Kinetic Properties of Phosphotransacetylase from Veillonella alcalescens

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The phosphotransacetylase of Veillonella alcalescens catalyzes a reversible reaction with Michaelis-Menten kinetics for all substrates. The rate of the reverse reaction (the synthesis of acetyl coenzyme A from acetyl phosphate) was 6.5 times greater than the rate of the forward reaction (the synthesis of acetyl phosphate from acetyl coenzyme A). The apparent $K_m$ values determined for the forward reaction were $8.6 \times 10^{-4}$ M for acetyl coenzyme A and $9.3 \times 10^{-3}$ M for phosphate. In the reverse reaction, the $K_m$ values were $3.3 \times 10^{-4}$ M for coenzyme A and $5.9 \times 10^{-4}$ M for acetyl phosphate. The results of an analysis of the inhibition by end products in the forward and reverse directions were compatible with a random bi-bi mechanism. The enzyme was inhibited by adenosine triphosphate and adenosine diphosphate but was not affected by reduced nicotinamide adenine dinucleotide or pyruvate. The inhibition by adenosine triphosphate was noncompetitive with respect to acetyl phosphate and competitive with respect to coenzyme A. MgCl$_2$ reversed the inhibition by adenosine triphosphate or adenosine diphosphate. The role of Mg$^{2+}$ and adenylylates in the regulation of phosphotransacetylase activity is discussed.

The major source of adenosine triphosphate (ATP) for the growth of Veillonella alcalescens (previously called Micrococcus lactilyticus) comes from the degradation of pyruvate in the following sequence: pyruvate + coenzyme A (CoA) + ferredoxin $\rightarrow$ acetyl CoA + CO$_2$ + reduced ferredoxin (A); acetyl CoA + phosphate $\rightarrow$ acetyl phosphate + CoA (B); acetyl phosphate + ADP $\rightarrow$ acetate + ATP (C). Regulation of this pathway may occur at the first (Pelroy and Whiteley, unpublished data) and last reactions. Acetokinase, the enzyme mediating reaction C, catalyzes a kinetically irreversible reaction which is activated by acetyl phosphate and strongly inhibited by ATP (13). As shown in the present investigation, the activity of phosphotransacetylase, the enzyme mediating reaction B, is also subject to inhibition by metabolites.

The same sequence of reactions occurs also in Escherichia coli, except that in this latter organism cleavage of pyruvate in reaction A involves a more complex mechanism in which nicotinamide adenine dinucleotide (NAD) serves as the electron acceptor (6). Reaction A is inhibited in E. coli by guanosine triphosphate (GTP) and by acetyl CoA but not by ATP (15). Reaction B may be inhibited by ATP, ADP, and reduced nicotinamide adenine dinucleotide (NADH) whereas pyruvate may stimulate phosphotransacetylase activity (22, 23). The phosphotransacetylase of Clostridium kluyveri, however, is not affected by these compounds (22, 23). In C. kluyveri acetyl CoA may be generated by the oxidation of acetaldheyde, and the subsequent production of ATP occurs via reactions B and C. The regulation of acetokinase in E. coli and C. kluyveri has not been examined.

We have found that the phosphotransacetylase from V. alcalescens also is not affected by NADH or pyruvate but is inhibited by the products of reaction B and by ATP and ADP. However, Mg$^{2+}$ reversed this inhibition and also reduced the inhibition of the E. coli phosphotransacetylase by ATP and ADP. This paper describes some of the kinetic properties of the V. alcalescens enzyme and discusses the regulation of phosphotransacetylase in relation to the synthesis and utilization of acetyl CoA.

MATERIALS AND METHODS

Preparation of enzymes. The purification and properties of phosphotransacetylase from V. alcalescens have been described (26). E. coli B was grown in a glucose nutrient broth medium with vigorous
Phosphotransacetylation was partially purified by the protocol used for the purification of the V. alcalescens enzyme through step 3 (chromatography on diethylaminoethyl cellulose). C. kluyveri was cultivated as described by Stadtmann and Burton (21). Mutually purified preacetate dehydrogenase was obtained from crude extracts (prepared by sonic disruption) by fractionation with ammonium sulfate (the enzyme was present in the fraction precipitated by 40-60% saturation) followed by sedimentation in sucrose gradients. The fractions from sucrose gradients containing acetdehyde dehydrogenase were free of phosphotransacetylation, adenylate kinase, and enzymes capable of degrading acetyl CoA or acetyl phosphate.

