Acetyl-CoA Synthase/Carbon Monoxide Dehydrogenase (ACS/CODH)

A Ni-Fe-S-containing Bifunctional Enzyme with a Bio-organometallic Reaction Mechanism

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Where are ACS/CODH’s found?

- Anaerobic Bacteria and Archaea
- Chemoautotrophic (grows on CO$_2$/H$_2$ or CO)
- Evolutionarily primitive (thermophiles)
- Major role in global C$_1$ cycle
- Three major classes
  - $\alpha_2\beta_2 + \text{CoFeSP (} \gamma\delta \text{)}$ *Moorella thermoacetica*
  - $(\alpha\beta\gamma\delta\varepsilon)_2$ *Methanosarcina thermophila*
  - $\beta_2$ *Rhodospirillum rubrum*

Prokaryotes:
  - anaerobes
  - chemiautotrophic
  - thermophilic

Earliest Living Organisms
Major role: Global C cycle
Reactions catalyzed by the 310 kDa $\alpha_2\beta_2$ tetramer
From *Moorella thermoacetica*:

**CO/CO$_2$ Redox (β Subunit):**

$$\text{CO}_2 + 2\text{H}^+ + 2\text{e}^- \underset{(\text{CODH})}{\overset{}{\rightleftharpoons}} \text{CO} + \text{H}_2\text{O}$$

**Acetyl-CoA Synthesis (α subunit):**

$$\text{CH}_3^-\text{-Co}^{3+}\text{FeSP} + \text{CO} + \text{CoA} \underset{(\text{ACS})}{\overset{}{\rightleftharpoons}} \text{CH}_3\text{-C}-\text{SCoA} + \text{Co}^{1+}\text{FeSP}$$

Recombinant $\alpha$ subunit also catalytic if incubate apo-$\alpha$ with Ni
Structure of ACS/CODH

Alpha Subunit
Open Conformation
A-Cluster (Active site for ACS reaction)

Beta Subunits
C-Cluster (Active site for CODH reaction)

Alpha Subunit
Closed Conformation

Doukov et al., Science 2002
Darnault et al, Nature Structural Biology, 2003
Mechanism of the CODH Reaction
Controversy: Role of Bridging Sulfide in C-cluster

![Chemical structures of Carboxydothermus hydrogenoformans and Moorella thermoacetica](diagram)

**Carboxydothermus hydrogenoformans**


**Moorella thermoacetica**


Proposal of Dobbek, Svetlitchnyi, Liss and Meyer (JACS 2004):

• Bridging Sulfide is Required for Activity
• Incubation in CO abstracts S, forming COS and inactivating CODH
We tried the same experiment...
**CO Oxidation Activity of CODH$_{Rr}$ in the Presence of Sodium Sulfide**

CO + 2MV$^{2+}$ $\rightarrow$ CO$_2$ + 2MV$^{1+}$ + 2H$^+$

- CODH$_{Rr}$ incubated in HS$^-$ then assayed
- HS$^-$ inhibited catalysis
- Inhibition was partial
- Lag phase evident
- HS$^-$ bound CODH prior to catalysis, Yielding an inhibited state
- HS$^-$ is expelled, and rebinds during turnover
EPR = Electron Paramagnetic Resonance
Can observe systems with $S = \frac{1}{2}, 3/2, 5/2$, etc

- E, Potential Energy
  - $S = 1/2$
  - $\Delta E = h\nu = g\beta H$
  - $M_s$ at $E_+ = +1/2g\beta H$
  - $M_s$ at $E_- = -1/2g\beta H$

- H, Magnetic Field
  - Axial Symmetry ($g_\parallel > g_\perp$)
  - Rhombic Symmetry ($g_x \neq g_y \neq g_z$)

- Abs
- $\frac{d\text{Abs}}{dH}$

- $\frac{\text{Abs}}{\text{Cu(II)EDTA standard}}$

- Principal g-value
  - $g = h\nu / \beta H$ (for e$^-$:
    - $g = 2.0023$)

