Biochimica et Biophysica Acta, 527 (1978) 239—248

BBA 68586

PURIFICATION AND PROPERTIES OF THE ATP SULPHURYLASE OF RAT LIVER

J.N. BURNELL * and A.B. ROY

Department of Physical Biochemistry, John Curtin School of Medical Research, Australian National University, P.O. Box 334, Canberra City, ACT 2601 (Australia)

(Received March 14th, 1978)

Summary

ATP sulphurylase (ATP:sulphate adenylyltransferase, EC 2.7.7.4) has been purified about 2500-fold from rat liver. It was free of ATPase, inorganic pyrophosphatase, adenosine phosphosulphate kinase and ADP sulphurylase activities. The enzyme was homogeneous to chromatography on Sepharose 4B and to density-gradient sedimentation; it was not homogeneous to acrylamide gel electrophoresis nor to sedimentation in the ultracentrifuge. Possible reasons for this heterogeneity are considered. The molecular weight of the enzyme is 410 000 as measured by chromatography on Sepharose 4B. The $\overline{v}$ is 0.80, suggesting that ATP sulphurylase is a lipoprotein. The enzyme activity is associated with a pigment having a λ_{max} of 410 nm.

Studies of the forward, reverse and ATP-PP_i exchange reactions catalysed by ATP sulphurylase have shown that these are sequential bi-bi reactions, with ATP being the first substrate bound and adenosine phosphosulphate the last product released. The results are incompatible with previous suggestions that the ATP sulphurylase of rat liver catalysed a bi-bi ping-pong reaction.

Introduction

ATP sulphurylase (ATP:sulphate adenylyltransferase, EC 2.7.7.4) catalyses the first step in the metabolism of sulphate, the formation of adenylyl sulphate (adenosine 5′-phosphosulphate):

$$ATP + SO_4^{2-} \overset{Mg^{2+}}{\rightleftharpoons} \text{Adenosine phosphosulphate} + PP_i$$

* Present address: School of Environmental and Life Sciences, Murdoch University, Murdoch, Western Australia 6153.

The reaction is freely reversible with the equilibrium much in favour of ATP. ATP sulphurylase has been purified from yeast [1,2] *Penicillium chrysogenum* [3,4] and spinach leaves [5], and partly purified from rat liver [6] and Furth mouse mastocytoma [7]. The enzymes from yeast, *Penicillium* and spinach have many properties in common; in particular they catalyse a sulphate-dependent, but not a sulphate-independent, ATP-PP_i exchange. The rat liver enzyme, on the other hand, has appeared to be distinct because Levi and Wolf [6] reported that it did catalyse a sulphate-independent ATP-PP_i exchange. They postulated that an adenylated form of the enzyme was an intermediate and proposed that the reaction mechanism was ping-pong in the nomenclature of Cleland [8].

The present paper describes the purification of the ATP sulphurylase from rat liver and a study of the kinetics of its reactions, in both the forward and reverse directions, using its physiological substrates. The exchange reactions catalysed by the preparation have also been studied.

Methods and Materials

Adenosine phosphosulphate was purchased from the Sigma Chemical Co. Ltd. The purity of adenosine phosphosulphate was calculated from the stoichiometric conversion of adenosine phosphosulphate to [^{32}P]ATP in the presence of excess [^{32}P]PP_i, Mg^{2+} and ATP sulphurylase; the adenosine phosphosulphate was generally 80—85% pure. Carrier-free $^{35}SO_4^{2-}$ was obtained from the Radiochemical Centre, Amersham, Bucks., U.K. $^{32}P_i$, in dilute HCl, was obtained from the Australian Atomic Energy Commission, Lucas Heights, N.S.W., Australia, and was pyrolysed to $^{32}P_i$. Sepharose 4B, Sephadex G-200 and DEAE-Sephadex were obtained from Pharmacia, Uppsala, Sweden; hydroxyapatite was obtained from BioRad Labs., Richmond, Calif., U.S.A. All other chemicals were of analytical reagent grade and were obtained from either The Sigma Chemical Co. Ltd. or British Drug Houses.

