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ATP Sulphurylase from *Spirulina platensis*—Some Properties

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Amongst the Group VI anions used as substrate, sodium molybdate (1-2 μmoles) was found to be optimal for ATP sulphurylase (ATP sulphate adenylyl transferase, E.C. 2.7.7.4) activity in cell-free extract of the blue green alga *Spirulina platensis*. With sulphate and sulphite as substrates the inorganic phosphate (Pi) released was very low. Maximum enzyme activity was recorded when ATP and Mg^{++} were in equimolar concentrations; increasing concentrations of free ATP being inhibitory. Of the divalent cations, Mg^{++} (1.5 μmoles) gave the best results, Ca^{++} being totally inhibitory. Amongst the thiol protectors tested, cysteine and glutathione enhanced the enzyme activity, whereas N-ethylamaleimide (NEM) was found to be inhibitory.

Introduction

Inorganic sulphate metabolism has been studied extensively in bacteria (Roy & Trudinger 1970, Varma & Nicholas 1970, 1971), fungi (Tweedie & Segel 1971), green algae (Hodson & Schiff 1968, 1969, Schiff & Hodson 1973), higher plants (Shaw & Anderson 1972) and animal tissue systems (Pannikar & Bachawat 1968). However, not much is known about the assimilatory sulphate metabolism in blue-green algae Schmidt (1977) while studying four blue-green algae has suggested that 3′ phospho adenosine 5′ phospho sulphate (PAPS) might be the primary sulphate donor in *Synechocystis*, *Synechococcus* and *Spirulina*, whereas it might be adenosine 5′ phosphosulphate (APS) in *Plectonema*. However, this does not necessarily imply that APS is not formed in the first three systems. Sawhney and Nicholas (1976a) have localized the enzyme ATP sulphurylase, which catalyzes the addition of ATP to inorganic sulphate to form APS, the primary step in the activation of sulphate, and characterized some of its properties in *Anabaena cylindrica*. We have earlier reported the presence and localization of ATP sulphurylase in *Spirulina platensis*, (Menon & Varma 1979). The present paper

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deals with some of the properties of this enzyme.

Materials and Methods

Tris buffer (*p*H 7-9), inorganic pyrophosphatase (615 units/mg protein) ATP (disodium salt), tungstic acid (sodium salt), L-cysteine, L-methionine, reduced glutathione (GSH), and N-ethylamaleimide (NEM) were obtained from the Sigma Chemical Company, USA. Folin's reagent was obtained from the V P Chest Institute, Delhi. All other reagents used were of analytical grade. Cultures of *S. platensis* were obtained from the Microbiology Division, IARI, New Delhi. The alga was grown in modified Chu 10 medium (Safferman & Morris 1964) with trace elements (Allen & Arnon 1955). The cultures were maintained at $28\pm2°C$; with constant bubbling of sterile air and subcultured after every 21 days by transferring 5 ml aliquots into fresh medium. 10-day-old cultures were harvested, washed and sonicated and the homogenate was centrifuged and dialyzed following the method of Menon and Varma (1979) to give the S_{144} dz extract which was used in all the experiments.

ATP sulphurylase activity was measured by molybdolysis method modified by Hawes and Nicholas (1973). The final reaction mixture in a volume of 0.5 ml contained in μmoles: Tris-HCl (*p*H 7.4), 50; $MgCl_2$, 1.5; ATP, 1; Na_2MoO_4, 2; inorganic pyrophosphatase 2 units; enzyme, 0.05 ml unless stated otherwise. In controls, Na_2MoO_4 was omitted. The tubes were equilibriated at 40°C for 5 minutes before addition of enzyme. The reaction was allowed to proceed for 20 min at 40°C and 0.5 ml of cold 10% TCA was added to stop the reaction. The reaction mixture was immediately centrifuged at 10,000 g for 10 min to allow the precipitate to settle down, after which 2 ml of cold distilled water was added to each tube. Phosphate was estimated by the method of Fiske and Subbarow (1925). To 2 ml of sample, 0.5 ml of ammonium molybdate and 0.1 ml of reducing reagent (reducing agent : 0.2 g aminonaptho sulfonic acid, 1.25 g sodium sulphite and 1.2 g sodium bisulphite were ground in a mortar, and 0.25 g of this mixture was dissolved in 10 ml of distilled water) were added and mixed well in a vortex mixer. The E 750 was taken after 20 min in a Carl Zeiss spectrophotometer. A standard for phosphate was determined each time.

