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PHOSPHOTRANSACETYLASE FROM *BACILLUS SUBTILIS*: PURIFICATION AND PHYSIOLOGICAL STUDIES

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SUMMARY

Phosphotransacetylase (acetyl-CoA:orthophosphate acetyl transferase, EC 2.3.1.8) was purified over 600-fold from crude extracts of *Bacillus subtilis*. The purified enzyme was activated by K^+ and NH_4^+ and inhibited by Mn^{2+} and Ca^{2+}. Apparent Michaelis constants for the substrates were determined. The equilibrium constant of the reaction was found to favor the formation of acetyl-CoA. The enzyme was capable of cleaving the short chain fatty acid esters propionyl-CoA and butyryl-CoA but the V and affinity for these substrates were less than that observed for acetyl-CoA. The enzyme was unable to cleave succinyl-CoA or palmityl-CoA but palmityl-CoA was a competitive inhibitor, with respect to CoA-SH, of the reaction. The adenine nucleotides, ATP, ADP, and AMP, were inhibitory at 1.0 mM concentration. No inhibition by pyruvate or the reduced pyridine nucleotides was observed. A molecular weight of 90 000 was calculated for the enzyme from chromatography on Sephadex G-150 columns. Physiological studies revealed that maximal enzyme activity was attained during the exponential phase of growth. Studies with citric acid cycle mutants were consistent with the conclusion that phosphotransacetylase is not regulated with the citric acid cycle.

INTRODUCTION

Phosphotransacetylase (EC 2.3.1.8, acetyl-CoA:orthophosphate acetyl transferase) has been shown[1] to catalyze the reversible reaction,

$$\text{acetyl-}P + \text{CoASH} \rightleftharpoons \text{acetyl-S-CoA} + P_i \qquad (1)$$

This enzyme has been isolated and purified from *Clostridium kluyveri* grown under special conditions[2], from *Escherichia coli*[3], from *Lactobacillus fermenti*[4], from *Veillo-*

Abbreviation: DTNB, 5,5'-dithiobis-2-nitrobenzoic acid.

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nella alcalescens[5], and observed in crude extracts of *Bacillus megaterium*[6]. The clostridial enzyme has been crystallized[7] and used as the subject of extensive kinetic studies[8]. Marked differences exist between the phosphotransacetylases derived from *E. coli* on the one hand and from *Lactobacillus* and *Clostridium* on the other. Evidence has been presented that the *E. coli* enzyme is allosterically affected by pyruvate and NADH, while the enzyme from *Clostridium* shows no such interaction[9]. In *Clostridium*, however, the highest levels of enzyme are observed in cells grown on a medium containing crotonate[1]. Physiological studies are largely lacking in other organisms.

The *in vivo* role played by the enzyme phosphotransacetylase has not been thoroughly investigated. In an effort to understand the physiological function of this enzyme we decided to study phosphotransacetylase in *Bacillus subtilis*, an organism in which regulation of the citric acid cycle has been studied[10] and a number of citric acid cycle mutants are known[11]. The work reported here deals with the partial purification of the enzyme, its kinetic properties, and the effect of various carbon sources and mutant blocks on enzyme levels.

MATERIALS AND METHODS

Organism and cultivation

B. subtilis strain JH403 is a glutamate-requiring derivative of wild type W168, and is deficient in isocitrate dehydrogenase[11]. 61141 is an acetate-requiring derivative of strain 60015 (*met*$^-$, *ind*$^-$) defective in pyruvate dehydrogenase[12]. This mutant was a gift of Dr Ernst Freese.

All strains except 61141 were grown in minimal medium[13] supplemented with 0.05% casein hydrolysate and 0.5% glucose. This medium was used both in flask cultivation and in a 150-l Fermentation Design mass culture apparatus. When noted in the text, 0.5% arabinose or maltose was substituted for glucose in this medium. 61141 was cultured in a Tris–minimal medium[14] containing 0.5% glucose and 4 mM sodium acetate.

Reagents

Coenzyme A (CoASH) and palmityl-CoA were obtained from P.-L. Biochemicals. The acetyl-, succinyl-, and propionyl-thioesters of CoASH were prepared nonenzymatically by reacting CoASH with an excess of the appropriate anhydride according to the method of Simon and Shemin[15]. The reaction was allowed to continue at 0 °C until no free thiol groups could be titrated with 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB). All other chemicals used were reagent grade.

