J|A|C|S
COMMUNICATIONS

Published on Web 03/19/2003

Thiolate-Bridged Nickel−Copper Complexes: A Binuclear Model for the Catalytic Site of Acetyl Coenzyme A Synthase?

Rangan Krishnan, Janis K. Voo, Charles G. Riordan,* Lev Zahkarov, and Arnold L. Rheingold

Department of Chemistry and Biochemistry, University of Delaware, Newark, Delaware 19716

Received February 13, 2003; E-mail: riordan@udel.edu

Acetyl coenzyme A synthase/CO dehydrogenase (ACS/COdH) is a bifunctional enzyme found in acetogenic, methanogenic, and sulfate-reducing archaea and bacteria which enables the organisms to grow autotrophically on CO_2 and H_2.[1−3] The ACS catalyzed reaction entails the reversible assembly of acetyl CoA via the coupling of a methyl substituent from enzyme-bound methylcobamide, CO, and the thiolate, CoA.[4] The methyl group and CO fixed during the synthesis of the thioester are derived from CO_2 reduction, the latter at a different active site in the protein, the C cluster. A molecular tunnel in the protein permits for shuttling of CO from the C cluster to the A cluster, the site of acetyl CoA synthesis.[5,6] The enzyme from the acetogen, *Moorella thermoacetica*, has been examined extensively by enzymatic, biophysical, and, most recently, crystallographic analysis.[7] The last experiments identified copper as an integral constituent of the A cluster previously identified as a Ni−Fe_4S_4 cluster.[8] The remarkable structure consists of a cuboidal Fe_4S_4 unit linked via a cysteine to a binuclear NiCu subcluster, Figure 1. In this respect, the active site bears resemblance to the H cluster of the Fe-only hydrogenase, which contains an Fe_4S_4 linked via a cysteine residue to an Fe_2 subcluster.[9] Further, amide backbone coordination at Ni was unanticipated, although the N_2S_2 environment was predicted by EXAFS spectroscopy.[10] Our continued interest in functional models for ACS[11,12] prompted the investigation of thiolate-bridged NiCu complexes as a new direction for understanding this fascinating enzyme. Herein we report the first preparation of thiolate-bridged nickel−copper binuclear complexes. Preliminary reactivity studies confirm that the binuclear Ni(II)/Cu(I) complexes bind CO reversibly, generating a Cu(CO) adduct resulting from rupture of the thiolate bridges.

Given the utility of metal complexes with *cis*-dithiolates to serve as robust "metalloligands",[13−15] it is surprising that only a single report of a thiolate-bridged NiCu(I) species preceded this study.[16] Reactions of the two $[N_2S_2]$Ni species[15,17] in Scheme 1 with $[Cu(CH_3CN)_4]BF_4$ provided insight into the challenges in preparing such species. The products are polynuclear complexes in which two copper ions are bridged by $[N_2S_2]$Ni "ligands". The structures were authenticated by diffraction methods with details contained in the Supporting Information. A key feature of the structures is that the adjacent thiolate donors are coordinated to different Cu ions, rather than serving as a chelate to a single metal as observed for $[N_2S_2]$Ni ligated to Ni,[18] Fe,[14,15] and Cu(II).[19] The different stoichiometry of **1** versus **2** results in disparate reactivity, vide infra, and may be attributed to the greater steric demands of the *gem*-dimethyl substituents adjacent to the bridging sulfurs in **2**.

Changing the source of Cu(I) to the less labile $[PhTt^{tBu}]Cu(CH_3CN)$[20] resulted in isolation of discrete binuclear complexes, **3** and **4**, Scheme 1. The molecular structure of **3** determined by diffraction methods is displayed in Figure 2. The N_2S_2 ligation at Ni is unchanged as a consequence of coordination to the $[PhTt^{tBu}]Cu$ moiety. The Cu(I) is ligated in a roughly T_d array of four sulfur

