Acetate Biosynthesis by Acetogenic Bacteria

EVIDENCE THAT CARBON MONOXIDE DEHYDROGENASE IS THE CONDENSING ENZYME THAT CATALYZES THE FINAL STEPS OF THE SYNTHESIS*

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The purified carbon monoxide dehydrogenase from Clostridium thermoaceticum is the only protein required to catalyze an exchange reaction between carbon monoxide and the carbonyl group of acetyl-CoA. This exchange requires that the CO dehydrogenase bind the methyl, the carbonyl, and the CoA groups of acetyl-CoA, then equilibrate the carbonyl with CO in the solution and re-form acetyl-CoA. CoA is not necessary for the exchange and, in fact, inhibits the reaction. These studies support the view that CO dehydrogenase is the condensing enzyme that forms acetyl-CoA from its component parts. Carbon dioxide also exchanges with the C-1 of acetyl-CoA, but at a much lower rate than does CO. At 50 °C and pH 5.3, the optimal pH, the turnover number is 70 mol of CO exchanged per min/mol of enzyme. Low potential electron carriers are stimulatory. The KmaPp for stimulation by ferredoxin is 50-fold less than the value for flavodoxin. Neither ATP or P, stimulate the exchange. The EPR spectrum of the CO-reacted enzyme is markedly changed by binding of CoA or acetyl-CoA. Arginine residues of the CO dehydrogenase appear to be involved in the active site, possibly by binding acetyl-CoA. Mersalyl acid, methyl iodide, S,S-dithiobis-(2-nitrobenzoate), and sodium dithionite inhibit the exchange.
purification. The most important value is the ratio of CO dehydrogenase activity to exchange activity, based on units/mg of protein, which has an average value of 2.3, with very little variation during the purification procedure. Almost identical results are obtained based on the ratios of counts/min of $^{63}$Ni/unit of CO dehydrogenase and per milliunit of exchange. These results indicate that the nickel-containing CO dehydrogenase is responsible for both activities. Both activities are linear with concentration of CO. The removal of this protein should cause an approximately 66% loss of activity; however, the exchange activity (150 units/mg, containing CO dehydrogenase) was totally lost. Therefore one can affect the exchange activity without necessarily affecting the CO dehydrogenase activity (see below for modification experiments).

**Proof That Minor Contaminants Are Not Required as Catalysts for the Exchange Reaction**

After hydroxylapatite chromatography, the minor contaminant (band 4) was approximately 33% of the protein (Lane 1 of Fig. 1). If the protein of band 4 was indeed stimulatory to the exchange reaction, then removal of this protein should cause a decrease in specific activity and addition of the protein back to the exchange reaction assay mixture should cause an increase in exchange activity. Gel filtration (Step 5) removed most of the protein giving rise to band 4 (Lane 3 of Fig. 1) and also caused an increase in specific activity of both the CO dehydrogenase and the exchange activities (Table I). Based on densitometry scanning, the protein, after Step 5, was 99% pure. Addition of the purified protein of band 4 (Lane 2 of Fig. 1) in fairly high amounts (33 and 66 mg) did not increase the activity. It is unlikely that the protein of band 4 was inactivated by the time of the assay, since the exchange activity was determined within 1 h after elution from the column. Therefore, the small amount of contaminant due to the protein of band 4 is not responsible for catalysis of the exchange reaction.

The other minor contaminant, giving rise to band 3, could be removed by agarose-CoA chromatography (Step 6), and, in fact, it binds very tightly to agarose-CoA. It is not removed with 2 M urea; however, 6 M urea elutes this protein. After elution, this protein also did not stimulate the exchange reaction and, as in the case of the removal of the protein of band 4 by gel filtration, the specific activity increased after this affinity chromatography step (Table I).

That CO dehydrogenase is the only catalyst necessary for the exchange reaction is in contrast to results found previously (8, 9). The major source of the discrepancy is that previously 2 mM sodium dithionite was added to all enzyme solutions.