Assay of phosphotransacetylase

Two assay methods were used to determine the activity of phosphotransacetylase in crude and partially purified samples. Method 1 measures the phosphorolysis of acetyl-CoA and depends on the reaction between DTNB and CoASH[16]. The standard reaction mixture contained 0.2 μmole acetyl-CoA, 20 μmoles potassium phosphate, pH 7.6, 10 μmoles ammonium chloride, 1.0 μmole DTNB, 66 μmoles Tris–HCl, pH 7.6, and enzyme in a final volume of 1.0 ml. The reaction was monitored by observing the increase in absorbance at 412 nm.

Method 2 permits direct observation of the breakage or formation of the

thioester bond[17]. The rate of the esterification reaction was determined by monitoring the increase in absorbance at 233 nm in a reaction mixture containing 0.2 μmoles CoASH, 2.5 μmoles acetylphosphate (acetyl-*P*), 10 μmoles ammonium chloride, 76 μmoles Tris–HCl, pH 7.6, and enzyme in a final volume of 1.0 ml. The disappearance of acetyl-CoA due to phosphorolysis was observed in a reaction mixture identical to that used in Method 1, except without DTNB. Enzyme activities are expressed according to the notation of Cooper *et al.*[18] in which a unit of activity equals that amount of enzyme required to catalyze the breakage or formation of 1 μmole of thioester bond per min.

Molecular weight determination

The method of Whittaker[19] was followed for molecular weight determinations using a Sephadex G-150 column (2.4 cm × 28 cm) equipped with upward flow adaptors. The following standards were used in calibrating the column: ribonuclease (13 700); soybean trypsin inhibitor (21 500); bovine serum albumin (68 000) and human γ-globulin (160 000). The last, a highly purified preparation, was a gift of Dr Bruce Clinton. The void volume of the column was determined with Blue Dextran 2000 each time standards or samples were run.

RESULTS

Purification of phosphotransacetylase

Phosphotransacetylase was purified from crude lysates of *B. subtilis* W168. The results of a typical purification are presented in Table I. 100 g of cells harvested in mid-log phase were collected by centrifugation, washed once in 1 l 0.1 M potassium phosphate–5 mM β-mercaptoethanol buffer (pH 6.0), and then suspended in the same volume of this buffer containing 100 mg of lysozyme, 5 mg of deoxyribonuclease and 0.5 mg ribonuclease. The suspension was made 0.01 M with respect to $MgCl_2$ and then incubated with occasional agitation for 1 h. The partially lysed cell suspension was then sonically disrupted for 10 min at 0 °C; after sonication no intact cells could be observed microscopically. The lysate was clarified by centrifugation at 16 000 × *g*

TABLE I

PURIFICATION OF PHOSPHOTRANSACETYLASE

Fraction	*Total Protein (mg)*	*Total units (μmoles acetyl-CoA cleaved/min)*	*Spec. Activity*	*Purification (-fold)*	*Yield (%)*
1. Crude lysate	9400	$2.03 \cdot 10^4$	2.16	1.0	100
2. 60–85% ammonium sulfate precipitate	3074	$1.39 \cdot 10^4$	4.52	2.1	70
3. 60 °C heated supernatant	1482	$1.46 \cdot 10^4$	9.81	4.6	75
4. DEAE pooled peak	248	$7.08 \cdot 10^3$	28.5	13	40
5. Hydroxylapatite pooled peak	10.6	$3.99 \cdot 10^3$	378	177	20
6. 85% ammonium sulfate precipitate	3.3	$2.78 \cdot 10^3$	841	395	14
7. 60 °C heated supernatant	2.2	$3.02 \cdot 10^3$	1371	644	15

and the pH was adjusted to 7.6 with 0.1 M KOH. All steps beyond this point were carried out at 0–4 °C unless otherwise noted. The lysate obtained by this procedure is Fraction 1.

Fraction 1 was brought to 60% saturation by the gradual addition of 36.1 g of ammonium sulfate/100 ml of lysate, according to the 0 °C corrected formula of Dawson *et al.*[20]. After 30 min of stirring the suspension was centrifuged at 16 000 × *g* and the precipitate was discarded. The supernatant solution was brought to 85% saturation by the addition of 16.4 g of ammonium sulfate/100 ml. The suspension was again stirred for 30 min and then centrifuged as before. The precipitate was redissolved in a minimal volume of 0.1 M potassium phosphate–5 mM β-mercaptoethanol buffer (pH 7.6) and the solution is Fraction 2.

