Cyclic Voltammetry of Aquocobalamin on Clay-Modified Electrodes

VLADIMIR BOREK AND
MATTHEW J. MORRA*

*Soil Science Division, University of Idaho,
Moscow, Idaho 83844-2339*

Halogenated synthetic compounds are widespread contaminants of the environment. Although corrinoids reductively dehalogenate synthetic contaminants in solution, the redox behavior of sorbed tetrapyrroles has received limited attention. Colloidal clay suspensions were prepared as Ca^{2+} forms of hectorite (SHCa-1), montmorillonite (SWy-1, Syn-1, and SAz-1), and vermiculite (VTx-1) and spin coated on platinum electrodes. Cyclic voltammetry was performed with the clay-modified electrodes immersed in buffered solutions containing 1.0 mM aquocobalamin. Aquocobalamin in the presence of vermiculite-coated electrodes displayed the same cathodic and anodic peak potentials as unmodified electrodes immersed in aquocobalamin solutions. All other clay-modified electrodes shifted cathodic peaks to more negative values, while anodic peak shifts varied with the clay. Hectorite caused the largest shift in formal redox potential (−104 mV) as compared to aquocobalamin in solution. The redox behavior of aquocobalamin as modified by sorption to clay minerals potentially affects dehalogenation rates of synthetic organic compounds in the environment. Clays lowering the formal redox potential of the tetrapyrrole create a potentially more efficient catalyst for pollutant degradation. However, thermodynamic data as obtained using cyclic voltammetry cannot be used to make definitive predictions about the kinetics of contaminant dehalogenation. Reductive dehalogenation will be a function of altered eletrochemical properties of the tetrapyrrole as well as rates of contaminant diffusion to the site of tetrapyrrole sorption.

Introduction

Halogenated synthetic organic compounds are widespread contaminants in air, surface waters, groundwaters, and soils as a result of their use in a diversity of products (*1–3*). Although transformations of halogenated hydrocarbons occur (*4–6*), halogen substituents render them relatively resistant to degradation. Dehalogenation in anaerobic environments (*7*) results in compounds more susceptible to oxidative degradation. The participation of living microorganisms in these reactions is well established; however, abiotic catalysis of reductive transformations also occurs. Very little is known concerning the actual reductants except that a large proportion of the reducing equivalents is associated with the solid phase (*8*). It has been suggested that direct reduction of an organic contaminant by a reduced

sulfur or iron component is too slow to account for observed kinetics and that electron carriers mediate electron transfer (*9*). Tetrapyrroles such as Fe-containing hematin, Ni-containing coenzyme F_{430}, and Co-containing vitamin B_{12} have been proposed as potential electron-transfer mediators in natural environments (*9, 10*).

A variety of metal-substituted porphyrins, thought to be derived from chlorophylls and hemes, have been identified in geological samples and sediments (*11*). Although porphyrins metalated with Ni and V are dominant tetrapyrroles in sediments (*12*), Cu, Fe, and free base porphyrins have also been identified in sedimentary deposits (*13–16*). It has also been suggested that tetrapyrroles of bacterial origin such as vitamin B_{12} and coenzyme F_{430}, although not yet identified, may also occur (*11*).

Extracellular tetrapyrroles potentially available for participation in reductive dehalogenation are likely associated with solid surfaces (i.e., minerals and organic matter). Zoro et al. (*17*) demonstrated dehalogenation of DDT by a heme in sewage sludge. More recently Ukrainczyk et al. (*18*) showed CCl_4 dechlorination in the presence of a cationic porphyrin exchanged on a variety of mineral surfaces. However, redox activity of the sorbed tetrapyrrole varies with the mineral and the mechanism responsible for sorption (*18–21*).

Extrapolation of studies conducted with tetrapyrroles in aqueous solution thus may not accurately represent catalytic activities of the same tetrapyrroles when associated with colloids in soils, sediments, or groundwaters. It is well established that corrins such as aquocobalamin (Figure 1) promote the reductive dehalogenation of a wide variety of compounds in homogeneous solutions (*22, 23*). We recently demonstrated that aquocobalamin remains redox active when in association with hectorite, a Li-substituted 2:1 type clay mineral (*24*). Sorbed aquocobalamin is therefore a potential electron transfer mediator of reductive dehalogenation in soils and sediments. However, sorption may alter the formal redox potential of the central metal of the tetrapyrrole, thus modifying its electron transfer characteristics and ultimately its ability to catalyze reductive dehalogenation.

