

## Cys-Gly-Cys Tripeptide Complexes of Nickel: Binuclear Analogues for the Catalytic Site in Acetyl Coenzyme A Synthase

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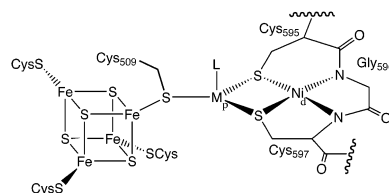
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The structure of the bifunctional enzyme acetyl coenzyme A synthase/CO dehydrogenase (ACS/COdH) from the anaerobic bacterium, *Moorella thermoacetica*, has been established recently by X-ray methods.<sup>1</sup> The  $\alpha_2\beta_2$  quaternary protein contains two copies of four different metal clusters, including the catalytic sites for the two disparate functions of the enzyme. The A cluster is the site for ACS activity, Figure 1. The cluster contains three components, a cuboidal  $\text{Fe}_4\text{S}_4$  unit, a square planar  $\text{Ni}_d$  (distal to the cuboid) ligated by two peptide amide nitrogens and two Cys side-chain thiolates, and a four-coordinate proximal metal site ( $\text{M}_p$ ) that connects the  $\text{Fe}_4\text{S}_4$  to the  $\text{Ni}_d$ . The metal in the central  $\text{Cys}_3$  site has been identified as either Cu, Ni, or Zn by multiwavelength anomalous dispersion (MAD) X-ray experiments, although it appears the active enzyme contains Ni in this site.<sup>2</sup> The unusual distal site bears resemblance to the Cys-Ser-Cys sequence at the nitrile hydratase active site that coordinates Co or Fe.<sup>3</sup> While short peptide complexes of Ni are well precedented, Gly-Gly-His representing a common sequence,<sup>4</sup> examples that incorporate Cys are rare.<sup>5</sup> In general, Ni tripeptide complexes are highly electron rich, permitting access to the  $\text{Ni}^{\text{III}}$  state.<sup>6</sup> However, further metalation of the Cys side chains provided by the proximal metal ( $\text{Ni}_p$ ), as found at the A cluster, should alter the redox properties, rendering the site suitable for catalysis. Herein, we seek to establish the fundamental properties of the Cys-Gly-Cys motif by exploring the Ni binding preference of this short peptide in the absence of the protein superstructure. Further, the resulting square planar  $\text{Ni}(\text{CGC})$  metallopeptide is a building block for the construction of larger structures related to the ACS active site. As the  $\text{Fe}_4\text{S}_4$  constituent of the A cluster likely tunes the electronic structure and redox properties of the binuclear site without binding substrate, we continue to focus on binuclear analogues,<sup>7</sup> specifically, NiNi species.<sup>8</sup>

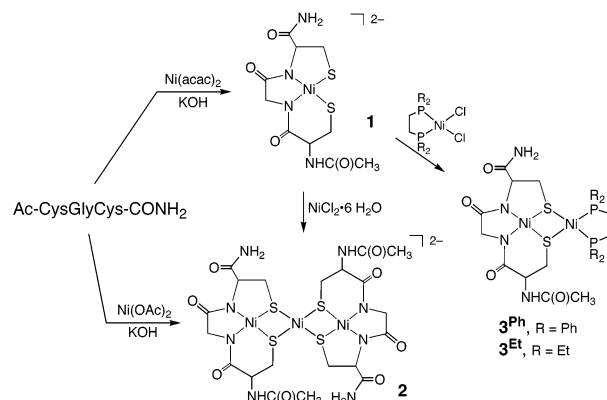
Addition of  $\text{Ni}(\text{acac})_2$  to  $\text{Ac-CysGlyCys-CONH}_2$  followed by 4 equiv of KOH yielded deep red  $\text{K}_2[\text{Ni}(\text{CGC})]$ , **1**, Scheme 1. Diamido dithiolato coordination to Ni as observed in the A cluster is supported by the similarity of the UV-vis spectra of diamagnetic **1** ( $\lambda_{\text{max}}$ , 550, 445, 275 nm) and diamidodithiolato nickel(II) complexes, such as  $\text{K}_2[\text{Ni}(\text{phmi})]$  (540, 425, 331, 270, 235 nm).<sup>9</sup> The red-shifting of the presumably d-d transitions in **1** reflects a slightly weaker ligand field provided by the tripeptide as compared to the phmi ligand. Proton NMR spectroscopy (1-D and COSY) is in accord with the diamidodithiolato coordination. Specifically, comparison of the  $^1\text{H}$  NMR spectra ( $d_6$ -DMSO) of the apo-peptide with **1** shows loss of the SH signals and two of the NH signals as well as upfield shifting of the remaining NH resonances upon metal binding. Confirmation of the mononuclear formulation of **1** is provided by ESI-MS analysis: the most intense isotope envelopes  $\{\text{H}[\text{Ni}(\text{CGC})]\}^-$  ( $m/z$ , 377.1, 92%) and  $\{\text{K}[\text{Ni}(\text{CGC})]\}^-$  ( $m/z$ , 415.1, 100%).