Fraction 2 was divided into 4.0-ml aliquots and placed in a 60 °C water bath for 12 min, followed by rapid cooling. The precipitate resulting from this treatment was removed by centrifugation and the supernatant was dialyzed overnight against 2 l of the same buffer. The dialysate is Fraction 3.

Fraction 3 was loaded onto a 2.4 cm × 13 cm column of DEAE-cellulose (Whatman DE-52) previously equilibrated with 0.1 M potassium phosphate–5 mM β-mercaptoethanol buffer (pH 7.6), and the same buffer was run through the column until no material absorbant at 280 nm was detectable in the effluent. The column was eluted with a linear gradient of 400 ml 0.1 to 0.7 M potassium phosphate buffer (pH 7.6) containing 5 mM β-mercaptoethanol. 3-ml fractions were collected. The enzyme was observed to elute from the column when the molarity of eluant was 0.32 M. The most active fractions (81–92) were pooled and dialyzed overnight against 2 l of 0.1 M potassium phosphate–5 mM β-mercaptoethanol buffer (pH 7.6). The dialysate is Fraction 4.

Fraction 4 was loaded onto a 2.4 cm × 12 cm column of hydroxylapatite (Bio-Rad HT) previously equilibrated with 0.1 M potassium phosphate–5 mM β-mercaptoethanol buffer (pH 7.6). The column was washed with the same buffer until the effluent showed no absorbance at 280 nm, and was eluted with a linear gradient of 300 ml of 0.1 to 0.7 M potassium phosphate buffer (pH 7.6) containing 5 mM β-mercaptoethanol with respect to phosphate. The enzyme was found to elute from the column when the molarity of buffer reached 0.28 M. 3-ml fractions were collected and the active fractions (70–77) were pooled. The pooled volume is Fraction 5.

Fraction 5 was brought to 85% saturation with ammonium sulfate, and the precipitate derived from this treatment was dissolved in 2.0 ml of 0.1 M potassium phosphate–5 mM β-mercaptoethanol buffer (pH 7.6). The solution, Fraction 6, was treated at 60 °C for 12 min as described above. The supernatant solution obtained from the heat step was dialyzed overnight against 200 ml of 0.1 M potassium phosphate–5 mM β-mercaptoethanol buffer (pH 7.6) containing 0.1 M ammonium sulfate. The dialysate is Fraction 7. This method provided a purification in excess of 700-fold over the crude lysate with a yield of 15% (Table I).

Effect of cations and pH on phosphotransacetylase activity

Using assay Method 2, the activity of the enzyme in the esterification reaction was determined in the presence of 200 μmoles Tris–HCl over a pH range of 6.0 to 10.0. The enzyme was maximally active in a tight peak centering at pH 7.6 (Fig. 1). The same optimum was also observed for the phosphorolysis reaction.

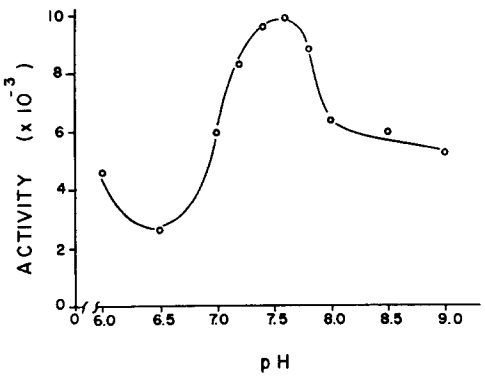

Fig. 1. Effect of pH on the transacetylation reaction. Assay method as described in the text.

The effect of mono- and divalent cations on the reaction rate was determined using a modification of Method 2 in which ammonium chloride was omitted from the assay mixture, and the ion being tested was substituted for it. The concentrations of the test ions are shown in Table II. It may be seen that K^+ and NH_4^+ at a concentration of 10 mM stimulated the reaction 3.4- and 5.2-fold, respectively, while the divalent ions, Mn^{2+} and Ca^{2+}, at 1 mM concentration, caused 50% inhibition. Barium was less effective in inhibiting the reaction than either calcium or manganese at 1 mM concentration and magnesium was observed to have no effect. Stadtman[21] reported no inhibition of the Clostridial enzyme in the presence of manganese and calcium, but did observe inhibition by lithium and sodium. As seen in Table II, the *B. subtilis* enzyme is unaffected by either lithium or sodium. In their study of the enzyme from *Lactobacillus fermenti* Nojiri *et al.*[4] observed low level stimulation of activity by NH_4^+ and K^+, and slight inhibition by calcium and magnesium.

