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PHOSPHOTRANSACETYLASE OF *ESCHERICHIA COLI* B, PURIFICATION AND PROPERTIES*

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SUMMARY

1. Phosphotransacetylase of *Escherichia coli* B has been purified 610 times over the crude extracts with 11% recovery, with the use of ammonium sulfate as a stabilizer. The purified enzyme was homogeneous in ultracentrifugal analysis with a $s_{20,w}$ of 8.1, and molecular weight has been tentatively estimated from the s value and from Sephadex G-200 gel filtration to be $1.6 \cdot 10^5$–$2.5 \cdot 10^5$.

2. K_m values of this enzyme were $3 \cdot 10^{-3}$–$4 \cdot 10^{-3}$ M for acetyl phosphate and $3.2 \cdot 10^{-4}$ M for CoA.

3. The enzyme was easily inactivated by dilution at 0° and by heat treatment at 46°, while sulfate protected the enzyme completely against both inactivations. The mechanism of action of sulfate as a stabilizer has been discussed in comparison with the action of phosphate.

4. Several differences in properties have been found between the enzyme of *E. coli* B and that of *Clostridium kluyveri*. These involve molecular weight and K_m values for acetyl phosphate and for CoA. There was no difference between these two enzymes with respect to their stabilities in the presence and the absence of ammonium sulfate.

INTRODUCTION

Phosphotransacetylase (acetyl-CoA:orthophosphate acetyltransferase, EC 2.3.1.8) catalyzes the formation of acetyl-CoA from acetyl phosphate (acetyl-*P*) and CoA. The enzyme is present in various bacteria[1–7], among which properties of the enzyme from *Clostridium kluyveri* have been notably described in detail[1–4]. We reported a highly specific method for determining CoA with the use of the partially purified phosphotransacetylase from *Escherichia coli* B. The enzyme was very unstable, but it was completely stabilized by the presence of 0.2 M ammonium sulfate[7]. This report deals with the purification of phosphotransacetylase of *E. coli* B, with the use

Abbreviation: acetyl-*P*, acetyl phosphate; PCMB, *p*-chloromercuribenzoate.

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of ammonium sulfate as a stabilizer throughout purification procedures. This paper also describes several properties of the purified enzyme. The enzyme of *E. coli* B has been found to be an allosteric enzyme which was activated by pyruvate and inhibited mainly by NADH. The allosteric character of this enzyme is to be described in the subsequent paper[8]. A part of these works was briefly communicated in another paper[9].

MATERIALS AND METHODS

Determination of the enzyme activity

Arsenolytic decomposition of acetyl-P. The enzyme activity was assayed by measuring acetyl-*P* arsenolyzed in the presence of CoA[7]. A reaction mixture, containing 6 μmoles of acetyl-*P*, 15.8 mμmoles of CoA (5 Lipmann units), 5 μmoles of cysteine, 20 μmoles of Tris–HCl (pH 8.0), 50 μmoles of potassium arsenate (pH 8.0) and the enzyme in a final volume of 1 ml was incubated at 25° for 15 min. The enzyme was diluted 10–250 times with 50 mM Tris–HCl (pH 8.0) before incubation, and 50 μl of the solution were added to the mixture. Acetyl-*P* remaining was determined by the hydroxamic acid method as reported previously[7]. This acetyl-*P*-determining system is designated as hydroxamic acid method I. One unit of phosphotransacetylase is defined as the amount of the enzyme which catalyzes the decomposition of 1 μmole of acetyl-*P* under the conditions described above.

K_m for acetyl-*P* was estimated in the reaction mixture described above, except that the reaction was carried out at 25° for 10 min with 20 μmoles of Tris–HCl (pH 7.8) and with varied concentrations of acetyl-*P* ranging from 0.4 μmole to 2.5 μmoles. Acetyl-*P* was measured by the following method. 0.6 ml of the hydroxylamine reagent, which was prepared before use by mixing 28% hydroxylamine–HCl with 14% NaOH and 0.5 M sodium acetate buffer (pH 5.2) (2.5:2.5:1, v/v/v), was added to the reaction mixture. The mixture was allowed to stand at room temperature for 15 min. The amount of acetyl-*P* was measured by colorimetry at 510 mμ after the addition of 0.2 ml each of 3 M HCl, 12% trichloroacetic acid and 10% $FeCl_3$ in 3 M HCl. This method is designated as hydroxamic acid method II.