### Comparison of the CO dehydrogenase and exchange reaction activities during the purification of CO dehydrogenase

| Protein                          | Units/mg | Milliunits/mg | $^{63}$Ni content | Units/milliuunits* |
|----------------------------------|----------|---------------|-------------------|-------------------|
|                                  |          |               | cpm/mg $\times 10^{-3}$ | cpm/unit | cpm/milliuunit |
| 1. Cell extract                  | 11,160   | 6.6           | ND*               | 3.5             | 540           | ND         | ND        |
| 2. DEAE-Sepharose                | 990      | 55            | 25                | 17              | 310           | 690        | 2.2       |
| 3. Phenyl-Sepharose              | 260      | 130           | ND                | 45              | 330           | ND         | ND        |
| 4. Bio-Gel HTP hydroxylapatite   | 100      | 290           | 115               | 95              | 320           | 820        | 2.5       |
| 5. Bio-Gel A-0.5mol gel filtration | 3       | 330           | 160               | 100             | 280           | 590        | 2.1       |
| 6. Agarose-CoA*                  | 7.6      | 390           | 170               | 118             | 300           | 700        | 2.3       |

*Units/milliuunits is defined as units of CO dehydrogenase (CO to CO$_2$) divided by milliunits of exchange activity.

* ND, we were unable to determine the exchange activity in the crude extract because the enzyme is dilute in pH 7.6 Tris-HCl buffer and it is necessary to add a large amount of extract to the assay mixture, which caused an increase in pH to greater than 7.0 (see later for pH optimum). The concentration of protein in the phenyl-Sepharose fractions was too dilute to determine the exchange activity.

* Only 5% of the HTP protein was applied to this column.

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**FIG. 1.** Sodium dodecyl sulfate-gel electrophoresis of carbon monoxide dehydrogenase. Lane 1, contains 0.02 mg of CO dehydrogenase after the Bio-Gel HTP step (Step 4). Lane 2 contains 0.02 mg of the purified band 4 protein after gel filtration (Step 5). Lane 3 contains 0.02 mg of CO dehydrogenase after gel filtration. Bands 1 and 2 are the CO dehydrogenase subunits ($M_r = 78,000$ and 71,000) (8). Bands 3 and 4 are impurities which are discussed in the text. The proteins were prepared and run in the sodium dodecyl sulfate/P$i_1$ system of Weber et al. (24) and stained with Coomassie Brilliant Blue R-250 (25).
during the purification of the CO dehydrogenase, and dithiobionate is a strong inhibitor of the exchange reaction. In addition, since electron carriers, which stimulate the exchange reaction were not added, an even lower percentage of the maximum amount of exchange activity/mg of added protein could be detected. These results show conclusively that CO dehydrogenase is the only protein that is required for catalysis of the exchange reaction between CO and the C-1 of acetyl-CoA.

**Stimulation of the Exchange Reaction by Low Molecular Weight Electron Carriers**

The exchange activity was approximately 5000-fold lower than the CO to CO₂ reaction, so a large excess of CO dehydrogenase was necessary to show that the exchange reaction occurred. Therefore, we attempted to purify a component that would stimulate the exchange activity.

**Isolation of Ferredoxin and Evidence That It Stimulates the Exchange**—An oxygen-sensitive factor which stimulated the exchange reaction was purified. The purified factor migrated on G-50 superfine gel with an elution volume similar to that of ferredoxin (Mᵣ = 6000). The exchange reaction occurs without addition of electron carrier, but a large increase in activity is obtained with addition of ferredoxin (Fig. 2). The amino acid composition of the factor corresponded to ferredoxin II (10) and was clearly different from ferredoxin I (11) (see “Experimental Procedures”). However, we have recently isolated two peaks of ferredoxin from DEAE-Sephacel and found that both proteins stimulated the exchange activity. Two ferredoxins, I and II, have previously been purified from *C. thermoaceticum* and extensively characterized (10, 11).

**Test of Other Electron Carrier Proteins**—Several different electron carriers, isolated from *Clostridium formicoaceticum* (12), were tested for stimulatory activity (Table III). Only the low potential carriers stimulated the exchange reaction. Non-linear Lineweaver-Burk plots of 1/concentration versus 1/activity were obtained, and the *Kₐ* values, determined from the linear portion of the graph (at high concentrations of electron carrier) were 22.2 μM for flavodoxin and 0.43 μM for ferredoxin (Fig. 2B). Thus the *Kₐ* for ferredoxin is 50-fold lower than the *Kₐ* for flavodoxin. The carriers were reduced upon addition of CO dehydrogenase to the assay mixture. This was most obvious in the flavodoxin reaction when the bright yellow color of flavodoxin was bleached, corresponding to the reduction of FMN to FMNH₂. No semiquinone intermediate was seen, indicating the low potential of the CO/CO₂ couple and the ability of CO dehydrogenase to use flavodoxin as a two electron acceptor (8, 9). Methyl viologen also stimulated the exchange reaction between [1-¹⁴C]acetyl-CoA and CO₂, however, at high concentrations (greater than 0.5 mM), it was found to inhibit.

