C. Elution from XAD-4 resin. For details see Methods.

borate anion exchange column, indicating that the C_2OH and C_3OH groups of the ribose are free to form a borate complex. This property clearly distinguishes MTA-sulfoxide from 2'-O-methyladenosine, with the latter compound passing through the anion exchange column under these circumstances. Both the isolated and authentic MTA-sulfoxides form an etheno derivative with chloroacetaldehyde, with excitation and emission spectra being identical with that of adenosine[3]. This reaction indicates that the adenine moiety has no substitutions on the amino group or on the N_1 nitrogen of the purine ring.

MTA-sulfoxide produces less color (absorbance at 670 nm) in the orcinol reaction for ribose-containing compounds than does adenosine (Table 2), although the absorption spectra of the products of the two compounds (and MTA) with orcinol are identical, with a maxima at 670 nm. Although all purine ribonucleosides react with orcinol under these circumstances, the amount of color produced under standard conditions varies considerably. Consequently, this test shows that the authentic and isolated MTA-sulfoxides have the same color yield at 670 nm within experimental error. Neither authentic or isolated MTA-sulfoxide nor

TABLE 1. Comparison of Other Properties of Isolated and Authentic MTA-Sulfoxide

	Spectral ratios in 1 N HCl				pH 10 Borate anion exchange column	Formation of etheno derivative *
	240/260	250/260	275/260	290/260		
Isolated MTA-Sulfoxide	.46	.85	.43	.03	Retained	Yes
Authentic MTA-Sulfoxide	.45	.85	.43	.03	Retained	Yes

*40 min. heating at 80°C with chloroacetaldehyde

TABLE 2. Comparison of Properties of Adenine Nucleosides

	Orcinol reaction for ribose#	Substrate for adenosine deaminase	$t_{1/2}$ for acid hydrolysis: 80°C HCl, 0.25 N	80°C HCl, 1.1 N	100°C Perchloric acid, 0.1 N
	%	%	min.	min.	min.
Adenosine	100	100	32 ± 2.2**		14
MTA	53	0*	71 ± 5		
Authentic MTA-Sulfoxide	68	0*	486 ± 32	74	49
Isolated MTA-Sulfoxide	76	0*	501 ± 37***		

*No deamination in 24 hr under conditions where 99% of adenosine was deaminated in one min.

**Standard error (SE) was calculated from the equation:

$$\frac{SE}{b} = r\sqrt{\frac{n-2}{1-r2}}$$ where b = slope, and r = correlation coefficient.

*** $p > 0.75$ when compared to authentic MTA-sulfoxide.

#Expressed as per cent of color yield of adenosine with 20 min heating at 100°C.

MTA showed the slightest activity with calf intestinal adenosine deaminase. This appears to be true of most 5'-substituted adenosines[10]. Adenosine and deoxyadenosine are readily deaminated by adenosine deaminase and many modified adenosines (e.g., 2'-O-methyladenosine and N^6-methyladenosine) are slowly deaminated by this enzyme[2]. MTA-sulfoxide proved to be much more resistant to acid hydrolysis than adenosine or MTA (Table 2). The same relative bond labilities were noted in both HCl and perchloric acids. The bond labilities of the isolated and authentic MTA-sulfoxides were identical within experimental error. The rate of hydrolysis of the purine-ribose bond has been shown by others to change with various substituent groups on the ribose moiety[11].

Air Oxidation of MTA to MTA-Sulfoxide.

In a subsequent study, we added MTA (0.28 μmols) to 30 ml of simulated urine (urea plus salts) and using the isolation procedure for MTA-sulfoxide described above, recovered 52% of the initial MTA as MTA-sulfoxide. We also confirmed the presence of MTA-sulfoxide in the urine of subject JRG when the antioxidant, thiodiglycol, was included in the isolation procedure, although the amount of MTA-sulfoxide noted was less than that found when the original isolation procedure was used.

Identification of MTA in Urine.