Methods

Assays of ATP sulphurylase. In each of the assays the reaction mixture (1 ml) was at pH 7.8 in phosphate buffer of the concentrations specified for the individual methods and it contained (in addition to reagents given below) 10 mM $MgCl_2$ and 0.05 mM dithiothreitol. The reaction was started by the addition of the enzyme (in phosphate buffer) to the remainder of the reaction mixture and, after incubation for 10 min at 37°C, it was stopped by the addition of the appropriate reagent. In the assays measuring the production of [^{32}P]ATP, this was identified by paper chromatography [5].

Forward reaction: formation of adenosine phosphosulphate. The reaction mixture contained 2 mM Na_2K_2ATP, 20 mM Na_2 $^{35}SO_4$ (specific activity 5 Ci/mol) and 2 units inorganic pyrophosphatase/0.1 M phosphate buffer (pH 7.8). The reaction was started by adding 0.3 mg enzyme and was stopped by adding 3 ml ice-cold 0.1 M phosphate buffer (pH 7.8)/0.1 M Na_2SO_4. The [^{35}S]adenosine phosphosulphate was separated from $^{35}SO_4^{2-}$ by the ethanol-precipitation technique of Reuveny and Filner [9] and the radioactivity was measured in a

Packard Tricarb 3255 scintillation counter. Assays were performed in duplicate.

Reverse reaction: formation of ATP. The reaction mixture contained 0.1 mM adenosine phosphosulphate and 0.2 mM [^{32}P]$Na_4P_2O_7$ in 0.05 M phosphate buffer. The reaction was started by adding 50 μg enzyme and stopped by adding 2 ml 7.5% (w/v) trichloroacetic acid. [^{32}P]ATP was separated from [^{32}P]PP_i by adsorption to charcoal and washing as described by Berg [10], before the radioactivity adsorbed on the charcoal was counted using a Nuclear Chigaco end-window planchette counter. ATP sulphurylase activity was expressed as the adenosine phosphosulphate-dependent synthesis of ATP in μmol/min.

Sulphate-dependent ATP-PP_i exchange. This was carried out as described by Shaw and Anderson [5]. The reaction mixture contained 4 mM Na_2K_2ATP, 2 mM [^{32}P]$Na_4P_2O_7$ and 10 mM K_2SO_4 in 0.05 M phosphate buffer. Control reaction mixtures contained 20 mM KCl in place of K_2SO_4. The reaction was started by adding 0.3 mg enzyme and stopped by adding 2 ml 7.5% (w/v) trichloroacetic acid. The [^{32}P]ATP was separated and determined as above.

Calculation of kinetic constants. Apparent values of K_m and V, with their associated standard errors, were computed by the method of Wilkinson [11]. Appropriate replots of the apparent constants were used to obtain values for K_m, V and K_i of the various substrates and inhibitors.

Protein determination. During the initial stages of the purification, proteins were determined by the method of Lowry et al. [12], using bovine serum albumin as standard. For more purified samples the concentration was determined spectrophotometrically, using a value of 13.5 for $A^{1\%}_{280nm}$. The latter value was determined using solutions of highly purified ATP sulphurylase, the concentrations of which were determined refractometrically (in a Bryce Phoenix differential refractometer) assuming a value of 0.0018 dl $\cdot$ g^{-1} for the specific refractive increment.

Polyacrylamide gel electrophoresis. This was carried out using the phosphate buffer of Weber and Osborn [13]. The gel concentration was 5% (w/v) and electrophoreses were run in 0.2 M phosphate buffer (pH 7.8) for 2 h at 15°C. The current was maintained at 8 mA per gel.