K_m for CoA was measured in 0.5 ml of reaction mixture being incubated for 10 min. The reaction mixture contained 2 μmoles of acetyl-*P*, 10 μmoles of cysteine, 10 μmoles of Tris–HCl (pH 7.8), 25 μmoles of potassium arsenate (pH 7.8), the enzyme and varied concentrations of CoA ranged from 25 to 200 nmoles. Acetyl-*P* was determined by hydroxamic acid method II.

Acetyl-CoA formation. K_m for acetyl-*P* was measured also by a modified method of BERGMEYER *et al.*[4]. To a cuvette of 1-cm light path were added 120 μmoles of Tris–HCl (pH 7.8), 0.5 μmole of CoA, 3 μmoles of dithiothreitol, 100 μmoles of NH_4Cl and varied concentrations of acetyl-*P* ranging from 1.2 to 32 μmoles; water was added to give a final volume of 2.95 ml. After standing 20 min at 22°, 50 μl of the enzyme solution which had been diluted with 0.4 M ammonium sulfate (pH 7.8) and kept in an ice bath were added. Increase in absorbance at 233 mμ was read every 10 sec.

General procedures

Protein was determined according to the method of LOWRY *et al.*[10]. Blue dextran and cytochrome *c* were estimated from absorbances at 675 mμ and at 520 mμ, re-

spectively. Fibrinogen and serum albumin were estimated by measuring absorbance at 240 mμ. Enzyme activities of catalase, alcohol dehydrogenase and intestinal alkaline phosphatase were measured from the decomposition of H_2O_2 (see ref. 11), from the reduction of NAD^+ (see ref. 12) and from the hydrolysis of *p*-nitrophenyl phosphate[13], respectively.

Reagents

Dilithium acetyl-*P*, crystalline phosphotransacetylase of *Cl. kluyveri*, beef-liver catalase, yeast alcohol dehydrogenase were obtained from Boehringer. Bovine-intestinal alkaline phosphatase and horse-heart cytochrome *c* type II were purchased from Sigma Chemical Co. Bovine fibrinogen was purified chromatographically from bovine fibrinogen, Fraction I (Armour Pharmaceutical Co.). Bovine serum albumin, Fraction V, was obtained from Daiichi Pure Chemical Co. Pure CoA which was synthesized chemically in this laboratory[14] was used.

RESULTS

Purification of phosphotransacetylase

As reported previously[7], the enzyme from *E. coli* B was too unstable to be subjected to dialysis or Sephadex gel filtration. However, 0.2 M ammonium sulfate was found to stabilize the enzyme completely. Preliminary experiment has shown that the enzyme could be dialyzed and passed through a Sephadex column without any loss of its activity in the presence of 0.2 M ammonium sulfate. Based on this finding, the purification of this enzyme has been achieved successfully with the use of ammonium sulfate throughout the purification steps. All operations were done in a cold room at 4°.

E. coli B cells were harvested at late log phase from an aerated glucose medium[7]. The enzyme specific activity of the cells at this stage was considerably higher than that of the cells at stationary phase. Among several carbon sources of the medium, such as acetate, pyruvate, crotonate and glucose, the latest one, so far being tested, was found to give the highest yield of the enzyme.

Step 1. Extraction of the enzyme. E. coli B cells which were harvested from 31 l of culture were washed twice with 0.14 M NaCl and lyophilized. The dried cells (27.2 g) were sonicated in 2-g portions suspended in 40 ml of 20 mM $KHCO_3$ at 20 Kcycles for 20 min under cooling and were centrifuged at 15 000 × *g* for 30 min. The resultant supernatant fluid was saved. The combined precipitates from the respective extractions were sonicated again with 100 ml of 20 mM $KHCO_3$ and centrifuged, and the supernatant fraction was combined with the former supernatant fluid (660 ml).