- Mixture of Signals

Quantified by Integration and comparison to Cu(II)EDTA standard
Redox, EPR of C-Cluster

\[
\begin{align*}
\text{C}_{\text{ox}} & \overset{1\text{e}^-}{\rightleftharpoons} \text{C}_{\text{red1}} \\
(S = 0) & (S = 1/2) \\
\text{[Ni}^{2+} & \text{Fe}^{2+}\]) & \overset{1\text{e}^-}{\rightleftharpoons} \text{["Ni}^{0} & \text{Fe}^{2+}\]) \\
\text{CO} + \text{H}_2\text{O} & \rightarrow \text{CO}_2 + 2\text{H}^+ \\
\end{align*}
\]

Anderson et al. *Biochemistry*, 1996, 8371-8380
Effect of Sodium Sulfide on \( C_{\text{red1}} \) EPR Spectra

Increasing concentrations of sulfide

\( C_{\text{red1}} \) signal shifts to \( g = 1.95, 1.85, 1.70 \)

\( C_{\text{red2}} \) signal does not shift

1 mM HS\(^-\)
Implication for Substrate Binding to C-cluster

Effect of HS\(^{-}\) on activity and EPR similar to those of CN\(^{-}\)
  - Partial Inhibition
  - Binds C\(_{\text{red1}}\) not C\(_{\text{red2}}\)
  - CO incubation reactivates

Similarity to substrate HO\(^{-}\) (ENDOR shows binding to C\(_{\text{red1}}\) not C\(_{\text{red2}}\))

Proposal: Substrate HO\(^{-}\) binds like HS\(^{-}\) --- bridging between [Ni and Fe\(_{a}\)] in C\(_{\text{red1}}\) only
Model of Sulfide Inhibition
CO Oxidation Activity of CODH\textsubscript{Mt} with HS\textsuperscript{-}

From the model we can derive the rate equation...

\[ v = \frac{8750K_I + 5190[HS^-]}{K_I + [HS^-]} \]

Using \( K_I = 60 \, \mu M \)…
They Conclude…

“…the S2 ligand between Ni and Fe1 is absent in catalytically competent enzyme”

“The structure-based mechanism outlined agrees in all central aspects with the bimetallic mechanism proposed on the basis of EPR, ENDOR, and Mössbauer spectroscopy.”
CODH Catalytic Mechanism

\[
\begin{align*}
&\text{[Ni}^{2+}\text{ Fe}_{a}^{2+}] \\
\text{[Ni}^{2+}\text{ Fe}_{a}^{2+}] &\xrightarrow{\text{H}^+} \text{[Ni}^{2+}\text{ Fe}_{a}^{2+}] \\
\text{[Ni}^{2+}\text{ Fe}_{a}^{2+}] &\xrightarrow{\text{CO}} \text{[Ni}^{2+}\text{ Fe}_{a}^{2+}] \\
\text{[Ni}^{2+}\text{ Fe}_{a}^{2+}] &\xrightarrow{\text{H}^+} \text{[Ni}^{2+}\text{ Fe}_{a}^{2+}] \\
\text{[Ni}^{2+}\text{ Fe}_{a}^{2+}] &\xrightarrow{2\text{e}^-} \text{H}_2\text{O} \\
\text{[Ni}^{0}\text{ Fe}_{a}^{2+}] &\xrightarrow{\text{CO}_2} \text{[Ni}^{2+}\text{ Fe}_{a}^{2+}] \\
\end{align*}
\]
Mechanism of the ACS Reaction: Role of the Tunnel
A-Cluster in Open Conformation

“Proximal” \( \text{Ni}_p \) is labile – can be removed by phen - Reversible

Distal Site, square planar – remains \( \text{Ni}^{2+} \)
Cu or Zn can replace Ni in Proximal Site
This inactivates the enzyme
Hydrophobic Tunnel connects A- and C-clusters as well as the two C-clusters.

