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A Different Mechanism for the Reductive Dechlorination of Chlorinated Ethenes: Kinetic and Spectroscopic Evidence

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Reductive dechlorination is the most common reaction in the remediation of groundwater and soils contaminated with chlorinated compounds. The reaction that occurs in anaerobic bacteria can also be catalyzed by vitamin B12 and titanium citrate. Reductive dechlorination without the release of chlorinated ethene intermediates from the chloroalkylcobalamin complexes is proposed as an alternate reaction pathway for the reductive dechlorination of chlorinated ethenes. The revised scheme is supported by (a) the identification of several chloroalkylcobalamin intermediates by direct liquid injection of the reaction mixtures into an electrospray mass spectrometer, (b) the simultaneous presence of all the dechlorination intermediates in the mixtures, and (c) gas chromatographic data showing rapid formation of ethene and acetylene in the presence of a large excess of the primary substrates. Homolytic cleavage and titanium-catalyzed elimination are presented as competing mechanisms for the formation of the products from the alkylcobalamin intermediates. The distribution of dechlorination products was dependent on the availability of titanium from different chelating agents. This means that it may be possible to favor the formation of the fully dechlorinated products and to reduce the release of undesirable intermediates such as vinyl chloride by adjusting the amount and type of titanium chelate used.

Introduction

The potential role of the reductive dechlorination reaction in the remediation of groundwater and soil contaminated by chlorinated organic solvents has led to renewed interest in its mechanism. Reductive dechlorination is the predominant reaction responsible for the transformation of chlorinated compounds by anaerobic bacteria. Vitamin B12 (cyanocobalamin) has been used, as a substitute for anaerobic bacteria, in biomimetic systems to conduct studies on the mechanism of reductive dechlorination (*1*−*7*). It has also been used as a groundwater remediation method (*8*−*10*). Reductive dechlorination occurs naturally at some, but not all, contaminated sites requiring remedial action. This can be due to a multitude of factors such as pH, lack of electron donors, or toxicity of the contaminants to bacteria. The addition of vitamin B12 and titanium citrate is potentially the best solution to overcome some of these problems. Successful application in the environment requires an understanding of the mechanism and of the rate-limiting parameters.

Most of the published mechanistic studies have used one-carbon substrates as models for the reductive dechlorination reaction (*1, 2*). Kinetic studies have shown the potential interest of the reaction for other molecules of environmental interest (*11*). Assaf-Anid et al. (*1*), who have studied the reductive dechlorination of CCl_4 catalyzed by vitamin B12 using dithiothreitol as a reducing agent, have proposed the formation of carbene intermediates and a cobalt(II) cobalamin species (B_{12r}) as the catalyst responsible for the reaction. They reported the UV/vis spectral changes associated with the formation of chloroalkyl cobalamins (*7*). Chiu and Rheinhard proposed a reaction mechanism for the reduction of CCl_4 in titanium citrate involving the formation of trichloromethyl radicals and carbene anions (*2*).

Until recently, it was generally believed that the observed differences in reaction rates for different reducing agents could be related to their ability to reduce the central cobalt atom. The formation of the alkyl cobalamin complex would only be possible for a reduced species. Many authors have proposed that the reaction occurs with cobalt(I) cobalamin (B_{12s}) whereas others contend that B_{12r} is sufficiently reduced for the reaction to occur (*1*−*4*). In the case of the reductive dechlorination of pentachlorophenol, only the fully reduced complex is capable of effecting the reaction (*6*). The effect of different reducing agents on the dechlorination of ethenes was therefore reexamined.

The established mechanism for the reductive dechlorination of tetrachloroethylene with a cobalamin and titanium citrate (*12, 13*) is also currently challenged by the discovery of a potential parallel pathway involving an elimination reaction leading to the formation of acetylene. In one of these papers (*12*), a chloroacetylene intermediate was identified. In the other (*13*), an intermediate where acetylene is formed while bound to the cobalamin molecule was postulated. Special attention was therefore given to the conditions leading to the formation of acetylene.

This paper describes a series of experiments that have led to the formulation of another possible pathway. In this study, the reactions were carried out in the presence of large excesses of chlorinated ethenes present initially as nonaqueous phases. This method gave a different perspective on the reaction kinetics, because the initial substrate remained in a large excess in solution, able to compete with the dechlorination products for the cobalamin. The reaction mixtures were analyzed by the electrospray mass spectrometry to identify the alkylcobalamins as they were formed. When combined with the mass spectrometric findings, the information on the rate of product formation provided evidence for the new reaction pathway. The spectral and kinetic data suggest that sequential reductive dechlorination of tetrachloroethylene occurs while the substrate is still bound on the cobalamin molecule. Moreover, titanium seems to play a role that is beyond the simple reduction of the cobalt center to Co(I).

Vitamin B12 has been measured by a variety of mass spectral techniques such as fast atom bombardment (*14*), laser desorption (*15*), plasma desorption (*16*), and more recently MALDI (matrix assisted laser desorption ionization) (*17*). However, these techniques were all used for the analysis of the pure isolated compounds and would not be suitable for the measurement of air-sensitive reaction products in aqueous solutions. Direct liquid injection (DLI) negative ion LC/MS (*18*) and the more recently developed electrospray (ES) LC/MS (*19*) had better potential. In these techniques,

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the reduced solution can be introduced directly into the mass spectrometer by a syringe-filled loop without further manipulations, which eliminates the chance of exposure to the atmosphere.