**Materials.** Acetyl phosphate and acetyl CoA were synthesized as described by Stadtmann (20) and Simon and Shemin (17), respectively. Ferredoxin (24) and rubredoxin (2) were isolated from *Micrococcus aerogenes* as described previously. Either protein can substitute for ferredoxin isolated from *V. alcalescens* in the oxidative decarboxylation of pyruvate (Garrard and Whiteley, unpublished data). Reduction of ferredoxin and rubredoxin was achieved by addition of Na$_2$S$_2$O$_4$.

**Assays.** Phosphotransacetylation was assayed by four methods: method I, which measures the "reverse reaction" (the formation of acetyl CoA from acetyl phosphate) by coupling phosphotransacetate to citrate synthetase and malate dehydrogenase; method II, which measures the "forward reaction" (the formation of acetyl phosphate from acetyl CoA) by coupling phosphotransacetylation with acetaldehyde dehydrogenase from *C. kluyveri*; method III, which determines residual acetyl CoA remaining after a specified time by coupling with citrate synthetase and malate dehydrogenase; and method IV, which measures the CoA-dependent arsenolysis of acetyl phosphate. The effects of adenylates and other compounds on the rates of reaction of the various indicator enzymes were determined in separate experiments. Unless otherwise specified, all kinetic experiments were performed with fractions having a specific activity of 400 to 600 or with completely homogeneous enzyme preparations (steps 4 and 5 or step 6 preparations from Table I of reference 26).

**Method I.** Acetyl CoA synthesis was measured via reactions D to F: acetyl phosphate + CoA = acetyl CoA + phosphate (D); malate + NAD → oxaloacetate and NADH (E); oxaloacetate + acetyl CoA → citrate + CoA (F).

The reaction mixture contained: 2.5 units of malate dehydrogenase (Calbiochem); 2.5 units of citrate synthetase (Calbiochem); 10 μmoles of β-mercaptoethanol; 10 μmoles of L-malate, 0.001 to 0.1 μmole of CoA (P-L Chemical, Inc.); 0.1 to 10 μmoles of acetyl phosphate; 50 μmoles of tris(hydroxymethyl)aminomethane (Tris)-hydrochloride buffer (pH 8.3); 0.01 to 0.1 units of phosphotransacetylation; and 0.3 μmole of NAD (Sigma Chemical Co.) in a total volume of 1 ml. All the components of the reaction mixture were contained except acetyl phosphate or CoA and incubated for 1 to 2 min at room temperature to allow the formation of NADH via reaction E to reach equilibrium. Acetyl phosphate or CoA was then added, and the changes in absorbancy at 340 nm were followed at room temperature with a Gilford cuvette positioner and recorder. It should be noted that CoA was cycled in this coupled assay and was also cycled in method II (described below) thus permitting the use of low concentrations of CoA. Accordingly, in some experiments, the amount of NADH formed was greater than the amount of CoA added. The rate of the reaction, however, was dependent on the amount of phosphotransacetylation added and was linear for at least the first 5 min after addition of the last substrate. When method I was used to measure the effect of ATP, corrections were made for the inhibition of citrate synthetase. Under the conditions of assay used, with acetyl CoA substituting for acetyl phosphate and phosphotransacetylation, there was a 25% inhibition in the rate of citrate synthetase in the presence of 5 mM ATP.

**Method II.** Acetyl CoA was generated in a CoA-dependent oxidation of acetaldehyde as shown in reactions G and H in the presence of phosphotransacetylation from *V. alcalescens* and acetaldehyde dehydrogenase from *C. kluyveri*: acetaldehyde + CoA + NAD → acetyl CoA + NADH (G); acetyl CoA + phosphate = acetyl phosphate + CoA (H).