Electron transfer in redox active systems can be directly determined using cyclic voltammetry. The introduction of clay-modified electrodes by Ghosh and Bard (*25*) has created an opportunity to study changes in redox behavior of molecules sorbed on mineral surfaces. Electrodes modified by forming films of inorganic microcrystalline structured materials (clays, zeolites, or related aluminosilicates) on a conductive surface have been used to study the redox behavior of many different sorbed organic molecules (*26*). Colloidal clays have attracted considerable attention because of their appreciable surface area and unusual intercalation properties. Clays are employed in many commercial processes and are important as catalysts or catalyst supports (*27*). The immobilization of complex catalysts in clay structures is an area of growing interest since clay colloids have been found to influence the specificity of catalysis (*28*).

Sorption to the surface of clay may significantly alter the redox activity of particular molecules. Previous studies indicate that adsorbed complexes remain electroactive when adsorption sites are crystal lattice edges or complexes are bonded to already adsorbed molecules as ionic paired species. Intercalated molecules are usually electroinactive; electroactivity can be reestablished if a charge shuttle is added or in some cases when interlayer swelling is increased by pillaring (*26, 29, 30*).

* To whom correspondence should be addressed; e-mail: mmorra@uidaho.edu; telephone: (208)885-6315; fax: (208)885-7760.
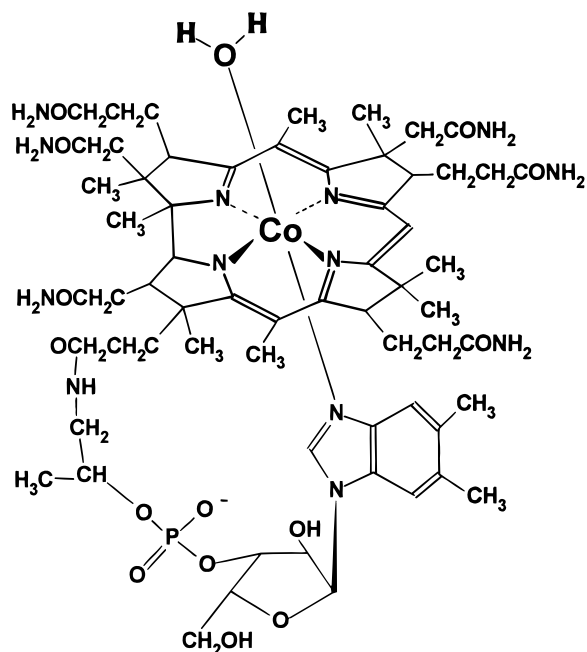
FIGURE 1. Structure of aquocobalamin.

Our objective was to determine changes in the redox behavior of tetrapyrroles when sorbed to various clay minerals. We focused our efforts on aquocobalamin, a microbially produced corrin substituted with a ligand expected in natural systems. We have concentrated on the Co(III)/Co(II) redox couple since aquocobalamin functions as an electron-transfer mediator of reductive dehalogenation in the Co(II) state (22, 31, 32), and this is a more likely redox transformation of aquocobalamin in anaerobic environments than complete reduction to Co(I). Cathodic and anodic peaks obtained using clay-modified electrodes and cyclic voltammetry allow the calculation of formal redox potentials for aquocobalamin sorbed to mineral surfaces. Redox potentials of sorbed aquocobalamin reflect the impact of sorption on the electrochemical properties of the central metal of the corrin and, thus ultimately, catalysis of reductive dehalogenation. This work has relevance to both the prediction of intrinsic contaminant modification as might occur in contaminated anaerobic environments or the use of immobilized tetrapyrroles for in situ remediation.

Materials and Methods

Chemicals and Clay Minerals. All chemicals were analytical grade reagents and used without further purification. Aquocobalamin was purchased from Fluka (Fluka Chemie AG, Buchs, Switzerland). Colloidal platinum was prepared by refluxing $PtCl_2$ (Aldrich, Milwaukee, WI) in a water–ethanol mixture containing 3% poly(vinyl alcohol) (average molecular mass of 50 000) (Aldrich, Milwaukee, WI) (33).

Clay minerals were obtained from the Source Clay Minerals Repository (University of Missouri, Columbia, MO). Colloidal clay suspensions of barasym SSM-100 (Syn-1, synthetic mica-montmorillonite), hectorite (SHCa-1), Na-montmorillonite (SWy-1), Ca-montmorillonite (SAz-1), and vermiculite (VTx-1) were prepared by pretreating to remove carbonates, saturating with Ca^{2+}, and size-fractionating using a method similar to that previously reported (24). Clays (50 g) were dispersed in 500 mL of water by sonication and mechanical stirring, carbonates were removed with a 1 M ammonium acetate–acetic acid buffer (pH 5.0), and the remaining material was centrifuged at 895g for 1 h. Colloidal suspensions of the clays were Ca^{2+} saturated by shaking with 1 M $CaCl_2$ and dialyzed against deionized water until no

reaction with silver nitrate was observed. After freeze-drying, clays were resuspended in deionized water at a concentration of 5 g 100 mL^{-1}.

