J. Am. Chem. Soc. **1982**, *104*, 4986–4989

Cooperativity in Chemical Model Systems: Ligand-Induced Subunit Dimerization

T. G. Traylor,* M. J. Mitchell, J. P. Ciccone, and S. Nelson

Department of Chemistry, University of California
San Diego, La Jolla, California 92093

Received September 23, 1981

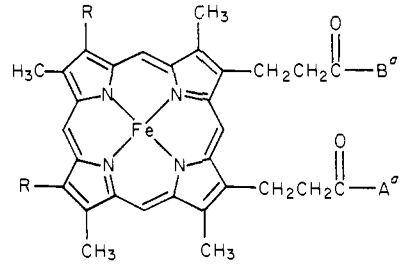
Cooperative reactivity in solution is common in biological systems but not in purely chemical systems.[1,2] In its simplest form, cooperativity is displayed when multiple identical chemical sites communicate by an allosteric mechanism so that reaction at one of the sites makes the same reaction at a second site more favorable.[3] This process can be accomplished either by ligand-induced aggregation or by ligand-induced conformational change in a preformed aggregate or other multisite system. The few attempts to model allosteric effects with small molecules have dealt with the latter mechanism.[4–6] We now describe a class of model metalloporphyrin compounds that display cooperativity through ligand-induced dimerization.

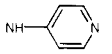
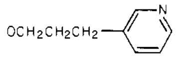
The most thoroughly studied cooperative system is hemoglobin, in which four almost identical heme sites react with either dioxygen or carbon monoxide as described above.[7] Hemoglobin has been treated as a two-state system in which its low-affinity (T-state) form is converted to the high-affinity (R-state) form by a conformational change that occurs as ligands sequentially bind to the hemes.[7–9] Plots of the fraction of hemoglobin sites occupied (Y) vs. concentration of ligand ([L]) are sigmoid shape as a result of cooperativity. The Hill plot ($\log (Y/(1-Y))$ vs. $\log$ [L]) gives a slope, n, of 2.8 for hemoglobin compared to 1.0 for myoglobin.[7] The Hill coefficient, n, is commonly used as a test for positive cooperativity ($n > 1$).

We have modeled this two-state cooperative binding with the protoporphyrin IX derivative **1** (Chart I). In this compound the side chain is too short to bind internally to form chelated heme.

In both the Fe^{III} and Fe^{II} states the affinity of four-coordinated iron porphyrins for the first added ligand is often lower than that for the second. This effect is especially large when the first and second ligands are donor–acceptor pairs such as imidazole, CO (with Fe^{II} porphyrin) or pyridine, CN^- (with Fe^{III} porphyrin). As a result of this effect the side chain base metalloporphyrin **1** (or $\mathbf{1}^+Cl^-$), which might exist in the four-coordinated state in solution, tends to aggregate or dimerize when a ligand (CO or CN^-) is added in order to make the base–Fe bond. This, along with the neighboring group effect, results in the two-state cooperative system described in eq 1–5 (Scheme I). The cyclization (eq 3b) results from the same proximity effect that makes the chelated (long base side chain) hemes assume a five-coordinated structure.[11,12] The equilibria connecting these species are shown in Scheme II, where H^+ is the form with B protonated.

Chart I. Protoporphyrin Derivatives[10]



compd^b	R	A	B
1	CH=CH$_2$	OCH$_3$	
2	Et	OCH$_3$	
3	CH=CH$_2$	OCH$_3$	OH

^{a} Compounds **1** and **2** are mixtures of isomers having these side chains interchanged. ^{b} The forms of these compounds are illustrated with **1**: **1** = Fe^{II} form; $\mathbf{1}^+Cl^-$ = $Fe^{III}Cl^-$ form; **1P** = free porphyrin derivative.

