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Dechlorination of Chlorinated Ethenes by Electrospray LC/MS

By:
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Several chloroalkyl intermediates formed during the reaction between tetrachloroethylene and aquocobalamin were identified by direct liquid injection in an Electrospray LC/MS. The concurrent presence of all reaction intermediates when combined with information on the dechlorination products formation, lead to a revised scheme on the possible pathways of the reductive dechlorination reaction catalysed by cobalamins (such as vitamin B12). The new proposed pathways involve reductive dechlorination occurring on the alkyl cobalamins, and homolytic cleavage and titanium catalysed elimination as competing mechanisms for the formation of the products from the alkylcobalamin intermediates.

MANAGEMENT PERSPECTIVE

The reductive dechlorination reaction catalysed by cyanocobalamin (vitamin B12) has been the subject of renewed interest recently because of its potential role in the remediation of chlorinated organic solvents contaminated groundwater and soil. Vitamin B12 has been used in biomimetic system as a substitute for anaerobic bacteria but also on its own, as a potential chemical catalyst. This paper presents the identification of the intermediates in the reaction as measured by the recently installed Electrospray LC/MS interface to the mass spectrometer at NWRI. Better understanding of the reaction mechanisms is essential in being able to use the method for remediation of contaminated water under various circumstances.

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Introduction

The reductive dechlorination reaction catalysed by cyanocobalamin (vitamin B12) has been the subject of renewed interest recently because of its potential role in the remediation of chlorinated organic solvents contaminated groundwater and soil. Vitamin B12 has been used in biomimetic system as a substitute for anaerobic bacteria (1-7) but also on its own, as a potential chemical catalyst (8-9).

Most of the mechanistic studies published have used one carbon substrates as models for the reductive dechlorination reaction (1,2) whereas kinetic studies have shown the potential interest of the reaction for other molecules of environmental interest (10). Assaf-Anid et al. (1) have studied the reductive dechlorination of CCl_4 catalysed by vitamin B12 using dithiothreitol as a reducing agent and proposed the formation of carbene intermediates and a Cobalt II cobalamin species (B_{12r})

as the catalyst responsible for the reaction. Their evidence was mostly based on visible spectral data or product distribution. They reported the UV/vis spectral changes associated with the formation of chloroalkyl cobalamins (7).

Until recently, it was generally believed that the observed differences in reactions rates between 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 with 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). Chiu and Rheinhard proposed a reaction mechanism for the reduction of carbon tetrachloride in titanium citrate (2).

More recently, the established mechanism of the reductive dechlorination of tetrachloroethylene by cobalamin using titatium citrate (11,12) has been challenged by the discovery of a potential parallel pathway involving an elimination reaction leading to the formation of acetylene. In one of the papers (11) a chloroacetylene intermediate is identified.

This paper describes the LC/MS analysis of the reaction mixture which leads to yet another possible pathway. In this study, the reactions were carried out in the presence of large excess of substrates present as non-aqueous phases. This method gives a different perspective on the reaction kinetics because the initial substrate remains in solution, able to compete with the dechlorination products for the cobalamin. When combined with the LC/MS findings, the information on the rate of product formation provide evidence for a new reaction pathway. The spectral and kinetic data suggest that the sequential reductive dechlorination of tetrachloroethylene occurs while the substrate is still bound on the cobalamin molecule. Moreover, titanium seems to play a role which 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 (13), Laser Desorption (14) Plasma Desorption (15) and more recently MALDI (Matrix Assisted Laser Desorption Ionization) (16). However these techniques were all used on the pure isolated compound and would not be suitable to measure air sensitive reaction products present in aqueous solutions. Direct Liquid Injection negative ion LC/MS (17) and the more recently developed Electrospray (ES) LC/MS (18) had better potential because in these techniques, the reduced solution can be introduced directly into the mass spectrometer by a syringe, without further manipulations, and therefore reduce the chance of exposure to the atmosphere. ES is a very soft ionization technique offering good resolution, which can detect very subtle changes in molecular weight. It has been applied to chlorins which bear some structural similarity to corrins (19). ES produces multiply charged ions which is useful in the analysis of large biomolecules because smaller, more easily measured fragments are produced (18).

Materials and Method

Chemicals The chemicals for this study were obtained from the following sources: L-ascorbic acid from Mallinckrodt; sodium borohydride from Anachemia; titanium 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; EDTA disodium salt dihydrate, sodium carbonate, sodium bisulfite, sodium dithionite, nitriloacetate from J.T. Baker; all chlorinated ethenes and gas chromatography standards from Supelco Canada Ltd. Oakville, Ontario.

