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Activation of Sulphate in *Anabaena cylindrica*

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Summary. Crude cell-free extracts of *Anabaena cylindrica* synthesized adenosine-5′-phosphosulphate ($AP^{35}S$) and 3′-phosphoadenosine-5′-phosphosulphate ($PAP^{35}S$) from $^{35}SO_4^{2-}$ in the presence of Mg^{2+}, ATP and inorganic pyrophosphatase. Maximum $AP^{35}S$ and $PAP^{35}S$ were produced at pH 7.15 and 8.05, respectively. APS kinase was detected in the supernatant of crude cell-free extracts by a spectrophotometric procedure. ATP-Sulphurylase had an absolute requirement for Mg^{2+} and less than 30% $AP^{35}S$ was formed when Mg^{2+} was replaced by either Mn^{2+} or Co^{2+}. Nucleotide triphosphates other than ATP and 2′-deoxyATP were ineffective in this reaction. Maximum enzyme activity was observed at equimolar concentrations of Mg^{2+} and ATP and excess of either of these was inhibitory. Other nucleotide triphosphates, like GTP, UTP, CTP, TTP, ITP, or 2′-deoxyATP also inhibited the enzyme activity. Inhibition by GTP was competitive with respect to ATP. ATP-sulphurylase activity was not affected by cysteine, methionine or glutathione.

Introduction

Sulphate assimilation has been studied in bacteria, fungi, algae and higher plants (Roy and Trudinger, 1970; Schiff and Hodson, 1973; Siegel, 1975). In all these organisms the initial step, which involves the activation of sulphate yielding APS, is catalysed by ATP-sulphurylase (ATP-sulphate adenylyltransferase, E.C. 2.7.7.4). In photosynthetic organisms, APS is further metabolized by APS-sulphotransferase to yield ultimately reduced sulphur compounds (Tsang and Schiff, 1975; Schmidt, 1975). However, in non-photosynthetic organisms, APS is further activated by APS kinase (ATP-adenylylsulphate-3′-phosphotransferase, E.C. 2.7.1.25) to PAPS which serves as a substrate for PAPS-sulphotransferase (Tsang and Schiff, 1975). The mechanism of sulphate reduction, including the types of sulphur nucleotides involved, has not been determined in blue-green algae. Although ATP-sulphurylases from higher plants (Ellis, 1969; Balharry and Nicholas, 1970; Shaw and Anderson, 1972; Onajobi et al., 1973) and non-photosynthetic organisms (Robbins and Lipmann, 1958; Varma and Nicholas, 1971; Tweedie and Segel, 1971a; Hawes and Nicholas, 1973) have been characterized, the enzyme has not been studied in algae. This paper deals with the production of sulphur nucleotides and some properties of ATP-sulphurylase from the blue-green alga, *Anabaena cylindrica*.

Abbreviations: APS, adenosine-5′-phosphosulphate; PAPS, 3′-phosphoadenosine-5′-phosphosulphate

Materials and Methods

Plant Material and Chemicals

Anabaena cylindrica (Strain 1403/2A) was obtained from the Culture Collection of Algae and Protozoa, Cambridge, U.K. Yeast inorganic pyrophosphatase, creatine phosphate, creatine phosphokinase and the nucleotides were purchased from Sigma Chemical Co., St. Louis, Mo., USA. Carrier-free $Na_2{}^{35}SO_4$ and $PAP^{35}S$ were from Radiochemical Centre, Amersham, U.K., and New England Nuclear, Boston, Mass., USA, respectively. $AP^{35}S$ was prepared enzymically from $^{35}SO_3^{2-}$ and 5′-AMP using extracts of *Thiobacillus denitrificans* as described by Adams et al. (1971).

Preparation of Cell-free Extracts

Procedures for growing the alga and preparation of cell-free extracts (S_3) and various subcellular fractions have been described previously (Sawhney and Nicholas, 1976). The crude extract (S_3) was centrifuged at $20,000 \times g$ for 20 min. The supernatant fraction (S_{20}) was heated in a water bath at 50° for 30 min and stored at −15°. After 12 h it was thawed and centrifuged at $20,000 \times g$ for 30 min. The supernatant fraction so obtained is designated

S_{20H}. The cell preparations (S_3, S_{20} and S_{20H}) were desalted before use by passing them through a Sephadex G-25 column (1.5 × 19 cm) and eluting with 0.1 M Tris-HCl (pH 7.1). The specific activity of ATP-sulphurylase in the heat-treated, desalted extract (S_{20H}) was 3 times greater than in the S_3 preparation and it was relatively free from 5′-nucleotidase and enzymes which degrade APS and PAPS (Sawhney and Nicholas, 1976).