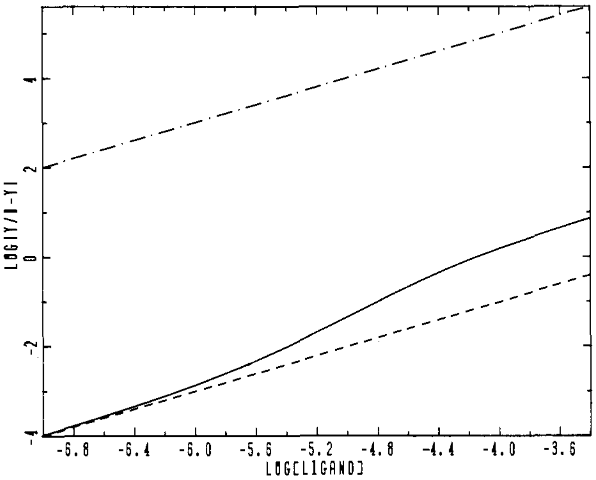


Figure 1. Plots of $\log (Y/(1-Y))$ vs. $\log$ [L] using Y calculated from eq 8 with [L] and K values in M: line 1 (–·–) total heme concentration $[H]_{tot} = 5 \times 10^{-7}$ M, $K_1 = 10^{-9}$, $K_2 = 10^{-6}$, $K_5 = 10^{-10}$, $K_a = 4 \times 10^{-9}$, pH = 7.0, slope = 1.0, $[L]_{1/2} = 10^{-9}$ M; line 2 (- - -) total heme concentration $[H]_{tot} = 5 \times 10^{-7}$ M, $K_1 = 10^{-3}$, $K_2 = 100$, $K_5 = 10^{-10}$, $K_a = 4 \times 10^{-9}$, pH = 2.0, slope = 1.0, $[L]_{1/2} = 9.3 \times 10^{-4}$ M; line 3 (—) total heme concentration $[H]_{tot} = 5 \times 10^{-7}$ M, $K_1 = 10^{-3}$, $K_2 = 100$, $K_5 = 10^{-10}$, $K_a = 4 \times 10^{-9}$, pH = 7.0, maximum slope = 1.7, $[L]_{1/2} = 6.9 \times 10^{-5}$ M.

We can write Y, the fraction of total heme in the ligated form, as a function of the free heme concentration [H], proton concentration, and the equilibrium constants as shown in eq 8. The free heme [H] used in eq 8 is calculated from the total heme, $[H]_{tot}$, according to eq 9. We have used published values[13,14] of ligation constants of four- and five-coordinated hemes for both [L] and base as approximations of K_1, K_2, and K_5 and have programmed eq 8 to explore the ranges of values of constants and heme concentrations that lead to the highest extent of cooperativity, represented by the highest n value. We assume that $K_5 = K_4$ as a result of the preference for the cyclic form of H_2.[12]

Figure 1 shows plots of $\log (Y/(1-Y))$ vs. $\log$ [L] from eq 8, with conditions considered to be attainable with the **1** + CO system.[13,14] One set of conditions, line 1, employed a very small dissociation constant, K_2, which results in no cooperativity, n = 1, and high affinity. Alternatively, very low pH values (e.g., pH ~2) also remove cooperativity by complete protonation of the base with and without carbon monoxide, but the affinity is low (line 2). A high dissociation constant, K_1, for the monomer, a high

---

[1] Walsh, C. "Enzymatic Reaction Mechanisms"; W. H. Freeman: San Francisco, 1979; pp 157–162.

[2] Rebek, J., Jr.; Wattley, R. V.; Costello, T.; Gadwood, R.; Marshall, L. *J. Am. Chem. Soc.* **1980**, *102*, 7398–7400.

[3] Wyman, J., Jr. *Adv. Prot. Chem.* **1964**, *19*, 223–286.

[4] Rebek, J., Jr.; Wattley, R. V.; Costello, T.; Gadwood, R.; Marshall, L. *Angew. Chem., Int. Ed. Engl.* **1981**, *20*, 605–606. The biphenyl bis(crown ether) described in this paper appears to be the only other solution study displaying a Hill coefficient greater than 1.

[5] Lehn, J. M. *Acc. Chem. Res.* **1978**, *11*, 49–57.

[6] Traylor, T. G.; Tatsuno, Y.; Powell, D. W.; Cannon, J. B. *J. Chem. Soc., Chem. Commun.* **1977**, 732–734.

[7] Antonini, E.; Brunori, M. "Hemoglobin and Myoglobin in Their Reactions with Ligands"; Elsevier: New York, 1971; (a) pp 381–414; (b) pp 179–184.

[8] Edelstein, S. J. *Ann. Rev. Biochem.* **1975**, *44*, 209–232.

[9] Perutz, M. F. *Ann. Rev. Biochem.* **1979**, *48*, 327–386.

