ACTIVATION AND INHIBITION OF PURIFIED PHOSPHOTRANSACETYLASE OF ESCHERICHIA COLI B BY PYRUVATE AND BY $NADH_2$ AND CERTAIN NUCLEOTIDES

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Phosphotransacetylase (EC 2.3.1.8 Acetyl-CoA:Orthophosphate acetyltransferase) from Escherichia coli B has been found to be very unstable and be easily inactivated by dilution, but the enzyme was found to be stabilized by the presence of ammonium sulfate(Abiko, Suzuki, Shimizu, 1967). The use of ammonium sulfate throughout purification steps has provided the highly purified enzyme preparation, which was homogeneous in ultracentrifugal analysis.

We described previously that the enzyme of partially purified state was inhibited by ATP(Suzuki, Abiko, Shimizu, 1967). The nature of the inhibition by ATP as well as by other nucleotides has been further investigated with highly purified phosphotransacetylase from E.coli B, and it was found that ADP and all kinds of nucleoside 5'-triphosphates inhibited the enzyme. The enzyme has been also found to be strongly inhibited by $NADH_2$ and to be stimulated by pyruvate. These compounds, however, did not affect the activity of phosphotransacetylase from Clostridium kluyveri.

This report describes kinetic studies on these inhibitions and activation, in addition to the purification of the enzyme.

The allosteric character of this enzyme is also discussed here. Details will be published in a subsequent paper.

METHOD

Phosphotransacetylase activity was assayed as reported previously(Abiko, Suzuki, Shimizu, 1967). This method involves measuring the decomposition of acetyl-P in the presence of CoA and arsenate. Phosphotransacetylase of Cl.kluyveri was purchased from Boehringer & Soehne GmbH.

RESULTS

Purification of the Enzyme —— Extracts from 27.5g of dried E.coli B cells were fractionated first by the acid ammonium sulfate method(Abiko, Suzuki, Shimizu, 1967). Pellet which had been harvested from between 30 and 35 % saturation of acid ammonium sulfate was fractionated again by ammonium sulfate at pH 8. The precipitates of 28 - 40 % saturation was passed through a Sephadex G-200 column in the presence of 0.2 M ammonium sulfate at pH 7.6, and the enzyme fraction was fractionated further by a DEAE-Sephadex A-50 column with ammonium sulfate gradient ranging 0.05 M to 0.2 M at pH 7.8. The enzyme preparation of this eluate was condensed by being precipitated from 50 % saturation of ammonium sulfate, and passed again through Sephadex G-200. The enzyme has been purified 610 times over the crude extracts with 11 % recovery and was homogeneous in ultracentrifugal analysis with a $S_{20,w}$ of 8.1(Fig. 1). Km values for acetyl-P and for CoA were 4×10^{-3} M and 3.2×10^{-4} M, respectively.

Effect of Nucleotides —— Phosphotransacetylase has been found to be inhibited by ADP and by all of ATP, ITP, UTP, CTP and GTP, while no inhibition was observed with other nucleoside

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Abbreviation: acetyl-P, acetylphosphate.

