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Characterization of Chlorovinylcobalamin, A Putative Intermediate in Reductive Degradation of Chlorinated Ethylenes

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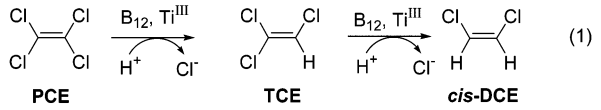
Perchloroethylene (PCE) and trichloroethylene (TCE) are persistent contaminants found in many terrestrial and groundwater environments.[1] Several anaerobic organisms use corrinoid-dependent enzymes to reductively dechlorinate these toxic compounds in a process that is coupled to energy metabolism.[2] Vitamin B_{12} itself has also been used for catalytic abiotic dechlorination of PCE and TCE in the presence of titanium(III)citrate (eq 1).[3]



(1)

Mechanistic studies on this latter process suggest that conversion of PCE to TCE occurs via electron transfer from cob(I)alamin to PCE,[3d,4] whereas further transformation of TCE to lesser chlorinated ethylenes and ultimately ethylene and acetylene involves organocobalamins. The latter hypothesis is supported by detection of di- and monochlorinated vinylcobalamins by mass spectrometry and by cobaloxime model studies.[5−7] At present, no information is available about the structure and chemical properties of chlorinated vinylcobalamins. Moreover, it is unclear whether these compounds could function as intermediates in the catalytic process because the Co−C bond in vinylcobalamins would be too strong for homolytic cleavage. Here we describe the synthesis of *cis*-chlorovinylcobalamin **1** and the first reported crystal structure of an organocobalamin with an sp^2-hybridized carbon ligand. Chlorovinylcobalamin **1** and vinylcobalamin **2** display interesting electrochemical properties that have important implications for the mechanism and scope of B_{12}-catalyzed reductive dechlorination.

Treatment of aqueous cob(I)alamin with chloroacetylene produced **1** in 89% yield without detection of the trans isomer. X-ray crystallographic analysis (Figure 1) revealed a Co−C bond length of 1.953 Å,[8] shorter than the corresponding bonds in methylcobalamin (MeCbl, 1.983 Å)[9a] and adenosylcobalamin (AdoCbl, 2.049 Å)[9b] as expected for a vinyl ligand. The Co−N bond length to the axial benzimidazole ligand is 2.128 Å in **1**, as compared to 2.195 Å for MeCbl and 2.234 Å for AdoCbl, providing an example of the "inverse" trans effect often observed in cobalamins and cobaloximes.[10]

As anticipated, complex **1** proved stable in solution, and thus we questioned its possible involvement in the catalytic process. However, when **1** was treated anaerobically with Ti(III)citrate, a slow decrease in the absorbance of **1** at 527 nm was observed (Figure 2), concomitant with formation of cob(I)alamin at 384 nm. After 1 h, the decrease in absorbance at 527 nm accelerated before leveling off, and vinylcobalamin **2**, characterized by X-ray analysis, was isolated in 50% yield.[11] The acceleration in the reduction of **1** after a lag period suggests that the cob(I)alamin initially formed

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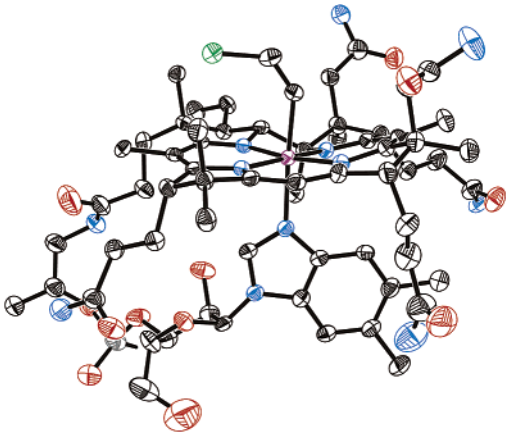

Figure 1. Thermal ellipsoid plot (35% probability) of the structure of *cis*-chlorovinylcobalamin **1** (ORTEP drawing).[8] The complex was crystallized from 40% aqueous PEG.