In open conformation, tunnel to A-cluster is blocked.

Tunnel controls delivery of CO to A-cluster.
ACS Activity vs [CO]

Plot for ACS/CODH:
• At [CO] < 100 μM, CO is a “normal” substrate
• At [CO] > 100 μM, CO is an inhibitor
• Inhibition is cooperative (more than 1 CO involved)
• Residual activity (10% of max) is insensitive to CO

Plot for α subunit:
• CO is a “normal” substrate
• No cooperative inhibition
• Max activity is ~ same as residual of WT
**Effect of Blocking Tunnel**

Blockage between A and C-clusters:
- CODH activity unaffected
- ACS activity (using CO$_2$) $\sim 0$
- ACS activity using CO – residual only
- No CO-cooperative inhibition

Blockage between C and C-clusters:
- Same as AC mutants but CODH activity $\sim 5\%$ of WT
- CO/CO$_2$ may enter at beta:beta interface

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Tan, Volbeda, Fontecilla-Camps, Lindahl, *JBIC* 2006
Two Migration Pathways for CO used in ACS Catalysis

Tunnel Pathway:
- CO enters at the $\beta\beta$ interface and migrates to the A-cluster
- Responsible for majority activity and CO cooperative inhibition

Direct-binding Pathway:
- CO from solvent binds $N_{i_p}$ of the A-cluster directly.
- Responsible for the residual activity
- Not associated with CO cooperative inhibition
Conclusions

- The tunnel delivers CO/CO$_2$ to the active sites; delivery is regulated by protein conformational change.
- The tunnel region between A- and C-clusters is exclusively used for ACS reaction, not CODH reaction.
- The tunnel region between the two C-clusters participates in the CODH reaction (and indirectly in the ACS reaction).
- CO/CO$_2$ may enter/exit the enzyme at the $\beta$-$\beta$ interface.
- The tunnel is involved in the cooperative inhibition by CO.
- CO used in “majority” activity approaches Ni$_p$ via tunnel.
- CO used in residual activity approaches Ni$_p$ via solvent.
Mechanism of the ACS Reaction:
Electronic Configuration of the A-cluster
Mössbauer Spectroscopy (Nuclear $\gamma$-Ray Resonance)

Useful for $^{57}$Fe ($I = \frac{1}{2}$) Systems

All electronic and magnetic states observed (no “Mössbauer-Silent Fe)

Intensity proportional to # of Fe atoms contributing

$I = \frac{3}{2}$

$\Delta E_Q$

$14.4 \text{ KeV}_\delta = \text{isomer shift (0 mm/s) (0.2–1 mm/s)}$

$I = \frac{1}{2}$

Change in Oxidation State

Change in Symmetry

Magnetic Field (external Or Internal e.g. $S = \frac{1}{2}$)

% Transmission

Energy (mm/s) “Quadrupole Doublet” “Magnetic”

Magnetic; e.g. 60%

Connected to EPR

40% Quadrupole Doublet

Rhombic symme Or Magnetic Mixture
Redox States of the A-cluster

**A<sub>ox</sub> State**

Obtained when potential > -500 mV vs. NHE
No EPR Signals (i.e. not Ni<sup>1+</sup> or Ni<sup>3+</sup>); Ni<sub>p</sub> is 2+
Mössbauer shows quadrupole doublet typical of S = 0 [Fe<sub>4</sub>S<sub>4</sub>]<sup>2+</sup>

Electronic Assignment: \[ A_{ox} = \{[Fe_4S_4]^{2+} Ni_p^{2+}\} \]
\[ S_{system} = 0 \]

