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Reductive Transformation of Trichloroethene by Cobalamin: Reactivities of the Intermediates Acetylene, Chloroacetylene, and the DCE Isomers

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The transformations of acetylene, chloroacetylene, 1,1-dichloroethene (DCE), and *cis*- and *trans*-DCE mediated by cobalamin in the presence of titanium(III) citrate were investigated at pH 8 and 22 °C. Acetylene quantitatively reacted to ethene via vinylcobalamin as the proposed intermediate. Chloroacetylene reacted to acetylene and vinyl chloride. Proposed intermediates are ethynylcobalamin and vinylcobalamin, respectively. The principal initial reaction of chloroacetylene formed ethynylcobalamin which decomposed to acetylene. The proposition for ethynyl- and vinylcobalamin formation is based on fitting reaction models to kinetic data. Kinetic modeling suggests half-lives for ethynyl- and vinylcobalamin of 1.4 and 251 h, respectively. 1,1-Dichloroethene reacted to approximately 20% volatiles (ethene, ethane, vinyl chloride, and acetylene) and 80% unidentified nonvolatile products. *cis*- and *trans*-DCE transformed slowly and produced small yields of vinyl chloride, ethene, and ethane.

Introduction

The capability of microbial cultures to reductively transform chlorinated aliphatic hydrocarbon compounds has been attributed to transition metal coenzymes, such as cobalamin (vitamin B_{12}), cofactor F_{430}, or heme (*1*−*4*). This hypothesis is supported by studies of pure cultures that reductively dechlorinate tetrachloroethene (PCE) and trichloroethene (TCE). These studies indicate corrinoids are involved in the dehalogenation reactions (*3, 5*−*10*). To gain insight into the chemistry of such biological dehalogenation reactions, model systems are studied using cobalamin (cbl) and bulk reductants such titanium(III) citrate or reduced sulfur compounds (*4, 11*−*18*). Titanium(III) citrate reduces cob(III)alamin (cbl^{III}) to cbl^{I}, a strong reductant and nucleophile (*19*). Cbl^{I} is capable of dechlorinating TCE to ethene (*4, 12, 13*). Two separate pathways leading to ethene have been identified: a pathway in which sequential hydrogenolysis leads directly to ethene through the intermediates *cis*-, *trans*-, and 1,1-dichloroethene (DCE) and vinyl chloride (*4*) and a pathway which is initiated by α,β-dihaloelimination of TCE to form chloroacetylene (*13*). Chloroacetylene can be transformed by hydrogenolysis to acetylene and hydrogenation of acetylene leads to ethene (*13*). The latter pathway is significant because it bypasses vinyl chloride, a relatively stable carcinogen (*20*). There are three potential links between the two pathways: (i) α,β-dihaloelimination of *cis*- and *trans*-DCE and (ii) α-dihaloelimination of 1,1-DCE forming acetylene or (iii) hydrogenation of chloroacetylene forming vinyl chloride.

The "super reduced" cobalamin (cbl^{I}) is both a powerful reductant and a nucleophile that has been shown to react with chlorinated substrates via single electron transfer (SET) (*12, 16*), nucleophilic substitution (S_N2) or addition reactions (*11, 21*−*23*). SET and S_N2 reactions can compete (*21, 24*). SET leads to reductive bond cleavage of the carbon−chlorine bond and the formation of a radical (*25*). Spectroscopic evidence has shown that reactions of cbl^{I} with alkylhalids can lead to relatively stable alkyl-, vinyl-, or ethynylcobalamins (*11, 12, 16*). These alkylcobalamin complexes can decompose by reductive cleavage of the cobalt−carbon bond with titanium(III) citrate as the reductant (*16*). For PCE and TCE, involvement of titanium(III) citrate in cobalt−carbon cleavage reactions has been inferred from the observed pH dependence of the product distribution found in TCE transformations (*12*). Reductive cleavage is facilitated by electron-withdrawing substituents (*26*) and is rapid compared to nonreductive cleavage reactions (*27*−*31*). Heterolytic reductive cleavage leading to cbl^{II} and the carbanion is favored over homolytic reductive cleavage which forms cbl^{I} and a radical (*26, 29, 32*). Thermally or sterically induced nonreductive cleavage of the cobalt−carbon bond yields cbl^{II} and a radical (*33, 34*). The radical intermediates are rapidly reduced followed by either β-elimination or protonation (*35, 36*) or, alternatively, abstract a hydrogen from hydrogen donors (*12, 16*). The formation and decomposition of intermediate cobalamin complexes from reactions of ethynes with cbl have not been investigated extensively in the present context.

In this paper, we present transformation data of chloroacetylene, acetylene, and the three DCE isomers, intermediates of the TCE transformation process. It is shown that chloroacetylene is a precursor for vinyl chloride and acetylene. The data reported here provide kinetic evidence for the formation of intermediate cobalamin complexes are used to quantify their stability and help to explain product distributions. The data serve as a basis for future efforts to model the TCE transformation process.

Materials and Methods

Chemicals and Reagents. All chemicals were from commercial sources (Aldrich) described previously (*16*) and used as received without further purification: aquocobalamin (vitamin B_{12a}, >96%, Fluka), TCE (99+%), *cis*-DCE (99%), *trans*-DCE (98%), and 1,1-DCE (99%). Acetylene (99.6%, Altair) was obtained as a compressed gas. The calibration standards for vinyl chloride, acetylene, and ethene, ranging from 1000 ppm to 10% in nitrogen, were purchased from Scott Specialty Gases. TCE, *cis*-DCE, *trans*-DCE, and 1,1-DCE spike solutions and a combined calibration standard solution containing TCE and the DCE isomers were prepared in methanol (99.9+%) and stored at −15 °C. All other reagent stock solutions were prepared and stored as previously described (*16*). Tris (ultrapure, Baker) was used as the pH buffer.

