as the initial absorbance of the solution, A_t is the absorbances at varying time throughout reaction, and A_∞ is the final absorbance. All absorbance values were recorded at a fixed wavelength in the MLCT region. The slope of the plotted line is equal to the reaction rate constant k_{obsd}. Reaction rates were determined to be independent of ligand concentration over the 10^{-3}–10^{-1} M range. Rate data have been obtained at several temperatures, and these are listed in Table II. Activation parameters calculated from Arrhenius-type plots are also shown in Table II.

Rate data for dissociation of CO from $M(CO)_5$(daf) follows the order Mo > Cr > W, which is congruent with that previously established for the reactivity of the parent hexacarbonyls[7] and that of the calculated M–C force constants of $M(CO)_6$ in solution.[8] It is also noted that the observed reaction rates are considerably greater than values previously reported for CO or L dissociation in $M(CO)_5L$ complexes in which the entering species is an amine, phosphine, phosphite, or arsenide ligand.[9] This rate increase is attributed to a substantial contribution to the CO dissociation reaction when the associating ligand is already attached to the metal center in a monodentate manner. These results are in agreement with those previously reported for $M(CO)_5L$ complexes, where M = Cr or Mo, and L = a series of bidentate sulfur ligands.[10] In contrast, a number of $M(CO)_5L$ complexes, where L = a bidentate phosphorus or arsenic ligand, have been shown to undergo chelation via a largely dissociative-type mechanism.[11]

Activation energy parameters indicate that the $M(CO)_5$(daf) chelation process is largely enthalpy controlled. Furthermore, it is notable that the positive ΔS^* value obtained for the reaction of $Mo(CO)_5$(daf) suggests an increased amount of dissociative character in the transition state consistent with that reported for thermal substitution reactions of other Mo carbonyl complexes.[7,12]

The reaction product $W(CO)_4$(daf) was isolated. Anal. Calcd for $W(CO)_4$(daf): C, 38.8; H, 1.74; N, 6.04. Found: C, 39.3; H, 1.92; N, 5.45. Infrared bands (in chloroform) at 2011, 1897, 1877, and 1832 cm^{-1} are consistent with the C_{2v} arrangement of the CO groups in the complex.[13] The bands are assigned as $A_1{}^{1a}$, B_1, $A_1{}^{1b}$, and B_2, respectively.

Photolysis of $M(CO)_6$ solutions at 293 K containing dabp in benzene results in the formation of species having absorption maxima in the 385–391-nm region. As noted above, this absorption is typical of $M(CO)_5L$ species and is therefore assigned as a $^1A(e^4b_2{}^2) \rightarrow {}^1E(e^3b_2{}^2a^1)$ transition. However, an important difference is reported here. The $M(CO)_5$(dabp) photoproduct does not further react thermally to form the corresponding bidentate product, but instead it is stable and remains in solution. Thus, it appears for the $M(CO)_5$(dabp) complex that one of the ligand nitrogen atoms coordinates with the metal center, while the other nitrogen atom remains free.

The $M(CO)_5$(dabp) complexes have been isolated as solid complexes following prior formation of the tetrahydrofuran adduct (see Experimental Section). The infrared spectrum of $W(CO)_5$(dabp) has been recorded in chloroform and indicates four bands in the CO region centered at 2069, 1981, 1932, and 1900 cm^{-1}, consistent with the C_{4v} arrangement of the CO ligands. These bands correspond to the $A_1{}^1$, B_2, E_1, and $A_1{}^2$ modes, respectively.[13b,c] However, the intensity of the normally forbidden B_2 mode is enhanced, indicating that the CO ligands are somewhat distorted from ideal C_{4v} group symmetry. Anal. Calcd for $W(CO)_5$(dabp): C, 37.7; H, 1.27; N, 5.86. Found: C, 38.2; H, 1.42; N, 6.03.