**Role of Electron Carriers in the Exchange Reaction**—When CO was replaced with CO₂ as the gas phase in the exchange reaction, 16% of the ¹⁴C from the C-1 of acetyl-CoA was exchanged with CO₂ as compared to 44% with CO as the gas phase. When N₂ was the gas phase, 3% of the counts were lost from the ¹⁴C during the reaction. These results indicate that CO and not CO₂ is the actual form of carbon undergoing exchange with the C-1 of acetyl-CoA and that the role of the low potential electron carriers is to stimulate an internal electron transfer that occurs during the cleavage of the methyl and CoA bonds to the carbonyl and the resynthesis of acetyl-CoA (see below for a possible mechanism).

**Effect of Modifying Reagents on the Exchange Reaction and the CO Dehydrogenase Reaction**

Phenylglyoxal, methylglyoxal, and butanedione, which are potent arginine modifiers (13, 14), all caused inhibition of the CO dehydrogenase exchange reaction (Table III). When arginine reagents were added directly to the assay mixture, strong inhibition occurred within the time course of the assay. When the enzyme was preincubated with 24 mM butanedione and then added to a complete assay mixture at specified times, approximately 50% inhibition occurred within 5 min, and no further inactivation occurred up to 1 h. Under these conditions, the presence of CO in the enzyme solution had no effect on the inhibition. The CO to CO₂ activity of the CO dehydrogenase was not affected by the arginine reagents, even in high concentrations under conditions that caused 90% inhibition of exchange activity. When ⁶⁵Ni-enzyme was reacted with 20 mM methylglyoxal and gel filtrated on the Bio-Gel P-6DG
containing enzyme was incubated with the enzyme's iron-sulfur clusters; however, when the 63Ni-demonstrated that approximately 6 arginines were modified. The actual number of arginines modified will be determined using radioactive phenylglyoxal. There are

A strong inhibitory effect on the exchange reaction either in the presence or absence of ferrous ammonium sulfate, which is in contrast to the results obtained by Hu et al. (6) with an impure preparation. It seemed likely that a phosphate source might be necessary so MES (Na salt) buffer was substituted for phosphate buffer in the exchange reaction. The replacement of phosphate by MES had absolutely no inhibitory effect on the exchange reaction.

Interaction of CO Dehydrogenase with CoA

EPR Spectra—When the CO dehydrogenase is treated with CO, a paramagnetic nickel-carbon species is formed which has g values at 2.08 and 2.02 (16). We have studied a number of preparations of CO dehydrogenase and found, in many cases, a third g value at g = 2.05 (Fig. 3). The relative intensity of the 3 components varies significantly among different preparations of the enzyme. In the spectra shown at the top reactions. At 40 mM concentration, methyl iodide inhibits both reactions as well. Interestingly, the nickel chelator, dimethylglyoxime, had no effect on either the exchange or the CO dehydrogenase activity after a 30-min incubation with 2 mM glyoxime. These results with the glyoxime reagent indicate that the nickel is chelated quite tightly in the enzyme.

ATP was found to have no effect on the exchange reaction either in the presence or absence of ferrous ammonium sulfate, which is in contrast to the results obtained by Hu et al. (6) with an impure preparation. It seemed likely that a phosphate source might be necessary so MES (Na salt) buffer was substituted for phosphate buffer in the exchange reaction. The replacement of phosphate by MES had absolutely no inhibitory effect on the exchange reaction.