Synthesis of Chloroacetylene. Chloroacetylene was synthesized from *cis*-DCE using the procedures described by Denis et al. (*37*). Chloroacetylene is a toxic gas and can be explosive under oxic conditions (*38, 39*). A diazomethane generator (MNNG diazomethane-generation apparatus, Al

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drich) placed in a nitrogen-flushed glovebag (Atmosbag, Aldrich) was used as a reaction apparatus A total of 10 mmol of *cis*-DCE in 2 mL of tetrahydrofuran (THF, anhydrous, 99.9%, inhibitor-free, Aldrich) was added to excess lithium hydride powder (95%, Aldrich) suspended in 1.5 mL of THF in the inner reaction tube of the closed and oxygen-free reaction apparatus. The outer tube of the reactor contained 5 mL of THF. The reactor was kept on an ice/salt slush bath during the reaction. A total of 10 μL of methanol (anhydrous, 99.8%, Aldrich) was injected into the inner tube to initiate the reaction. After 45 min, another 300 μL of methanol was slowly injected. The reaction was terminated after 4 h and the solution from the outer tube was transferred under nitrogen into an amber vial and stored at −15 °C. The chloroacetylene solution served as the standard stock solution. The chloroacetylene concentration (27 mM) was determined using the approach described below.

Kinetic Experiments. All experiments were conducted at 22 ± 1 °C and pH 8.0 under anaerobic and light-excluded conditions. The experimental procedures used were similar to those described previously (*16*) except as follows: in each 64 mL amber borosilicate bottle, a 32 mL aqueous solution was placed which contained 15 mM titanium(III) citrate, 10 μM cbl, and 60 mM Tris, leaving 32 mL of headspace (N_2/H_2). The reaction was initiated by injecting microliter amounts of substrate spike solution into the reaction solution and equilibrated by vigorous manual shaking of the bottles. Preliminary tests indicated equilibration to be complete in less than 3 min (data not shown). During the entire reaction period, the bottles were kept on a shaker table at 400 rpm. Conditions for all experiments were the same except for the initial amounts of the substrate and cbl, which in some cases were varied. Identical solutions containing the substrate without cbl served as controls. The pH of the reaction mixture was measured using an EA 940 Expandable Ion Analyzer (Orion, Boston, MA). Concentrations of volatiles were determined by sampling 50 μL aliquots of the gaseous headspace using GC/FID.

The analytical methods used were adapted from Burris et al. (*13*). Headspace aliquots of 50 μL were injected into an HP 5890 series II gas chromatograph (GC)/flame ionization detector (FID). Calibration factors were calculated based on the total amount of compounds added to the reactor bottle. For chloroacetylene, an indirect method was used as described below. The freshly synthesized chloroacetylene was identified using an HP 5890 GC/MSD (5970 mass selective detector) under EI mode, scanning from 40 to 200 amu, and a 30 m × 0.32 mm DB-5 capillary column (0.25 μm film thickness, J&W Scientific) at an isothermal temperature of 40 °C. To verify chloroacetylene as a reaction intermediate of the TCE transformation process, 560 μmol of TCE was reacted with 0.32 μmol of cobalamin under standard conditions. The reaction mixture was analyzed by GC/FID as described above. Chloroacetylene was identified by retention times comparing the reaction mixture with a control and the freshly prepared chloroacetylene standard.

Data Analysis. The reactions were studied in two phase reactors consisting of a liquid and a gas volume, denoted V_l and V_g, respectively. The data were evaluated assuming that (i) transformations occurred only in the liquid phase, (ii) equilibration between the two phases was rapid relative to the liquid-phase reactions, and (iii) the ratio between the gas and liquid-phase concentrations, c_g/c_l, was given by Henry's law constant, H_c. The total amount of a compound i, $\{S_i\}$, in the reactor is given by

$$\{S_i\} = c_{gi}(V_g + V_l/H_{ci}) \quad (1)$$

Pseudo-first-order and zero-order substrate removals were expressed in terms of the measured total amount of the substrate, $\{S_i\}$, using eqs 2 and 3, respectively:

$$-d\{S_i\}/dt = k'_{obs}\{S_i\} \quad (2)$$

$$-d\{S_i\}/dt = k_{obs} \quad (3)$$

where k'_{obs} and k_{obs} are the observed mass-based apparent first-order and zero-order rate constants in units of inverse hours and micromoles per hour, respectively. Using standard statistical techniques, 95% confidence intervals were obtained.

Kinetic data were evaluated using the kinetic modeling software package, Scientist (MicroMath, Scientific Software, Salt Lake City, UT). The models were described by sets of ordinary differential equations (ODEs) representing the following rate laws:

$$-d\{S_i\}/dt = \sum k''_j\{S_i\}\{C\} \quad (4)$$

$$-d\{IC_i\}/dt = \sum k'_j\{IC_i\} - \sum k''_j\{S_i\}\{C\} \quad (5)$$

$$d\{P_i\}/dt = \sum k''_j\{S_i\}\{C\} + \sum k'_j\{IC_i\} \quad (6)$$

where k''_j and k'_j are the second-order and first-order mass-based rate constants, $\{C\}$ and $\{IC_i\}$ are the total amounts of the reduced catalyst, cbl^I, and the intermediate cobalamin complex, respectively, and $\{P_i\}$ is the total molar mass of product. The subscripts i and j indicate the different compounds and reactions involved. To obtain solutions for a given time interval, the ODEs were solved by numerical integration using EPISODE, an initial value ODE solver provided by the software package Scientist.

Only volatile compounds were measured. The intermediate cobalamin complexes and unknown products were obtained by fitting the postulated models to the data sets assuming mass conservation (Table 1). The experimental data were fitted using a least-squares minimization algorithm (Scientist) constrained to the ODEs with the fitting parameters k''_j and k'_j, j = 1, 2, 3, ..., r, for each second- or first-order reaction r of the kinetic model m, respectively (Table 1), and to the mass conservation equation for the catalyst C (cbl^I):

$$\{C\} = \{C\}_0 - \sum\{IC_i\} \quad (7)$$

where $\{C\}_0$ is the initial amount of catalyst present in the reactor. This mass conservation is based on the assumption that (1) all oxidized free cobalamin (cbl^{II}) is rapidly recycled to cbl^I by titanium(III) citrate and (2) no cobalamin consumption reactions exist other than intermediate complex formation. Experimentally, it was found that cbl^{II} and cbl^{III} are reduced within seconds (data not shown). Standard deviations for the best-fit parameters k''_j and k'_j and overall correlation coefficients, r_m^2, were calculated by the software package Scientist. The consistency of the kinetic models was tested by incorporating the rate constants derived from the simplest reaction model into successively more complex reaction models.