Protein was estimated by the method of Lowry and Folin (1951), using BSA as standard.

Results

The effect of varying incubation periods on the enzyme activity revealed that up to a period of 20 min there was a linear increase in the amount of Pi release, after which it levelled (figure 1). Hence for the rest of the experiments the reaction mixture was incubated for 20 min.

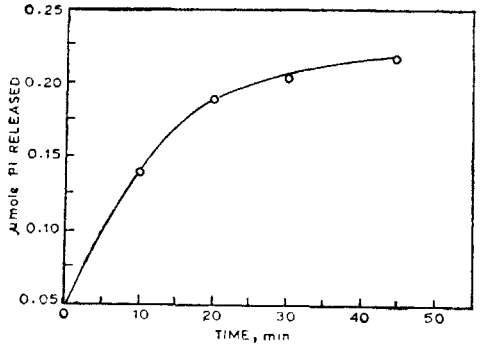

Figure 1

With increasing amounts of enzyme added there was a linear increase in the amount of Pi released. The enzyme activity was linearly proportional to protein concentration in the range of 0 μg to 432 μg (figure 2).

Sodium molybdate, 1-2 μmoles, was optimal for enzyme activity and higher

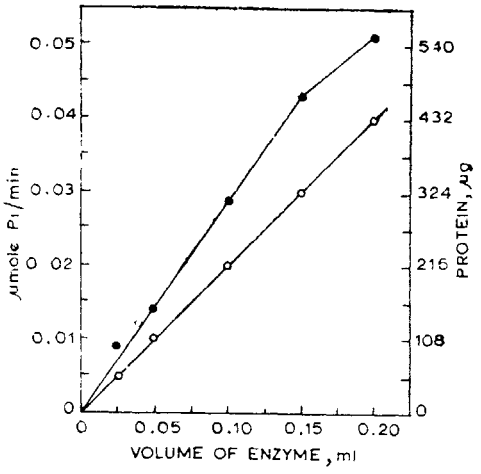

Figure 2

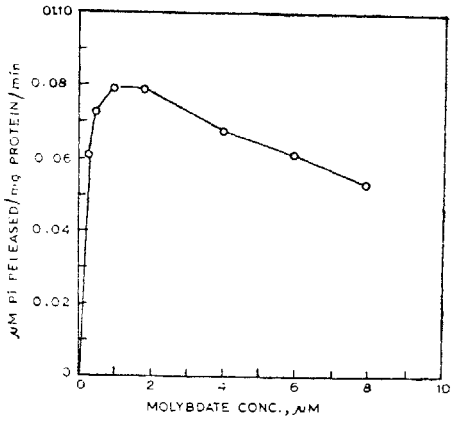

Figure 3

concentrations were inhibitory (figure 3). Other Group VI anions, such as sulphate, sulphite and tungstate were tested for their effect on enzyme activity (table 1). With tungstate as substrate the enzyme retained about 80% of its activity as compared to molybdate but with sulphite and sulphate as substrates the activity was only in the range of 10-20% as compared to molybdate (table 2).

