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Effect of Zn on Acetyl Coenzyme A Synthase: Evidence for a Conformational Change in the α Subunit during Catalysis

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Acetyl coenzyme A synthase/carbon monoxide dehydrogenase (ACS/CODH ≡ ACS) is a bifunctional enzyme found in anaerobic archaea and bacteria that grow chemo-autotrophically on simple inorganic compounds such as CO_2/H_2.[1] The enzyme from *Moorella thermoacetica* is a 310 kDa $\alpha_2\beta_2$ tetramer in which the α subunits contain the active-site *A-cluster* and are used to synthesize acetyl-CoA from CO, CoA, and a methyl group donated by a corrinoid-iron−sulfur protein (CoFeSP). *β* subunits contain clusters required for CO/CO_2 redox catalysis. The A-cluster is composed of an Fe_4S_4 cubane bridged by a cysteine thiolate to a binuclear site.[2] The metal of the binuclear site distal to the cubane is a square-planar Ni coordinated to two bridging thiolates and two amide nitrogens from the protein backbone. The proximal metal of the binuclear site can be Ni, Cu, and Zn. The α subunits can be stabilized in *open* and *closed* conformations. Reported structures contain Ni in the proximal site (Ni_p) when the conformation is open, and Cu and Zn when closed.[2] Ni_p is selectively removed by 1,10-phenanthroline (phen).[3] Consistent with this lability, Ni_p is surface-exposed, as probably required for methyl group transfer from CoFeSP; in contrast, the Cu and Zn in the closed conformation are buried. Consensus is emerging that the Ni_p form is active, while Cu is inhibitory.[4,5] Occupancy by Zn is negatively correlated to activity,[6] but more direct evidence for this is required. Also uncertain is the role, if any, of the α-subunit conformations during catalysis. In this communication, we provide evidence that Zn inhibits catalysis, methyl group transfer, and NiFeC signal development when it replaces Ni_p. Importantly, this replacement occurs only when the enzyme functions catalytically, suggesting that α undergoes a conformational change during catalysis and that Zn replaces Ni_p only when the conformation is open.

Native ACS[7] was examined for activity as a control (Figure 1, ◆). As expected,[3,4] phen-treatment completely inactivated the enzyme, and ∼70% of the original activity was recovered upon subsequent treatment with $NiCl_2$. No activity developed upon incubating phen-treated ACS in 10 equiv/α of $ZnSO_4$ (Figure 1, ●), nor did activity develop after subsequently treating with 10 equiv/α of $NiCl_2$ (Figure 1, *). These results suggest that: (a) a Zn ion bound to the proximal site of phen-treated ACS, (b) the Zn-bound form was inactive, and (c) Ni could not displace Zn once bound at the proximal site. Similar results were observed using Cu,[4] but the effect of Zn appears restricted to ACS activity, as it had no effect on CO oxidation activity (Table 1). In contrast, Cu inhibited both CODH and ACS activities.

Binding Zn at the proximal site prevented methyl group transfer from CoFeSP and abolished the NiFeC EPR signal. Using stopped-flow, Ti^{3+}citrate-reduced ACS (Figure 2A) accepted a methyl group in accordance with a second-order rate constant of 11 $\mu M^{-1}\ s^{-1}$, similar to previous reports.[6,8] A phen-treated sample was unable to accept a methyl group before or after Zn was added to it (Figure 2C).