The mass-based second-order rate constants k''_j derived from the model fits were converted to liquid-phase concentration-based rate constants k''^{liq}_j assuming that

$$-d\{S_i\}/dt = V_l d[c_i]/dt \quad (8)$$

$$-d[c_i]/dt = V_l \sum k''^{liq}_j [c_i][C] \quad (9)$$

where the edged brackets designate liquid-phase concentrations. On the basis of eqs 1, 4, 8 and 9, relation 10 can be derived, which relates the mass-based rate constant to the constant expressing the reaction rate in solution:

TABLE 1. Rate Laws[a] Given as Sets of ODEs Describing the Rate-Determining Reactions of Acetylene, Chloroacetylene, and 1,1-DCE Transformations Catalyzed by cbl^{I} [b]

schemes	reactions	ODE[c]
acetylene (Scheme 1)	1, 2	$-d\{ACT\}/dt = (k_1' + k_2')\{ACT\}\{cbl^{I}\}$
	2, 3, 4	$d\{vinyl\text{-}cbl^{III}\}/dt = k_2'\{ACT\}\{cbl^{I}\} - (k_3 + k_4)\{vinyl\text{-}cbl^{III}\}$
	1, 3, 4, 5	$d\{ETH\}/dt = (k_3 + k_4)\{vinyl\text{-}cbl^{III}\} + k_1'\{ACT\}\{cbl^{I}\}$
chloroacetylene (Schemes 1 and 2)	6, 7, 8	$-d\{CAT\}/dt = (k_6' + k_7' + k_8')\{CAT\}\{cbl^{I}\}$
	8, 9, 10	$d\{ethynyl\text{-}cbl^{III}\}/dt = k_8'\{CAT\}\{cbl^{I}\} - (k_9 + k_{10})\{ethynyl\text{-}cbl^{III}\}$
	1, 2, 7, 9, 10,	$d\{ACT\}/dt = k_7'\{CAT\}\{cbl^{I}\} + (k_9 + k_{10})\{ethynyl\text{-}cbl^{III}\}$ $-(k_1' + k_2')\{ACT\}\{cbl^{I}\}$
	6, 12	$d\{VC\}/dt = k_6'\{CAT\}\{cbl^{I}\}$
1,1-DCE (Scheme 3)	14, 15, 16	$-d\{1,1DCE\}/dt = (k_{14}' + k_{15}' + k_{16}')\{1,1DCE\}\{cbl^{I}\}$
	15, 17, 18	$d\{R\text{-}cbl^{III}\}/dt = k_{15}'\{1,1DCE\}\{cbl^{I}\} - (k_{17} + k_{18}')\{R\text{-}cbl^{III}\}$
	16, 18	$d\{volatiles\}/dt = k_{16}'\{1,1DCE\}\{cbl^{I}\} + k_{18}'\{R\text{-}cbl^{III}\}$
	14, 17	$d\{unknown\}/dt = k_{14}'\{1,1DCE\}\{cbl^{I}\} + k_{17}'\{R\text{-}cbl^{III}\}$

[a] Mass-based notation is dependent on reactor configuration. [b] ACT = acetylene, ETH = ethene, vinyl-cbl^{III} = vinylcobalamin, CAT = chloroacetylene, VC = vinyl chloride, ethynyl-cbl^{III} = ethynylcobalamin, R-cbl^{III} = intermediate cobalamin complex, where R represents an alkyl-, alkenyl-, or alkynyl group, unknown = nonvolatile products, proposed intermediate cobalamin complex. [c] Steady-state approximations were used for radical species.

$$k_j''^{liq} = V_l\, k_j'' f_c \tag{10}$$

where the conversion factor f_c is defined as

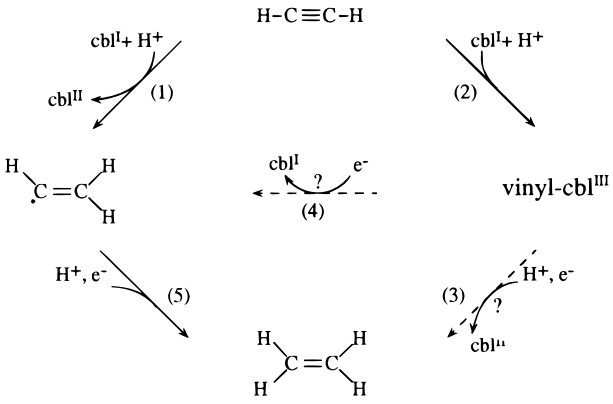
$$f_c = 1 + H_c(V_g/V_l) \tag{11}$$

For the chlorinated ethenes, the H_c values at 22 °C were taken from the literature (*40*). For acetylene, ethene, and ethane, H_c values were estimated as 0.93, 7.96, and 19.88, respectively, using aqueous solubility data measured at 1 atm constant pressure (*41*). For chloroacetylene, no solubility data was available. The H_c value of chloroacetylene was estimated using eq 1. The total amount of chloroacetylene, $\{S_{CAT}\}$, was obtained from transformation data under conditions where chloroacetylene was assumed to be quantitatively converted into acetylene and vinyl chloride (shown below). Gas-phase concentrations c_{gCAT} were estimated assuming that acetylene and chloroacetylene have the same FID response factors. The estimated H_c value of chloroacetylene was 1.2. The following f_c values were used: f_c(TCE) = 1.34, f_c(*cis*-DCE) = 1.14, f_c(*trans*-DCE) = 1.34, f_c(1,1-DCE) = 1.97, f_c(vinyl chloride) = 2.01, f_c(acetylene) = 1.93, and f_c(chloroacetylene) = 2.18.