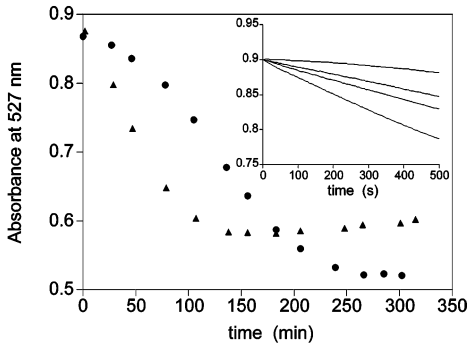

Figure 2. Time-dependent changes of Abs_{527} during reaction of cobalamin **1** (0.14 mm) with 5 mm Ti(III)citrate at pH 9 in the absence (●) and presence of 0.014 mM cob(I)alamin (▲). Inset: initial rates with (from top to bottom) 0%, 20%, 40%, and 80% cob(I)alamin. The initial absorbances were normalized to account for the increase in Abs_{527} due to the added catalyst (Supporting Information).

may act as a catalyst. To test this hypothesis, **1** was treated with excess Ti(III)citrate and a catalytic amount of cob(I)alamin (10%). This reaction did not display a significant lag phase, and increased concentrations of cob(I)alamin enhanced the initial rate (inset, Figure 2).[12] On the other hand, vinylcobalamin **2** subjected to the same conditions was not dealkylated over 6 h.[13] The Co(I)-catalyzed reduction of **1** was unexpected because the potentials required to reduce alkylcobalamins ($E° < -1.5$ V vs NHE)[14] are typically much more negative than the Co(I)/Co(II) couple of B_{12} (−0.61 V)[15] or the redox potential of Ti(III)citrate ($E° \approx -0.6$ V at pH 8).[5,16] To gain insights into the redox properties of **1**, a cyclic voltammogram was recorded in DMF, exhibiting an irreversible cathodic peak potential at −1.23 V (Figure 3). The return scan as well as the second forward scan show the appearance of the Co^{I}/Co^{II} couple

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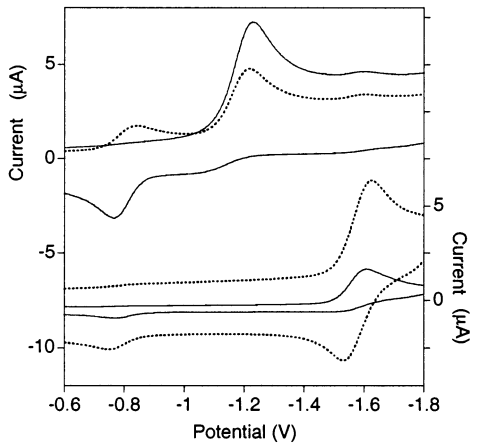

Figure 3. Top: CV of 1 mM **1** in DMF at 25 °C (0.1 V/s); second forward scan is shown as a dashed line. Bottom: CV of **2** at 0.1 (solid line) and 1 V/s (dashed line). Potentials were measured against an internal standard (Supporting Information) and are reported versus NHE.

of unalkylated B_{12} ($E_{1/2}$ −0.80 V in DMF), indicating cleavage of the Co−C bond upon electron transfer. The CV of **2** was obtained for comparison, displaying a cathodic E_p at −1.61 V. Surprisingly, CVs recorded at faster scan rates (1 V/s) resulted in a quasi-reversible wave.[17] To the best of our knowledge, **2** is the first reported organocobalamin to give reversible reduction behavior at ambient temperature and without the need for ultrafast scan rates.[14a] The high stability of the one-electron-reduced intermediate is most likely due to the stronger Co−C bond in **2** as compared to alkylcobalamins previously investigated electrochemically.

