## Adenosine 5'-triphosphate sulphurylase from *Spirulina platensis*

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*Summary.* ATP-sulphurylase from an unicellular blue-green alga, *Spirulina platensis* was localized in the soluble fractions of cell-free homogenate, and it was stable for over 3 weeks at −6°C.

Assimilatory sulphate metabolism has been studied extensively in bacteria[1-3], fungi[4], and green algae[5,6], but comparatively little is known in blue-green algae[7,8]. Recently, PAPS, (3'-phosphoadenosine-5'-phosphosulphate) has been implicated as an intermediate in the assimilatory sulphate reduction in *Spirulina, Synechococcus* and *Synechocystis,* whereas *Plectonema* has sulphotransferase activities specific for APS similar to those in green algae[9]. The present communication reports the localization and some properties of an active ATP-sulphurylase system in *Spirulina platensis.* This alga is of special interest due to the high protein content, and it is being widely used as a rich source of single cell protein[10].

*Materials and methods.* Tris buffer (pH 7–9), inorganic pyrophosphatase, (615 units/mg protein) and ATP (disodium salt) were obtained from Sigma Chemical Company, Missouri, USA. Folin's reagent was obtained from V.B. Patel Chest Institute, Delhi. All other chemicals used were of analytical grade. Culture of *Spirulina platensis* was obtained from the Culture Collection, Microbiology Division, I.A.R.I., New Delhi.

Alga was grown in a prescribed medium[11] with constant bubbling of sterile air (incubation temperature 28 °C and light intensity 1000 lux). 10 g (fresh weight) washed cells were suspended in 30 ml cold 0.1 M Tris-HCl buffer, (pH 7.4) and were disrupted at 4 °C using a MSE ultrasonic disintegrator (20 Kcys/sec) fitted with a titanium probe. The supernatant obtained after centrifuging (Zanetski K 24) the cell homogenate at 13,000 × g for 1 h was used as the crude extract ($S_{13}$). The subcellular fractions were obtained by further centrifuging at 100,000 × g for 2 h, and then at 144,000 × g for 4 h ($S_{144}$). The pellet obtained was suspended in 2 ml 0.1 M Tris-HCl buffer pH 7.4 (P144). The fraction ($S_{144}$) was then dialyzed against 2 changes of 0.1 M Tris-HCl in a cold room ($S_{144dz}$).

ATP-sulphurylase activity was assayed by the molybdolysis method, modified by Hawes and Nicholas[12]. The reaction mixture contained (total vol. 0.5 ml): Tris-HCl buffer, pH 7.4 50 µmoles; $MgCl_2$, 1.5 µmoles; ATP, 1 µmole; $Na_2MoO_4$, 2 µmoles; inorganic pyrophosphatase, 2 units; enzyme, 0.1 ml. Controls without $Na_2^-$ $MoO_4^{2-}$ and with boiled enzyme were included. The tubes were equilibrated at desired temperature for 10 min before the reaction was started by adding the enzyme. Incubation was for 30 min in a reciprocating water-bath, and 0.5 ml of ice-cold trichloroacetic acid (10%, w/v) was added to stop the reaction. The tubes were placed in ice to minimize acid-catalysed break-down of ATP. Phosphate ($P_i$) was determined immediately in the reaction mixture. The resultant protein precipitate was centrifuged for 10 min at 8000 × g and 0.5 ml sample was taken for the phosphate assay.

A modification of the method of Fiske and SubbaRow[13] was used to determine inorganic phosphate. The reaction mixture was diluted to 2.0 ml with water, and then 0.5 ml of $(NH_4)_6$ $Mo_7O_{24}$, 4 $H_2O$ and 0.1 ml of the test reagent were quickly mixed into it. After the mixture had been standing

Localization of adenosine 5'-triphosphate sulphurylase

| Fraction | Protein (mg/ml) | Specific activity (Pi, released µmoles/30 min/ mg protein | Relative purity |
|---|---|---|---|
| Supernatant ($S_{13}$) | 6.32 | 1.02 | 1 |
| Supernatant ($S_{144}$) | 4.88 | 2.29 | 2.2 |
| Supernatant ($S_{144}$) dialyzed for 22 h against 0.1 M Tris-HCl buffer (pH 7.4) | 4.08 | 2.6 | 2.5 |
| Pellet (144) | 1.64 | 0.03 | - |

10-day-old algal culture was used for the preparation of cell-free extracts. Incubation of the reaction mixture was completed at 40 ± 1 °C in a reciprocating water-bath. The molybdolysis assay was used to determine enzyme activity.

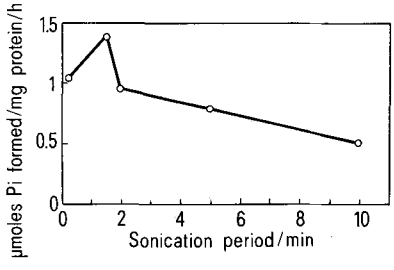


Fig. 1. Effect of sonication period on ATP sulphurylase activity.

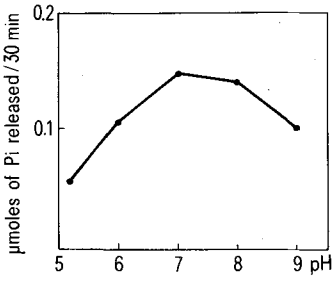


Fig. 2. Effect of pH on the activity of ATP sulphurylase.