ES is a soft ionization technique where the transfer ions from solution to the gas phase occur with little decomposition. The ES spectrum usually contains an ion (m/z) representative of the molecular weight of the compound (m) divided by the number of charges it carries once ionized (z). ES produces multiply charged ions, which is useful in the analysis of large biomolecules, because smaller fragments are produced (*19*) that are detected more easily by the commercial quadrupole instruments that have a limited mass range. In the case of cobalamins, a doubly charged ion is formed, which means that most fragment ions appear at half the molecular weight. The process of ion formation in ES has been paralleled to the electrochemical processes in solution. Molecules that are either already ionic in solution or that can be ionized by the addition of acids are the most suited for ES/MS (*20*). The technique has been used in the measurement of air-sensitive organometallic compounds (*21*). Ikonomou et al. (*22*) and Cheng et al. (*23*) have used ES/MS to investigate ionic metal complexes in aqueous solution. The method has also been applied to chlorinated organoplatinum anticancer agents (*24*) that bear functional group similarities (nitrogen−metal bonds and the presence of chlorine) to the chloroalkyl cobalamin intermediates formed in the reductive dechlorination reaction.

Materials and Methods

Chemicals. The chemicals for this study were obtained from the following sources: L-ascorbic acid from Mallinckrodt; sodium borohydride from Anachemia; titanium(III) chloride from Fisher Scientific; tris(hydroxymethyl)aminomethane (Tris), sodium citrate, acetic acid, and methanol from Caledon Laboratories, Georgetown Ontario; aquocobalamin acetate salt and cyanocobalamin from Sigma Chemicals; ethylenediaminetetraacetate disodium salt dihydrate (EDTA), sodium carbonate, sodium bisulfite, sodium dithionite, and nitriloacetate from J. T. Baker; all chlorinated ethenes and gas chromatography standards from Supelco Canada Ltd., Oakville, Ontario.

Chelated titanium was prepared from $TiCl_3$ (8 mL, 20% in HCl), buffered at pH 8 by the addition of sodium carbonate and Tris, to which either sodium citrate (6 g of sodium citrate in 60 mL of Tris), ascorbate (5.3 g of ascorbic acid in 80 mL of water and brought pH to >9 with NaOH), nitriloacetate (NTA, 10.3 g in 60 mL of Tris) or EDTA (7.5 g in 80 mL of Tris) was added under an argon atmosphere.

Other Reductants. Sodium borohydride (1.2 g) was added to 200 mL of Tris at pH 8 containing 25 mg of cyanocobalamin. Sodium sulfide (250 mg) was added to 40 mL of Tris buffer at pH 8 containing 2.5 mg of cyanocobalamin.

Degradation Experiments. The reactions were carried out with an excess of the substrate in sealed vials with a headspace. The headspace was monitored by gas chromatography to measure the concentration of nonchlorinated gases. Then, at different time intervals, selected on the basis of the known reaction rates of each compound, a subsample of the aqueous phase was removed, purified rapidly through a sorbent cartridge, and analyzed in the MS. The aqueous phase concentrations varied with the solubility (*25*) of each component: PCE, 149 mg/L; TCE, 763 mg/L; *cis*-DCE, 4000 mg/L; *trans*-DCE, 6260 mg/L; VC, 2763 mg/L.

Each chlorinated ethene substrate, as a pure compound in a separate phase (40 μL), was incubated with a solution of the reductant in a 125-mL serum bottle, sealed with a butyl rubber septum, and containing 2 mg of aquocobalamin in 20 mL of 0.1 M Tris buffer and 10 mL of a chelated titanium at pH 8. In this study, aquocobalamin was used instead of cyanocobalamin because it is more easily reduced and does not produce cyanide, a potential interfering reactant. Further 10-mL additions of chelated titanium were made when the aquocobalamin looked oxidized. Aquocobalamin solutions are pink when oxidized, orange when reduced to Co(II), and gray when reduced to Co(I). Titanium chelates also change color depending on their oxidation state, which allow the use of visual inspection as an indicator of redox conditions.

The formation of hydrocarbon gases was followed by analyzing 50 μL of headspace using a SRI 8610 gas chromatograph equipped with two columns: a DB-624 75m, 0.53 mm i.d., 3 μm film column leading to an ECD detector for the chlorinated compounds and a GS-Q 30m, 0.53 mm i.d. FID column for the lesser chlorinated or nonchlorinated compounds, installed into a single injector. Helium was used as the carrier gas. An isothermal 35 °C temperature program was used for separation of gases and a ramp to 135 °C at 5 °C/min for the other analytes.