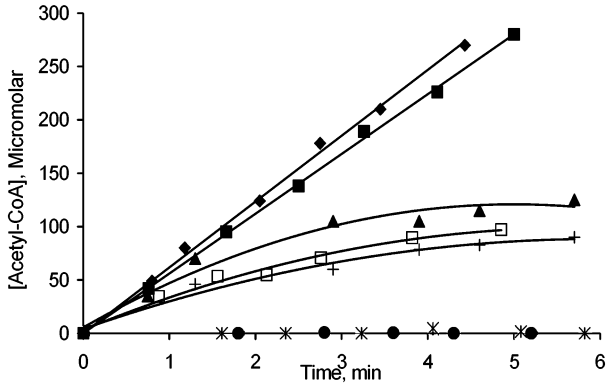

Figure 1. Acetyl-CoA synthase activity (performed as described[9] using CH_3-THF, CoA, CoFeSP, CO_2 in 50 mM MES buffer pH 6.3, 25 °C). (◆), native ACS (0.3 μM, final); (■), ACS treated with 10 equiv/α of $ZnSO_4$, passed through Sephadex G25 and assayed in a solution containing 100 μM EDTA; (▲), ACS treated with 10 equiv/α of $ZnSO_4$ and passed through Sephadex G25; (□), ACS treated with 10 equiv/α $ZnSO_4$; (+), ACS treated with 100 equiv/α of $ZnSO_4$; (●), ACS treated with phen, passed through G25, and then treated with 10 equiv/α of $ZnSO_4$; (*), ACS treated with phen, passed through G25, treated with 10 equiv/α of $ZnSO_4$, and then 10 equiv/α of $NiCl_2$.

Table 1. Summary of CO Oxidation Activity, Methyl Group Transfer, and ACS Activity

sample	CODH ($\mu M\ min^{-1}\ mg^{-1}$)	methyl transfer (s^{-1})	ACS (min^{-1})
ACS	484	26	203
ACS + Zn (with EDTA)	397	25	183
ACS + phen	383	0[a]	0[a]
ACS + phen + Zn	392	0	0
ACS + phen + Ni	351	16[a]	120[a]
ACS + phen + Cu	13	0	0
ACS + phen + Zn + Ni	326	0	0

[a] Data from previous work.[4]

Subsequent addition of Ni to this sample had no effect (Figure 2D). EPR spectra of CO-treated ACS exhibited the NiFeC signal (Figure 3, I) while phen-treated ACS that was subsequently incubated in Zn (and then in CO) did not exhibit this signal (Figure 3, III). When another sample treated similarly with phen and Zn was subsequently incubated in Ni (and then CO), no NiFeC signal developed (Figure 3, IV). These experiments indicate that the Zn form of the A-cluster cannot be reduced and bound with CO as is required for the development of this signal. They also indicate that the Zn form cannot be methylated; like Cu^{1+}, the Zn^{2+} ion appears insufficiently nucleophilic to accept a methyl group from CoFeSP.

To determine whether added Zn could replace Ni_p, a sample of ACS was incubated for 3 h in 100 equiv/α of $ZnSO_4$ and then used in the synthesis of acetyl-CoA. Surprisingly, during the first minute of reaction, the catalytic rate of the Zn-treated ACS sample

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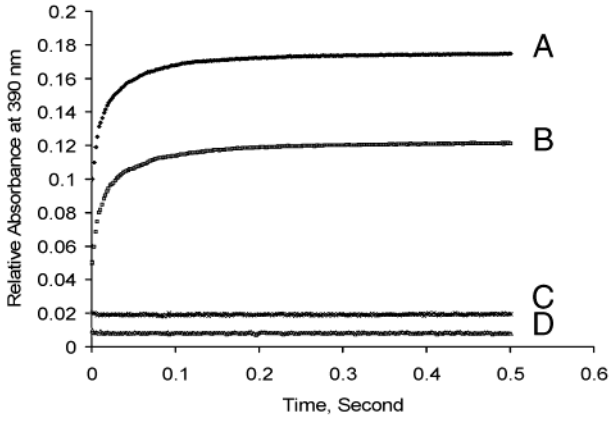

Figure 2. Methyl group transfer from CH_3-CoFeSP to ACS prepared in different states monitored at 390 nm, 25 °C, as described.[8] (A) ACS (10 μM final concentration, in 50 mM Tris buffer pH 8.0) was preincubated with Ti^{3+}-citrate (1 mM, final) for 20 min, then mixed with CH_3-CoFeSP (5 μM, final) that had been similarly preincubated; (B) same as A, except that ACS was additionally preincubated with 10 equiv/α of $ZnSO_4$; (C) same as A, except that ACS was treated with 10 equiv/α of phen, passed through G25, and then incubated in 10 equiv/α of $ZnSO_4$; (D) same as C, except that the phen-G25-Zn-treated ACS sample was subsequently incubated in 10 equiv/α of $NiCl_2$.