Results and Discussion

Acetylene. The kinetic data and the model fits of the acetylene transformation experiment is shown in Figure 1. The proposed acetylene transformation pathway is indicated in Scheme 1. During the first 22 h, the acetylene mass decreased relatively rapidly by 0.28 μmol, nearly the total amount of cbl added (0.32 μmol). After 22 h, ethene accounted only for 11% of the acetylene transformed. The unaccounted mass was tentatively attributed to the formation of vinyl-cbl^{III}. Thereafter, the acetylene mass decreased at a constant rate [$k_{obs} = (0.80 \pm 0.07) \times 10^{-3}$ μmol/h] while the total mass of volatiles (acetylene + ethene) remained constant, indicating quantitative transformation of acetylene into ethene. These observations are qualitatively consistent with a kinetic model in which cbl^{I} catalyzes the transformation of acetylene into ethene via vinyl-cbl^{III} as the relatively stable intermediate (reaction 2). The time profiles for free cbl^{I} and vinyl-cbl^{III} were not measured but were calculated using the fitted k_1'', k_2'' and $k_3' + k_4'$, assuming that the missing mass was equal to the mass of vinyl-cbl^{III}. According to this model, cbl^{I} decreases rapidly during the first 22 h and reaches a steady state after 40 h. Vinyl-cbl^{III} can decompose into to ethene and the free catalyst via reaction 3 or 4. Close examination of the initial ethene formation rate, however, suggests that a second acetylene transforming pathway exists besides the addition of cbl^{I} to acetylene form vinyl-cbl^{III}. Figure 1b shows model simulations that include or exclude the SET reaction. The appearance of ethene is immediate and not delayed by the formation of a stable intermediate as predicted by the kinetic model which only considers the addition of cbl^{I} (reaction 2) as the initial transformation. To account for the rapid appearance of ethene, the pathway that includes SET (reaction 1) followed by reduction of the radical (reaction 5) was postulated.

SCHEME 1



Fitting the data shown in Figure 1 to the rate laws of Table 1 resulted in the kinetic constants (converted to liquid-phase conditions) summarized in Table 2. The initial transformation was reported to be first order in the proton concentration (*11, 12*), and k_1'' and k_2'' are expected to be pH dependent. The vinylcobalamin decomposition (via reactions 3 and/or 4) is represented by the sum of k_3' and k_4'. These two constants cannot be evaluated individually with the available data. Although the contribution of SET to the overall ethene formation appears to be small ($k_1'' \ll k_2''$), the formation of radicals in biological systems could potentially lead to toxicological effects.

Chloroacetylene. The kinetics of the chloroacetylene transformation was studied in the presence of 0.32 μmol of cbl^{I} at two different initial chloroacetylene amounts. In the first case, initial chloroacetylene amount was 2.05 μmol, 6.4 times in excess of the cbl^{I} concentration. In the second case, the initial chloroacetylene amount was 0.54 μmol, approximately equal to the amount of cbl^{I}. The results of these two experiments are shown in Figures 2 and 3, respectively. The proposed pathway is shown in Scheme 2.

In the experiment shown in Figure 2, two distinct reaction regimes are evident: chloroacetylene decreased rapidly for the first 20 min and more slowly thereafter. During the rapid chloroacetylene decline, the total mass of volatiles declined by 0.44 μmol and then remained constant. Of this mass loss, 0.32 μmol was tentatively attributed to the formation of ethynyl-cbl^{III} (reaction 8). Acetylene and vinyl chloride were formed without an apparent delay, suggesting a pathway with very reactive intermediates. The unaccounted mass

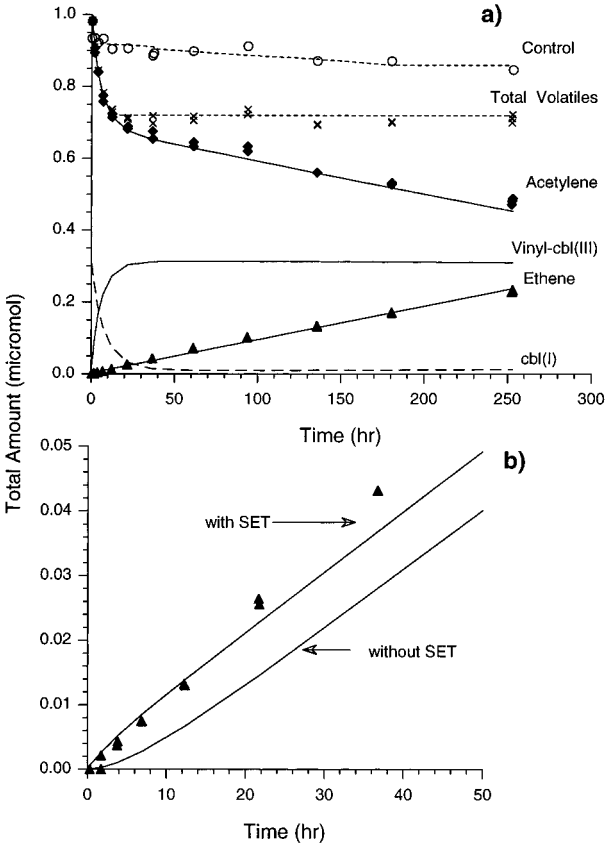


FIGURE 1. Reductive transformation of 0.98 μmol of acetylene in the presence of 0.32 μmol of cobalamin, under standard conditions [15 mM Ti(III) citrate at pH 8]. (a) Lines represent least-mean-square data fits constrained to the kinetic model indicated in Scheme 1 and rate laws indicated Table 1. The experimental data originate from replicate experiments. Cbl^{I} and vinylcobalamin profiles were determined from the constrained model calculations. Total volatiles = sum of acetylene and ethene in the reactor (liquid and gas phases). (b) Model fit for ethene formation with and without SET (reaction 1).

(6%) is tentatively attributed to the formation of unknown products during the initial phase of the reaction. The formation of ethynyl-cbl^{III}, the concomitant decrease in cbl^{I}, and the slow formation of vinyl- cbl^{III} predicted by the kinetic model is indicated in Figure 2b. After 20 min, the mass of total volatiles remained constant, suggesting quantitative conversion of chloroacetylene into acetylene and vinyl chloride. During this time, acetylene reacted slowly to vinyl-cbl^{III} as discussed above. Ethene was not detected. The final yields of acetylene and vinyl chloride were 66 and 10%, respectively. The proposed pathway is consistent with data reported by Johnson et al., who suggested the formation of ethynyl-cbl^{III} in reactions of cbl^{I} with bromoacetylene (*23*).

