$W(CO)_6$-photocatalyzed reactions of olefins.[3,4]

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Registry No. $W(CO)_6$, 14040-11-0; $W(CO)_5$, 30395-19-8; $W(CO)_5(\eta^2\text{-}C_2H_4)$, 93117-80-7; $W(CO)_4(\eta^2\text{-}C_2H_4)$, 97073-99-9; $W(CO)_5(\eta^2\text{-}C_3H_6)$, 93223-97-3; $W(CO)_4(\eta^2\text{-}C_3H_6)$, 97074-00-5; *cis*-$W(CO)_4(\eta^2\text{-}C_3H_6)_2$, 94920-28-2; $W(CO)_5(\eta^2\text{-}1\text{-}C_5H_{10})$, 53261-74-8; $W(CO)_4(\eta^2\text{-}1\text{-}C_5H_{10})$, 97074-01-6; $W(CO)_5(\eta^2\text{-}1,4\text{-pent})$, 97074-02-7; *cis*-$W(CO)_4(\eta^2\text{-}1\text{-}C_5H_{10})_2$, 97074-03-8; *trans*-$W(CO)_4(\eta^2\text{-}C_5H_{10})_2$, 97133-84-1; $W(CO)_5(\eta^2\text{-}C_5H_6)$, 97102-14-2; $W(CO)_4(\text{nor})$, 12129-25-8; $Cr(CO)_6$, 13007-92-6; $Cr(CO)_5$, 26319-33-5; $(\eta^5\text{-}C_5H_5)W(CO)_3CH_3$, 12082-27-8; $W(CO)_5NH_3$, 15133-64-9; CO, 630-08-0; ethylene, 74-85-1; propylene, 115-07-1; 1-pentene, 109-67-1.

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Transport of Hydrogen Ions by a 4Fe–4S Model Compound in a Directional Electron-Transport System[†]

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The ability of $Fe_4S_4(SC_6H_5)_4^{2-/3-}$ 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 $(CH_3N((CH_2)_7CH_3)_3)_2Fe_4S_4(SC_6H_5)_4$ in toluene solution mediated the terminal reduction of methylviologen in aqueous solution. That $Fe_4S_4(SC_6H_5)_4^{2-/3-}$ mediated the electron transfer was demonstrated by using optical and EPR spectroscopies. The average molar ratio of electrons/hydrogen ions transported for 11 trials was found to be 1.18 ± 0.24. Valinomycin, a potassium ionophore, did not diminish the cotransport of hydrogen ions.

Iron–sulfur proteins are found in plants, bacteria, and mammals. They function in a wide range of reactions, including electron transport, enzymatic functions, and perhaps in regulation as well. Iron–sulfur proteins may be involved in proton translocation in energy-transducing membranes.[1,2] This possible energy conservation role, with consideration that some hydrogenases contain 4Fe–4S centers, suggests that reduction-linked hydrogen ion binding may be a functionally important property for some iron–sulfur proteins.

Soluble low-molecular-weight iron–sulfur proteins generally exhibit some pH dependence in their midpoint reduction potentials.[3,4] Previous studies[5] have shown that *Clostridium pasteurianum* ferredoxin can exhibit reduction-linked hydrogen ion binding in a manner consistent with two equivalent proton binding sites with $pK_{ox} = 7.4$ and $pK_{rd} = 8.9$. Whether the peptide or the $Fe_4S_4Cys_4$ cluster provides the site(s) of proton binding in this protein has not yet been established.

pH-dependent behavior of several $Fe_4S_4(SR)_4^{n-}$ model complexes has been reported. Job and Bruice[6] have reported an optically detected pK_a of 7.4 for the water-soluble complex $Na_5(n\text{-}C_4H_9)_4NFe_4S_4(SCH_2CH_2COO)_4$. Additionally, Bruice et al.[7] have reported a kinetically determined pK_a of 3.92 for $((n\text{-}C_4H_9)_4N)_2Fe_4S_4(SCH_2CH(CH_3)_2)_4$ in 60/40 (v/v) *N*-methylpyrrolidinone/H_2O. A pH dependence of approximately 60 mV/pH unit has been reported[8] for the reduction potential of $((n\text{-}C_4H_9)_4N)_2Fe_4S_4(SC_6H_4(n\text{-}C_8H_{17})\text{-}p)_4$ and two related complexes in aqueous micellar solution, although a species adsorbed onto the mercury electrode may actually have been observed.

