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Structural Models of the Bimetallic Subunit at the A-Cluster of Acetyl Coenzyme A Synthase/CO Dehydrogenase: Binuclear Sulfur-Bridged Ni−Cu and Ni−Ni Complexes and Their Reactions with CO

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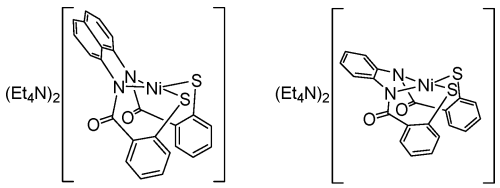
The bacterial metalloenzyme acetyl coenzyme A synthase/CO dehydrogenase (ACS/CODH) catalyzes two very important biological processes, namely the reduction of atmospheric CO_2 to CO and the synthesis of acetyl coenzyme A from CO, CH_3 from a methylated corrinoid iron−sulfur protein, and the thiol coenzyme A.[1] The structure of the A-cluster, where ACS synthesis occurs, has recently been determined by crystallography.[2,3] Two separate A-cluster structures of ACS/CODH from the bacterium *Moorella thermoacetica* reveal a multimetallic active site containing a cuboidal Fe_4S_4 unit bridged to a bimetallic subunit through one Cys-S moiety. The bimetallic site contains a square-planar $Ni(II)_d$ site (distal to the cubane) coordinated to two deprotonated carboxamido nitrogens from the peptide backbone and two cysteine sulfurs. The other metal center, designated as the proximal metal (M_p), contains either a Cu,[2] Ni,[3] or Zn[3] ion and is bridged to the Ni_d site through two Cys-S residues and to the Fe_4S_4 unit through one other Cys-S, affording an $M_p(Cys\text{-}S)_3$ coordination sphere. In addition to the three bridging Cys-S moieties, a fourth, still unidentified, ligand is also bound to complete the coordination sphere. An intense debate over the roles of three different metal ions has recently been subsided following careful biochemical studies[4] and publication of a third A-cluster structure from the hydrogenogenic bacterium *Carboxydothermus hydrogenoformans*.[5] It is now evident that Ni occupies the M_p site in the catalytically active enzyme; both Cu and Zn found in the crystal had their origin in the promiscuity of adventitious metal ion capture by the Ni_d site.[4]

The quest for insight into the mechanism of acetyl coenzyme A synthesis on the basis of the crystal structures has prompted us[6] and others[7] to synthesize structural analogues of the active site. In these attempts, the Fe_4S_4 unit has often been ignored (unlikely substrate binding site) and the focus has been primarily on the M_p-Ni_d bimetallic site. In this Communication, we report the results of our modeling attempt(s) that utilizes the Ni(II)−dicarboxamido-dithiolato complexes $(Et_4N)_2$[Ni(NpPepS)] (**1**)[6b] and $(Et_4N)_2$-[Ni(PhPepS)] (**2**) as Ni_d synthons in the construction of Ni−Cu and Ni−Ni dinuclear analogues (Figure 1).

Treatment of an MeCN solution of **1** with 1 equiv of [Cu(neo)-Cl] (neo = 2,9-dimethyl-1,10-phenanthroline) afforded the brown dinuclear Ni−Cu complex (Et_4N)[Cu(neo)Ni(NpPepS)] (**3**) (Figure 2) in 70% yield. Crystallographic analysis of **3** reveals a square planar Ni(II) ion bridged to a distorted tetrahedral Cu(I) center via the thiolato-S donors of **1**. The average Cu−S and Ni−S distances of **3** (2.2934 and 2.1950 Å, respectively) compare well with those observed in the Cu form of ACS/CODH.[2] The Cu−Ni distance of **3** (3.0740 Å) is somewhat longer than that of the enzyme (2.792 Å). Steric constraints imposed by the $NpPepS^{4-}$ ligand frame are presumably responsible for the longer metal−metal distance.



(1) (2)

Figure 1. Ni(II) synthons (Ni_d mimics) used in this study.

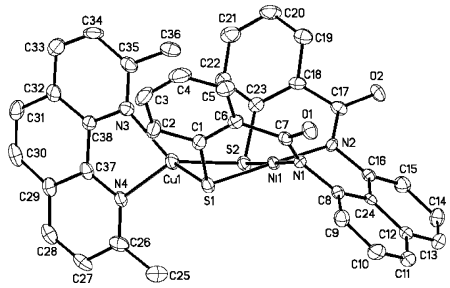


Figure 2. ORTEP diagram of the anion of (Et_4N)[Cu(neo)Ni(NpPepS)] (**3**) (50% probability) with the atom-labeling scheme. H atoms are omitted for the sake of clarity. Selected bond distances (Å) and bond angles (°): Cu−N(3) 2.037(5), Cu−S(1) 2.2670(16), Ni−N(1) 1.889(5), Ni−S(1) 2.1876(15), Ni−Cu 3.0740(11), N(3)−Cu−N(4) 82.66(18), N(3)−Cu−S(1) 129.79(14), N(4)−Cu−S(2) 115.91, S(1)−Cu−S(2) 87.46(6), N(1)−Ni−N(2) 88.75(19), N(2)−Ni−S(1) 177.80(15), S(1)−Ni−S(2) 92.49(6), C(1)−S(1)−Ni 101.17(18).