Step 2. Acid-ammonium sulfate fractionation. 165 ml of a saturated ammonium sulfate solution were added to the extracts at 4° to give 20% saturation. The solution was brought to 30% saturation by further addition of the acid-saturated ammonium sulfate solution which was prepared by mixing 3 ml of H_2SO_4 with 1 l of the saturated ammonium sulfate solution. The precipitates formed were removed by centrifugation at 8 000 × *g* for 15 min. 73 ml of the acid-saturated ammonium sulfate solution were added to the supernatant fraction (810 ml) to make 35% saturation. The precipitates were collected by centrifugation and dissolved in 0.1 M Tris–HCl (pH 8) to a final volume of 342 ml.

Step 3. Alkaline ammonium sulfate fractionation. 137 ml of the saturated am-

monium sulfate solution were added to the solution of Step 2 to give 28% saturation, being adjusted to pH 8 with 1 M Tris solution. The precipitates formed were removed by centrifugation at 11 000 × *g* for 15 min, and the supernatant fluid (505 ml) was brought to 40% saturation by the addition of 51 ml of the saturated ammonium sulfate solution. The precipitates formed were harvested by centrifugation and redissolved in a small volume of 50 mM Tris–HCl (pH 8). The solution was dialyzed against 10 mM Tris–HCl containing 0.2 M ammonium sulfate (pH 7.6).

Step 4. Sephadex G-200 chromatography. The dialyzed solution of Step 3 (14 ml) was divided into two parts and each of them was passed through a Sephadex G-200 column, which was prewashed with 0.2 M ammonium sulfate (pH 7.6). Fig. 1 shows

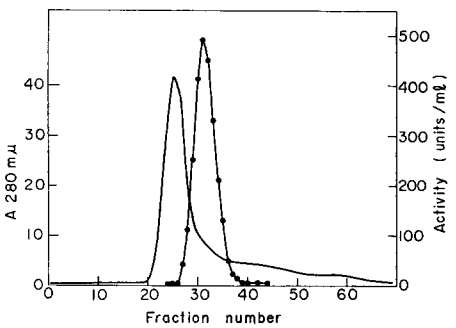

Fig. 1. Sephadex G-200 chromatography (first). The enzyme preparation from Step 3 as described in the text (volume, 7 ml) was added to a column (2.2 cm × 57 cm) of Sephadex G-200 equilibrated in 0.2 M ammonium sulfate (pH 7.6) at 4°. The enzyme was eluted with the same buffer at a flow rate of 9 ml/h, and fractions of 3 ml each were collected. $A_{280\ m\mu}$, ———; phosphotransacetylase activity ,●—●.

the elution profile of the enzyme. Tube numbers 29–35 were combined, and the enzyme was condensed by being precipitated from 50% saturation of ammonium sulfate. The condensed filtrates from 2 runs of the gel filtration were combined (8.5 ml).

Step 5. DEAE-Sephadex chromatography. The solution of Step 4 was dialyzed overnight against 0.2 M ammonium sulfate in 10 mM Tris–HCl (pH 7.6). Thereafter, the dialyzed solution was diluted exactly 4 times with 10 mM Tris–HCl (pH 8.5) and was applied to a DEAE-Sephadex column which was equilibrated with 0.05 M ammonium sulfate (pH 7.8). The enzyme was eluted from the column with an ammonium sulfate gradient ranging from 0.05 M to 0.2 M (pH 7.8). As shown in Fig. 2, the enzyme was eluted at tube numbers 80–98. These fractions were combined and condensed by lyophilization and by ammonium sulfate precipitation (2.1 ml).

Step 6. Second Sephadex G-200 chromatography. The condensed DEAE-eluate was passed again through a Sephadex G-200 column which was equilibrated in 0.2 M ammonium sulfate (pH 7.6). Elution profile of the enzyme is shown in Fig. 3. Tubes 18–22 were combined (12.5 ml), and the enzyme was condensed by ammonium sulfate precipitation and was dialyzed against 0.2 M ammonium sulfate in 10 mM Tris–HCl (pH 7.6). The purified enzyme preparation was kept frozen at −20°.

Summary of the purification of the enzyme is presented in Table I. The enzyme has been purified 610 times over the crude extracts with 11% recovery.