**CH<sub>3</sub>-A<sub>ox</sub>: Methylated State**

Mössbauer shows S = 0 [Fe<sub>4</sub>S<sub>4</sub>]<sup>2+</sup>
EPR silent (i.e. not Ni<sup>1+</sup> or Ni<sup>3+</sup>) i.e. Ni<sub>p</sub><sup>2+</sup>
Many examples of Ni<sup>2+</sup>-CH<sub>3</sub> model complexes
Ni<sub>p</sub> is required for methyl transfer; methylation blocks Ni<sub>p</sub> removal
Electronic Assignment: \[ CH_3-A_{ox} = \{[Fe_4S_4]^{2+} Ni_p^{2+} \text{-CH}_3\} \]
\[ S_{system} = 0 \]

\[ \text{A}_{\text{red}}\text{-CO State} \]

\[ A^{\text{ox}} + 1\text{e}^- + \text{CO} \rightleftharpoons A^{\text{red}}\text{-CO} \]

S=0 \quad S=1/2

NiFeC EPR signal \( (g_\perp = 2.08, \, g_\parallel = 2.03) \)

Mössbauer shows magnetic \([\text{Fe}_4\text{S}_4]^{2+}\)

Electronic Assignment: \( A^{\text{red}}\text{-CO} = \{[\text{Fe}_4\text{S}_4]^{2+} \, \text{Ni}_p^{1+}\text{-CO}\} \)

Catalytic Intermediate or Inhibitor of catalysis?

A_{red-Act}: The Reductively Activated State

\[ A_{ox} + CH_3-Co^{3+}FeSP \rightarrow \text{No Reaction} \]

But in presence of low-potential reductant (e.g. dithionite, Ti^{3+} citrate)…

\[ (A_{ox} + ne^{-}) + CH_3-Co^{3+}FeSP \leftrightarrow CH_3-A + Co^{1+}FeSP \]

390 nm
Mechanistic Implications

Step 1: Reductive Activation:
\[ \{[Fe_4S_4]^{2+} \text{ Ni}_{p}^{2+}\} + n e^- \leftrightarrow A_{\text{red-act}} \{?\} \]
\( S_{\text{system}} = 0 \) (or 1)

Step 2: Methylation:
\[ A_{\text{red-act}} \{?\} + \text{CH}_3^+ \leftrightarrow \{[Fe_4S_4]^{2+} \text{ Ni}_{p}^{2+} \text{-CH}_3\} \]
\( S_{\text{system}} = 0 \) (or 1)

Implications:
\( S_{\text{system}} \) for \( A_{\text{red-act}} = 0 \) (or 1, 2 etc)
and
\( n = 2 \)

Steps 3 and 4: CO Insertion, CoASH attack:
\[ \{[Fe_4S_4]^{2+} \text{ Ni}_{p}^{2+} \text{-CH}_3\} + \text{CO} + \text{CoASH} \leftrightarrow A_{\text{red-act}} \{?\} \]
Acetyl-CoA
The Heterogeneity Puzzle:
For all labs, enzymes, preps…

Spin concentration of NiFeC EPR signal 0.2 - 0.3 spin/α
Quantification of methyl group transfer: 0.3 – 0.5 Me/α
Quantification of labile Ni removed and inserted: ~ 0.2 Ni/α
Mössbauer of $A_{\text{red}}$-CO:
~30% is $S = \frac{1}{2} [\text{Fe}_4\text{S}_4]^{2+}$; ~70% is $S = 0 [\text{Fe}_4\text{S}_4]^{2+}$

What does this mean?

