Partial Reactions Catalyzed by Protein Components of the Acetyl-CoA Decarbonylase Synthase Enzyme Complex from Methanosarcina barkeri*

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In methanogens, the acetyl-CoA decarbonylase synthase (ACDS) complex, which has five different subunits, catalyzes synthesis and cleavage of acetyl-CoA according to the reaction: CO₂ + 2H⁺ + 2e⁻ + CH₃H₂SPT + CoA ⇌ acetyl-CoA + H₂SPT + H₂O, where H₂SPT and CH₃H₂SPT are tetrahydrosarcinapterin and N⁵-methyl-tetrahydrosarcinapterin, respectively. We have dissociated the ACDS complex into three protein components by limited proteolytic digestion. Catalysis of acetyl-CoA synthesis was lost in parallel with the loss of the intact β subunit; however, no decrease in activity was detected in any of the three partial reactions found to be catalyzed by distinct protein components of the proteolyzed ACDS complex: (a) CO dehydrogenase, catalyzed by the αε component; (b) CH₃H₂pteridineneob(II)amide-protein methyltransferase, catalyzed by the intact γ subunit and fragments of the δ subunit; and (c) acetyltransferase, catalyzed by a truncated form of the β subunit. The results indicated that the β subunit is responsible for binding CoA and acetyl-CoA and suggested that acetyl-enzyme formation occurs on the β subunit. A value of 5.5 × [H⁺]⁻¹ M⁻¹ was determined for the equilibrium constant of the following reaction at pH 7.5 and 25 °C: CH₃H₂SPT + cob(I)amide-protein + H⁺ ⇌ H₂SPT + CH₃cob(III)amide-protein.

The acetyl-CoA decarbonylase synthase (ACDS) complex has been detected in a variety of methanogens including species of Methanosarcina, Methanobrevibacter (i.e. Methanoseta), and Methanococcus (1, 2). The multienzyme complex from Methanosarcina barkeri is composed of five different subunits, possibly arranged in an α₂γδε₂ structure with the individual subunits of molecular masses of 89, 60, 50, 48, and 20 kDa, respectively (3). The complex contains CO-acceptor oxidoreductase, Co-β-methylcobamidetetrahydrotetrapyrrole methyltransferase, and acetyl-CoA synthase activities (3–5). In the past, the ACDS complex has been referred to as the carbon monoxide dehydrogenase complex and/or the carbon monoxide dehydrogenase-corrinoid enzyme complex. Previous investigations of acetyl-CoA synthesis and cleavage (3, 5) have established that the purified, intact enzyme complex catalyzes the following reaction:

\[\text{CoA} + 2\text{Fd}_{\text{red}}(\text{Fe}^{III}) + 2\text{H}^{+} + \text{CH}_{3}\text{H}_{2}\text{SPT} + \text{CoA} \rightleftharpoons \text{acetyl-CoA} + \text{H}_{2}\text{SPT} + 2\text{Fd}_{\text{ox}}(\text{Fe}^{II}) + \text{H}_{2}O\]

**REACTION 1.**

where Fd stands for ferredoxin, and CH₃H₂SPT and H₂SPT denote N⁵-methyl-tetrahydrosarcinapterin and tetrahydrosarcinapterin, respectively. The sarcinapterin compounds are used in M. barkeri in place of the corresponding tetrahydrofolate derivatives. The structures of CH₃H₂SPT and H₂SPT are shown in Fig. 1.