The purified enzyme preparation was found to be almost homogeneous in ultra-

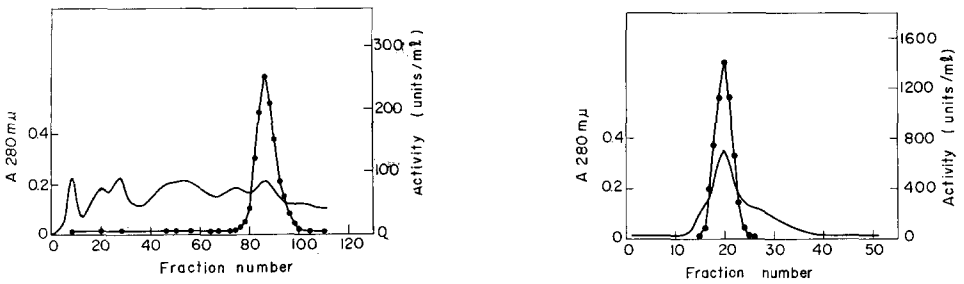

Fig. 2. DEAE-Sephadex chromatography. DEAE-Sephadex A-50 (3.5 mequiv./g) was prewashed, first with 0.5 M NaOH, followed with 0.5 M HCl and finally with redistilled water. The washed DEAE-Sephadex was equilibrated with 0.05 M ammonium sulfate in 10 mM Tris–HCl at pH 7.8, and a column (1.7 cm × 33 cm) was prepared. The column was washed with 400 ml of the same buffer as described above, and then the sample from Step 4 (see text) was added to the column. Elution was done at 4° with a linear gradient obtained with 400 ml of 0.05 M ammonium sulfate in 10 mM Tris–HCl (pH 7.8) in a mixing chamber and 400 ml of 0.2 M ammonium sulfate in 10 mM Tris –HCl (pH 7.6) in a second container. The flow rate was 25 ml/h and the fraction size was 8 ml ———, $A_{280\ m\mu}$; ●—●, phosphotransacetylase activity.

Fig. 3. Sephadex G-200 chromatography (second). The condensed DEAE-Sephadex eluate of Step 5 (see text) was passed through a column (1.7 cm × 53 cm) of Sephadex G-200, equilibrated in 0.2 M ammonium sulfate (pH 7.6) at 4°, and the enzyme was eluted at a flow rate of 3 ml/h, and the fraction size was 2.5 ml. ———, $A_{280\ m\mu}$; ●—●, phosphotransacetylase activity.

centrifugal analysis as shown in Fig. 4. $s_{20,w}$ of the enzyme was 8.1 in 10 mM Tris–HCl (pH 7.6) containing 0.2 M ammonium sulfate and at a protein concentration of 2 mg/ml.

Kinetic parameters of phosphotransacetylase

The enzyme of *E. coli* B had an optimum pH between 7.6 and 8.0.

K_m for acetyl-*P* has been estimated to be $3 \cdot 10^{-3}$ M in the presence of $1.67 \cdot 10^{-4}$ M CoA, being measured from the reaction of acetyl-CoA formation, and v_{max} was 9.8 mole of acetyl-CoA formed per mg protein per min at 22°. In the arsenolytic system, the K_m was $4 \cdot 10^{-3}$ M in the presence of $1.58 \cdot 10^{-5}$ M of CoA. Substrate inhibition has been observed with respect to acetyl-*P* at the concentrations above $4 \cdot 10^{-3}$ M in the arsenolytic system and above $1 \cdot 10^{-2}$ M in the acetyl-CoA-forming system, although the inhibition was weaker in the latter system.

K_m values for acetyl-*P* of the enzyme of *Cl. kluyveri* were $6.4 \cdot 10^{-4}$ M (*cf.* ref. 4)

TABLE I

SUMMARY OF PURIFICATION OF PHOSPHOTRANSACETYLASE

Purification step	*Vol. (ml)*	*Total activity (units)*	*Total protein (mg)*	*Specific activity (units/mg)*	*Recovery (%)*
1. Crude extract	660	119 000	14 500	8.2	100
2. Acid $(NH_4)_2SO_4$ 30–35%	342	92 000	8 200	11.3	78
3. Alkaline $(NH_4)_2SO_4$ 28–40%	25	62 500	1 750	33.6	53
4. Sephadex filtrate, first	8.5	40 000	259	153	34
5. DEAE-Sephadex eluate	2.1	14 700	8.7	1700	13
6. Sephadex filtrate, second	12.5	13 000	2.6	5000	11