Studies are described here which demonstrate that the model compound $(CH_3N((CH_2)_7CH_3)_3)_2Fe_4S_4(SC_6H_5)_4$ in toluene solution can mediate electron transfer between two aqueous phases and in concert translocate hydrogen ions stoichiometrically.

Experimental Section

Cr^{II}edta Solution. Solid $CrCl_2 \cdot 4H_2O$ was prepared according to Holah and Fackler,[9] with use of pure Cr metal obtained from Alfa Inorganics. Because of the tendency of Cr(II) to polymerize in solution near neutrality, Cr^{II}edta was prepared for each experiment from a stock 0.5 M solution of $CrCl_2$ in 0.1 M HCl. A typical solution of Cr^{II}edta was prepared by adding 750 μL of 0.2 M edta (pH 5.1) to 300 μL of 0.5 M $CrCl_2$, followed by addition of 3.0 mL of 0.1 M Tris-Cl/0.1 M KCl buffer, pH 8.5. The final solution contained 37 mM Cr^{II}edta, pH 7.5.

Synthesis of the Iron–Sulfur Complex. A methanolic solution of $Na_2Fe_4S_4(SC_6H_5)_4$ was prepared as previously described.[10] $(CH_3N((CH_2)_7CH_3)_3)_2Fe_4S_4(SC_6H_5)_4$ was prepared by the addition of 2 mol of $(CH_3N((CH_2)_7CH_3)_3)Cl$ (Aldrich)[11] per mole of cluster in methanol. The complex was isolated after storage at −40 °C. The resulting black solid was recrystallized twice from warm CH_3CN/MeOH and dried in vacuo.[12] The complex is freely soluble in toluene, and as such is the first reported $Fe_4S_4(SR)_4^{n-}$ complex soluble in a non-water-miscible solvent.

Spectral Studies. Electron paramagnetic resonance (EPR) spectra were recorded with a Varian V-4500 X-band spectrometer equipped with a Heli-Tran liquid helium transfer system (Air Products). The frequency was measured with a Hewlett-Packard 532A frequency meter, and α,α′-diphenyl-β-picrylhydrazyl (DPPH) was used as a *g* marker.

Optical spectra were obtained with use of quartz cells of 1-, 2-, or 10-mm path length fitted with cotton plugs containing aqueous $CrCl_2$ and sealed from the atmosphere with rubber septums. Spectra were recorded with a Cary 219 recording UV–visible spectrometer.

The Electron-Transport System

In these experiments a sequential system for electron transfer was used, where aqueous Cr^{II}edta was the electron donor, and $(CH_3N((CH_2)_7CH_3)_3)_2Fe_4S_4(SC_6H_5)_4$ (Fe–S complex) in toluene solution mediated the terminal reduction of methylviologen in aqueous solution. Because of the air sensitivity of these species, all reactions and transfers were conducted in a glovebox fitted

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(11) The cation is a mixture of C_8–C_{10} chains, with C_8 predominating.
(12) Anal. Calcd. for $C_{74}H_{128}Fe_4N_2S_8$: Fe, 14.64; N, 1.84; S, 16.81. Found: Fe, 13.56; N, 1.82; S, 15.69.[11]

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Table I. Relationship between the Concentration of Reduced Methylviologen Produced and the Generated Hydrogen Ion Concentration in Several Electron-Transport Experiments[a]

expt	concn of Fe–S complex, mM	pH in the methylviologen phase		concn of H^+ generated, mM	concn of reduced methylviologen, mM	ratio of reduced methylviologen to hydrogen ion
		initial	final			
1	5.55	8.12	7.33	0.19	0.15	0.79
2	4.44	7.58	6.83	0.13	0.10	0.77
3	4.35	8.16	7.52	0.18	0.25	1.4
4	4.35	8.89	8.16	0.13	0.18	1.4
5	4.40	8.12	7.43	0.18	0.35	1.9
6	5.10	8.14	7.40	0.19	0.29	1.5
7	1.95	8.13	7.55	0.16	0.21	1.3
8	1.95	8.15	7.68	0.13	0.14	1.1
9	1.95	8.15	7.83	0.09	0.09	1.0
10	2.23	8.15	6.83	0.26	0.27	1.0
11[b]	1.52	8.26	7.65	0.17	0.13	0.76