Adsorption Experiments. Solutions of aquocobalamin with suspensions of each of the clay minerals were equilibrated for 24 h and repeatedly centrifuged at 1680g for 30-min intervals until the supernatant was clear. Concentration of unadsorbed aquocobalamin in the supernatant was determined by measuring solution absorbance at 365 nm using an Aminco DW-2a UV/VIS spectrophotometer (computer software and reconditioning by Olis, Bogart, GA). Sorption isotherms for aquocobal(III)amin were determined in solutions buffered at pH values of 4.2 using 0.1 M solutions of acetate. Five different substrate concentrations were used to determine each of the Langmuir sorption isotherms. We used general linearized model dummy variable regressions to determine the uniqueness of sorption as represented by isotherm slopes.

Cyclic Voltammetry. Due to strong adsorption of cobalamines on the surface of platinum electrodes, only freshly cleaned electrodes were used for measuring, and in most cases only data from the first three potential cycles were collected. Electrodes were uniformly cleaned following procedures recommended by the manufacturer (34). Cleaning involved mechanical polishing with 15-, 3-, and 1-μm diamond slurries, washing with acetone and methanol, polishing with Al_2O_3, polishing on a nylon pad, cleaning in an ultrasonic bath, and finally washing with deionized water.

We used two methods of casting the clay film on electrode surfaces. The first method was similar to the original method employed by Ghosh and Bard (25). A colloidal clay suspension (10 μL) was mixed with 20 μL of the colloidal Pt suspension containing 1% poly(vinyl alcohol) (PVA), and 25 μL of the resulting mixture was applied to the previously cleaned Pt electrode surface and dried at room temperature. The clay layer had an estimated thickness of 3 μm. A spin coating method (26) was also employed to obtain a stable casting of clay suspensions on Pt electrode surfaces without the presence of colloidal Pt and PVA. Spin coating involved inserting a Pt electrode through a hole cut in the bottom of a centrifuge tube, filling the tube with a clay suspension, and centrifuging the tube–electrode assembly at 895g for 20 min. This procedure produced electrodes consistently coated with clay layers also having an estimated thickness of 3 μm.

Cyclic voltammetry was carried out with a BAS-100B Electrochemical Workstation (Bioanalytical Systems, West Lafayette, IN). All electrochemical experiments were performed in an undivided three-electrode cell having a 20-mL volume. The working electrode was a Pt disk (1.6 mm diameter, 2.00 mm^2 area), the counter electrode was a Pt wire, and the reference was a saturated Ag/AgCl electrode ($E^\circ = 0.199$ mV). All voltammetric experiments were carried out in 0.1 M buffer (acetate, pH 4.2; phosphate, pH 6.8; borate, pH 9.0) solutions, and the concentration of aquocobalamin in the electrochemical cell was 1.0 mM. The working electrode was polarized at a rate 1–5 mV s^{-1}. Each value presented for anodic and cathodic scans represents the mean of five replications, with each replication representing a cyclic voltammogram generated using a different freshly polished, clay-coated electrode.

Results and Discussion

Adsorption Experiments. Sorption isotherms of aquocobalamin on the clay minerals were determined since the extent and type of interaction will influence redox activity as monitored using cyclic voltammetry. Equilibrium aquocobalamin concentrations were fit with a linearized Langmuir equation in the form of $C/n = C/b + 1/Kb$ in which n is moles solute sorbed per gram of mineral, C is the equilibrium solute concentration, b is the maximum amount sorbed (monolayer

TABLE 1. Sorption of Aquocobalamin on Clay Mineral Suspensions Buffered at pH 4.2[a]

clay	b (μmol g^{-1})	K (mL μmol^{-1})	significance[b]
vermiculite (VTx-1)	31.4	4.5	a
montmorillonite (Syn-1)	84.0	288	a
hectorite (SHCa-1)	267	2020	b
montmorillonite (SAz-1)	265	2327	b
montmorillonite (SWy-1)	269	2068	b

[a] A linearized form of the Langmuir equation ($C/n = C/b + 1/Kb$) was used to estimate maximum sorption (b) and the sorption constant (K). C is the equilibrium solute concentration (μmol mL^{-1}), and n is the amount of aquocobalamin sorbed per unit mass of mineral (μmol g^{-1}). [b] Different letters within this column indicate that the isotherms are different ($P \leq 0.05$) according to single degree of freedom contrasts on slopes ($1/b$) generated from the linearized Langmuir model.