The data shown in Figure 3a provides additional kinetic evidence for the pathway proposed in Scheme 2. Here, the total volatiles decreased rapidly by 0.34 μmol by the time of the first measurement (6 min) consistent with the rapid formation of ethynyl-cbl^{III}. After 6 min, two different reaction regimes are evident. First, while chloroacetylene was present, the total molar mass of volatiles remained constant, indicating that chloroacetylene was quantitatively converted into acetylene and vinyl chloride. These conditions correspond to the phase in the above experiment where chloroacetylene is slowly declining. After 1 h, when chloroacetylene was completely transformed, vinyl chloride remained constant at 0.04 μmol, while acetylene formation continued. After 5 h, traces of ethene were observed (data not shown).

Taken together, these observations indicate that chloroacetylene is transformed into acetylene (66%) and vinyl chloride (10%). Interestingly, vinyl chloride was formed only when chloroacetylene was present. Acetylene appears to be formed via reductive cleavage of ethynyl-cbl^{III}, presumably with titanium(III) citrate as the reductant. Three possible initial transformation reactions can be proposed: (i) SET and protonation (reaction 6) to yield the vinyl chloride radical, (ii) SET concomitant with reductive chloride cleavage to form the ethynyl radical (reaction 7), and (iii) nucleophilic chloride substitution with cbl^{I} to form the ethynyl-cbl^{III} (reaction 8). The ethynyl-cbl^{III} may react further via reaction 9 forming acetylene or via reaction 10 forming the ethynyl radical which can be reduced to acetylene. The proposed vinyl chloride formation involves two sequential SETs (reactions 6 and 12) although other pathways are also conceivable. For instance, a short-lived chlorovinyl-cbl^{III} intermediate may form which is then reductively cleaved (not shown in Scheme 2).

The experimental data shown in Figures 2a and 3a were fitted to the kinetic model considering three parallel (pseudo)-second-order chloroacetylene removal reactions (reactions 6, 7, and 8), the slow transformation of the ethynyl-cbl^{III} into acetylene, and the formation of vinyl chloride via reaction 6 (Table 1). The reaction of ethynyl-cbl^{III} to acetylene via reactions 9 and 10 was modeled by a pseudo-first-order process with a rate constant of $k'_9 + k'_{10}$. In reactions 9 and 10, titanium(III) citrate is assumed to be the reductant. The time profiles of the three postulated cobalamin species, cbl^{I}, ethynylcobalamin, and vinylcobalamin were calculated based on mass balance considerations, assuming rapid reduction of cbl^{II} to cbl^{I}, assuming rapid mass transfer of the volatiles between the liquid and gaseous phases, and incorporating the model parameters derived from the acetylene transformation experiments (Figure 2b and 3b). To quantitatively compare the model results, the different kinetic parameters obtained from fitting the data were converted to liquid-phase rate constants as described above. The kinetic constants (converted to liquid-phase conditions) are summarized in Table 3. Although the model was able to reflect the time course of the different species that were measured, some significant discrepancies are evident: (1) the initial amounts of chloroacetylene obtained by fitting were approximately 10% below the analytically determined amounts; (2) the rate constants for the two chloroacetylene transformation experiments disagreed for all reactions except the transformation of ethynylcobalamin to acetylene ($k'_9 + k'_{10}$). These discrepancies are unexplained but could be due to rate-limiting gas−liquid equilibration, slow cbl^{I} regeneration or cbl^{I} concentration-dependent decomposition of intermediate cobalamin complexes. The reported chloroacetylene transformation rates are, therefore, system dependent and considered "apparent". The ratios k''_7/k''_6 and k''_2/k''_1 are approximately 0.5, and a ratio of 0.025 for chloroacetylene and acetylene, respectively, suggests chloroacetylene reacts more readily via SET than acetylene. This is consistent with the Marcus theory which predicts increased reduction rates with increasing one-electron reduction potentials, i.e., degree of halogenation (*25*). The formation of chloroacetylene in cells and its formation of radicals is likely to lead to toxic effects and could therefore be significant.

As shown in Figure 2b and 3b, cbl^{I} is rapidly bound as ethynyl-cbl^{III} thus lowering the overall activity of cbl^{I}. Over time, as acetylene is formed, it reacts to vinyl-cbl^{III}. Because vinyl-cbl^{III} is more stable than ethynyl-cbl^{III} (by a factor of 120−185) with time, the former is becoming a more significant deactivating species. The model predicts steady-state conditions are reached in 25 h with residual amounts of 0.05 μmol of cbl^{I} (results not shown). This is in agreement with the kinetic model for the acetylene transformation. At the low chloroacetylene amounts, the steady-state conditions are reached after approximately 60 h.

TABLE 2. Liquid-Phase Rate Constants Based on Kinetic Parameters k_j'' and k_j' Constrained to the Kinetic Models Describing Acetylene, Chloroacetylene, and 1,1-DCE Transformation (Standard Conditions: 15 mM Ti(III) Citrate, pH 8)