Interestingly, the cathodic cyclic voltammogram of **3** in DMF does not exhibit any reduction wave up to −1.8 V (vs SCE). It is therefore evident that the Ni(II) center of **3** (Ni_d mimic) is very resistant to reduction. The metal centers of **3** show no affinity toward CO,[7c] and passage of CO through solutions of **3** results in no breakdown of the complex. This latter behavior of **3** contrasts that of similar Ni(II)−Cu(I) sulfur-bridged complexes.[7b]

The reaction of $[Ni(terpy)Cl_2]$ (terpy = 2,2′:6′,2″-terpyridine) and **1** (1:1 ratio) in MeCN resulted in the isolation of neutral [Ni(terpy)Ni(NpPepS)] (**4**) as a red-brown powder (75%). Since the A-cluster exhibits an EPR spectrum (NiFeC signal) upon one-electron reduction and CO binding (A_{red}-CO), we explored the possibility of reduction and CO binding with the Ni−Ni dimer **4**. Upon reduction of **4** to $\mathbf{4_{red}}$ with $Na_2S_2O_4$, an axial EPR spectrum (100 K, DMF glass) is observed ($g = 2.226, 2.125$). This spectrum is characteristic of five-coordinate Ni(I) in a trigonal bipyramidal geometry.[8] Reaction of CO with $\mathbf{4_{red}}$ in DMF results in $\mathbf{4_{red}}$**-CO** adduct with a terminal Ni(I)−CO band at 2044 cm^{-1}. In DMF glass, $\mathbf{4_{red}}$**-CO** displays a rhombic EPR spectrum with $g = 2.223, 2.128, 2.019$ (Figure S1, Supporting Information), typical of similar six-coordinate Ni(I) compounds with terminally bound CO molecule.[8] Since **4** does not exhibit any affinity toward CO, it is evident that the Ni_p mimic in this model only binds CO in the reduced (+1)

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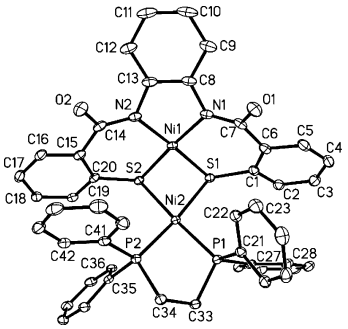

Figure 3. ORTEP diagram of [Ni(dppe)Ni(PhPepS)] (**6**) (50% probability) with the atom-labeling scheme. H atoms are omitted for the sake of clarity. Selected bond lengths (Å) and bond angles (°): Ni1−Ni2 2.8255(4), Ni1−N1 1.8909(17), Ni1−N2 1.8895(17), Ni1−S1 2.1558(5), Ni1−S2 2.1438(5), Ni2−S1 2.2354(6), Ni2−S2 2.2413(5), Ni2−P1 2.1781(6), Ni2−P2 2.1816(6), S2−Ni1−S1 79.29(2), P1−Ni2−P2 86.18(2), Ni1−S1−Ni2 80.077(19), C1−S1−Ni 111.15(17).

state. The Ni_d mimic **1** shows no affinity toward CO under similar reducing conditions, and no EPR signal is observed.

