Phosphate-Stimulated Breakdown of 5'-Methylthioadenosine by Rat Ventral Prostate

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A soluble enzyme preparation catalysing the release of adenine from 5'-methylthioadenosine was purified some 30-fold from extracts of the rat ventral prostate. This reaction was completely dependent on addition of inorganic phosphate ions to the assay medium. This absolute requirement for phosphate ions suggests a phosphorolytic cleavage mechanism. After acid treatment, the other product of the reaction appeared to be 5-methylthioribose. The actions of some other well-characterized enzymes of nucleoside metabolism of 5'-methylthioadenosine were also investigated; purified purine nucleoside phosphorylases from calf spleen and human erythrocytes did not attack 5'-methylthioadenosine. The role of 5'-methylthioadenosine in mammalian tissues is discussed.

Pegg & Williams-Ashman (1968*a*, 1969) have delineated the properties of a partially purified soluble enzyme system from rat ventral prostate that catalyses the overall reaction:

With crude prostatic extracts, this stoichiometry was not apparent because of rapid destruction of 5'-methylthioadenosine. The enzymic degradation of 5'-methylthioadenosine by rat ventral prostate would also account for the observation (Rhodes & Williams-Ashman, 1964) that this organ in sexually mature rats contained less than 0.2 μmole of 5'-methylthioadenosine/g. fresh weight of tissue, despite the presence of 5–7 μmoles of both spermidine and spermine/g. (a considerable proportion of these polyamines may be present extracellularly in prostatic secretion). In addition to being a by-product of the biosynthesis of spermidine, and apparently of spermine as well (Pegg & Williams-Ashman, 1969), 5'-methylthioadenosine is produced during the enzymic formation of homoserine lactone from *S*-adenosylmethionine by some micro-organisms (Shapiro & Mather, 1958; Mudd, 1959).

The mechanism(s) involved in the enzymic breakdown of 5'-methylthioadenosine in mam-

malian tissues do not appear to have been examined previously. A variety of bacteria contain nucleosidases that catalyse the hydrolytic degradation of 5'-methylthioadenosine to yield adenine and other products (Shapiro & Mather, 1958; Duerre, 1962). A partially purified preparation from *Escherichia coli* also hydrolysed *S*-adenosyl-L-homocysteine to give adenine and *S*-ribosyl-L-homocysteine (Duerre, 1962). Evidence presented by Schlenk & Ehninger (1964) suggested that in *Candida utilis* *S*-adenosyl-L-methionine can be formed from 5'-methylthioadenosine without cleavage of the latter molecule. In this connexion it is noteworthy that Geffer, Hausmann, Gold & Hurwitz (1966) demonstrated a feeble rate of non-enzymic condensation of γ -aminobutyrolactone with 5'-methylthioadenosine to form *S*-adenosylmethionine.

The experiments described below show that 5'-methylthioadenosine is rapidly broken down to yield adenine and other products by a soluble rat ventral-prostate enzyme, which was purified about 30-fold. This enzymic degradation of 5'-methylthioadenosine exhibits a marked requirement for inorganic orthophosphate or arsenate. A possible explanation for the findings is that the enzyme catalyses a phosphorolytic cleavage of 5'-methylthioadenosine to yield adenine and 5-methylthioribose 1-phosphate. It is noteworthy that previously described purine nucleoside phosphorylases (purine nucleoside-orthophosphate ribosyl-transferase, EC 2.4.2.1) do not promote any fast phosphorolytic cleavage of adenine-containing

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nucleosides (Friedkin & Kalckar, 1961; Kim, Cha & Parks, 1968*a,b*; Krenitsky, Elion, Henderson & Hitchings, 1968).

MATERIALS AND METHODS

Materials. L-[*Me*-¹⁴C]Methionine (11 mc/m-mole) was purchased from the New England Nuclear Corp., Boston, Mass., U.S.A., and [8-¹⁴C]ATP (32 mc/m-mole) was obtained from Schwarz BioResearch Inc., Orangeburg, N.Y., U.S.A. S-Adenosyl-L-methionine labelled in the methyl group or in the adenine moiety was prepared as previously described (Pegg & Williams-Ashman, 1969). 5'-Methylthioadenosine was prepared from S-adenosyl-L-methionine by the procedure described by Schlenk & Ehninger (1964). 5'-Methylthioribose was obtained by the acid hydrolysis of 5'-methylthioadenosine (Smith & Schlenk, 1952).