[10] Compound $\mathbf{1}^+Cl^-$ was prepared by coupling 4-aminopyridine with protohemin chloride monomethyl ester, $\mathbf{3}^+Cl^-$, by methods previously described.[12] Compound $\mathbf{2}^+Cl^-$ was obtained from a previous study.[11]

[11] Traylor, T. G.; Chang, C. K.; Geibel, J.; Berzinis, A.; Mincey, T.; Cannon, J. *J. Am. Chem. Soc.* **1979**, *101*, 6716–6731.

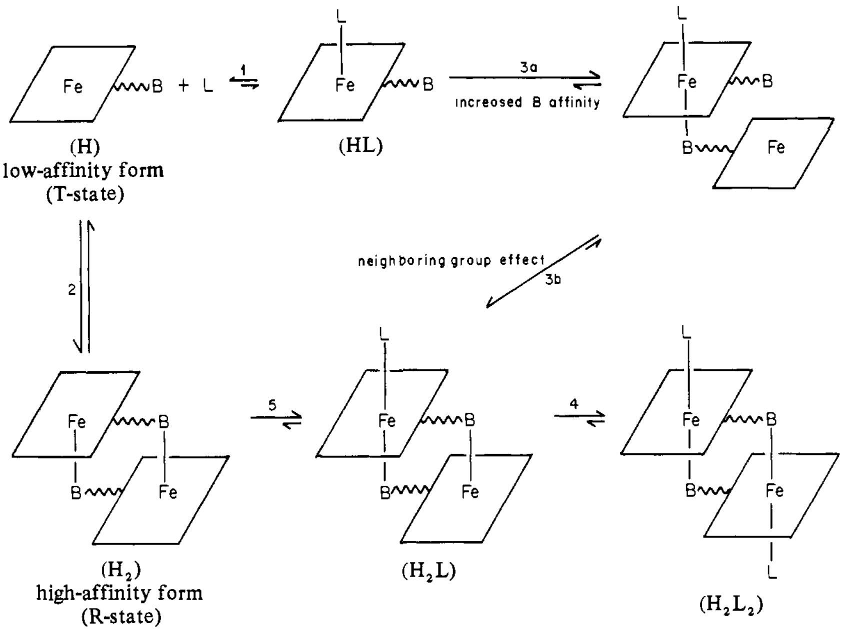
[12] Cannon, J.; Geibel, J.; Whipple, M.; Traylor, T. G. *J. Am. Chem. Soc.* **1976**, *98*, 3395–3396.

[13] Rougee, M.; Brault, D. *Biochemistry* **1975**, *14*, 4100–4106.

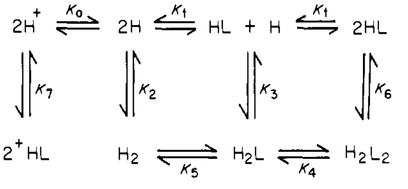
[14] White, D. K.; Cannon, J. B.; Traylor, T. G. *J. Am. Chem. Soc.* **1979**, *101*, 2443–2454.

0002-7863/82/1504-4986$01.25/0

Scheme I



Scheme II



$$Y = \{([H][L]/K_2K_5)(1 + [L]/2K_5) + ([L]/K_1) \times (1 + [H^+]/K_a)\}/\{(2[H]/K_2)(1 + [L]/2K_5)^2 + (1 + [H^+][L]/K_1K_a + [H^+]/K_a + [L]/K_1)\} \quad (8)$$

$$[H] = \left\{-\left(1 + \frac{[H^+][L]}{K_1K_a} + \frac{[H^+]}{K_a} + \frac{[L]}{K_1}\right) + \left[\left(1 + \frac{[H^+][L]}{K_1K_a} + \frac{[H^+]}{K_a} + \frac{[L]}{K_1}\right)^2 + \frac{8[H]_{tot}}{K_2}\left(1 + \frac{[L]}{2K_5}\right)^2\right]^{1/2}\right\} \Big/ \frac{4}{K_2}\left(1 + \frac{[L]}{2K_5}\right)^2 \quad (9)$$

dissociation constant for the dimer, K_2, and a low value of K_5 result in cooperativity for two sites, $n = 1.7$ (line 3). Intermediate values of these constants result in n values ranging between 1 and 2. These computer simulations clearly predict cooperativity for such nonchelated tail base hemes.

This prediction is realized in the titration of 1^+Cl^- with cyanide ion. Titration of protohemin with cyanide was previously studied both in water and in cetyltrimethylammonium bromide (CTAB).[15,16] These studies showed that pyridine binds very poorly to hemin and that addition of pyridine to hemin greatly increases CN^- affinity while addition of CN^- to hemin greatly increases pyridine affinity. These are the conditions required for cooperativity according to Scheme II ($K_1 >> K_5$, $K_2 >> K_3$).