Kinetic parameters of phosphotransacetylase

Apparent Michaelis constants for the four substrates of Reaction 1 were

TABLE II

EFFECT OF MONO AND DIVALENT CATIONS ON THE REACTION CATALYSED BY PHOSPHOTRANSACETYLASE

The transesterification reaction was assayed according to Method II. All cations were used as the chloride.

Addition	*Concn (mM)*	*Relative enzyme activity*
None		100
Ammonium	10	520
Potassium	10	343
Sodium	10	100
Lithium	10	100
Magnesium	10	109
Manganese	1	44
Calcium	1	55
Barium	1	85
Barium	10	24

TABLE III

APPARENT MICHAELIS CONSTANTS FOR PHOSPHOTRANSACETYLASE SUBSTRATES

The assays were made using Method II. Numbers in parentheses refer to references.

	K_m (*M*)			
Substrate	*B. subtilis*	*E. coli (3)*	*L. fermenti (4)*	*C. kluyveri (1)*
CoASH	$9.6 \cdot 10^{-5}$	$3.2 \cdot 10^{-4}$	$8.7 \cdot 10^{-5}$	$5.6 \cdot 10^{-4}$ (7)
Acetyl-*P*	$4.8 \cdot 10^{-4}$	$3 \cdot 10^{-3}$	—	$6.6 \cdot 10^{-4}$ (7)
Acetyl-S-CoA	$6.0 \cdot 10^{-5}$	—	—	$1.2 \cdot 10^{-3}$ (25)
PO_4	$1.0 \cdot 10^{-2}$	—	—	$2.4 \cdot 10^{-1}$ (25)

determined. The results of these determinations are shown in Table III. In determining these values, the non-varied substrate was at a concentration at least twice its K_m. The value for acetyl-CoA was determined in the presence of potassium phosphate concentrations ranging from 0.5 mM to 80 mM. In all cases, acetyl-CoA showed substrate inhibition, and the apparent K_m was approximated using the graphic method of Cleland[22] as seen in Fig. 2. These figures are in reasonable agreement with the findings for the enzyme from *C. kluyveri* and *L. fermenti*[7,4] but differ significantly from observations on the *E. coli* enzyme[3], whose affinity for acetyl-*P* and CoASH is lower by at least an order of magnitude.

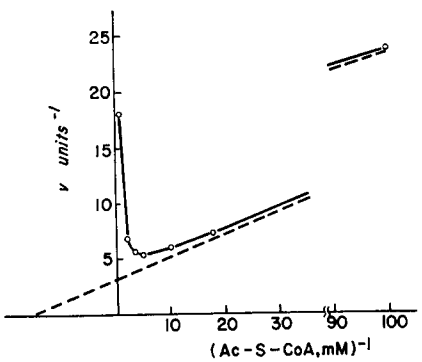

Fig. 2. Double-reciprocal plot of the effect of varying acetyl-CoA concentration on reaction rate. — — —, construction arrived at according to the method of Cleland[21] for determination of apparent K_m in the presence of substrate inhibition. P_i was kept constant at 0.05 M. The reaction was observed using Method 2.

The equilibrium constant, K_{eq}, determined using assay Method 2 for the phosphorolysis of acetyl-CoA, was in close agreement with the findings of Klotzsch for the Clostridial enzyme[2] and of Nojiri *et al.*[4] for the *Lactobacillus* enzyme. From the expression

$$\frac{[\text{Acetyl-S-CoA}] \times [P_i]}{[\text{CoASH}] \times [\text{Acetyl-}P]} = K_{eq}$$

it was found that $K_{eq} = 154 \pm 14$ when acetyl-S-CoA was varied over the range of 0.2 to 2 mM, and P_i from 0.2 to 20 mM.