Reaction mixtures contained: 10 units of partially purified *C. kluyveri* acetaldehyde dehydrogenase; 0.06 to 1.0 units of phosphotransacetylation; 50 μmoles of acetaldehyde; 10 μmoles of β-mercaptoethanol; 0.10 to 0.30 μmole of CoA; 1 to 10 μmoles of potassium phosphate (pH 8.3); and 50 μmoles of Tris-hydrochloride buffer (pH 8.3) in a total volume of 1 ml. The reaction was initiated by the addition of 0.3 μmole of NAD, and the increase in absorbancy at 340 nm was followed at room temperature as described above. The reaction was linear for at least 3 min.

**Method III.** A reaction mixture containing 0.01 to 1.0 μmole of acetyl CoA, 10 μmoles of β-mercaptoethanol, 10 μmoles of potassium phosphate (pH 8.3), 50 μmoles of Tris-hydrochloride buffer (pH 8.3), and 0.10 to 1.0 unit of phosphotransacetylation in a total volume of 1 ml was incubated at 25 C for 1 to 10 min depending on the experiment and then heated in a boiling water bath for 5 to 10 min to inactive phosphotransacetylation. The amount of residual acetyl CoA which is stable under these conditions (21) was estimated from the total amount of NADH formed when citrate synthetase, malate, malate dehydrogenase, and NAD were added at the concentrations used in method I. Controls containing the initial quantities of acetyl CoA used in each experiment were included to provide a direct measure of the maximum amount of NADH which could be produced under the conditions of each experiment. Separate experiments showed that the rates of reaction observed with synthetic acetyl CoA were very similar to those observed with equivalent amounts of acetyl CoA generated enzymatically from acetyl phosphate or from acetaldehyde. Acetyl phosphate did not inhibit the indicator enzymes used in these assays.

**Method IV.** Reaction mixtures contained: 10
μmoles of acetyl phosphate, 0.1 μmole of CoA, 10 μmoles of Tris-hydrochloride buffer (pH 8.3), 200 μmoles of potassium arsenate (pH 8.3), 10 μmoles of β-mercaptoethanol and phosphotransacetylase in a total volume of 1.0 ml. The reaction mixtures were incubated at 37 C for 15 to 60 min, samples were withdrawn from the reaction mixtures, and the residual acetyl phosphate was determined by the hydroxamate method (20). Ten to twenty times more enzyme was required for this assay than for assays using method I or II.

One unit of phosphotransacetylase activity is defined as that amount of protein which catalyzed the utilization of 1 μmole of substrate/min in either the forward or the reverse reaction. Specific activity was expressed as units/milligram of protein. The protein content of various fractions was determined by the method of Lowry et al. (9).

Acetokinase, adenylate kinase, acetothiokinase, and the hydrolysis of acetyl phosphate and acetyl CoA either by phosphatase activity, by deacylase activity, or by nonspecific degradation were measured as described earlier (13).

RESULTS

Comparison of two assay methods. Because some of the kinetic properties of the enzyme were measured by coupling with acetaldehyde dehydrogenase from C. kluyveri (method II), it was essential to determine if phosphotransacetylase was the only rate-limiting component in this assay—i.e., that the assays were performed in the presence of sufficient aldehyde dehydrogenase to ensure almost instantaneous conversion of CoA to acetyl CoA. This point was investigated by determining phosphotransacetylase activity in the forward direction by two different methods as a function of CoA or acetyl CoA concentration. The results of these experiments are presented in Fig. 1. The left hand ordinate of this figure indicates initial velocity measured at several CoA concentrations under conditions where acetyl CoA was generated by acetaldehyde dehydrogenase. The right hand ordinate shows initial velocity of the phosphotransacetylase-mediated conversion of chemically synthesized acetyl CoA to acetyl phosphate. The fact that the values obtained from each method fall on a common curve indicates rapid conversion of CoA to acetyl CoA in the aldehyde-coupled assay. Some of the kinetic experiments described below have been performed with acetyl CoA generated from acetaldehyde dehydrogenase. On the basis of the experiments shown in Fig. 1, the assumption was made that, under these conditions, acetyl CoA concentration was equivalent to the initial CoA concentration.