Identification of Products of Sulphate Activation

The reaction mixture used for identifying the products of sulphate activation, in a final volume of 1 ml, contained in μmoles: ATP (neutralized with sodium bicarbonate), 6; $MgCl_2$, 10; yeast inorganic pyrophosphatase, 0.2 units; $Na_2{}^{35}SO_4$, 3 μCi; extract (S_3), 0.2–0.4 ml; and 250 μmole of an appropriate Tris-HCl buffer to adjust pH of the reaction mixture to the required value. After incubating at 30° for 30 min, the reaction was stopped with 1 ml of 95% (v/v) ice-cold ethanol. Then 100-μl aliquots were spotted onto 3MM Whatman paper and ^{35}S-labelled products were separated by high-voltage electrophoresis, identified by comparing their electrophoretic mobilities with authentic standards, and radioassayed as described previously (Sawhney and Nicholas, 1976).

ATP-sulphurylase Activity

The enzyme was assayed by following the production of $AP^{35}S$ from $Na_2{}^{35}SO_4$. The composition of the reaction mixture and the details of the experimental procedures used have been described above. ATP-sulphurylase activity is expressed as cpm × 10^3 of $AP^{35}S$ produced per ml of incubation mixture in 30 min.

APS Kinase Activity

The activity was assayed spectrophotometrically by following the rate of NADH oxidation as described by Burnell and Whatley (1975). The reaction mixture, in a final volume of 1.5 ml, contained the following in μmoles: Tris-HCl (pH 8.0), 350; $MgCl_2$, 15; KCN, 13; NaF, 13; ATP, 3.5; phosphoenolpyruvate, 0.6; NADH, 0.4; APS, 1.0; pyruvate kinase, 13 units; lactate dehydrogenase, 13 units; and an appropriate amount of the extract. The reaction was started by adding APS and the decrease in absorbance at 340 nm was recorded in a Shimadzu spectrophotometer. The enzyme activity is expressed in nmoles NADH oxidized per min.

Protein was determined by the method of Lowry et al. (1951), using bovine serum albumen as a standard.

Results

Products of Sulphate Activation and Effect of pH

When extracts (S_3) of the alga were incubated with $Na_2{}^{35}SO_4$ and Mg^{2+}, ATP and inorganic pyrophosphatase, both $AP^{35}S$ and $PAP^{35}S$ were formed. However, at pH 7.15 very small amounts of $PAP^{35}S$ were produced, namely, less than 10% of the total radioactivity in the ^{35}S-nucleotides (Table 1). At pH 8.05, this proportion increased from 20% after 15 min to 50% after a 60 min incubation. Maximum amounts of $AP^{35}S$ and $PAP^{35}S$ were produced at pH 7.15 and 8.05 respectively (Fig. 1A, B). Relatively smaller amounts of $AP^{35}S$ formed at pH values higher than 7.15 did not result from a further utilization of this sulphur nucleotide. Had this been the case, then the diminished production of $AP^{35}S$ at pH values higher than 7.15 would have been accompanied by a corresponding increase in $PAP^{35}S$ formation. The results indicate that the pH optimum for ATP-sulphurylase was around 7.1 and its activity was negligible at pH 9.0. The pH optimum for the production of $PAP^{35}S$ from $Na_2{}^{35}SO_4$ was about pH 8.05.