The presence of one chloride on the vinyl ligand shifts the peak potential by a remarkable 0.4 V.[18] This effect is much more pronounced than that observed for the corresponding cobaloximes ($\Delta E_p = 0.15$ V).[6] The E_p of the vinylcobalamins is expected to shift to even less negative potentials when the number of chlorides on the vinyl ligand is increased,[6] and hence reduction of di- and trichlorinated vinylcobalamins would occur readily. The reduction potential for trichlorinated vinylcobalamin is probably close to or less negative than the Co^{I}/Co^{II} couple of B_{12}, which explains why trichlorinated vinylcobalamin has never been detected. Our findings also explain why B_{12}-catalyzed dechlorination of PCE does not stall as a result of formation of chlorinated vinylcobalamins. However, they also highlight a weakness as the dechlorination of **1** efficiently produces **2**.[19] The resistance of **2** toward reductive dealkylation prevents the rapid regeneration of the active catalyst, and this sequestering presents an impediment for efficient B_{12}-catalyzed dechlorination of chloroethylenes.

These results suggest an interesting possibility for the enzymatic dechlorination. The dehalogenases characterized to date contain a corrinoid and two or more iron sulfur clusters.[2] When the fully reduced protein from *Dehalobacter restrictus* was reacted with PCE, Co(II) formation was observed, suggesting a one-electron-transfer mechanism[20] analogous to the abiotic reaction.[3d,4] Our results offer an alternative explanation featuring formation of a trichlorovinylcobalamin, which is subsequently rapidly reduced by electron transfer from one of the reduced FeS clusters resulting in Co(II) and a trichlorovinyl anion.[21]

In summary, chlorovinylcobalamins observed previously by mass spectrometry in abiotic dechlorination reactions may be generated by reaction of cob(I)alamin with chloroacetylene, a compound detected as an intermediate in the B_{12}-catalyzed dechlorination of PCE.[3e,g] Return of chlorinated vinylcobalamins to the active form of the catalyst can be achieved via reductive dealkylation promoted by cob(I)alamin. This reductive dealkylation may be particularly facile for multichlorinated vinylcobalamins, but not for vinylcobalamin.

Acknowledgment. This work was supported by the Roy J. Carver Charitable Trust (01-154). The Materials Chemistry Laboratory at the University of Illinois was supported in part by NSF (CHE 95-03145). Any opinions, findings, and conclusions expressed are those of the authors and do not necessarily reflect the views of the Carver Trust or NSF.

Supporting Information Available: Synthetic procedures for **1** and **2**, simulations of Figures 2 and 3, CV data referenced versus ferrocene, and crystallographic data (PDF and CIF). The structure of **1** has been deposited in the Cambridge Crystallographic Data Center. This material is available free of charge via the Internet at http://pubs.acs.org.

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(11) A manuscript comparing the structures of **1** and **2** is in preparation.
(12) Because of the occurrence of multiple simultaneous processes (reduction of **1** by cob(I)alamin and Ti(III)citrate, reduction of Co^{II} by Ti^{III}, and reaction of cob(I)alamin with vinyl chloride/acetylene), it was not possible to directly extract meaningful rate constants. For kinetic simulations supporting the proposed mechanism, see the Supporting Information.
(13) Over long periods of time, vinylcobalamin is reduced under these conditions. By simulating the kinetics of dechlorination of PCE, Semadeni et al. estimated the $t_{1/2}$ of vinylcobalamin as 250 h,[3g] consistent with our observations with authentic **2**.
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(17) Although the wave of **2** is quasi reversible ($\Delta E_p \approx 90$ mV), some of the one-electron-reduced intermediate undergoes Co−C bond cleavage as a small peak due to reoxidation of cob(I)alamin occurs around −0.77 V versus NHE.
(18) Part of this difference is due to the irreversible follow up reaction that cleaves the Co−C bond for **1**, which shifts the peak potential to a less negative value. See the Supporting Information for simulations of the CV that estimate the magnitude of this shift to be 120 mV or less.
(19) The conversion of **1** to **2** likely involves formation of acetylene and/or vinyl chloride, which is known to react with cob(I)alamin. Naumberg, M.; Duong, K. N. V.; Gaudemer, A. *J. Organomet. Chem.* **1970**, *25*, 231−242. Johnson, A. W.; Mervyn, L.; Shaw, N.; Smith, E. L. *J. Chem. Soc.* **1963**, 4146.
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(21) The advantage of such a mechanism is that it would not involve vinyl radicals if reduction generates cob(II)alamin and the vinyl anion (as observed for methoxycarbonylcobalamin).[14d]

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