Structures and Energetics of Models for the Active Site of Acetyl-Coenzyme A Synthase: Role of Distal and Proximal Metals in Catalysis

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Acetyl-coenzyme A synthases/carbon monoxide dehydrogenases (ACS/CODH) are a family of enzymes found in anaerobic chemo-autotrophic bacteria and archaea. ACS/CODH from the homoacetogen *Moorella thermoacetica* is a bifunctional 310 kDa $\alpha_2\beta_2$ tetramer that in the *A-cluster* of the α subunit synthesizes acetyl-CoA from carbon monoxide (CO), CoA, and CH_3^+ (delivered by CH_3-$Co^{III}FeSP$, a corrinoid/iron–sulfur methyl-transfer protein) and in the *C-cluster* of the β subunit generates CO (which migrates to the A-cluster through a protein-encapsulated tunnel) by reducing carbon dioxide.[1,2] The active site of the A-cluster (depicted in the left-most drawing of Scheme 1) has a distal Ni, Ni_d, coordinated to two carboxyamido N (from the protein backbone) and two cysteinyl S; a proximal metal, M_p, coordinated by the same two S_{cys} as Ni_d; and a third cysteinyl S, which bridges to an Fe_4S_4 cubane.

Several recent[2] studies on ACS/CODH have produced conflicting views of the mechanism and contradictory reports on the nature of M_p and its role in the mechanism. Although biomimetic studies[3] have also proven helpful in proposing the active-site structure, function, and mechanism, they have not been able to resolve a number of important issues discussed below.

(1) Ni, Cu, and Zn have been reported for M_p; Zn is adventitious and inactive, but there has been controversy about the activity of Ni and Cu. (2) Reductive activation of the ($[Fe_4S_4]^{2+}$:Ni_p^{II}:Ni_d^{II}) A-cluster appears to require $2e^-$, but the site(s) to which these electrons localize and the step(s) of catalysis at which they add remain controversial. Ni_p^{II} may be reduced to Ni_p^0 prior to substrate addition, or it may be reduced to Ni_p^I with CO binding and then be reduced by a second e^- concomitant with CH_3^+ addition. Both approaches ultimately afford Ni_p^{II}–acetyl, the species presumably attacked by CoA to form product. (3) Whether CoA binds directly to the metal ion of this species (prior to attacking the carbonyl carbon) or whether it attacks the carbonyl carbon without prior metal coordination is another uncertainty. (4) A proposed bimetallic mechanism has CO binding to M_p and the methyl group binding to Ni_d.[1b,4] In contrast, a proposed monometallic mechanism has CO and methyl both binding to Ni_p.[2b]

This work utilizes density functional calculations[5] to address these controversies. The active-site model constructed for our calculations has Ni_d bound to two carboxyamido N and two thiolato S (modeling two cysteinyl S) and M_p bound to a bridging-Fe–thiolato S (methyl sulfide modeling the bridging-Fe_{cubane}–cysteinyl S) and the same two thiolato S as Ni_d; the Fe of the cubane is modeled as a high-spin Fe^{II} tetrathiolate (Scheme 1).[5] Proximal metal (M_p) was modeled alternatively as Ni or Cu. The relevant geometries are tetrahedral (T_d) for Ni^0, Ni^I, high-spin (HS, $S = 1$) Ni^{II}, and Cu^I; square planar (SPL) for low-spin (LS, $S = 0$) Ni^{II} and Cu^{II}; and trigonal planar (TPL) for three-coordinate Ni and Cu. Addition of CO occurs without changing the metal oxidation states, while CH_3^+ addition requires a formal increase of 2 in the oxidation state. An appropriate active-site model begins with a four-coordinate square planar distal Ni^{II} and a reduced M_p, either Ni^0/Cu^I or Ni^I/Cu^{II}. A