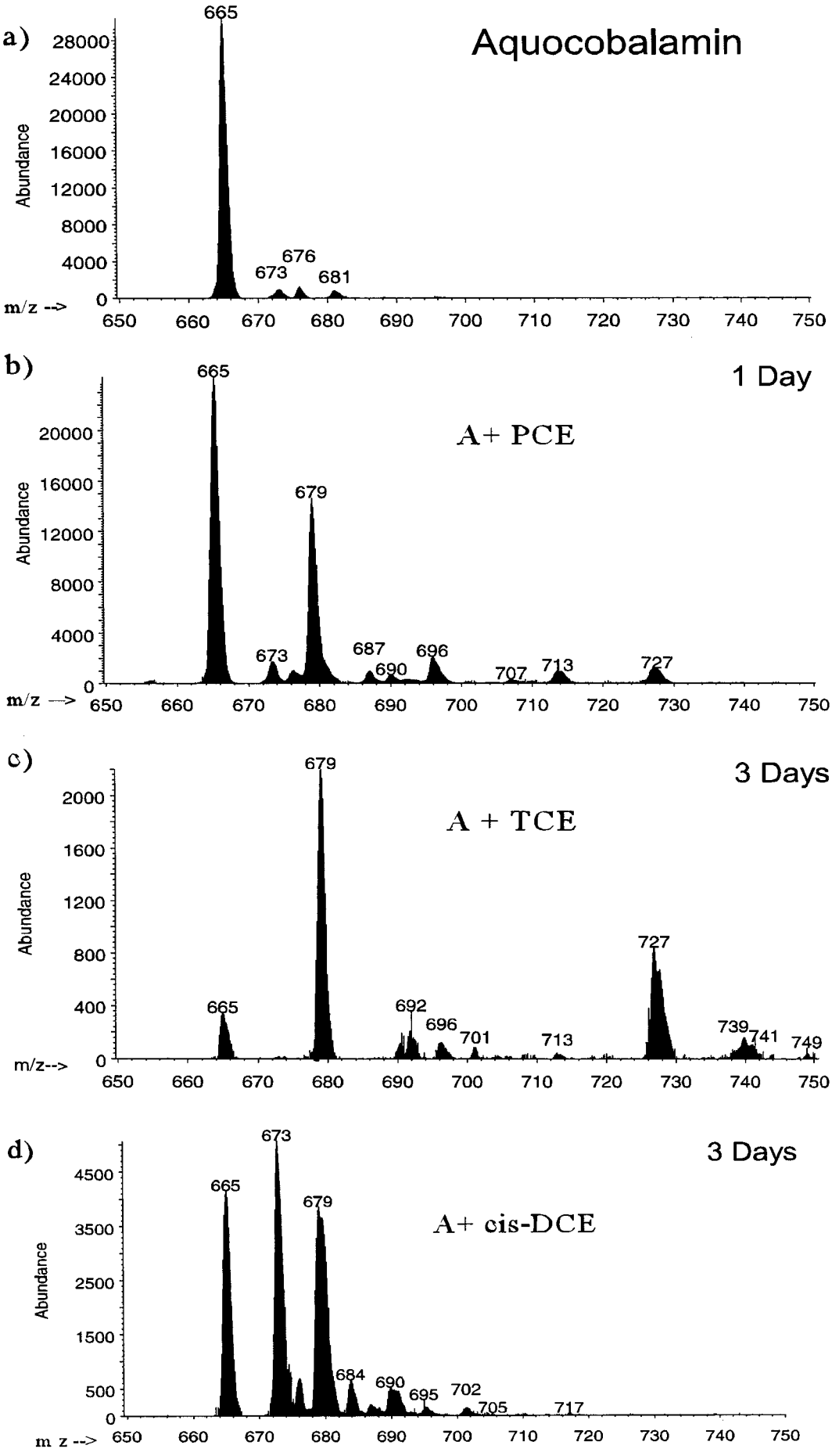
A 10-mL subsample of the aqueous phase was removed for ES/MS analysis. The sample was passed through a C18 Sep-Pak cartridge (Waters/Millipore, Mississauga, Ontario) wrapped in aluminum foil to exclude light. The cartridge was rinsed with 10 mL of degassed water (Milli-Q purification system, Waters/Millipore, Mississauga, Ontario) to remove salts that could interfere in the electrospray process (*23*), and the sample eluted with 2 mL of methanol. A 0.4-mL portion was transferred into another covered vial containing 1.1 mL water and 0.5 mL of 10% acetic acid. This solution was then injected into the sample injection loop of the ES/LC/MS.

Mass Spectrometry. The LC/MS instrument was a Hewlett-Packard 59987A with an electrospray interface and 5989A mass spectrometer, equipped with a HP 1050 series HPLC. In this case, no LC separation was performed. The sample was loaded into the 1 mL sample loop and introduced by flow injection into the mass spectrometer by the LC pump delivering an 80:20 water/methanol solution containing 2% acetic acid at 0.05 mL/min. The drying gas flow was set at 30 mL/min and the drying gas temperature was 200 °C. The nebulizing gas pressure was set at 80 psi.

Results

The ES mass spectra resulting from the reaction between aquocobalamin and the known products of the reductive dechlorination of tetrachloroethene (PCE) are shown in Figure 1. The spectral region shown corresponds to the peaks for the doubly charged ions produced by acidified cobalamins. Because the resolution of the instrument is at unit mass, all measured fragment ions are approximated to the closest integral m/z value. Mass assignments are made by comparing the apex of the peaks to the mass spectral standards. A peak at m/z 1345 (not shown) was identified as the protonated molecular ion $[M + H]^+$ of aquocobalamin. The base peak at m/z 673 can be attributed to $[M + 2H]^{2+}$. The base peak in the spectra of all reduced species is at m/z 665. This peak represents the addition of two hydrogens to aquocobalamin less its axial ligand, i.e., cobalamin $[(M - OH) + 2H]^{2+}$.

Possible structural assignments of the mass spectral peaks shown in Figure 1 are listed in Table 1. In the reaction mixture of aquocobalamin with PCE (Figure 1b), it was most interesting to find together alkyl cobalamins representing the full range of dechlorination products, and this in the presence of a large excess of the substrate. The peaks at m/z 679, 696/697, and 713/714 were assigned to the ethenyl, chloroethenyl, and dichloroethenyl cobalamins, respectively. There is no measurable trichloroethenyl cobalamin (peak expected at m/z 730), presumably because it is too unstable. The possibility that the losses of chlorine atoms were occurring in the source of the mass spectrometer was ruled


a)
Aquocobalamin
Abundance
m/z -->
665
676
673
681
b)
1 Day
A+ PCE
Abundance
m/z -->
665
679
673
687
696
690
707
713
727
c)
3 Days
A + TCE
Abundance
m/z-->
679
727
665
692
696
701
713
739
741
749
d)
3 Days
A+ cis-DCE
Abundance
m z -->
673
665
679
684
690
695
702
705
717