Density gradient centrifugation. Purified samples of ATP sulphurylase were sedimented for 16 h at 3°C in a 5—20% (w/v) gradient of sucrose in 0.05 mM phosphate buffer (pH 7.8) at 35 000 rev./min in a Spinco Model L2 Centrifuge, rotor SW56. Catalase, alcohol dehydrogenase and lysozyme were included as markers. After sedimentation, 0.5 ml samples of the gradient were collected, the enzyme localised by its activity and the marker proteins by their enzyme activity or absorbance.

Ultracentrifugation. This was carried out in a Spinco Model E ultracentrifuge at temperatures close to 20°C using Schlieren or interference optics as appropriate. Sedimentation velocity measurements were made in double-sector cells in an An-E rotor at 52 000 rev./min. In all cases the buffer was 0.025 M phosphate (pH 7.8)/1 mM EDTA/1 mM dithiothreitol. Sedimentation coefficients were corrected to standard conditions using a $\bar{v}$ of 0.80 (see below).

Preparation of ATP sulphurylase. All steps were carried out at about 5°C. Unless otherwise specified, the buffer used during the purification procedure

was 0.025 M phosphate buffer (pH 7.8)/1 mM EDTA/1 mM dithiothreitol.

Livers of male Wistar rats (200—300 g) were removed and rinsed in extracting medium (0.25 M sucrose/0.025 M KCl/1 mM EDTA) before being homogenised, in a Sorvall blendor, in 3 vols. of the same medium. The homogenate was centrifuged at 9000 × *g* for 30 min and the supernatant solution decanted and stored at 5°C. The sediment was rehomogenised in extracting medium and centrifuged at 9000 × *g* for 60 min. The supernatant solution was removed and combined with the first extract and the sediment discarded. The combined supernatant fractions were centrifuged at 15 000 × *g* for 60 min and the sediment discarded. Solid $(NH_4)_2SO_4$ was added to the supernatant solution according to the table of Green and Hughes [14] to precipitate the enzyme at 35—65% saturation. The precipitated protein was dissolved in and dialysed against buffer.

The enzyme was then precipitated at 20—40% (w/v) ethanol at −10°C, then dissolved in and dialysed against buffer. It was then precipitated as before at 30—60% saturated $(NH_4)_2SO_4$, dissolved in buffer and dialysed.

The resulting solution was applied to a column (90 × 5 cm) of Sephadex G-200 and eluted with buffer in 6-ml fractions. The enzyme was eluted at the void volume.

The active fractions were pooled and loaded on to a column (24 × 3 cm) of DEAE-Sephadex A-50 and eluted in 6-ml fractions with a linear ionic strength gradient formed from 250 ml buffer and 250 ml buffer containing 0.5 M KCl. The enzyme was eluted in a single sharp peak and the appropriate fractions were pooled and dialysed against buffer.

The dialysed solution was loaded on to a column (9 × 3.5 cm) of hydroxyapatite which was then washed with 50 ml buffer and then with 100 ml 0.1 M phosphate buffer (pH 7.8)/1 mM EDTA/1 mM dithiothreitol. The enzyme was then eluted in 6-ml fractions with linear gradient in phosphate (100 ml of the latter buffer and 100 ml 0.3 M phosphate buffer (pH 7.8)/1 mM EDTA/1 mM dithiothreitol). The enzyme appeared as a single sharp peak and the active fractions were pooled, dialysed against buffer, concentrated by ultrafiltration to about 2 ml and again dialysed against buffer.

This solution was then loaded on to a column (70 × 2.6 cm) of Sepharose 4B and eluted with buffer in 1.5-ml fractions. The enzyme was eluted as a sharp symmetrical peak.

The active fractions were pooled, concentrated by ultrafiltration and exhaustively dialysed against buffer to give an enzyme solution which, at 5—10 mg/ml, was stable for several months at 5°C. It was not stable to freezing and thawing.