~ 30% functional α subunits
• Catalytically active
• Labile Ni
• capable of NiFeC EPR

~70% nonfunctional α subunits
* inactive
* no labile Ni (Zn? Cu?)
4.2 K Mössbauer Spectra of $\alpha$; ca. 2006

For $A_{\text{red-act}}$:
- $\sim 30\%$ $S = 0 \text{[Fe}_4\text{S}_4]\text{]^{2+}}$ (functional form)
- $\sim 70\%$ $S = 3/2 \text{[Fe}_4\text{S}_4]\text{]^{1+}}$ (nonfunctional form)

Heterogeneity present in apo-alpha form

Bramlett, Stubna, Tan, Surovtsev, Munck, Lindahl, Biochemistry 2006
Conclusions Regarding $A_{\text{red-act}}$

70% Component represents nonfunctional A-clusters
\{[$Fe_4S_4]^{1+} X\}
30% Component represents functional A-clusters
$A_{\text{red-act}} = S = 0, \{[Fe_4S_4]^{2+} Ni_p^0\}$

Objections to a $Ni_p^0$ State

Ligands are inconsistent (not phosphines)
But bridging thiolates might mimic phosphines…

Shouldn’t a zero-valent Ni reduce $[Fe_4S_4]^{2+}$?
But Mössbauer study suggests $E^0_{\text{cube2+/1+}} < -800 \text{ mV}$

Two DFT computational studies disfavor $\{[Fe_4S_4]^{2+} Ni_p^0\}$ state.
But $E^0$ is very sensitive to environment ∴ difficult to model accurately

Two redox titrations suggest $n = 1$ (not $n = 2$) for reductive activation

Previously reported $n = 1$ fit to Nernst Equation

Our refitting (2-term global optimization)
Best-Fit values:

$n = 1.7 \pm 0.2$

$E^0 = -479$ (pH 6.5)

$E^0 = -495$ (pH 7.2)

$E^0 = -524$ (pH 7.9)
Shouldn’t the Fe₄S₄ be reduced in A_{red-act}??

Let’s monitor reduction of [Fe₄S₄]²⁺ cubane by Ti³⁺ citrate…

Reduction is slow relative to methylation rate
(Nonfunctional form is probably becoming reduced)

Tan…Lindahl, JACS 2003
Competition Experiment:
(Add reductant and CH$_3$-Co$_3^+$FeSP Simultaneously to $\alpha_{ox}$)

Monitor at 390 nm (sensitive to both $[\text{Fe}_4\text{S}_4]^{2+}$ reduction and Co$^{1+}$)
CH$_3$ transfer (and reductive activation) > 100X faster than cubane reduction

Cubane NOT reduced fast enough to be the site of reductive activation
But heterogeneity could complicate interpretation…
A-cluster model complexes support \( n = 2 \), \( \text{Ni}_p^0 \)-based Mechanism

Ito, Kotera, Matsumoto, Tatsumi (PNAS, 2009)

\[
\begin{align*}
&[\text{Ni}_d^{2+} \text{Ni}_p^0] \\
&\text{A}_{\text{red-act}}
\end{align*}
\]

\[
\begin{align*}
&[\text{Ni}_d^{2+} \text{Ni}_p^{2+}-\text{CH}_3] \\
&\text{CH}_3\text{-A}_{\text{ox}}
\end{align*}
\]

Also… Riordan, Rauchfuss, Holm, Daresbourg, Mascharak

Structurally Relevant Model Complexes display same essential chemistry!
In 2007, we stopped adding Ni to E coli and started running α on FPLC (FPLC; Superdex gel filtration).

<table>
<thead>
<tr>
<th>Condition</th>
<th>Monomer</th>
<th>Dimer</th>
<th>Tetramer</th>
</tr>
</thead>
<tbody>
<tr>
<td>apo-α</td>
<td>88%</td>
<td>12%</td>
<td></td>
</tr>
<tr>
<td>apo-α + Ni</td>
<td>15%</td>
<td>38%</td>
<td>47%</td>
</tr>
<tr>
<td>Dimer peak; Concentrated, Re-run</td>
<td>48%</td>
<td>49%</td>
<td>3%</td>
</tr>
</tbody>
</table>

Dimer and tetramer have Activity; not monomer.