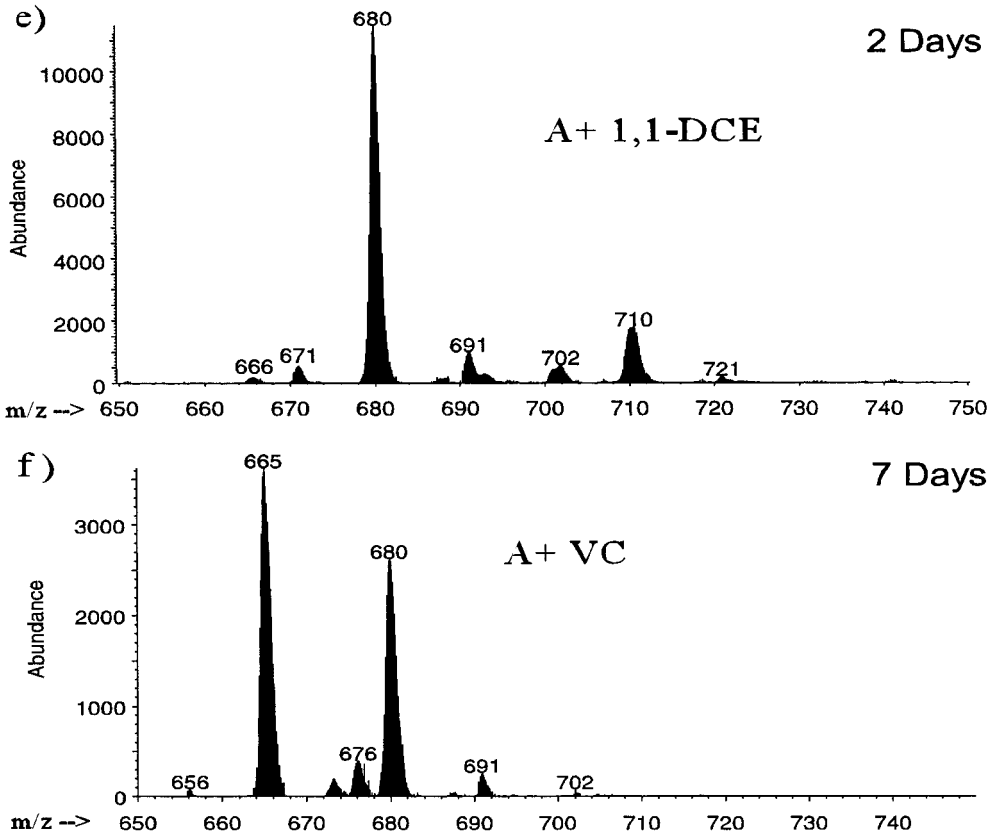


FIGURE 1. Electrospray mass spectra of the alkyl cobalamins in the reaction mixtures of aquocobalamin with the chlorinated substrates PCE, TCE, C-DCE 1,1-DCE, and VC after the indicated reaction times.

TABLE 1. Spectral Assignment of Alkyl Substituents in Chloroalkylcobalamins Formed from Reduced Cobalamins and Chlorinated Ethenes

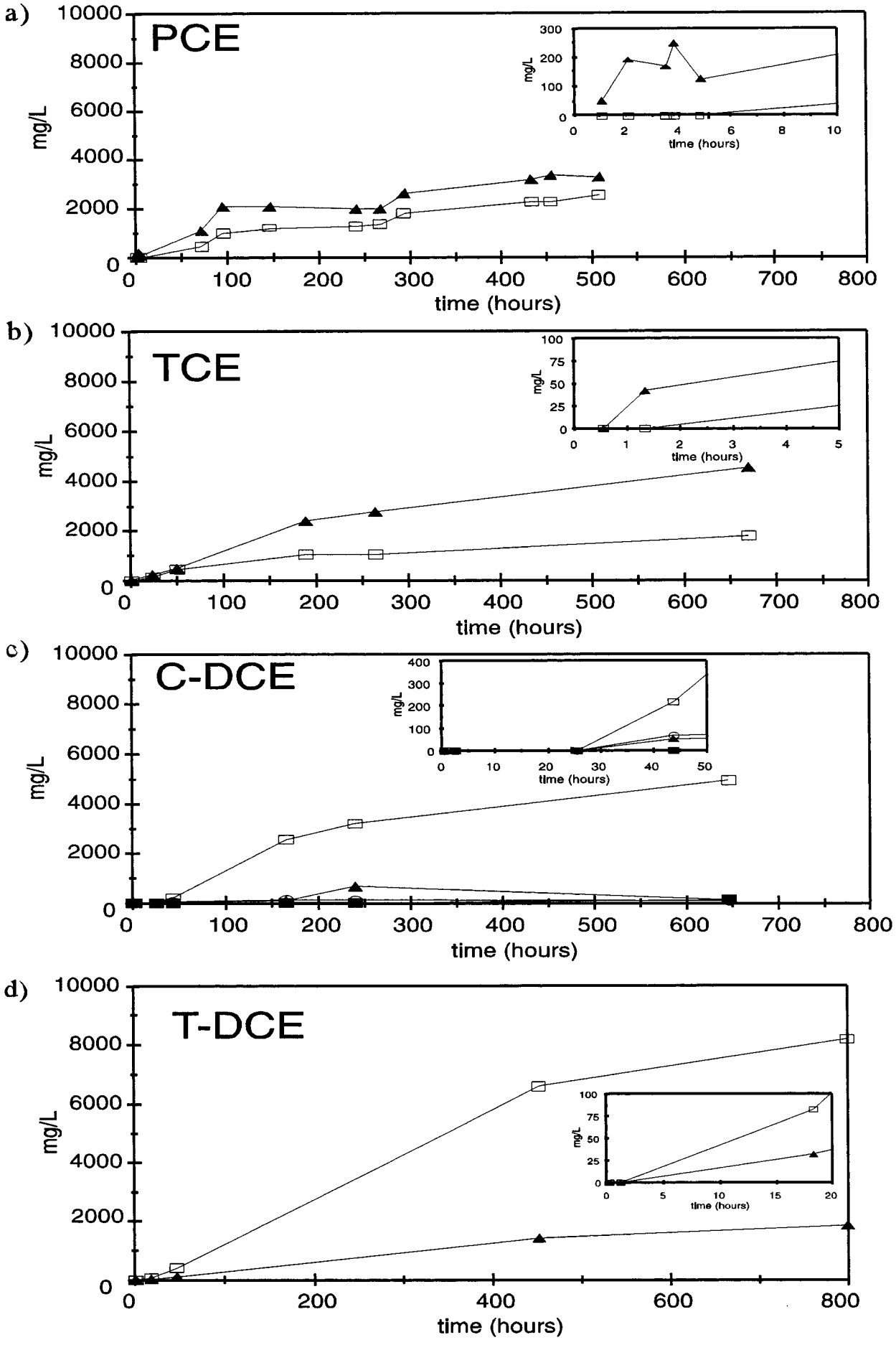
observed *m/z*	R in $[M + 2H + R]^{2+}$
Aquocobalamin + PCE (Spectrum after 1 Day Shown in Figure 1b)	
679	$-CH{=}CH_2$
687	$-C_2H_3O$
696/697	$-CCl{=}CH_2$ or $-CH{=}CHCl$
713/714	$-CCl{=}CHCl$
727/728	$-C_4H_5Cl_2$
Aquocobalamin + TCE (Spectrum Shown in Figure 1c)	
679	$-CH{=}CH_2$
692	$-C_4H_7$
696/697	$-CCl{=}CH_2$ or $-CH{=}CHCl$
701	$-C_4H_7O$
713/714	$-CCl{=}CHCl$
727/729	$-C_4H_5Cl_2$
739/741	$-C_6Cl_2H_5$
Aquocobalamin + *cis*-DCE (Spectrum Shown in Figure 1d)	
673	$-CH_4$
679	$-CH{=}CH_2$
680	$-CH_2{-}CH_3$
691	$-C_4H_5$
702	$-C_4H_9O$
Aquocobalamin + 1,1-DCE (Spectrum Shown in Figure 1e)	
680	$-CH_2{-}CH_3$
691	$-C_4H_5$
695 (not found)	$-C{\equiv}C{-}Cl$
Aquocobalamin + VC (Spectrum Shown in Figure 1f)	
680	$-CH_2{-}CH_3$
691	$-C_4H_5$
702	$-C_4H_9O$