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of Fig. 3, the \( g = 2.05 \) signal is the major component and in the bottom spectra, it is the minor component. A remarkable change occurs in the EPR spectrum when the enzyme is treated with CoA, and the symmetry becomes more axial (Fig. 3). The same change is seen upon treatment of CO dehydrogenase with acetyl-CoA (not shown). Enzyme incubated with CoA or acetyl-CoA in the absence of CO is EPR silent. When the enzyme is treated with CoA, gel filtrated to remove all the nonbound CoA, and then treated with CO, the resulting spectrum has three \( g \) values and is identical to the untreated enzyme. Thus the change in EPR properties is reversible. Theory predicts that each line produced by paramagnets associated with \( ^{13}C \) \( (I = 0) \) will be broadened or split into a doublet when the carbon is replaced by \( ^{12}C \) \( (I = 1/2) \) (17). Substitution of \( ^{13}CO \) for \( ^{12}CO \) causes a substantial broadening of the line widths of all three components of the nickel-carbon signal, as was found earlier for the \( g = 2.08 \) and 2.02 components (16). The \( g = 2.08, 2.05, \) and 2.03 line widths are broadened by 2, 5, and 13 G, respectively. The \( g = 2.03 \) component \( \pm 0.03 \) clearly showed a splitting into a doublet as was found earlier (16). These results indicate that CoA and acetyl-CoA bind to the CO dehydrogenase near the paramagnetic nickel-carbon center.

**Other Evidence of Acetyl-CoA Binding to CO Dehydrogenase**—We have found that CO dehydrogenase binds to hexane-CoA-Sepharose 4B. The enzyme is eluted with approximately 0.5 m NaCl; whereas, most other proteins elute in the wash (50 mM KP, or Tris-HCl buffer).

The \( ^{2}H \) of [acetyl-\( ^{2}H \)]acetyl-CoA, after incubation with CO dehydrogenase is clearly separated from the enzyme peak by gel filtration (Fig. 4). When [\( ^{13}C \)]acetyl-CoA is reacted with CO dehydrogenase in the presence of CO, a significant amount of radioactivity is retained in the enzyme peak (Fig. 4). This complex between \( ^{13}C \) of C-1 acetyl-CoA and CO dehydrogenase is, therefore, not due to binding of acetyl-CoA to CO dehydrogenase, but is apparently due to binding of \( ^{13}CO \) which is formed from \( ^{13}C \) acetyl-CoA by the exchange reaction. The lack of complete coincidence of the radioactivity and the CO dehydrogenase may be due to partial dissociation which occurs during the chromatography. Previously, Peczak and Wood (7) showed that \( ^{13}CO \) plus \( H_{2} \) and the C-1 of pyruvate formed a C-1 complex with CO dehydrogenase that could be isolated by gel filtration. We have been unable to detect, by gel filtration techniques, an analogous complex of \( ^{14}CO \) with CO dehydrogenase by directly reacting CO dehydrogenase with \( ^{14}CO \).

**DISCUSSION**

Based on the results presented here, a new scheme for the biosynthesis of acetate is proposed (Fig. 5) which, in contrast to previous schemes (2–4, 6), places CO dehydrogenase as the central enzyme in the pathway. This proposal is based on the observation that CO dehydrogenase is the only enzyme that is required for catalysis of the exchange between \( ^{13}C \) acetyl-CoA and CO. The reactions involved in the exchange are enclosed by the dotted lines of Fig. 5. For the C-1 to be converted to CO, the bonds between C-2 and C-1 and between C-1 and CO must be cleaved forming separate methyl, carbonyl (C=), and CoA groups which are bound to the enzyme as shown schematically in Equation 2, where X, Y, and Z are sites on the CO dehydrogenase.

\[
CH_{2}CO-SCoA \Leftrightarrow X-CH_{2} + Y-CO + Z-CoA \tag{2}
\]

addition, the Y-CO must equilibrate with CO in the solvent. CO has been shown to bind to CO dehydrogenase and form a paramagnetic nickel-carbon species (10, 16; Fig. 3). Although not shown in Fig. 5, the "Y" in Equation 2 apparently is the nickel site of the CO dehydrogenase and the paramagnetic complex is speculated to be the C1 intermediate referred to as Y-CO. Since ATP and P, are not required for the exchange reaction, this Ni-Co species is probably not phosphorylated, as had been suggested by Hu et al. (6). Ferredoxin is postulated to be involved in this step of Fig. 5 since electron carriers stimulate the exchange and since this step involves a redox change in the nickel. We were able to trap this C1 intermediate by gel filtration by using the C-1 of acetyl-CoA as the source of CO. However, we have been unable to detect an intermediate by gel filtration when CO dehydrogenase is reacted directly with \( ^{14}CO \). This is apparently due to the low solubility of CO and to exchange between solvent CO and enzyme-bound CO.