The overall reaction of acetyl-CoA synthesis or cleavage may be divided into several possible partial reactions. One of these is CO-acceptor oxidoreductase (CO dehydrogenase). This reaction may be written as follows,

\[\text{CO}_{2} + 2\text{H}^{+} + 2\text{e}^{-} \rightleftharpoons \text{CO} + \text{H}_{2}O\]

**REACTION 2.**

and is carried out by an α₂δ₂ component, containing nickel and iron-sulfur centers, as shown in studies (6) that preceded those on the multienzyme complex. This partial reaction is catalyzed also by the intact ACDS complex from the genus Methanosarcina (1, 3), from Methanothrix strain CALS (2), and from Methanococcus vannielii, as well as by the α₂δ₂ component isolated from the multienzyme complexes from M. barkeri (2, 6), M. vannielii (7), and Methanosarcina thermophila (8). The δ₂ protein in Methanophyrum soehnengii has been reported to exhibit an oxygen-stable CO dehydrogenase activity and has also been the subject of several detailed studies (9–12). Interestingly, evidence for a high molecular mass enzyme complex in this organism has not yet been presented.

A discrete, 102 kDa, γδε₁ corrinoid/iron-sulfur protein subcomponent of the ACDS complex from M. thermophila has been identified in experiments in which the complex was dissociated by treatment with a cationic detergent (8). In these studies, a portion of the enzyme complex remained undissociated, and recovery of the β subunit was not reported. It was shown that the reduced corrinoid protein became methylated in the presence of methyl iodide. However, methylation by CH₃H₂SPT was not described, and it was unknown whether or not a

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1. The abbreviations used are: ACDS, acetyl-CoA decarbonylase synthase; CH₃H₂SPT, N⁵-methyl-tetrahydrosarcinapterin; H₂SPT, tetrahydrosarcinapterin; Fd, ferredoxin; HPLC, high pressure liquid chromatography; MOPS, 3-(N-morpholino)propanesulfonic acid; PAGE, polyacrylamide gel electrophoresis.

2. S. H. Zinder and D. A. Grahame, unpublished results.

3. D. A. Grahame and E. DeMoll, unpublished results.

4. D. A. Grahame and E. DeMoll, unpublished results.
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Fig. 1. Structures of tetrahydroaroscinapterin (H₄SPT), R = H; and N₃-methyl-tetrahydroaroscinapterin (CH₃H₄SPT), R = CH₃ (20).

Acetyl-CoA Decarboxylase Synthase Complex—The ACDS complex was isolated from acetate-grown cells of M. barkeri by anaerobic gel filtration on Sepharose CL-6B, as described previously (3). Preparations of the enzyme complex were stored in liquid N₂. Protein was assayed by the method of Bradford (16), with the dye reagent supplied by Bio-Rad and with bovine γ-globulin as standard.

Proteolysis and Dissociation of the ACDS Complex into Subcomponents—Preparation of the enzyme complex, 5.3 mg, was treated under anaerobic conditions with 0.2 mg of chymotrypsin for 60 min at 24°C in a reaction mixture (1.0 ml) that contained 50 mM Tris-HCl buffer, pH 7.5. This procedure resulted in complete loss of the ability to catalyze acetyl-CoA synthase. However, no decrease was observed in activities of CO dehydrogenase, CO-β-methylcob(II)amide tetrahydropteridine methyltransferase, or acetyltransferase. After the incubation with chymotrypsin, the mixture was applied to an anion-exchange column (Bio-Rad Econo-Pac Q cartridge). Elution was carried out at 1 ml/min with a linear gradient (80 ml) of 0–1 M NaCl in 50 mM Tris-HCl buffer, pH 7.5. Fractions were collected at 1-min intervals. The time chosen for proteolysis (60 min) was not critical because samples incubated for either 20 or 60 min produced ion-exchange elution profiles that were indistinguishable. Therefore, no special treatment was found necessary to terminate the reactions prior to chromatography.

Assay of Acetyltransferase Activity in Resolved Fractions of the Proteolyzed ACDS Complex—Development of a new assay for acetyltransferase was based on the analogous reaction of 3⁻[¹⁴C]CoA/acetoyl-CoA exchange, first described for carbon monoxide dehydrogenase from C. thermoautotrophicum (17, 18). The exchange reaction has been shown to be strictly dependent on redox potential and does not proceed to a significant extent in the absence of a strong reductant (18). The method developed herein also uses an HPLC separation step; however, radioactively labeled coenzyme A is not required.