out by conducting a time series (spectra not shown). When the mass spectral analysis was conducted after a longer reaction time, the proportion of the less chlorinated alkylcobalamins increased. Also, more dimers (4-carbon compounds) were formed by the recombination of the chloroethenyl radicals (*m*/*z* 692, 710, 727, 741, and above).

When starting the reaction with trichloroethene (TCE), a similar product distribution was expected. The spectrum shown in Figure 1c, taken after 3 days, shows a small peak for dichloroethenylcobalamin (*m*/*z* 713), a monochloroethenyl derivative (*m*/*z* 696/697), and an ethenyl (*m*/*z* 679) derivative, as well as dimers (*m*/*z* 727 and above). The less chlorinated ethenes, *cis*-dichloroethene (*cis*-DCE) (Figure 1d), 1,1-dichloroethene (1,1-DCE) (Figure 1e), and chloroethene (vinyl chloride, VC) (Figure 1f) showed similar product distributions. Both an ethenyl (*m*/*z* 679) and an ethyl (*m*/*z* 680) cobalamin were present in the spectrum with *cis*-DCE, but only the peak at 680 was present in the reaction with 1,1-DCE and VC. There was no evidence of the presence of a chloroethynyl cobalamin (*m*/*z* 695) as the precursor to chloroacetylene.

The rate of formation of the nonchlorinated gases was measured by gas chromatography (Figure 2a–f). When aquocobalamin was incubated with a large excess of PCE (Figure 2a) or TCE (Figure 2b), acetylene was found in the reaction mixture within 4 h. The production of acetylene was somewhat slower with *cis*- or *trans*-DCE (Figure 2c,d) as starting products. It did not occur at all with 1,1-DCE or VC as starting materials (Figure 2e,f). The information is summarized in Table 2. Ethene was the predominant gas formed from *cis* and *trans*-DCE, 1,1-DCE, and VC. Ethane was formed from 1,1-DCE and VC, but not from the other compounds. Some methane was found in the reaction mixture containing *cis*-DCE.

When sodium borohydride or sodium sulfide were used to reduce aquocobalamin, no products were formed after 2 days. When titanium(III) was used, the amount of acetylene


a)
PCE
mg/L
time (hours)
b)
TCE
mg/L
time (hours)
c)
C-DCE
mg/L
time (hours)
d)
T-DCE
mg/L
time (hours)