Table 1. Synthesis of ^{35}S-nucleotides by cell-free extract (S_3)
Values are cpm × 10^3 of ^{35}S-nucleotide produced per ml incubation mixture; values in parentheses represent counts in $PAP^{35}S$ as percent of total counts in ^{35}S-nucleotides

The reaction mixture, in a final volume of 1 ml, contained the following: ATP, 6 μmole; $MgCl_2$, 10 μmole; yeast inorganic pyrophosphatase, 0.2 units; $Na_2{}^{35}SO_4$ (carrier-free), 3 μCi; 0.4 ml extract (S_3) containing 2.72 mg protein and 250 μmole of an appropriate Tris-HCl buffer for adjusting the pH of the reaction mixture to the required value. The reaction was stopped with 1 ml of 95% (v/v) ethanol. ^{35}S-Labelled compounds were separated, identified and radioassayed as described in Materials and Methods

Time of incubation	pH of reaction mixture			
	7.15		8.05	
(min)	$AP^{35}S$	$PAP^{35}S$	$AP^{35}S$	$PAP^{35}S$
15	31.2	2.5 (7.4)	12.3	3.4 (21.6)
30	52.6	3.4 (6.0)	24.7	9.8 (28.4)
45	74.2	5.2 (6.5)	20.0	11.1 (35.6)
60	91.1	8.8 (8.8)	17.6	17.4 (49.7)

Activity of APS kinase in cell extracts (S_{20}) was also confirmed using a spectrophotometric procedure for the assay of enzyme. In this method, ADP formed during the APS kinase reaction is determined by coupling it through pyruvate kinase and lactate dehydrogenase to the oxidation of NADH. Results in Table 2 show that NADH was oxidized only when the reaction mixture contained both APS and the extract. The rate of APS-dependent NADH oxidation was proportional to the amount of the extract used. In the presence of ATP, NADH oxidation was three times greater than that without added ATP. Because the extract (S_{20}) was assayed without desalting, APS kinase activity in the absence of added ATP was probably associated with an endogenous level of the nucleotide in the extract. On substituting AMP for APS in the reaction mixture, the rate of NADH oxidation was negligible, indicating that the extract was free of adenylate kinase. This enzyme would have interfered with the assay by generating ADP from ATP and AMP should the latter nucleotide be produced by chemical or enzymic hydrolysis of either APS or ATP.

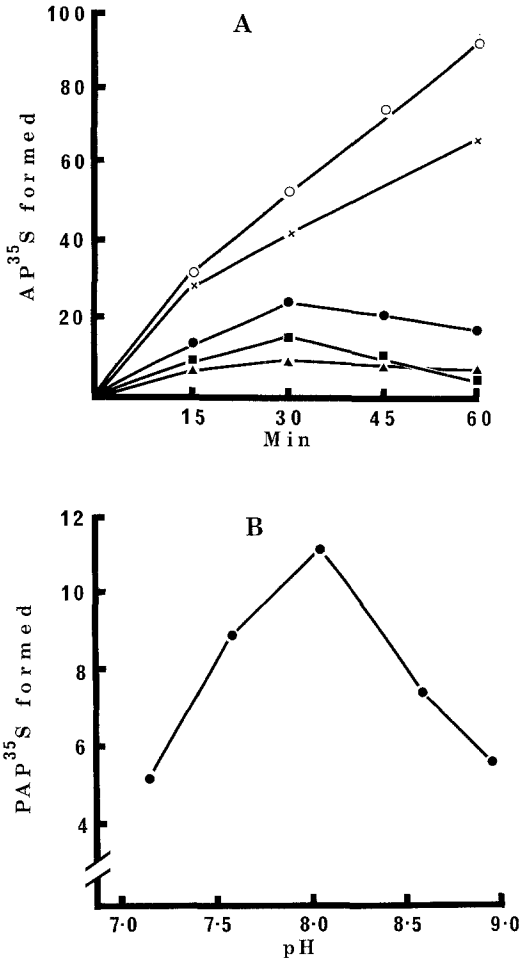

Fig. 1 A and B. Effect of pH on the production of $AP^{35}S$ and $PAP^{35}S$ in cell extracts (S_3). The experimental details are as described in Table 1. **A** Amount of $AP^{35}S$ formed represents $AP^{35}S$ (cpm $\times 10^3$) produced per ml of incubation mixture during the incubation time specified in the figure. ○, 7.15; ×, 7.6; ●, 8.05; ■, 8.6; ▲, 8.95. **B** $PAP^{35}S$ formed is expressed as $PAP^{35}S$ (cpm $\times 10^3$) produced per ml of incubation mixture in 45 min

Table 2. APS-kinase activity in cell extracts (S_{20})

Complete reaction mixture contained in 1.5 ml the following in μmoles: Tris-HCl (pH 8.0), 350; $MgCl_2$, 15; KCN, 13; NaF, 13; ATP, 3.5; phosphoenolpyruvate, 0.6; NADH, 0.4; APS, 1.0; pyruvate kinase, 13 units; lactate dehydrogenase, 13 units; 0.1 ml extract (S_{20}) containing 0.7 mg protein. The preparation S_{20} was used without desalting (see Materials and Methods). The decrease in absorbance at 340 nm was measured in a Shimadzu spectrophotometer.