Prior to assay for acetyltransferase activity, samples from the fractions were reduced by incubation at one-tenth of their original concentration for 10 min in the presence of 2 mM Ti³⁺–EDTA in 50 mM MOPS buffer, pH 7.2, in a total volume of 100 μl. Aliquots (5–10 μl) of the incubation mixtures were then added to a solution (97–92 μl) containing 18 nmoles of acetyl-CoA, 0.8 nmoles of ferredoxin, 460 nmoles of Ti³⁺–EDTA, and 6 μM of MOPS buffer, pH 7.2. The acetyl transfer reaction was initiated by the addition of 18 μl of 1 mg ml⁻¹ 3⁻dephospho-coenzyme A. The reaction mixtures (final volume of 120 μl) were maintained at 23°C for 20 min. Thereafter, the reactions were stopped by addition of 120 μl of a solution containing 20 mM sodium 2-mercaptoethanol, 0.15 M sodium citrate, pH 4.0. The final mixtures were stored frozen in liquid nitrogen prior to analysis. Quantitative determination of the products coenzyme A and S-acetyl-3⁻dephospho-CoA was carried out on samples, 100 μl, analyzed by reversed phase HPLC under anaerobic conditions. HPLC analysis was carried out as described previously (3, 5) with modification of the solvent gradient to allow separation of the four derivatives: coenzyme A, 3⁻dephospho-CoA, acetyl-CoA, and S-acetyl-3⁻dephospho-CoA. In the modified procedure, the column (APEX octadecyl, 250 × 4.6 mm diameter, from J ones Chromatography, Inc.) was equilibrated in 50 mM tetramethylammonium phosphate, pH 4.7, and a linear gradient of 0–20% acetonitrile in the same solution was applied at 1 ml/min over a period of 35 min. As shown in Fig. 2, the rate of product formation declines steadily as the reaction proceeds. Therefore, the reaction time and amount of enzyme employed for single time point assays of fractions was chosen so that none of the reactions were

Cross-reactivity observed in the antibodies against the e subunit was markedly reduced by affinity purification. Preimmune sera drawn from the animals did not react with any of the five ACDS subunits.

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allowed to proceed to more than approximately 30% completion. The rate of acetyl-CoA hydrolysis was found to be negligible in control reactions carried out in the absence of 3'-dephospho-CoA. Formation of S-acetyl-3'-dephospho-CoA was not detected in reaction mixtures that lacked Ti₃⁵²⁺-EDTA. Since Co-β-methylcob(III)amide-protein methyltransferase, or acetyltransferase.

The ability of limited proteolytic digestion to cause dissociation of the M. barkeri ACDS complex was investigated by reaction of the enzyme complex with chymotrypsin followed by anion-exchange chromatography. As shown in Fig. 3, this procedure resulted in resolution of three major peaks of protein. The three protein components emerged from the column following a peak of unbound material that contained peptides too small to be resolved on a 12% acrylamide SDS gel. Each of the three protein peaks possessed characteristic subunit compositions, as shown by SDS-PAGE analysis (Fig. 3). Unaltered subunit bands as well as modified subunits were identified by Western blot analyses using subunit-specific antibodies (data not shown). As shown in Fig. 3, the first protein peak contained the γ subunit and four other polypeptides corresponding to partially degraded forms of the δ subunit (δ₄). The second protein component contained the α and ε subunits. The final peak contained a truncated form of the β subunit (β₄). Evidence for the presence of iron-sulfur centers in each of the three peaks was found both by direct determinations of iron and by measurement of the UV-visible absorption spectra of the three protein components. No fraction was recovered that contained detectable quantities of the unresolved enzyme complex. Therefore, the overall dissociation of the complex was judged to be efficient and quantitative.

