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Capture of Ni^{II}, Cu^{I} and Zn^{II} by thiolate sulfurs of an N_2S_2Ni complex: A role for a metallothiolate ligand in the acetyl-coenzyme A synthase active site†

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The metal binding affinity of an $(N_2S_2)Ni$ bridging metallothiolate ligand (Zn^{2+} < Ni^{2+} < Cu^{+}) gives precedent for the observed heterogeneity in ACS/CODH.

Although uncommon for the metal binding sites of cysteine-rich metalloproteins, peptide sequences of Cys-X-Cys which bind metals into the protein "backbone" through thiolate sulfurs and amido nitrogens, N_2S_2M, are important components of the active sites of nitrile hydratase (X = serine; M = Co or Fe) and acetyl-coenzyme A (CoA) synthase/carbon monoxide dehydrogenase, ACS/CODH (X = glycine, M = Ni).[1–4] The former demonstrates "post-translational" (after assembly) modification *via* oxygen capture converting the thiolato sulfurs into sulfinato/sulfenato sulfur donors.[5] A case can be made that the electron-rich sulfurs of the $(N_2S_2)Ni$ site in the latter are also post-translationally modified by the capture of Cu, Zn, and Ni metal ions.[3,4] This report offers chemical precedent for such a process as well as an affinity ranking of $(N_2S_2)Ni$ for exogeneous metal ions.‡

Independent reports of two X-ray crystal structures of the bifunctional enzyme ACS/CODH derived from the same bacterial source, *Moorella thermoacetica*, have confirmed the protein to exist as a heterodimer of dimers, $\alpha_2\beta_2$.[3,4] The ACS active sites, *i.e.*, the multimetallic catalyst(s) for the CO/CH_3 coupling reaction were found in the A clusters of the α subunits. Both structures reveal the metal distal to the 4Fe4S cluster to be nickel, Ni_d, in *ca.* square planar N_2S_2 coordination bound by two amido nitrogens and two cysteine sulfurs. In the study by Doukov, *et al.*,[3] X-ray absorption/anomalous scattering experiments established the proximal metal to be copper in a pseudo tetrahedral array of three (μ-SR) and one undefined ligand. A similar approach by Darnault, Volbeda, *et al.*,[4] uncovered two forms of the α subunits, one of which contained a tetrahedral zinc, Zn_p, rather than copper in the proximal metal site; the other contained a nickel, Ni_p, in a roughly square planar geometry, Figure 1. Important for distinguishing Ni_p *vs.* Zn_p was the observation that the α subunits also differ by a rotation with respect to each other that renders the A cluster of one subunit more exposed to the surface and thus solvent accessible. This α-open subunit contains the $[Ni_dNi_p]Fe$ cluster while α-closed form is composed of $[Ni_dZn_p]Fe$. The importance of these forms for enzyme function and for understanding the heterogeneity in the bifunctional CODH/ACS enzymes is described in reference 4.

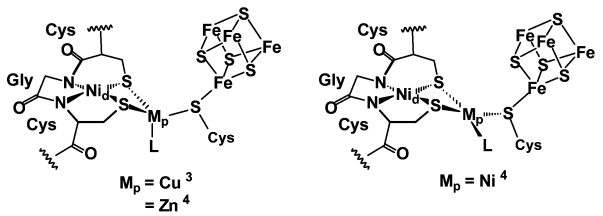


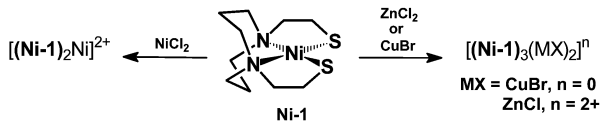
Fig. 1 The A clusters of ACS.[3,4]

† Electronic supplementary information (ESI) available: X-ray data, experimental procedures and vis–UV spectroscopic monitor data. See http://www.rsc.org/suppdata/cc/b3/b304884p/

