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Requirement for Coenzyme A in the Phosphoroclastic Reaction of Anaerobic Bacteria

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Various bacteria which degrade pyruvate by the phosphoroclastic reaction were examined with respect to the role of coenzyme A (CoA) in this reaction. The strictly anaerobic bacteria, which cleave pyruvate by the phosphoroclastic reaction characteristic of *Clostridia*, required catalytic levels of CoA for the CO_2-pyruvate exchange and acetoin-forming portions of the phosphoroclastic reaction. These reactions were reversibly inhibited by the CoA analogue, desulfo-CoA. In contrast, using cell-free extracts of bacteria which degrade pyruvate by the coliform phosphoroclastic reaction (pyruvate formate-lyase), no requirement for CoA could be observed for the formate-pyruvate exchange reaction. It is suggested that CoA serves a regulatory function in the early portion of the clostridal type of phosphoroclastic reaction.

Reactions which are termed phosphoroclastic are used by certain bacteria for the anaerobic catabolism of pyruvate. The phosphoroclastic reaction of the genus *Clostridium* results in the cleavage of pyruvate to yield carbon dioxide, hydrogen, and acetyl phosphate (8). The phosphoroclastic reaction which is associated with fermentation by the coliforms produces formate and acetyl phosphate as products (25). Coenzyme A (CoA), thiamine pyrophosphate, and divalent metals are cofactors for both types of phosphoroclastic reactions, and acetyl CoA has been identified as an intermediate in both cases (8, 9, 14, 18, 22, 24, 27). Chase and Rabinowitz recently suggested the name pyruvate-formate lyase for the reaction involving the cleavage of pyruvate to acetyl CoA and formate (4), and Lindmark et al. have purified the enzyme (11).

Exchange reactions have been shown to be associated with both types of phosphoroclastic reaction. A rapid carbon dioxide-pyruvate exchange has been demonstrated to occur with cell-free extracts from several clostridia as well as other microorganisms possessing the clostridial type of phosphoroclastic reaction (10, 19, 24, 27, 28). A formate-pyruvate exchange reaction has been found using organisms such as *Escherichia coli* (23), *Streptococcus faecalis* (31), and *Micrococcus lactilyticus* (14). Thiamine pyrophosphate and divalent metals have been demonstrated to stimulate both types of exchange reactions (19, 23, 24, 27, 29). In addition, CoA has been found to stimulate carbon dioxide-pyruvate exchange by cell-free extracts from several microorganisms (17, 19, 24, 27, 29). Since this rapid exchange reaction is believed to occur prior to the formation of acetyl CoA, these data imply a role of CoA in addition to that of acyl acceptor.

We have reexamined the phosphoroclastic reactions of several microorganisms with special attention given to the CoA requirements. The effects of CoA and the CoA analogue, desulfo-CoA, on acetyl phosphate formation from pyruvate, carbon dioxide-pyruvate exchange, and acetoin formation, using cell-free extracts prepared from *Desulfovibrio vulgaris*, *M. lactilyticus*, *Sarcina maxima*, *Clostridium pasteurianum*, and *C. butyricum*, are described in this report. The effect of these compounds on the pyruvate formate-lyase reaction of *E. coli* and *S. faecalis* are also examined.

MATERIALS AND METHODS

Organisms and growth conditions. The organisms, the sources of the strains used, and the methods of growth are summarized in Table 1, except for *E. coli*. *E. coli* strain B was obtained from C. B. Thorne (University of Massachusetts, Amherst). The growth medium consisted of 1.0% glucose, 0.2% peptone, 0.2% yeast extract, 0.8% nutrient broth, and 1.4% each of KH_2PO_4 and K_2HPO_4. Broth cultures (20-liter) were inoculated with 250 ml of an aerobically grown culture and incubated anaerobically for 25 hours at 37 C; the cells were harvested with a Sharples centrifuge. Cell suspensions of all organisms were stored at −25 C under nitrogen until used.