The side-chain pyridine heme 1^+Cl^- was titrated with cyanide in pH 6.85 buffered solutions of myristyltrimethylammonium bromide (MTAB) suspension. This compound was converted cleanly with isosbestic points to the pyridine–hemin–CN^- dimer as indicated by its characteristic UV–visible spectrum. Figure 2 shows a series of difference spectra for this titration, described by eq 10. The concentration of free cyanide ion at half conversion, $[CN^-]_{1/2}$, was 4×10^{-7} M. Further addition of cyanide resulted in a second isosbestic titration to the dicyanide (eq 11) (Figure 3) with $[CN^-]_{1/2} = 10^{-5}$ M. Hill plots of the first and second titration are shown in Figure 4 along with a similar titration of pyridine-chelated mesohemin 2^+Cl^-. This compound also displayed a clean two-step titration according to eq 12 and 13. Although

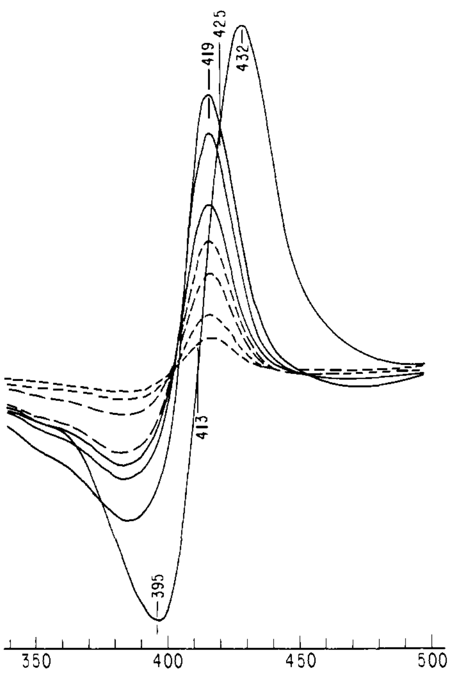
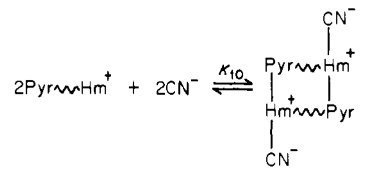


Figure 2. Difference spectra in the titration of 7.1×10^{-6} M tailed pyridine hemin 1^+Cl^- in 2% myristyltrimethylammonium bromide–0.1 M phosphate buffer at pH 6.85. The spectrum was taken in the absence of NaCN, stored, and subtracted from each of the subsequent spectra in the microprocessor-controlled Uvikon spectrophotometer. Increasing peaks at 419 nm correspond to the following concentrations of total added NaCN: 3.1×10^{-5}, 4.8×10^{-5}, 7.1×10^{-5}, 8.6×10^{-5}, 11×10^{-5}, 17×10^{-5}, 45×10^{-5} M. The curve with the 432-nm maximum is at 0.1 M NaCN.

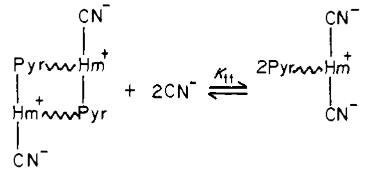
---

[15] Simplicio, J.; Schwenzer, K. *Biochemistry* **1973**, *12*, 1923–1929. These authors obtain spectra like those we observe for the Fe^{III} forms and indicate that this form is the Fe^{III}–OH species in CTAB. All three compounds studied here are in this state at pH 7.

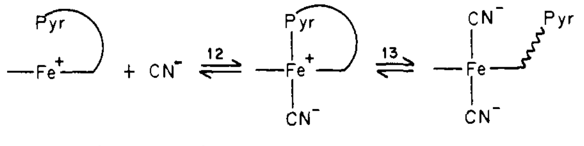
[16] Phillips, J. M. *Rev. Pure Appl. Chem.* **1960**, *10*, 35–60.



$$2\,\mathrm{Pyr{\sim}Hm^+} + 2\,\mathrm{CN^-} \overset{K_{10}}{\rightleftharpoons} \begin{array}{c} \mathrm{CN^-}\\ | \\ \mathrm{Pyr{\sim}Hm^+} \\ |\qquad | \\ \mathrm{Hm^+{\sim}Pyr}\\ | \\ \mathrm{CN^-}\end{array} \qquad (10)$$