Extensive literature exists for monomeric square planar $(N_2S_2)Ni$ complexes, including amino- and deprotonated amido-nitrogen donors.[6–8] While tetraanionic amidothiols most similar to peptide backbone N_2S_2 binding sites demonstrated success at stabilizing the $Ni^{II/III}$ couple at biologically-relevant potentials, they permit reduction ($Ni^{II/I}$) only at highly negative potentials.[6] Even neutral $(N_2S_2)Ni$ complexes show a $Ni^{II/I}$ couple at *ca.* −2 V.[8] It is therefore unlikely that $(N_2S_2)Ni$ sites can accommodate a fundamental requirement of ACS reactivity: the oxidative addition of CH_3^+, forming from M^n a M^{n+2}–CH_3, followed by CO insertion.[6] The $(N_2S_2)Ni$ model complexes do, however, serve as versatile metallothiolate ligands, binding through sulfur to a range of metal moieties. For example, the square planar bismercaptoethanediazacyclooctanenickel(II), (bmedaco)Ni, or the **Ni-1** complex,[9] serves as an S-donor, monodentate metallothiolate ligand in (**Ni-1**)$Fe^0(CO)_4$, analogous to phosphine derivatives, $(R_3P)Fe(CO)_4$.[10] On oxidation to Fe^{II}, **Ni-1** binds as a bidentate ligand in (**Ni-1**)$_2Fe^{II}(CO)_2^{2+}$ as would a diphosphine ligand.[10] The diphosphine analogy is repeated in the nucleation of two **Ni-1** by a Ni^{II} ion into the diamagnetic, blood-red $[(\textbf{Ni-1})_2Ni]^{2+}$ trimetallic shown in Schemes 1 and 2.[11] A complex of formulation $[(\textbf{Ni-1})_3(ZnCl)_2]^{2+}$, in which **Ni-1** is a bidentate bridging ligand results from exposure of **Ni-1** to solutions of $ZnCl_2$.[12] In the presence of excess Cu^{I} salts, large neutral clusters of $[(\textbf{Ni-1})_2(Cu^{I}Cl)_4]$ form.[13] With excess **Ni-1**, a pentanuclear $[(\textbf{Ni-1})_3(CuBr)_2]$ results, Schemes 1 and 2, which is first reported herein and characterized by X-ray crystallography.

As in the isostructural $[(\textbf{Ni-1})_3(ZnCl)_2]^{2+}$ analogue, two CuBr units of $[(\textbf{Ni-1})_3(CuBr)_2]$ form a linear X-M–M-X axis, while three bidentate bridging **Ni-1** units form dihedral planes between the $(N_2S_2)Ni$ units at *ca.* 120° to each other. The Zn^{2+} or Cu^{+} metal ions are in pseudo-tetrahedral environments in each, with Zn–Zn and Cu–Cu distances of 4.35(1) Å and 4.05(1) Å, respectively. Important for the studies below, the zinc complex is fuchsia colored in solution, while the copper analogue is orange-yellow to yellow-brown. While the polymetallic complexes are not structural analogues of the Ni(μ-$SR)_2M$ active sites of ACS, the affinity of the $(N_2S_2)Ni$ terminal thiolates for various metals may indicate the point at which the specificity of metal-ion delivery might relate to the active site construction. A binuclear NiCu synthetic analogue has recently been reported.[14]

A qualitative ranking of the binding ability of **Ni-1** with Zn^{II}, Cu^{I}, and Ni^{II} was established by a metal ion displacement experiment using pre-formed clusters, Scheme 2. The experimental protocol was to mix MeOH solutions of the cluster complexes with the metal salts (CuBr in CH_3CN; $ZnCl_2$ and $NiCl_2$ in CH_3OH) in aliquots up to the amount necessary to achieve stoichiometric or formula requirements of the metal-



Scheme 1

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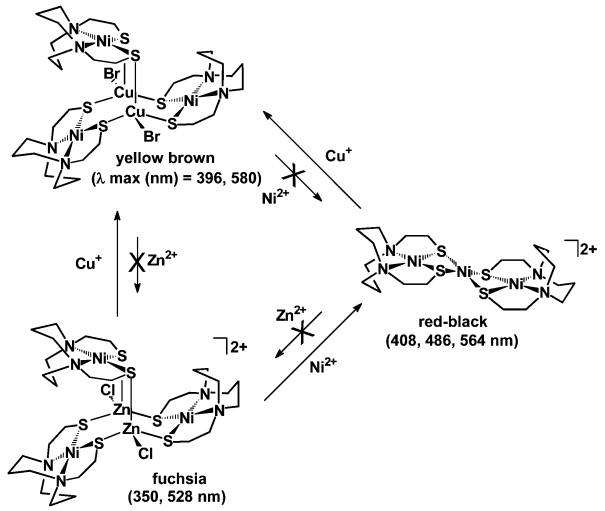
exchanged cluster. Reactions were monitored by visual color changes (which occurred, if at all, on time of mixing at 22 °C) and by changes in the d–d transitions in the visible region of the electronic spectra, listed as λ_{max} in Scheme 2. In this manner it was found that both Ni^{2+} and Cu^{+} replace Zn^{2+} in the [(**Ni-1**)$_3$(ZnCl)$_2$]$^{2+}$ complex, and Zn^{2+} does not react with the [(**Ni-1**)$_3$(CuBr)$_2$] or [(**Ni-1**)$_2$Ni]$^{2+}$ clusters. The yellow-brown solution of [(**Ni-1**)$_3$(CuBr)$_2$] showed no reaction with added $NiCl_2$, however the opposite reagent arrangement, *i.e.*, the mixing of red-black [(**Ni-1**)$_2$Ni]$^{2+}$ with CuBr, resulted in conversion to [(**Ni-1**)$_3$(CuBr)$_2$], Scheme 2.