The extent of proteolytic digestion was varied by incubating samples of the enzyme complex at a fixed concentration with different concentrations of chymotrypsin. SDS-PAGE was used to monitor the progress of digestion of each of the subunits. The band corresponding to the β subunit was found to be highly susceptible to proteolytic attack (Fig. 4). As the concentration of chymotrypsin was increased, the β subunit band intensity decreased markedly. Complete loss of the intact β subunit band occurred under conditions in which intense bands were still found for all other subunits. Densitometric analyses showed that loss of the β band occurred with concomitant loss of the ability to carry out overall synthesis of acetyl-CoA, as shown in Fig. 4. Further digestion at the levels greater than those shown in Fig. 4 then resulted in selective loss of the band corresponding to the intact δ subunit. Samples that contained residual amounts of the intact δ band were found to be only partially resolved by subsequent ion-exchange chromatography. These findings indicated that activity is lost prior to extensive dissociation of the complex.

Although overall acetyl-CoA synthesis activity was abolished by proteolytic treatment of the enzyme complex, no decrease in activity was detected for CO dehydrogenase, CH₂-H₄pteridine:cob(I)amide-protein methyltransferase, or acetyltransferase. Assays for total cobamide content were carried out on fractions obtained in resolution of the protein subcomponents, as shown in Fig. 5A. The major peak of cobamide was closely associated with the first protein peak, as shown in Fig. 5A. Peak 1 contained 74% of the total cobamide. The remaining 26% eluted over a broad region of the gradient and did not coincide with any of the three major protein peaks.

Since Co-β-methylcob(III)amide-tetrahydropteridine transmethylase had not been reported with isolated components of the ACDS complex, tests were performed to determine whether or not the reduced peak 1 corrinoid protein was capable of
undergoing direct reaction with CH$_3$-H$_4$SPt. The Co(I) form of the corrinoid protein was first generated by reaction with 1 mM Ti$_3$-EDTA. As described previously for detection of the reduced cobamide in the intact complex (4), reduction to the Co(I) level was indicated by the development of absorption spectra that showed a prominent peak at 394 nm. The reduction reached 50% completion in approximately 35 s, as shown in Fig. 6. In contrast, under otherwise similar conditions, the undigested enzyme complex required tens of minutes to become reduced (5). Addition of CH$_3$-H$_4$SPt to the reduced corrinoid protein resulted in immediate loss of the 394-nm absorbance peak, and concomitant increase in absorbance around 456 nm. The spectral changes were characteristic of formation of the base-off Co$_3$-methyl cobamide, as observed previously in the intact complex (4). This finding indicated that methyl group transfer from CH$_3$-H$_4$SPt does not require additional subunit proteins (α, β, or ε) and that methyltransferase activity is an intrinsic property of the γδ corrinoid protein subcomponent.

CO dehydrogenase was also measured in the fractions derived from anion-exchange chromatography of the digested enzyme complex. As shown in Fig. 5B, CO dehydrogenase was associated exclusively with peak 2. These results constitute the first demonstration that the αε carbon monoxide dehydrogenase subcomponent may be released from the enzyme complex by the action of a protease.

Acetyltransferase activity of the intact complex from M. thermophila was previously detected based on the exchange of 3$_2^{32}$P-CoA with acetyl-CoA (19). However, the subunit location of this activity was not investigated. In order to identify the protein component responsible for acetyltransferase activity, fractions obtained during resolution of the digested enzyme complex were assayed for acetyltransferase (reaction 3), as described under "Materials and Methods." As shown in Fig. 5C, acetyltransferase activity was found to be associated with the protein peak containing the truncated β subunit. The results establish a previously unrecognized activity of the β subunit and indicate that the binding site for CoA and acetyl-CoA reside within a domain that remains intact in the truncated β subunit.