This study has demonstrated the vast difference in coordination abilities of a closely related group of diimine ligands. The rapid bidentate coordination of phen, proceeding with no observable monodentate species, is significantly altered by the removal of a bridging C–H group to form daf. Apparently, the methylene bridge in daf distorts the bipyridine portion of the molecule so as to reduce the N–metal overlap, and this leads to a relatively slow chelation process. Removal of the remaining methylene bridge of daf to form dabp has further reduced the coordination abilities of the second nitrogen, so the $M(CO)_5$(dabp) complexes remain as the stable products. This observation may also be interpreted in terms of a reduction of the N–metal overlap in the $M(CO)_5$(dabp) complex. Furthermore, the relatively low basicity of the dabp ligand (pK_a 1.92)[14] compared to phen (pK_a 4.84)[15] may also be contributing to the lack of chelation in the $M(CO)_5$(dabp) complexes.

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Registry No. $Cr(CO)_5$(daf), 106213-19-8; $Mo(CO)_5$(daf), 106213-20-1; $W(CO)_5$(daf), 106213-21-2; $Cr(CO)_5$(dabp), 106213-22-3; $Mo(CO)_5$(dabp), 106213-23-4; $W(CO)_5$(dabp), 106213-24-5; $Cr(CO)_4$(phen), 14168-63-9; $Mo(CO)_4$(phen), 15740-78-0; $W(CO)_4$(phen), 14729-20-5; $Cr(CO)_4$(daf), 106213-25-6; $Mo(CO)_4$(daf), 106213-26-7; $W(CO)_4$(daf), 106213-27-8; $Cr(CO)_6$, 13007-92-6; $Mo(CO)_6$, 13939-06-5; $W(CO)_6$, 14040-11-0.

(7) Darensbourg, D. J. *Adv. Organomet. Chem.* **1982**, *21*, 113.
(8) Jones, L. H.; McDowell, R. S.; Goldblatt, M. *Inorg. Chem.* **1969**, *8*, 2349.
(9) (a) Darensbourg, D. J.; Brown, T. L. *Inorg. Chem.* **1968**, *7*, 1679. (b) Ignemanson, C. M.; Angelici, R. J. *Inorg. Chem.* **1968**, *7*, 2646. (c) Covey, W. D.; Brown, T. L. *Inorg. Chem.* **1973**, *12*, 2820. (d) Darensbourg, D. J.; Ewen, J. A. *Inorg. Chem.* **1981**, *20*, 4168.
(10) Connor, J. A.; Hudson, G. A. *J. Chem. Soc., Dalton Trans.* **1975**, 1025.
(11) (a) Connor, J. A.; Day, J. P.; Jones, E. M.; McEwen, G. K. *J. Chem. Soc., Dalton Trans.* **1973**, 347. (b) Connor, J. A.; Hudson, G. A. *J. Organomet. Chem.* **1974**, *73*, 351. (c) Connor J. A.; Riley, P. I. *J. Organomet. Chem.* **1975**, *94*, 55.
(12) Howell, J. A. S.; Burkinshaw, P. M. *Chem. Rev.* **1983**, *83*, 557.
(13) (a) Chatt, J.; Watson, H. R. *J. Chem. Soc.* **1961**, 4980. (b) Orgel, L. E. *Inorg. Chem.* **1962**, *1*, 25. (c) Kraihanzel, C. S.; Cotton, F. A. *Inorg. Chem.* **1963**, *2*, 533.
(14) (a) MacBride, J. A. H.; Wright, P. M.; Hull, R. *J. Chem. Res., Synop.* **1984**, *10*, 328. (b) Deroski, B. R.; MacBride, J. A. H.; Markgraf, J. H.; Ricci, J. S., Jr. *Can. J. Chem.* **1984**, *62*, 2235.
(15) *CRC Handbook of Chemistry and Physics*, 62nd ed.; Weast, R. C., Ed.; CRC: Boca Raton, FL, 1981; p D140.