Dimer:
- 0.5 Me/α transferred
- 0.4 spin/α NiFeC spin intensity

Dimer is heterogeneous (one functional subunit; one nonfunctional subunit)

(Tan, Kagiampakis, Surovtsev, Demeler, Lindahl, Biochemistry 2007)
Properties of Ni-activated Alpha Subunit Dimers

Methyl Group Acceptor Ability:
Dimer accepts ~ 0.5 Me/α
Monomer ~ inactive

Why only 0.5 Me/α?
Incomplete Reaction?

Change [α] and see if Me/α changes
20 μM
10 μM
5 μM
2.5 μM
1.5 μM

~ 0.5 Me/α at all [α]

What does this mean?
Asymmetric Subunits in Ni-activated Dimer

EPR of reduced/CO dimer: NiFeC signal had 0.4 spin/α
(EPR of monomer apo-α showed only residual signal)

Mössbauer Spectra of reduced/CO dimer:
  40% associated with $S = ½ A_{\text{red}}$-CO state
  60% associated with $S = 0$ $[\text{Fe}_4\text{S}_4]^{2+}$ inactive clusters

When considered with methyl group transfer quantification, (0.5 Me/α)

Indicates that alpha dimers consist of **asymmetric subunits**
  * One “catalytic” subunit (accepts Me group; exhibits NiFeC)
  * One “structural” subunit

Could heterogeneity be functionally required??
Is Dimerization Specific for Ni?

- Ni$^{2+}$
- Cu$^{2+}$
- Zn$^{2+}$
- Co$^{2+}$
- Pd$^{2+}$
- Pt$^{2+}$

Higher-Mol. Wt Oligomer
Tentative Conclusions Regarding Dimerization

Alpha subunits dimerize when Ni binds to the proximal site of the A-cluster

Dimerization (Oligomerization) is specific for $M^{2+}$ ions that prefer square-planar geometries (Ni, Pd, Pd)

This geometry at the proximal site enforces a particular subunit conformation (i.e. open) that is conducive to dimerization

One subunit of the dimer is catalytic and in the open conformation while the other is structural and in the closed conformation
Apo-α + Ti(III) citrate ca. 2008

\[\sim 100\% \text{ is } S = 1/2 \ [\text{Fe}_4\text{S}_4]^{1+} \text{ cluster – no redox heterogeneity}\]
4.2 K Mössbauer of $[\alpha(\text{Ni})]_2 + \text{Ti(III)}$ Citrate

$20\% \text{ S } = \frac{1}{2} [\text{Fe}_4\text{S}_4]^{1+}$
$52\% \text{ S } = 0 \{[\text{Fe}_4\text{S}_4]^{1+} \text{ Ni}^{1+}\}$
$22\% \text{ S } = 0 \{\text{Fe}_4\text{S}_4]^{1+} \text{ Ni}^{1+}\}$

$\delta = 0.56 \text{ mm/s } \Delta E_Q = 1.25 \text{ mm/s}$
$\delta = 0.55 \text{ mm/s } \Delta E_Q = 0.47 \text{ mm/s}$

Coupled state proposed by Brunold and Field, based on DFT

4.5 K 0.05T Mössbauer of A-Cluster containing subunit from *Methanosarcina thermophila*

\[ \text{A}_{\text{ox state}} \]

\[ \sim 100\% \ S = 0 \ [\text{Fe}_4\text{S}_4]^{2+} \]
\[ (\delta = 0.46 \text{ mm/s}; \Delta E_Q = 1.1 \text{ mm/s}) \]

\[ \text{A}_{\text{red-act state}} \]

\[ \sim 80\% \ S = 0 \ \{[\text{Fe}_4\text{S}_4]^{2+}\text{Ni}_{p}^{2+/0}\} \]
\[ (\delta = 0.46 \text{ mm/s}; \Delta E_Q = 1.1 \text{ mm/s}) \]

\[ \sim 20\% \ S = 0 \ \{[\text{Fe}_4\text{S}_4]^{1+}\text{Ni}_{p}^{1+}\} \]
\[ (\delta = 0.53 \text{ mm/s}; \Delta E_Q = 1.1 \text{ mm/s}) \]