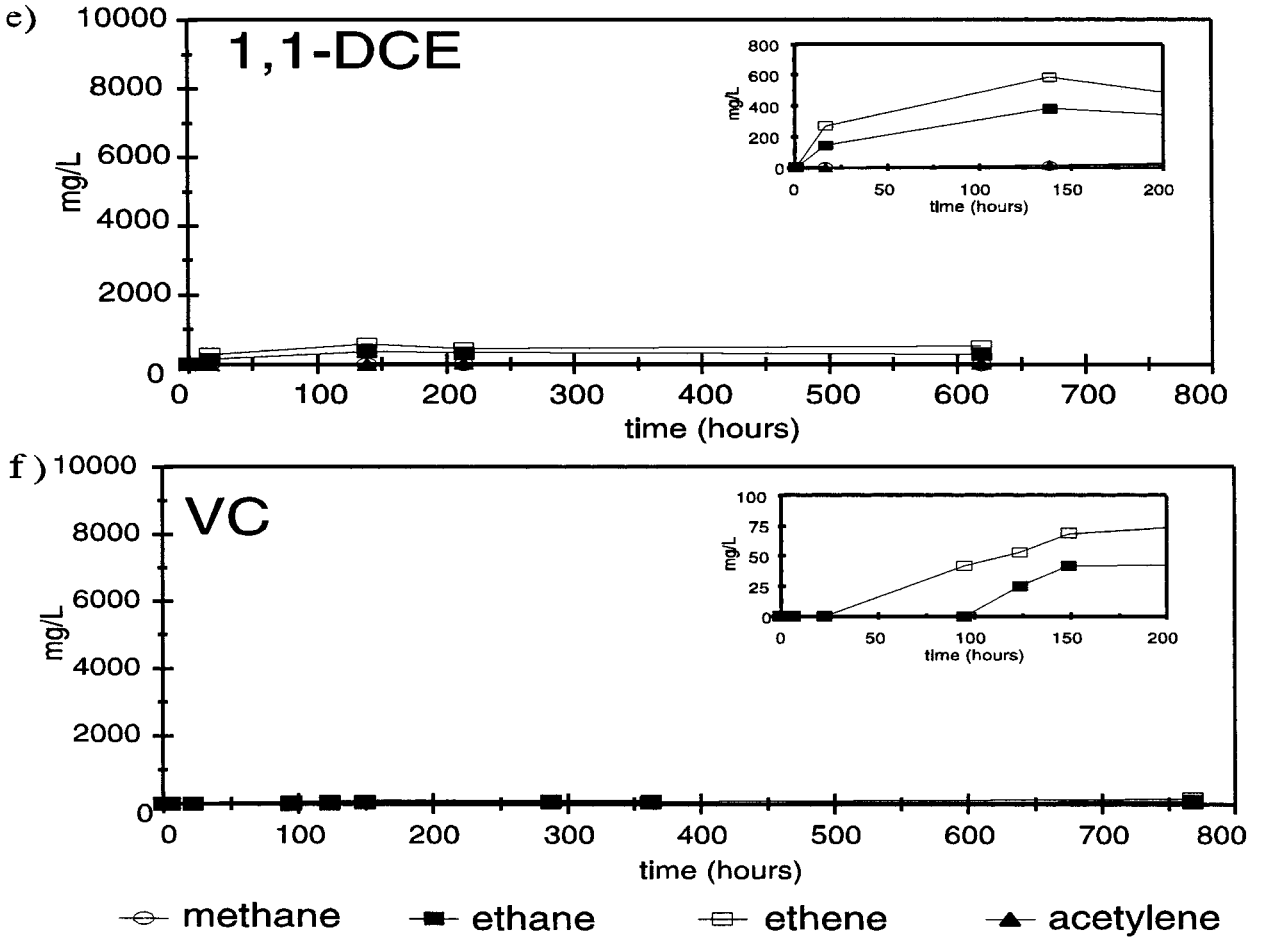


FIGURE 2. Rate of formation of hydrocarbon gases from the reaction of chlorinated ethenes with aquocobalamin and titanium citrate. Insets are enlargements of the initial portions of the graphs.

TABLE 2. Acetylene Production from Chloroethenes Using Titanium Citrate

substrate	alkylcobalamin formed (see Figure 3)	acetylene formed
PCE	**I**	yes
TCE	**II** and **III**	yes
trans-DCE	**IV**	yes
cis-DCE	**V**	very little
1,1-DCE	**VII**	no
VC	**VI**	no

formed depended on the chelating agent used. The production of ethene and acetylene from PCE was fastest with titanium citrate (2−3 h). With Ti−NTA, an equivalent amount of ethene and acetylene were formed, except that the reaction was slower than with titanium(III) citrate (17 h). With titanium(III) ascorbate, ethene and ethane were formed within a few hours, but very little acetylene was formed. With Ti−EDTA, the reaction was also slow (17 h), but mostly acetylene was formed.

Discussion

The goal of this paper was to elucidate the reaction mechanism of vitamin B12-catalyzed reductive dechlorination of chlorinated ethenes. The most widely accepted mechanism involves binding of the chorinated ethene to the cobalamin, followed by homolytic cleavage to form a chloroalkyl radical, which produces a compound containing one chlorine atom less than the starting material. Further dechlorination involves binding of the first formed product and a repeat of the reaction. Two factors led us to reexamine this pathway: (a) the concurrent presence of alkyl cobalamins in various stages of dechlorination in the reaction mixtures and (b) the immediate formation of acetylene in a saturated solution of PCE.

The proposed pathway for the degradation of PCE catalyzed by cobalamins is shown as Figure 3, where the dashed lines show potential reactions that were not supported by experimental evidence. After the initial formation of the trichloroethenyl cobalamin (**I**), three potentially competing reactions may occur: (a) the homolytic cleavage (HC) of the carbon−cobalt bond leading to the formation of a trichloroethenyl radical, quenched by the medium to form TCE; (b) further reductive dechlorination (RD) to a dichloroethenyl (**II** and **III**), chloroethenyl (**IV** and **V**), and ethenylcobalamin (**VI**); (c) elimination reactions (E) leading to acetylene formation, potentially via chloroacetylene.

The simultaneous presence of the dechlorination intermediates (**II**−**VI**) in the mass spectra (Figure 1) support the possibility for reductive dechlorination to occur on the chloroalkyl cobalamins. In addition, because the rates of dechlorination are known to decrease with the degree of chlorination of ethenes (*11, 13*) and because a vast excess of starting material was used in these experiments, it is difficult to account otherwise for the formation of acetylene and ethene from PCE within a few hours (Figure 2a). Indeed, if each dechlorinated ethene formed (TCE, then DCE, and VC in sequence) had to be released to the medium and then had to compete with PCE to bind again to the cobalamin molecule to be further degraded, products such as ethene and acetylene