Reaction mixture[a]	nmole NADH oxidized min^{-1}
Complete (0.05 ml of extract S_{20})	28.6
Complete (0.10 ml of extract S_{20})	57.1
Omit extract	4.7
Omit APS	4.7
Omit ATP	19.4
Omit APS, with AMP (5 μmole)	4.7
Omit ATP and APS	0

[a] Unless otherwise specified, 0.1 ml of extract (S_{20}) was used

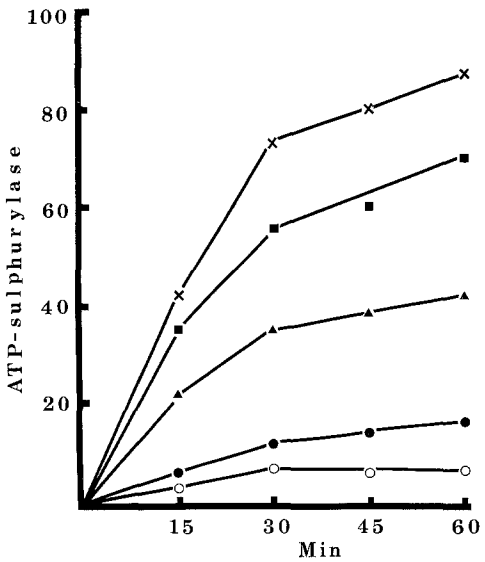

Fig. 2. Effect of incubation time and amounts of extract (S_{20}) on $AP^{35}S$ production. Reaction mixture was the same as described in Table 1 except that Tris-HCl (pH 7.1) and the following amounts of extract (S_{20}) were used: ○, 0.05 ml; ●, 0.1 ml; ▲, 0.2 ml; ■, 0.3 ml; ×, 0.4 ml. The extract contained 0.25 mg protein in 0.1 ml of the preparation. ATP-sulphurylase activity represents $AP^{35}S$ (cpm $\times 10^{-3}$) produced per ml incubation mixture

Properties of ATP-Sulphurylase

All the experiments described in this section were carried out at pH 7.15. On incubating an algal extract (S_{20}) with $^{35}SO_4^{2-}$, the amount of $AP^{35}S$ formed was linear up to 30 min and thereafter its rate of production gradually declined (Fig. 2). The results also show that correspondingly more $AP^{35}S$ was synthesized on increasing the amount of extract in the incubation mixture. Carrier-free sulphate was used in these experiments because the production of $AP^{35}S$ was linear with time as well as with graded amounts of enzyme. In an experiment in which carrier sulphate (3 μCi/0.4 μmole Na_2 SO_4) was added, 3.2 nmole of $AP^{35}S$ (44.7×10^3 cpm per ml of incubation mixture) were produced in 30 min. The corresponding counts obtained with carrier-free Na_2 $^{35}SO_4$ were 86.51×10^3 cpm per ml of incubation mixture in 30 min.

The effect on enzyme activity of incubating the extract at 57° is presented in Figure 3. The enzyme was stable up to 15 min and after 90 min it still retained about 53% of its original activity.

