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PURIFICATION AND PROPERTIES OF ATP-SULFURYLASE FROM FURTH MOUSE MASTOCYTOMA

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SUMMARY

1. The enzyme, ATP-sulfurylase (ATP: sulfate adenylyltransferase, EC 2.7.7.4) which catalyzes the reaction, ATP + inorganic sulfate $\rightleftharpoons$ adenylyl sulfate + inorganic pyrophosphate, was purified from a high-speed supernatant of mouse mastocytoma (Furth) by $(NH_4)_2SO_4$ fractionation, hydroxylapatite column chromatography, and Geon resin electrophoresis. The purification resulted in a 545-fold increase in specific activity.

2. The purified enzyme exhibited a pH optimum of 8.5, and it was free of inorganic pyrophosphatase (EC 3.6.1.1), ATP phosphohydrolase (EC 3.6.1.3), adenylylsulfate kinase (EC 2.7.1.25) and adenylylsulfate sulfohydrolase.

3. The enzyme did not show an absolute requirement for metal ions, but its activity was increased by Mn^{2+}, Mg^{2+}, and Zn^{2+}. EDTA was a powerful inhibitor of the ATP-sulfurylase; this inhibition was reversed by Mn^{2+}, Mg^{2+}, Cu^{2+}, Co^{2+} and Zn^{2+}. 2 mM solutions of Ni^{2+}, Ba^{2+} and Ca^{2+} inhibited the enzyme.

4. The enzyme reacted only with adenylylsulfate or deoxyadenylylsulfate and inorganic pyrophosphate as substrates (ATP sulfurylase reaction in reverse). Nucleotidylsulfates other than adenylylsulfate inhibited the reaction, when added to the latter as substrate.

5. The reaction catalyzed by the mastocytoma ATP-sulfurylase exhibited saturation phenomena, but it did not strictly follow Michaelis–Menten kinetics.

INTRODUCTION

It was reported recently that the enzyme ATP-sulfurylase (ATP: sulfate adenylyltransferase, EC 2.7.7.4) of mouse mastocytoma (Furth), partially purified by $(NH_4)_2SO_4$ fractionation and gel filtration, was resolved into two distinct peaks upon

Abbreviations: APS, adenylylsulfate; dAPS, deoxyadenylylsulfate; PAPS, 3'-phosphoadenylylsulfate; GPS, guanylylsulfate; IPS, inosylylsulfate.

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chromatography on DEAE-cellulose[1]. In the meantime, a technique for the purification of this enzyme was developed, and some of the properties of the purified ATP-sulfurylase were studied. The results of these experiments are summarized in the present communication.

MATERIALS AND METHODS

Chemicals

Chemicals were C. P. or reagent grade, or the purest preparations available. ADP, ATP, IMP, ECTEOLA-cellulose (Cellex-E), hydroxylapatite (Bio-Gel-HTP), L-cysteine hydrochloride, glutathione (reduced), β-mercaptoethanol, Cleland's reagent and histamine (free base) were obtained from Calbiochem; AMP, Tris (Trizma Base) and firefly luciferase extract from Sigma Chemical Co.; dAMP, GMP, CMP, UMP and cyclic AMP from P-L Biochemicals, Inc.; cupferron, dithizone and 8-hydroxyquinoline from Matheson, Coleman and Bell; 2,2'-bipyridine hydrochloride ("bipyridyl") from G. F. Smith Chemical Co.; EDTA from Eastman Organic Chemicals; Geon resin from Goodrich Chemical Co.; serotonin creatinine sulfate from Mann Research Laboratories; adenosine from Nutritional Biochemicals; inorganic pyrophosphatase (pyrophosphate phosphohydrolase, EC 3.6.1.1) from Worthington Biochemical Corp.; ^{32}P-labeled sodium pyrophosphate and $Na_2{}^{35}SO_4$ from New England Nuclear. Heparin sodium, U.S.P. (161 units/mg) was a gift of Riker Laboratories.