Effect of nucleotides and other compounds on phosphotransacetylase

Phosphotransacetylase from *E. coli* has been shown to be an allosteric enzyme whose activity is stimulated by pyruvate and inhibited strongly by NADH and less strongly by ADP and ATP[20]. The enzyme from *Lactobacillus* was unaffected by the nicotinamide adenine phosphates in either the oxidized or the reduced form[4]. Using assay Method 2, we tested the effects of NAD, NADH, NADP, and NADPH on the reaction rates. In the concentration range between 0.1 and 1.0 mM, none of these compounds had any significant effect. Experiments with AMP, ADP and ATP, however, suggested that the relationship between the *B. subtilis* enzyme and the adenosine phosphates may be different than that observed either in *E. coli* or in *L. fermenti*. At a concentration of 1.0 mM, all three adenine nucleotides inhibited the esterification reaction. AMP and ATP caused a 33% inhibition rate, while ADP produced 37% inhibition. At the same concentrations, AMP stimulated the phosphorolysis reaction by 10% while ADP and ATP had no appreciable effect. Pyruvate, which stimulates the *E. coli* enzyme[8] and phosphoenolpyruvate were also tested for effect, as were the citric acid cycle intermediates, isocitrate, succinate and malate. None of the compounds in this last series was found to produce any change in enzyme activity.

Substrate specificity

Using DTNB to monitor the production of CoASH, it was found that the partially purified enzyme was unable to cleave palmityl- or succinyl-CoA. However, propionyl-, and butyryl-CoA were able to serve as substrates. The concentrations of these compounds required for half-maximal velocity of the phosphorolysis reaction (K_m) and the calculated V for these substrates are shown in Table IV. Although

TABLE IV

SUBSTRATE SPECIFICITY

The assays were made using Method 1.

Substrate	*V (μmoles ester cleaved/min)*	K_m *(M)*
Acetyl-CoA	3.12	$6.0 \cdot 10^{-5}$
Propionyl-CoA	0.91	$1.5 \cdot 10^{-4}$
Butyryl-CoA	0.55	$5.8 \cdot 10^{-5}$

palmityl-CoA was not cleaved by the enzyme, we found that it inhibited the enzymatic formation of acetyl-CoA. Examination of the kinetics of inhibition by palmityl-CoA (Fig. 3) showed that this compound acts as a competitive inhibitor of the interaction between CoASH and the free enzyme. A K_i of $2.78 \cdot 10^{-5}$ M was calculated from the data.

Determination of molecular weight

A crude lysate, and a sample of Fraction VII preparations, previously dialyzed against 0.1 M potassium phosphate–5 mM β-mercaptoethanol buffer (pH 7.6) containing 0.05 M ammonium sulfate were chromatographed on a Sephadex G-150

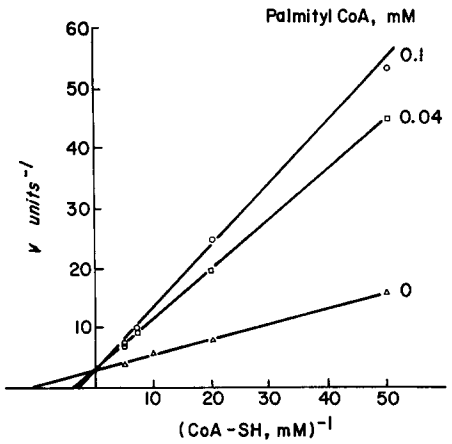

Fig. 3. Kinetics of inhibition of palmityl-CoA. Using assay Method 2, the effect of two concentrations of palmityl-CoA on the phosphorolysis of acetyl-CoA was determined. ○—○, 10^{-4} M palmityl-CoA; ×—×, $4 \cdot 10^{-5}$ M palmityl-CoA; △—△, no palmityl-CoA added. In all experiments, inorganic phosphate was kept constant at 0.05 M. The concentration of protein in the assay mixture was 90 μg/ml, and the enzyme had a specific activity of 1300 units/gm.

column equilibrated with the same buffer. The column was calibrated for molecular weight determination by observing the elution volume (V_e) of a series of standard proteins and the absolute exclusion volume (V_0) of Blue Dextran 2000. Commercial phosphotransacetylase from *C. kluyveri* (Sigma) which has a reported weight of 68 000 (ref. 3) was calculated to have a weight of 73 000. Enzymatic activity from the crude *B. subtilis* lysate and from Fraction VII, (Fig. 4) were calculated to have a molecular weight of 90 000 (ref. 15). This figure is intermediate between the values reported for the *L. fermenti* enzyme[4] and the *E. coli* enzyme[3].