The results in Table 3 indicate that $AP^{35}S$ and $PAP^{35}S$ were not formed in the absence of either Mg^{2+} or ATP. The omission of inorganic pyrophos-

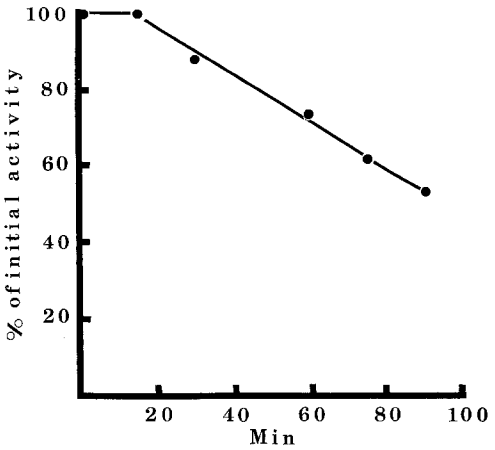

Fig. 3. Effect of heat treatment at 57° on ATP-sulphurylase activity. Cell extract (S_{20}) was maintained at 57° and, at the specified periods, aliquots were withdrawn and placed in ice. The enzyme activity was determined as described in Table 1, except that the reaction mixture contained Tris-HCl (pH 7.1). Extract (0.4 ml) containing 1.6 mg protein was used for the assay

Table 3. Requirements for the production of $AP^{35}S$ and $PAP^{35}S$ in cell extracts (S_{20})

Complete reaction mixture, in a final volume of 1 ml, contained in μmoles: Tris-HCl (pH 7.1), 50; $MgCl_2$, 10; ATP, 6; creatine phosphate 15; inorganic pyrophosphatase, 0.2 units; creatine phosphokinase, 0.2 units; $Na_2{}^{35}SO_4$ (carrier-free), 3 μCi; 0.4 ml of extract (S_{20}) containing 0.67 mg protein. Other experimental details are given in Materials and Methods

Reaction mixture	^{35}S-nucleotide produced (cpm × 10^3 per ml incubation mixture in 30 min)	
	$AP^{35}S$	$PAP^{35}S$
Complete	100.00	3.52
Boiled extract	4.99	0.30
Omit $MgCl_2$	0.13	0.13
Omit ATP and ATP-generating system	0.45	0.10
Omit ATP-generating system	115.99	2.40
Omit inorganic pyrophosphatase	98.31	4.20

phatase was without effect, presumably because the reaction might not have reached equilibrium in the absence of carrier sulphate or because an adequate amount of this enzyme was already present in the extract (S_{20}). About 15% more $AP^{35}S$ was formed in the absence of an ATP-generating system comprising creatine phosphate and creatine phosphokinase. Subsequent experiments showed that an ATP-generating system had a variable effect depending upon the concentration of ATP used (Table 4). Smaller amounts of $AP^{35}S$ were formed in the presence of an ATP-generating system than with ATP alone when

Table 4. Effect of ATP-generating system on $AP^{35}S$ production in extracts (S_{20H})

Experimental details as in Table 3. Each assay mixture contained 0.5 ml of the S_{20H} fraction containing 1.8 mg protein.

Concn. of ATP	ATP-generating system	$AP^{35}S$ produced (cpm × 10^3 per ml incubation mixture)	
(mM)		20 min	40 min
5	−	53.16	78.12
5	+	36.79	57.16
10	−	26.91	40.83
10	+	37.78	59.16

Table 5. Effect of various amounts of Mg^{2+} and ATP on ATP-sulphurylase activity in cell extract (S_{20H})

Values are cpm × 10^3 of $AP^{35}S$ per ml incubation mixture in 30 min.

Experimental details as in Table 3, except that creatine phosphate and creatine phosphokinase were omitted from the reaction mixture and different amounts of ATP and Mg^{2+} were used.

Concn. of ATP (mM)	Concentration of Mg^{2+} (mM)				
	4	8	12	20	30
4	*61.98*[a]	51.63	51.10	28.23	19.59
8	20.52	*65.99*	68.15	46.57	27.02
12	13.95	38.00	*67.56*	52.38	34.92
20	7.29	16.71	31.83	*56.29*	52.94
30	3.51	9.85	18.15	38.56	*53.84*

[a] In italics, enzyme activity at equimolar concentrations of Mg^{2+} and ATP

5 mM ATP was used. The generating system enhanced $AP^{35}S$ production in the presence of 10 mM ATP although this amount, without the generating enzyme, depressed the activity compared to that with 5 mM ATP.

Chloride salts of divalent cations like Ni^{2+}, Fe^{2+}, Cu^{2+}, Zn^{2+}, or Ca^{2+} did not replace Mg^{2+}, while 30% of $AP^{35}S$ was produced with either Mn^{2+} or Co^{2+} compared to that with Mg^{2+}. Ca^{2+} (10 mM), when included with 10 mM Mg^{2+}, diminished $AP^{35}S$ production to about half of that with Mg^{2+} alone. The maximum activity of ATP-sulphurylase was obtained with Mg^{2+}.