Green, trinuclear **2** is accessible either from **1** or, alternatively, via direct metalation of the apo-peptide, Scheme 1. The former



**Figure 1.** The A cluster of ACS.  $\text{M}_p$  has been identified as Ni, Cu, or Zn by MAD experiments; L is an unknown, nonproteinaceous ligand.

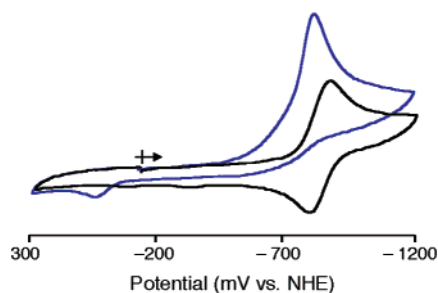
### Scheme 1



route should prove useful for the preparation of trinuclear species with different metals in the central position. The ESI mass spectrum of **2** contains two prominent isotope envelopes derived from the parent trinuclear complex,  $m/z$ , 810.9 ( $\{\text{H}[\text{Ni}(\text{CGC})]_2\text{Ni}\}^-$ ) and  $m/z$ , 848.9 ( $\{\text{K}[\text{Ni}(\text{CGC})]_2\text{Ni}\}^-$ ). **2** is diamagnetic, consistent with a planar central metal.<sup>10</sup>

The demonstrated utility of **1** as a precursor for construction of higher nuclearity complexes motivated the preparation of binuclear NiNi species derived from reaction of **1** with a suitable reagent. Reaction of **1** with  $(\text{R}_2\text{PCH}_2\text{CH}_2)_2\text{NiCl}_2$  ( $\text{R} = \text{Ph}, \text{Et}$ )<sup>11</sup> proceeded in DMF yielding orchid **3Ph** or **3Et**, Scheme 1. ESI-MS analysis supports the solution compositions as binuclear,  $m/z$ , 833.2 (**3Ph** +  $\text{H}^+$ ), 641.2 (**3Et** +  $\text{H}^+$ ). The  $^{31}\text{P}$  NMR spectra show inequivalent phosphorus environments due to the asymmetry of the tripeptide as expected for the dithiolato bridged structure.

Reductive activation of the A cluster is required for catalysis, although it is unclear if carbonylation is commensurate with reduction and whether the spectroscopically observed  $S = 1/2$  state ( $\text{A}_{\text{red}}-\text{CO}$ ) is a catalytic intermediate or inhibitor.<sup>12</sup> The cathodic cyclic voltammograms of **2** and **3** show metal-based reductions. Trinuclear **2** displays a reversible reduction at  $-995$  mV assigned to  $\text{Ni}^{\text{II/I}}$  for the outer two  $\text{Ni}(\text{N}_2\text{S}_2)$  sites. Darensbourg and co-workers have observed similar effects upon metalation of neutral diaminodithiolato  $\text{Ni}^{\text{II}}$  complexes.<sup>13</sup> **3** exhibit two one-electron reductive processes: a reversible reduction at  $-531$  mV (**3Ph**),  $-828$  mV (**3Et**) and a quasi-reversible event at  $-1360$  mV (**3Ph**),  $-1641$  mV (**3Et**). The first event most likely represents reduction to a



**Figure 2.** Cyclic voltammograms of  $3^{\text{Et}}$  under Ar (black) and CO (blue) in the cathodic region.  $\text{CH}_3\text{CN}$  solutions contained 10 mM  $3^{\text{Et}}$ , 0.1 M  $[\text{Bu}_4\text{N}]\text{-PF}_6$ ; data collected with a scan rate of 200 mV/s.