![Figure 1](http://jb.asm.org/)

**Fig. 1.** A comparison of two assay methods for the determination of the velocity of the forward reaction as a function of acetyl CoA concentration. A, Filled circles: micromoles of NADH formed per minute per milligram of protein, assayed by method II (described in Materials and Methods) in the presence of 10 mM potassium phosphate. The reaction was started by adding 0.125 μg of phosphotransacylase having a specific activity of 600. B, Open circles: micromoles of acetyl CoA converted per minute per milligram of protein assayed by method III (see Materials and Methods) in the presence of 10 mM potassium phosphate. The reaction was started by adding 0.036 μg of phosphotransacylase having a specific activity of 600.

**Determination of apparent K_m values.** The curve presented in Fig. 1 is hyperbolic, characteristic of an enzyme having Michaelis-Menten kinetics. Similar hyperbolic curves were obtained when the velocity of the reaction was measured as a function of each of the substrates in the forward and reverse directions. Lineweaver-Burk plots of initial velocity measurements as a function of substrate concentration are shown in Fig. 2 through 4 for each of the substrates in the forward and reverse directions. Figure 2A (velocity as a function of acetyl phosphate concentration at three concentrations of acetyl phosphate) presents data in the form of reciprocal plots for the synthesis of acetyl CoA from acetyl phosphate. It should be noted that the assays used in these experiments permitted the cycling of CoA. When the intercept values were replotted according to Cleland (6), in Fig. 2B and 3B, the V_max values (estimated from the intercepts on the ordinate) were very similar. The apparent K_m constants for CoA and acetyl phosphate were 3.3 x 10^-4 M and 5.9 x 10^-4 M, respectively. These values are not significantly dif-
ferent from those determined for phosphotransacetylase from *E. coli* (16) and *C. kluyveri* (3).

Similar experiments for the forward reaction (the synthesis of acetyl phosphate from acetyl CoA) yielded data which are plotted in reciprocal form in Fig. 4 and 5. As stated earlier, the concentration of acetyl CoA in these experiments, and also in those shown in Fig. 6 and 7, was assumed to be equivalent to the concentration of CoA. Figure 4 (velocity as a function of phosphate concentration determined at three concentrations of acetyl CoA and Fig. 5A (velocity as a function of acetyl CoA concentration at three concentrations of phosphate) show that the reciprocal plots yield lines which intersect at a point on the abscissa, thus permitting a direct calculation of apparent $K_m$. The $K_m$ for acetyl CoA was $8.6 \times 10^{-4}$ M and that for phosphate was $9.3 \times 10^{-2}$ M. The $V_{max}$ values for the forward and reverse reactions as determined from Fig. 2B and 5B were 0.177 and 0.027 umole/min, respectively. Thus, phosphotransacetylase catalyzed a freely reversible reaction characterized by Michaelis-Menten kinetics for all substrates. As found earlier for other phosphotransacetylases (22), the maximum velocity of the reverse reaction was significantly greater than the rate of the forward reaction. For *V. alcalescens*, the $V_{max}$ of the reverse reaction was 6.5 times greater than that of the forward reaction.

**Inhibition by end products.** The effects of acetyl phosphate and CoA on the velocity of the forward reaction are indicated in reciprocal

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**Fig. 2. Reciprocal velocity of the reverse reaction as a function of acetyl phosphate concentration determined at three concentrations of CoA.** A, Data obtained by using assay method I (see Materials and Methods). The reaction was started by adding 0.125 µg of phosphotransacetylase having a specific activity of 600. B, Intercept values from Fig. 2A as a function of the reciprocal of CoA concentration. Velocity in A and B is expressed as micromoles of NADH formed/minute.