Preparation of cell-free extracts. Cell-free extracts of *C. pasteurianum* and *C. butyricum* were prepared as described by the freeze-thaw method of Koepsell

TABLE 1. *Strains, sources, and growth conditions of microorganisms*

Microorganism	Strain	Source	Growth conditions (reference)
Clostridium pasteurianum	ATCC6014	SC[a]	17
C. butyricum	ATCC6013	SC	17
Desulfovibrio vulgaris	*D. desulfricans* Hildenborough	J. M. Akagi, University of Kansas	24
C. acidi-urici	Soil isolate	D. Kupfer, University of Massachusetts	19
Micrococcus lactilyticus	Strain 221	H. R. Whiteley, University of Washington	27
Sarcina maxima	Strain 11	E. Canale-Parola, University of Massachusetts	10
Streptococcus faecalis	Strain 10C1	N. P. Wood, University of Rhode Island	31

[a] Stock culture collection of Department of Microbiology, University of Massachusetts, Amherst.

and Johnson (8) or by sonic disruption. The frozen cell suspension was thawed in an equal volume of oxygen-free, 0.05 M phosphate buffer (*p*H 6.6) containing 0.003 M dithiothreitol. Approximately 40-ml samples were sonically disrupted for 10 min in a Raytheon model DF101, 10-kc sonic oscillator. The whole cells and cellular debris were removed from the extracts by centrifugation at 37,000 × *g* for 20 to 30 min. Whenever possible, all steps were carried out at 0 to 5 C under a nitrogen atmosphere. Due to the lability of the phosphoroclastic enzymes, stringent conditions to maintain anaerobiosis were employed throughout all experimental procedures.

Cell-free extracts of *C. acidi-urici*, *M. lactilyticus*, *D. vulgaris*, *E. coli*, and *Sarcina maxima* were prepared by the method of Kupfer and Canale-Parola (10). *Streptococcus faecalis* cell-free extracts were prepared by procedures similar to those used for *C. acidi-urici*, with the exception that the thawed cells were disrupted in a Hughes press which had been chilled to −25 C.

Removal of CoA. The endogenous CoA was removed from the cell-free extracts by procedures similar to those described by Stadtman (21). The extract was placed on an anaerobic, chromatographic column of Dowex (Dowex 1-X9, Cl^-, 50 to 100 mesh) and quickly passed through the column by using distilled water as the eluting fluid. The first 70% of the protein-containing eluate was collected and this constituted the CoA-free, cell-free extract.

Preparation of ferredoxin-free extracts and ferredoxin. Ferredoxin-free extracts were prepared by slight modifications of the method of Mortenson (15).

Exchange assays. The carbon dioxide-pyruvate exchange assays were conducted at 37 C under a nitrogen atmosphere using Warburg flasks. The method was slightly modified from that described by Wolfe and O'Kane (28). The assay mixture (3.2 ml) contained the indicated amount of protein, 10 μmoles of thiamine pyrophosphate, 0.01 μmole of CoA, 10 μmoles of $MnCl_2$, 10 μmoles of dithiothreitol, 100 μmoles of potassium pyruvate, 100 μmoles of KH_2PO_4, 100 μmoles of $NaH^{14}CO_3$ or $H^{14}COOH$, and the electron acceptor at the indicated concentration. In formate-pyruvate exchange measurements, 4.0 μmoles of 2,3-dimercaptopropanol and 10.0 μmoles of $FeSO_4$ replaced the dithiothreitol (30). The acid phosphate and pyruvate were contained in one sidearm, the sodium bicarbonate-^{14}C or formate-^{14}C in the other sidearm, and the remaining assay components in the main flask. The reaction was initiated by tipping the contents of the sidearms into the main flask. The reaction was terminated by the addition of 500 μmoles of H_2SO_4.

Protein was precipitated by the addition of 300 μmoles of trichloroacetic acid and was removed by centrifugation. The residual pyruvate was recovered from the supernatant liquid as the 2,4-dinitrophenylhydrazone precipitate. This precipitate was washed three times with 10.0 ml of distilled water, collected by filtration, and dried, and the radioactivity was determined with a thin-window gas-flow Geiger counter equipped with a scaler (Nuclear-Chicago Corp., Des Plaines, Ill.). Corrections for self-adsorption and background were applied in the determination of the specific activity (counts per minute per μmole) of the pyruvate phenylhydrazone. The $NaH^{14}CO_3$ and formate-^{14}C used in this assay procedure contained sufficient radioactivity (5 to 10 nc/μmole) so as to detect at least 0.05 units of exchange activity (approximately 300 counts per min per μmole).

Colorimetric assays. The method of Lipmann and Tuttle (12) was used to determine acetyl phosphate and acetyl-CoA. The assay was similar to that used in the exchange assays, but the $NaH^{14}CO_3$ or $Na^{14}COOH$ was deleted. Phosphotransacetylase activity was measured by the disappearance of acetyl phosphate by arsenolysis (21).

The acetoin assays were similar to the exchange assays, but 500 μmoles of acetaldehyde replaced the $NaH^{14}CO_3$ or $Na^{14}COOH$. Acetoin content was determined by the method of Westerfeld (26).