reactants and init. liquid-phase conc.	reaction path	rate constants[a,b] (liquid phase)
16.0 μM acetylene[c]	acetylene → ethenyl radical	$k_1''^{liq} = (0.3 \pm 0.3) \times 10^{-3}\ \mu M^{-1}\ h^{-1}$
10.0 μM cobalamin	acetylene → vinylcobalamin	$k_2''^{liq} = (12.2 \pm 1.3) \times 10^{-3}\ \mu M^{-1}\ h^{-1}$
	vinylcobalamin → ethene	$(k_3'^{liq} + K_4^{liq}) = (2.9 \pm 0.3) \times 10^{-3}\ h^{-1}$
29.4 μM chloroacetylene[c]	chloroacetylene → chlorovinyl radical	$k_6''^{liq}$(app) $= (0.4 \pm 0.2) \times 10^{-1}\ \mu M^{-1}\ h^{-1}$
10.0 μM cobalamin	chloroacetylene → ethynyl radical	$k_7''^{liq}$(app) $= (1.6 \pm 0.5) \times 10^{-1}\ \mu M^{-1}\ h^{-1}$
	chloroacetylene → ethynylcobalamin	$k_8''^{liq}$(app) $= (1.5 \pm 0.8) \times 10^{-1}\ \mu M^{-1}\ h^{-1}$
	ethynylcobalamin → acetylene	$(k_{10}'^{liq} + K_{11}^{liq}) = (5.4 \pm 1.4) \times 10^{-1}\ h^{-1}$
7.7 μM chloroacetylene[c]	chloroacetylene → chlorovinyl radical	$k_6''^{liq}$(app) $= (4.9 \pm 1.9) \times 10^{-1}\ \mu M^{-1}\ h^{-1}$
10.0 μM cobalamin	chloroacetylene → ethynyl radical	$k_7''^{liq}$(app) $= (10.3 \pm 3.1) \times 10^{-1}\ \mu M^{-1}\ h^{-1}$
	chloroacetylene → ethynylcobalamin	$k_8''^{liq}$(app) $= (51.6 \pm 20.1) \times 10^{-1}\ \mu M^{-1}\ h^{-1}$
	ethynylcobalamin → acetylene	$(k_{10}'^{liq} + K_{11}^{liq}) = (3.4 \pm 0.6) \times 10^{-1}\ h^{-1}$
12.7 μM 1,1-DCE[c]	1,1-DCE → unknown	$k_{14}''^{liq} = 8.8 \times 10^{-3}\ \mu M^{-1}\ h^{-1}$
10.0 μM cobalamin	1,1-DCE → R-cbl^{III}	$k_{15}''^{liq} = (9.4 \pm 2.1) \times 10^{-3}\ \mu M^{-1}\ h^{-1}$
	1,1-DCE → volatiles	$k_{16}''^{liq} = 2.4 \times 10^{-3}\ \mu M^{-1}\ h^{-1}$
	R-cbl^{III} → unknown	$k_{17}'^{liq} = 7.3 \times 10^{-3}\ h^{-1}$
	R-cbl^{III} → volatiles	$k_{18}'^{liq} = 2.5 \times 10^{-3}\ h^{-1}$
200.0 μM 1,1-DCE[c]	1,1-DCE → unknown	$k_{14}''^{liq} = 1.7 \times 10^{-3}\ \mu M^{-1}\ h^{-1}$
100.0 μM cobalamin	1,1-DCE → R-cbl^{III}	$k_{15}''^{liq} = 2.0 \times 10^{-3}\ \mu M^{-1}\ h^{-1}$
	1,1-DCE → volatiles	$k_{16}''^{liq} = 0.4 \times 10^{-3}\ \mu M^{-1}\ h^{-1}$
	R-cbl^{III} → unknown	$k_{17}'^{liq} = 3.6 \times 10^{-3}\ h^{-1}$
	R-cbl^{III} → volatiles	$k_{18}'^{liq} = 3.2 \times 10^{-3}\ h^{-1}$

[a] The second-order kinetic parameters were converted using the corresponding factors f_c and V_l. [b] Errors represent two standard deviations derived from the model fits. Omitted error limits are >65%. [c] Correlation coefficient of model fit: $r_m^2 > 0.99$.

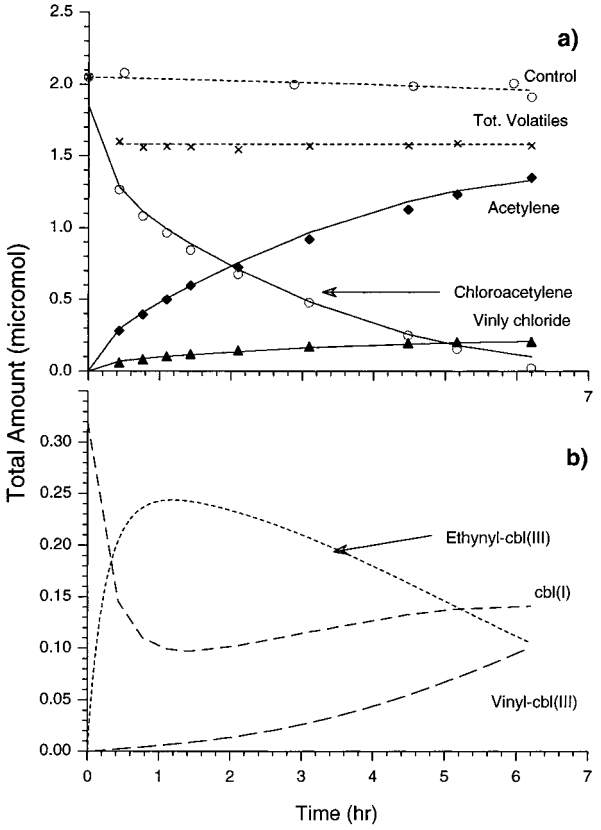


FIGURE 2. (a) Reductive transformation of 2.05 μmol of chloroacetylene in the presence of 0.32 μmol of cobalamin, under standard conditions [15 mM Ti(III) citrate at pH 8]. Lines represent least-mean-square data fits constrained to the kinetic model (Scheme 2) including the kinetic coefficients of the acetylene transformation (Scheme 1) shown in Table 2. Total volatiles = sum of acetylene, chloroacetylene, and vinyl chloride in the reactor. (b) Cbl^{I} and ethynylcobalamin profiles were determined from the model calculation based on the initial chloroacetylene amount of 1.82 μmol.