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Preparation of Sulfheme Derivatives from Reconstituted Myoglobins Lacking Heme Vinyl Substituents

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Sulfmyoglobin is a green form of myoglobin in which the heme prosthetic group is modified by reduction of one of the pyrrole rings and incorporation of one sulfur atom into the macrocycle.[1,2] The structure of the resulting prosthetic group is that of a sulfur-containing chlorin,[3] although the identity of the reduced pyrrole ring is unknown, and several different models for the nature of sulfur attachment have been suggested.[4,5] On the basis of nuclear magnetic resonance (NMR) studies, Timkovich and Vavra have recently concluded that either heme ring A or B is probably modified in the formation of sulfmyoglobin.[6] This conclusion

(1) Berzofsky, J. A.; Peisach, J.; Blumberg, W. E. *J. Biol. Chem.* **1971**, *246*, 3367–3377.
(2) Berzofsky, J. A.; Peisach, J.; Horecker, B. L. *J. Biol. Chem.* **1972**, *247*, 3783–3791.
(3) Chang, C. K. In *Biological Chemistry of Iron*; Dunford, H. B., Dolphin, D., Raymond, K. N., Sieker, L., Eds.; Reidel: Boston, 1982; pp 313–334.
(4) Berzofsky, J. A.; Peisach, J.; Alben, J. O. *J. Biol. Chem.* **1972**, *247*, 3774–3782.
(5) Scheer, H.; Inhoffen, H. H. In *The Porphyrins*; Dolphin, D., Ed.; Academic: New York, 1978; Vol. 2, pp 45–90.

0020-1669/87/1326-0622$01.50/0

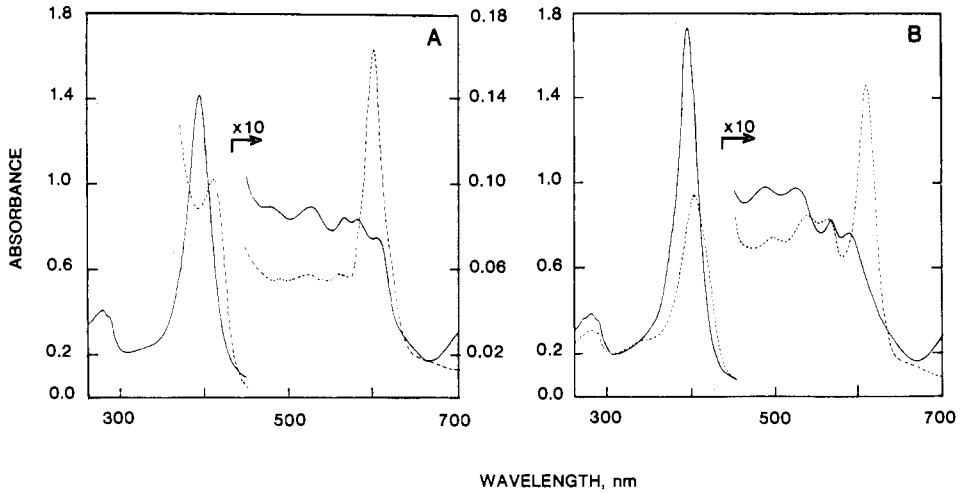

Figure 1. Electronic absorption spectra of heme-substituted sulfmyoglobin derivatives: (A) iron(III) sulfdeuteromyoglobin (—) (pH 6.0, phosphate (0.1 M)), iron(II) sulfdeuteromyoglobin (---) reduced with $Fe(EDTA)^{2-}$ (pH 8.0, phosphate (0.1 M)); (B) iron(III) sulfmesomyoglobin (—) (pH 6.0, phosphate (0.1 M)), iron(II) and iron(III) sulfmesomyoglobins (---) (pH 8.0, phosphate (0.1 M)).