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

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

Degradation experiments In this study, aquocobalamin was used instead of cyanocobalamin because it is more easily reduced and to remove cyanide as one potential interfering reactant.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 containing 2 mg aquocobalamin in 20 ml 0.1M TRIS buffer and 10 ml of a chelated titanium at pH 8. Further 10 mL addition of chelated titanium were made when the aquocobalamin looked oxidized. The formation of hydrocarbon gases was followed by analysing 50 µL of headspace using an SRI 8610 Gas Chromatograph equipped with two columns: a DB-624 75m, 0.53 mm i.d., 3µm film leading to an ECD detector for the chlorinated compounds and a GS-Q 30m, 0.53mm i.d. FID column for the lesser chlorinated or non-chlorinated compounds, hooked 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 ramping to 135°C at 5°C/min for the rest.

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

LC/MS The LC/MS was a Hewlett Packard 59987A with an electospray interface and 5989A mass spectrometer equipped with a HP 1050 series HPLC. In this case, no LC separation was done, the whole sample was injected directly into the mass spectrometer. The drying gas flow was set at

30 and the drying gas temperature was 200°C. The nebulizing gas flow was set at 80. The sample was injected through a 1mL sample loop at 0.05 ml/min.

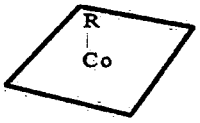
Results

The ES LC/MS spectra resulting from the reaction between aquocobalamin and the known products of the reductive dechlorination of tetrachloroethene (PCE) are shown in Figure 1. Acidified cobalamins produce a doubly charged ion. The more prominent peaks in the spectra are therefore at half mass, which is the region shown in the figure. Because of the limit of resolution at unit mass of the instrument, all measured fragments are approximated to the closest unit mass . Aquocobalamin itself shows a molecular ion at 1345, which is consistent with its protonated molecular ion. The base peak at m/e 673 can be attributed to $M^{++}/2$. The base peak for all spectra of reduced species is at m/e 665, which can be assigned to $M+2^{++}/2$, that comes from the addition of two protons to the vitamin B12 less the axial ligand, i.e. cobalamin. The possible mass assignments of the axial ligands are shown in Table 1.

It was most interesting to find in the reaction of aquocobalamin with PCE, the simultaneous presence of adducts representing the full range of dechlorination products, and this, in the presence of a large excess of the substrate. The peaks at m/e 679,696, 713/15 and 727/29 were assigned to the ethenyl, chloroethenyl, dichlorethenyl and trichloroethenyl cobalamins. The possibility that the losses of chlorines were occurring in the source of the mass spectrometer was ruled out by conducting a time series which resulted in a larger amount of less chlorinated alkylcobalamins formed with time. Also, losses in the mass spectrometer would be 35, not 34 (substitution of H for Cl). After a reaction period of several days, the more highly chlorinated intermediates disappeared in favour of the less chlorinated ones and more evidence of the formation of dimers was observed (692, 710, 741 and above).When starting the reaction with TCE, similar product distribution was expected, except for the most highly chlorinated intermediate. The spectrum shown in Figure 1 was taken after 3 days and only shows a monochloroethenyl (696) and an ethenyl (679) ligand as well as evidence of dimers (727 and above). The less chlorinated ethenes (cis-DCE, 1,1-DCE and VC)

showed similar product distribution with an ethyl (680) rather than an ethenyl ligand formed and a butadienyl (691) rather than the butenyl (692) ligand seen with the more chlorinated compounds.

Table 1. Spectral assignment of alkyl substituents in chloroalkylcobalamins formed from reduced cobalamins and chlorinated ethenes.

Calculated Mass (Observed when not identical)	Fragment R= 	Found in (reaction time-days)
673	$-CH_3$	c-DCE (3d)
678.5 (679)	$-CH=CH_2$	PCE (1d,13d), TCE (3d), c-DCE (3d)
679.5 (680)	$-CH_2-CH_3$	c-DCE (3d), 1,1-DCE (2d), VC(7d)
691	$-C_4H_5$	c-DCE (3d), 1,1-DCE (2d), VC (7d)
692	$-C_4H_7$	PCE (13d), TCE (3d)
694.5 (not found)	$-C\equiv C-Cl$	not found
695.5 (696)	$-CCl=CH_2$ or $-CH=CHCl$	PCE (1d), TCE (3d)
702.5 (702)	C_2H_3Ti ?	c-DCE (3d), 1,1-DCE (2d), VC (7d)
710	C_4H_6Cl	1,1-DCE (2d)
712.5 (713/715)	$-CCl=CHCl$	PCE (1d)
727	$-C_4H_5Cl_2$	PCE (1d, 13d), TCE (3d)
729.5 (727/729)	$-CCl=CCl_2$	PCE (1d), TCE (3d)
741	$-C_4Cl_3H_2$	PCE (13d), TCE (3d)

These findings added to the recently reported formation of acetylene by Burris and Glod (11,12), question the currently accepted pathways. In fact, when aquocobalamin was incubated with a large excess of PCE or TCE, acetylene was found within an hour in the reaction mixture. The formation of non-chlorinated gases from a series of successively less chlorinated intermediates are shown on Figure 2. While the production of acetylene was almost immediate with PCE and TCE, it was somewhat slower with *cis*- or *trans*-DCE as starting products. It did not occur at all with 1,1-DCE or vinyl chloride (VC) as starting materials (Table 2).