Xanthine oxidase (EC 1.2.3.2), adenosine deaminase (EC 3.5.4.4), 5'-AMP deaminase (EC 3.5.4.6), ATP, S-adenosyl-L-methionine, adenosine and adenine were obtained from the Sigma Chemical Co., St Louis, Mo., U.S.A. Purine nucleoside phosphorylase (EC 2.4.2.1) from calf spleen was purchased from Boehringer Corp., New York, N.Y., U.S.A., and crystalline purine nucleoside phosphorylase from human erythrocytes (Agarwal & Parks, 1969) was a generous gift from Dr R. E. Parks, jun. Hepes* was a product of the California Corporation for Biochemical Research, Los Angeles, Calif., U.S.A.

Analytical procedures. Protein concentration was determined by the method of Lowry, Rosebrough, Farr & Randall (1951) with crystalline bovine serum albumin as a standard. Descending paper chromatograms were run for 14 hr. on strips of Whatman 3MM paper, 45 cm. long and 4 cm. wide. Solvent A was butan-1-ol-acetic acid-water (12:3:5, by vol.). Solvent B was propan-2-ol-formic acid-water (7:1:2, by vol.). Solvent C was isobutyric acid-5M-NH₃ (5:3, v/v). U.v.-absorbing substances were detected with a Mineralight, model UVS11, and sulphur-containing components were determined by spraying with potassium iodoplatinate (Winegard & Toennies, 1948). After chromatography of radioactive compounds, the dried chromatogram was cut into strips 1 cm. wide, which were placed in vials with 15 ml. of scintillation fluid (Pegg & Williams-Ashman, 1969), and their radioactivity was counted with the Nuclear-Chicago no. 722 liquid scintillation system. The counting efficiency for ¹⁴C was approximately 55%.

Enzyme assays. Decomposition of 5'-methylthioadenosine by prostatic extracts was followed by measuring the formation from 5'[*Me*-¹⁴C]-methylthioadenosine of labelled material not retained by Dowex 50 (H⁺ form) in 0.3M-trichloroacetic acid. The assay medium contained 25 μmoles of sodium Hepes buffer, pH 7.5 (adjusted with 5M-NaOH), 2.5 μmoles of KH₂PO₄ (adjusted to pH 7.5 with 5M-KOH), 0.5 μmole of 5'[*Me*-¹⁴C]-methylthioadenosine (0.5 c.p.m./pmole) and the enzyme protein in a total volume of 0.5 ml. After incubation at 37° for 30 min., the reaction was terminated by the addition of 0.5 ml. of 0.6M-trichloroacetic acid. Any protein precipitate was removed by centrifugation. The supernatant was applied to a small column (3 cm. × 0.2 cm.) of Dowex 50 (H⁺ form); [the resin Dowex 50W (X2; 100–200 mesh) was converted into the H⁺ form by wash-

ing with 20 vol. of 5M-HCl followed by washing with water until the pH of the effluent approached neutrality]. After application of the sample, the column was washed with 5 ml. of 0.3M-trichloroacetic acid. A 0.5 ml. sample of the combined eluates was then added to 15 ml. of scintillation fluid and its radioactivity was counted. Results were expressed as nmoles of 5'-methylthioadenosine decomposed/mg. of protein added, and were corrected for the small amount of radioactivity (less than 100 c.p.m.) present in the eluate from the Dowex column when no enzyme was added.

Formation of adenine from 5'-methylthioadenosine was measured by two methods: first, by measurement of the increase in *E*₃₀₅ in the presence of excess of xanthine oxidase (Klenow, 1952), which converts the adenine formed into 2,8-dihydroxyadenine; secondly, 5'-methylthioadenosine labelled with ¹⁴C in the adenine moiety was prepared and used as substrate. After incubation at 37° for 30 min., the reaction was terminated by the addition of an equal volume of 0.3M-trichloroacetic acid. The protein precipitate was removed by centrifugation and the supernatant was extracted twice with 5 ml. of ether to remove the trichloroacetic acid. A 0.1 ml. sample of the solution was applied to Whatman 3MM paper and chromatographed in solvent A, which separates adenine from 5'-methylthioadenosine (Schlenk & Ehninger, 1964). The dried chromatogram was cut into 1 cm. strips and its radioactivity was counted.