Titration of the reduced corrinoid protein with CH$_3$-H$_4$SPt was carried out in order to determine the equilibrium constant for methyl transfer in reaction 3. As shown in Fig. 6, each successive addition of CH$_3$-H$_4$SPt made to the reduced corrinoid protein resulted in an immediate decrease in the absorbance function (A$_{394}$ nm−A$_{456}$ nm). The addition of excess H$_4$SPt to the methylated corrinoid protein caused an immediate increase in the absorbance function, indicating that the reaction was freely reversible. The ratio of methylated to demethylated...
Corrinoid protein, \([\text{CH}_3\text{-cob(III)amide-protein}] / [\text{cob(I)amide-protein}]\), was determined from the spectrophotometric data. Corresponding ratios of \([\text{H}_4\text{SPt}] / [\text{CH}_3\text{-H}_4\text{SPt}]\) were measured by HPLC analysis (5) of aliquots that were removed after each addition of \(\text{CH}_3\text{-H}_4\text{SPt}\), as described in the legend to Fig. 6. Both sets of ratios are plotted in Fig. 7 as a function of the total concentration of \(\text{CH}_3\text{-H}_4\text{SPt}\) added. The equilibrium product/substrate ratio, \([\text{H}_4\text{SPt}] / [\text{CH}_3\text{-cob(III)amide}]\), was found to be independent of the amount of \(\text{CH}_3\text{-H}_4\text{SPt}\) added, as shown in Fig. 7. The \(K_{eq}\) value measured at pH 7.5 was \(5.5 \pm 0.3\).

**DISCUSSION**

It was demonstrated previously that the ACDS complex catalyzes the synthesis and cleavage of acetyl-CoA (reaction 1) (3, 5); however, unambiguous assignment of the catalytic roles of the various protein subcomponents of the enzyme complex has not been reported. In order to obtain information on the quaternary structure of the ACDS complex and to identify catalytic properties of individual protein subunits or subcomponents, we developed a new procedure for dissociation of the enzyme complex. Important advantages over the previously described detergent fractionation protocol (8) are (a) that three protein components are resolved instead of two and (b) that dissociation of the enzyme complex is quantitative and does not produce a fraction corresponding to the residual unresolved enzyme complex. The three separate protein components so obtained display distinct subunit compositions and exhibit characteristic catalytic activities.

Component 1. \(N^3\text{-Methyl-tetrahydropteridinecob(I)amide-protein}\)—The first of the three protein peaks produced by chromatographic resolution of the digested enzyme complex is a corrinoid protein that contains the 50-kDa \(\gamma\) protein Methyltransferase—The first of the three protein peaks produced by chromatographic resolution of the digested enzyme complex is a corrinoid protein that contains the 50-kDa \(\gamma\)
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**Figure 6. Reduction of the protein-bound cobamide with Ti\(^{3+}\)-EDTA and reaction with CH\(_3\)H\(_4\)SPT.** Reduction of the ACDS corrinoid protein subcomponent and titration with CH\(_3\)H\(_4\)SPT was carried out at 23°C with a solution (800 μl) that contained 50 mM Tris-HCl, pH 7.5, and the corrinoid protein (3.55 μM cobamide) obtained from ion-exchange chromatography of the proteolytically digested enzyme complex (peak 1 in Fig. 3). The processes of reduction by Ti\(^{3+}\) and methylation by CH\(_3\)H\(_4\)SPT were monitored spectrophotometrically. At the indicated time point, 12 μl of 64 mM Ti\(^{3+}\)-EDTA was added. Addition of the indicated amounts of CH\(_3\)H\(_4\)SPT was made from a 0.485 mM stock solution. After each addition of CH\(_3\)H\(_4\)SPT, two 60-μl samples were removed from the cuvette, mixed with 60 μl each of a solution containing 20 mM 2-thioethanesulfonate and 0.5 mM sodium citrate, pH 4.0, and frozen in liquid nitrogen. In each sample, the [H\(_4\)SPt]/[CH\(_3\)H\(_4\)SPt] concentration ratio was subsequently determined by HPLC analysis according to the procedure described previously (5). Absorbance data have been corrected for dilution resulting from reagent additions.