Since the Ni_p site in ACS is four-coordinate in the resting state, we attempted synthesis of appropriate Ni−Ni models with a second Ni_p synthon, $[Ni(dppe)Cl_2]$ (dppe = 1,2-bis(diphenylphosphino)-ethane). This Ni_p synthon is expected to favor an easier reduction to the Ni(I) oxidation state and further facilitate binding of CO. However, due to the geometric constraints imposed by the ligand frame in **1**, all attempts to synthesize the Ni−Ni complex with $[Ni(dppe)Cl_2]$ eventually led to the formation of trimeric $(Et_4N)_2$-$[Ni(DMF)_2\{Ni(NpPepS)\}_2]$ (**5**, Scheme S1, Supporting Information). We therefore synthesized the second Ni_d synthon $[Ni(PhPepS)]^{2-}$ (anion of **2**, structure shown in Figure S2, Supporting Information), employing a ligand with less steric demands. Reaction of **2** with 1 equiv of $[Ni(dppe)Cl_2]$ in MeCN afforded [Ni(dppe)Ni(PhPepS)] (**6**, Figure 3) as a blue-green crystalline solid in 85% yield. In this Ni−Ni model, the two Ni(II) centers are bridged through both thiolato-S donors of the $PhPepS^{4-}$ ligand frame, and both exist in square planar geometry. The dihedral angle between the two square planes is 111.4°, and the Ni- - -Ni separation is 2.8255(4) Å. The metric parameters of the bridged Ni_d synthon in **6** are very similar to that noted with **2**. This suggests that sulfur metalation does not change any structural feature of this Ni_d mimic. Complex **6** can be easily reduced with $Na_2S_2O_4$ or $NaBH_4$, and $\mathbf{6_{red}}$ exhibits a strong Ni(I) EPR spectrum similar to other Ni(I)-P_2S_2 complexes.[9] Since **2** does not exhibit any reduction wave up to −1.8 V vs SCE in solvents such as DMF, it is clear that the reduction occurs at the Ni_p site of **6**. Although **6** displays no reactivity toward CO, the one-electron-reduced species $\mathbf{6_{red}}$ is different. Passage of CO through a DMF solution of $\mathbf{6_{red}}$ generates the CO adduct $\mathbf{6_{red}}$**-CO** (EPR spectrum shown in Figure S3, Supporting Information) that displays a strong ν_{CO} band at 1997 cm^{-1}, consistent with a terminal Ni(I)-CO unit.[10] One must note that this ν_{CO} value is very close to the enzyme value of 1996 cm^{-1}.[11] Rauchfuss and co-workers have reported a dinuclear Ni complex in which two molecules of CO are bound to a Ni(0) center in terminal fashion.[7c] This species displays two ν_{CO} bands at 1948 and 1866 cm^{-1}. Since these ν_{CO} values are lower than that of the enzyme, it is quite possible that the Ni_p site in ACS does not attain the 0 oxidation state during catalysis. Recently, two groups have reported sulfur-bridged dinuclear Ni complexes with P_2S_2 coordination at the bridged Ni center that exhibit low reduction potentials.[7a,e] However, no spectroscopic data are available on the CO adducts of these complexes in the reduced state. Complex **6** is therefore the first structurally characterized Ni−Ni model that includes dicarboxamide-dithiolate ligation (Ni_d mimic) with a bridged Ni(II) center that can be reduced to the Ni(I) state and binds CO in terminal fashion (Ni_p mimic).

Several groups have shown that the ACS activity and the NiFeC signal of the enzyme diminish upon treatment with 1,10-phenanthroline (phen), whereupon part of the Ni is removed (labile Ni).[1,12] Treatment of **1** or **2** with excess phen (up to 100 equiv) does not lead to the formation of $[Ni(phen)_3]^{2+}$. However, when **6** is treated with excess phen (~25 equiv) in CH_2Cl_2, the electronic absorption spectrum of **6** changes to that of the monomer **2** and $[Ni(phen)_3]^{2+}$ over a time period of 35 min, with $k_{obs} = 1.502 \times 10^{-4}\ s^{-1}$ (Figure S4, Supporting Information). This reaction confirms that the thiolato-S bridges between the Ni_d and the Ni_p centers in models such as **6** are vulnerable to phen treatment. Since phen does not remove Ni from the Ni_d synthons **1** and **2**, it is apparent that the labile Ni in the enzyme arises from the Ni_p site. Interestingly, addition of excess (up to 100 equiv) neocuproine, a Cu(I) chelator, to **6** does not result in any Ni loss. These observations are in line with those observed with the enzyme.[4a,c]

In summary, we report two Ni−Ni models, namely **4** and **6**, that exhibit structural features and chemical properties very similar to those of the binuclear active site of ACS/CODH. These models are the first examples of sulfur-bridged dinuclear Ni complexes that bind CO at the bridged Ni(I) center, as proposed in the mechanism of acetyl coenzyme A synthesis.[3] Also, in one case, the corresponding Ni−Cu model has been characterized.

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Supporting Information Available: Spectroscopic and analytical data for the complexes; Scheme S1 summarizing the synthetic reactions; X-band EPR spectra of $\mathbf{4_{red}}$ and $\mathbf{4_{red}}$**-CO** (Figure S1) and $\mathbf{6_{red}}$**-CO** (Figure S3); ORTEP diagram of $(Et_4N)_2[Ni(PhPepS)]$ (**2**) (Figure S2); changes in the electronic absorption spectrum of **6** upon phen addition (Figure S4); and X-ray crystallographic files in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

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