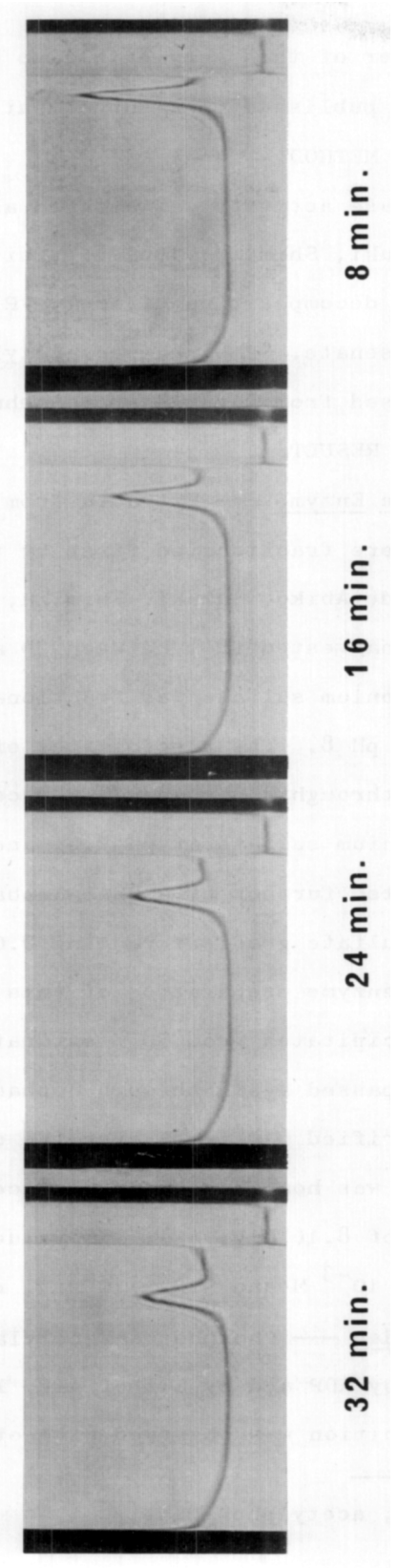

Fig. 1. Ultracentrifuge Schlieren pattern of phosphotransacetylase from E.coli B. The centrifugal speed was 47,660 rpm at 13.7°C and the photographs were taken at 8 minutes' intervals. Protein concentration was 2.0 mg per ml. of 0.2 M ammonium sulfate at pH 7.6. Sedimentation proceeds from right to left.

di- and mono-phosphates. ADP-ribose exerted a slight inhibition. Of these nucleotides tested, ADP had the strongest inhibitory effect and next to it was placed ATP.

Fig. 2 shows the pH-activity curves of the enzyme reaction in the presence and the absence of ADP. Inhibitory rates by ADP at pHs above 6.8 were almost constant: 55 % inhibition with 0.2 mM of ADP. The Lineweaver-Burk plot of the reaction with respect to CoA concentrations showed that ADP was a non-competitive inhibitor with a Ki value of 3.3×10^{-4} M. The enzyme was, however, not affected by ADP at pHs below 6.0. These results strongly suggest that ADP site was different from an active site of the enzyme. ATP was also a non-competitive inhibitor with a Ki value of 8×10^{-4} M. ADP and ATP were found to inhibit the reaction with varied concentrations of acetyl-P in sigmoidal curves.

ADP was recovered quantitatively from the reaction mixture which had been incubated with the enzyme, and phosphotransacetylase from Cl.kluyveri was not affected by ADP, indicating that ADP was not involved in the transacetylation reaction.

Effect of $NADH_2$ —— $NADH_2$ was found to have more potent inhibitory effect than ADP: 46 % inhibition at 0.036 mM of $NADH_2$ and the inhibitor was not consumed during the reaction. $NADPH_2$ had a slight inhibitory effect: 3 % as active as $NADH_2$. However, NAD and NADP had no effect even at 0.5 mM and 0.46 mM, respectively. Phosphotransacetylase from Cl.kluyveri was not affected by $NADH_2$.

In the presence of $NADH_2$, sigmoidal relationship was found between the reaction velocity and concentration of acetyl-P. The Lineweaver-Burk plot in respect of CoA concentrations indicated that $NADH_2$ was a non-competitive inhibitor with a