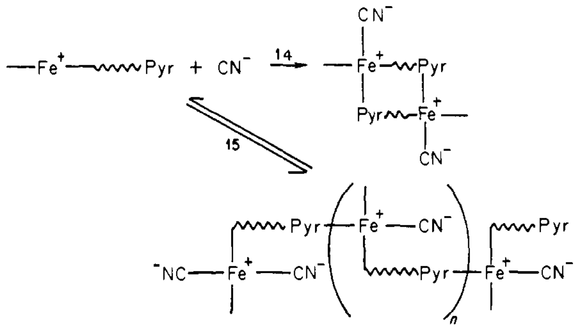
$$\begin{array}{c} \mathrm{CN^-}\\ | \\ \mathrm{Pyr{\sim}Hm^+} \\ |\qquad | \\ \mathrm{Hm^+{\sim}Pyr}\\ | \\ \mathrm{CN^-}\end{array} + 2\,\mathrm{CN^-} \overset{K_{11}}{\rightleftharpoons} \begin{array}{c} \mathrm{CN^-}\\ | \\ 2\,\mathrm{Pyr{\sim}Hm^+} \\ | \\ \mathrm{CN^-}\end{array} \qquad (11)$$



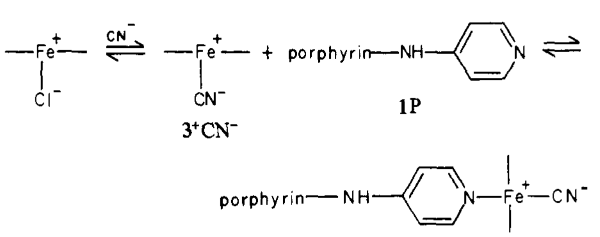
titration of 1^+Cl^- and 2^+Cl^- both proceeded in two well-separated steps through a pyridine–Fe^{III}–CN to the $Fe^{III}(CN^-)_2$ species, titration of protohemin dimethyl ester (3^+Cl^-) went directly to the $Fe^{III}(CN^-)_2$ species without an intermediate. The latter result resembles that obtained by Simplicio and Schwenger[15] using protohemin itself.

The slopes obtained for the chelated hemin, 2^+Cl^- ($n = 1.1$), and the tailed hemin, 1^+Cl^- ($n = 2.1$), indicate maximum cooperativity for the latter. The result of this cooperativity on CN^- affinity is interestingly similar to the effect in hemoglobin cooperativity. The high-affinity state, represented by 2^+Cl^-, has $[CN^-]_{1/2} = 1.2 \times 10^{-8}$ M; the low-affinity, four-coordinated state, protohemin dimethyl ester, has a second K larger than the first and thus $[CN^-]_{1/2} > 1.6 \times 10^{-6}$ M for the first CN^- addition. The cooperative system, 1^+Cl^-, has $[CN^-]_{1/2} = 4 \times 10^{-7}$ M.

The six-coordinated Pyr–Hm^+–CN^- species obtained upon titration of 1^+Cl^- could be either a dimer or a long-chain polymer, shown in eq 14 and 15. The polymerization requires that the



cyanopyridine compound 1^+CN^- combine with the pyridine of a second 1^+Cl^- or 1^+CN^- without the cyclization of eq 14. An equivalent combination would be the cyanoprotohemin monomethyl ester 3^+CN^- plus the protoporphyrin 4-pyridylamide **1P** (eq 16).



1P

3^+CN^-

(16)