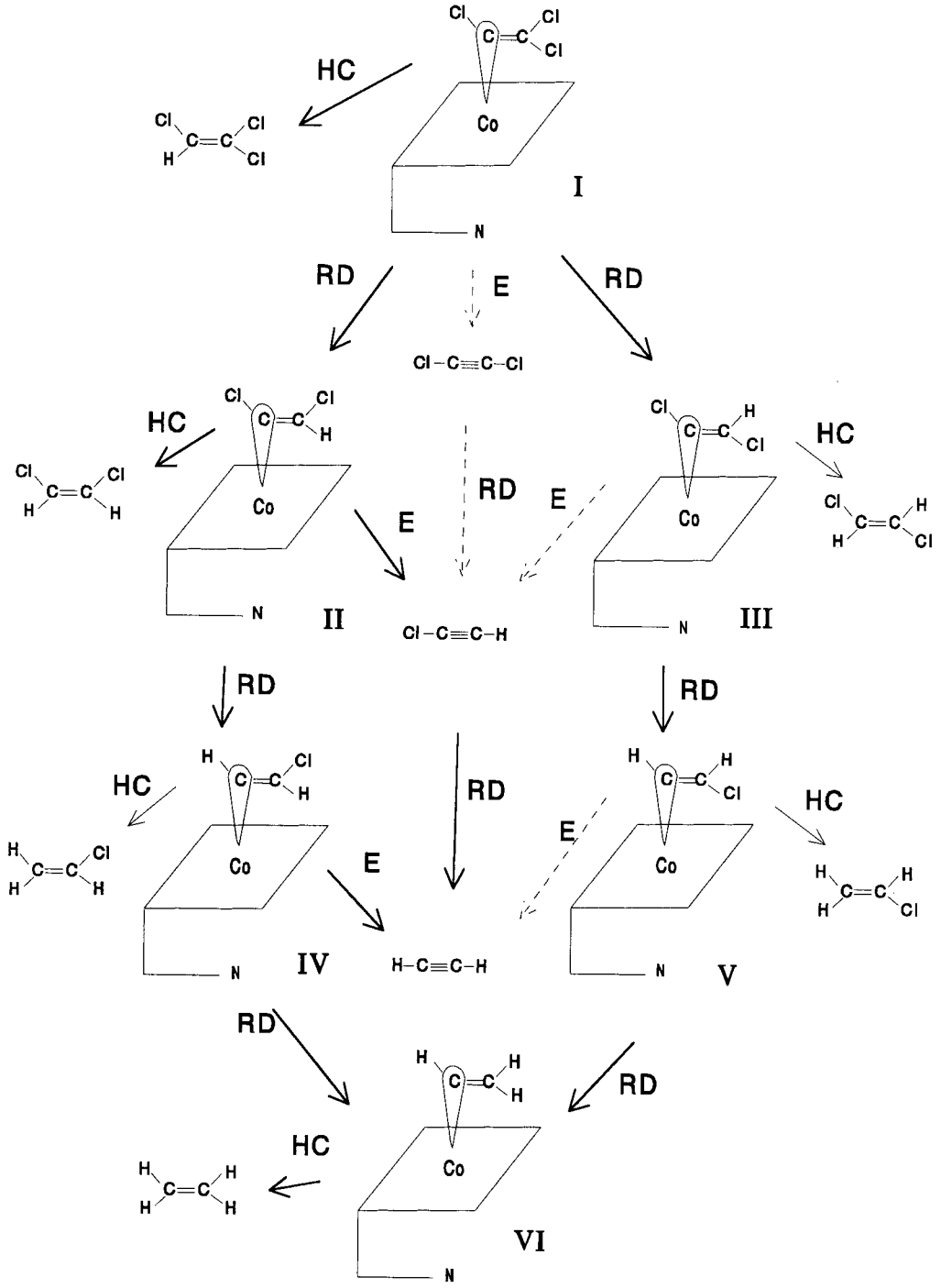


FIGURE 3. Reaction pathway for the reductive dechlorination of PCE with cobalamin. RD, reductive dechlorination; HC, homolytic cleavage; E, elimination. The dashed arrows indicate potential reactions for which there is no experimental evidence.

would only be formed after all the starting material had disappeared.

The homolytic cleavage reactions to form the corresponding chloroalkyl radicals and the formation of successively less chlorinated products are well documented (*11*–*13*, *26*, *27*). The free trichloroethenyl and dichloroethenyl radicals have been trapped with phenyl-*tert*-butylnitrone (PBN) and identified by GC/MS (*28*). What remains to be elucidated are the factors that govern the relative rates of homolytic cleavage and reductive dechlorination for each intermediate.

Burris et al. and Glod et al. (*12*, *13*) have also suggested the formation of acetylene by elimination reactions either occurring in solution or via an alkynyl cobalamin. There were no peaks in the mass spectra supporting the formation of an alkynyl cobalamin (m/z 678). The difference in the present scheme is that the elimination is depicted as a means of removal from the cobalamin rather than an independent process. The distinction is important because, in this case, elimination is a process that competes with further reductive dechlorination and that can potentially be modulated.

The scheme also implies that sequential reductive dechlorination occurs on the alkylcobalamin molecule. This means that once PCE is bound, it is transformed to a series of reductive dechlorination and elimination products in proportions that are dependent on the reaction conditions. Therefore, it may be possible to select the chelating agents, their concentration, or the pH, such as to favor the formation