Results

Purification

The course of a typical purification is shown in Table I. A 2500-fold purification was achieved from the first $(NH_4)_2SO_4$ precipitation and the final specific activity, measured by the adenosine phosphosulphate-dependent synthesis of ATP, was 2 $\mu mol \cdot min^{-1} \cdot mg^{-1}$. This method of assay was used throughout the purification procedure.

TABLE I

THE PURIFICATION OF ATP SULPHURYLASE FROM RAT LIVER

The table shows the course of a typical preparation from 315 g of rat liver, as described in the text. Units are defined as μmol ATP synthetised per min.

Stage	Protein (mg)	Enzyme activity (U)	Specific activity (U/mg)
First $(NH_4)_2SO_4$ precipitation	$31 \cdot 10^3$	23.8	0.0008
Ethanol precipitation	$7.9 \cdot 10^3$	22.8	0.0029
Second $(NH_4)_2SO_4$ precipitation	$1.8 \cdot 10^3$	20.8	0.012
Sephadex G-200	763	19.6	0.026
DEAE-Sephadex	150	18.1	0.12
Hydroxyapatite	30	16.0	0.53
Sepharose 4B	6.7	14.2	2.1

The final preparation contained no detectable ATPase, adenosine phosphosulphate kinase, ADP sulphurylase or inorganic pyrophosphatase activities. It was also free from aminoacyl-tRNA synthetase activity.

Physical properties

The purified enzyme was consistently associated with a yellow pigment having a λ_{max} of 410 nm. In four independent preparations of the enzyme the ratio A_{280}/A_{410} ranged from 22 to 24 with a mean of 22.7. The partial specific volume of ATP sulphurylase was calculated from the densities of its solutions (3—8 mg/ml) in 0.025 M phosphate buffer (pH 7.8) measured in a DMA 02D Density Meter (Anton Paar, Graz, Austria). The mean value of $\bar{v}$ was 0.80.

The enzyme preparation was homogeneous with respect to protein and to ATP sulphurylase activity during chromatography on Sepharose 4B and its elution volume from a calibrated column gave a molecular weight of 410 000. It also behaved as a homogeneous species on sedimentation in a sucrose density gradient, with an $s_{20,w}$ of 4.8 (Fig. 1). Electrophoresis on polyacrylamide gel gave two major and one minor band of protein (Fig. 2). Attempts to locate the enzyme activity on the intact gel by the method of Skyring et al. [15] were not

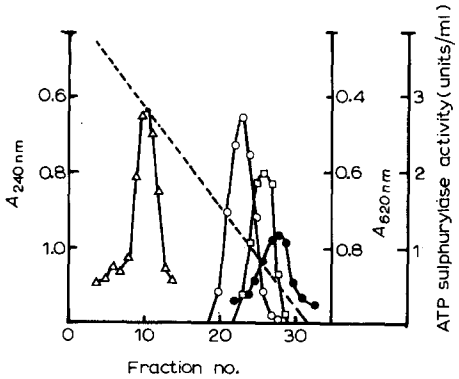

Fig. 1. Sucrose density gradient centrifugation of ATP sulphurylase. Catalase (△), alcohol dehydrogenase (□) and lysosyme (●) were used as marker proteins. ATP sulphurylase (○) was located by measuring the adenosine phosphosulphate-dependent synthesis of ATP.

Fig. 2. Polyacrylamide gel electrophoresis of purified rat liver ATP sulphurylase. 61.4 μg of protein was applied to the gel.

successful due to the presence of phosphate in the buffer, but assays on slices of gel suggested that at least the two major protein bands showed ATP sulphurylase activity. The positions of these two bands corresponded to molecular weights of 500 000 and 1000 000.