The protein content of solutions was estimated by

the biuret-phenol method of Lowry et al. (13) with bovine serum albumin as a standard.

Synthesis of compounds. Desulfo-CoA was prepared by the method of Chase et al. (3); acetyl phosphate was prepared by the methods described by Avison (1).

Chemicals. CoA, thiamine pyrophosphate, potassium pyruvate, dithiothreitol, and ^{14}C-sodium formate were purchased from Calbiochem (Los Angeles, Calif.). The ^{14}C-sodium bicarbonate was obtained from Volk Radiochemical Company (Burbank, Calif.). Medium constituents were purchased from Amber Laboratories (Milwaukee, Wisc.) and from Difco.

RESULTS

Preliminary experiments with lyophilized, CoA-depleted extracts confirmed a *p*H of 6.5 to 6.7 as optimal for CO_2-pyruvate exchange (27, 29) in the presence of either phosphate or arsenate buffers. Dialyzed extracts were stimulated in exchange activity by divalent metals at concentrations of 2.0×10^{-3} M. Experiments with extracts which either had been treated with Dowex chloride resin or dialyzed resulted in only slight stimulation of activity (20 to 30%) upon the addition of thiamine pyrophosphate. When *C. pasteurianum* extracts were used which had been treated to reduce the ferredoxin content, the addition of ferredoxin stimulated acetyl phosphate formation, acetoin formation, and CO_2-pyruvate exchange, as previously reported for *M. lactilyticus* by Whiteley and McCormick (27).

Requirement of CoA for exchange. In 1959, Mortlock et al. (17) demonstrated a CoA stimulation of the carbon dioxide-pyruvate exchange portion of the clostridial phosphoroclastic reaction. These authors demonstrated with CoA-depleted extracts of *C. butyricum*, which were incapable of forming acetyl phosphate, that catalytic concentrations of CoA (2.0×10^{-5} M) were required for optimal exchange activity. Higher concentrations of CoA (4.0×10^{-5} to 8.5×10^{-5} M) were inhibitory to the carbon dioxide-pyruvate exchange. Similar CoA effects have since been reported for the carbon dioxide-pyruvate exchange catalyzed by extracts of *M. lactilyticus* (27), *D. vulgaris* (24), *Sarcina maxima*, and *C. acidi-urici* (19). Typical data for the CoA stimulation of carbon dioxide-pyruvate exchange by CoA-depleted extracts of *C. pasteurianum* are presented in Fig. 1. The addition of low amounts of CoA (2.0×10^{-5} M) resulted in a 70% increase in exchange activity, but greater amounts (9.0×10^{-5} M) caused inhibition. The data for *C. butyricum* (Fig. 4, curve A) and for *M. lactilyticus* (Fig. 5, curve A) were in very close agreement with those previously reported by Mortlock et al. (17) and by Whiteley and McCormick (27), respectively. The optimal exchange activity for both organisms occurred at a CoA concentration of 3.0×10^{-6} M. Although only a slight increase in exchange activity resulted when CoA was added to Dowex-treated extracts of *D. vulgaris* (Fig. 6, curve A), maximal exchange activity was seen as 10^{-5} M CoA, a

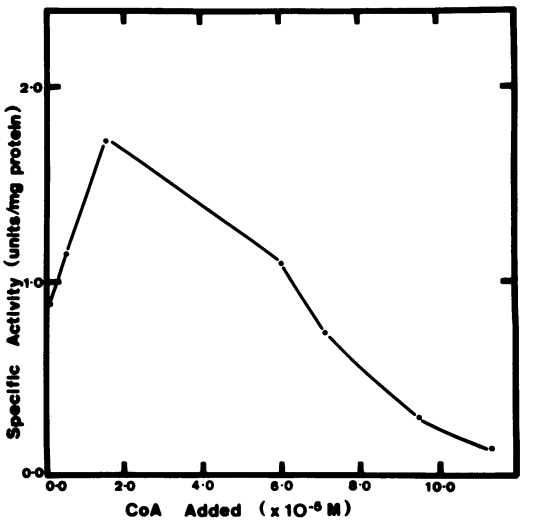

FIG. 1. *Effects of CoA on the carbon dioxide-pyruvate exchange of C. pasteurianum extracts. The pH of the phosphate buffer was 6.6, and each flask contained 2.2 mg of a CoA-depleted extract.*