Our results demonstrate that the C1, but not the methyl or CoA group, form a tightly bound complex with the CO dehydrogenase that can be isolated by gel filtration. Nevertheless, the methyl and CoA groups must bind to the CO dehydrogenase since there are no other acceptors for these groups during the exchange. That the exchange is inhibited by CoA is
Evidence that CoA is bound to the CO dehydrogenase. In addition, EPR results demonstrate binding of CoA and acetyl-CoA to the enzyme. Acetyl-CoA and CoA modify the environment around the nickel-CO paramagnetic center from a rhombic to a more axially symmetric species (Fig. 3). Since the nickel in the enzyme is labile to mer-salyl, sulfhydryl ligands to the nickel in CO dehydrogenase seem likely. Nickel (III) peptide complexes with all nitrogen or all sulfur ligands have EPR spectra with axial symmetry; whereas, the mixed N,S donor sets have rhombic spectra (18). It is possible, therefore, that the CoA sulfhydryl (formed from free CoA or acetyl-CoA via the exchange) binds directly to the nickel creating a more symmetric environment around the nickel and the observed changes in the EPR spectrum. CoA analogues will be tested for similar effects on the EPR spectrum in order to determine which moieties of the coenzyme are primarily responsible for the change in the EPR spectra. Interestingly, acetyl-CoA has no effect on either the \( K_\text{c} \) or \( V_\text{max} \) of the CO oxidation reaction (8). We propose that the CoA may be bound to arginine groups of CO dehydrogenase. This is based on the inhibition of the exchange, but not the CO dehydrogenase reaction, by butanediol, methylglyoxal, and phenylglyoxal. Detailed studies by other workers (13, 14) have shown that the pyrophosphate bridge of coenzymes can be bound via the guanidino linkage of arginine residues and that butanediol, methylglyoxal, and phenylglyoxal prevent this binding.

Therefore, we propose that, in the exchange reaction, \( ^{14}\text{C} \)acetyl-CoA binds to the CO dehydrogenase and is separated into the methyl, \( ^{14}\text{C} \), and CoA moieties. In the next step, the \( ^{14}\text{C} \) (Y-CO) exchanges with CO in the solution, and, in the final step, the groups recombine to form \( ^{14}\text{C} \)acytetyl-CoA.

In the synthesis of acetyl-CoA from CO and \( \text{H}_2 \), the required reductive capacity is supplied by the \( \text{H}_2 \) and, with CO, is supplied in the formation of CO\(_2\) by the CO dehydrogenase. The CO\(_2\) is converted via formate to formyltetrahydrofolate which is reduced to methyltetrahydrofolate. The reactions have been completely characterized by Ljungdahl and co-workers (2, 19). A methyl transferase catalyzes the synthesis of a methylated corrinoid protein from methyltetrahydrofolate (3). This methyl group is then transferred to CO dehydrogenase. Then CO and CoA bind to the CO dehydrogenase, as shown in Fig. 5, and are condensed with the methyl group to form acetyl-CoA, which is utilized in anabolic pathways. We do not know if free CoA or the methyl group of the methylated corrinoid protein are transferred directly to the CO dehydrogenase, or if the protein, Fm, may function in one of these roles (20). This protein is required in acetate synthesis, but is not required or even stimulatory for the exchange reaction. Thus CO dehydrogenase plays four roles in acetate biosynthesis: (i) the oxidation of CO to CO\(_2\) with generation of required reducing capacity, (ii) the reduction of CO\(_2\) to CO, (iii) the formation of a \( \text{C}_1 \) intermediate from CO\(_2\), and (iv) the condensation of this \( \text{C}_1 \) intermediate with the methyl group and the CoA group to form acetyl-CoA. These roles for CO dehydrogenase are much greater than had been previously imagined. Our results directly contradict the recent hypothesis of Diekert et al. (21), based on whole cell studies that the CO dehydrogenase plays only the first and second but not the third and fourth roles.

