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PURIFICATION AND SOME PROPERTIES OF ATP-SULFURYLASE FROM DEVELOPING SEA URCHIN EMBRYOS

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Summary

ATP-sulfurylase (ATP:sulfate adenylyltransferase, EC 2.7.7.4), purified about 200-fold from sea urchin embryos, was free of ATPase and inorganic pyrophosphatase. The molecular weight of the enzyme was approx. 280 000 measured by gel filtration. The enzyme was activated by Mg^{2+}, Ca^{2+} or Zn^{2+}; EDTA and *p*-chloromercuriphenylsulfonate inhibited the enzyme activity. The inhibition was reversed by addition of Mg^{2+} and dithiothreitol, respectively. The enzyme activity increased continuously as the pH was raised from 5.6 to 10.6. The K_m values for the enzyme were calculated to be 13 μM for adenosine 5'-phosphosulfate and 23 μM for pyrophosphate.

Introduction

It has been reported that during early embryogenesis of sea urchin, SO_4^{2-} are incorporated into the acid mucopolysaccharide moiety of proteoglycans [1,2], which are considered to play some important roles in development [3—5]. In previous papers [6,7], we have reported that the rapid increase in activity of sulfate-activating system, between the blastula and the gastrula stage, coincides with the accumulation of 3'-phosphoadenosine 5'-phosphosulfate and the synthesis of acid mucopolysaccharide in the embryos [8]. In the present paper, the purification and properties of ATP-sulfurylase, which catalyzes the first step of sulfate activation, from sea urchin embryos are described.

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Materials and Methods

Materials. Embryos of *Anthocidaris crassispina* were cultured at 20°C for 48 h with gentle stirring and collected with brief centrifugation. To 100 ml packed embryos were added 80 ml glycerol, 10 mg soybean trypsin inhibitor, 45 mg dithiothreitol and 100 ml 10 mM potassium phosphate buffer (pH 7.5), 50 mM KCl, 2 mM $MgCl_2$, 1 mM EDTA, 50% glycerol. The suspension was stored at −20°C until use. There was no significant loss of enzyme activity after 6 months.

ATP-sulfurylase assay. The enzyme activity was assayed by estimation of ATP produced from adenosine 5′-phosphosulfate and PP_i in the presence of 1 mM Mg^{2+}. The standard conditions of assay were described previously [6]. When the effects of varying concentration of substrates were studied, the volume of the first incubation mixture was scaled up to 0.8 ml in order to supply enough amount of the substrate at low concentration. One unit of the activity was defined as 1 μmol ATP produced/h and specific activity as 1 μmol ATP produced/h per mg protein.

Enzyme purification. Stored embryo suspension was homogenized with a Waring Blendor in an equal volume of 0.1 M K_2HPO_4, 2 mM 2-mercaptoethanol. The homogenate was centrifuged at 100 000 × *g* for 1 h, and 0.5 mg/ml protamine sulfate was added to the supernatant. After centrifugation, the supernatant was adsorbed on hydroxyapatite and eluted stepwise with phosphate buffer (pH 7.5). To the active fraction eluted with 0.2 M phosphate buffer, solid $(NH_4)_2SO_4$ was added and the precipitate formed at 35—65% saturation was collected, dissolved and dialyzed against buffer A (50 mM potassium phosphate buffer (pH 7.5), 2 mM 2-mercaptoethanol, 10% glycerol) containing 0.5 mM EDTA.

The dialyzed solution was applied on to a Sepharose 6B column (2 × 80 cm) and eluted with buffer A containing 0.5 mM EDTA. The enzyme was eluted in a single peak. The peak fractions were combined and adsorbed on a hydroxyapatite column (1.5 × 20 cm) and eluted with a linear gradient of phosphate buffer (0.05—0.4 M, pH 7.5). The active fractions were pooled and dialyzed