Deamination of adenine nucleosides was measured by recording the decrease in *E*₂₆₅ (Kalckar, 1947*a*) with a Gilford recording spectrophotometer. The temperature of the cuvette was maintained at 25°.

Animals. Adult male rats of the Sprague-Dawley strain weighing 250–350 g. were fed on a diet of rat cake and water *ad libitum*.

Enzyme preparations. Rats were killed by cervical dislocation and the ventral prostates dissected free of fat and connective tissue *in situ*. The ventral prostate tissue was then minced with scissors and homogenized, in a glass apparatus equipped with a Teflon pestle, with 4 vol. of ice-cold 0.25M-sucrose containing 0.3 mM-EDTA (disodium salt, adjusted to pH 7.4 with M-NaOH). All operations were carried out at 2°. The homogenate was centrifuged at 10000*g* for 10 min. and the precipitate discarded. The supernatant was centrifuged at 45000 rev./min. for 90 min. in the no. 50 rotor of a Spinco model L-2 ultracentrifuge. After ultracentrifugation, the pellet was removed and the supernatant passed through a glass funnel containing a plug of glass wool.

The ultracentrifuged prostatic extract was fractionated by the addition of solid (NH₄)₂SO₄ of low heavy-metal content (obtained from Mann Research Laboratories Inc., New York, N.Y., U.S.A.) to give 40% saturation. The solution was stirred for 30 min. and then centrifuged at 12000*g* for 15 min. The precipitate was discarded and additional (NH₄)₂SO₄ added to bring the solution up to 55% saturation. After stirring for 30 min. the precipitate was collected by centrifugation at 12000*g* for 15 min. The solutions were maintained at 0° during (NH₄)₂SO₄ fractionation and the pH was kept at 6.8 by the addition of M-NH₃ when necessary. The protein precipitate was then dissolved in 10 mM-Hepes buffer, pH 7.4 (adjusted with M-NaOH), containing 1 mM-2-mercaptoethanol and passed through a small (25 cm. × 1 cm.) desalting column of Bio-Gel P-10, previously equilibrated with the same buffer. The

* Abbreviation: Hepes, 2-(*N*-2-hydroxyethyl)piperazin-*N'*-yl)ethanesulphonic acid.

protein concentration was adjusted to approx. 20 mg./ml. Aged alumina C_γ gel (Sigma), 1 g. in 10 ml. of water, was added for each 10 ml. of solution and the mixture slowly stirred at 0° for 30 min. After centrifugation at 2000g for 10 min. the supernatant was discarded and the gel washed with 20 ml. of 0.1 M-NaH₂PO₄ adjusted to pH 7.4 with 5 M-NaOH. The enzyme activity was then eluted from the gel by two washes with 10 ml. of 0.25 M-NaH₂PO₄ of pH 7.4. During each wash, the gel was slowly stirred for 30 min. and then separated by centrifugation at 2000g for 10 min. The eluates in 0.25 M-NaH₂PO₄, pH 7.4, were combined and solid (NH₄)₂SO₄ was added to give 60% saturation. After standing for 1 hr. the precipitate was centrifuged off at 15000g for 30 min. and stored as a frozen pellet at -20°. Under these conditions the activity was stable for at least 3 weeks. Before being tested, the material was dissolved in 10 mM-Hepes buffer, pH 7.4, containing 1 mM-2-mercaptoethanol, and desalted by passage through a Bio-Gel column as described above.

RESULTS

As shown in Table 1, extracts of the rat ventral prostate readily catalysed the decomposition of 5'-methylthioadenosine. Over 80% of the activity contained in the prostate homogenates was present in the supernatant fraction after centrifugation at 100000g for 90 min. Fractionation of the enzyme by precipitation with ammonium sulphate and adsorption on calcium phosphate gel resulted in a 30-fold purification (Table 1). This preparation was stable for at least 3 weeks when stored at -20° and catalysed the decomposition of approximately 1.6 μmoles of 5'-methylthioadenosine/hr./mg. of protein.