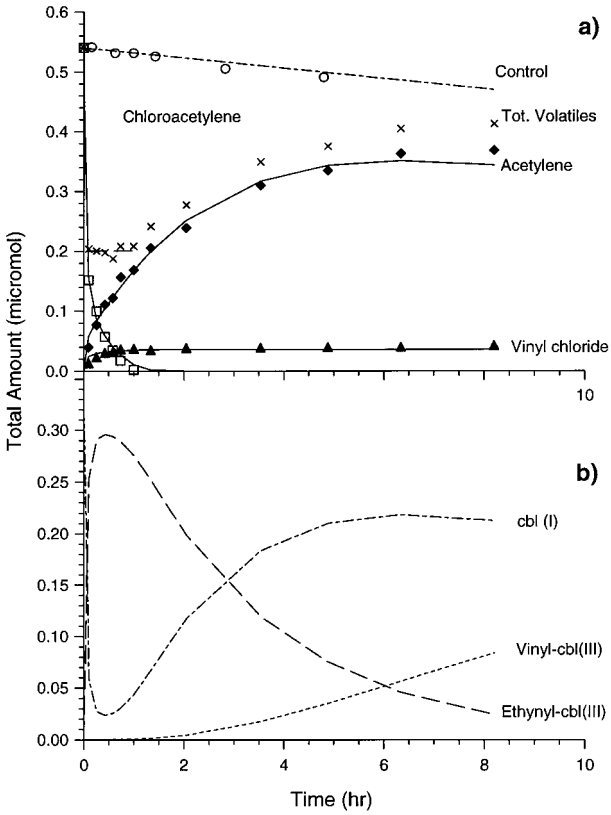


FIGURE 3. (a) Reductive transformation of 0.54 μmol of chloroacetylene in the presence of 0.32 μmol of cobalamin, under standard conditions [15 mM Ti(III) citrate at pH 8]. Lines represent model fits to the averaged data of replicate experiments (Scheme 2) including the kinetic coefficients of the acetylene transformation (Scheme 1) shown in Table 2. Total volatiles = sum of acetylene, chloroacetylene, and vinyl chloride in the reactor. (b) Cbl^{I} and ethynyl-cbl^{III} profiles were determined from the model calculation based on the initial chloroacetylene amount of 0.49 μmol (kinetic coefficients for acetylene transformation were 90% of the values in Table 2).

***cis*- and *trans*-DCE.** The transformation of *cis*- and *trans*-DCE isomers was studied under standard conditions. In the first experiment, the initial amounts of *cis*- and *trans*-DCE

SCHEME 2

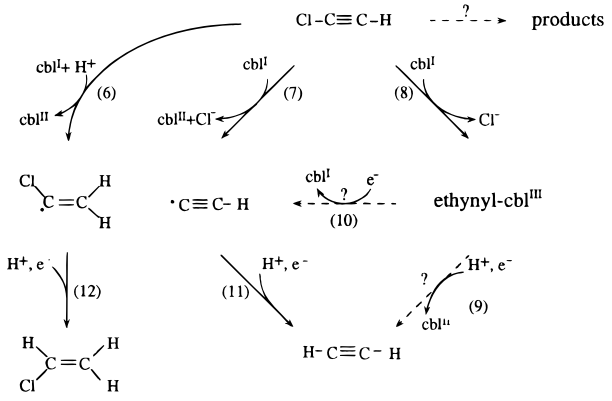


TABLE 3. Product Distributions of the Reductive Transformation of the DCE Isomers Catalyzed by Cob(I)Alamin (Standard Conditions: 15 mM Ti(III) Citrate, pH 8)

reactants and initial amounts	intermediates and products	final yields[a] (%)	conversion[a] (%)	reaction time (h)
0.8 μmol of *trans*-DCE 0.32 μmol of cobalamin	vinyl chloride	2.2	13.0	120
0.8 μmol of *cis*-DCE 0.32 μmol of cobalamin	vinyl chloride	0.9	8.8	120
13.7 μmol of *cis*-DCE	vinyl chloride	1.4	19.5	159
3.2 μmol of cobalamin	ethene	0.5		
	ethane	0.05		
13.4 μmol of *trans*-DCE	vinyl chloride	3.2	27.9	159
3.2 μmol of cobalamin	ethene	1.4		
	ethane	0.1		
12.6 μmol of 1,1-DCE	ethene	9.5	94.1	159
3.2 μmol of cobalamin	vinyl chloride	6.9		
	acetylene	2.0		
	ethane	0.3		
	nonvolatile	75.4[c]		
0.8 μmol of 1,1-DCE	ethene	6.3	98.5	121
0.32 μmol of cobalamin	vinyl chloride	1.9		
	acetylene	1.8		
	ethane	8.7		
	nonvolatile	79.9[c]		

[a] If not otherwise stated, values are derived from measurements at the indicated time. [b] Values derived from model calculations. [c] Total molar mass loss derived from experiments.

were 13.7 μmol and in the second initial amounts were 0.8 μmol. Conditions, conversion rates, products, and yields are summarized in Table 3. Both *cis*-DCE and *trans*-DCE transformed very slowly, mainly to vinyl chloride. After 5 days, substrate conversions were 8.8 and 13%, respectively, with yields of vinyl chloride of 0.9 and 2.2%, respectively. When the amounts of cobalamin, *cis*- and *trans*-DCE were increased to 3.2, 13.7, and 13.4 μmol, respectively, small amounts of ethene and ethane were detected in addition to vinyl chloride. The unaccounted mass is tentatively attributed to alkyl-, alkenyl-, or alkynylcobalamin intermediates. Alkyl- and vinylcobalamins have been found recently in similar transformation studies (*11*). Vinyl chloride has been shown to react to ethene with vinyl-cbl^{III} and ethene−cobalamin π-complex as possible intermediates (*11*). Ethane was found previously in similar experiments (*11*) and was speculated to form via hydrogenation of the ethene-cobalamin π-complexes. Acetylene was not detected.

1,1-DCE. The transformation of 0.8 μmol of 1,1-DCE initiated by 0.32 μmol of cbl was relatively rapid and complete within approximately 121 h. The data and the model fits are indicated in Figure 4, and Scheme 3 summarizes the pathway. 1,1-DCE transformation was first-order rate [$k'_{obs} = (33.9 \pm 1.6) \times 10^{-3}$ h^{-1}]. Deactivation of cbl was not apparent. The volatile products formed were vinyl chloride, ethene, ethane, and acetylene (Table 3) and accounted for 19% of the added 1,1-DCE. When the initial amounts of cbl and 1,1-DCE were increased to 3.2 and 12.6 μmol, respectively, 1,1-DCE