Neither $AP^{35}S$ nor any other ^{35}S-labelled compound was formed when other nucleotide triphosphates (UTP, GTP, CTP, TTP or ITP) were substituted for ATP in the reaction mixture. The activity with 2′-deoxyATP was only 37% that of ATP.

The amount of $AP^{35}S$ formed increased proportionally with increasing concentrations of ATP up to 6 mM but beyond this, enzyme activity declined

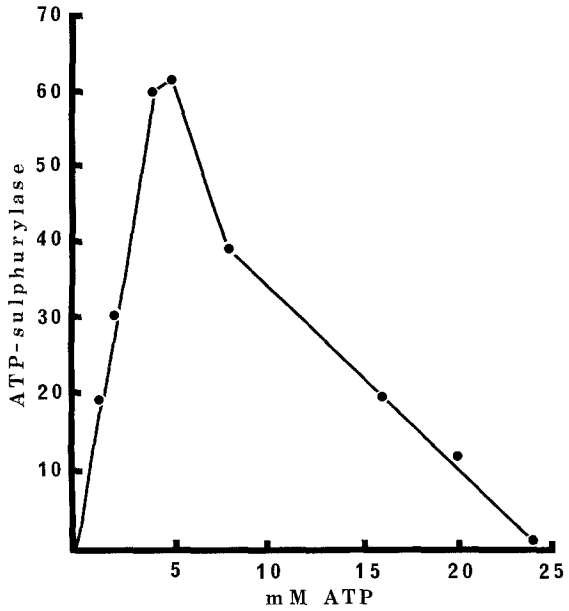

Fig. 4. Effect of different concentrations of ATP on ATP-sulphurylase activity in cell extracts (S_{20H}). The enzyme activity, determined as described in Figure 2, is expressed as $AP^{35}S$ ($cpm \times 10^3$) produced per ml of incubation mixture in 30 min

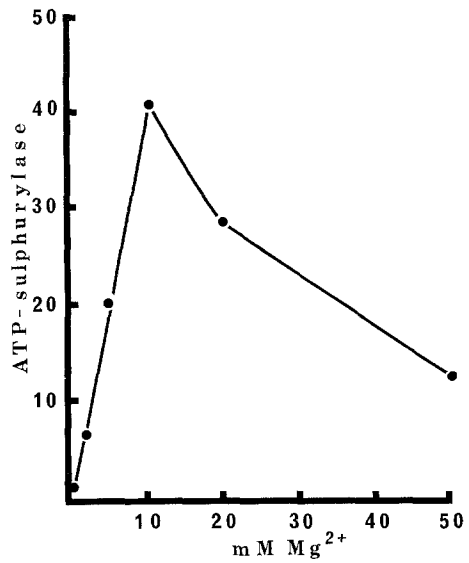

Fig. 5. Effect of different concentrations of Mg^{2+} on ATP-sulphurylase activity in cell extracts (S_{20H}). ATP-sulphurylase activity was determined as given in Figure 2 and is represented as $AP^{35}S$ ($cpm \times 10^3$) produced per ml of incubation mixture in 30 min

sharply (Fig. 4). The optimum amount of Mg^{2+} for the enzyme activity was 10 mM (Fig. 5). The data in Table 5 show that the inhibition by ATP could be reversed by increasing the concentration of Mg^{2+} and the maximum activities were obtained with equimolar quantities of Mg^{2+} and ATP. The inhibition by ATP, however, was not specific because other nucleotide triphosphates produced similar effects (Table 6). Thus, when GTP, UTP, CTP, TTP or 2′-deoxyATP, each at 12 mM, was added to the incubation

Table 6. Effect of nucleotide triphosphates[a] on ATP-sulphurylase activity (S_{20H})

The control tube contained, in a final volume of 1 ml, the following in μmoles: Tris-HCl (pH 7.1), 50; ATP, 6; $MgCl_2$, 10; inorganic pyrophosphatase, 0.2 units; $Na_2{}^{35}SO_4$ (carrier-free), 3 μCi; 0.4 ml of extract (S_{20H}) containing 1.45 mg protein. After 30 min incubation at 30°, radioactivity in $AP^{35}S$ was determined as given in Materials and Methods