Sample was \( \sim 100\% \) active in accepting methyl group
Kinetic Model for the ACS Reaction:
Kinetic Modeling of the Acetyl-CoA Synthesis Mechanism

Tan, Surovtsev, Lindahl, JACS 2007
Monitoring the Kinetics of Methyl Group Transfer – Relatively Easy

\[ \text{Ni}^0 + \text{CH}_3\text{-Co}^{3+} \rightleftharpoons \text{Ni}^{2+}\text{-CH}_3 + \text{Co}^{1+} \]

\[ k_{+\text{met}} = 15 \ \mu\text{M}^{-1}\text{s}^{-1} \]
\[ k_{-\text{met}} < 0.05 \ \mu\text{M}^{-1}\text{s}^{-1} \]
\[ K_{\text{met}} > 300 \]
Monitoring the Thermodynamics of CO Insertion - *not so easy*

\[ \text{Ni}^{2+} \text{-C(O)CH}_3 + \text{Co}^{1+} \rightleftharpoons \text{No Reaction} \]

\[ \text{CO} + \text{Ni}^{2+} \text{-CH}_3 + \text{Co}^{1+} \rightleftharpoons \text{Ni}^{0} + \text{CH}_3\text{-Co}^{3+} \]

\[ K_{D(ins)} = 0.3 \, \mu\text{M} \]
Competition Reaction to Monitor Kinetics of CO Insertion

- More difficult

\[
\text{Ni}^{2+} \text{-CH}_3 + \text{Co}^{1+} \rightleftharpoons \text{Ni}^0 + \text{CH}_3 \text{-Co}^{3+} + \text{CO}
\]

\[ \text{Ni}^{2+} \text{-C(O)CH}_3 \]

When \( k_{\text{ins}} = 100 \ \mu\text{M}^{-1}\text{s}^{-1} \)

Not a good fit

\( k_{\text{ins}} = 0.1 \ \mu\text{M}^{-1}\text{s}^{-1} \)
Monitoring Acetyl Group Transfer - Difficult

\[
\text{Ni}^{2+} - \text{C}(O)\text{CH}_3 + \text{CoA} \rightleftharpoons \text{Ni}^0 + \text{CH}_3\text{C}(O)\text{-CoA} \\
+ \text{CH}_3\text{-Co}^{3+} \\
\downarrow \\
\text{Ni}^{2+} - \text{CH}_3 \\
+ \text{Co}^{1+} \text{(detect this)}
\]

Use methyl Transfer Rxn as a reporter

Incubate Ni\textsuperscript{2+}-CH\textsubscript{3} + CO
Works – but simulations must include CO inhibition

![Graph showing absorbance over time with different CoA and CO concentrations.](image)

Best-Fit Kinetic Parameters

- $k_{+CoA} = 4 \mu M^{-1}s^{-1}$
- $k_{-CoA} = 6 \mu M^{-1}s^{-1}$  \( K_{CoA} = 0.66 \)
- $k_{+CO} = 12 \mu M^{-1}s^{-1}$
- $k_{-CO} = 65 s^{-1}$  \( K_{CO} = 0.19 \mu M^{-1} \)
Predictions of Model…

\[ \begin{align*}
&\text{Ni}^0 + \text{CH}_3\text{-Co}^3\text{FeSP} \quad \rightleftharpoons \quad \text{Ni}^{2+}\text{-CH}_3 + \text{Co}^{1+}\text{FeSP} \quad K_{\text{met}} \\
&\text{Ni}^{2+}\text{-CH}_3 + \text{CO} \quad \rightleftharpoons \quad \text{Ni}^{2+}\text{-C(O)CH}_3 \quad K_{\text{ins}} \\
&\text{Ni}^{2+}\text{-C(O)CH}_3 + \text{CoA} \quad \rightleftharpoons \quad \text{CH}_3\text{C(O)-CoA} + \text{Ni}^0 \quad K_{\text{CoA}} \\
&\text{CO} + \text{CoA} + \text{CH}_3\text{-Co}^3\text{FeSP} \quad \rightleftharpoons \quad \text{CH}_3\text{-C(O)-CoA} + \text{Co}^{1+}\text{FeSP} \quad K_{\text{ACS}}
\end{align*} \]