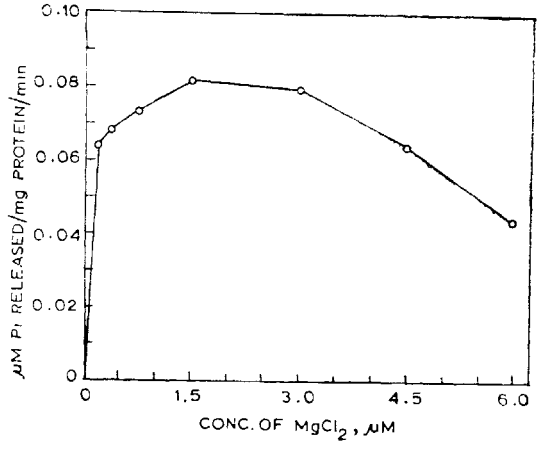

Figure 4

Table 1 *Effect of some Group VI anions on ATP sulphurylase activity*

Group VI anion	Concentration (μmoles)	μmoles Pi released/ mg protein/min
Molybdate	0.5	0.076
	1.0	0.089
Tungstate	0.5	0.065
	1.0	0.074
Sulphate	0.5	0.019
	1.0	0.012
Sulphite	0.5	0.0073
	1.0	0.0073

Reaction mixture contained in μmoles: Tris-HCl (*p*H 7.4) 50; $MgCl_2$—1.5; ATP—1; Inorganic pyrophosphotase—2 units; Enzyme—0.05 ml; Group VI anions added were as shown in table

μM = μmoles

Table 2 *Effect of divalent cations Co^{++}, Mn^{++}, Mg^{++} and Ca^{++} on ATP sulphurylase activity*

Cation	Concentration (μmoles)	μmoles Pi released/ mg protein/min
Mg^{++}	0.75	0.078
	1.50	0.092
	3.0	0.08
Co^{++}	0.75	*
	1.5	0.78
	3.0	0.58
Mn^{++}	0.75	0.0708
	1.5	0.0708
	3.0	0.067
Ca^{++}	0.75	0.00
	1.5	0.00
	3.0	0.00

*Not done

Reaction on mixture contained in μmoles: Tris-HCl (*p*H 7.4)—50; sodium molybdate —2; Inorganic pyrophosphotase—2 units; ATP—1, enzyme—0.05 ml and divalent cations as indicated in table

$MgCl_2$ at a concentration of 1.5 μmoles was optimal for enzyme activity, higher concentration being inhibitory (figure 4). Enzyme activity was highest when ATP and $MgCl_2$ were in equimolar concentrations. Increasing the concentration of free ATP caused a sharp decline in the enzyme activity (figure 5).

The effect of three divalent cations Co^{++}, Mn^{++} and Ca^{++} was compared with that of Mg^{++}. The highest activity was recorded when Mg^{++} (1.5 μmoles) was incorporated into the reaction mixture. With Mn^{++} and Co^{++} (1.5 μmoles) the enzyme activity was 75% and 85% respectively, as compared to Mg^{++}, Ca^{++} however, completely inhibited enzyme action (table 2).

Table 3 shows the effect of various thiol protectors on the enzyme. Cysteine (0.5 μmoles and glutathione (1.0 μmoles) produced the maximum enhancement of 23% and 40% respectively, as compared to controls which were without any thiol protector. Methionine did not alter the enzyme activity significantly, whereas, NEM caused inhibition (table 3).

Table 3 *Effect of various thiol protectors on ATP Sulphurylase activity*

Thiol Protector	Concentration (μmoles)	Amount of Pi (μmoles) released/ mg protein/ minute	% Activity
None		0.088	100
Cysteine	0.5	0.109	123.86
	1.0	0.091	103.4
	2.0	0.088	100
Methionine	0.5	0.097	110.22
	1.0	0.092	104.5
	2.0	0.092	104.5
GSH	0.5	0.091	103.4
	1.0	0.123	139.7
	2.0	0.088	100
NEM	0.5	0.082	93.18
	1.0	0.057	64.77
	2.0	0.038	43.18

Reaction mixture contained in a final volume of 0.5 ml: Tris-HCl (*p*H 7.4)—50 μmoles; $MgCl_2$—1.5 μmoles; ATP—1 μmoles; sodium molybdate—2 μmoles; Inorganic pyrophosphatase —2 units ; enzyme—0.05 ml; and thiol protector at concentrations mentioned in table