The preparation was not homogeneous with respect to sedimentation coefficient. Ultracentrifugation gave a very complex Schlieren pattern of resolved and partly resolved peaks (Fig. 3) and the measurement of the sedimentation coefficients, at least of the latter, was difficult. In a number of preparations, the mean values of $s_{20,w}$ were 5.3, 8.6, 11, 18 and 20. The proportions of these species varied from preparation to preparation but in all cases the major component was the 5.3- or 8.6-S material: considerable amounts of 11-S material were frequently present but never more than traces of the faster-sedimenting components. The above values of $s_{20,w}$ are means from 5 preparations: a typical Schlieren pattern is shown in Fig. 3. In this particular experiment the values of $s_{20,w}$ were 5.3, 7.9, 12 and 18.

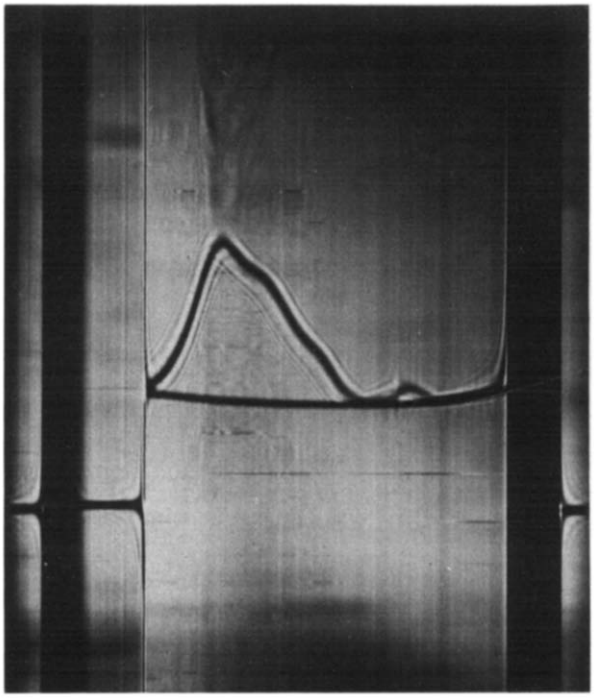

Fig. 3. Sedimentation velocity pattern of ATP sulphurylase at 20°C in 0.025 M phosphate buffer (pH 7.8)/1 mM EDTA/1 mM dithiothreitol.

The sedimentation behaviour was too complex for easy interpretation, but it was clear that it did not show the existence of ATP sulphurylase as a rapidly equilibrating polymerising system because there was no change in the proportions of the different components on dilution. Attempts to modify the sedimentation behaviour by exhaustive dialysis of the ATP sulphurylase against buffers containing the substrates 0.01 M Mg · ATP or 0.1 M Na_2SO_4, or the disulphide-splitting reagent 0.067 M Na_2SO_3 were unsuccessful: none of these treatments altered the Schlieren patterns. The complexity of the patterns was not due to denaturation of the enzyme during sedimentation: the enzyme could be recovered from the cell with no change in specific activity.

Sedimentation equilibrium [16] also showed that the enzyme was not homogeneous with respect to mol. wt. but contained components of molecular weight from 10^5 or less to 10^6 and more.

Kinetic properties

ATP sulphurylase is unstable in Tris, diethylbarbiturate, glycine, triethanolamine and bicarbonate buffers (pH 7.8) but is stable in phosphate buffers of the same pH. It is also unstable in phosphate buffers containing Tris. At the pH optimum of 7.8—8.0 in 0.1 M phosphate buffer the ATP sulphurylase reaction was of zero order for at least 20 min and the velocity was directly related to the enzyme concentration in all 3 of the assays described above.

In contrast to the findings of Levi and Wolf [6], purified rat liver ATP sulphurylase did not catalyse the incorporation of $[^{32}P]PP_i$ into ATP in the absence of SO_4^{2-}. However, in preliminary experiments with partially purified ATP sulphurylase, obtained by batch-wise treatment with hydroxyapatite as described by Levi and Wolf [6], a sulphate-independent PP_i-ATP exchange, similar to that described by these authors, was noted. This activity was eliminated on further purification and was never seen in preparations of the enzyme eluted from a column of hydroxyapatite by a phosphate gradient, as described above.