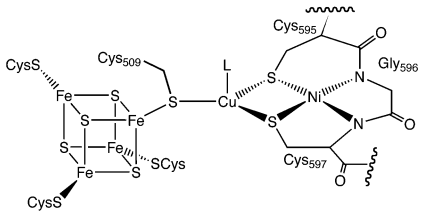
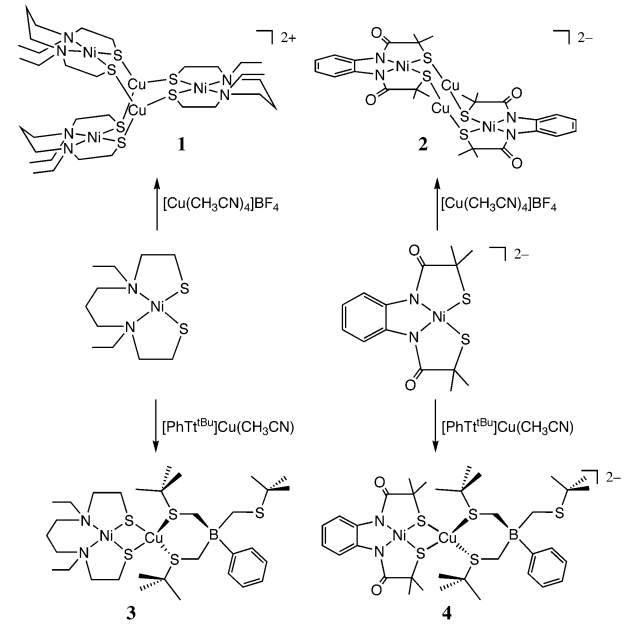


Figure 1. Active site of acetyl coenzyme A synthase (A cluster). L is an unknown, nonproteinaceous ligand.[7]

Scheme 1



ligands, two thiolate and two thioether donors, the latter a consequence of the κ^2-coordination of the borato ligand. There is one rather long Cu(I)−S(thiolate) bond distance at 2.515(1) Å, while the remaining three Cu−S distances are in the range observed for

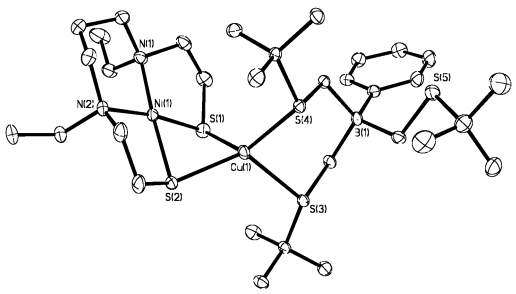


Figure 2. Thermal ellipsoid representation of **3**. Selected distances (Å) and angles (°), Cu(1)−S(1), 2.515(1); Cu(1)−S(2), 2.264(1); Cu(1)−S(3), 2.367(1); Cu(1)−S(4), 2.274(1); Ni(1)−Cu(1), 2.9173(8); S(1)−Ni(1)−S(2), 84.62(5); S(1)−Cu(1)−S(2), 75.66(4); S(3)−Cu(1)−S(4), 88.87(4).

10.1021/ja0346577 CCC: $25.00

other Cu(I) complexes, including the average Cu−S length in **1** of 2.284(1) Å. A similar asymmetry in the bridging cysteinates is apparent in the A cluster structure.[21] The Cu- - -Ni distance of 2.917-(1) Å is only slightly longer than that determined for the A cluster, 2.79 Å. Proton NMR spectral data indicate that the κ^2-[PhTt$^{t\mathrm{Bu}}$] coordination in **3** and **4** is maintained in solution as evidenced by distinct signals for the ligated and free thioether substituents. The cyclic voltammograms of **3** and **4** display processes assigned to nickel-based redox events and oxidation of the [PhTt$^{t\mathrm{Bu}}$] ligand as deduced by comparison with the starting reagents. Coordination of the [PhTt$^{t\mathrm{Bu}}$]Cu moiety has only a modest effect on the [N_2S_2]-Ni redox potentials, resulting in anodic shifts due to metalation of the thiolates. No copper-based oxidation is available in these complexes with sulfur-only coordination in accord with the finding that N_2O-treated ACS does not exhibit a Cu(II) ESR signal.[8]