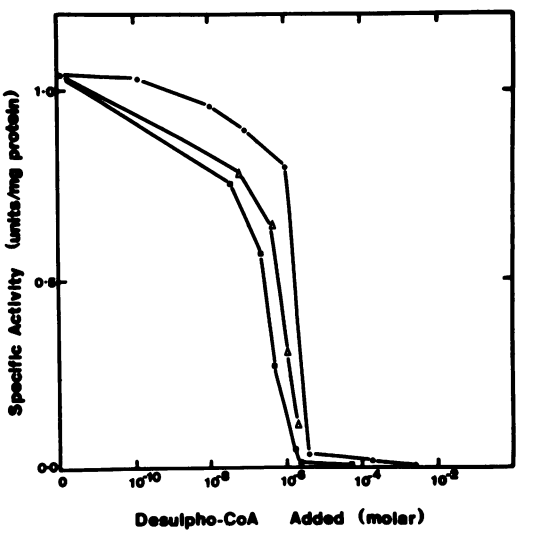

FIG. 2. *Effects of desulfo-CoA on the carbon dioxide-pyruvate exchange of CoA-depleted C. pasteurianum extract. Assay conditions were similar to those given in Fig. 1. Curves represent the data obtained in three experiments on the same extract.*

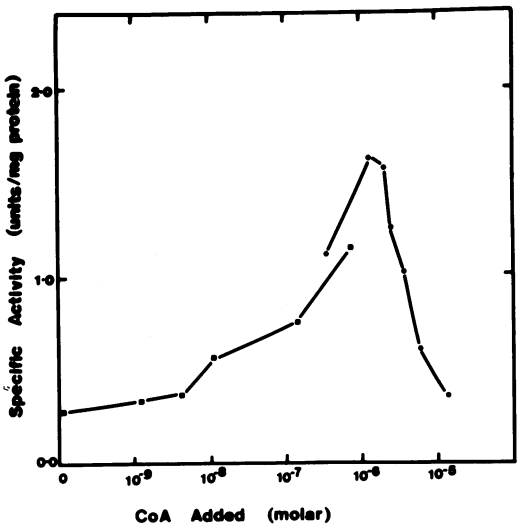

FIG. 3. *Effects of CoA on the desulfo-CoA inhibition of carbon dioxide-pyruvate exchange with C. pasteurianum extracts. Assay conditions were similar to those given in Fig. 1. Each flask contained 3.57 × 10^{-7} M desulfo-CoA which caused 60% inhibition of maximal exchange. CoA was added to each flask to give the indicated final concentration.*

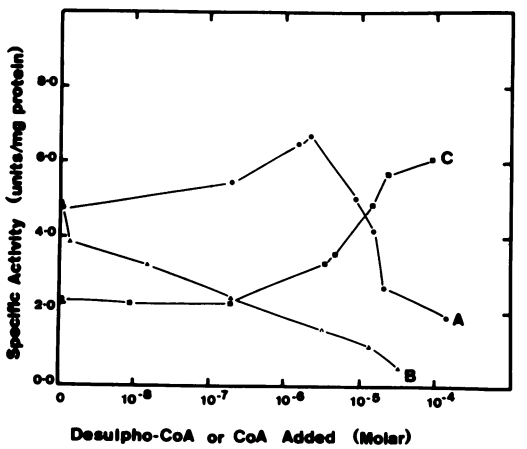

FIG. 4. *Effects of CoA and desulfo-CoA on the carbon dioxide-pyruvate exchange with C. butyricum extracts. (A) Added CoA varied. (B) Added desulfo-CoA varied. (C) Added CoA varied in the presence of 1.75 × 10^{-5} M desulfo-CoA. Assay mixture contained 4.23 mg of a CoA-depleted C. butyricum extract. Reaction time was 15 min at 37 C.*

value comparable to that found by Suh and Akagi (24) for this organism. Similar CoA effects were found also for the carbon dioxide-pyruvate exchange catalyzed by extracts of *S. maxima* and *C. acidi-urici*.

The stimulation of CO_2-pyruvate exchange by CoA has been difficult to interpret since the exchange reaction is believed to occur prior to the energy-yielding oxidative reaction and prior to the formation of acetyl CoA (17). Although

