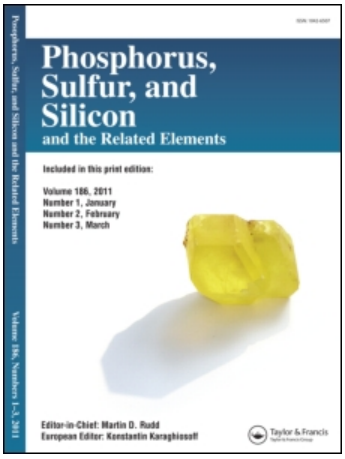

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THE ROLE OF SELENOCYSTEINE IN Ni, Fe HYDROGENASES: BIOPHYSICAL AND SYNTHETIC MODEL STUDIES

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The active site of Ni,Fe hydrogenases contains a heterodinuclear cluster where the Ni and Fe centers are bridged by two cysteine thiolate S-donor atoms. Two terminal cysteine thiolates are also present in the Ni coordination environment. In a few of the enzymes, one of the terminal thiolates is substituted by a selenocysteine. This substitution has an effect on the redox chemistry of the enzyme and on the catalytic mechanism. The presence of selenocysteine provides a spectroscopic probe of a specific Ni ligand. X-ray absorption spectroscopy was used to probe the Ni and Se environments in *Desulfomicrobium baculatum* hydrogenase in the oxidized and reduced states. These studies reveal that the Se environment is sensitive to the redox poise of the enzyme, while no changes in the Ni environment were observed. Crystallographically characterized model Ni complexes featuring alkylthiolate and alkylselenolate ligation are used explore the changes in structure and redox properties that might arise in the biological systems.

Keywords: hydrogenase, selenocysteine, XAS

INTRODUCTION

Hydrogenases (H_2ases) are enzymes that catalyze the reversible, two-electron oxidation of H_2. By far the most common class of H_2ases

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contain multiple Fe atoms (mostly as various Fe, S clusters) and a single Ni atom. The crystal structure of the H_2ase from *Desulfovibrio gigas*,[1] reveals that the active site is composed of a Ni, Fe heterodinuclear cluster. This cluster features two cysteinate bridges between the metals and two terminal cysteinate ligands to Ni. The structure of the enzyme active site is shown in figure 1. In a few Ni, Fe H_2ases (e.g.,

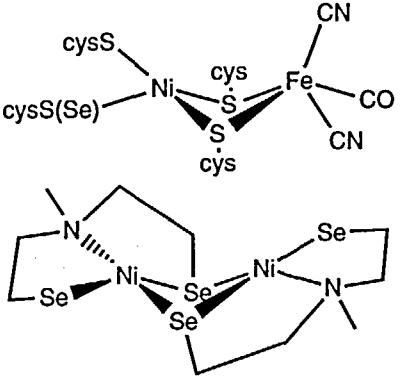

FIGURE 1 Core Structure Comparison: The H_2ase structure (top) and the structure of a Ni(II) selenolate model complex (bottom) are shown.

Desulfomicrobium baculatum H_2ase), one of the terminal cysteinate ligands to Ni is substituted by selenocysteine.[2, 3] This substitution has an effect on the redox chemistry of the enzyme (the enzyme is more stable to oxidation and may be isolated in air in a state that does not require reductive activation) and on the catalytic mechanism (H/D exchange is altered).[4, 5] Here we address the origin of these effects by examining changes in the Se and Ni environments in the selenocysteine containing H_2ase from *D. baculatum*, and by examining the structures and redox properties of analogous thiolate and selenolate complexes.

RESULTS AND DISCUSSION

Changes in electron density at atomic centers can be monitored by examining the effect of redox processes on the energy of the x-ray absorption edge.[6] Previous studies of the K-edge energy of Ni centers of several Ni,Fe H_2ases reveal that the Ni center is not very sensitive to the redox state of the enzyme.[7] This lack of sensitivity is even more pronounced in the selenocysteine containing enzyme from *D. baculatum*. Measurement of the Ni K-edge energy for an oxidized (as isolated) sample and an H_2-reduced sample show no shift in the edge energy within experimental error (± 0.2 eV). In contrast, the energy of the Se K-edge shifts -0.8 eV upon reduction of the enzyme, demonstrating that this atom is sensitive to the redox state change (figure 2).

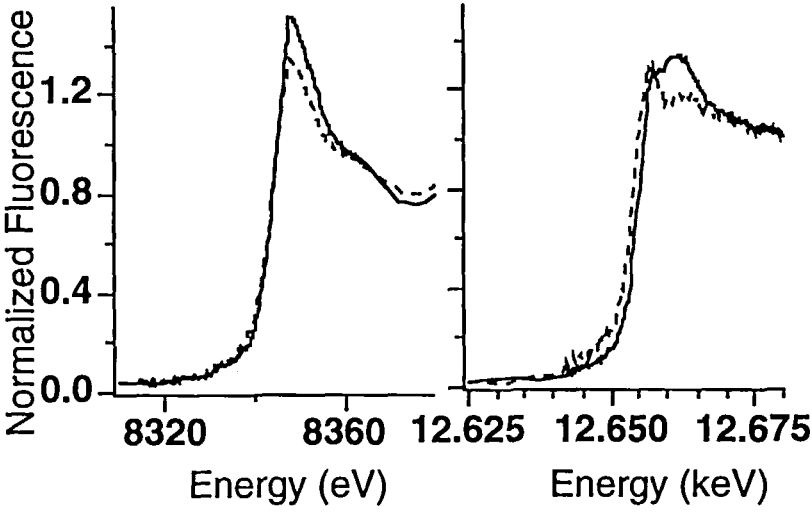

FIGURE 2 XANES Spectra from *D. baculatum* Hydrogenase (oxidized = solid line, reduced = dashed line). The Ni-K-edge spectra are shown on the left, the Se K-edge spectra are shown on the right.