Scheme 1. Diagrammatic Representation of the ACS Active Site
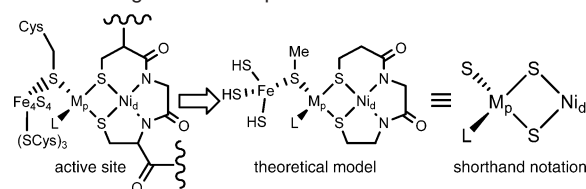
Scheme 2. Calculated Energies of Optimized Structures for Intermediates and Transition States in a Proposed Mechanism[a]
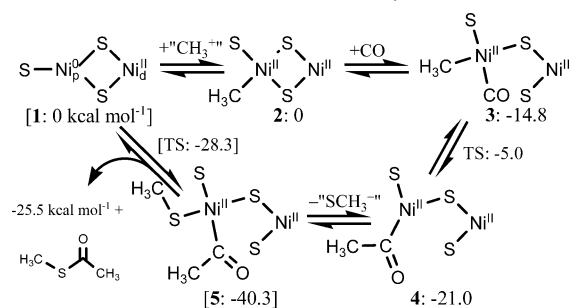
[a] Labels refer to the species as discussed in the text. All species contain high-spin Fe^{II}. Two sets of relative energies are presented because **1** and **5** (in brackets) have a different charge than **2**, **3**, and **4** (not in brackets). (This diagrammatic representation of the model is described in Scheme 1.)

number of species were examined (see Supporting Information), but only those of reasonable stability or with low-energy transition states are described here.

The first issue investigated was whether Cu in the proximal site could support the binding of CO and/or CH_3^+. A $Cu^I(CO)$ species is stable, but calculations with the appropriate charge and number of unpaired electrons for $Cu^{II}(CO)$ converged to a Cu^I species by oxidizing the sulfurs of the $Fe(SH)_3(SMe)$ unit (a Cu^{III} species would be less likely). Although methylation of Cu_p produces stable species, neither Cu^I- nor $Cu^{II}-(CH_3)$ species binds CO exothermically: the $Cu^I(CO)(CH_3)$ species has a CO bridging to one of the S_{Ni_d} and is less stable than the separated species, CO and the $Cu-CH_3$ complex, by 5.1 kcal mol^{-1}, and calculations for $Cu^{II}(CO)-(CH_3)$ only converge to a species with CO loss. The Cu^I-acetyl complex is calculated to be more stable than the separated CO and Cu^I-CH_3, but the instability of the $Cu^I(CO)(CH_3)$ species suggests CO loss (after methylation) is a competitive, unproductive route. Thus, we conclude that the Cu_p form of ACS/CODH is not functional. Equivalent calculations with Ni_p better modeled the catalytic activity (vide infra). Furthermore, recent calculations of the EPR hyperfine interaction of a model of the $S = 1/2$ $A_{red}-CO$ species (called NiFeC) have been interpreted as favoring proximal Ni (rather than Cu); and a recent spectroscopic study of CO binding also supports $M_p = Ni$.[6]

The next issue investigated was whether activation of the A-cluster might require $2e^-$ or $1e^-$ reduction from {Ni_p^{II}:Ni_d^{II}}, and to which species these reductions might lead, {Ni_p^0:Ni_d^{II}}, {Ni_p^I:

Ni_d^I}, or {Ni_p^I:Ni_d^{II}}. According to our calculations, the $2e^-$ activated species is {Ni_p^0:Ni_d^{II}} (**1**), not {Ni_p^I:Ni_d^I}. The addition of CO to Ni_p^0 leads to a species that dissociates by breaking the Ni_p-S_{Fe} bond, while the addition of CH_3^+ leads to a species with two SPL Ni^{II} (**2**). Addition of CO to this SPL Ni_p^{II}–SPL Ni_d^{II} species causes Ni_p to break one of its μ_2 cysteinyl–S_{Ni_d} bonds, while strengthening the remaining $Ni_p-S_{Ni_d}$. This deligation is necessary to maintain a low-energy SPL geometry while both substrates are bound to the same Ni_p^{II} metal. From this point, the SPL $Ni_p^{II}(CO)$-(CH_3) species (**3**) can form an acetyl group through a low-barrier ($\sim$10 kcal mol^{-1}), CO insertion/CH_3-migration transition state, producing a TPL Ni_p^{II}–acetyl species (**4**). The stability of the acetyl complex has been noted experimentally.[7]