FIGURE 2. Time sequence of cyclic voltammograms determined after immersion of a clay-modified electrode into a 0.1 M solution of aquocobalamin.

coverage), and K is a constant. Estimated maximal adsorbed amounts of aquocobalamin as determined from the isotherms are shown in Table 1. Affinities of oxidized aquocobal-(III)amin to the clays was greatest for hectorite, montmorillonite SWy-1, and montmorillonite SAz-1. Substantially less sorption occurred on montmorillonite Syn-1 and vermiculite.

The greatest amount of sorption occurred with the three expandable clays having large internal surface areas. This is consistent with the observation that aquocobalamin, cyanocobalamin, and dicyanocobinamide intercalate Ca^{2+}-saturated hectorite, expanding the basal spacing from 14.5 to 22.1 Å (*24*). External surface areas for hectorite and the two montmorillonites, SWy-1 and SAz-1, range from 31 to 92 m^2 g^{-1}, whereas total surface areas which include the interlayer regions range from 486 to 820 m^2 g^{-1} (*35*). Although corrin orientation on mineral surfaces was not determined, an estimate of surface coverage can be made using an average value for maximum sorption on the three expansible clays (267 μmol g^{-1}) and planar dimensions of the corrin ring (9 Å) (*36*). Calculations indicate that an approximate surface area of 130 m^2 g^{-1} clay was occupied by the corrin. In contrast to the large surface area clays, vermiculite VTx-1 is only slightly expandable with an external surface area of 45.9 and a total surface area of only 91.5 m^2 g^{-1} (*37*). Similarly, montmorillonite Syn-1 is only partially expandable in that it is formed from a random interlayering of nonexpandable mica-like and expandable montmorillonite-like spacings (*38*).

Cyclic Voltammetry. We have concentrated our efforts on the Co(III)/Co(II) redox couple of aquocobalamin, the most likely redox transformation of aquocobalamin in anaerobic environments. The first step of aquocobal(III)-amin reduction is slow due to slow kinetics associated with displacement of the aquo ligand. Voltammograms therefore show reversibility of Co(III)/Co(II) electron transfer only when moderate sweep rates are used. Moreover, cyclic voltammetry is complicated by aquocobalamin adsorption to the electrode surface. For these reasons, it was necessary to work only with freshly cleaned electrodes and use sweep rates less than 10 mV s^{-1}.

We observed a difference in the behavior of clay-modified electrodes prepared by the two methods, air-drying and spin-coating. Electrodes prepared by air-drying clay suspensions containing colloidal Pt and PVA directly on the electrode surface were electrochemically inactive in the presence of aquocobalamin even after a 30-min presoaking period in buffer. Electrodes regained electroactivity in the presence of aquocobalamin usually within 1 h and only after 20−50 potential cycles, but the cathodic peak potential was absent or poorly developed. Peak currents of air-dried clay-modified electrodes were much lower than currents observed for unmodified el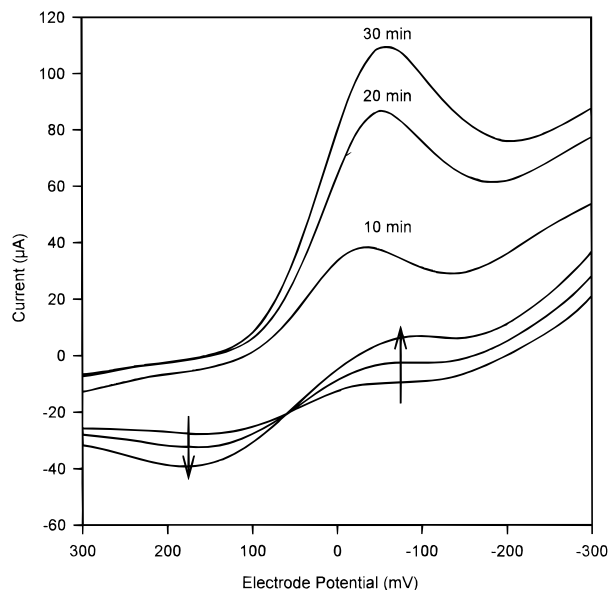ectrodes in aquocobalamin solutions. In contrast, aquocobalamin in the presence of spin-coated electrodes was electroactive with the first scan (Figure 2). Cathodic and anodic peak currents increased up to 30 min where they remained relatively constant. Peak currents observed with spin-coated electrodes were under all experimental conditions lower or comparable with currents observed for unmodified electrodes in aquocobalamin solutions. Previous investigations indicate that only 10−30% of the species incorporated into the clay film by immersion in a solution of the species is electroactive; however, the electroactive species may concentrate in the clay film and produce higher peak currents than occur when a bare electrode is immersed in the same solution (*26*).