We also devised a separative procedure for MTA which included thiodiglycol as antioxidant (Fig. 5). This procedure is not as sensitive

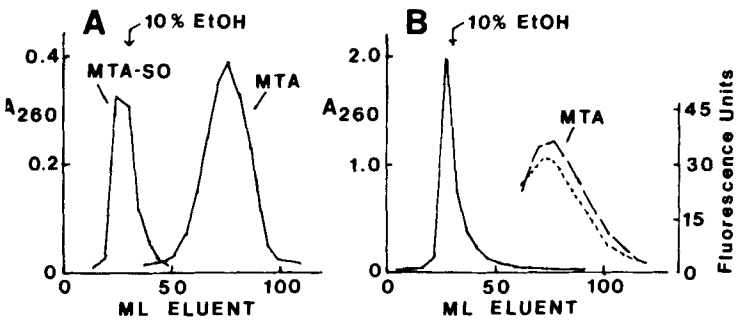

FIG. 5. Separation and analysis of urinary MTA. A. Known compounds in 4 ml of simulated urine (urea + salts). B. JRG urine (2 ml). Fluorescence was measured at 425 nm with excitation at 280 nm (----) or 305 nm (— —). Fluorescence readings are corrected for the initial fluorescence of the fractions from the columns. For additional experimental details, see Methods.

as the isolation procedure used for MTA-sulfoxide, and we were not able to isolate and purify the MTA. Nevertheless, we were able to detect MTA in three of the urine samples from immunodeficient children, but the amount of MTA found was only ca. 60% of that previously noted for MTA-sulfoxide. The identification of the MTA in these samples was confirmed by oxidation of the MTA to MTA-sulfoxide with hydrogen peroxide, followed by column chromatography of the MTA-sulfoxide on a cation exchange column (ammonium acetate + acetic acid elution). We were unable to detect MTA by this procedure in normal urine samples, but the sensitivity of the assay was limited by the presence of interfering fluorescing materials.

DISCUSSION

We have identified an unknown adenine ribonucleoside that is present in increased levels in two of six severe combined immunodeficiency patients as MTA-sulfoxide. Although the compound is obviously the oxidation product of MTA, the mechanism of oxidation is presently unknown. Three possibilities exist: (a), oxidation by liver microsomes as a normal mechanism of biotransformation; (b), auto-oxidation in vivo by endogenous hydrogen peroxide, superoxide or molecular oxygen; and (c), oxidation that occurs during the isolation procedures used to purify the compound prior to identification. At present, we have not been able to establish which of the above possibilities, or a combination of them, is responsible for our finding of MTA-sulfoxide in urine by our isolation procedure.

The studies on the identification of MTA in urine and on the oxidation of MTA to MTA-sulfoxide are consistent with the interpretation that some, but not all, of the MTA-sulfoxide isolated from urine is formed from MTA during the isolation procedure. Prior to the present report, neither MTA nor MTA-sulfoxide had been identified in urine. Whether the MTA-sulfoxide noted in the urine is excreted into the urine as MTA-sulfoxide or whether it is excreted as MTA and converted to MTA-sulfoxide during the isolation procedure, the isolated MTA-sulfoxide is still clearly a urinary end-product of endogenous MTA.

The preliminary studies on MTA in urine are being continued in order to devise a more sensitive assay for MTA, and one that might be used for its detection in normal urine. A sensitive MTA assay might

prove particularly useful, since a deficiency of MTA phosphorylase has been noted recently in some human tumor cell lines[12].

If oxidation of MTA occurs enzymatically, the MTA-sulfoxide should be stereospecific since thioether sulfoxides exhibit optical activity. Thioethers have been shown to undergo stereospecific oxidation to sulfoxides by rat liver microsomes in vitro[13]. At present, we have not had a sufficient amount of our isolated MTA-sulfoxide to compare its optical activity with that of MTA-sulfoxide produced by chemical oxidation of MTA. Consequently, we have not been able to use this unique property of an enzymatically produced compound as a means of clarifying the mechanism of its production.