Preparation of adenylylsulfate (APS)

APS was prepared from AMP and pyridine sulfurtrioxide according to the method of BADDILEY *et al.*[2], and purified by chromatography on ECTEOLA-cellulose. 4 ml of reaction mixture[2] was diluted to 200 ml and applied to a column of ECTEOLA-cellulose (2.5 cm — 35 cm), previously equilibrated with 0.02 M NH_4HCO_3. The column was then washed with 500 ml of the same buffer solution, and the nucleotides were eluted with an NH_4HCO_3 gradient[3] (1 l 0.6 M NH_4HCO_3 in reservoir, 1 l 0.02 M NH_4HCO_3 in mixing bottle). The flow rate was adjusted to 60 ml/h, and

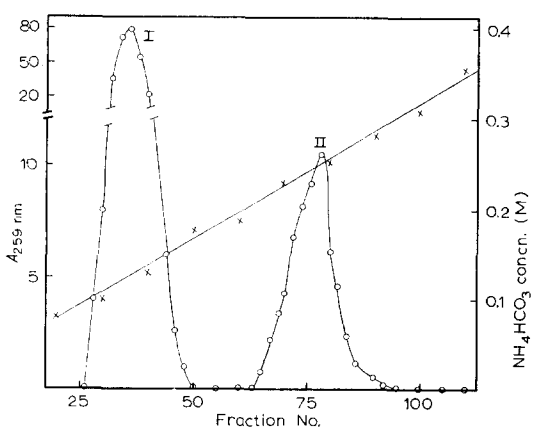


Fig. 1. Separation of APS from AMP by chromatography on ECTEOLA-cellulose. Gradient elution with NH_4HCO_3. Experimental details are described in text. ○—○, $A_{259\ nm}$; ×—×, NH_4HCO_3 concentration. Peak I, AMP; Peak II, APS.

10-ml fractions were collected. The elution profile (Fig. 1) shows that AMP (Peak I) and APS (Peak II) were completely separated. Fractions 65–85 (Peak II) were pooled, and NH_4HCO_3 was removed from the APS preparation by lyophilization[3]. APS was characterized by paper chromatography using isobutyric acid–0.5 M ammonia (5:3, v/v) and *n*-propanol–ammonia (*d*, 0.88)–water (6:3:1, by vol.) as solvents, or by electrophoresis in 0.05 M ammonium acetate, pH 8.5 (ref. 2). Authentic samples of AMP, ADP ond ATP were used as reference standards. The identity of APS was further established by determining its ultraviolet absorption spectrum (at pH 2 and 12, λ_{max} was found to be 257 and 259 nm, respectively). The APS was found to contain adenine, P_i and sulfate in the ratio of 1.00:1.02:0.93. The other analogs of APS, such as guanylylsulfate (GPS), deoxyadenylylsulfate (dAPS) and inosylylsulfate (IPS) were prepared and purified in a similar manner.

Determination of ATP-sulfurylase activity

ATP-sulfurylase activity was measured by following the ATP formation from APS and PP_i (ATP-sulfurylase reaction in reverse which is favored energetically)[4]. The reaction mixture, containing 20 μmoles Tris–HCl (pH 8.5), 0.4 μmole APS, 0.75 μmole PP_i, 0.4 μmole $MnCl_2$, and enzyme, in a total volume of 0.2 ml, was incubated at 37° for 10–30 min. These incubation periods lay within the linear portion of the time curve (Fig. 4). In the control tube, either boiled enzyme was used, or the enzyme was added after incubation. The reaction was stopped by immersing the tubes in boiling water for 60 sec. After chilling in ice water, the sample was diluted to 25 ml with 0.02 M P_i buffer, pH 7.4. An aliquot was taken to determine ATP. In some experiments the disappearance of PP_i was used as an index of enzyme activity[4]; in this case, the reaction mixture, after incubation, boiling for 60 sec and chilling in ice, was further incubated with an excess of inorganic pyrophosphatase at 37° for 15 min, and liberated P_i was measured.

Chemical determinations and characterizations

NH_4HCO_3 was estimated by measuring NH_3 according to the method of CHANEY AND MARBACH[5]. Adenine was determined on the basis of its ultraviolet absorbance, P_i according to the method of AMES[6] or that of FISKE AND SUBBAROW[7], sulfate according to that of ANTONOPOULOS[8], and protein according to that of LOWRY *et al.*[9]. ATP was measured using firefly luciferase and a liquid scintillation counter[10–12]. Counting intervals of 6 sec were employed, starting 14 sec after addition of the luciferase. A standard ATP solution was always included to correct for variations in activity of the firefly extract.