Table I. Soret Maxima of Fe(II) and Fe(III) Derivatives of Sulfdeutero- and Sulfmesomyoglobins

	sulfdeuteromyoglobin		sulfmesomyoglobin		sulfmyoglobin[a]	
derivative	λ_{max}, nm	ϵ, mM^{-1}	λ_{max}, nm	ϵ, mM^{-1}	λ_{max}, nm	ϵ, mM^{-1}
Fe^{II}	408	120			421	86
$Fe^{III}(H_2O)$	397	140	396	138	404	117
$Fe^{III}(OH^-)$	402	107	400	102	408	100
$Fe^{III}(CN^-)$	407	114	405	91	412	73.7

[a] From ref 1.

raised the possibility that the vinyl groups associated with these pyrroles might also be involved in sulfmyoglobin formation.[6]

To assess heme vinyl group participation in the generation of sulfmyoglobin, we have prepared derivatives of myoglobin in which the heme vinyl groups are replaced with protons (deuteroheme IX substituted myoglobin) or ethyl substituents (mesoheme IX substituted myoglobin) and have determined the ability of these modified proteins to form the corresponding sulfheme derivatives.

Experimental Section

Ferrideuteroporphyrin IX chloride and ferrimesoporphyrin IX chloride were purchased from Porphyrin Products, Inc. (Logan, UT). Horse heart myoglobin (Sigma Type III) was purified as described by Tomoda et al.[7] Apomyoglobin was prepared by the method of Teale.[8] The apoprotein was reconstituted with heme by a modification of the method described by Tamura et al.[9] After addition of the heme solution to the apoprotein, the mixture was allowed to stand on ice for 15 min and then centrifuged at 9200*g* for 20 min (4 °C). The supernatant solution was concentrated by ultrafiltration (Amicon YM-5 membrane). The resulting solution (90 mg/2 mL) was eluted over a 2 × 33 cm column of Sephadex G-25 fine resin (Pharmacia) at 4 °C with 0.1 M potassium phosphate buffer (pH 8.0 at 25 °C). Protein fractions possessing Soret/A_{280} ratios of 5.8/1 (deuterohememyoglobin) or 6.5/1 (mesohememyoglobin) in the acid-met form were pooled, concentrated by ultrafiltration, and used for sulfmyoglobin preparation. Concentrations of metdeuteromyoglobin and metmesomyoglobin were determined at pH 6.0 on the basis of their Soret absorbances (ϵ_{392} = 128 mM^{-1} and ϵ_{395} = 172 mM^{-1}, respectively[9]). Molar absorptivities of sulfheme derivatives were determined by the pyridine hemochrome method.[10]

Sulfdeutero- and sulfmesomyoglobin were prepared by the sequential addition of hydrogen peroxide, catalase, and sodium sulfide as described for the native protein by Berzofsky et al.[1] Sulfdeuteromyoglobin was reduced fully in ice-cold phosphate buffer (pH 8.0) with concentrated $Fe(EDTA)^{2-}$ solution.[11] Ferrosulfdeuteromyoglobin fractions with A_{601}/A_{551} ratios of 3.0 or greater were collected and concentrated. Both sulfmyoglobins were oxidized with bis(dipicolinato)cobaltate(III) ($Co(dipic)_2NH_4$),[12] and any excess oxidizing agent was subsequently removed by ultrafiltration. The $Fe^{III}(CN^-)$ complexes of both proteins were prepared by addition of KCN dissolved in potassium phosphate buffer at pH 8.0. The $Fe^{III}(OH^-)$ derivatives of both proteins were obtained in 0.1 M borate buffer at pH 10 (25 °C). Electronic absorption spectra were obtained with a Cary 219 spectrophotometer.