At all stages of purification, and after the enzyme preparations had been desalted, the breakdown of 5'-methylthioadenosine was dependent on the

addition of phosphate ions. Fig. 1 shows that arsenate could partially replace phosphate, but that sulphate, citrate and maleate were inactive. The concentration of arsenate added in this experiment was saturating; it was not possible to obtain the rate of decomposition of 5'-methylthioadenosine observed in the presence of phosphate by adding further quantities of arsenate. In the presence of saturating concentrations of phosphate, the rate of cleavage of 5'-methylthioadenosine was linear with time for up to 30 min. and then declined. In other experiments (not shown), in which large amounts of enzyme were added, at least 85% of the added 5'-methylthioadenosine was degraded. A maximal rate of reaction was obtained in the presence of 5 mM-NaH₂PO₄ and the approximate *K_m* for phosphate was found to be 0.6 mM (Fig. 2).

The optimum pH for breakdown of 5'-methylthioadenosine by the prostatic enzyme was about 7.5. The rate of reaction decreased considerably below pH 6.5 and above pH 8.5. No requirement for a metal ion was observed; Mg²⁺, Mn²⁺ and Ca²⁺ were tested at concentrations from 1.0 to 20.0 mM.

Table 1. *Partial purification of 5'-methylthioadenosine-cleaving enzyme*

About 5 g. wet wt. of ventral prostate was obtained from 12 rats and treated as described in the Materials and Methods section. The enzyme activity of the various fractions was assayed in the standard assay medium, with incubation at 37° for 15 min.

Fraction	Protein (mg.)	Specific activity (μmole of 5'-methylthioadenosine split/mg. of protein/15 min.)	Recovery (%)
Ultracentrifuged extract	245	0.014	100
(NH ₄) ₂ SO ₄ ppt. (40-55%)	48	0.053	74
Alumina C _γ gel eluate	6	0.350	61
(NH ₄) ₂ SO ₄ ppt. (0-60%)	4	0.425	49

Fig. 1. Effect of various anions on the decomposition of 5'-methylthioadenosine by prostatic extracts. The assay medium contained 25 μmoles of sodium Hepes buffer, pH 7.5, 0.5 μmole of 5-[Me-¹⁴C]-methylthioadenosine (0.5 c.p.m./pmole), about 0.01 mg. of enzyme protein and the addition shown, in a total volume of 0.5 ml. The additions were 5 μmoles of NaH₂PO₄ (●), 12.5 μmoles of Na₂HAsO₄ (○), 12.5 μmoles of Na₂SO₄ (△), 12.5 μmoles of trisodium citrate (▲) or 12.5 μmoles of disodium maleate (□). All of these solutions were adjusted to pH 7.5 with M-NaOH or M-HCl as required before addition to the assay medium. The tubes were then incubated at 30° for the times shown.

Fig. 2. Effect of phosphate concentration on cleavage of 5'-methylthioadenosine by prostate enzyme. (a) The assay medium contained 25 μ moles of sodium Hepes buffer, pH 7.5, 0.5 μ mole of 5' [$Me-^{14}C$] methylthioadenosine (0.5 c.p.m./p mole), about 0.02 mg. of enzyme protein and the concentration of NaH_2PO_4 (adjusted to pH 7.5 with NaOH) shown, in a total volume of 0.5 ml. The tubes were incubated for 15 min. at 37°. (b) Double-reciprocal plot of the same data, giving K_m (phosphate) approx. 0.6 mM.

Fig. 3. Effect of concentration of 5'-methylthioadenosine on its decomposition by prostatic enzyme. (a) The assay medium contained 5 μ moles of NaH_2PO_4 , 25 μ moles of sodium Hepes buffer, pH 7.5, 0.02 mg. of enzyme protein and 5' [$Me-^{14}C$] methylthioadenosine (0.5 c.p.m./p mole) at the concentrations shown in a total volume of 0.5 ml. Incubation was for 15 min. at 37°. (b) Double-reciprocal plot of the same data, giving K_m (5'-methylthioadenosine) approx. 0.3 mM.

A maximal rate of reaction occurred in the presence of 1.0 mM-5'-methylthioadenosine and an approximate K_m of 0.3 mM was obtained (Fig. 3). The addition of 1 mM-S-adenosyl-L-methionine had no effect on the decomposition of 5'-methylthioadenosine. However, L-methionine was a weak inhibitor of the reaction, causing 20% inhibition at 50 mM. High concentrations of 2-mercaptoethanol (20 mM) resulted in approx. 50% inhibition when added to the standard assay medium.