**Fig. 3. Reciprocal velocity of the reverse reaction as a function of CoA concentration determined at three concentrations of acetyl phosphate.** A, Data obtained by using assay method I, as described in Fig. 2A. B, Intercept values from Fig. 3A as a function of the reciprocal of acetyl phosphate concentration. Velocity in A and B is expressed as micromoles of NADH formed/minute.

**Fig. 4. Reciprocal velocity of the forward reaction as a function of phosphate concentration determined at three concentrations of acetyl CoA.** Data obtained by using assay method II as described in Fig. 1A. Velocity is expressed as micromoles of NADH formed/minute.
plots shown in Fig. 6 and 7. Parallel experiments on the effect of phosphate on the velocity of the reverse reaction measured as a function of acetyl phosphate and CoA concentrations are presented in Fig. 8 and 9. It is noteworthy that at the concentrations tested all four paired combinations gave results indicating competitive inhibition—i.e., reciprocal plots of the data yielded straight lines whose intercepts were independent of inhibitor concentration. The slopes varied inversely with the concentration of the inhibitor. When the slope values from the lines shown in Fig. 6 through 9 were plotted as a function of the inhibitor concentration, straight lines were obtained (not shown).

Although the analysis is incomplete, since only four of the eight possible product inhibition patterns have been investigated, the data shown in Fig. 6 through 9 are compatible with a rapid equilibrium random addition mechanism ("random, bi-bi-"); 5). However, the finding that all product inhibitions were competitive was unexpected since noncompetitive inhibition by one or two of the products is usual for this mechanism. If it is assumed that the product competes with the substrate because both have common structural moieties, then the following pairs of compounds would be expected to be mutually exclusive with respect to phosphotransacetylase: (i) acetyl...
phosphate and acetyl CoA, (ii) acetyl CoA and CoA, and (iii) acetyl phosphate and phosphate.

As indicated in Fig. 6, 7, and 8, inhibition by these pairs of compounds was competitive. In addition, the substrate pairs (acetyl phosphate and CoA; acetyl CoA and phosphate) which might be present simultaneously on the enzyme and thus could form a ternary complex with the enzyme might also be expected to exhibit competitive inhibition. However, saturation of the enzyme with CoA would not be expected to prevent the binding of phosphate. The finding that phosphate and CoA inhibited the enzyme competitively (Fig. 9) suggests that the active site has been specialized to prevent the presence of both compounds on the enzyme simultaneously.

Effects of ATP, ADP, and other compounds. Phosphotransacetylase was sensitive to inhibition by ATP and ADP but not by AMP (Fig. 10A). The enzyme was not affected by the addition of either ethylenediaminetetraacetate (EDTA) (not shown) or MgCl₂. The presence of 1 mM EDTA had no effect on the inhibition by ATP or ADP. As seen in Fig. 10A, rather high concentrations of nucleotides (1.5 mM ATP and 6 mM ADP) were required to achieve 50% inhibition. The most interesting observation, however, was that inhibition by either adenylate was reversed by the addition of equimolar amounts of MgCl₂. Other divalent cations also reversed the inhibition, but Mg²⁺ was the most effective. Several other enzymes have been reported to be more sensitive to ATP than to the MgATP complex—e.g., hexokinase (11), fumarase (14), phosphofructokinase (8), aldolase (19), and citrate synthetase (7).

Similar results with respect to the effect of Mg²⁺ and ATP were obtained with a partially purified phosphotransacetylase fraction isolated from E. coli. In agreement with the findings of Suzuki et al. (22, 23), the enzyme was found to be sensitive to ATP, ADP, and NADH. However, as seen from the data shown in Fig. 10B, the inhibition by ATP and ADP was almost completely reversed by the addition of MgCl₂. Suzuki et al. (22, 23) reported that pyruvate increased the inhibition of the E. coli phosphotransacetylase by ADP, ATP, and NADH and proposed that all four compounds regulated phosphotransacetylase activity in vivo. Thus, regulation would be achieved by some of the components of the reaction which generates acetyl CoA (i.e., pyruvate and NADH) and of the components of the reaction which converts acetyl phosphate to acetate (i.e., ADP and ATP). These experiments were performed in the absence of Mg²⁺. The present experiments (Fig. 10B) show that Mg²⁺ had no effect on the inhibition of the E. coli enzyme by NADH where addition of pyruvate partially reversed the inhibition. The failure to observe reversal of ATP inhibition by pyruvate might be due to the fact that the fractions used in the present