Analyses of XANES features provide information about the overall structure of a metal atom.[6] No features associated with a 1s -> $4p_z$ transition were visible in spectra obtained from *D. baculatum* H_2ase, indicating that the Ni site is not a planar, four-coordinate one. A peak assigned to a 1s -> 3d transition was visible and had an intensity appropriate for a six-coordinate Ni complex in both the oxidized and reduced samples, indicating that no change in coordination number occurs. While the Se K-edge XANES spectra do not exhibit either of these transitions, the post-edge XANES clearly indicates that the structure of the Se-donor atom has changed in the reduced enzyme. Model studies suggest that one possibility is protonation of the Se atom in reduced forms of the enzymes.

Crystal structures of dinickel(II) model complexes show that they resemble the basic core structure of the H_2ase active site in that they feature two metals with a combination of di-μ-thiolato/selenolato and terminal thiolato/selenolato ligation (Figure 1).[8] The largest structural difference between the thiolate and selenolate analogs is a lengthening of the metal-chalcogenide bonds in the selenolate complex (Ni - S_{ave} = 2.18 Å; Ni - Se_{ave} = 2.30 Å). The Ni-Ni distance is slightly longer in the selenolate complex (2.72 Å vs. 2.68 Å) and the fold angle (the dihedral angle formed by the $Ni(S(Se))_2$ planes) decreases slightly in the selenolate complex (104° vs. 108°). Neither system can be easily reduced to a formal Ni(II)/(I) system, but each complex may be oxidized by one electron at modest potentials to give an S = 1/2 cation.[8] This cation radical exhibits an epr spectrum nearly identical to the spectra obtained from redox intermediates observed in H_2ases. Theoretical and spectroscopic experiments suggest that much of the spin density resides on the S/Se-donors in the oxidized models. Given the structural similarities between the model and enzyme Ni sites, the similarity in their spectral properties indicates a similar electronic structure.

Despite the similarity of the dinickel(II) structures and the spectral similarities of the cation radicals, there are differences in the stability of the thiolate and selenolate analogs with respect to oxidation by O_2. While the thiolate complexes undergo oxidation to complexes containing sulfinate ligands, no such oxidation occurs in the selenolate complexes.[8] Such a change is consistent with an antioxidant role for selenocysteine in the enzyme. This role is consistent with the increased stability to O_2 observed in the enzymes containing selenocysteine.

Both analogs undergo protonation to form stable thiol and selenol products. Complexes with one or two added protons have been characterized by elemental analysis in the solid state and spectroscopically in solution. Elemental analysis reveals that addition of one or two protons is accompanied by the addition of one or two counterions (ClO_4^-, PF_6^-, etc.), which confirms the stoichiometry of the reaction. Addition of a proton to the thiolate dimer gives rise to a new feature at 2690 cm^{-1} that shifts to 2630 cm^{-1} in the selenolate complex and is assigned to a S(Se)-H stretch. The presence of a solvent exchangeable proton in this product was confirmed by ^2H NMR, which revealed a single resonance at 1.93 ppm. The nuclearity of the complex was confirmed by ^{13}C-NMR. The dimer contains only five unique C atoms yielding five ^{13}C-NMR resonances. Monoprotonation destroys the symmetry of the complex, and nine of the ten possible ^{13}C-NMR resonances were observed.

Structural analyses of the thiol and selenol complexes were carried out by XAS analysis and in analogy with crystallographically characterized alkylated analogs. Analysis of XANES and EXAFS features reveal little difference between the structures of the thiolate/selenolate dimers and their protonated analogs. This was confirmed by the crystal structures of the monobenzylated and dibenzylated thiolate dimers. These structures show that the sites of alkylation and protonation are the terminal thiolate/selenolate ligands.

Further, the changes observed in the Ni-S and Ni-Ni distances in the alkylated complexes are ≤ 0.02 Å, which is the accuracy of the EXAFS analysis.

Acknowledgments

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References

[1.] A. Volbeda, et al., *J. Am. Chem. Soc.*, **118**, 12989 (1996).
[2.] M. K. Eidsness, et al., *Proc. Natl. Acad. Sci. U S A* , **86**, 147 (1989).
[3.] S. H. He, et al., *J. Biol. Chem.*, **264**, 2678 (1989).
[4.] S. P. J. Albracht, *Biochim. Biophys. Acta* , **1188**, 167 (1994).
[5.] O. Sorgenfrei, E. C. Duin, A. Klein, S. P. J. Albracht, *J. Biol. Chem.*, **271**, 23799 (1996).
[6.] G. J. Colpas, et al., *Inorg. Chem.*, **30**, 920 (1991).
[7.] Z. Gu, et al., *J. Am. Chem. Soc.*, **118**, 11155 (1996).
[8.] S. B. Choudhury, M. A. Pressler, S. A. Mirza, R. O. Day, M. J. Maroney, *Inorg. Chem.*, **33**, 4831 (1994).