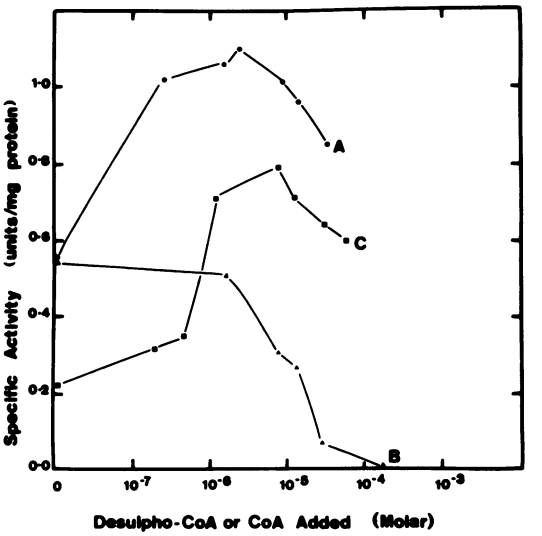

FIG. 5. *Effects of CoA and desulfo-CoA on the carbon dioxide-pyruvate exchange with M. lactilyticus extracts. (A) Added CoA varied. (B) Added desulfo-CoA varied. (C) Added CoA varied in the presence of 7.0 × 10^{-6} M desulfo-CoA. A CoA-depleted M. lactilyticus extract (4.0 mg) was used. The reaction time was 20 min at 37 C.*

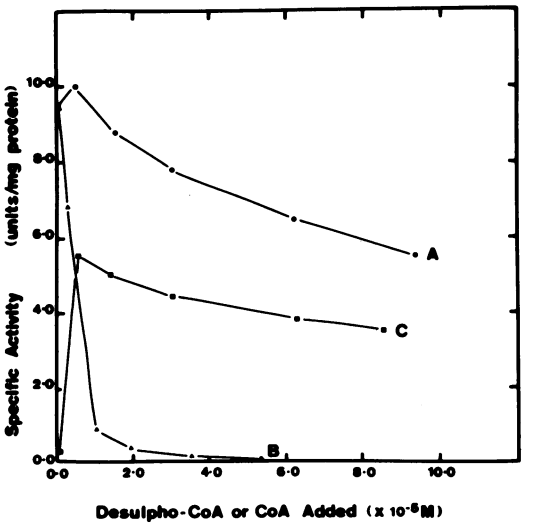

FIG. 6. *Effects of CoA and desulfo-CoA on the carbon dioxide pyruvate exchange with D. vulgaris extracts. (A) Added CoA varied. (B) added desulfo-CoA varied. (C) CoA varied in the presence of 9.0 × 10^{-6} M desulfo-CoA. A CoA-depleted D. vulgaris extract (0.91 mg) was used.*

past reports have also indicated a CoA stimulation of the formate-pyruvate exchange reaction (pyruvate formate-lyase system), Lindmark et al. (11) have now shown that CoA is not required for the purified formate-pyruvate exchange enzyme of *Streptococcus faecalis* (11).

Effects of CoA and desulfo-CoA on acetyl phosphate formation. In 1966, the synthesis of the CoA analogue, desulfo-CoA, was reported by Chase et al. (3). The structural difference between CoA and desulfo-CoA was that the sulfhydryl group of the terminal β-aminoethanthiol portion was replaced by a hydrogen atom. Since desulfo-CoA was shown to be a competitive inhibitor of reactions involving CoA (3), it seemed a useful tool for determining CoA requirements of enzyme-catalyzed reactions. Desulfo-CoA, prepared by the method of Chase et al. (3) was found to act as a competitive inhibitor of phosphotransacetylase from *C. pasteurianum*. Therefore, desulfo-CoA would be predicted to inhibit acetyl phosphate formation in those phosphoroclastic degradations of pyruvate which synthesize acetyl CoA as an intermediate. Desulfo-CoA was effective at concentrations of 2.4×10^{-6} M or greater in inhibiting acetyl phosphate production from pyruvate by extracts of *C. butyricum*.

The desulfo-CoA inhibition that was observed could be relieved by the addition of an equimolar concentration of CoA to the reaction mixture. Analogous results for the effects of CoA and desulfo-CoA on acetyl phosphate production from pyruvate were obtained in experiments with CoA-depleted extracts of *C. pasteurianum*, *M. lactilyticus*, *Sarcina maxima*, and *Streptococcus faecalis*. These results indicated that desulfo-CoA exerted an inhibitory action on the phosphoroclastic synthesis of acetyl phosphate from pyruvate by extracts of diverse organisms.

Desulfo-CoA was also found to be an extremely powerful inhibitor of the carbon dioxide-pyruvate exchange reaction of *C. pasteurianum*. A 15% inhibition could be observed at 10^{-9} M (Fig. 2). The addition of CoA to the reaction mixture could completely relieve the desulfo-CoA inhibition of exchange (Fig. 3). A concentration of 3.8×10^{-7} M desulfo-CoA caused a 60% inhibition of exchange which could be reversed by 10^{-7} M CoA. Greater concentrations of CoA showed inhibition similar to that displayed in Fig. 1.