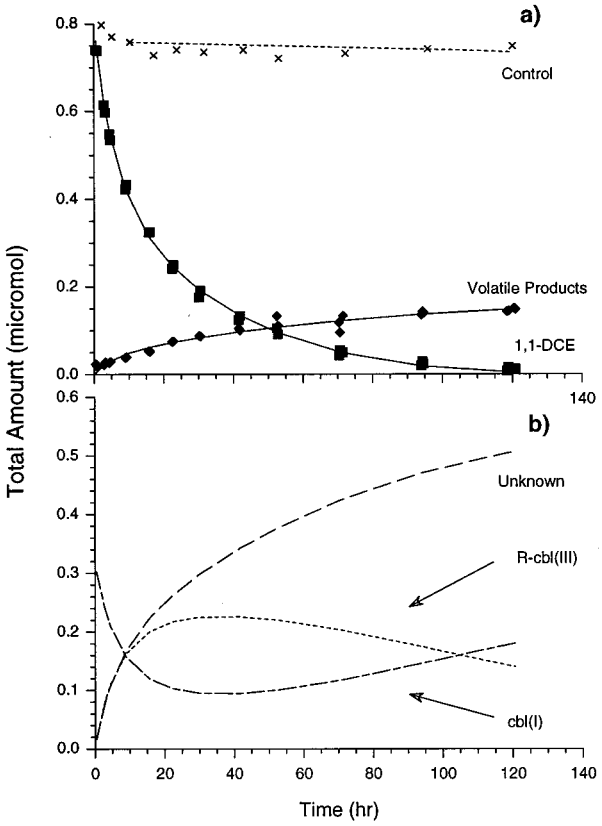
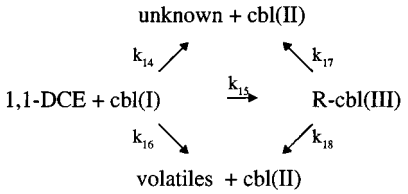


FIGURE 4. (a) Reductive transformation of 0.8 μmol of 1,1-DCE in the presence of 0.32 μmol of cobalamin, under standard conditions [15 mM Ti(III) citrate at pH 8]. Lines represent least-mean-square data fits constrained to the kinetic model (Scheme 3). The experimental data were obtained from replicate experiments. (b) Nonvolatile unknown products, cbl^{I} and R-cbl^{III} profiles, were determined from the model calculation based on the given initial conditions. Total volatiles is the sum of ethene, vinly chloride, acetylene, and ethane. R represents an alkyl, alkenyl-, or alkynyl group.

SCHEME 3



disappearance was first order during the first 20 h [$k'_{obs} = (14.4 \pm 1.8) \times 10^{-3}$ h^{-1}] (Figure 5a). Thereafter, the rate slowed, indicating catalyst inhibition, probably due to the formation of relatively stable intermediates binding cbl. Approximately 20% of the 1,1-DCE reacted to volatiles (Table 3). All observed products formed concurrently and without apparent delay (data not shown) suggesting that their pathways did not involve stable intermediate. After 121 h, 75−80% of the 1,1,-DCE was transformed into nonvolatile products.

Since the major portion of products formed is unknown, mechanistic conclusions are difficult. The kinetic model shown in Scheme 3 summarizes the apparent transformations. According to this scheme, 1,1-DCE is concurrently transformed into unknown nonvolatile products (reaction 14), R-cbl^{III} compounds (reaction 15) (R represents an alkyl-, alkenyl-, or alkynyl group) and volatiles (reaction 16). The volatiles include ethene, vinyl, chloride, and acetylene. Rapid reduction of cbl^{II} to cbl^{I} is assumed. The model is indicated in Tables 1 and 3 and Figures 4 and 5. The rate constants

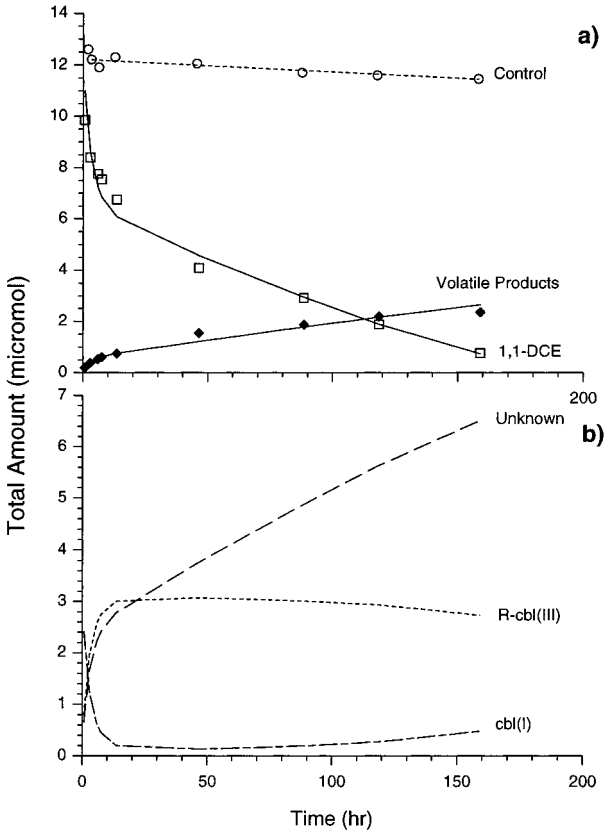


FIGURE 5. (a) Reductive transformation of 12.6 μmol of 1,1-DCE in the presence of 3.2 μmol of cobalamin, under standard conditions [15 mM Ti(III) citrate at pH 8]. Lines represent least-mean-square data fits constrained to the kinetic model (Scheme 3). (b) Unknown products, cbl^{I} and R-cbl^{III} profiles, were determined from the model calculation based on the given initial conditions. Total volatiles is the sum of ethene, vinly chloride, acetylene, and ethane. R represents an alkyl, alkenyl-, or alkynyl group.

derived from the experimental data with higher initial cbl^{I} and 1,1-DCE concentrations were smaller by a factor of 4.5−6, except for the rate constants of the R-cbl^{III} decomposition reactions, which were comparable. The difference in reactivity and product distributions of the *cis*- and *trans*-DCE isomers and 1,1-DCE merits further investigation. Perhaps, 1,1-DCE undergoes SET reactions much more readily than the *cis*- and *trans*-DCE isomers, forming the chlorovinyl radical which is then further reduced to vinyl chloride (as indicated in reaction 12). Glod et al. suggest that the different alkylcobalamins formed with 1,1-DCE react into polar products such as acetaldehyde (*11*). Highly reactive C_2-carbenes stabilized by cobalamin coordination may lead to the formation of acetylene through a 1,2-hydrogen shift, yield ethene by the addition of hydrogen, and may account for the formation of water-soluble products such as acetaldehyde.

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