**Figure 7. Determination of the equilibrium constant K\(_{eq}\) for the CH\(_3\)H\(_4\)SPt: cob(ll)amide-protein methyl transfer reaction (Reaction 3).** The K\(_{eq}\) for the CH\(_3\)H\(_4\)SPt: cob(ll)amide methyl transferase reaction was determined by analysis of the ([CH\(_3\)-cob(ll)amide]:cob(ll)amide) and [H\(_4\)SPt]:[CH\(_3\)H\(_4\)SPt] concentration ratios that resulted during titration of the reduced corrinoid protein with CH\(_3\)H\(_4\)SPT as described under “Materials and Methods,” and in the legend to Fig. 6. The ([CH\(_3\)-cob(ll)amide]:cob(ll)amide) concentration ratios (●) were obtained from the spectrophotometric data, and the corresponding K\(_{eq}\) values (○) obtained as the product [CH\(_3\)-cob(ll)amide]:cob(ll)amide × [H\(_4\)SPt]:[CH\(_3\)H\(_4\)SPt] are shown plotted as a function of the total added CH\(_3\)H\(_4\)SPt.

Subunit and fragments of the 48-kDa δ subunit. A similar γδ subcomponent was dissociated previously by the detergent treatment procedure (8); however, we have extended the characterization of the corrinoid protein to investigate reactivity of this component with the physiological methyl donor substrate CH\(_3\)H\(_4\)SPT. Although evidence for methyl group transfer from CH\(_3\)H\(_4\)SPT to the corrinoid cofactor contained in the intact complex has been presented earlier (3), analogy to the acetyl-CoA synthesizing system of C. thermoaerium might suggest that a separate methyltransferase (possibly one of the subunits other than γ or δ) would be required for methylation of the corrinoid protein by CH\(_3\)H\(_4\)SPT. However, we now show that in the absence of other proteins the isolated, reduced γδ* cob(ll)amide protein reacts very rapidly with CH\(_3\)H\(_4\)SPT. This demonstrates that methyltransferase activity is an intrinsic property of the γδ corrinoid component. Although this differs from the clostridial corrinoid/sulfur protein, the intrinsic activity is analogous to the ability of the active form of methionine synthase to catalyze methylation of the bond B\(_{12}\) cofactor by reaction with N\(^5\)-methyl-H\(_4\)folate.

Equilibrium studies of methyl transfer between the enzyme-bound corrinoid moiety and the reduced pteridine substrate indicate that the process is freely reversible under physiologically relevant conditions. The equilibrium constant for CH\(_3\)-H\(_4\)SPT: cob(ll)amide-protein methyl transfer (Reaction 3) was 5.5 [H\(^+\)]\(^{-1}\) M\(^{-1}\) (Fig. 7). Therefore, in the process of acetyl-CoA deavage, methyl group transfer from the enzyme to H\(_4\)SPT is slightly thermodynamically unfavorable under standard state conditions (K\(_{eq}\) = 1/5.5, ΔG\(^{0}\) = +1.0 kcal/mol). However, very low levels of CH\(_3\)H\(_4\)SPT are detected during purification of H\(_4\)SPT from cell extracts, and based on the amounts of ACDS complex and H\(_4\)SPT obtained during purification from an equal amount of cell paste, the ratio of H\(_4\)SPT to enzyme cobamide is estimated to be approximately 50:1. These findings indicate that at equilibrium in the presence of physiological concentrations of enzyme and CH\(_3\)H\(_4\)SPT, approximately 91% of the enzyme-bound methyl groups would be transferred to the cellular pool of H\(_4\)SPT. Demethylation of the enzyme corrinoid is also exceedingly rapid. Therefore, it is unlikely that methyl group transfer to H\(_4\)SPT presents either a kinetic or thermodynamic barrier of significance in the overall process of acetyl-CoA deavage in vivo.