Results and Discussion

Both sulfdeutero- and sulfmesomyoglobin prepared as described above elute from the gel filtration column as a mixture of the iron(II) and iron(III) sulfhemeproteins. This behavior contrasts with that of native sulfmyoglobin, which elutes from the G-25 column in the fully reduced state. Attempts to reduce either mixture with dithionite or to oxidize either mixture with potassium ferricyanide resulted in the formation of substantial amounts of the corresponding metheme derivatives. The mixture of iron(II) and iron(III) sulfdeuteromyoglobins can be fully reduced (λ_{max} = 601 nm) with $Fe(EDTA)^{2-}$ and fully oxidized with $Co(dipic)_2^-$ to produce the electronic absorption spectra shown in Figure 1 A. Metsulfdeuteromyoglobin was found to revert to metdeuteromyoglobin more readily than metsulfmyoglobin converts back to metmyoglobin, even at 0 °C.

Attempts to reduce the mixture of iron(II) and iron(III) sulfmesomyoglobins with $Fe(EDTA)^{2-}$ at pH 8.0 (14 °C) produced a decrease in absorbance at 607 nm and the appearance of two maxima in the Soret region, one at 395 nm and the other at 417 nm (data not shown). Thus, we were unable to reduce sulfmesomyoglobin completely, even with this mild reducing agent. The metsulfmesomyoglobin derivatives differ from the metsulfdeutero derivatives in that they are stable for several hours at 14 °C. The electronic absorption spectra of ferrisulfmesomyoglobin and the iron(II) and iron(III) sulfmesomyoglobin mixture are shown in Figure 1B, and the spectroscopic parameters estimated for the sulfmyoglobin derivatives described above are compared

(6) Timkovich, R.; Vavra, M. R. *Biochemistry* **1985**, *24*, 5189–5196.
(7) Tomoda, A.; Takizawa, T.; Tsuji, A.; Yoneyama, Y. *Biochem. J.* **1981**, *193*, 181–185.
(8) Teale, F. W. J. *Biochim. Biophys. Acta* **1959**, *35*, 543.
(9) Tamura, M.; Asakura, T.; Yonetani, T. *Biochem. Biophys. Acta* **1973**, *295*, 467–479.
(10) Antonini, E.; Brunori, M. In *Hemoglobin and Myoglobin in Their Reactions with Ligands*; Neuberger, A., Tatum, E. L., Eds.; North-Holland: London, 1971; p 11.
(11) Wherland, S.; Holwerda, R. A.; Rosenberg, R. C.; Gray, H. B. *J. Am. Chem. Soc.* **1975**, *97*, 5260–5262.
(12) Mauk, A. G.; Coyle, C. L.; Bordignon, E.; Gray, H. B. *J. Am. Chem. Soc.* **1979**, *101*, 5054–5056.

in Table I to the published values for native sulfmyoglobin. Owing to the relative instability of the new sulfmyoglobin derivatives, we estimate that our proteins are 80–90% pure, so that the values listed in Table I are best regarded as lower limits of the true values.

As neither deuteroheme IX or mesoheme IX possesses vinyl substituents, the present results establish that vinyl groups are not required for sulfmyoglobin formation. Nearly 50 years ago, Michel reported that hematoheme-substituted myoglobin, another derivative ostensibly devoid of heme vinyl groups, can also form sulfmyoglobin.[13] However, as hematoheme is now known to contain contaminants of vinyl-containing species, the present findings eliminate this source of ambiguity and are consistent with resonance Raman results for native sulfmyoglobin that suggest the presence of both vinyl substituents.[14,15]

Our finding that the heme-substituted sulfmyoglobin derivatives are initially obtained as a mixture of Fe(II) and Fe(III) species (i.e., that they are more readily autoxidized than native sulfmyoglobin) suggests that the reduction potentials of deuterosulfmyoglobin and mesosulfmyoglobin are significantly lower than the reduction potential of native sulfmyoglobin. As the reduction potentials of mesohememyoglobin and deuterohememyoglobin are reported to be only 6–22 mV lower than that of native myoglobin,[16] it may be that the magnitude of heme-substituent effects on the reduction potentials of sulfheme proteins is significantly different from the corresponding effects on the native heme prosthetic group.