[a] Iron–sulfur complex in toluene solution was vigorously agitated with 37 mM Cr^{II}edta in 74 mM Tris-Cl/74 mM KCl aqueous buffer, pH 7.5, in a v/v ratio of approximately 1. The toluene phase, containing reduced iron-sulfur complex, was then transferred to a tube containing 10 mM methylviologen in 0.5 mM Tris-Cl/100 mM KCl aqueous buffer, pH ~8, and agitated. The reduction of methylviologen was quantitated optically, and the concentration of hydrogen ion generated was determined by pH measurement and comparison to a previously determined titration curve. Experiment 11 contained, in addition to the iron-sulfur complex, 6.5 mM valinomycin in the toluene phase with 74 and 100 mM KCl in the electron-donor and electron-acceptor aqueous phases, respectively. [b] Valinomycin control.

with an oxygen scrubbing atmosphere recirculation system, under a 90% nitrogen/10% hydrogen atmosphere.

Cr^{II}edta was chosen as the reductant because of its demonstrated ability to rapidly reduce $Fe_4S_4(SR)_4^{2-}$ complexes.[13] Methylviologen was chosen as the terminal electron acceptor because of the change from colorless to blue on reduction of an aqueous solution and because of the pH independence of its reduction potential.[14]

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 was used, although the exact concentrations and volumes used varied between experiments. After a wait of 10–20 min 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 methylviologen in 0.5 mM Tris/100 mM KCl buffer (pH ~8). This mixture was agitated for approximately 3 min, and the immiscible phases were allowed to separate. The toluene phase was then removed from the top of the methylviologen solution. Reduction was evidenced by appearance of a deep blue color in the methylviologen phase. The number of moles of reduced methylviologen generated was measured optically ($\epsilon^{600} = 11.8$ cm^{-1} mM^{-1} [15]). The number of moles of hydrogen ions generated in the methylviologen aqueous phase was determined by pH measurements before and after the experiment, followed by comparison to an experimental titration curve.

The results of several experiments indicating the number of moles of methylviologen reduced and the number of moles of protons generated are listed in Table I. The efficiency of electron transport averaged approximately 55% of the theoretical maximum for the 11 trials. While the numbers for proton transport varied among experiments, in all cases a drop in the pH was observed to be coincident with the reduction of methylviologen. The average molar ratio of reduced methylviologen to transported hydrogen ions (MV/H^+) for these 11 experiments was 1.18 ± 0.24. The reduction potential of methylviologen is pH independent,[4,14] so it is assumed that methylviologen does not bind hydrogen ions on reduction.

Evidence of Electron Transport Mediated by the Iron–Sulfur Complex

Evidence suggesting that electron transport between Cr^{II}edta and methylviologen was actually mediated by the Fe–S complex and not by a contaminant or by solubilized Cr^{II}edta was obtained several ways. After exposure of the Fe–S model to the aqueous Cr^{II}edta phase, an EPR spectrum characteristic of reduced 4 Fe–4 S complex was obtained. While the *g* values for this approximately axial spectrum ($g_{\parallel} = 2.02$, $g_{\perp} = 1.90$) are not identical with reported values obtained in other solvents, some variation between solvents has been previously noted.[16] This observation of reduced Fe–S complex is consistent with optical experiments which show the characteristic bleaching expected for reduction of $Fe_4S_4(SR)_4^{2-}$ complexes.[17,18] After reoxidation by exposure to methylviologen, 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 methylviologen. If $CH_3N((CH_2)_7CH_3)Cl$ 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 methylviologen observed. Atomic absorption analysis of the toluene phase in the presence of Fe–S complex and after exposure to aqueous Cr^{II}edta indicates that the chromium concentration is only 1 or 2 μM, too low to contribute significantly to the observed electron transport.