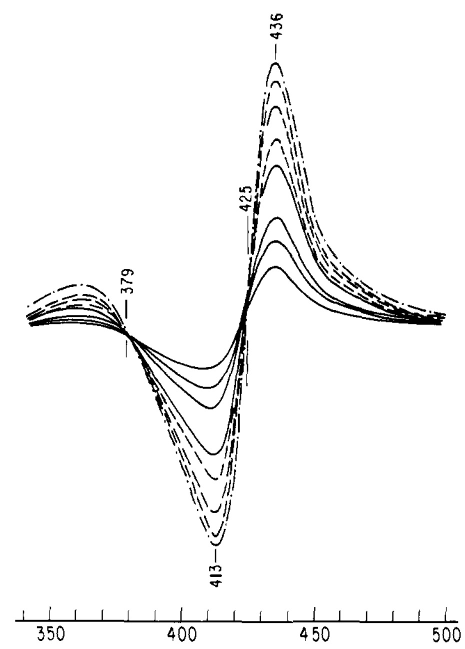


Figure 3. Difference spectra for titration of 8.5×10^{-6} M 1^+Cl^- from $(1^+CN^-)_2$ to $1^+(CN^-)_2$ obtained as above. The spectrum at 4.5×10^{-4} M NaCN (total concentration), the end point for the first titration, was recorded and subtracted from subsequent spectra taken at 1.0×10^{-3}, 1.5×10^{-3}, 2.0×10^{-3}, 3.0×10^{-3}, 3.9×10^{-3}, 4.9×10^{-3}, 9.7×10^{-3}, and 100×10^{-3} M total NaCN, corresponding to increases at 436 nm. The actual Soret maxima for 1^+Cl^-, $(1^+CN^-)_2$, and $1^+(CN^-)_2$ are 400, 415, and 430 nm, respectively.

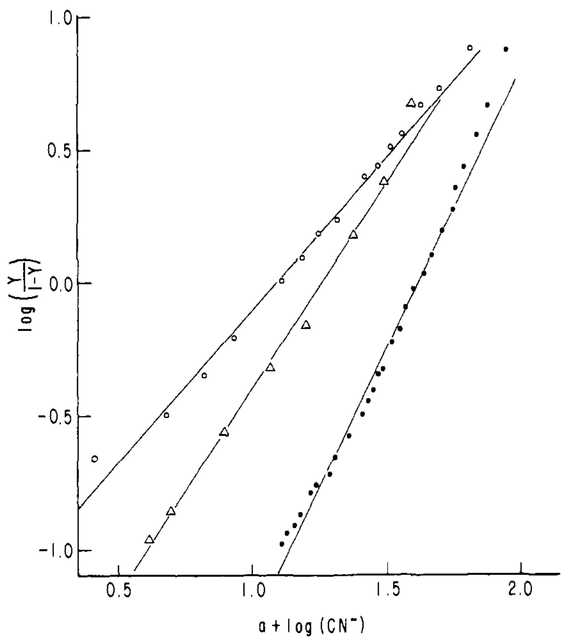
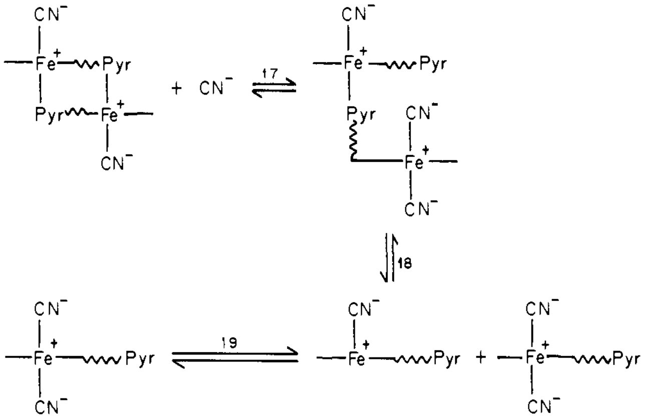


Figure 4. Plots of log $(Y/(1 - Y))$ vs. log (CN^-) for titrations with NaCN at pH 6.85 in 2% myristyltrimethylammonium bromide in 0.1 M phosphate buffer: $(CN^-) \equiv$ free cyanide ion concentration calculated by subtracting that bound to hemin and dividing the resultant by the ratio of HCN to CN^- at the appropriate pH; (○) titration of 6.8×10^{-6} M 2^+Cl^-, $a = 9$, slope 1.1; (●) titration of 7.4×10^{-6} M 1^+Cl^-, $a = 8$, slope = 2.1; (△) titration of 7.4×10^{-6} M 1^+Cl^-, $a = 5$, slope = 1.5 (pH 7.1). The slight curvature in these plots is due to errors at the low and high saturation. Other plots do not show this curvature.

Compounds 3^+CN^- and **1P** should have solubility (activity) characteristics similar to that of 1^+Cl^-, but the mixture cannot form the cyclic dimer. The mixture of 5 μM 3^+Cl^- and 5 μM **1P** was titrated with sodium cyanide at pH 6.85 in MTAB suspension. Except for a slight early appearance of a species whose Soret absorption resembles that of Pyr–Hm^+–CN^-, the titration resembled that of protohemin dimethyl ester, going almost directly to hemin$(CN^-)_2$ with a $[CN^-]_{1/2} = 1.3 \times 10^{-6}$ M compared to 1.6×10^{-6} M for the protohemin monomethyl ester. The failure of this mixture to form the six-coordinated Pyr–Hm^+–CN^- species indicates that the noncyclic formation of the same species (eq 15) in earlier experiments is highly unlikely.