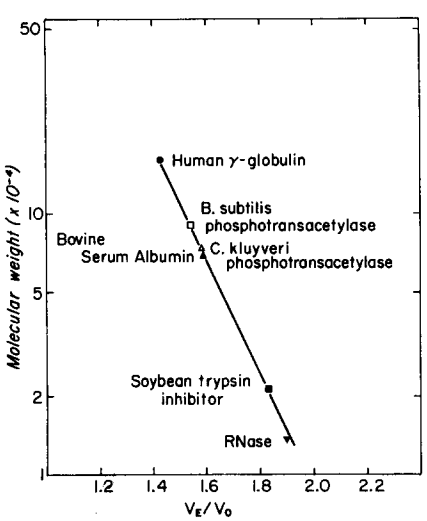

Fig. 4. Determination of molecular weight of phosphotransacetylase using Sephadex G-150 chromatography. The absolute exclusion volume, V_0, of the column was determined as described in the text.

Physiological studies on phosphotransacetylase

It was of interest to determine the relationship between phosphotransacetylase activity and growth phase since the enzymes of the citric acid cycle are known to be most active late in the logarithmic phase of growth and during the onset of sporulation. A curve of growth versus enzyme activity for the wild type strain growing in minimal medium of glucose is shown in Fig. 5. The specific activity of phosphotrans-

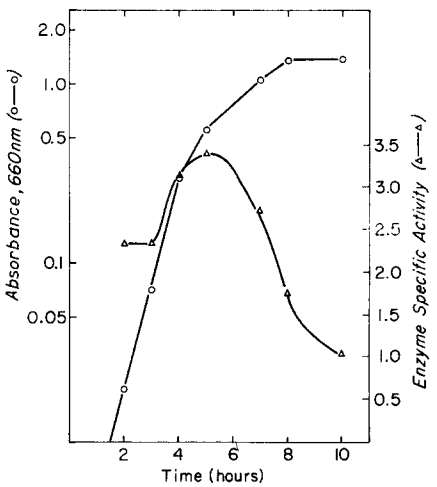

Fig. 5. Growth of wild type strain W168 and time of appearance of phosphotransacetylase. Cells were grown from a 2-l inoculum in 100 l of minimal medium containing 0.5% glucose and 0.05% casein hydrolysate. At intervals of 1 hour after inoculation, samples of one liter each were withdrawn; turbidity was assayed in a Klett-Sumerson colorimeter at 660 nm. Growth (○—○) is expressed as log absorbance value. Enzymatic activity was assayed according to Method 1 and is expressed as μmoles acetyl-CoA hydrolyzed /min per mg protein (△—△).

acetylase was seen to be highest in the late log phase and to decline sharply as the culture entered stationary phase. The decline in phosphotransacetylase at the approach of stationary phase is the inverse of that found for the citric acid cycle enzymes during this period. In order to further test whether the synthesis of phosphotransacetylase was in any way connected with the induction of the citric acid cycle, we performed experiments to determine if cultural conditions had any effect on the level of phosphotransacetylase. A portion of these experiments are shown in Table V. It may be seen that the wild type strain growing in either rich medium or on minimal medium with glucose as carbon source has a specific activity of phosphotransacetylase around 3.5. An isocitric dehydrogenase mutant, JH403, growing under these same conditions, has about a 2-fold higher level of phosphotransacetylase in the enriched medium. In minimal medium, however, the level is the same as that found in the wild type. Under these conditions, in either medium, the synthesis of aconitase in this mutant is highly derepressed. Thus, the derepression of aconitase does not appear to have any effect on the level of phosphotransacetylase. A mutant, 61141, defective in the pyruvate dehydrogenase complex was starved for acetate in order to determine whether a low level of either acetyl-CoA or acetyl phosphate might lead to derepression of the phosphotransacetylase. This mutant, when starved for acetate

TABLE V

EFFECT OF CULTURE MEDIUM ON PHOSPHOTRANSACETYLASE LEVELS IN SELECTED MUTANTS AT MID LOG GROWTH

Strain	*Medium*	*Phosphotransacetylase spec. act. (μmoles acetyl-CoO cleaved/min. per mg protein)*
W168	Penassay broth: Minimal Salts and 0.5% glucose and 0.05% acid-hydrolysed casein	3.61 3.39
JH403	As above	6.29 3.40
61141	As above *plus* 0.4 mM sodium acetate	488 1.06

(Table V), has lower levels of phosphotransacetylase than those found when the strain is grown in enriched medium. However, the levels are not drastically reduced from those seen in the wild type. Growth of the wild type strain on carbon sources other than glucose, *e.g.* maltose or arabinose, did not lead to significant changes in the level of phosphotransacetylase, nor did starvation for any of the common amino acids in auxotrophic mutants. Thus, although the level of this enzyme is subject to some change in its specific activity, in none of the experiments that we have tried, were we able to show significant derepression or induction.