In addition to the insight that this work provides into the involvement of heme vinyl groups in sulfmyoglobin formation, it is our expectation that the new sulfmyoglobin derivatives described here should be of use in subsequent NMR and resonance Raman studies and should help to facilitate rigorous structural characterization of the sulfheme prosthetic group.

Addendum

While this paper was being reviewed, a related report appeared that reached conclusions consistent with those reported here.[17]

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Registry No. Heme, 14875-96-8.

(13) Michel, H. O. *J. Biol. Chem.* **1938**, *126*, 323–348.
(14) Andersson, L. A.; Loehr, T. M.; Lim, A. R.; Mauk, A. G. *J. Biol. Chem.* **1984**, *259*, 15 340–15 349.
(15) Andersson, L. A.; Loehr, T. M.; Chang, C. K.; Mauk, A. G. *J. Am. Chem. Soc.* **1985**, *107*, 182–191.
(16) Brunori, M.; Saggese, U.; Rotilio, G. C.; Antonini, E.; Wyman, J. *Biochemistry* **1971**, *10*, 1604–1609.
(17) Chatfield, M. J.; La Mar, G. N.; Balch, A. L.; Smith, K. M.; Parish, D. W.; Le Page, T. J. *FEBS Lett.* **1986**, *206*, 343–346.

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Anion-Exchanged Hydrotalcite-like-Clay-Modified Electrodes

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There has been considerable interest in modified electrodes using inorganic layers such as clay,[1-4] zeolite,[5] and transition-metal cyanides.[6] Modification of electrodes with a thin layer of clay has been reported by Bard et al.[1] and other groups.[2-4] In addition to the studies of modified electrodes, it has recently been shown that free-standing pillared clay films behave as cation-exchange membranes by measurements of the membrane potential.[1e] The clay colloid used in the previous works[1-4] was sodium montmorillonite, one of the members in the smectic group of clays.[7] Montmorillonite has an expanding 2:1 layer lattice structure and has a cation-exchange capacity due to unbalanced charges in the interior of the layers.[7] Cations such as $Ru(bpy)_3^{2+}$ (bpy = 2,2′-bipyridine), $Fe(bpy)_3^{2+}$, $Os(bpy)_3^{2+}$, or MV^{2+} (methylviologen) incorporated by the cation-exchange reaction into montmorillonite films were found to be electroactive.[1-4]