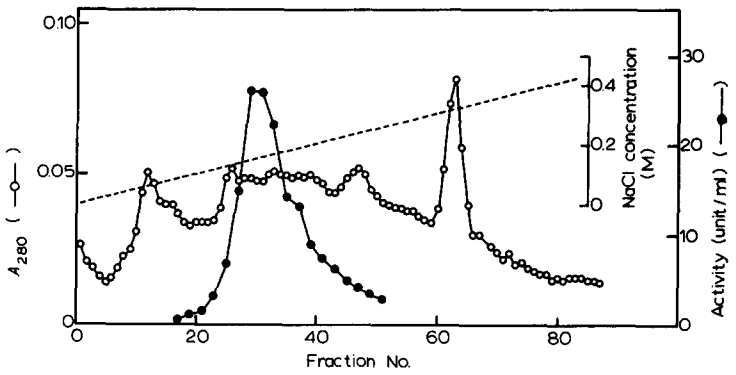


Fig. 1. Elution profile of ATP-sulfurylase from DEAE-Sephadex column. The second ammonium sulfate fraction (3.8 ml) was applied to a DEAE-Sephadex column, and after washing with 100 ml of buffer B, the enzyme was eluted with a linear gradient of NaCl (0—0.5 M) in buffer B collecting fractions of 2 ml.

against buffer A containing $(NH_4)_2SO_4$ (60% saturation). The precipitate was collected and dissolved in and dialyzed against buffer B (20 mM potassium phosphate buffer (pH 7.5), 2 mM 2-mercaptoethanol, 10% glycerol). The solution was applied to a DEAE-Sephadex column (0.9 × 10 cm) and eluted with a linear gradient of NaCl (0—0.5 M) in buffer B (Fig. 1). The peak fractions of the enzyme activity were pooled and dialyzed against buffer A.

The DEAE-Sephadex fraction as well as the second ammonium sulfate precipitate, was used for characterization of the enzyme. Neither ATPase nor inorganic pyrophosphatase activity was detected in both fractions.

Others. Adenosine 5'-phosphosulfate was prepared according to the method described by Baddily et al. [9]. Protein was determined by the method of Lowry et al. [10] using bovine serum albumin as standard.

Results

Enzyme purification

The purification steps were summarized in Table I. Approx. 200-fold purification was achieved compared with the initial homogenate, and the final specific activity was approx. 780 μmol ATP per h per mg protein.

Effects of divalent cations

Although the enzyme preparation exhibited activity without addition of exogeneous divalent cations, considerable activation was observed when they were added in the incubation mixture (Fig. 2). Mg^{2+} activated the enzyme by 90% while Mn^{2+} and Co^{2+}, by 25% at appropriate concentration. Ca^{2+} and Zn^{2+} activated the enzyme at optimal concentration of 0.5 and 0.13 mM, respectively. EDTA inhibited the enzyme activity almost completely at 5 mM, and this effect was reversed by further addition of $MgCl_2$ at 10 mM.

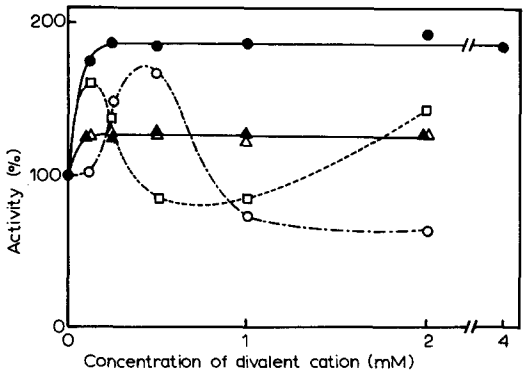
Effects of SH blocker

The enzyme retained 19% of original activity when treated with *p*-chloromercuriphenyl sulfonate at 1 mM and 4% at 5 mM. The enzyme treated with

TABLE I

PURIFICATION STEPS OF ATP-SULFURYLASE

	Protein (mg)	Specific activity (unit/mg protein)	Total activity (unit)	Recovery (%)	-fold
Homogenate	5060	3.9	$199 \cdot 10^2$	100	1
100 000 × *g* supernatant	2140	6.8	145	73	1.7
Hydroxyapatite stepwise fraction	506	19.5	99	50	5.0
First ammonium sulfate precipitate	275	29.6	81	41	7.5
Sepharose 6B fraction	140	44.9	63	32	11
Hydroxyapatite column fraction	29	113	33	17	29
Second ammonium sulfate precipitate	11	178	19	9.5	45
DEAE-Sephadex fraction	1.0	778	7.9	4.0	198