MTA is a product of polyamine synthesis, being produced in stoichiometric amounts during the biosynthesis of spermidine and spermine. Intracellular concentrations of MTA appear to be far below those of polyamines as a consequence of the rapid metabolism of MTA[14]. MTA is converted to methionine and adenine by the successive action of (a), MTA phosphorylase, (b), an unidentified enzyme which converts 5-methylthioribose phosphate to 2-keto-4-methylthiobutyrate[15], and (c), glutamine aminotransferase. Backlund, et al.[15] also noted that when MTA was incubated in the absence of Mg^{++} in their rat liver homogenate incubation system, an unknown compound accumulated. This compound may have been MTA-sulfoxide since it had an R_f value of 0.34 during paper chromatography in butanol/acetic acid/water. Schlenk, et al.[4] have reported an R_f value of 0.36 for MTA-sulfoxide by paper chromatography using this same solvent system. These studies would be consistent with the possibility that MTA-sulfoxide may be produced endogenously.

Both the unknown compound identified by Hirschhorn, et al.[2] and MTA-sulfoxide are converted to adenine by acid hydrolysis and both of these compounds have chromatographic properties that are similar in many respects to those of 2'-O-methyladenosine. Since both compounds form complexes with borate indicating that they contain cis-diol groups, they clearly differ in this respect from 2'-O-methyladenosine. However, MTA-sulfoxide is clearly distinguished from the unknown nucleoside of Hirschhorn, et al. by the difference in acid lability. They reported that their unknown compound was much more acid labile than adenosine, whereas, MTA-sulfoxide is hydrolyzed in acid one-twelfth as rapidly as adenosine. The two compounds also differ in susceptibility to deamina-

tion by calf intestinal adenosine deaminase. The adenine ribonucleoside of Hirschhorn and co-workers was deaminated by adenosine deaminase, albeit more slowly than adenosine. We found no detectable deamination of MTA-sulfoxide in 24 hr under conditions where an equivalent amount of adenosine was completely deaminated in one minute. These two differences in properties clearly indicate that the adenine nucleoside of Hirschhorn, et al. is not MTA-sulfoxide.

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REFERENCES

1. Mills, G.C., Schmalstieg, F.C., Koolkin, R.J. and Goldblum, R.M. (1982) Biochem. Med. 27, 37.
2. Hirschhorn, R., Ratech, H., Rubinstein, A., Papageorgiou, P., Kesarwala, H., Gelfand, E. and Roegner-Maniscalco, V. (1982) Pediat. Res. 16, 362.
3. Mills, G.C., Schmalstieg, F.C., Trimmer, K.B., Goldman, A.S. and Goldblum, R.M. (1976) Proc. Nat. Acad. Sci. USA 73, 2876.
4. Schlenk, F., Zydek-Cwick, C.R. and Hutson, N.K. (1971) Arch. Biochem. Biophys. 142, 144.
5. Brown, A.H. (1946) Arch. Biochem. 11, 269.
6. Mills, G.C. (1982) J. Chromat. 242, 103.
7. Mills, G.C., Goldblum, R.M., Newkirk, K.E. and Schmalstieg, F.C. (1978) Biochem. Med. 20, 180.
8. The isolation and quantitation of 1-methylguanine and four other modified nucleosides from these urine samples will be described in a separate communication.
9. Mills, G.C., Foster, N.G. and Goldblum, R.M. (1981) Biochem. Med. 26, 90.
10. Pegg, A.E. and Williams-Ashman, H.G. (1969) J. Biol. Chem. 244, 682.
11. Gin, J.B. and Dekker, C.A. (1968) Biochemistry 7, 1413.
12. Kamatani, N. and Carson, D.A. (1980) Cancer Res. 40, 4178.
13. Hajjar, N.P. and Hodgson, E. (1982) Biochem. Pharmacol. 31, 745.
14. Williams-Ashman, H.G., Seidenfeld, J. and Galletti, P. (1982) Biochem. Pharmacol. 31, 277.
15. Backlund, P.S., Chang, C.P. and Smith, R.A. (1982) J. Biol. Chem. 257, 4196.

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