![Graph](http://jb.asm.org/)

**Fig. 9.** Reciprocal velocity of the reverse reaction as a function of CoA concentration measured in the presence of phosphate. Data obtained in the presence of 0.6 mM acetyl phosphate by using assay method I as described for Fig. 2A. Velocity is expressed as micromoles of NADH formed/minute.
experiments were not as highly purified as those used by Suzuki et al. (22, 23). Also, the arsenolysis assay used in these experiments was less sensitive than that used by Suzuki et al. It should be pointed out, however, that the same results were obtained by a more sensitive assay (method I, tested in the presence of 1 to 5 mM ATP).

Neither pyruvate nor NADH had any effect on phosphotransacetylase from *V. alcalescens* nor did either compound alter the inhibition by ATP. Since NADH cannot serve as an electron acceptor in the oxidative decarboxylation of pyruvate by this anaerobe (25), suitable acceptors, ferredoxin and rubredoxin, were tested. Neither the oxidized nor the reduced forms of ferredoxin or rubredoxin had any effect on phosphotransacetylase activity or the inhibition of this activity by ATP.

**Kinetic analysis of ATP inhibition.** Although it is not known what proportion of the ATP and ADP of *V. alcalescens* exists in the free form in vivo and, hence, the possible physiological significance of the inhibition by these compounds cannot be evaluated, it was of interest to determine the nature of the inhibition as a possible means of analyzing the kinetic properties of the enzyme. Accordingly, the velocity of the reaction was measured in the presence of two concentrations of ATP and several concentrations of acetyl phosphate (Fig. 11) and CoA (Fig. 12). The data, presented in the form of reciprocal plots, indicate that the inhibition by ATP was competitive with respect to CoA and noncompetitive with respect to acetyl phosphate. Similar observations were made when ADP was substituted for ATP. These results suggest that ATP and ADP compete with CoA for the same part of the active site on the enzyme whereas acetyl phosphate does not. The velocity curves from which these data were derived were hyperbolic, indicating that cooperative effects probably were not involved.

The effects of ATP and ADP on the *V. alcalescens* phosphotransacetylase differ from those observed with a highly purified *E. coli* enzyme (22, 23). First, the latter enzyme was reported to be more sensitive to the adenylates (50% inhibition at 0.8 mM ATP and 0.35 mM ADP); secondly, the inhibition by ATP was noncompetitive with respect to CoA; and, lastly, plots of the velocity curves obtained in the presence of ATP when measured as a function of acetyl phosphate concentration were slightly sigmoidal. These results were obtained in the absence of Mg$^{2+}$; as stated earlier (Fig. 10B), addition of Mg$^{2+}$ reversed the inhibition.