For further characterization of the ATP formed from APS and PP_i by the action of ATP sulfurylase, the triphosphate was identified by paper chromatography of the reaction mixture in *n*-propanol–ammonia (*d*, 0.88)–water (6:3:1, by vol.)[2], and by paper electrophoresis (Whatman No. 1) in 0.05 M P_i buffer, pH 8.5 (8 V/cm, 4.5 h). In the electrophoresis experiments, PP_i labeled with ^{32}P was used as one of the substrates incubated with the enzyme. In these experiments, one radioactive peak corresponding to [^{32}P]ATP was found after incubation with ATP sulfurylase. In control samples incubated without enzyme, ^{32}P-labeled PP_i remained unchanged.

Inorganic sulfate, the second product of the reversed ATP sulfurylase reaction, was characterized in a similar manner by incubating ^{35}S-labeled APS as one of the

substrates with enzyme, and then subjecting the reaction mixture to paper electrophoresis under conditions as described above. In samples incubated with enzyme, one radioactive spot corresponding to ^{35}S-labeled sulfate was identified, but in control samples without enzyme, or with boiled enzyme, [^{35}S]APS was the only radioactive compound detected.

Purification of ATP sulfurylase

Furth mouse mast cell tumors were carried and harvested as reported earlier from this laboratory[13]. The following operations were carried out at 0–4°, unless otherwise mentioned.

Extraction: 75 g mast cell tumor tissue was homogenized with 150 ml of 0.25 M sucrose in 0.02 M Tris, pH 7.4. The homogenate was centrifuged at 20 000 × *g* for 20 min. The pellet was discarded. The supernatant was centrifuged at 105 000 × *g*

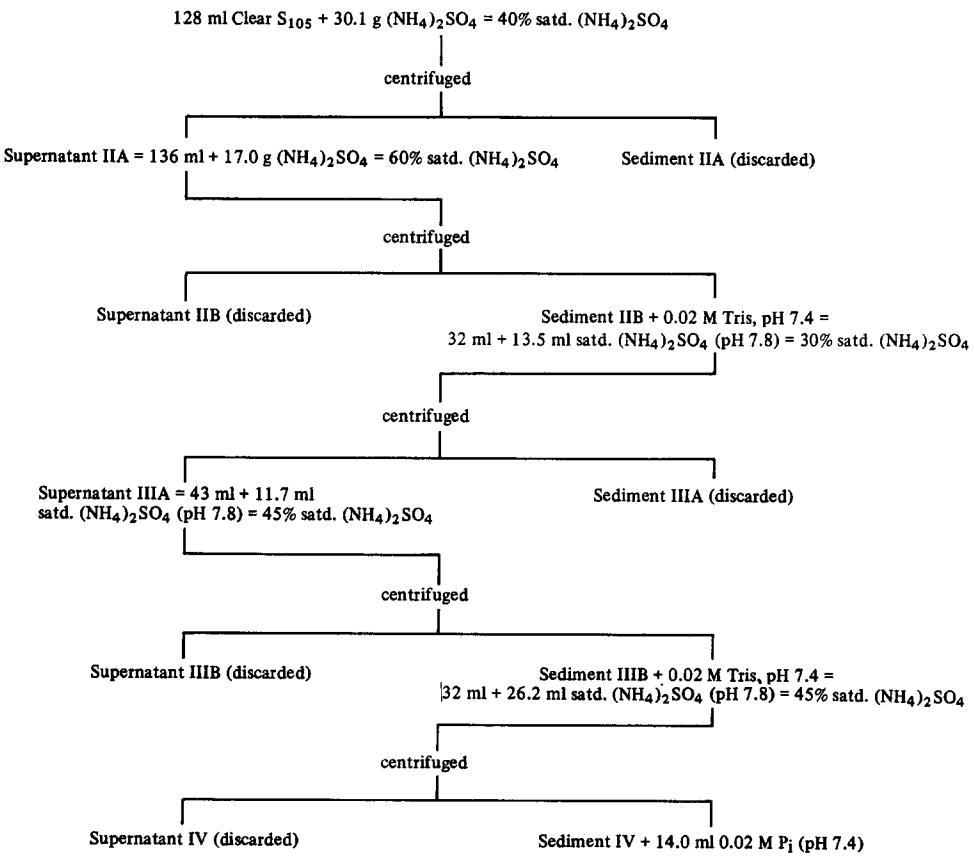


Fig. 2. Purification of ATP-sulfurylase; flow diagram of $(NH_4)_2SO_4$ fractionation. Experimental details are described in the text.

for 2 h. The clear supernatant (designated, Clear S_{105}) was carefully removed; it contained 1.92 g protein in 128 ml.