The labelled product of the decomposition of [$Me-^{14}C$]5'-methylthioadenosine, isolated after passage through Dowex 50 H^+ from in 0.3 M-trichloroacetic acid, was subjected to paper chromatography (solvents A and B) and exhibited R_F values similar to those of authentic 5-methylthioribose in each case. The material also gave a positive test

with the iodoplatinate spray for sulphur-containing components, and did not absorb u.v. light. The material was therefore tentatively identified as 5-methylthioribose. In view of the complete requirement for phosphate ions for the decomposition of 5'-methylthioadenosine, it seemed likely that a phosphorolytic splitting mechanism was involved. The product from such a mechanism would be expected to be 5-methylthioribose 1-phosphate. The acid conditions used to isolate the 5' [$Me-^{14}C$] methylthioribose after decomposition of 5' [$Me-^{14}C$] methylthioadenosine by the prostatic enzyme would be likely to cause hydrolysis of the putative acid-labile 5' [$Me-^{14}C$] methylthioribose 1-phosphate (Kalckar, 1947b). In experiments designed to attempt to isolate this product, the reaction was halted by the addition of 2 ml. of ice-

cold water and the tubes were placed in an ice bath. As quickly as possible the diluted reaction mixtures were applied to small columns (3cm. $\times$ 0.2cm.) of Dowex AG1 (X10; formate form; 200–400 mesh; Bio-Rad Laboratories, Richmond, Calif., U.S.A.), which would bind the negatively charged putative 5[Me- 14 C]-methylthioribose 1-phosphate. The column was then washed with 10ml. of water, which eluted 5'-methylthioadenosine and 5-methylthioribose. Further elution with 0.1M-sodium formate (adjusted to pH 5.0 with formic acid) eluted other labelled material, which on treatment with 0.5M- H_2SO_4 for 10min. at 37° was completely converted into a compound with similar chromatographic properties to 5-methylthioribose. However, the amount of material binding to the AG 1 column was rather variable from one experiment to another and comprised at most only 30% of the radioactivity found in 5-methylthioribose under the standard assay conditions. Owing to the instability of this compound and to the limited amounts obtainable, it was not possible to identify it definitively as 5-methylthioribose 1-phosphate. The small yield of this material even under mild extraction conditions may well be due to the presence in the prostatic enzyme preparation of phosphatases capable of removing the phosphate from 5-methylthioribose 1-phosphate. It is well known that the rat prostate exhibits pronounced acid- and alkaline-phosphatase activities (Price & Williams-Ashman, 1961).

The nature of the other product of breakdown of 5'-methylthioadenosine by prostatic extracts was investigated by using substrate labelled in the adenine moiety. After incubation the assay medium was deproteinized and a portion subjected to paper chromatography with solvents A and C. The only radioactive material detected other than 5'-methylthioadenosine had R_F value corresponding to adenine in each solvent system. The appearance of this product was completely dependent on the addition of phosphate ions to the assay medium. After elution of the labelled product from the paper by 1ml. of 5mM- NaH_2PO_4 , pH 7.0, the u.v.-absorption spectrum was similar to that of an authentic adenine standard, with a maximum at 260nm. There was no trace of hypoxanthine, which would be formed if the 5'-methylthioadenosine were deaminated before removal of the sugar. The formation of adenine from 5'-methylthioadenosine could also be demonstrated in a coupled assay system involving addition of excess of xanthine oxidase to the assay medium. Xanthine oxidase has been shown to oxidize adenine to 2,8-dihydroxyadenine, which has a maximum extinction at 305nm. (Klenow, 1952). At this wavelength, 5'-methylthioadenosine barely absorbs light and the molar extinction coefficient for 2,8-dihydroxy-

Table 2. *Stoichiometry of formation of adenine and 5-methylthioribose from 5'-methylthioadenosine by prostatic enzyme*

This table shows the results of three separate experiments with the same concentration of reactants and enzyme. [8- 14 C]Adenine released from 5'-methylthio[8- 14 C]adenosine was measured by chromatographic separation (method A). Adenine released from 5'-methylthioadenosine was determined by the increase in E_{305} in the presence of excess of xanthine oxidase (method B). [Me- 14 C]-Methylthioribose formed from 5[Me- 14 C]-methylthioadenosine was determined by method C. The assay media contained 50mM-sodium Hepes buffer, pH 7.5, 10mM- NaH_2PO_4 (adjusted to pH 7.5 with M-NaOH), 0.01mg. of enzyme protein and 1.0mM-5'-methylthioadenosine. Approx. 0.3 unit of xanthine oxidase was added to the medium of method B, which was assayed in a cuvette maintained at 30° by a circulating water bath. A molar extinction coefficient of 15500 for 2,8-dihydroxyadenine at 305 nm. was assumed (Klenow, 1952). The tubes in methods A and C were incubated at 30° in a shaking water bath.