Scheme 2

From the above study a qualitative ranking of metal ion affinity by the nickel dithiolate ligand is $Zn^{2+} < Ni^{2+} < Cu^{+}$. To confirm, mixtures of dissolved CuBr and $NiCl_2$ in various ratios were added to MeOH solutions of **Ni-1**. With sufficient CuBr, only the [(**Ni-1**)$_3$(CuBr)$_2$] complex was observed; with CuBr in deficiency, a mixture of [(**Ni-1**)$_3$(CuBr)$_2$] and [(**Ni-1**)$_2$Ni]$^{2+}$ resulted.

Early biochemical studies by Lindahl, *et al.* demonstrated the existence of a "labile" nickel associated with the α subunit of ACS/CODH.[15] Its removal by 1,10-phenanthroline, phen, resulted in loss of ACS activity and the correlating spectroscopic signals, even though substantial amounts of non-labile nickel remained.[15] To model this, we checked for lability of Ni^{2+} in the two nickel binding sites of [(**Ni-1**)$_2$Ni]$^{2+}$ by reaction with phen. On addition of 3 equiv of phen to red-black [(**Ni-1**)$_2$Ni]$^{2+}$ a color bleaching indicated formation of the salmon-pink Ni(phen)$_3^{2+}$ as a mixture with **Ni-1**.[16] A separate attempt to remove Ni from the N_2S_2 binding site of **Ni-1** with three equivalents of phen were unsuccessful. Neither does a six-fold excess of phen degrade the [(**Ni-1**)$_3$(CuBr)$_2$] complex.

Our studies model a metal-ion capture event of a peptide-backbone, non-labile, $N_2S_2Ni_d$ unit, and establish the capability of such a nickel dithiolate to bind the exogeneous metals observed in the X-ray crystal structures of ACS/CODH. The affinity ranking of $Zn^{2+} < Ni^{2+} < Cu^{+}$ and the lability of both Zn and Ni when bound by the N_2S_2Ni model complex indicates that the chemically most reasonable Ni_dNi_p ACS active site could be compromised by copper replacement. Nevertheless conflicting reports of copper *vs*. nickel requirements for enzyme activity exist, and provide, as of this writing, a continuing controversy.[17,18] It is our contention that the preponderance of evidence regarding nickel chemistry, including the metal-ion capture studies shown here, leads to agreement with earlier proposals by Darnault, Volbeda, *et al.*,[4] and with the report of Grahame and Gencic[18] that a dinuclear nickel site confers activity on the ACS enzymes. Our studies provide precedence for Lindahl's enzymatically active labile nickel, the proximal nickel of Figure 1. In an S-donor environment softened by the distal Ni^{2+}, Ni_p is expected to be able to access lower oxidation states, Ni^{I} or Ni^{0}, as if in a diphosphine ligating environment, and perform the established organonickel chemistry (oxidative-addition of Me^{+}, CO binding and migratory insertion, and reductive displacement by SR^{-}) required of the enzyme.[19,20] That square planar Ni^{II} can undergo the latter processes has ample precedence in the literature,[20,21] and there are as well precedents for oxidative addition of CH_3^{+} to reduced Ni.[19]

The mechanism whereby adventitious copper might be excluded and nickel delivered in a specific manner to the ACS enzyme within a complete organism is doubtless to be found in the many nickel-processing steps[22] upstream of the capture of the proximal metal by the $(N_2S_2)Ni_d$. As expressed by Hausinger, the expulsion of incorrectly associated metals is among the possibilities for ultimately achieving functioning active sites in nickel enzymes.[22] In this vein, the observation that nickel will displace zinc in the (N_2S_2)Ni–Zn model studies could suggest a role for zinc in bioassembly of the sulfur-rich active site. Whether the NiZn binding site is important to enzyme activity, or simply a precursor to a NiNi site is not yet known.

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Notes and references

‡ *[(**Ni-1**)$_3$(CuBr)$_2$]:* Crystals were obtained from ether vapor diffusion into a CH_2Cl_2 solution. *Crystal data*: $C_{38}H_{80}Br_2Cu_2N_6Ni_3O_2S_6$, M = 1308.47, a = 26.1834(13), b = 26.1834(13), c = 15.5725(9) Å, V = 10676.0(10) Å^3, T = 110(2) K, *I*4/*m*, Z = 8, μ = 3.592 mm^{-1}, reflections collected = 27161, independent collections = 4799, R_{int} = 0.1329, final R values: R_1 = 0.0519, *wR*2 = 0.1447. CCDC 211927. See http://www.rsc.org/suppdata/cc/b3/b304884p/ for crystallographic data in .cif format.

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