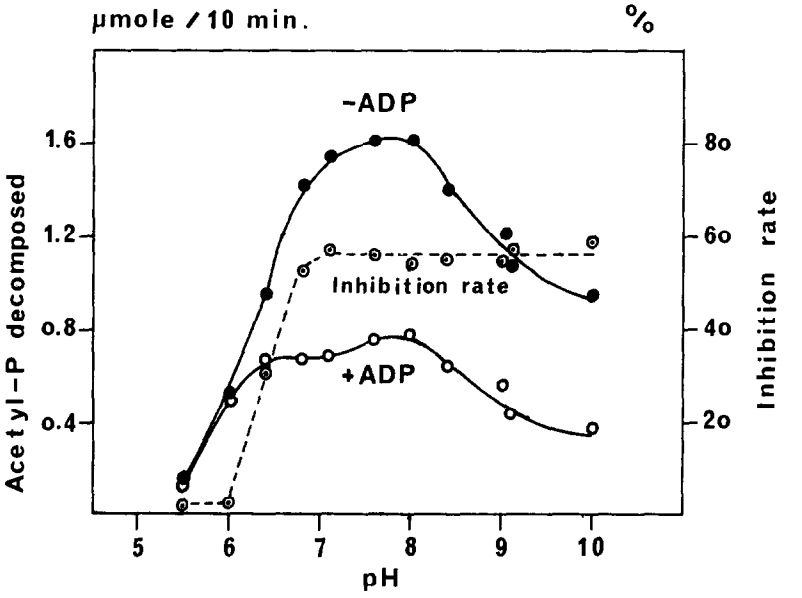

Fig. 2. Effect of ADP on the enzyme activities at varied pHs. The enzyme(1.1 unit) was incubated at 25°C for 10 min. in the reaction mixtures containing 2.0 μmoles of acetyl-P, 15.8 mμmoles of CoA, 10 μmoles of cysteine, 50 μmoles of K-arsenate of various pHs, 20 μmoles of buffers with varied pHs, in the presence and the absence of 0.2 mM of ADP; final volume was 1.0 ml. Buffers used were K-acetate(pH 5.5 - 6.4), Tris-HCl(pH 6.8 - 9 0) and glycine-KOH(pH 9.1 - 10.0), respectively. Acetyl-P was determined by the hydroxamic acid method(Abiko, Suzuki, Shimizu, 1967), except that reduced volume with increased concentration of each reagent was used.

Ki value of 6.6×10^{-5} M.

Effect of Pyruvate —— Pyruvate was found to stimulate the enzyme reaction at pH 7.8, while it did not affect at pH 5.8, suggesting that a stimulatory site of pyruvate was distinct from an active site of the enzyme. Pyruvate was not utilized in the enzyme reaction, in accordance with an analogous result that the enzyme from Cl.kluyveri was not affected by pyruvate. There was no marked effect on the enzyme activity in the following substances: α-ketoglutarate, oxaloacetate, glyoxylate, lactate, phosphoenolpyruvate, citrate, succinate, propionate, acetate, palmitate, alanine and glucose-6-phosphate.

Double reciplocal plots of the reaction velocity and substrate concentration showed that pyruvate increased Vmax without changing Km for CoA, and it lowered Km for acetyl-P without change in Vmax. Further kinetic studies have revealed a remarkable feature of the reaction that pyruvate, more effectively, activated the enzyme which had been inhibited by inhibitors than that without inhibitor: 0.5 mM of pyruvate stimulated the reaction by 80 % in the presence of 0.05 mM of $NADH_2$, while in the absence of inhibitor stimulation rate was only 12 %. As described above, in the presence of inhibitors the enzyme reactions proceeded in sigmoidal curves with respect to acetyl-P, while the above reactions to which pyruvate was added were also sigmoidal, suggesting that a stimulatory site and an inhibitory site were different from each other.

DISCUSSION

Phosphotransacetylase catalyzes the formation of acetyl-CoA from acetyl-P and CoA. The enzyme from E.coli B has been shown to be an allosteric enzyme which was activated by pyruvate and inhibited mainly by $NADH_2$. This prominent feature was observed with the enzyme from E.coli B cells harvested at log-phase from aerated glucose medium, but not with the enzyme from Cl.kluyveri. A plausible biological meaning of the control by pyruvate and by $NADH_2$ would be present in a concominant regulation of the tricarboxylic acid cycle by phosphotransacetylase along with phosphoenolpyruvate carboxylase. The former enzyme is involved in acetyl-CoA formation from pyruvate via acetyl-P in cooperation with the phosphoroclastic split of pyruvate(Strecker, 1955), or with the phosphate-linked pyruvate oxidation(Hager, Lipmann, 1955), while the latter which catalizes the formation of oxaloacetate from phosphoenol-

pyruvate was reported to be activated by acetyl-CoA(Canovas, Kornberg, 1965).

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