The effect of CoA and desulfo-CoA on the exchange reactions of *C. butyricum*, *M. lactilyticus*, and *D. vulgaris* are shown in Fig. 4–6.

As shown in curve B in Fig. 4–6, the addition of catalytic amounts (10^{-6} M to 5.0×10^{-4} M) of desulfo-CoA resulted in a substantial decrease in exchange activity. In most cases, a concentration of 2.0×10^{-5} M desulfo-CoA caused more than a 90% inhibition of carbon dioxide-pyruvate exchange activity, which was more pronounced than the CoA inhibition of exchange at this concentration. As can be seen, the desulfo-CoA inhibition with extracts of *C. butyricum*, *M. lactilyticus*, and *D. vulgaris* were qualitatively similar. As shown by curve C in Fig. 4–6, the addition of an approximately equimolar amount of CoA to the reaction mixture restored more than 80% of the original exchange activity in experiments with *C. butyricum* (Fig. 4, curve C), *M. lactilyticus* (Fig. 5, curve C), and *D. vulgaris* (Fig. 5, curve C). With extracts of *Sarcina maxima*, the addition of 1.8×10^{-5} M desulfo-CoA resulted in 95% inhibition of exchange activity. Activity was restored to 54% of the original value by the further addition of 3.0×10^{-6} M CoA. With extracts of *C. acidi-urici*, desulfo-CoA at 10^{-6} M inhibited exchange activity 83%. Activity was completely restored by CoA at a concentration of 10^{-5} M.

A product inhibition of pyruvate degradation by acetyl phosphate with extracts from nitrogen- or ammonium-grown *C. pasteurianum* cells has been reported by Biggins and Dilworth (2). The CO_2-pyruvate exchange activity and pyruvate degradation by our extracts, prepared from peptone-grown cells, were slightly inhibited (10 to 20%) by high acetyl phosphate concentrations (8×10^{-3} M) only if phosphotransacetylase activity was also present in the extracts. The acetyl phosphate inhibition could be relieved by the addition of CoA (10^{-4} M), indicating that inhibitory effects of acetyl phosphate might result from the accumulation of acetyl-CoA with an overall decrease in the free CoA concentration.

In contrast to data obtained with the above organisms, experiments performed with extracts of *Streptococcus faecalis* gave results which were in agreement with the observations of previous workers in that CoA did not stimulate and desulfo-CoA did not inhibit the formate-pyruvate exchange reaction (11, 30). Similar results were found with CoA for the formate-pyruvate exchange catalyzed by *E. coli* extracts. Although a slight stimulation of exchange activity by CoA with crude extracts was occasionally observed, this observation could not be demonstrated with Dowex-treated extracts.

Effects of CoA and desulfo-CoA on acetoin formation. The degradation of pyruvate via the clostridial phosphoroclastic reaction is believed to involve a hydroxyethylthiamine pyrophosphate intermediate, as has been proposed for other types of pyruvate degradation (5–7). Crude extracts and fractions obtained from organisms

possessing phosphoroclastic reaction form acetoin (acetyl methylcarbinol) when both pyruvate and acetaldehyde are available as substrates. The acetoin is believed to originate from a condensation of the hydroxyethyl intermediate with free acetaldehyde (17).

In experiments with CoA-depleted extracts of the various organisms, acetoin formation was stimulated in all cases by the addition of CoA (Table 2). Desulfo-CoA inhibited acetoin formation by extracts of all five organisms, and this inhibition could be overcome by the addition of CoA to the reaction mixtures (Table 2).

TABLE 2. *Effects of Coenzyme A (CoA) and Desulfo-CoA on acetoin formation*[a]

Addition	Acetoin formed				
	Clostridium pasteurianum	*C. butyricum*	*Micrococcus lactilyticus*	*Sarcina maxima*	*Desulfovibrio vulgaris*
None	1.15	9.60	4.01	4.56	9.94
CoA	3.70	15.40	11.05	9.60	24.80
Desulfo-CoA	0.60	<0.20	1.12	2.15	5.27
CoA + Desulfo-CoA	3.00	8.76	9.45	5.30	14.75

[a] CoA concentration was 1.56 μM and the desulfo-CoA concentration was 1.20 μM, except for *C. butyricum* and *C. pasteurianum* which concentrations were increased two- and four fold, respectively. Values are expressed as micrograms of acetoin formed per milligram of protein.