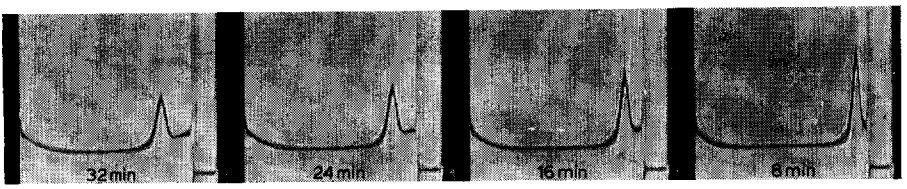

Fig. 4. Ultracentrifuge schlieren pattern of phosphotransacetylase of *E. coli* B. The photographs were taken at 8-min intervals after a maximum speed of 47 660 rev./min was reached at 13.7°. Protein concentration was 2.0 mg per ml of 0.2 M ammonium sulfate in 10 mM Tris–HCl (pH 7.6). Sedimentation proceeds from right to left.

and $1.25 \cdot 10^{-3}$ M, being estimated from the acetyl-CoA formation at $1.67 \cdot 10^{-4}$ M of CoA and from the arsenolysis reaction at $1.58 \cdot 10^{-5}$ M of CoA, respectively.

K_m values for CoA of the enzyme of *E. coli* B and of the enzyme of *Cl. kluyveri* were measured from the arsenolysis reaction in the presence of $4 \cdot 10^{-3}$ M of acetyl-*P*. The former enzyme had a K_m value of $3.2 \cdot 10^{-4}$ M, while the latter had a K_m value of $1.2 \cdot 10^{-4}$ M.

Molecular weight estimation of phosphotransacetylase using Sephadex G-200 column

For the estimation of molecular weight, phosphotransacetylase from *E. coli* B and that from *Cl. kluyveri* were passed through a Sephadex G-200 column along with standard proteins, after being dialyzed against 0.2 M ammonium sulfate (pH 7.6). The proteins used as references are described in the legend for Fig. 5, and their molecular weights and chromatographic behaviors in Sephadex G-200 gel filtration were reported in detail by ANDREWS[15].

Fig. 5 shows the elution profiles of phosphotransacetylases and those of the standard proteins. From the figure, the molecular weight of the enzyme from *E. coli* B and that from *Cl. kluyveri* have been estimated to be around $4.5 \cdot 10^5$ and $6 \cdot 10^4$,

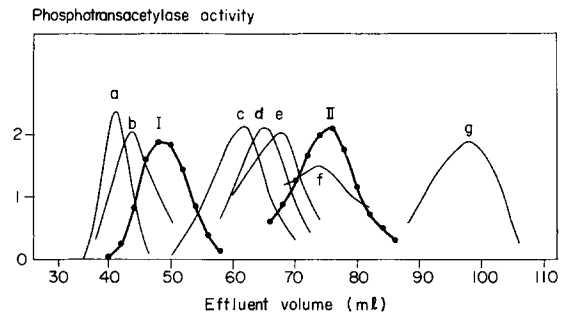

Fig. 5. Sephadex G-200 gel filtration of phosphotransacetylases along with several proteins as references. Sephadex G-200 (lot No. 2008, fine grade, Pharmacia) was added to a 0.2 M ammonium sulfate solution and was allowed to swell for 5 days. Thereafter, the gel suspension was washed with 0.2 M ammonium sulfate by repeated decantation. A column (0.75 cm × 67 cm) was prepared and was equilibrated with 0.2 M ammonium sulfate (pH 7.6) at 4°. Phosphotransacetylase from *E. coli* B and that from *Cl. kluyveri* were passed through the column along with standard proteins. Curves I and II represent the elution profiles of phosphotransacetylase from *E. coli* B and that from *Cl. kluyveri*, respectively. The standard proteins used as references were: a, blue dextran; b, bovine fibrinogen; c, beef-liver catalase; d, yeast alcohol dehydrogenase; e, intestinal alkaline phosphatase; f, bovine-serum-albumin Fraction V; g, horse-heart cytochrome *c*.

respectively, based on the restricted assumption that molecular shapes of both enzymes were globular[15].