Incubation time (min.)	Adenine formed (μ mole)		5-Methylthioribose formed (μ mole) Method C
	Method A	Method B	
8	0.012	0.009	0.012
16	0.025	0.020	0.023
24	0.034	0.033	0.035
32	0.047	0.043	0.045
40	0.051	—*	0.055
48	0.058	—*	0.064

* Not determined owing to limited solubility of 2,8-dihydroxyadenine.

adenine at pH 7.4 is 15500. In the presence of the prostatic enzyme xanthine oxidase and 5'-methylthioadenosine, the addition of phosphate led to an increase in the E_{305} that could be followed on a recording spectrophotometer. The increase in the E_{305} was greater than that in the E_{290} , which provides additional evidence that adenine rather than hypoxanthine was being oxidized by the xanthine oxidase. Table 2 shows the results of parallel experiments with similar amounts of the same enzyme preparation and similar concentrations of reagents to measure both products of 5'-methylthioadenosine decomposition. There was close stoichiometry between the amounts of 5-methylthioribose produced and adenine formed. The latter was measured both by the formation of [8- 14 C]-adenine from 5'-methylthio[8- 14 C]adenosine and by the xanthine oxidase method. There was excellent agreement between the two methods. Unfortunately, the apparently simple coupled assay (which does not require labelled substrates) has two major

disadvantages: the reaction of xanthine oxidase with adenine is slow, necessitating the addition of a large amount of xanthine oxidase, and the product 2,8-dihydroxyadenine, has very limited solubility (Bendich, Brown, Philips & Thiersch, 1950).

The possibility that 5'-methylthioadenosine was a substrate for some well-characterized mammalian enzymes acting on nucleosides was also investigated. Adenosine deaminase from calf intestine catalysed no detectable deamination of 5'-methylthioadenosine under conditions at which a rate 750-fold less than that of the deamination of comparable concentrations of adenosine could have been detected. 5'-AMP deaminase from rabbit muscle also did not attack 5'-methylthioadenosine at a rate more than one-three hundredth of the rate of deamination of 5'-AMP. Purine nucleoside phosphorylases from human erythrocytes and from calf spleen did not catalyse any phosphorolytic cleavage of 5'-methylthioadenosine. The rate of breakdown of 5'-methylthioadenosine that could have been detected in the present experiments was 500-fold less than the rate of cleavage of inosine. In all of these studies the concentration of 5'-methylthioadenosine was 0.2 mM. Schaedal, Waldvogel & Schlenk (1947) have also reported that 5'-methylthioadenosine is not attacked by adenosine deaminase or by a partially purified purine riboside phosphorylase from rat or rabbit liver.

The homogenates of the rat ventral prostate exhibit considerable adenosine deaminase activity (1 mg. of protein from the ultracentrifuged supernatant extract catalysed the deamination of 0.02 μ mole of adenine/hr. at 25°). And although phosphate-dependent cleavage of adenosine did occur with crude prostate extracts, it is conceivable that the adenosine was first deaminated. However, the prostatic adenosine deaminase was similar to the highly purified enzyme from intestine in that neither preparation attacked 6'-methylthioadenosine.