Addition to reaction mixture	ATP-sulphurylase activity[b]	Inhibition (%)
Control	60.59	–
+ATP	23.49	62
+2′-deoxy ATP	13.90	78
+ GTP	23.69	60
+ CTP	25.33	59
+ UTP	19.55	68
+ TTP	24.93	59
+ ATP and $MgCl_2$ (8.0 mM)[c]	59.17	2
+ GTP and $MgCl_2$ (8.0 mM)[c]	34.33	42
+ CTP and $MgCl_2$ (8.0 mM)[c]	31.43	47

[a] Concentration 12 mM

[b] $cpm \times 10^3$ of $AP^{35}S$ produced per ml incubation mixture in 30 min

[c] In addition to that normally present (10 mM) in the reaction mixture

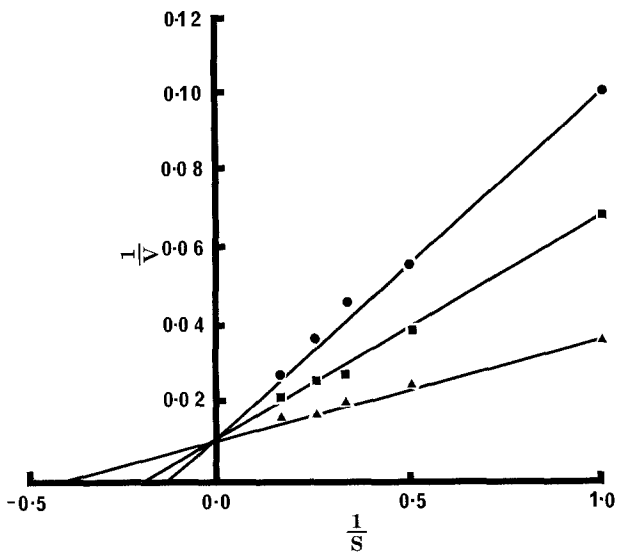

Fig. 6. Double-reciprocal plot of effect of GTP on ATP-sulphurylase activity in the presence of different concentrations of ATP. The experimental conditions are given in Table 6, except that the following amounts of GTP were added: ▲, control (without GTP); ■, 5 mM; ●, 10 mM. 1/V represents the reciprocal of $AP^{35}S$ ($cpm \times 10^3$) formed per ml of incubation mixture after 30 min incubation and 1/S is the reciprocal of mM concentration of ATP in the reaction mixture

Table 7. Intracellular distribution of ATP-sulphurylase

10 ml of crude extract (S_3) was centrifuged sequentially at $10,000 \times g$ for 30 min, $23,000 \times g$ for 30 min, and $75,000 \times g$ for 45 min. The pellet obtained after each centrifuging (designated as P_{10}, P_{23} and P_{75}, respectively) was suspended in 10 ml of 0.1 M Tris-HCl (pH 7.1). The supernatant from the final centrifuging is denoted as S_{75}. ATP-sulphurylase in these fractions was assayed in the backward reaction by measuring the amount of ATP formed from APS and inorganic pyrophosphate using the luciferin-luciferase method of Balharry and Nicholas (1970)

Fraction	ATP-sulphurylase activity (nmole of ATP produced per min)	
	per ml of the fraction	per mg protein
S_3	65	30.0
P_{10}	2	8.0
P_{23}	2	10.5
P_{75}	2	8.0
S_{75}	48	33.0

mixture (which already contained 6 mM ATP), the inhibition of enzyme activity was around 60%. The inclusion of 18 mM ATP in the reaction mixture produced a similar inhibitory effect. However, unlike ATP, the inhibition by the other nucleotide triphosphates was not readily reversed by increasing the concentration of Mg^{2+} (Table 6). A Lineweaver-Burk plot (Fig. 6) of the effect of GTP on ATP-sulphurylase indicates that this nucleotide, and possibly the others, are competitive inhibitors of ATP.

Preincubation of the extract (S_{20H}) with 10 mM of cysteine, methionine or glutathione at 7° for 1 h did not affect the enzyme activity.