Component 2, CO:Acceptor Oxidoreductase—A previous study showed that a portion of the CO dehydrogenase αε component could be dissociated from the M. thermophila enzyme complex in the presence of a cationic detergent (8). We now show that complete release of the CO dehydrogenase component occurs as a result of limited proteolytic digestion of the enzyme complex (Fig. 5B). Two different forms of CO dehydrogenase have been noticed previously in extracts of M. thermophila (1,3) and M. barkeri (2,3). The ability to generate a second form of the enzyme by proteolytic action now suggests an explanation for the presence of the different forms.

Component 3, Acetyltransferase—Whereas previous investigations demonstrated acetyl transfer/exchange activity of the intact enzyme complex (19), we now show that the site of redox-dependent acetyltransferase is located on the 60-kDa β subunit. As revealed by SDS-PAGE, low levels of chymotrypsin act selectively on the 60-kDa β subunit to produce a truncated subunit (β*) of about 50 kDa (Fig. 2) that possesses a high specific activity of acetyltransferase (Fig. 5). Similar results were also found with other proteases such as bromelain and trypsin (data not shown). Thus, the data suggest that a region of the β polypeptide is highly susceptible to general proteolytic attack. In experiments in which the extent of digestion is varied, overall acetyl-CoA synthetic activity is lost in direct proportion to the loss of the intact β subunit (Fig. 4). However, more extensive digestion is required to bring about dissociation.
of the complex. Loss of the intact 48-kDa \(\delta\) subunit occurs as the level of digestion is further increased and correlates with the ability to obtain high resolution of the three protein components (and with the absence of residual undissociated enzyme). The results allow us to formulate the hypothesis that integrity of the \(\delta\) subunit may be essential for maintaining the quaternary structure of the enzyme complex. Furthermore, since loss of acetyl-CoA synthetic activity occurs without overall dissociation of the enzyme complex, the involvement of the \(\beta\) subunit apparently extends beyond that of a structural role or that of the ability to carry out acetyl transfer. Further investigations are needed to explain the precise mechanism for the decline in overall ACDS activity coinciding with the loss of the intact \(\beta\) subunit.

In summary, the results demonstrate catalytic roles for the \(\gamma\delta\) (or possibly \(\gamma\) alone), \(\alpha\epsilon\), and \(\beta\) protein subcomponents of the ACDS complex. We may now write reactions 5–7 to include the enzyme subcomponents with a subscript denoting the relevant subunits.

\[
E_{\alpha} + CO_2 + 2H^+ + 2e^- \rightarrow E_{\alpha} - CO + H_2O \\
\text{REACTION 5.}
\]

\[
E_{\gamma\delta} - Co \rightarrow I \rightarrow 1CH_3-H_4SPt + H^+ \rightarrow E_{\gamma\delta} - Co \rightarrow III \rightarrow -CH_3 + H_4SPt \\
\text{REACTION 6.}
\]

\[
E_{\gamma} + acetyl-CoA \rightarrow E_{\gamma} - acetyl + CoA \\
\text{REACTION 7.}
\]

The \(\beta\) subunit catalyzes the exchange/transfer of the acetyl group of acetyl-CoA. Consequently a likely role for the \(\beta\) subunit in acetyl-CoA synthesis in the intact ACDS complex would be to catalyze the reversible synthesis of acetyl-CoA from CoA and an acetyl group bound to the \(\beta\) subunit. The role of the \(\gamma\) subunit in the intact ACDS complex would be to catalyze the reversible transfer of the methyl group from CH\(_3\)-H\(_4\)SPt to the bound cobamide cofactor, and perhaps ultimately to the site of acetyl synthesis. The exact role of the \(\delta\) subunit is unknown, however, our data suggest that integrity of the \(\delta\) subunit could be involved in maintaining the overall quaternary structure. During the process of acetyl-CoA cleavage, the transfer of the methyl group from the corrinoid protein to the cellular pool of H\(_4\)SPt is rapid and thermodynamically favorable under conditions likely to exist in vivo.

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