Behavior of air-dried and spin-coated electrodes can be explained by differences in clay hydration, with drier films requiring longer soaking times prior to the development of any electrochemical response (*39*). This is true although PVA addition promotes a random organization of clay on the electrode surface, typically increasing the diffusion coefficient and reducing the time necessary for appearance of an electrochemical response from some species (*39*). Diffusion of the electrochemically reactive species may be inhibited in spin-coated electrodes prepared in the absence of PVA because clay particles are oriented in a parallel fashion with the electrode surface. However in the present case, the electrochemical response promoted by the maintenance of water in the preparation supersedes any diffusional limitation imposed by clay orientation.

In addition to direct effects of clay modification, the electrochemical properties of clay-modified electrodes are affected by numerous experimental parameters. These include the clay type, method of clay preparation, thickness of the clay film, concentration of the supporting electrolyte, and chemical qualities of the electroactive species. An attempt was made to standardize all these variables, altering only the type of clay coating the electrode and system pH. The determination of aquocobalamin redox potentials in the presence of buffers having pH values of 6.8 and 9.0 was not possible because of poorly developed cyclic voltammograms. The peaks were broad and exhibited high variability making any assessment of changes in the electrochemical behavior of aquocobalamin unreliable. A number of possible factors contributed to this observation including increased sorption of aquocobalamin on the electrode surface and the presence
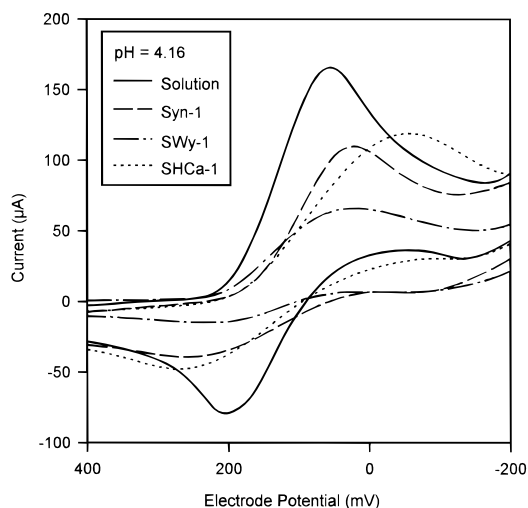
FIGURE 3. Cyclic voltammograms of aquocobalamin (0.1 M) on a bare platinum electrode and clay-modified platinum electrodes in acetate buffer solutions (pH 4.2) using a scan rate of 5 mV s^{-1} and a range from +500 to −500 mV.

TABLE 2. Potentials (mV) Determined for the Cyclic Voltammetry of Aquocobalamin on Clay-Modified Platinum Electrodes Immersed in Solutions Buffered at pH 4.2[a]

clay mineral	E_c	E_a	$|E_c - E_a|$	$E^{o\prime}$	shift
none	114 (1.89)a	179 (3.97)c	65	147	
vermiculite (VTx-1)	118 (3.70)a	181 (6.69)c	63	150	3
hectorite (SHCa-1)	−32 (3.97)e	116 (6.84)e	148	42	−105
montmorillonite (Syn-1)	52 (6.70)d	146 (7.71)d	94	99	−48
montmorillonite (SAz-1)	91 (4.13)b	230 (7.60)b	139	161	14
montmorillonite (SWy-1)	78 (2.50)c	266 (2.50)a	188	172	25

[a] E_c is the cathodic peak potential, E_a is the anodic peak potential, $|E_c - E_a|$ is the absolute difference of cathodic and anodic peak potentials, $E^{o\prime}$ is the formal redox potential calculated as an average of E_c and E_a, and potential shift is the difference of $E^{o\prime}$ of aquocobalamin in solution and $E^{o\prime}$ of aquocobalamin in the presence of a clay-modified electrode. Standard errors of the means for E_c and E_a are included in parentheses. Potentials were measured against a saturated Ag/AgCl reference electrode in 1.0 mM solutions of aquocobalamin (0.1 M acetate buffer) at a scan rate of 5 mV s^{-1} over a range from +350 to −150 mV. Different letters within the same column indicate that the mean values presented differ ($P \leq 0.05$) according to the Student−Newman−Keuls multiple comparison procedure.