$(NH_4)_2SO_4$ fractionation: The ATP sulfurylase present in Fraction Clear S_{105} was purified further by a course of successive $(NH_4)_2SO_4$ precipitations, as outlined in Fig. 2. After each addition of $(NH_4)_2SO_4$ indicated, the mixture was centrifuged at 10 000 × *g* for 20 min. The final pellet (Sediment IV) was dissolved in 14 ml of 0.02 M P_i buffer, pH 7.4, and dialyzed overnight against 1 500 ml of the same buffer. It contained 215 mg protein.

Hydroxylapatite column chromatography: The dialyzed protein solution was applied to a hydroxylapatite column (2.2 cm × 16.5 cm) previously equilibrated with 0.02 M P_i buffer, pH 7.4, and the column was washed with 300 ml of 0.11 M P_i buffer of the same pH. The adsorbed material was eluted from the column with 300 ml 0.15 M P_i buffer, pH 7.4; 10-ml fractions were collected and assayed for ATP-sulfurylase activity. Fractions 4–19 which contained enzyme activity were pooled, concentrated by ultrafiltration, and the concentrate was made to 1.0 ml containing 0.1 M P_i buffer, pH 7.4; the final solution contained 31.6 mg protein.

Resin electrophoresis: 250 g of Geon resin was washed several times with water, and then with 0.1 M P_i buffer, pH 7.4. A slurry of the resin in the same buffer was poured into a plastic tray (9 cm × 30 cm). Excess buffer was removed from the resin surface with filter paper. A groove was made in the resin bed about 10 cm from the negative end, and the eluate of the hydroxylapatite column was placed in the groove with a fine-tipped capillary pipette. The groove was then filled with resin and the bed covered with a sheet of Dow Saran wrap to reduce evaporation. For electrophoresis, a potential of 175 V was applied to the resin bed *via* filter paper dipping into electrode chambers containing 0.1 M P_i buffer, pH 7.4, at 4° for 24 h. Then appropriate parts of the resin were cut into 1.5-cm sections, and each segment was eluted with 10 ml of 0.02 M P_i buffer, pH 7.4. The fractions exhibiting activity were pooled and concentrated by ultrafiltration. The concentrate contained 7.5 mg protein.

RESULTS

Some properties of purified ATP sulfurylase

When the tumor was homogenized and the homogenate fractionated as de-

TABLE I

PURIFICATION OF ATP-SULFURYLASE

Step	*Fraction*	*Volume (ml)*	*Protein (mg)*	*Enzyme (units*)*	*Specific activity***
I	Clear S_{105}	128	1920	979	0.510
II	Sediment IIB (40–60% satd. $(NH_4)_2SO_4$)	32	696	5150	7.40
III	Sediment IIIB (30–45% satd. $(NH_4)_2SO_4$)	32	350	5350	15.3
IV	Sediment IV (0–45% satd. $(NH_4)_2SO_4$)	16	215	5920	27.5
V	Hydroxylapatite column chromatography Eluate, 0.15 M potassium phosphate buffer, pH 7.4	1	31.6	3640	115
VI	Resin electrophoresis	4	7.5	2090	279

* Enzyme unit = 1 μmole ATP formed in 30 min under assay conditions.
** Specific activity = enzyme units/mg protein.

scribed above, an ATP sulfurylase preparation was obtained exhibiting a specific activity of 279 enzyme units per mg protein; this represents a 545-fold increase in specific activity, as compared to the high-speed supernatant of the original tumor homogenate (Table I). During the first $(NH_4)_2SO_4$ fractionation (Step II, Sediment IIB) the total enzyme activity increased more than 5-fold (Table I). For this reason, it was not possible to determine the overall yield. The total enzyme activity recovered in the final fraction amounted to about 40% of that found in the product of Step II (Sediment IIB, Table I and Fig. 2). The purified ATP-sulfurylase was found to be free of inorganic pyrophosphatase (EC 3.6.1.1), ATPase (EC 3.6.1.3), APS kinase (EC 2.7.1.25) and APS sulfohydrolase. When subjected to gel filtration using Sephadex G-200, the ATP-sulfurylase was excluded. Experiments using disc gel electrophoresis yielded inconsistent results. In some experiments, only two distinct bands were seen, in others a few additional fractions were detected.