DISCUSSION

In past studies of the phosphoroclastic reactions, a number of different compounds have been implicated as possible cofactors. In several cases, the stimulation in activity which had been observed was subsequently shown to be due to the reducing ability of the added compound, serving to protect the enzymes against inactivation by oxidation (17, 29, 31). However, the requirement of the carbon dioxide-pyruvate exchange reaction for CoA can be demonstrated in the presence of high concentrations of other reducing sulfhydryl compounds. Furthermore, the inhibition of exchange activity resulting from the addition of low concentrations of the CoA analogue, desulfo-CoA, indicates a true requirement for CoA in the carbon dioxide-pyruvate portion of the phosphoroclastic reaction.

Speckman and Collins (20) and Chuang and Collins (5) have presented evidence that acetyl CoA is used for diacetyl formation by certain bacteria. The formation of acetoin by the reaction described in this paper appears to result from a condensation of enzyme-bound hydroxyethylthiamine pyrophosphate with free acetaldehyde, as originally described by Juni (6), since (i) there is no evidence for diacetyl formation under the assay conditions used in this paper, (ii) acetoin formation is dependent upon the addition of both pyruvate and acetaldehyde as substrates, and (iii) there is no correlation between the ability of cell-free extracts to produce acetyl CoA from pyruvate and their ability to form acetoin. Acetoin formation from acetaldehyde and pyruvate does appear to be correlated with the ability of extracts to catalyze the carbon dioxide-pyruvate exchange reaction.

Current models for the clostridial phosphoroclastic reaction postulate the decarboxylation of pyruvate with the formation of an hydroxyethylthiamine pyrophosphate intermediate (14, 17). This portion of the reaction is fast and reversible and represents the carbon dioxide-pyruvate exchange portion of the system. In the presence of added acetaldehyde, a slow condensation occurs between the hydroxyethyl moiety and acetaldehyde, resulting in the formation of acetoin. The competitive inhibition of both of these reactions by desulfo-CoA establishes a requirement for CoA in these reactions. Acetyl CoA cannot replace this CoA requirement. Cell-free extracts containing limiting inorganic phosphate accumulate acetyl CoA upon incubation with pyruvate. The rapid conversion of the endogenous supply of CoASH to the acyl ester results in severe inhibition of the CO_2-pyruvate exchange reaction (17). The addition of low levels of CoA will relieve this inhibition in cell-free extracts, as will the addition of other substances which can serve as acyl acceptors.

It is possible that CoA may simply serve a regulatory function in initiating the degradation of pyruvate by the phosphoroclastic reaction. With CoA rather than acetyl CoA required as an activator of the enzyme, the initial steps in the degradation of pyruvate would not occur in the absence of a sufficient endogenous supply of CoA to act as the acceptor of the acetyl moiety. In vivo accumulation of acyl esters of CoA would lead to decreased activity of the phosphoroclastic reaction, with pyruvate diverted to other pathways.

In contrast to the data obtained with the carbon dioxide-pyruvate exchange reaction, desulfo-CoA showed no significant inhibition of the formate-pyruvate exchange systems of *E. coli* strain B or *S. faecalis*. These results are in agreement with the recent observations of Lindmark et al. (11) and Wood (30) that CoA was not required for formate-pyruvate exchange by a purified enzyme fraction from *S. faecalis*.