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 methylviologen ultimately produced. This was done to demonstrate that the only major species mediating electron transport between Cr^{II}edta and methylviologen was the Fe–S complex. For these estimates the extinction coefficient reported for oxidized $((CH_3CH_2)_4N)_2Fe_4S_4(SC_6H_5)_4$ in DMF solution[19] was used, $\epsilon^{457} = 17.7$ cm^{-1} mM^{-1}. Further, on the basis of results of sequential treatment of the Fe–S complex with Cr^{II}edta to ensure full reduction, the ratio $A_{ox}/A_{rd} = 0.61$ at 457 nm was used for the Fe–S complex. Thus the concentration of the Fe–S complex in toluene solution was optically measured, and the extent of reduction after treatment with Cr^{II}edta was calculated on the basis of the optical absorbance at 457 nm to determine the number of moles of reduced iron–sulfur complex produced. The reduced Fe–S complex was reoxidized with use of aqueous methylviologen, and the number of moles of reduced methylviologen produced was determined optically. In this

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manner, ratios of reduced Fe–S complex to reduced methylviologen of 0.88 and 1.01 were estimated in two experiments. If the Fe–S complex were the sole electron transfer agent, a ratio of 1 would be expected. In view of the uncertainty in the values of the extinction coefficients of the oxidized and reduced Fe–S complex, the experimentally obtained ratios of 0.88 and 1.01 agree quite well with the expected value of 1.0. This indicates that the number of moles of electrons transferred to methylviologen can be reasonably accounted for by the number of moles of reduced Fe–S complex present.

The optical and EPR evidence demonstrating the presence of reduced Fe–S complex, in conjunction with the control electron-transfer experiments and the quantitative optical studies, demonstrates that the Fe–S complex was the predominant mediator of electron transfer between Cr^{II}edta and methylviologen.

Evidence of Reduction-Linked Proton Transport

As shown in Table I, the average molar ratio of electrons transported (as represented by reduced methylviologen) to hydrogen ions generated was 1.18 ± 0.24 for the 11 experiments.

This observed change in pH suggests that the Fe–S complex is cotransporting electrons and hydrogen ions by a mechanism of reduction-linked proton binding. However, two other possible origins for the observed pH changes should be considered: (1) uptake of hydroxide by the Fe–S complex upon reoxidation; (2) proton transport by the Fe–S complex driven by separation of charge between the toluene and aqueous phases.

The first possibility, hydroxide uptake, is improbable. The site of association of the hydroxide anion would presumably be an iron atom. The iron atoms in this complex are in tetrahedral sulfur environments and therefore do not provide a likely site for hydroxide binding. Additionally, the X-ray structures of several $Fe_4S_4(SR)_4^{2-}$ complexes have been reported,[10a,20,21] and in no case has a bound hydroxide been reported.

The second possibility, that of forced proton transfer, can be tested by using valinomycin. The electron-transport system, as constructed, would generate a separation of charge between each phase boundary if the electron transfers were not electrically neutral. If no cation were transported with the electron, only a small amount of electron transport would occur before a substantial electric potential was generated. In effect, this generated potential could act as a driving force to artificially couple electron and proton transport. For example, a hydrated proton might be electrostatically pulled into the toluene phase, forming a noncovalent association with the Fe–S complex. On reoxidation of the Fe–S complex and passage of the electron into the methylviologen aqueous phase, the hydrated proton would follow. To test this hypothesis, the antibiotic valinomycin was used. Valinomycin is a ring carrier ionophore that can permit K^+ ion to pass into the hydrophobic toluene phase. This prevents the buildup of a substantial electrical potential due to separation of charge between the toluene and aqueous phases. 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. Table I (experiment 11) gives the results of a trial where 74 and 100 mM KCl were present in the Cr^{II}edta and the methylviologen phases, respectively, and the toluene phase contained 6.5 mM valinomycin and 1.5 mM Fe–S complex. Because no significant increase was observed either in the ratio of electron/protons transferred or in the number of moles of electrons transported, it is concluded that the second possibility, that of proton transport driven by the separation of charge, is not the dominant mechanism.

Thus the 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.

Conclusion

In the directional electron transport system, as constructed, the Fe–S complex can act to transport electrons between aqueous phases. Further, electron transport is coupled to a directional proton transport. These results suggest that the $Fe_4S_4Cys_4$ cluster in iron–sulfur proteins may have an intrinsic reduction-linked hydrogen ion binding behavior. These clusters may provide the site of such binding in *Clostridium pasteurianum* ferredoxin[5] and further may be functionally important sites of proton binding in some iron–sulfur systems.

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Registry No. $(CH_3N((CH_2)_7CH_3)_3)_2Fe_4S_4(SC_6H_5)_4$, 97135-58-5; $Na_2Fe_4S_4(SC_6H_5)_4$, 97135-59-6; $(CH_3N((CH_2)_7CH_3)_3)$, 5137-55-3; H^+, 12408-02-5.

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