Influence of the composition of the incubation medium on ATP-sulfurylase activity

The enzyme exhibited a pH optimum at 8.5 (Tris–HCl buffer). An absolute requirement for metal ions was not demonstrable, but Mn^{2+} was observed to cause a considerable stimulation of ATP-sulfurylase (Table II and Fig. 3). In the presence

TABLE II

EFFECT OF METAL IONS ON ATP-SULFURYLASE ACTIVITY

The incubation mixture, consisting of 0.75 μmole PP_i, 0.4 μmole APS, 20 μmoles Tris–HCl, pH 8.5, 2 μg ATP-sulfurylase, and 2 mM metal ion, in a total volume of 0.2 ml, was incubated for 10 min at 37°, and the ATP formed was determined as described in text.

Addition	*ATP formed (nmoles)*
None	90.2
Mn^{2+}	224
Mg^{2+}	146
Zn^{2+}	118
Cu^{2+}	109
Co^{2+}	106
Fe^{2+}	89.9
Hg^{2+}	76.2
Ca^{2+}	56.0
Ba^{2+}	50.0
Ni^{2+}	39.9

of Mg^{2+} and Zn^{2+}, the enzyme was activated to a lesser extent. Ni^{2+}, Ba^{2+} and Ca^{2+} depressed the reaction rate. The other metal ions examined had only minor or insignificant effects, at a concentration of 2 mM (Table II).

The stimulation of ATP-sulfurylase by Mn^{2+} was greatest at concentrations of 1–3 mM; higher Mn^{2+} concentrations depressed the reaction rate (Fig. 3). Monovalent ions such as Na^+, K^+, and NH_4^+ did not show any significant effects on the enzyme activity, up to concentrations of 10 mM, in the presence as well as in the absence of Mn^{2+}.

EDTA was a powerful inhibitor of ATP-sulfurylase (Table III). The inhibition by 1 mM EDTA was reversed by 2 mM solutions of a number of divalent cations.

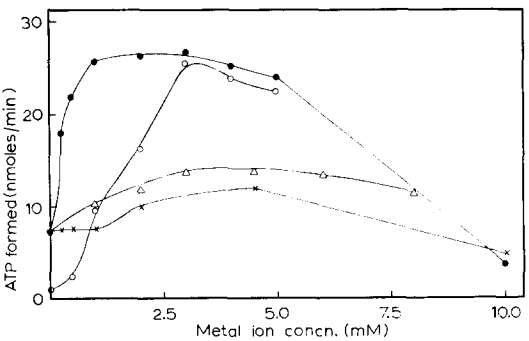


Fig. 3. Influence of metal ion concentration on the velocity of the ATP-sulfurylase reaction measured by determining ATP formation. Experimental conditions as described in text, except for addition to the incubation medium of 2 μg ATP-sulfurylase, and of varying concentrations of metal ions and 1 mM EDTA as indicated. Incubation period, 10 min. ●—●, Mn^{2+}; ○—○, Mn^{2+} + EDTA; △—△, Mg^{2+}; ×—×, Zn^{2+}.

However, different metal ions varied greatly with respect to the magnitude of this reversal of the inhibition by EDTA (Table III). In the presence of Mn^{2+} concentrations above 3 mM, a 1 mM EDTA solution had practically no effect (Fig. 3). Other chelating agents such as bipyridyl, cupferron, 8-hydroxyquinoline and dithizone had practically no effect on enzyme activity at a concentration of 1 mM.

The rate of the reaction catalyzed by ATP-sulfurylase was not modified by any of the following substances, when added to the incubation medium at a final concentration of 1 or 10 mM: Sulfhydryl compounds, such as cysteine, reduced glutathione, Cleland's reagent or β-mercaptoethanol; adenosine, AMP, ADP or cyclic AMP; histamine, serotonin, or heparin (the latter at a final concentration of 60 μg/0.2 ml).

Substrate specificity of ATP-sulfurylase

APS could not be replaced as substrate by GPS, IPS, ADP or AMP. But the

TABLE III

REVERSAL OF EDTA INHIBITION OF ATP-SULFURYLASE BY SOME DIVALENT METAL IONS

Experimental conditions as described in Table II, except for addition of 1 mM EDTA where indicated.

