

4. Models for Metalloproteins



PS4.1 — MO

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TRANSPORT OF HYDROGEN IONS BY A 4Fe-4S MODEL COMPOUND IN A DIRECTIONAL ELECTRON TRANSPORT SYSTEM.

The ability of $[\text{Fe}_4\text{S}_4(\text{SC}_6\text{H}_5)_4]^{2-/3-}$ to cotransport electrons and hydrogen ions in a directional electron transport system has been examined. A sequential electron transport system was used, where aqueous Cr(II)edta was the electron donor, and $(\text{CH}_3\text{N}((\text{CH}_2)_7\text{CH}_3)_3)_2[\text{Fe}_4\text{S}_4(\text{SC}_6\text{H}_5)_4]$ in toluene solution mediated the terminal reduction of methyl viologen in aqueous solution. The iron-sulfur complex, $(\text{CH}_3\text{N}((\text{CH}_2)_7\text{CH}_3)_3)_2[\text{Fe}_4\text{S}_4(\text{SC}_6\text{H}_5)_4]$ was prepared by the addition of $(\text{CH}_3\text{N}((\text{CH}_2)_7\text{CH}_3)_3)\text{Cl}$ (Aldrich), to the sodium salt of the cluster in methanol. The complex was isolated after storage at -40°C and recrystallized from warm $\text{CH}_3\text{CN}/\text{MeOH}$. The complex is freely soluble in toluene, and as such is the first reported $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{n-}$ complex soluble in a water immiscible solvent.

Reduction of the Fe-S complex was accomplished by vigorously agitating a mixture of aqueous Cr(II)edta with a toluene solution of the model for several minutes. In a typical experiment, 4.05 ml of 37 mM Cr(II)edta (pH 7.5) and 0.3 ml of 3 mM Fe-S complex were used, although the exact concentrations and volumes used varied between experiments. After waiting 10-20 minutes to allow

the immiscible aqueous and toluene phases to separate, a portion of the toluene phase was carefully transferred to a tube containing 3.00 ml of 10 mM methyl viologen in 0.5 mM tris/100 mM KCl buffer (pH 8). This mixture was agitated for approximately three minutes, and the immiscible phases were allowed to separate. The toluene phase was then removed from the top of the methyl viologen solution. Reduction was evidenced by the appearance of a deep blue color in the methyl viologen phase. The number of moles of reduced methyl viologen generated was measured optically. In all cases a drop in the pH was observed to be coincident with the reduction of methyl viologen. The average molar ratio of reduced methyl viologen to transported hydrogen ions (MV/H^+) for these eleven experiments was 1.18 ± 0.24 . The reduction potential of methyl viologen is pH independent, so it is assumed that methyl viologen does not bind hydrogen ions on reduction.

Evidence suggesting that electron transport between Cr(II)edta and methyl viologen was actually mediated by the Fe-S complex was obtained in several ways. After exposure of the Fe-S model to the aqueous Cr(II)edta phase, an EPR spectrum characteristic of reduced 4Fe-4S complex was obtained. While the g-values for this approximately axial spectrum ($g = 2.02$, $g = 1.90$) are not identical with reported values obtained in other solvents, some variation between solvents has been previously noted [1]. This observation of reduced Fe-S complex is consistent with optical experiments which show the characteristic bleaching expected for reduction of $[\text{Fe}_4\text{S}_4(\text{SR})_4]^{2-}$ complexes [2]. After reoxidation by exposure to methyl viologen, the original optical spectrum was regained, with recoveries of the original absorbance typically between 85 and 100%.

Electron transfer experiments were performed in the absence of the Fe-S complex, and these yielded no reduction of methyl viologen. If $\text{CH}_3\text{N}((\text{CH}_2)_7\text{CH}_3)\text{Cl}$ is included in the toluene phase (as a test of possible solubilization of the negatively charged Cr(II)edta complex), there still is no reduction of methyl viologen observed.

Optical studies were performed to quantitate the number of moles of reduced model compound after exposure to Cr(II)edta, and the number of moles of reduced methyl viologen ultimately pro-

duced. The experimentally obtained ratios of 0.88 and 1.01 agree quite well with the expected value of 1.0. This indicates that the only major species mediating electron transport between Cr(II)edta and methyl viologen was the Fe-S complex.

The observed change in pH suggests that the Fe-S complex is cotransporting electrons and hydrogen ions by a mechanism of reduction-linked proton binding. Another possible origin for the observed pH changes that should be considered is proton transport by the Fe-S complex driven by separation of charge between toluene and aqueous phases. This can be tested using valinomycin, a ring carrier ionophore which can permit K^+ ion to pass into the hydrophobic toluene phase. If under normal experimental conditions a potential was developed, addition of valinomycin should collapse the potential. Thus the rates of electron transport and the ratio of electron/proton transported would be greatly increased on addition of valinomycin. Because no significant increases were observed, it is concluded that proton transport driven by the separation of charge is not the dominant mechanism.

These results indicate that the Fe-S complex does cotransport protons and electrons. Further, the transport of the proton is not a process driven solely by the separation of charge between the aqueous and toluene phases. The actual site of binding of the hydrogen ion to the reduced Fe-S complex is not yet established. Studies are currently in progress to determine the site or sites of association.

ACKNOWLEDGEMENTS

We wish to thank M.A. Greaney, P. Mandel, and E.I. Stiefel for technical assistance and helpful discussions.

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PS4.2 — TU

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SYNTHESIS AND STUDY OF AN ANALOG FOR THE $[Fe_4S_4]^{3+}$ CENTER OF OXIDIZED HIGH POTENTIAL IRON-SULFUR PROTEINS

Although many examples of synthetic analogs for the $[Fe_4S_4]^{n+}$ ($n=1,2$) oxidation levels of iron-sulfur proteins have been prepared and characterized, no previous attempt to synthesize a compound with the $n=3$ oxidation level has been successful. Electrochemical studies have shown that $[Fe_4S_4(S-2,4,6-(i-Pr)_3C_6H_2)_4][(n-Bu)_4N]_2$, (**1**) and $[Fe_4S_4(S-2,3,5,6-Me_4C_6H)_4][(n-Bu)_4N]_2$, (**2**), can be reversibly oxidized in CH_3CN and CH_2Cl_2 by one electron to the corresponding $[Fe_4S_4(SR)_4]^{1-}$ compounds, **3** and **4**. The $-1/-2$ and $-2/-3$ redox couple potentials measured by normal and reverse pulse voltammetry in CH_2Cl_2 versus SCE are respectively: -0.12 V and -1.20 V for **1** and -0.05 V and -1.10 V for **2**. The oxidation of **1** with $[(C_5H_5)_2Fe]BF_4$ produces $[Fe_4S_4(S-2,4,6-(i-Pr)_3C_6H_2)_4][(n-Bu)_4N]$, (**3**), in 35% yield. Formulation of **3** as the first example of a synthetic compound containing the $[Fe_4S_4]^{3+}$ core, was confirmed by an X-ray crystal structure. The $[Fe_4S_4-(S-\alpha-C)_4]$ unit of **3** (Fig. 1) has crystallographic C_2 symmetry and approximate D_{2d} symmetry. The average Fe-S (2.26 Å) and Fe-SR (2.21 Å) bond distances are similar to the distances reported for the X-ray structure of the $[Fe_4S_4]^{3+}$ center of the oxidized high potential iron-sulfur protein from *Chromatium vinosum*. Also, the $[Fe_4S_4]$ core of **3** is tetragonally compressed with four short Fe-S bonds and eight long Fe-S bonds. The observed shift to longer wave-