The enzyme is clearly sensitive to sulphydryl reagents; 10 mM *N*-ethylmaleimide and 5 mM *p*-chloromercuribenzoate inhibited the reverse reaction. The enzyme is also inhibited by EDTA but this inhibition is presumably due to the chelation of Mg^{2+} because it is reversed by the addition of an excess of Mg^{2+}.

Forward reaction. ATP sulphurylase, in the presence of inorganic pyrophosphatase, catalysed the synthesis of $[^{35}S]$adenosine phosphosulphate from ATP and $^{35}SO_4^{2-}$. Both AMP and ADP were linear competitive inhibitors with respect to ATP, while NO_3^- and ClO_3^- were linearly competitive with respect to SO_4^{2-} and uncompetitive with respect to ATP.

Reverse reaction. The enzyme catalysed the formation of $[^{32}P]$ATP from $[^{32}P]PP_i$ and adenosine phosphosulphate. The reaction was absolutely dependent upon the presence of Mg^{2+}. The effects of adenosine phosphosulphate concentration (5—200 μm) and of PP_i concentration (0.01—0.4 mM) were investigated: with adenosine phosphosulphate as variable substrate the slopes and intercepts of reciprocal plots were dependent upon the concentration of PP_i, and with the latter as variable substrate they were dependent upon the concentration of adenosine phosphosulphate. The double reciprocal plots were families of straight lines intersecting on, or very close to, the abscissa. This is

obviously inconsistent with ATP sulphurylase catalysing a ping-pong reaction, as has been suggested by Levi and Wolf [6], but is consistent with it catalysing a sequential reaction.

Both the reaction products, ATP and SO_4^{2-}, were inhibitors. The inhibition by ATP was competitive with respect to adenosine phosphosulphate and non-competitive with respect to PP_i, while the inhibition by SO_4^{2-} was noncompetitive with respect to both adenosine phosphosulphate and PP_i. In all of these experiments the concentrations of the non-variable substrate were nonsaturating (0.1 mM adenosine phosphosulphate ($4 \cdot K_m$) and 0.2 mM PP_i ($10 \cdot K_m$)).

Sulphate-dependent ATP-PP_i exchange. The rate of this reaction varied with the concentration of both ATP and SO_4^{2-}, and both were required for the reaction to occur. There was also an absolute requirement for Mg^{2+}. In all assays the concentration of Mg^{2+} was held constant at 10 mM. The effects of ATP concentration (0.5—4.0 mM) and SO_4^{2-} concentration (0.5—20.0 mM) were investigated in detail. The double reciprocal plots were families of straight lines intersecting on the abscissa, and gave values of 0.38 mM ATP and 2.5 mM SO_4^{2-} respectively for the appropriate K_m (Table II).

Purified ATP sulphurylase did not catalyse PP_i exchange when ATP was replaced by ADP or AMP, nor did it catalyse P_i exchange with AMP, ADP or ATP when the PP_i in the normal reaction mixture was replaced by P_i. Sulphate could not be replaced by CrO_4^{2-}, MoO_4^{2-}, ClO_3^- or NO_3^- although it could by SeO_4^{2-} which was a true alternate substrate for the enzyme. The ratio of SO_4^{2-}-dependent to SeO_4^{2-}-dependent ATP-PP_i exchange remained constant during the purification of the ATP sulphurylase from liver. The K_m for SeO_4^{2-} was 0.61 mM, considerably less than that for SO_4^{2-} (2.5 mM), but the maximum velocity with SeO_4^{2-} was only about 27% of that with SO_4^{2-}. The effect of SeO_4^{2-} on the SO_4^{2-}-dependent ATP-PP_i exchange is consistent with both substrate competing for a single site [17].