#### Scheme 2



valence localized  $\text{Ni}^{\text{II}}\text{Ni}^{\text{I}}$  binuclear state in which the  $\text{Ni}(\text{S}_2\text{P}_2)$  site is reduced as the phosphine ligands support the lower oxidation states of Ni more effectively than the electron-rich peptide. That  $3^{\text{Et}}$  is more difficult to reduce, by  $\sim 300$  mV for both processes, is expected due to the greater donor ability of depe and further suggests that the second reduction is centered also on the  $\text{Ni}(\text{S}_2\text{P}_2)$  site, giving rise to a  $\text{Ni}^{\text{II}}\text{Ni}^{\text{0}}$  species. The  $\text{Ni}^{\text{II}}\text{Ni}^{\text{0}}$  mixed valence state has been proposed for the reduced form of the A cluster and preceded synthetically, albeit in a complex containing a  $\text{Ni}(\text{CO})_2$  site to further stabilize the  $\text{Ni}^{\text{0}}$  oxidation state.<sup>8</sup> However, an alternative valence description of the reduced A cluster,  $\text{Ni}^{\text{II}}\text{Ni}^{\text{I}}\text{-}[\text{Fe}_4\text{S}_4]^+$ ,<sup>14</sup> is attractive as it places the second electron on the cuboidal cluster.  $[\text{Fe}_4\text{S}_4]$  clusters have reduction potentials more accessible than the second reduction of  $3$ , even as the latter are more biased toward  $\text{Ni}^{\text{0}}$  by virtue of phosphine coordination. Preparation of analogues of  $3$  affixed to an  $[\text{Fe}_4\text{S}_4]$  cluster would provide insight into the fundamental redox properties and electronic structure descriptions of the A cluster.

Although  $3^{\text{Ph}}$  and  $3^{\text{Et}}$  do not interact with CO in the  $\text{Ni}^{\text{II}}\text{Ni}^{\text{II}}$  states, the reduced forms bind CO as assayed by cyclic voltammetry, Figure 2. The cathodic scan of  $3^{\text{Et}}$  under CO (1 atm) shows a single irreversible wave 60 mV positive of  $E_c^{\circ}$  under Ar (the second reductive wave under Ar is absent under CO). The current passed in the presence of CO indicates a two-electron process. The observation suggests an ECE electrochemical process, Scheme 2. CO ligation to the  $\text{Ni}^{\text{II}}\text{Ni}^{\text{I}}$  represents the chemical (C) step in this process with such coordination facilitating the second reduction (E) to  $\text{Ni}^{\text{II}}\text{Ni}^{\text{0}}$ . Efforts to isolate and characterize reduced CO-bound forms of  $3$  are in progress.<sup>15</sup>

In sum, the Cys-Gly-Cys complex of Ni is a useful precursor for the synthesis of analogues of the binuclear component of the A cluster, that is, complexes  $3$ , which are close synthetic representations of the  $\text{Ni}^{\text{II}}\text{Ni}^{\text{II}}$  state of the enzyme. The different coordination spheres of the metals in these complexes bias the redox properties, resulting in two-electron reduction to the  $\text{Ni}^{\text{II}}\text{Ni}^{\text{0}}$  state. Reductive

carbonylation further stabilizes the  $\text{Ni}^{\text{II}}\text{Ni}^{\text{0}}$  state. It remains to be demonstrated if the reduced forms of  $3$  are potent nucleophiles for transmethylation reaction requisite in ACS or if CO binding in fact renders the reduced states incompetent for this process.

**Acknowledgment.** We thank the NIH for financial support, Profs. Joel Schneider and Dennis Evans for helpful discussions, and Ms. Sowmya Subramanian for peptide synthesis.

**Supporting Information Available:** Synthetic procedures and characterization data (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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- (15) While Scheme 2 shows a single CO bound to reduced forms of  $3$ , the stoichiometry is not yet known.<sup>8</sup>

JA038086U