When sodium borohydride or sodium sulfide were used to reduce aquocobalamin, in spite of the fact that a cobalt (I) species was formed (grey colour, and typical UV/vis spectrum), no products were formed after 2 days. In the presence of titanium, the amount of acetylene formed depended on the chelating agent used. The production of hydrocarbon gases from PCE was fastest with titanium citrate and ascorbate (2-3 hours), but ethene and ethane and little acetylene were formed with titanium ascorbate. With NTA, an equivalent amount of ethene and acetylene were formed, which is similar to what is observed in citrate, except that the reaction was slower (17 hours before any acetylene was observed). With EDTA, the reaction was also slow (17 hours), but mostly acetylene was formed.

Table 2. Acetylene production from chloroethenes.

Substrate	Alkyl cobalamin 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

DISCUSSION

The results of this investigation complement the knowledge that has been gathered thus far with the goal of elucidating the reaction mechanism of vitamin B12-catalysed reductive dechlorination. The concurrent presence of alkyl cobalamins in various stages of dechlorination and the almost immediate formation of acetylene in the presence a saturated solution of PCE, a substrate

was similar in both systems, although the rates of formation differed. In the presence of ascorbate, which produced the lowest Eh (-700mV), mostly ethene and ethane were formed, but the rate of dechlorination was the fastest. With EDTA, the rate was similar to that in NTA, but mostly acetylene was formed. This would indicate that reductive dechlorination rates may be Eh dependent, but that elimination rates may depend more on the availability of titanium and on steric considerations.

The LC/MS data also showed the formation of what seemed like radical recombination products (R= C4 and up). The formation of dimers has been the subject of discussion over the potential structure of the alkyl cobalamin intermediates. An intermediate where two alkyl cobalamin complexes are arranged β face to β face has been proposed (24). The formation of alkyl cobalamins where the alkyl group is C_4, as were observed in the LC/MS spectra, support this hypothesis. 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 B12 molecules.

CONCLUSION

A new reaction scheme for the reaction between cobalamins and chlorinated ethenes was proposed based on the Electrospray LC/MS analysis of the reaction mixtures. This new scheme is based on the fact that the sequential reductive dechlorination must occur while the chorinated alkene is still bound to the cobalamin. This is supported kinetically by the early appearance of acetylene in the mixture in the presence of a large excess of tetrachlorethene which is known to bind preferentially. In addition, the simultaneous presence of all in the partially chlorinated intermediates in the LC/MS spectra supports this hypothesis. Titanium was also found to play a key role in the formation of elimination products.

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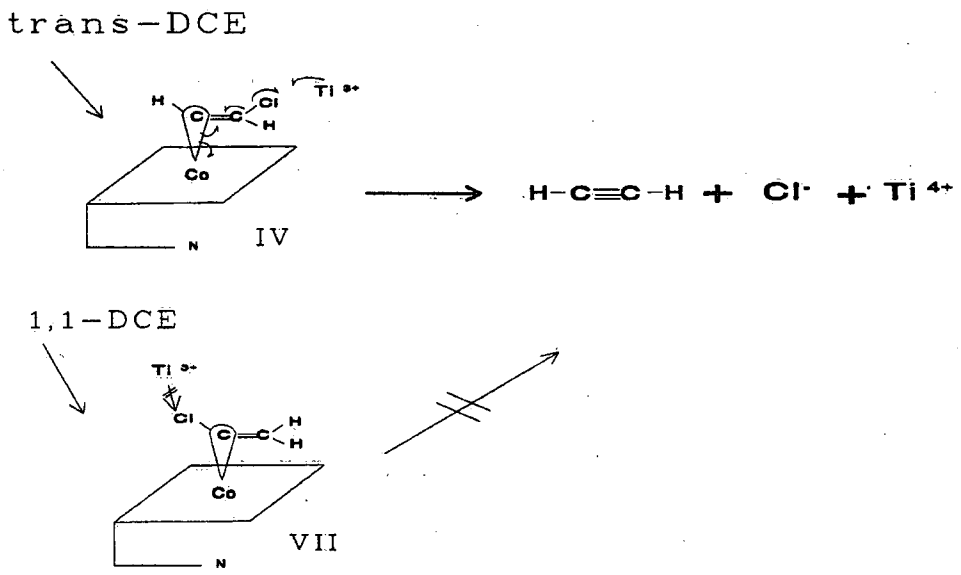

Figure 4 Titanium catalysed elimination is favored by *trans* configuration.

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