of trace contaminants in the system. Also of consequence is the change in electrochemical behavior of aquocobalamin with system pH (*40*). There are two pH regions with constant electrochemical potentials for the Co(III)/Co(II) couple: a region below pH −2.4 and a region between pH 2.9 and pH 7.8. In other pH ranges, the formal redox potentials decrease linearly with rising pH (slope 60 mV pH^{-1}). A lower redox potential is thus required for Co(III) reduction at pH 9.01 as compared to those needed at pH values of 4.2 and 6.8. Potentials necessary for Co(III) reduction at pH 9.0 concomitantly result in the reduction of water and a cathodic peak that interferes with that for the Co(III)/Co(II) couple.

Finally, a pH-mediated change in aquocobalamin sorption to clays is predicted. Decreased sorption is expected with increased pH as a result of pH-dependent changes in aquocobalamin ligation. Aquocobalamin in the Co(III) form is a six coordinate complex with ligands in both the α (lower) and β (upper) positions (Figure 1). In this form the molecule possesses two negative charges, one contributed from phosphate in the benzimidazol tail and the other from one of the nitrogens coordinating Co. The overall charge on the molecule is thus +1. Above pH 7.8, the water molecule in the β position is converted to OH$^-$, eliminating any net charge on the molecule (*40*). Decreased sorption is likely because of reduced electrostatic attraction of the neutral molecule for the negatively charged clays. Previous research has shown substantially greater sorption of positively charged aquocobalamin as compared to neutral cyanocobalamin on Ca^{2+}-saturated hectorite (*24*). Since both molecules have similar molecular dimensions, charge characteristics do indeed appear to play a role in corrinoid sorption. These quantitative and associated qualitative changes in aquocobalamin sorption with increased pH undoubtedly influence electrochemical behavior of the molecule as determined using cyclic voltammetry.

Typical cyclic voltammograms for aquocobalamin in solution and aquocobalamin sorbed to hectorite and montmorillonites SWy-1 and Syn-1 are shown in Figure 3 for systems buffered at pH 4.2. Even when we paid maximal attention to uniformity of electrode preparation, positions of cathodic and anodic peak potentials varied from one electrode to another. The average positions of peak potentials for aquocobalamin in the presence of each of the clays and a pH 4.2 buffer are given in Table 2. The calculated formal redox potential ($E^{o\prime}$) of −52 mV (vs SHE) for aquocobalamin in a pH 6.8 sodium phosphate buffer is in general agreement

with data obtained by others for the Co(III)/Co(II) couple (*40, 41*).

Vermiculite-coated electrodes displayed the same cathodic and anodic peak potentials as aquocobalamin solutions. All other clay-modified electrodes shifted cathodic peaks to more negative values with montmorillonite SAz-1 having the least effect and hectorite having the greatest. Anodic peak shifts varied with the clay; montmorillonites SAz-1 and SWy-1 shifting aquocobalamin peak potentials to more positive potentials, and hectorite and montmorillonite Syn-1 having the opposite effect. Aquocobalamin in the presence of hectorite experienced the greatest overall change in formal redox potential with a shift of −104 mV as compared to aquocobalamin in solution. Montmorillonite Syn-1 was the only other clay to cause a negative shift in the formal redox potential of aquocobalamin.

Shifts in the formal redox potentials of other organic cations in the presence of clay-modified electrodes have also been observed (*39, 42*). Changes in peak potentials depend on the supporting electrolyte concentration and altered reaction equilibria induced by the interaction of the redox active species with the clay film (*26*). Formal redox potentials for aquocobalamin were determined at a consistent electrolyte concentration. Altered potentials thus reflect the effect sorption has on the ability to reduce or oxidize the central metal of the tetrapyrrole. Negative shifts in cathodic peak potentials most likely reflect stabilization of the oxidized and positively charged tetrapyrrole species by interaction with negatively charged clays. However, the positive charge of aquocobalamin is located on the cobalt ion in the center of the ring system and is therefore shielded by several angstroms of organic structure. In contrast to cationic porphyrins (*21*), previous investigations showed no obvious changes in the FT-IR or UV/VIS spectra of aquocobalamin with its sorption to hectorite (*24*). Aquocobalamin interaction with the clays is therefore facilitated by charge characteristics but cannot be explained solely by a cation exchange mechanism. Hydrophilic amide and acid groups of the side chains (Figure 1) participate in hydrogen bonding, thus promoting the interaction of tetrapyrroles with other molecules such as proteins (*43*). The association of organic molecules with mineral surfaces is often through carbonyl or amine groups. Lone pair electrons of these groups may be protonated at

the mineral surface or linked to a water molecule on the exchange cation (*44*). It is likely that these groups also play a role in corrinoid sorption on minerals.