Addition	*ATP formed (nmoles)*
None	89.5
EDTA	9.9
EDTA + Mn^{2+}	248
EDTA + Mg^{2+}	135
EDTA + Cu^{2+}	109
EDTA + Zn^{2+}	106
EDTA + Co^{2+}	96.8
EDTA + Fe^{2+}	85.1
EDTA + Ca^{2+}	78.3
EDTA + Ba^{2+}	50.1
EDTA + Ni^{2+}	47.2
EDTA + Hg^{2+}	30.4

TABLE IV

EFFECT OF APS ANALOGS ON ATP-SULFURYLASE ACTIVITY

Incubation conditions as described in text, except for addition of 2 μg ATP sulfurylase and of nucleotidylsulfates as indicated. The reaction velocity was determined by measuring the disappearance of PP_i as described in text. Incubation period, 10 min.

Addition	*PP_i disappeared (nmoles)*
None	176
1 mM dAPS	143
1 mM IPS	140
1 mM GPS	144

enzyme acted when dAPS was substituted for APS in the reaction mixture. When either dAPS or GPS or IPS, respectively, were added to the incubation medium together with APS, an inhibition of the enzymatic ATP formation by about 20% was observed (Table IV). When P_i was substituted for PP_i, no disappearance of P_i was observed; apparently, P_i was unable to replace PP_i as substrate in the reverse ATP-sulfurylase reaction.

Stoichiometry of the reaction

When the disappearance of PP_i and the formation of ATP and inorganic sulfate in the presence of ATP-sulfurylase were determined quantitatively, 1.06 molecule ATP was found to be formed and 1.16 molecule sulfate liberated for each molecule PP_i disappearing. These results confirm the stoichiometry of the reaction established by earlier investigations[14,15].

Some kinetic properties of ATP sulfurylase

The influence of incubation time on the reaction rate is shown in Fig. 4. A linear relationship was noted during the initial 40 min, corresponding to a period during which about 80–90% of the APS initially present had reacted, under the conditions of these experiments.

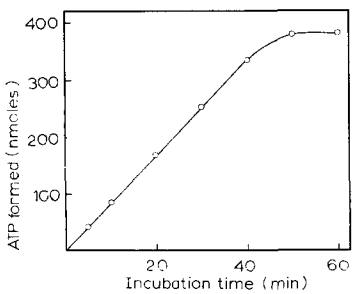


Fig. 4. Influence of incubation time on ATP-sulfurylase reaction measured by determining ATP formation. Experimental conditions as described in text, except for addition to the incubation medium of 1 μg ATP-sulfurylase, and variation of incubation period as indicated.

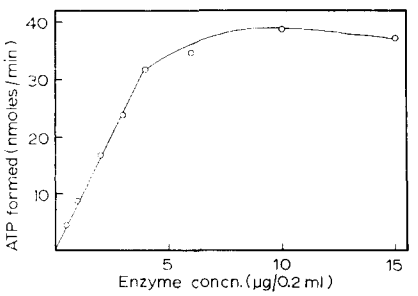


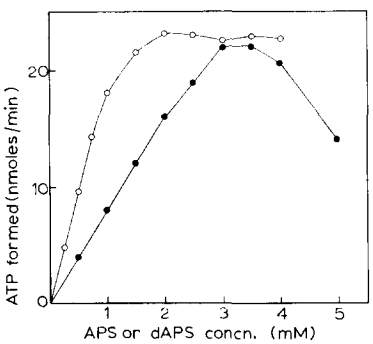
Fig. 5. Influence of ATP-sulfurylase concentration on the reaction velocity measured by determining ATP formation. Experimental conditions as described in text, except for ATP-sulfurylase concentration as indicated. Incubation period, 10 min.

The reaction velocity was observed to increase linearly with rising enzyme concentration up to 4 μg purified enzyme protein per 0.2 ml. Above that level, the substrate concentration became limiting, and the reaction rate approached a plateau, under the conditions of these experiments (Fig. 5).

When the concentrations of APS, dAPS and PP_i, respectively, were modified (dAPS replacing APS as substrate), with the levels of the second substrates being kept constant, saturation phenomena were observed (Figs. 6 and 7). However, the reactions did not strictly follow Michaelis-Menten kinetics under the conditions of those experiments. This was true even when the concentrations of the co-substrates were maintained in excess of the saturation levels.

The rate of the reaction was decreased when APS was replaced by its deoxy analog as substrate, and a higher dAPS concentration was required to reach the same level of saturation of the enzyme (Fig. 6). Still higher concentrations of dAPS (above 3.5 mM) were found to inhibit the reaction, as measured by the disappearance of PP_i (Fig. 6).