Both AMP and ADP were inhibitors of the exchange reaction, noncompetitive with respect to SO_4^{2-} and competitive with respect to ATP. On the other hand, ClO_3^- and NO_3^- were competitive inhibitors with respect to SO_4^{2-} and uncompetitive with respect to ATP. The concentrations of the non-variable substrates in these experiments were maintained at 20 mM PP_i ($100 \cdot K_m$) and 10 mM SO_4^{2-} ($4 \cdot K_m$). Values of the appropriate kinetic constants are given in

TABLE II

KINETIC CONSTANTS FOR THE ATP SULPHURYLASE OF RAT LIVER

Substrate or inhibitor	K_m (mM)	K_i (mM)	Reaction
ATP	0.38		Exchange
SO_4^{2-}	2.5		Exchange
	3.2		Forward
PP_i	0.018		Reverse
Adenosine phosphosulphate	0.025		Reverse
SeO_4^{2-}	0.61	0.50	Exchange
AMP		0.60	Exchange
ADP		0.06	Exchange
NO_3^-		2.2	Exchange
ClO_3^-		0.25	Exchange

Table II. These last results suggest that AMP, ADP, ClO_3^- and NO_3^- are dead-end inhibitors of rat liver ATP sulphurylase, and that the reaction catalysed by the enzyme is a sequential one. Again the inhibition patterns are inconsistent with the ping-pong mechanism proposed by Levi and Wolf [6].

Discussion

The method of purification described above is simple and reliable, and gives a preparation of ATP sulphurylase whose specific activity (Table I) is twice that obtained by Levi and Wolf [6].

The $\overline{v}$ of ATP sulphurylase is remarkably high, 0.80, and this seems to show that the enzyme must be a lipoprotein. Scarcity of material has so far prevented further investigation of this point, or of the apparent constant association of the enzyme with a pigment absorbing at 410 nm. Assuming the molecular weight of the enzyme to be 410 000 (see below) and a 1:1 molar ratio of pigment to protein, then the ϵ_{410nm} of the pigment must be 70 000. This is a very high figure, but the values of ϵ for many carotenoids are of this order of magnitude, a point of some interest in view of their lipid nature and the long-standing suggestions of a relationship between vitamin A and ATP sulphurylase. Although a direct relationship now seems to be excluded (see ref. 18) the situation is still by no means clear and further work would be useful.

Unfortunately some doubt must remain about the purity of the preparation. Although it behaved as a homogeneous protein during chromatography on Sepharose 4B and during sedimentation in a sucrose density gradient, it did not do so during gel electrophoresis or sedimentation in the ultracentrifuge. The former showed two major and one minor band, and the latter showed multiple peaks in the Schlieren patterns. Chromatography showed that the molecular weight of ATP-sulphurylase was 410 000, a value consistent with that of one of the major bands obtained in gel electrophoresis. This is likely to be the molecular weight of the species with an $s_{20,w}$ of 4.8, as measured in density gradient sedimentation, which probably corresponds to the species with an $s_{20,w}$ of 5.3 in classical sedimentation, having regard to the possible errors in both these measurements, especially in multi-component systems. On the basis of a simple model assuming spherical molecules the expression $s_p^\circ = s_m^\circ \cdot n^{2/3}$, where s_m° and s_p° are the sedimentation coefficients at infinite dilution of a monomer and polymer respectively and n the degree of polymerisation, can be derived. If the species having an $s_{20,w}$ of 5.3 (5.19—5.34 in 4 preparations) be regarded as a monomer then the predicted values of $s^\circ_{20,w}$ are 5.3, 8.4, 11.0, 13.4, 15.5, 17.5, 19.4 and 21.2 for the monomer to the octamer. Species with sedimentation coefficients of 5.3, 8.6, 11, 18 and 21 have been detected experimentally and it is therefore tempting to suggest that the Schlieren patterns show the sedimentation of a series of polymers of a monomeric unit of molecular weight about 400 000 and $s_{20,w}$ about 5.3. This would also be consistent with the apparent distribution of molecular weight seen in equilibrium sedimentation. If this be the case, the polymeric species are not in rapid equilibrium because dilution makes no difference to the behaviour during sedimentation. Further speculation is unwarranted at this stage because it must be admitted that the reason for the discrepancy between the various methods remains unknown. Perhaps it