In addition to multiple bonding mechanisms and the heterogeneity of sorption sites, changes in aquocobalamin ligation undoubtedly influence cathodic and anodic peak potentials. Redox potentials for the Co(III)−Co(II)−Co(I) system are controlled by the electron-donating character of both the α and β ligands of the aquocobalamin molecule (*40*). This may be especially true in the case of anodic scans based on the fact that aquocobalamin oxidation to the Co-(III) form requires ligation at both positions. Reaction or replacement of aquocobalamin ligands with functional groups on mineral surfaces would alter anodic peak potentials, possibly contributing to the rather broad anodic peaks and anodic peak shifts among the minerals.

The redox behavior of aquocobalamin is thus differentially modified with sorption to clay minerals. This is significant since abiotic catalysis of reductive dehalogenation with extracellular tetrapyrroles is controlled by the redox active center of the molecule. Those clays lowering the formal redox potential of the tetrapyrrole from that observed in aqueous solution are potentially better reductants and, possibly, more efficient catalysts of pollutant degradation. From the opposite perspective, decreased catalysis is possible because lower redox potentials are required for Co reduction and the formation of a species capable of participating in reductive dehalogenation.

These findings are relevant to both intrinsic rates of contaminant transformation and potential in situ remediation strategies. Tetrapyrroles released in the environment from indigenous microbial populations or exogenous additions will be sorbed by colloidal materials such as clays. As shown here, sorption differentially alters redox activity of the tetrapyrrole and thus its potential to participate in abiotic catalysis of reductive dehalogenation. However, overall rates of reductive dehalogenation will be a function of both altered eletrochemical properties of the tetrapyrrole as well as contaminant diffusion to the site of catalysis. Consideration of diffusional limitations is especially important given the fact that aquocobalamin occupies interlayer spaces of expandable 2:1 clays. Assays are therefore necessary to determine the overall impact of tetrapyrrole sorption on dehalogenation rates of environmental pollutants.