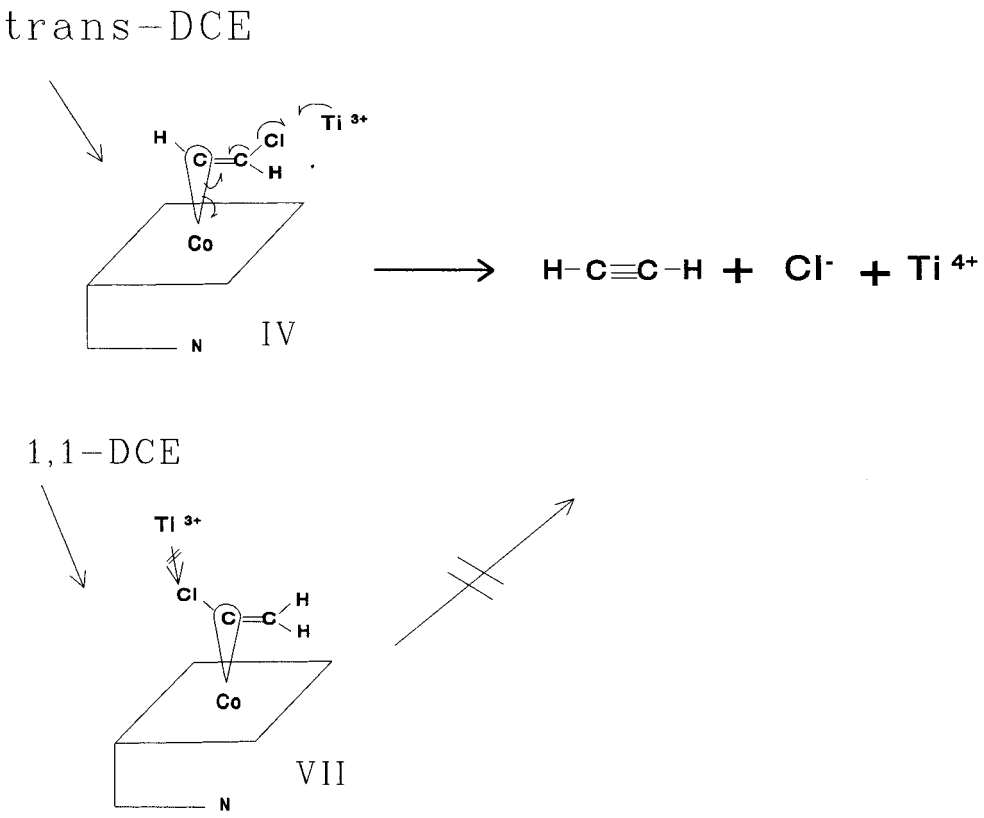


FIGURE 4. Titanium-catalyzed elimination is favored by a trans configuration.

of fully dechlorinated products and to minimize the formation of undesirable compounds such as VC.

When considering the rates of gas formation for the series of chlorinated ethenes (Figure 2), it is apparent that acetylene was formed preferentially from some substrates and not at all from others (Table 2). The largest yield of acetylene was observed from PCE, followed by that from TCE. The reactions of the dichloroethenes were most distinct. No acetylene was formed from 1,1-DCE, little from *cis*-DCE, and more from *trans*-DCE. The predominance of the reaction from the *trans*-DCE isomer can be explained by examining the chloroalkyl intermediate formed (structure IV, Figure 4). The chlorine atom is on the β-carbon from the carbon−cobalt bond in a trans position with respect to the cobalt, a position that is know to be favorable for a concerted elimination reaction. The cis isomer becomes intermediate **V**, which is not as favorably configured and leads to little acetylene formation. In the case of 1,1-DCE, the remaining chlorine is bound to the same carbon atom as the cobalt (structure **VII**), precluding a β-elimination and the production of acetylene. Similarly, VC does not lead to the formation of any acetylene. In this case, it is because no chlorine is left on the ethenyl cobalamin. More acetylene is formed from PCE than TCE, presumably because there are two chlorines to choose from on the β-carbon. Because *trans*-DCE forms an intermediate that favors elimination, it seldom accumulates in this reaction medium. In summary, the specificity of the acetylene formation from the various substrates supports a reaction scheme where elimination occurs from the alkyl cobalamin complex.

The role played by Ti(III) in the reaction has been investigated in the past with respect to the pH and redox potential (E_h) produced. Holliger et al. (*5*) noted that cysteine and sodium dithionite did not sustain the dechlorination of 1,2-dichloroethane, and the reaction was slow with dithiothreitol. The influence of titanium(III) concentrations and the effectiveness of the different reagents were ascribed to the E_h produced. Although a cobalt(I) species was formed (gray color and typical UV/vis spectrum), no reaction was observed with either sodium sulfide or borohydride. The E_h and reaction produced are summarized in Table 3. In the present work, the mass spectrum of aquocobalamin reduced by various reagents was recorded (spectra not shown). In addition to the peak at m/z 665, the spectrum of cobalamin reduced with dithionite had a prominent peak at m/z 706. This can be ascribed to an $-SO_3$ ligand to the cobalt atom of cobalamin. The potential of a dual role for sulfur anions, acting as reducing agents and as ligands to cobalamin, has been documented in the early literature (*29*). According to this reference, dithionite had been reported to reduce vitamin B12, via a "sulfoxylatocobalamin" intermediate, which is presumably what was observed here. The addition of the reagent to the cobalt atom competes with the binding of the substrate, thus explaining the lack of reactivity.

TABLE 3. Effect of Reducing Agents on the Vitamin B12-Catalyzed Reductive Dechlorination

reagent	color	E_h[a]	CCl_4	PCE
bisulfite	pink/peach	+100	no	no
$NaBH_4$	orange	−570	no	no
dithionite	orange	−600	no	no
DTT	orange	−330	yes	no
sulfide	dark brown	−570	yes	no
Ti(III)	very dark brown	−630	yes	yes

[a] E_h varies with pH. These were measured at pH 8.

The effect of different chelating agents for titanium could be also considered as a means of providing different redox conditions. Glod et al. (*13*) reported a slower dechlorination rate for titanium−NTA as compared to that of titanium citrate. This is also what was observed here. The ethene/acetylene ratio was similar in both systems, although the rates of formation differed. With titanium(III) ascorbate, which produced the lowest E_h (−700 mV), mostly ethene and ethane

were formed. However, the rate of dechlorination was the fastest of all the chelating agents. With titanium(III)−EDTA, the rate was similar to that in NTA, but mostly acetylene was formed. This would indicate that reductive dechlorination rates may be E_h dependent, but elimination rates may depend more on the availability of titanium and on steric considerations.

The mass spectrometric data also showed the formation of what seemed like radical recombination products (R= C4 and up). The formation of dimers has been considered as arising from the arrangement of the alkyl cobalamin intermediates in solution. An intermediate where two alkyl cobalamin complexes are arranged β-face to β-face could favor this process, because the chloroalkyl radicals are in close proximity. They can therefore combine to form four-carbon compounds, despite the overwhelming amount of solution surrounding the molecules (*30*). Butenes and chlorobutadiene have been identified in some of the reaction mixtures. The formation of dimers occurs in solution and is rarely seen in vivo because the presence of a surrounding protein precludes the formation of an intermediate involving two cobalamin molecules (*27*).

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