Effect of ammonium sulfate on phosphotransacetylase

Stabilizing effect of ammonium sulfate. As reported previously, phosphotransacetylase from *E. coli* B in a partially purified state was easily inactivated by dilution, whereas the enzyme was stabilized completely by the presence of 0.2 M ammonium sulfate[7]. Fig. 6 illustrates the stability of the purified enzyme under various conditions. Dilution of the enzyme preparation with 50 mM Tris–HCl (pH 7.8) resulted in a considerable loss of the activity during storage at 0° (Fig. 6, Curve d), and the addition of mercaptoethanol did not affect the stability. However, the enzyme was fairly stable in the presence of 0.2 M ammonium sulfate as observed with the partially purified

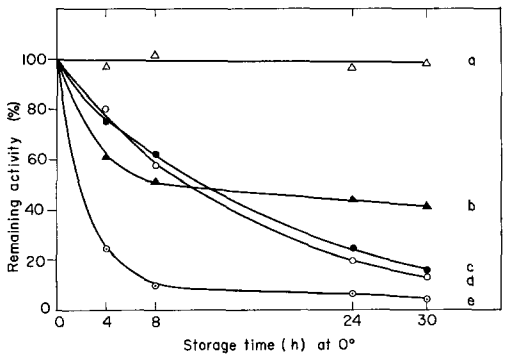

Fig. 6. Inactivation of phosphotransacetylase during storage at 0°. The purified enzyme (1.4 mg protein per ml of 0.2 M ammonium sulfate at pH 7.6) was diluted 100 times with several buffers described below. The diluted enzyme solutions were kept at 0°, and aliquots (50 μl each) of the solutions were assayed for the remaining activity at indicated time. Buffers used for diluting the enzyme were: a, 0.2 M ammonium sulfate in 50 mM Tris–HCl buffer (pH 7.8); b, 0.2 M ammonium sulfate in 50 mM Tris buffer containing 0.1 mM PCMB; c, 1 mM mercaptoethanol in 50 mM Tris buffer; d, 50 mM Tris buffer; e, 0.1 mM PCMB in 50 mM Tris buffer.

enzyme[7]. These results are likely to indicate that the inactivation by dilution occurred due to something other than oxidation of the enzyme. On the other hand, *p*-chloromercuribenzoate (PCMB) accelerated the inactivation of the enzyme as shown by Curve e in Fig. 6, but the inactivation was protected by about 50% in the presence of 0.2 M ammonium sulfate (Curve b). These results indicate that in the presence of ammonium sulfate SH groups of the enzyme protein became partially resistant against modification by PCMB.

The enzyme preparation of *E. coli* B diluted with 50 mM Tris–HCl (pH 7.8) was stable up to 20 min at 25°–37°. On heating at 46° for 10 min, the diluted enzyme lost 80% of its activity. When ammonium sulfate was added at the concentrations of 0.05 M and 0.2 M, the diluted enzyme preparation retained 56% and 100% of its initial activity, respectively, after the heat treatment. Potassium phosphate was also found to protect the enzyme against the heat inactivation. The diluted enzyme preparations, containing 0.05 M and 0.2 M potassium phosphate (pH 7.8), retained 49% and 98% of the initial enzyme activity, respectively, on heating at 46° for 10 min.

The enzyme from *Cl. kluyveri* was also inactivated by dilution and was stabilized

by the presence of ammonium sulfate. The enzyme of this source was kept at 0° for 24 h, after being diluted with 50 mM Tris–HCl (pH 7.8) containing varied concentrations of ammonium sulfate, and thereafter the remaining activity was assayed. The enzyme preparation diluted with Tris buffer to a final ammonium sulfate concentration of 0.02 M (protein concentration, 13.5 μg/ml) was found to have only 40% of its initial activity. In the presence of ammonium sulfate at 0.05 M, 0.1 M and 0.2 M, however, the enzyme was found to retain its activity by 70%, 85% and 100%, respectively, in accordance with the result observed with the enzyme of *E. coli* B (ref. 7).