should be noted that Shoyab and Marx [19] have reported that the ATP sulphurylase of mouse mastocytoma gives multiple peaks in the ultracentrifuge, and that two forms of the enzyme can be separated by chromatography on DEAE-cellulose [20]. No such fractionation of the rat enzyme was found on DEAE-Sephadex.

The results of the kinetic investigations are interesting because they are incompatible with the reaction mechanism proposed by Levi and Wolf [6]. Initial velocity studies of the reverse reaction clearly show it to be sequential and not ping-pong, as previously suggested [6]. For the reverse reaction, with the fixed substrates non-saturating, the inhibition by SO_4^{2-} was noncompetitive with respect to both adenosine phosphosulphate and PP_i while the inhibition by ATP was competitive with respect to adenosine phosphosulphate and non-competitive with respect to PP_i: this inhibition pattern conforms to that of an ordered bi-bi reaction [8] in which adenosine phosphosulphate is the first substrate bound and ATP the last product released.

It should be pointed out that the exact role of Mg^{2+} in the reaction has not been elucidated because of the necessity of carrying out the assays in phosphate buffer but there is no doubt that Mg^{2+} is required for all the reactions catalysed by the ATP sulphurylase of rat liver. It therefore seems justifiable to assume that the true substrates for this enzyme are Mg · ATP and Mg · $P_2O_7^{2-}$ as they are for the plant [21] and microbial [3] ATP-sulphurylases.

References

1 Robbins, P.W. and Lipmann, F. (1958) J. Biol. Chem. 233, 686—690
2 Hawes, C.S. and Nicholas, D.J.D., (1973) Biochem. J. 133, 541—550
3 Tweedie, J.W. and Segel, I.H. (1971) J. Biol. Chem. 246, 2438—2446
4 Farley, J.R., Cryns, D.F., Joy, Y.H. and Segel, I.H. J. Biol. Chem. 251, 4389—4397
5 Shaw, W.H. and Anderson, J.W. (1972) Biochem. J. 127, 237—247
6 Levi, A.S. and Wolf, G. (1969) Biochim. Biophys. Acta 172, 262—282
7 Shoyab, M., Su, L.Y. and Marx., W. (1972) Biochim. Biophys. Acta 258, 113—124
8 Cleland, W.W. (1963) Biochim. Biophys. Acta 67, 104—137
9 Reuveny, Z. and Filner, P. (1976) Anal. Biochem. 75, 410—428
10 Berg, P. (1956) J. Biol. Chem. 222, 1025—1034
11 Wilkinson, G.N. (1961) Biochem. J. 80, 324—332
12 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
13 Weber, K.M. and Osborne, M. (1975) J. Biol. Chem. 244, 4406—4412
14 Green, A.A. and Hughes, W.L. (1955) Methods Enzymol. 1, 67
15 Skyring, G.W., Trudinger, P.A. and Shaw, W.H. (1972) Anal. Biochem. 48, 259—265
16 Yphantis, D.A. (1964) Biochemistry 3, 297—317
17 Pocklington, T. and Jeffrey, J. (1969) Biochem. J. 112, 331—334
18 Roy, A.B. and Trudinger, P.A. (1970) The Biochemistry of Inorganic Compounds of Sulphur, pp. 100—103, Cambridge University Press, Cambridge, U.K.
19 Shoyab, M. and Marx, W. (1972) Biochim. Biophys. Acta 258, 125—132
20 Shoyab, M. and Marx, W. (1970) Life Sci. 9, 1151—1158
21 Shaw, W.H. and Anderson, J.W. (1974) Biochem. J. 139, 27—35