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**Fig. 10. Effect of adenylates, Mg$^{2+}$, pyruvate, and NADH on the activities of phosphotransacetylase from *V. alcalescens* and *E. coli*.** A, Phosphotransacetylase from *V. alcalescens* assayed by method IV. The reaction was started by adding 2.5 µg of protein having a phosphotransacetylase specific activity of 400. B, Phosphotransacetylase from *E. coli* was assayed by method IV. The reaction was started by adding 20 µg of protein having a phosphotransacetylase specific activity of 50. Velocity in A and B is expressed as micromoles of acetyl phosphate utilized/20 min. Curve A, ATP; B, varying amounts of ATP plus 10 mM pyruvate or 1 mM NADH; C, ADP; D, AMP; E, equimolar mixtures of ATP and MgCl$_2$; F, MgCl$_2$ or pyruvate; G, NADH; H, varying amounts of NADH plus 10 mM pyruvate.
these inhibitions in the presence of 10 mM acetokinase in crude extracts of V. alcalescens (13) indicated that the activities of the two enzymes were approximately equal. As stated earlier, acetokinase catalyzes a kinetically sluggish reaction in the direction of acetyl phosphate synthesis whereas, for phosphotransacetylase, the rate of the reverse reaction was 6 to 10 times greater than the rate of the forward reaction. If the in vivo kinetic properties are similar to those determined in vitro, synthesis of ATP would be minimal and acetyl CoA would accumulate. However, on the basis of the apparent \( K_m \) values for acetyl CoA and CoA, synthesis of acetyl phosphate (and hence of ATP) would be favored at low CoA concentrations. Furthermore, during growth, removal of ATP by biosynthetic reactions would presumably "pull" the three-step sequence in the direction of ATP synthesis. If ATP accumulated, however, or if CoA were available in higher concentrations, a shift in the reactions would be expected in the direction of acetyl CoA synthesis. This could be achieved by virtue of the inhibitory effect of ATP on acetokinase and phosphotransacetylase.

Any discussion of possible regulation by ATP or ADP in vivo must take into consideration the interconversion of adenylates by adenylic kinase. Thus, the effect of changes in the ratio (ATP + 1/2 ADP)/(ATP + ADP + AMP) or "energy charge" (1) should be evaluated rather than the effect of specific concentrations of individual nucleotides. However, the present investigation indicates that the concentration of Mg\(^{2+}\) may be as important in regulating the production of ATP from pyruvate by V. alcalescens as the over-all availability of ATP and ADP.

**DISCUSSION**

Although only a limited range of inhibitor concentrations was tested, the observation that inhibition of phosphotransacetylase in the forward and reverse directions by products was competitive for all end products suggests that nonproductive ternary or "dead-end complexes" between products and substrates were not formed. In contrast, other enzymes known to catalyze rapid equilibrium random addition mechanisms (e.g., creatine kinase; reference 12) generally display one or sometimes two noncompetitive product inhibitions. A possible explanation for the apparent exclusion of substrates and products from the active site is that the enzyme undergoes local deformation due to binding of one ligand regardless of which ligand of the substrate pair is bound first. In this regard, it is of interest that inhibition of the enzyme by ATP (in the absence of Mg\(^{2+}\)) is noncompetitive with respect to acetyl phosphate and competitive with respect to CoA. If it is assumed that the latter occurs because of structural similarity between ATP and the nucleotide portion of the CoA molecules, then the portion of the active site which binds the sulfhydryl-containing pantetheinyl moiety of CoA may be involved in the mutual exclusion of substrates and end products since these inhibitions are all competitive.

A comparison of the maximum velocities of phosphotransacetylase and acetokinase in crude extracts of V. alcalescens (13) indicated
Phosphotransacetylase does not require Mg\(^{2+}\) for activity, and, as shown in this study, the enzyme is inhibited by ATP but not by MgATP. In contrast, acetokinase is sensitive to inhibition by MgATP and Mg\(^{2+}\) is required for catalysis (13). Mg\(^{2+}\) would presumably be required for the activity of the V. alcalescens adenylate kinase. Moreover, an analysis of the effect of various concentrations of Mg\(^{2+}\) on the formation of the several chelated species of adenylates which participate in the reaction catalyzed by muscle adenylate kinase has led to the proposal that Mg\(^{2+}\) may serve as an important feedback signal for the activity of this enzyme (4). The concentration of intracellular MgATP would also be affected by pH (14).

The intracellular concentration of Mg\(^{2+}\) in V. alcalescens is not known. It has been estimated that the concentration of free Mg\(^{2+}\) in E. coli is approximately 4 mM (10) and that the total concentration of Mg\(^{2+}\) in bacteria is approximately 10 mM (18). If the intracellular concentration of Mg\(^{2+}\) in V. alcalescens is of the same order of magnitude, it is apparent that relatively small fluctuations in the availability of this cation could have significant effects on the regulation of phosphotransacetylase and acetokinase and thus on the production of energy from pyruvate.

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LITERATURE CITED