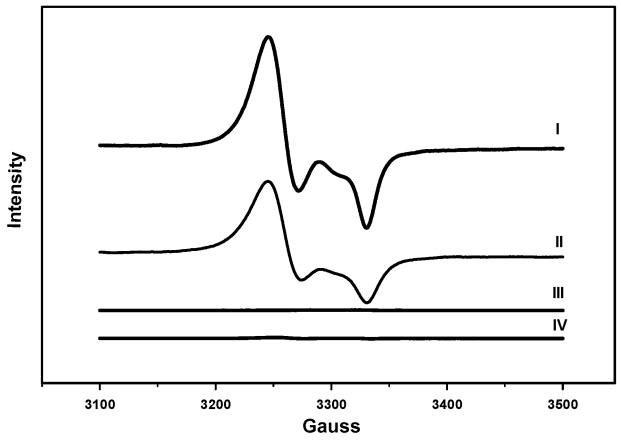

Figure 3. X-band EPR of ACS at 130 K (essentially performed as described[3]). (I) ACS (0.27 spin/αβ); (II) ACS plus 10 equiv/α of $ZnSO_4$ (0.25 spin/αβ); (III) ACS incubated in 10 equiv/α of phen, passed through Sephadex G25, and treated with 10 equiv/α of $ZnSO_4$; (IV) ACS as in III but subsequently treated with 10 equiv/α of $NiCl_2$.

was similar to that of native ACS (Figure 1, +). Within ~5 min, activity declined and approached zero activity. A similar experiment using 10 equiv/α of Zn afforded similar results (Figure 1, □). Also unexpected was that a Zn-treated ACS sample accepted a methyl group from CoFeSP at the same rate and overall intensity as native enzyme (Figure 2B). Consistent with this, EPR of an equivalent Zn-treated ACS sample exhibited the NiFeC signal (Figure 3 II) with spin intensity comparable to that of native ACS. Given these differences relative to the effects of Cu, we suspect that the strength of binding to the proximal site is ordered Cu > Zn > Ni.

To better understand the effects of Zn, a sample of ACS was treated for 5 h in 10 equiv/α of $ZnSO_4$ and then passed through a Sephadex G25 column to remove excess Zn. This Zn-treated-and-excess-Zn-removed sample exhibited stronger activity than that which had been treated with Zn only, but the overall behavior was similar (Figure 1, ▲). A similarly treated sample assayed for catalysis in the presence of EDTA exhibited activity (Figure 1, ■) corresponding to 90% of that from native ACS.

We interpret these results as follows. Simply incubating Zn in ACS for an extended period is NOT sufficient to replace Ni_p. Rather, Zn replaces Ni_p only after the enzyme engages in catalysis; under the conditions used, this replacement reaction occurred with $k_{app} \approx 0.6$ min^{-1}. Once Zn replaces Ni_p the enzyme irreversibly inactivates. The rate of Zn attack is too slow to quantitatively replace Ni_p during the period required for methyl group transfer; thus, incubation in Zn had no noticeable effect on methyl group transfer rate, but it slowly attenuated the rate of acetyl-CoA synthesis. Treatment with Sephadex G25 removes some but not all Zn; thus, EDTA functions to chelate excess Zn and prevent its attack on Ni_p.

We have attempted to understand the conditions required for Zn to replace Ni_p. Incubating Zn-treated ACS with CO had no effect on NiFeC signal intensity (Figure 3, II). Samples of Zn-treated ACS were separately incubated with reduced methyl viologen, CoFeSP, and CoA for 2 h prior to CO treatment and EPR analysis. In all cases, the NiFeC signal was observed, with intensities comparable to that of native ACS. A sample of ACS treated with all substrates required for catalysis (with CH_3-THF limiting) was incubated 3 h and then analyzed by EPR spectroscopy. The NiFeC signal was observed in this positive control. However, when Zn was included in a similar assay mixture, no such signal was observed.

What phenomenon would allow Zn to effectively remove Ni_p when the enzyme is engaged in catalysis but not when it is disengaged? Zn appears to replace Ni_p when the enzyme is in one or more intermediate states of catalysis but this(these) state(s) must not be present when ACS is reduced in CO alone or in the presence of CoA, CoFeSP, or MV^{1+}. The most reasonable conclusion is that Ni_p is susceptible to Zn-attack in the open conformation and protected from attack in the closed conformation and that the conformation of the α subunit changes during catalysis. However, more than just the conformational change observed by X-ray might be required for metal exchange (e.g. reduction of Ni_p might also be required). Nevertheless, this is the first evidence that the structurally characterized conformations of the α subunit serve a mechanistic role in ACS catalysis.

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