Inhibitory effect of ammonium sulfate on phosphotransacetylase. As reported previously[7], ammonium sulfate was a potent inhibitor of phosphotransacetylase of *E. coli* B. Inhibitory effects of ammonium sulfate as well as of potassium phosphate were further studied using the purified enzyme of *E. coli* B, and it was found that potassium phosphate was also as potent an inhibitor as ammonium sulfate. The former salt at 2 mM, 4 mM, 10 mM and 20 mM inhibited the activity by 12%, 22%, 33% and 44%, while at those concentrations the latter inhibited it by 12%, 23%, 35% and 48%, respectively.

DISCUSSION

Phosphotransacetylase of *E. coli* B was very unstable. However, the use of ammonium sulfate to stabilize this enzyme has provided the highly purified enzyme preparation, which was homogeneous in ultracentrifugal analysis, with a value of 8.1 S in the presence of 0.2 M ammonium sulfate (Fig. 4). On the assumption that the enzyme was globular, molecular weight of this enzyme has been calculated to be $1.6 \cdot 10^5$ from its s value[16] and $4.5 \cdot 10^5$ from Sephadex G-200 gel filtration (Fig. 5). This contradiction in molecular weight between the two estimates is likely due to an anomalous shape of the enzyme molecule. The above possibility seems quite feasible, since conformation of phosphotransacetylase was suggested to be changed by the presence of ammonium sulfate, as described below. Moreover, it was reported that the molecular weight of fibrinogen, which has an enormously high axial ratio, was $3.4 \cdot 10^5$ with an s value of 7.8 (see ref. 17), whereas the value estimated by a Sephadex G-200 column was $7.7 \cdot 10^5$ (see ref. 15). Fetuin and γ-globulin, which have high axial ratios, were reported to be eluted from a Sephadex column faster than expected[15]. Supposing that phosphotransacetylase of *E. coli* B might have an axial ratio as high as that of fibrinogen, the molecular weight of this enzyme has been estimated to be around $2.5 \cdot 10^5$ from the elution profile in Sephadex G-200 gel filtration shown in Fig. 5. From these considerations, the molecular weight of phosphotransacetylase of *E. coli* B might be in a range between $1.6 \cdot 10^5$ and $2.5 \cdot 10^5$.

It was obvious, however, that there was a great difference in molecular weight between the enzymes from *E. coli* B and from *Cl. kluyveri*, since the molecular weight of the latter was estimated to be in a range between $4 \cdot 10^4$ (see ref. 4) and $6 \cdot 10^4$ (Fig. 5). Different behaviors between these two enzymes in acid ammonium sulfate fractionation might also reflect different protein species. The enzyme from *E. coli* B was precipitated between 30% and 35% saturation as described above, while the enzyme from *Cl. kluyveri* was reported to be precipitated between 49% and 70% saturation[2]. There also existed a slight difference in K_m values for CoA and for acetyl-*P* between these two enzymes; K_m of the enzyme from *E. coli* B was $3.2 \cdot 10^{-4}$ M for CoA and $3 \cdot 10^{-3}$ M

for acetyl-*P*, while that of the Clostridium enzyme was $1.2 \cdot 10^{-4}$ M and $6.4 \cdot 10^{-4}$ M, respectively. However, no difference has been observed between the two enzymes with respect to their stability in the presence and the absence of ammonium sulfate.

On examining the effects of various cations and anions, we found that SO_4^{2-} was effective for stabilizing the enzyme of *E. coli* B and for inhibiting the enzyme activity. Moreover, the dual effect of sulfate as a stabilizer and an inhibitor was suggested to be based on a common mechanism, since the effect of sulfate on the stabilization of the enzyme was found to be parallel with its inhibitory effect on the enzyme activity[7]. From the experiment using the purified enzyme, phosphate has been also shown to be a stabilizer and to be an inhibitor as effective as sulfate, presumably indicating that the mechanism of action of sulfate was the same as that of phosphate. It was, therefore, suggested that sulfate might exert its action on the enzyme as an analogue of phosphate, which is a member of the substrates of phosphotransacetylase.

The enzyme from *E. coli* B has been found to be inactivated drastically in the presence of PCMB. In the presence of ammonium sulfate, however, the PCMB-induced inactivation of the enzyme did not exceed about 50% (Fig. 6, Curve b), indicating that ammonium sulfate caused a partial resistance of SH groups of the enzyme against modification by PCMB. These results are likely to suggest that a conformational change in the enzyme molecule might occur in the presence of sulfate.

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