An intriguing, yet unresolved, aspect of the enzymology of ACS concerns the nature of the intermediate resulting from reductive carbonylation of the A cluster. The so-called NiFeC state (alternatively, A_{red}−CO) results upon exposure of the isolated protein with CO and exhibits ESR line-broadening upon isotopic perturbation with ^{57}Fe, ^{61}Ni, or ^{13}CO.[22] This intermediate is characterized further by a ν_{CO} band (1995 cm^{-1}) consistent with a terminal M−CO.[23] In light of the recent structural evidence for Cu in the A cluster, a Cu(I)−CO adduct has been proposed as an intermediate on the pathway of acetate formation.[7,8] To pursue this possibility by establishing relevant small molecule transformations, solutions of **1**−**4** were exposed to an atmosphere of CO. While **1** is unreactive under these conditions, the other complexes are sensitive to CO, yielding adducts characterized by terminal ν_{CO} modes, 2042 cm^{-1} (for **2**) and 2078 cm^{-1} (**3** and **4**), that exhibited the expected shifts following incubation with ^{13}CO ($\nu_{^{12}CO}/\nu_{^{13}CO}$, 1.023; calcd, 1.023). Spectral analysis confirmed that the carbonylation of **3** and **4** results in rupture of the thiolate bridges generating the respective mononuclear species, [N_2S_2]Ni and [PhTt$^{t\mathrm{Bu}}$]Cu(CO), the latter also available via the carbonylation of [PhTt$^{t\mathrm{Bu}}$]Cu(CH_3CN) under comparable conditions. Interestingly, the carbonylations of **3** and **4** are reversible as purging solutions with N_2 results in the disappearance of the 2078 cm^{-1} IR band that reappears upon subsequent cycling with CO. At 25 °C and 1 atm of CO, **3** is fully carbonylated, while **4** shows only partial product formation.

As the identification of Cu within the A cluster was unanticipated, its role in ACS catalysis remains to be elucidated. The ligation environment of the copper site is consistent with the Cu(I) oxidation level as is the postulated CO binding.[7,8] However, the isotope sensitivity of the NiFeC ESR signal is difficult to interpret in the context of the X-ray structure. The direct (or through bond) distance between Ni and the Fe_4S_4 cluster appears too great to account for the spin delocalization required to account for the hyperfine line-broadening attributed to both ^{57}Fe and ^{61}Ni, although this clearly warrants a detailed spectroscopic and computational investigation. Furthermore, the structure does not appear to account for the observation that phen removes some of the Ni in the protein ("labile Ni").[24] Alternatively, it is possible the native enzyme functions with Ni in the "Cu site" as has been suggested for the methanogenic protein.[25] Specifically, the A cluster may be of Ni_2-[Fe_4S_4] composition with the trigonal Cys_3 metal binding site coordinating Ni under native conditions. Such a low coordinate site should be susceptible to demetalation by phen, accounting for the Ni lability, whereas the diamidodithiolato [N_2S_2]Ni appears much less accessible to chelating agents. Further, the former site possesses the coordination flexibility to facilitate acetyl CoA dis/assembly. To explore this hypothesis, we are preparing binuclear Ni_2 complexes akin to **3** and **4** in an attempt to elicit relevant reactivity to understand the fundamental chemistry of this novel binuclear system.[11]

Acknowledgment. We thank the National Institutes of Health for financial support.

Note Added in Proof. Lindahl and Fontecilla-Camps have just reported an X-ray structure of the COdH/ACS from *M. thermoacetica* that shows two different conformations of the α subunit, each contains an A cluster of distinct metal composition: Ni_2-[Fe_4S_4] and NiZn-[Fe_4S_4]. The authors propose that only the former cluster is active; Darnault, C.; Volbeda, A.; Kim, E. J.; Legrand, P.; Vernede, X.; Lindahl, P. A.; Fontecilla-Camps, J. C. *Nat. Struct. Biol.*, published on March 10, 2003.