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LITERATURE CITED

1. Avison, A. W. D. 1955. The synthesis of acyl phosphates in aqueous solutions. J. Chem. Soc. **1955**:732–738.
2. Biggins, D. R., and M. J. Dilworth. 1968. Control of the phosphoroclastic activity in extracts of *Clostridum pasteurianum* by ADP and acetyl phosphate. Biochim. Biophys. Acta **156**:285–296.
3. Chase, J. F. A., B. Middleton, and P. K. Tubbs. 1966. A coenzyme A analogue, desulpho-CoA; preparation and effects on various enzymes. Biochem. Biophys. Res. Commun. **23**:208–213.
4. Chase, T., and J. C. Rabinowitz. 1968. Role of pyruvate and *S*-adenosylmethionine in activating the pyruvate formate-lyase of *Escherichia coli*. J. Bacteriol. **96**:1065–1078.
5. Chuang, L. F., and E. B. Collins. 1968. Biosynthesis of diacetyl in bacteria and yeast. J. Bacteriol. **95**:2083–2089.
6. Juni, E. 1952. Mechanisms of the formation of acetoin by bacteria. J. Biol. Chem. **195**:715–726.
7. Juni, E. 1952. Mechanisms of formation of acetoin by yeast and mammalian tissues. J. Biol. Chem. **195**:727–734.
8. Koepsell, H. J., and M. J. Johnson. 1942. Dissimilation of pyruvic acid by cell-free preparations of *Clostridium butylicum*. J. Biol. Chem. **145**:376–386.
9. Koepsell, H. J., and M. J. Johnson. 1944. Role of phosphate in pyruvic acid dissimilation by cell-free extracts of *Clostridium butylicum*. J. Biol. Chem. **154**:535–547.
10. Kupfer, D. G., and E. Canale-Parola. 1967. Pyruvate metabolism in *Sarcina maxima*. J. Bacteriol. **94**:984–990.
11. Lindmark, D. G., P. Paolella, and N. P. Wood. 1969. The pyruvate formate-lyase system of *Streptococcus faecalis*. I. Purification and properties of the formate-pyruvate exchange. J. Biol.Chem. **244**:3605–3612.
12. Lipmann, F., and L. C. Tuttle. 1945. On the condensation of acetyl phosphate with formate or carbon dioxide in bacterial extracts. J. Biol. Chem. **158**:505–519.
13. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
14. McCormick, N. G., E. J. Ordal, and H. R. Whiteley. 1962. Degradation of pyruvate by *Micrococcus lactilyticus*. I. General properties of the formate-exchange reaction. J. Bacteriol. **83**:887–898.
15. Mortenson, L. E. 1964. Purification and analysis of ferredoxin from *Clostridium pasteurianum*. Biochim. Biophys. Acta **81**:71–77.
16. Mortenson, L. E., R. C. Valentine, and J. E. Carnahan. 1962. Ferredoxin in the phosphoroclastic reaction of pyruvic acid and its reaction to nitrogen fixation in *Clostridium pasteurianum*. J. Biol. Chem. **238**:794–800.
17. Mortlock, R. P., R. C. Valentine, and R. S. Wolfe. 1959. Carbon dioxide activation in the pyruvate clastic system of *Clostridium butyricum*. J. Biol. Chem. **234**:1653–1656.
18. Oster, M. O., and N. P. Wood. 1964. Formate-pyruvate exchange reaction in *Streptococcus faecalis*. II. Reaction conditions for cell extracts. J. Bacteriol. **87**:104–113.
19. Raeburn, S., and J. C. Rabinowitz. 1964. Pyruvate synthesis by a partially-purified enzyme from *Clostridium acidiurici*. Biochem. Biophys. Res. Commun. **18**:303–307.
20. Speckman, R. A., and E. B. Collins. 1968. Diacetyl biosynthesis in *Streptococcus diacetilicus* and *Leuconostoc citrovorum*. J. Bacteriol. **95**:174–180.
21. Stadtman, E. R. 1955. Phosphotransacetylase from *Clostridium kluyveri*, p. 596–599. *In* S. P. Colowick and N. O. Kaplan (ed.), Methods in enzymology, vol. I. Academic Press Inc., New York.
22. Still, J. L. 1941. Pyruvic dehydrogenase of *Bacterium coli*. Biochem. J. **35**:380–391.
23. Strecker, H. J. 1951. Formate fixation in pyruvate by *Escherichia coli*. J. Biol. Chem. **189**:815–830.
24. Suh, B., and J. M. Akagi. 1966. Pyruvate-carbon dioxide exchange reaction of *Desulfovibrio desulfuricans*. J. Bacteriol. **91**:2281–2285.
25. Utter, M. F., and C. H. Werkman. 1943. Formation and reactions of acetyl phosphate in *Escherichia coli*. Arch. Biochem. **5**:413–422.
26. Westerfeld, W. W. 1945. A colorimetric determination of blood acetoin. J. Biol. Chem. **161**:495–502.
27. Whiteley, H. R., and N. G. McCormick. 1963. Degradation of pyruvate by *Micrococcus lactilyticus*. III. Properties and cofactor requirements of the carbon dioxide-exchange reaction. J. Bacteriol. **85**:382–393.
28. Wolfe, R. S., and D. J. O'Kane. 1953. Cofactors of the phosphoroclastic reaction of *Clostridium butyricum*. J. Biol. Chem. **205**:755–765.
29. Wolfe, R. S., and D. J. O'Kane. 1955. Cofactors of the carbon dioxide-pyruvate exchange reaction of *Clostridium butyricum*. J. Biol. Chem. **215**:637–643.
30. Wood, N. P. 1965. Formate-pyruvate exchange system: *Streptococcus faecalis*, p. 718–722. *In* S. P. Colowick and N. O. Kaplan (ed.), Methods in enzymology, vol. IX. Academic Press Inc., New York.
31. Wood, N. P., and D. J. O'Kane. 1963. Formate-pyruvate exchange reaction in *Streptococcus faecalis*. I. Factor requirements for intact cells. J. Bacteriol. **87**:97–103.