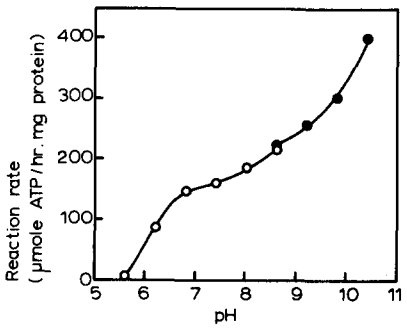


Fig. 2. Effects of divalent cations on ATP-sulfurylase. The activity of the second ammonium sulfate fraction (6.4 μg protein per assay) was determined under the standard condition except for divalent cations indicated. ●———●, $MgCl_2$; ○———○, $CaCl_2$; ▲———▲, $CoCl_2$; △———△, $MnCl_2$; □———□, $ZnCl_2$.

Fig. 3. Effects of pH on ATP-sulfurylase. The activity of the second ammonium sulfate fraction (2.1 μg protein per assay) was assayed under the standard condition except for the buffers indicated. The concentration of each buffer was 0.27 M. ●———●, glycine-NaOH; ○———○, Tris-maleate-NaOH.

the reagent at 1 mM restored its activity to 60% through successive incubation with 1.0 or 5.0 mM dithiothreitol.

pH dependence of ATP-sulfurylase

The pH dependence curve of ATP-sulfurylase was found to be unusual (Fig. 3). As the pH value was raised up from 5.6 to 10.6, the activity of the enzyme increased continuously. In order to exclude the possibility that the increase at higher range of pH was caused by loss of unknown component(s) or by modification of the enzyme during the purification process, the same experiment was carried out using crude extract as an enzyme source. The result was quite comparable with Fig. 3.

Molecular weight of ATP-sulfurylase

Molecular weight of ATP-sulfurylase was determined by Sepharose 6B gel filtration using ferritin, catalase and bovine serum albumin as standards. Molecular weight of the enzyme was calculated to be 280 000 from two independent experiments.

Kinetic constants

The effects of increasing adenosine 5′-phosphosulfate and pyrophosphate concentration on the rate of ATP-production were investigated. With double reciprocal plot of the data, K_m values were calculated to be 13 μM for adenosine-5′-phosphosulfate and 23 μM for pyrophosphate.

Discussion

In the present study, ATP-sulfurylase was purified approx. 200-fold from sea urchin embryos. This preparation exhibited specific activity of 780 μmol ATP

per h per mg protein, which is highest among animal enzymes [11—13]. It is remarkable that the specific activity of the crude extract of sea urchin embryo is 10—100-fold higher than those of rat liver [11,12]. This may be due to the fact that sea urchin embryo actively incorporates SO_4^{2-} [1,2].

The sea urchin ATP-sulfurylase required divalent cations for maximum activity. This observation is generally consistent with those on the mouse enzyme [13], but in contrast to the results of Tweedie and Segel [14] and Shaw and Anderson [15], who reported the absolute requirement of Mg^{2+}. The results of EDTA experiment of the present study indicate that trace amount of divalent cation is essential for the activity.

pH optima reported for ATP-sulfurylase from various organisms were between 7.5 and 8.5 [11,13,15,16] except for *Penicillium* enzyme which is active even at pH 10 [14]. A strange activation at high pH was observed on sea urchin enzyme. This is probably due to the change in higher order structure of the enzyme molecule induced by alkaline solution. It is reasonable to suppose that the activity around pH 8, where the slope of pH curve was gentle, corresponds to a physiological condition. Therefore ATP-sulfurylase was assayed at pH 8 throughout purification and characterization.

The molecular weight of the sea urchin ATP-sulfurylase (280 000) is remarkably smaller than that of rat liver enzyme. Burnell and Roy [12] have recently reported a molecular weight of 410 000 for their preparation.

K_m values for adenosine 5′-phosphosulfate (13 μM) and pyrophosphate (23 μM) are comparable with those of rat liver enzyme reported by Burnell and Roy [12] and are lower than in another report [11].

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