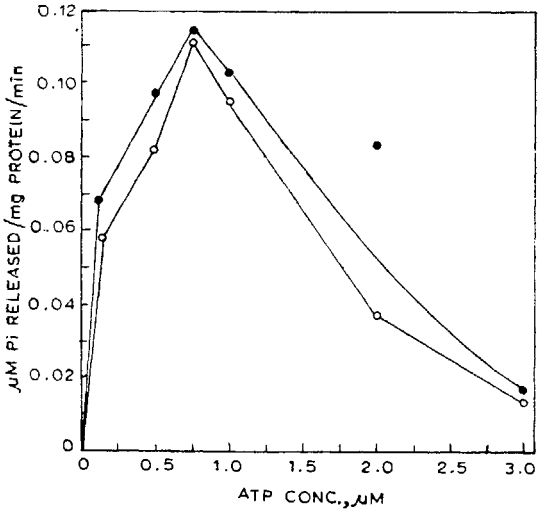

Figure 5

Discussion

ATP sulphurylase has been characterized in yeast (Hawes & Nicholas 1973), *Nitrobacter agilis* (Varma & Nicholas 1971), *Pencillium chrysogenum* (Tweedie & Segel 1971 a,b) and *Anabaena cylindrica* (Sawhney & Nicholas 1976). Our studies indicate that ATP sulphurylase from *S. platensis* has essentially the same properties as those in the above mentioned systems. However, it differs in certain important respects.

Concentrations of 1-2 μmoles of sodium molybdate were found to be optimal for activity, and further increase in concentration was inhibitory. This is in contrast to the

situation in yeast (Hawes & Nicholas 1973) where sodium molybdate concentrations upto 60 mM were not inhibitory. As in other systems, ATP sulphurylase in the present investigation showed an absolute requirement for a divalent cation.

With Mn^{++} and Co^{++} the enzyme retained 75% and 85% of its activity as compared to that of Mg^{++}. In spinach (Shaw & Anderson 1972) and yeast (Robins & Lipman 1958) Co^{++} was reported to be as effective as Mg^{++}, while in mouse mascocytoma (Shoyab et al. 1972) Mn^{++} was the most effective divalent cation. In *Anabaena cylindrica* (Sawhney & Nicholas 1976) an absolute requirement for Mg^{++} as with Co^{++} was noted; with Mn^{++}, however, the enzyme showed only 30% activity. Ca^{++} completely inhibited ATP sulphurylase activity in *S. platensis*. Although Ca^{++} inhibition has not been studied in other systems, in *Anabaena cylindrica* (Sawhney & Nicholas 1978) it completely inhibits the enzyme glutamine synthetase.

Of the Group VI anions tested for their effect on the enzyme, molybdate (1-2 μmoles) gave the best results. With tungstate there was 80% of Pi release as compared to molybdate. With sulphate and sulphite as substrates, the Pi release recorded was very low. Wilson and Bandurski (1958) suggested that when sulphate was used as the substrate the backward reaction (1) proceeded faster than the forward reaction:

$$ATP + SO_4 \rightleftharpoons APS + PPi \quad (1)$$
$$PPi \rightarrow 2Pi \quad (2)$$

As a result of this, there was very little accumulation of PPi and hence the Pi release was very low. However, when molybdate and tungstate were used as substrates the backward reaction was very much slower than the forward reaction, with the result that there was an accumulation of PPi, and hence high amounts of Pi were released. At the moment we do not offer any explanation for the release of low amounts of Pi when sulphite was used as substrate, which is in contrast to the findings of Wilson and Bandurski (1958) in yeast.

Amongst thiol protectors tested, cysteine and glutathione produced maximum enhancement in enzyme activity (23% and 40%, respectively) suggesting that—SH groups of the enzyme were essential for its activity. This also suggests that enzyme activity is not controlled by end product repression. Methionine did not alter the enzyme activity, whereas NEM was inhibitory. These results are in conformity with the reports on *Nitrobacter agilis* (Varma & Nicholas 1971). In *Anabaena cylindrica* cysteine and glutathione did not produce any enhancement in enzyme activity (Sawhney & Nicholas 1978).

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