This conclusion is strengthened by a second cooperative reaction, seen in the titration of the dimer $(Hm^+CN^-)_2$ to the monomer $Hm^+(CN^-)_2$ of $\mathbf{1}^+Cl^-$. The slope for this titration gives a Hill coefficient of 1.5, consistent with reactions 17–19 (Figure 4).



Reaction 17 is a low-affinity equilibrium due to the neighboring-group effect, which resists the addition of the first but not the second CN^-. It thus appears that the highly stabilized dimer form, $(\mathbf{1}^+CN^-)_2$, dissociates either CN^- or pyridine in a highly cooperative fashion.

The availability of these simple chemical systems makes possible studies of allosteric effects and the nature of cooperativity. Further studies of the effect of pH, base affinities, etc. on both ligand-induced dimerization and ligand-induced conformational change[6] are underway.

Acknowledgment. We are grateful to the National Institutes of Health (Grant HL-13581) for support of this research and to Professor Jack Kyte for helpful discussions.

Registry No. $\mathbf{1}^+Cl^-$, 82374-44-5; $\mathbf{2}^+Cl^-$, 72172-03-3.

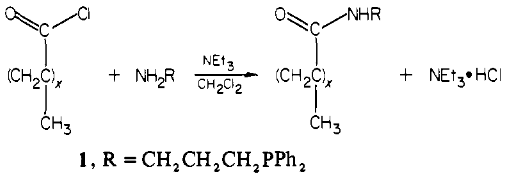
Preparation and Spectroelectrochemical Characterization of Silicon Electrodes Modified with Molybdenum Dinitrogen Complexes

Daniel L. DuBois* and John A. Turner

Photoconversion Research Branch
Solar Energy Research Institute
Golden, Colorado 80401

Received April 26, 1982

In this paper we report the modification of silicon electrodes with polymer-bound molydenum dinitrogen complexes and the characterization of these electrodes by cyclic voltammetry and Fourier transform–infrared spectroscopy. The condensation of (3-aminopropyl)bis(2-(diphenylphosphino)ethyl)phosphine,[1] **3**, with poly(methacryloyl chloride) produces a methacrylamide polymer with pendant tridentate phosphine ligands (eq 1).[2] In a similar manner reactions of poly(methacryloyl chloride) with Ligands **1** and **2** yield polymers containing monodentate and bidentate phosphine ligands, poly-P_1 and poly-P_2, respectively. Infrared spectra of the products of these condensation reactions have a strong amide carbonyl band at 1668 cm^{-1}. In addition there are acid and acid chloride carbonyl bands at 1710 and 1770 cm^{-1}, respectively, due to the failure of some of the sites of the original polymer to react with the amine functional group of the ligand.[3] These unreacted polymer sites do not interfere with subsequent chemistry, however.



$$(CH_2\overset{\overset{O\diagdown C \diagup Cl}{|}}{\underset{\underset{CH_3}{|}}{C}})_x + NH_2R \xrightarrow[CH_2Cl_2]{NEt_3} (CH_2\overset{\overset{O\diagdown C \diagup NHR}{|}}{\underset{\underset{CH_3}{|}}{C}})_x + NEt_3{\bullet}HCl \qquad (1)$$