ATP-sulphurylase activity was measured in various sub-cellular fractions, prepared by differential centrifugation of the broken cells as described earlier (Sawhney and Nicholas, 1976). About 75% of the total activity was recovered in the supernatant fraction obtained after centrifuging the extract at $75,000 \times g$ for 45 min (Table 7).

Discussion

Extracts of *Anabaena cylindrica* activate SO_4^{2-} to APS and under appropriate conditions PAPS is also produced. Except in a few instances (Mercer and Thomas, 1969; Schmidt, 1975; Burnell and Anderson, 1973; Stanley et al., 1975), attempts by several investigators to demonstrate the synthesis of PAPS in higher plants have not been successful (Asahi, 1964; Ellis, 1969; Balharry and Nicholas, 1970). The production of PAPS in the green algae, *Chlorella* (Hodson et al., 1968; Hodson and Schiff, 1969) and *Euglena* (Abraham and Bachhawat, 1963; Davies et al., 1966), however, is well established. Using a spectrophotometric procedure (Burnell and Whatley, 1975), we find that APS kinase is present in extracts (S_{20}) of *A. cylindrica*. Tsang and Schiff (1975) and Schmidt (1975) have proposed that photosynthetic organisms utilize APS during sulphate reduction. However, it is still not clear whether APS or PAPS is the active form of sulphate in this alga.

The properties of ATP-sulphurylase from *A. cylindrica* resembled those for the enzyme from other sources. Thus the enzyme from a variety of organisms requires a divalent cation for its activity (Wilson and Bandurski, 1956; Adams and Johnson, 1968; Panikkar and Bachhawat, 1968; Levi and Wolf, 1969; Ellis, 1969). ATP-sulphurylase from mouse mastocytoma had maximum activity with Mn^{2+} (Shoyab et al., 1972), whereas Co^{2+} was as effective as Mg^{2+} for the enzyme prepared from spinach (Shaw and Anderson, 1972) and yeast (Robbins and Lipmann, 1958). The enzyme from *A. cylindrica* had a requirement for Mg^{2+} but Mn^{2+} and Co^{2+} substituted for it to the extent of about 30%. About a third of normal activity was obtained when deoxyATP was used instead of ATP; other nucleotide triphosphates were without effect, in agreement with the results of Robbins and Lipmann (1958), Shaw and Anderson (1972), and Hawes and Nicholas (1973) with the enzyme from other organisms. The ATP-sulphurylase of the S_{20H} fraction was not affected by the end products of the pathway, e.g. cysteine, methionine and glutathione, as was also found with the enzyme from fungi (Tweedie and Segel, 1971b; Hawes and Nicholas, 1973), higher plants (Ellis, 1969), and animal tissues (Shoyab et al., 1972). ATP-sulphurylase from *A. cylindrica* was more heat stable than that from *Penicillium chrysogenum* (Tweedie and Segel, 1971a), yeast (Hawes and Nicholas, 1973), *Nitrobacter agilis* (Varma and Nicholas, 1971) and *Desulfovibrio desulphuricans* (Akagi and Campbell, 1962). In the present studies, maximum enzyme activity was obtained at pH 7.15, which differs from the broad pH optimum of 7–9 for the enzyme from other sources (Robbins and Lipmann, 1958; Panikkar and Bachhawat, 1968; Adams and Johnson, 1968; Levi and Wolf, 1969; Tweedie and Segel, 1971a; Shaw and Anderson, 1972; Hawes and Nicholas, 1973).

The observation that equimolar concentrations of Mg^{2+} and ATP were required for maximal enzyme activity is in accord with the conclusions of Tweedie and Segel (1971b) and Hawes and Nicholas (1973) that Mg^{2+}-ATP complex is the substrate for the enzyme. It has been shown that free ATP acts as a competitive inhibitor with respect to Mg^{2+}-ATP (Tweedie and Segel, 1971b). The present studies indicate that other nucleotide triphosphates also inhibited

ATP-sulphurylase and this inhibition was not readily reversed by increasing the concentration of Mg^{2+}. The observation that GTP was a competitive inhibitor of ATP without producing guanosine-5′-phosphosulphate, indicates that nucleotide triphosphates interact reversibly with the ATP-binding site of the enzyme, without producing the corresponding nucleotide-5′-phosphosulphate.

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