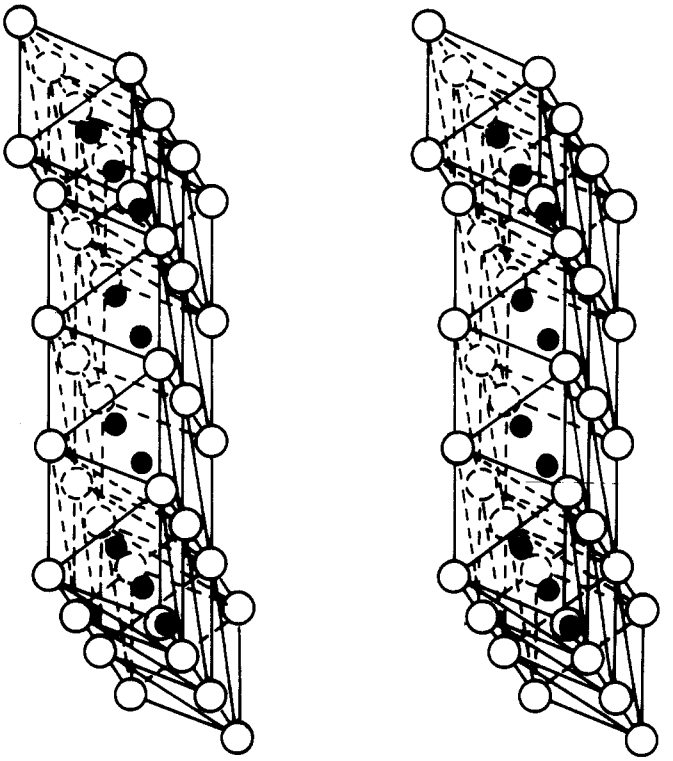

Figure 1. Perspective structure of positively charged brucite-like layers. Anions located in the interlayer are omitted for the sake of clarity: (○) hydroxyl; (●) Al^{3+} or Mg^{2+}.

On the other hand, it is well-known that the rhombohedral hydrotalcite $Mg_6Al_2(OH)_{16}CO_3{\cdot}4H_2O$ consists of positively charged brucite-like layers $[Mg_6Al_2(OH)_{16}]^{2+}$ and negatively charged interlayers $[CO_3{\cdot}4H_2O]^{2-}$, as shown in Figure 1.[7-9] A series of synthetic hydrotalcite-like compounds (HT) has been prepared by reactions of NaOH with mixed Mg–Al solutions.[9,10] It was reported that anions such as Cl^-, NO_3^-, or SO_4^{2-} located in the interlayer of HT would be substituted with other anions by anion-exchange reactions.[9d,11]

On the basis of the above background, we report in this paper a new type of clay-modified electrodes using HT, demonstrating

(1) (a) Ghosh, P. K.; Bard, A. J. *J. Am. Chem. Soc.* **1983**, *105*, 5691. (b) Ghosh, P. K.; Mau, A-W. H.; Bard, A. J. *J. Electroanal. Chem. Interfacial Electrochem.* **1984**, *169*, 315. (c) Ege, D.; Ghosh, P. K.; White, J. R.; Equey, J. F.; Bard, A. J. *J. Am. Chem. Soc.* **1985**, *107*, 5644. (d) White, J. R.; Bard, A. J. *J. Electroanal. Chem. Interfacial Electrochem.* **1986**, *197*, 233. (e) Itaya, K.; Bard, A. J. *J. Phys. Chem.* **1985**, *89*, 5565.
(2) Yamagishi, A.; Aramata, A. *J. Chem. Soc., Chem. Commun.* **1984**, 119.
(3) Liu, H. Y.; Anson, F. C. *J. Electroanal. Chem. Interfacial Electrochem.* **1985**, *184*, 411.
(4) Kamat, P. V. *J. Electroanal. Chem. Interfacial Electrochem.* **1984**, *163*, 389.
(5) Murray, C. G.; Nowak, R. J.; Rolison, D. R. *J. Electroanal. Chem. Interfacial Electrochem.* **1984**, *164*, 205.
(6) Itaya, K.; Uchida, I.; Neff, V. D. *Acc. Chem. Res.* **1986**, *19*, 162.
(7) Van Olphen, H. *An Introduction to Clay Colloid Chemistry*; Wiley: New York, 1977.
(8) Frondel, C. *Am. Mineral.* **1941**, *26*, 295.
(9) (a) Miyata, S. *Clays Clay Miner.* **1975**, *23*, 369. (b) Miyata, S.; Okada, A. *Clays Clay Miner.* **1977**, *25*, 14. (c) Miyata, S.; Hirose, T. *Clays Clay Miner.* **1978**, *26*, 441. (d) Miyata, S. *Clays Clay Miner.* **1983**, *31*, 305.
(10) (a) Brindley, G. W.; Kikkawa, S. *Clays Clay Miner.* **1981**, *28*, 87. (b) Kikkawa, S.; Koizumi, M. *Mater. Res. Bull.* **1982**, *17*, 191.
(11) Ikeda, T.; Amoh, H.; Tasunaga, T. *J. Am. Chem. Soc.* **1984**, *106*, 5772.

0020-1669/87/1326-0624$01.50/0