Literature Cited

(1) Hoff, R. M.; Muir, D. C. G.; Grift, N. P. *Environ. Sci. Technol.* **1992**, *26*, 266−275.
(2) Goodrich, J. A.; Lykins, B. W. J.; Clark, R. M. *J. Environ. Qual.* **1991**, *20*, 707−717.
(3) Boyd, S. A.; Mikesell, M. D.; Lee, J.-F. In *Reactions and Movement of Organic Chemicals in Soils*; Sawhney, B. L., Brown, K., Eds.; SSSA Special Publication 22; SSSA and ASA: Madison, WI, 1989; pp 209−228.
(4) Wolfe, N. L.; Mingelgrin, U.; Miller, G. C. In *Pesticides in the Soil Environment: Processes, Impacts, and Modeling*; Cheng, H. H., Ed.; SSSA: Madison, WI, 1990; pp 103−168.
(5) Chaudhry, G. R.; Chapalamadugu, S. *Microbiol. Rev.* **1991**, *55*, 59−79.
(6) Vogel, T. M.; Criddle, C. S.; McCarty, P. L. *Environ. Sci. Technol.* **1987**, *21*, 722−736.
(7) Kuhn, E. P.; Suflita, J. M. In *Dehalogenation of Pesticides by Anaerobic Microorganisms in Soils and Groundwater—A Review*; Sawhney, B. L., Brown, K., Eds.; SSSA and ASA: Madison, WI, 1989; pp 111−180.
(8) Macalady, D. L.; Tratnyek, P. G.; Grundl, T. J. *J. Contam. Hydrol.* **1986**, *1*, 1−28.
(9) Schwarzenbach, R. P.; Stierli, R.; Lanz, K.; Zeyer, J. *Environ. Sci. Technol.* **1990**, *24*, 1566−1574.
(10) Tratnyek, P. G.; Macalady, D. L. *J. Agric. Food Chem.* **1989**, *37*, 248−254.
(11) Callot, H. J.; Ocampo, R.; Albrecht, P. *Energy Fuels* **1990**, *4*, 635−639.
(12) Sundararaman, P.; Boreham, C. J. *Geochim. Cosmochim. Acta* **1993**, *57*, 1367−1377.
(13) Verne-Mismer, J.; Ocampo, R.; Bauder, C.; Callot, H. J.; Albrecht, P. *Energy Fuels* **1990**, *4*, 639−643.
(14) Clayton, J. L.; Michael, G. E. *Energy Fuels* **1990**, *4*, 644−646.
(15) Bonnett, R.; Czechowski, F.; Latos-Grazynski, L. *J. Chem. Soc. Chem Commun.* **1990**, 849−851.
(16) Bonnett, R.; Czechowski, F.; Latos-Grazynski, L. *Energy Fuels* **1990**, *4*, 710−716.
(17) Zoro, J. A.; Hunter, J. M.; Eglinton, G.; Ware, G. C. *Nature* **1974**, *247*, 235−236.
(18) Ukrainczyk, L.; Chibwe, M.; Pinnavaia, T. J.; Boyd, S. A. *Environ. Sci. Technol.* **1995**, *29*, 439−445.
(19) Barloy, L.; Battioni, P.; Mansuy, D. *J. Chem. Soc. Chem. Commun.* **1990**, 1365−1367.
(20) Battioni, P.; Bartoli, J. F.; Mansuy, D.; Byrun, Y. S.; Traylor, T. G. *J. Chem. Soc. Chem. Commun.* **1992**, 1051−1053.
(21) Ukrainczyk, L.; Chibwe, M.; Pinnavaia, T. J.; Boyd, S. A. *J. Phys. Chem.* **1994**, *98*, 2668−2676.
(22) Lewis, T. A.; Morra, M. J.; Brown, P. D. *Environ. Sci. Technol.* **1996**, *30*, 292−300.
(23) Chiu, P.-C.; Reinhard, M. *Environ. Sci. Technol.* **1995**, *29*, 595−603.
(24) Kliewer, K. A.; Morra, M. J. *Soil Sci. Soc. Am. J.* In press.
(25) Ghosh, P. K.; Bard; A. J. *J. Am. Chem. Soc.* **1983**, *105*, 5691−5693.
(26) Bard, A. J.; Mallouk; T. In *Molecular Design of Electrode Surfaces*; Murray, R. W., Ed.; John Wiley: New York, 1992; pp 271−312.
(27) Laszlo, P. *Acc. Chem. Res.* **1986**, *19*, 121−127.
(28) Pinnavaia, T. J. *Science* **1983**, *220*, 365−371.
(29) King, R. D.; Nocera; D. G.; Pinnavaia; T. J. *J. Electroanal. Chem.* **1987**, *236*, 43−53.
(30) Fitch, A. *Clays Clay Miner.* **1990**, *38*, 391−400.
(31) Assaf-Anid, N.; Hayes, K. F.; Vogel, T. M. *Environ. Sci. Technol.* **1994**, *28*, 246−252.
(32) Chiu, P.-C.; Reinhard, M. *Environ. Sci. Technol.* **1996**, *30*, 1882−1889.
(33) Hirai, H.; Nakao; Y.; Toshima; N. *J. Macromol. Sci. Chem.* **1979**, *A13*, 727−750.
(34) van Olphen, H. *An Introduction to Clay Colloid Chemistry,* 2nd ed.; Wiley: New York, 1963.
(35) *Electrode Polishing and Care, A-1302*; Bioanalytical Systems: West Lafayette, IN, 1992.
(36) Glusker, J. P. In B_{12}, *Vol. 1, Chemistry*; Dolphin, D., Ed.; Wiley: New York, 1982; pp 24−106.
(37) Dandurand, L.-M.; Morra, M. J.; Chaverra, M. H.; Orser, C. S. *Soil Biol. Biochem.* **1994**, *26*, 1423−1430.
(38) Wright, A. C.; Granquist, W. T.; Kennedy, J. V. *J. Catal.* **1972**, *25*, 65−80.
(39) Ege, D.; Ghosh; P. K.; White, J. R.; Equey, J.-F.; Bard, A. J. *J. Am. Chem. Soc.* **1985**, *107*, 5644−5652.
(40) Lexa, D.; Saveant, J. M. *Acc. Chem. Res.* **1983**, *16*, 235−243.
(41) Daas, P. J.; Keltjens, J. T.; Hagen, W. R.; van der Drift, C. *Arch. Biochem. Biophys.* **1995**, *319*, 244−249.
(42) Liu, H. Y.; Anson, F. C. *J. Electroanal. Chem.* **1985**, *184*, 411−417.
(43) Pratt, J. M. *Inorganic Chemistry of Vitamin B_{12}*; Academic Press: New York, 1972.
(44) Harter, R. D. In *Minerals in Soil Environments*; Dixon, J. B., Weed, S. B., Kittrick, J. A., Milford, M. H., White, J. L., Eds.; SSSA: Madison, WI, 1977; pp 709−739.