Surprisingly, low potential electron carriers, like ferredoxin and flavodoxin, are stimulatory to the exchange reaction. It seemed likely that the electron carrier could play one of two roles: (i) if CO\(_2\) was the actual form of C-1 that exchanged with the C-1 of acetyl-CoA, then the electron carriers could stimulate the exchange by oxidizing the CO to the more active form of C-1, CO\(_2\), or (ii) that the electron carrier could act by stimulating an internal electron transfer that occurred in the exchange reaction sequence. Since CO was more active in the exchange than CO\(_2\), the stimulation by low potential electron carriers may occur by aiding in an internal electron transfer that occurs during the exchange reaction. As shown in Table I, the ratio of the rates of the CO dehydrogenase (at pH 7.6) and exchange reactions (at pH 5.3), is approximately 2200. However, when the activity of CO oxidation to CO\(_2\) is measured at 50 °C at pH 5.3 with 1 \( \mu \)M ferredoxin II as electron carrier, the turnover number is approximately 780 mol/mol of CO dehydrogenase, which is 1/200 the rate with 100 mM methyl viologen at pH 7.6 (see Refs. 9 and 22). The exchange reaction, under these conditions, has a turnover number of 70.
mol of CO exchanged per min/mol of enzyme. Thus under the physiological conditions of growth in fairly acidic media, the two rates are more similar. Since the binding and the two electron oxidation of CO and release of CO₂ is rapid in comparison to the exchange reaction, the rate-limiting step of the exchange is not the Y-CO ⇌ CO + Y reaction of Equation 2. We have been unable to detect other factors in the ammonium sulfate 0–40%, 40–60% saturation fractions, or the 60% supernatant which stimulate the CO dehydrogenase exchange reaction in an assay mixture containing ferredoxin. It should be noted that rubredoxin is an excellent carrier for the low potential CO/CO₂ reaction but does not stimulate the exchange reaction.

It is interesting to compare this model (Fig. 5) with the proposed mechanism for industrial synthesis of acetate from methanol and CO (23, 33). Methyl iodide is an intermediate in this conversion. In this process, a rhodium catalyst is proposed to bind the methyl and iodide groups of methyl iodide and carbon monoxide. Then, by an insertion reaction, acetyl iodide and then hydrolyzed to acetate. The rhodium undergoes a +1/+3 redox interconversion during the catalysis. Thus the Monsanto process for acetate formation may have an analogue in nature and it is tempting to propose an acetyl nickel intermediate in the CO dehydrogenase reaction. The model in Fig. 5 makes several readily testable predictions: that labeled CoA should exchange with unlabeled acetyl-CoA and that the methyl group of acetyl-CoA should exchange with the methylated corrinoid protein. These possibilities are under current investigation.

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**CO Dehydrogenase as Catalyst of Acetyl-CoA Synthesis**

**Supplemental Material to Acetate Biogenesis by Aqueous Bacteria: Evidence that Carbon Monoxide Dehydrogenase Is the Catalyzing Enzyme That Catalyzes the Final Step of the Synthesis**

**Steve W. Nagasawa and Harold G. Wood**

**Experimental Procedures**

**Materials**

*Clostridium thermosulfurigenum* (DSM 1163) was grown in 20 l of medium as described earlier (26), and with the inclusion of [1-14C]acetyl-CoA from ICN Radiochemicals, [1-14C]acetate-CoA was synthesized from CoA and [1-14C]acetyl-CoA as described by Williams (27) or purchased from ICN Radiochemicals. The concentration of acetyl-CoA was determined by the following method: carbon monoxide dehydrogenase, nitrate synthase, coupled enzyme assay (28). Sodium dodecyl sulfate (Sequenaly grade) was from Pharmacia Fine Chemicals, and pyruvate hydrolyze from New England Nuclear. Carbon monoxide (99.9% purity) was obtained from Air Products Inc. (Detroit, MI), and CO was from BDH Isotopes (Div. of Merck & Co., Inc., NJ). Tris or Tris/dithiothreitol buffer was used in the presence of sodium dodecyl sulfate (Fig. 1).

**Enzyme and Protein Determination**

Protein was determined by a low-basal dye-binding assay (29), carbon monoxide dehydrogenase (CO to CO2) activity was determined at 550°C with 0.1 M sodium phosphate as an electron acceptor (26). Background activity for CO oxidation, when substrate was not present, was subtracted from the mixture. The standard assay mixture contained 0.80 mH/P, nN, 500 mU acetyl-CoA, and 0.1 M CO2. After addition of substrate, the reaction mixture was incubated in the presence or absence of 24 mH/P to determine the in vitro activity of ferredoxin. Due to the presence of C1, the latter eluting from the Sephadex G-50 superfine column (2.0 by 146 cm) was reconstituted with 100 mM NaP, 6.0, 0.02 M Na3P2O4, 100 mg/ml C1-P, 50 mM dithiothreitol, and 0.1 M CO2 as determined on the 3H channel of the spectrometer and corrected before presentation of the data (ref. 10).}

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