The $1e^-$ activated species is {Ni_p^I:Ni_d^{II}}; it binds CO and forms a stable species analogous to the well-known $S = \frac{1}{2}$ A_{red}–CO species, the role of which is controversial.[1c–f] Our computational study finds that CH_3^+ addition to this Ni_p^I–CO species does not result in a stable $Ni_p^{III}(CO)(CH_3)$ species but in a "dissociated" $Ni_p^{II}(CO)(CH_3)$ species that has broken the Ni_p-S_{Fe} bond (taking one e^- from S_{Fe} and leaving an unpaired e^- on the sulfurs of the dissociated Fe tetrathiolate). If the Fe_4S_4 cubane was in the $[Fe_4S_4]^+$ oxidation state, it could provide this electron without breaking the Ni_p-S_{Fe} bond, producing the same $Ni_p^{II}(CO)(CH_3)$ species as one finds starting from {Ni_p^0:Ni_d^{II}} and $[Fe_4S_4]^{2+}$,[6a] the oxidation state of the cubane for the remainder of the reaction.[1d] These results suggest that activation requires $2e^-$ overall and argues against a Ni^I/Ni^{III} "paramagnetic" mechanism. Experimentally, the paramagnetic A_{red}–CO species will react with CH_3^+, ultimately producing a diamagnetic species,[1g] possibly the SPL $Ni_p^{II}(CO)(CH_3)$ species (vide supra).

The TPL Ni_p^{II}–acetyl (**4**) could form a slightly more stable SPL Ni_p^{II}–acetyl species (not pictured, 3 kcal mol^{-1} lower) by reforming the second $Ni_p-S_{Ni_d}$ bond, but this SPL species cannot bind a thiolate group to later reductively eliminate the thioester. However, the trigonal planar Ni_p^{II}–acetyl species can bind the thiolate to form either $Ni_p^{II}(acetyl)(thiolate)$ species, a low-spin SPL species (**5**) or a high-spin T_d species (not pictured, 2.9 kcal mol^{-1} lower). This $Ni_p^{II}(acetyl)(thiolate)$ species proceeds through a transition state ($\sim$12 kcal mol^{-1} barrier) to produce a bound thioester Ni_p^0 species. If thiolate binds to Ni_d, the binuclear reductive elimination to form the thioester is quite high in energy ($\sim$33 kcal mol^{-1} barrier). Direct nucleophilic attack of bound acetyl by thiolate is also possible, but this route has a $\sim$20 kcal mol^{-1} barrier. Thus, nickel-assisted thioacetyl reductive elimination seems best.[8]

With respect to the question of a binuclear mechanism for acetyl formation, Ni_d^{II} does not add CO, according to our calculations, and addition of CH_3^+ to Ni_d^{II} does not lead to a stable species. The opposite arrangement $Ni_d^{III}(CH_3)$ and $Ni_p^I(CO)$ is predicted by theory to be a higher-energy species. The binuclear coupling transition state has not yet been located; however, (1) it must be higher in energy than the $Ni_d^{III}(CH_3)$–$Ni_p^I(CO)$ species, and (2) the large separation between CH_3 and CO groups suggests a large intrinsic barrier to coupling, as was calculated for the bimetallic coupling of Ni_p^I–acetyl and Ni_d^{III}–thiolate, which had a 33 kcal mol^{-1} barrier. Overall, these results support a mononuclear Ni_p-based mechanism in which the methyl group binds before CO.

In summary, the current work favors a mechanism in which methylation occurs first to Ni_p^0– or Ni_p^I–$[Fe_4S_4]^+$, followed by coordination of CO to form $Ni_p^{II}(CO)(CH_3)$ which breaks one of the S_{Ni_d} bonds (forming the bis square planar Ni^{II} species, as if the $Ni_dN_2S_2$ unit were acting as a biological pseudodiphosphine, mimicking behavior common to a bidentate phosphine[4]). The CO-insertion/CH_3-migration occurs on one metal (the proximal site)

forming the trigonal planar Ni_p^{II}–acetyl intermediate. Finally, addition of thiolate produces the thioester. This work disfavors the unprecedented bimetallic, CO-insertion/CH_3-migration mechanism (both in its diamagnetic and paramagnetic guise) and disfavors CO, CH_3^+, or thiolate (CoA) binding to the distal Ni. Finally, Ni in the proximal site produces a better catalyst than does Cu, a result consistent with a developing consensus.[1a,b,2a,e,6]

Supporting Information Available: Details of calculations and the species examined in the current study, including various modes of ligand binding and other various calculated minima and transition states. This material is available free of charge via the Internet at http://pubs.acs.org.

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