When the influence of a variation of the PP_i concentration on the reaction rate was examined, practically the same value was observed for the maximal velocity, as was found for APS and dAPS (Fig. 7).



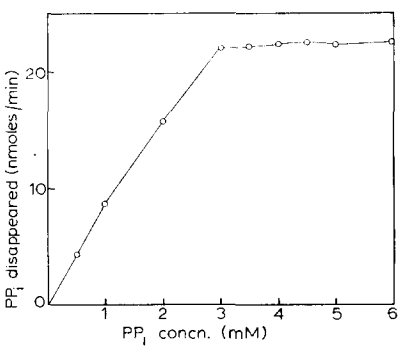


Fig. 6. Influence of APS and dAPS concentration on the velocity of the ATP-sulfurylase reaction measured by determining ATP formation. Experimental conditions as described in text, except for addition to the incubation medium of 2 μg ATP-sulfurylase, varying concentrations of APS and dAPS as indicated (dAPS replacing APS as substrate), and 3.8 mM PP_i. Incubation period, 10 min. ○—○, APS; ●—●, dAPS.

Fig. 7. Influence of PP_i concentration on the velocity of the ATP-sulfurylase reaction measured by determining disappearance of PP_i. Experimental conditions as described in text, except for addition to the incubation medium of 2 μg ATP-sulfurylase, varing concentrations of PP_i as indicated, and 2 mM APS. Incubation period, 10 min.

Stability of ATP-sulfurylase

The purified enzyme in solution (1 mg protein/ml) retained about 85% of its original activity after storage for 5 weeks at 0–4°. However, freezing and thawing inactivated the purified ATP-sulfurylase. Less highly purified preparations, such as the undialyzed product of the $(NH_4)_2SO_4$ fractionation (Sediment IV, Table I and Fig. 2) could be stored at −18° for 4 weeks without significant loss of activity. When Sediment IV was desalted by Sephadex G-200, using 0.02 M P_i buffer, pH 7.4, as eluent, the desalted fraction, dissolved in the same buffer, retained practically all

enzyme activity during storage at −18° for 2 weeks. However, when P_i buffer was replaced by other buffer salts at the same molarity and pH, such as Tris, Tris containing 10^{-4} M ATP, Tris containing 10^{-4} M ATP and 10^{-3} M $MgCl_2$, or borate or glycine containing 10^{-4} M ATP, practically the entire enzyme activity was lost after one course of freezing and thawing.

DISCUSSION

Several investigators described the purification of APS using Dowex-1 column chromatography followed by adsorption of the nucleotide on charcoal for desalting. A considerable loss of APS was reported to occur owing to the lability of the phosphosulfate linkage[16–18]. In our laboratory, too, the charcoal treatment was found to result in a marked degradation of APS. Therefore, we used ECTEOLA-cellulose, a more weakly basic anion exchanger than Dowex-1, which enabled us to elute the nucleotide at a lower salt concentration. For elution, NH_4HCO_3 was employed which could then be removed by lyophilization[3]. As a result, APS could be purified with an improved yield.

The enzyme, ATP-sulfurylase, has been purified from yeast by ROBBINS AND LIPMANN[4] and WILSON AND BANDURSKI[19], from *Nitrobacter agilis* by VARMA AND NICHOLAS[20], and from spinach by BALHARRY AND NICHOLAS[21*]. More recently ATP-sulfurylase was obtained from mammalian tissues also. PANIKKAR AND BACHHAWAT[22] and LEVI AND WOLF[23] reported on enzyme preparations derived from sheep liver and rat liver, exhibiting specific activities of 96 μmoles PP_i consumed per h per mg protein, and 45 μmoles ATP formed per h per mg protein, respectively. On the other hand, the ATP-sulfurylase preparation obtained in our alboratory from murine mast cell tumor was found to catalyze the formation of 279 μmoles ATP/30 min per mg protein, exhibiting the highest specific activity reported to date for mammalian ATP-sulfurylase.

During Step II of the purification procedure developed in our laboratory (first $(NH_4)_2SO_4$ fractionation), the total recovered enzyme activity appeared to increase approximately 5-fold (Sediment IIB, Table I); the possibility is considered that a removal of an ATP-sulfurylase inhibitor, or of another enzyme degrading or utilizing one of the substrates was responsible for the apparent increase in ATP-sulfurylase activity.