DISCUSSION

The polyamines spermine and spermidine are ubiquitous constituents of mammalian cells and in many tissues occur in appreciable quantities. We have shown that the utilization of putrescine and (-)-S-adenosyl-L-methionine for spermidine synthesis discovered by Tabor & Tabor (1964) in extracts of *E. coli* also occurs in mammalian tissues, and that spermine biosynthesis can take place by a similar mechanism (Pegg & Williams-Ashman, 1968a,b, 1969). These reactions form 1 mole of 5'-methylthioadenosine/mole of spermidine, and 2 moles of 5'-methylthioadenosine/mole of spermine. However, the occurrence of large amounts of this nucleoside in mammalian tissues has not been

reported. On the contrary, Smith, Anderson, Overland & Schlenk (1953) stated that the concentration of 5'-methylthioadenosine in muscle, liver and blood is small or insignificant, and that even in rabbits fed with large quantities of methionine the concentration of 5'-methylthioadenosine is only 0.3 μ mole/g. of liver. Normal rat liver contains about 1–2 μ moles of spermidine/g. and 0.7 μ mole of spermine/g. (Jänne, Raina & Siimes, 1964). Rhodes & Williams-Ashman (1964) found less than 0.2 μ mole of 5'-methylthioadenosine/g. and 5–7 μ moles of both spermine and spermidine/g. in the rat ventral prostate. The existence of one or more mammalian enzymes capable of further metabolism of 5'-methylthioadenosine is therefore to be expected, and would be of importance in the conservation of the adenine nucleoside pool, which might otherwise become severely depleted under conditions of sustained polyamine synthesis.

As shown above, adenosine deaminase and 5'-AMP deaminase do not catalyse the deamination of 5'-methylthioadenosine; moreover, purine nucleoside phosphorylase from two mammalian sources does not split this nucleoside. The latter finding is in agreement with the demonstration by numerous other workers that a 6-amino group on the purine moiety leads to a very large decrease in the affinity of purine nucleoside phosphorylase for the substrate and in the rate of reaction catalysed by the enzyme (Friedkin & Kalekar, 1961; Kim *et al.* 1968a,b; Krenitsky *et al.* 1968). Our experiments provide strong evidence for the presence in rat ventral prostate of an enzyme catalysing the phosphorolytic cleavage of 5'-methylthioadenosine, yielding adenine and 5-methylthioribose 1-phosphate. The complete dependence on the addition of phosphate ions for the reaction to proceed suggests a phosphorolytic rather than a hydrolytic splitting, but is not completely conclusive. Wang (1955) has described a pyrimidine nucleosidase from *Lactobacillus pentosus* that is stimulated by phosphate ions but does not form ribose 1-phosphate. In this case, however, other multivalent anions were as effective as phosphate in increasing nucleosidase activity, probably by stabilization of the enzyme, whereas the prostatic enzyme shows a specific requirement for phosphate or arsenate. The partially purified enzyme preparation described in this paper contains appreciable phosphatase activity (A. E. Pegg & H. G. Williams-Ashman, unpublished work), which may at least in part account for the low yield of material with the properties of 5-methylthioribose 1-phosphate.

Hurwitz, Heppel & Horecker (1957) have described an enzyme of microbial origin that catalyses the hydrolysis of 5'-AMP to adenine and ribose 5-phosphate. This reaction required ATP, which did not, however, appear to function as a reactant.

This conversion of 5'-adenylic acid into adenine and ribose 5-phosphate is hardly analogous to the phosphate-dependent splitting of 5'-methylthioadenosine described in the present paper. Also, Kornberg, Lieberman & Sims (1955) have described an inorganic pyrophosphate-dependent degradation of certain purine nucleoside 5'-phosphates, including 5'-AMP, to the free base and 5-phosphoribosyl 1-pyrophosphate. We did not examine the possible pyrophosphorolytic cleavage of 5'-methylthioadenosine in the prostate gland or other mammalian tissues. This may be worthy of experimental scrutiny.

When assayed under optimum conditions, the rate of decomposition of 5'-methylthioadenosine by crude rat ventral-prostate extracts corresponds to approx. $25 \mu\text{mole/hr./g. wet wt. of tissue at } 37^\circ$. This value is much greater than the activities of the enzyme(s) forming spermidine and spermine (Pegg & Williams-Ashman, 1968a,b, 1969) and could account for the lack of accumulation of significant amounts of 5'-methylthioadenosine in this gland. However, additional enzymic pathways for degradation of 5'-methylthioadenosine may exist in this and other mammalian tissues. The further metabolism of the sugar, 5-methylthioribose (formed from 5'-methylthioadenosine), does not appear to have been investigated in mammalian or bacterial cells. The possible occurrence of large quantities of 5-methylthioribose in the prostate and its secretions, or in other tissues rich in polyamines, is worthy of further investigation.

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