\[ K_{\text{met}} \cdot K_{\text{ins}} \cdot K_{\text{CoA}} = K_{\text{ACS}} \]

\[ > 300 \cdot 3.3 \mu M^{-1} \cdot 0.66 = > 660 \mu M^{-1} \]

Actual lower limit \( K_{\text{ACS}} > 0.03 \mu M^{-1} \)
Simulated effect of CO shows inhibition effect similar to that observed experimentally.

Predicitions of the Model...

Simulation

Data
Simulation shows decay of inhibitory Ni:CO state reaching a non-zero steady-state upon reacting with CH₃-Co³⁺, CO and CoA as observed experimentally.

Nonzero steady-state said to “prove” intermediacy of Ni:CO state.
Predictions of the Model…

Distribution of Enzyme States During Steady-State Catalysis

Fixed conditions as commonly used
Calculated % in each intermediate state

Found:
- Ni\(^0\), 0.2%
- Ni\(^{2+}\)-CH\(_3\), 95%
- Ni\(^{2+}\)-C(O)CH\(_3\), 0.3%
- Ni:CO, 4.4%

According to these computations, CO Insertion is Rate Limiting Step

<table>
<thead>
<tr>
<th>Rate Coefficient (k)</th>
<th>(\Delta v_0 / v_0) (\Delta k / k)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(k_{+\text{met}})</td>
<td>0.339</td>
</tr>
<tr>
<td>(k_{-\text{met}})</td>
<td>-0.002</td>
</tr>
<tr>
<td>(k_{+\text{ins}})</td>
<td>0.657</td>
</tr>
<tr>
<td>(k_{-\text{ins}})</td>
<td>-0.0004</td>
</tr>
<tr>
<td>(k_{+\text{CoA}})</td>
<td>0.002</td>
</tr>
<tr>
<td>(k_{-\text{CoA}})</td>
<td>0.001</td>
</tr>
<tr>
<td>(k_{+\text{CO}})</td>
<td>-0.332</td>
</tr>
<tr>
<td>(k_{-\text{CO}})</td>
<td>0.317</td>
</tr>
</tbody>
</table>
Acetyl-CoA Synthesis Mechanism (*circa* May 2006)
Conclusions

ACS/CODH catalyzes the synthesis of Acetyl-CoA from CO, CoA and a methyl group donated by a corrin protein.

Active site A-cluster is a novel \([\text{Fe}_4\text{S}_4]\)-Ni\(_p\) Ni\(_d\) cluster.

- \(A_{\text{ox}}\): \([\text{Fe}_4\text{S}_4]^{2+} \text{Ni}_p^{2+} \text{Ni}_d^{2+}\)
- \(A_{\text{red-act}}\): \([\text{Fe}_4\text{S}_4]^{2+} \text{Ni}_p^{0} \text{Ni}_d^{2+}\) or \([\text{Fe}_4\text{S}_4]^{1+} \text{Ni}_p^{0} \text{Ni}_d^{2+}\)
- \(\text{CH}_3-A_{\text{ox}}\): \([\text{Fe}_4\text{S}_4]^{2+} \text{Ni}_p^{2+}-\text{CH}_3 \text{Ni}_d^{2+}\)

Heterogeneity and batch-to-batch variations cause confusion. Ni-dependent oligomerization.

Chemical kinetic study:
- reductive activation
- Methyl group transfer
- CO insertion (slow step, probably involves protein conf. change)
- CoA attack, forming product
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