Since the enzyme was resolved into two active forms upon DEAE-cellulose chromatography, as reported recently[1], the possibility was considered that a similar separation into two components was accomplished in some of the disc gel electrophoresis experiments described above.

The results of gel filtration studies were interpreted to indicate that the molecular weight of ATP-sulfurylase was not less than 200 000, in agreement with observations by LEVI AND WOLF[23] who reported a value of 800 000–900 000 for the rat liver enzyme.

Mastocytoma ATP-sulfurylase was found to have a pH optimum at 8.5. Sheep liver enzyme was reported to exhibit maximum activity at the same pH (ref. 22), rat liver ATP-sulfurylase at pH 8.0 (ref. 23), and yeast enzyme at pH 7.5–9.0 (ref. 4).

* See *Note Added in Proof*, p. 123.

The murine ATP-sulfurylase did not show an absolute requirement for an exogenous metal ion; however, the enzyme activity was increased by Mn^{2+} and Mg^{2+}. This is in agreement with observations on the yeast enzyme by ROBBINS AND LIPMANN[4], but in contrast to results of PANIKKAR AND BACHHAWAT[22] who reported that ATP-sulfurylase of sheep liver had an absolute requirement for Mg^{2+}. EDTA was a powerful inhibitor of mast cell tumor ATP-sulfurylase; this inhibition could be reversed by Mn^{2+} or Mg^{2+}. These observations confirm findings on the rat liver enzyme[23]. The other chelating agents examined had no significant effect on enzyme activity.

Mastocytoma ATP-sulfurylase was quite specific toward its substrates. PP_i could not be replaced by P_i, nor could ADP, AMP, GPS or IPS substitute for APS. Of the nucleotidyl sulfates examined, only dAPS was found to be able to serve as substrate for the mastocytoma enzyme, aside from APS. This finding confirms observations indicating that, in addition to ATP, dATP, too, can form relatively stable complexes with ATP-sulfurylase of mast cell tumor[24].

The yeast enzyme appears to exhibit a similar specificity towards adenine nucleotidyl sulfates[4]; it was also found to act on dAPS or released PP_i from dATP in the presence of molybdate (M. SHOYAB AND W. MARX, unpublished observations). ABRAHAM AND BACHHAWAT[25] reported that a 3'-phosphoadenylylsulfate (PAPS) synthesizing system from *Euglena gracilis* too, was capable of utilizing dATP.

Although dAPS could serve as substrate for murine ATP-sulfurylase, a 1 mM solution of this nucleotide was observed to inhibit the enzyme by about 20%, when added to the reaction mixture together with APS; GPS and IPS showed a similar depressing action (Table IV). ATP, on the other hand, had no effect. In contrast, the sheep liver enzyme was reported to be inhibited strongly by 10^{-4} M ATP[22].

Although saturation phenomena were observed for each of the substrates, APS, dAPS and PP_i, the reaction catalyzed by murine ATP-sulfurylase was found not to follow strictly Michaelis–Menten kinetics. In contrast, K_m values were published for ATP-sulfurylases from other sources: For APS, K_m was reported to be $2 \cdot 10^{-3}$ M (sheep liver)[22], $2.5 \cdot 10^{-4}$ M (rat liver)[23], and $2.5 \cdot 10^{-5}$ M (*Nitrobacter agilis*)[20], respectively; for PP_i, K_m values ranging from $1.7 \cdot 10^{-3}$ to $3.7 \cdot 10^{-5}$ M were observed for these enzymes by the same authors.

Murine mast cell tumor ATP-sulfurylase was observed to be stimulated significantly by $5 \cdot 10^{5}$ M *p*-hydroxymercuribenzoate[26]. At higher concentrations, however, the mercurial inhibited the enzyme, in agreement with results of LEVI AND WOLF[23] dealing with the inhibition of rat liver ATP-sulfurylase by *p*-chloromercuribenzoate.

Although PAPS formation catalyzed by a high-speed supernatant fraction of mast cell tumor was observed to be enhanced by histamine and inhibited by serotonin in some experiments[1], it was not possible to relate these phenomena to ATP-sulfurylase. Neither histamine nor serotonin exerted a significant effect on the activity of purified ATP-sulfurylase.

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TWEEDIE AND SEGAL[27] recently reported the purification of ATP-sulfurylase from *Penicillium chrysogenum*.

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