1, $R = CH_2CH_2CH_2PPh_2$
2, $R = CH_2CH_2CH_2P(Ph)CH_2CH_2PPh_2$
3, $R = CH_2CH_2CH_2P(CH_2CH_2PPh_2)_2$

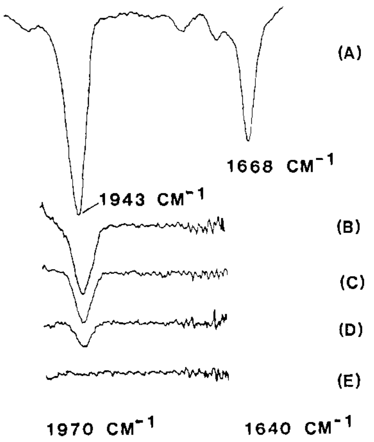


Figure 1. Transmission infrared spectra of a polymer-bound molybdenum dinitrogen complex prepared from 2 equiv of poly-P_1, 1 equiv of poly-P_3, and 1 equiv of $Mo(N_2)_2(PPh_2Me)_4$ on a 0.1 Ω cm *n*-type Si electrode. Spectrum A was taken in the absence of a supporting electrolyte solution. Spectra B–E are of the same electrode immersed in 0.3 N $LiClO_4$/methanol solution. Spectrum B was recorded before any cyclic voltammograms were recorded. Spectra C–E were taken after cyclic voltammograms 2, 5, and 6 of Figure 2. The cell path length was 0.5 mm, and each spectrum is the Fourier transform of 200 interferograms.

The lability of the phosphine ligands in $Mo(N_2)_2(PPh_2Me)_4$[4,5] provides a convenient method for introducing molydenum dinitrogen fragments into poly-P_1, poly-P_2, and poly-P_3. Reaction of poly-P_3 with $Mo(N_2)_2(PPh_2)Me)_4$ is accompanied by a shift in the infrared stretching frequency of the dinitrogen ligand from 1925 to 1943 cm^{-1}, indicating bonding between the pendant triphosphine ligands and molybdenum. Similar reations of $Mo(N_2)_2(PPh_2Me)_4$ with poly-P_2, poly-P_1, and a 2:1 mixture of poly-P_1 and poly-P_3 result in the formation of polymer pendant complexes with ν_{N_2} 1952 cm, 1925, and 1943 cm^{-1} respectively. Solutions of these reaction mixtures have been evaporated on silicon and carbon electrodes. These electrodes were then washed with hexanes and methanol to remove dimethylphenylphosphine and any unreacted $Mo(N_2)_2(PPh_2Me)_4$. This procedure results in electrodes coated with polymers containing molybdenum dinitrogen complexes.

A number of spectroscopic methods have been used previously to characterize derivatized electrodes including Auger,[6] ESCA,[7] and ultraviolet and visible spectroscopy,[7,8] We have taken advantage of the strong infrared absorption of coordinated dinitrogen and the infrared transparency of silicon to characterize our de-

---

(1) Uriarte, R.,Mazanee, T. J.; Tau, K. D.; Meek, D. W. *Inorg. Chem.* **1980**, *19*, 79.

(2) Reaction of poly(methacryloyl choride) with amines and alcohols has been used to generate other polymers of interest for electrode modification. Itaya, K.; Bard, A. J. *Anal. Chem.* **1978**, *50*, 1487. Degrand, C.; Miller, L. L. *J. Am. Chem. Soc.* **1980**, *102*, 5728. Fukni, M.; Kitani, A.; Degrand C.; Miller L. L. *Ibid* **1982** *104*, 28.

(3) From infrared absorption data it can be calculated that the mole percents of acid chloride, acid, and amide are 8.4%, 12.3%, and 79.3%, respectively. Anal. Calcd for C_4H_5OCl (8.4%), $C_4H_6O_2$ (12.3%), and $C_{35}H_{40}NOP_3$ (79.3%): C, 71.21; H, 6.87; N, 2.30; P, 15.29; Cl, 0.62. Found: C, 71.33; H, 6.69; N, 2.31; P, 15.07; Cl, 0.69. The ^{31}P NMR spectrum of poly-P_3 is a broad doublet (δ −12.9 relative to external H_3PO_4, J_{pp} = 27 Hz, $CDCl_3$) and a broad triplet (δ 22.1); the broadness of the resonances presumably result from slow tumbling of the phosphorus atoms upon incorporation into the polymer.

(4) George, T. A.; Kovar, R. A. *Inorg. Chem.* **1981**, *20*, 285.

(5) Chatt, J.; Heath, G. A.; Richards, R. L. *J. Chem. Soc., Dalton Trans.* **1979**, 2074.

(6) Bruce, J. A.; Wrighton, M. S. *J. Am. Chem. Soc.* **1982**, *104*, 74.

(7) William, K. W.; Rocklin, R. D.; Nowak, R.; Kuo, K.-N.; Schultz, F. A.; Murray, R. W. *J. Am. Chem. Soc.* **1980**, *102*, 7629.

(8) Scott, N. S.; Oyama, N.; Anson, F. C. *J. Electroanal. Chem.* **1980**, *110*, 303.

0002-7863/82/1504-4989$01.25/0