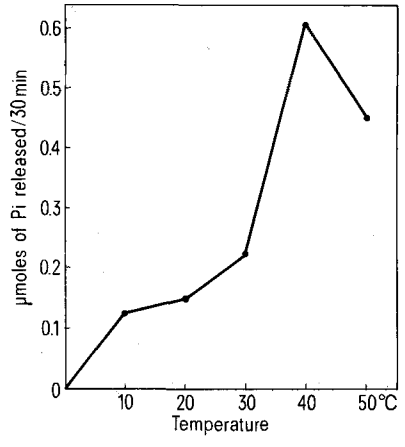


Fig. 3. Effect of temperatures on the activity of ATP sulphurylase.

for 20 min, the $E_{750}$ was determined in a spectrophotometer (1 cm light-path cell). A standard series for $P_i$ (0.04 µmoles of $P_i$) was determined each time. Protein was determined by the method of Lowry et al.[14] using bovine serum albumin as standard.

*Results and discussions.* ATP-sulphurylase was found in the supernatant fractions ($S_{13}$, $S_{144}$) (table). Cell-free preparations from 10-day-old cultures showed the maximum enzyme activity. (Figure 1 shows that maximum activity was obtained when the cell paste was disrupted by ultrasonicator (20 Kcys/sec) for 90 sec. The optimum pH of the enzyme was between 7 and 8 (figure 2). From 30 to 40 °C, there was a rapid increase in the enzyme activity, after which it declined (figure 3). Boiled enzyme (2 min) was completely inactive. The enzyme activity was stable for over 21 days when kept at − 6 °C.

The localization of ATP-sulphurylase in soluble subcellular fractions of cells, activity over the broad pH range of 7–8, are in general agreement with earlier studies[8] on enzyme from *Anabaena cylindrica.* Contrary to a report on *A. cylindrica*[8], the enzyme fraction from *S. platensis* is labile at temperatures higher tahn 50 °C.


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11 Algal Medium (g/l): $NaHCO_3$, 18; $K_2HPO_4$, 0.5; $NaNO_3$, 2.5; $K_2SO_4$, 1.0; NaCl, 0.1; $MgSO_4$. 7 $H_2O$, 0.2; $CaCl_2$, 0.04; $FeSO_4$, 7 $H_2O$, 0.01; EDTA, 0.08 and $A_5$ Sol., 1 ml pH adjusted between 9 and 10.
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## Effect of phytic acid on diamine oxidase activity in germinating pea seeds


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*Summary.* Diamine oxidase present in the cotyledons of germinating pea seeds is induced by phytic acid but the embryo enzyme is not affected. Polyamines have no effect on phytase activity of the cotyledon or embryo.


Diamine oxidase (E.C.1.4.3.6) of plants, which oxidizes several di- and polyamines as well as primary amines[3–5], has been extensively characterized from pea seedlings[3–6]. Studies from this laboratory have shown[7] that the pea enzyme, which may have a role in the synthesis of indole-3-acetic acid, is regulated in a feed-back manner by auxins and is induced by its substrates. On feeding 2,4-D to intact pea seedlings, the activity of the cotyledon diamine oxidase is reduced[7]. This effect is mediated through the embryo since its removal after soaking the seeds in 2,4-D for 14 h abolished the inhibitory effect, suggesting the elaboration of an inhibitor of diamine oxidase activity or synthesis in the embryo. The inhibitory effect of 2,4-D on the enzyme activity in the cotyledon, which may be mediated through ethylene, is reversed by exposure of seeds to red light[8].

Polyamines being cationic compounds are known to form complexes with polyanions such as nucleic acids[9,10]. It seemed worth investigating whether phytic acid, another polyanionic compound, has any control on the polyamine metabolism. The results of this study are reported in the present communication.

*Materials and methods.* Pea seeds *(Pisum sativum)* were surface sterilized with lysol and throughly washed with water and then soaked for 14 h in distilled water or other test material as specified. The seeds were then kept for germination at 22 °C in the dark in Petri dishes on moist filter papers. In cases where the embryo was removed before or after soaking, the cotyledons were kept in Petri dishes on moist filter papers for the same period of time. The time of the commencement of treatment or soaking of the seeds was considered as zero time. At specified periods cotyledon and embryo were separated and a 10% extract of the tissue was prepared by grinding with a pestle in a chilled mortar using 60 mM phosphate buffer, pH 7. The extract after passing through 2 layers of cheese cloth was used for enzyme assays. Diamine oxidase activity was determined as described earlier[7]. Phytase activity was determined according to the method of Mandal et al.[11]. 1 unit of diamine oxidase has been defined as the amount of enzyme required to form 1 µmole of $\Delta'$-pyrroline/min under the assay conditions.

*Results and discussion.* In whole seeds where the emryo was present during soaking and germination, the cotyledon diamine oxidase was induced by phytic acid (table 1). The induction of cotyledon enzyme was not affected by the removal of the embryo after soaking but its removal before soaking induced the cotyledon enzyme to a lesser extent, when compared with that obtained in cotyledons from

Table 1. Effect of phytic acid on pea cotyledon and embryo diamine oxidase activity

| Concentration of phytic acid (ppm) | Enzyme units/g fresh tissue in Cotyledon | | | | | | Embryo | |
|---|---|---|---|---|---|---|---|---|
| | Seeds soaked and germinated with embryo | | Embryo removed from seeds before soaking | | Embryo removed from seeds after soaking | | | |
| | 38 h | 62 h | 38 h | 62 h | 38 h | 62 h | 38 h | 62 h |
| 0 | 0.02 | 0.45 | 0 | 0 | 0.07 | 0.48 | 0.25 | 0.69 |
| 200 | 0.28 | 0.65 | 0 | 0.07 | 0.25 | 0.66 | 0.28 | 0.71 |
| 400 | 0.32 | 0.73 | 0.04 | 0.16 | 0.38 | 0.78 | 0.30 | 0.68 |

Values are means from duplicate samples.