Supporting Information Available: Synthetic procedures and characterization data (PDF); crystallographic information for compounds **1**, **2**, and **3** (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Ferry, J. G. *Crit. Rev. Biochem. Mol. Biol.* **1992**, *27*, 473−503.
(2) Ragsdale, S. W.; Kumar, M. *Chem. Rev.* **1996**, *96*, 2515−2540.
(3) Lindahl, P. A. *Biochemistry* **2002**, *41*, 2097−2105.
(4) Ragsdale, S. W.; Riordan, C. G. *J. Biol. Inorg. Chem.* **1996**, *1*, 489−493.
(5) Maynard, E. L.; Lindahl, P. A. *J. Am. Chem. Soc.* **1999**, *121*, 9221−9222.
(6) Seravalli, J.; Ragsdale, S. W. *Biochemistry* **2000**, *39*, 1274−1277.
(7) Doukov, T. I.; Iverson, T. M.; Seravalli, J.; Ragsdale, S. W.; Drennan, C. L. *Science* **2002**, *298*, 567−572.
(8) Seravalli, J.; Gu, W.; Tam, A.; Strauss, E.; Begley, T. P.; Cramer, S. P.; Ragsdale, S. W. *Proc. Natl. Acad. Sci. U.S.A.*, published on Feb. 14, 2003.
(9) Peters, J. W.; Lanzilotta, W. N.; Lemon, B. J.; Seefeldt, L. C. *Science* **1998**, *282*, 1853−1858.
(10) Russell, W. K.; Stålhandske, C. M. V.; Xia, J. Q.; Scott, R. A.; Lindahl, P. A. *J. Am. Chem. Soc.* **1998**, *120*, 7502−7510.
(11) Ram, M. S.; Riordan, C. G.; Yap, G. P. A.; Liable-Sands, L.; Rheingold, A. L.; Marchaj, A.; Norton, J. R. *J. Am. Chem. Soc.* **1997**, *119*, 1648−1655.
(12) Craft, J. L.; Mandimutsira, B. S.; Fujita, K.; Riordan, C. G.; Brunold, T. C. *Inorg. Chem.* **2003**, *42*, 859−867.
(13) Lai, C.-H.; Reibenspies, J. H.; Darensbourg, M. Y. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2390−2393.
(14) Colpas, G. J.; Day, R. O.; Maroney, M. J. *Inorg. Chem.* **1992**, *31*, 5053−5055.
(15) Osterloh, F.; Saak, W.; Pohl, S. *J. Am. Chem. Soc.* **1997**, *119*, 5648−5656.
(16) Jicha, D. C.; Busch, D. H. *Inorg. Chem.* **1962**, *1*, 878−883.
(17) Kruger, H. J.; Peng, G.; Holm, R. H. *Inorg. Chem.* **1991**, *30*, 734−742.
(18) Farmer, P. J.; Solouki, T.; Mills, D. K.; Soma, T.; Russell, D. H.; Reibenspies, J. H.; Darensbourg, M. Y. *J. Am. Chem. Soc.* **1992**, *114*, 4601−4605.
(19) Kaur, P. *Indian J. Chem., Sect. A* **1997**, *36*, 605−607.
(20) Ohrenberg, C.; Riordan, C. G.; Liable-Sands, L. M.; Rheingold, A. L. *Inorg. Chem.* **2001**, *40*, 4276−4283.
(21) Protein Data Bank code 1MJG; there are four A clusters in the asymmetric unit, two per $\alpha_2\beta_2$ heterotetramer. Consequently, the values reported are the distances averaged over the four unique sites.
(22) Gorst, C. M.; Ragsdale, S. W. *J. Biol. Chem.* **1991**, *266*, 20687−20693.
(23) Kumar, M.; Ragsdale, S. W. *J. Am. Chem. Soc.* **1992**, *114*, 8713−8715.
(24) Shin, W.; Lindahl, P. A. *J. Am. Chem. Soc.* **1992**, *114*, 9718−9719.
(25) Gencic, S.; Grahame, D. A. *J. Biol. Chem.* **2003**, *278*, 6101−6110.

JA0346577