DISCUSSION

Phosphotransacetylase from *B. subtilis* appears to be similar to the enzymes isolated from *Clostridium kluyveri*[8] and *Lactobacillus fermenti*[4] and also, quite recently from *Veillonella alcalescens*[5,24]. All of these enzymes are similar in their cation requirements and kinetic parameters. They differ from the *Escherichia coli* enzyme in that this enzyme appears to be under allosteric control[3]. This similarity is borne out in determinations of the molecular weight of the *B. subtilis* enzyme. On Sephadex G-150 columns we find a molecular weight of approx. 90 000, which is closer to that found for the *L. fermenti* enzyme and Clostridial enzyme and differs significantly from the high molecular weight *E. coli* enzyme.

The *B. subtilis* phosphotransacetylase was found to be sensitive to the adenine nucleotides, ATP, ADP, and AMP. In this respect, it resembles the enzyme from *V. alcalescens* since this enzyme has been shown to be sensitive to ADP and ATP, but not AMP[24]. The inhibition by ATP in this latter organism was shown to be competitive with respect to CoA and not competitive with respect to acetylphosphate. In the case of *B. subtilis*, a detailed analysis of the kinetics of inhibition has not been undertaken. Both of these enzymes differ from the *E. coli* enzyme in that NADH and pyruvate, inhibitors of the *E. coli* enzyme, have no effect on the activity of the enzyme from the former organisms.

The substrate specificity of the *B. subtilis* phosphotransacetylase has shown that the enzyme is capable of cleaving other short chain CoA esters besides acetyl-CoA. Thus, propionyl-CoA and butyryl-CoA are cleaved and the V decreases with increasing chain length, however, the K_m of the enzyme for these substrates does not

appear to be a function of chain length. The enzyme was unable to hydrolyse either succinyl-CoA or the long chain fatty acyl CoA, palmityl-CoA. Palmityl-CoA, however, was found to be a competitive inhibitor of the reaction with respect to CoA. Thus in most kinetic and molecular properties the *B. subtilis* enzyme belongs in the *C. kluyveri, L. fermenti, V. alcalescens* group.

It has been suggested that phosphotransacetylase has the function either of supplying active acetate for the production of diverse metabolites such as *O*-acetylglucose[5] or of playing a regulatory role in supplying acetyl-CoA for use in the citric acid cycle[9]. Pelroy and Whiteley[24] suggest that the phosphotransacetylase from *V. alcalescens* is mainly involved in the generation of ATP *via* the phosphoroclastic cleavage of pyruvate to acetyl-Coenzyme A and the formation of ATP from acetyl phosphate by acetyl kinase. The phosphoroclastic reaction in either *B. subtilis* or *E. coli* must not be able to supply sufficient acetyl-CoA since mutants in either of these organisms can be obtained in the pyruvate dehydrogenase complex and these mutants have an absolute requirement for acetate. The acetate requirement of the pyruvate dehydrogenase mutant, 61141, is not overcome by attempts to use pyruvate as a carbon source, nor is it overcome if the pentoses are used as carbon sources. The latter results suggest that the phosphoketolase reaction[23] may not occur in this organism, or if it does, it is unable to supply sufficient acetyl phosphate. The fact that the specific activity of phosphotransacetylase declines as the culture approaches stationary phase suggests that the enzyme is not co-regulated with the citric acid cycle since this is the time of maximal induction of these enzymes. All or our experiments on the regulation of phosphotransacetylase are most consistent with the hypothesis of Pelroy and Whitely[24] who suggest that phosphotransacetylase functions mainly to provide acetyl phosphate for ATP synthesis by acetyl kinase.

The inhibition of phosphotransacetylase by palmityl-CoA might suggest a role for long chain acyl-CoA esters in the regulation acetyl-CoA synthesis. A regulatory significance for such esters might be envisioned if phosphotransacetylase were primarily responsible for the generation of acetyl-CoA for fatty acid biosynthesis. Further mutant and kinetic studies should allow an assessment of the role of specific inhibitors.

ACKNOWLEDGEMENTS

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