G. Diekert

Tetrachloroethene Respiration

Abstract

Dehalospirillum multivorans is a strictly anaerobic bacterium, which is able to utilize H_2 as electron donor and tetrachloroethene (PCE) as electron acceptor in its energy metabolism. Energy is derived from PCE reduction *via* electron transport phosphorylation (tetrachloroethene respiration). PCE is reductively dechlorinated via trichloroethene (TCE) to *cis*-1,2-dichloroethene (DCE). This process was characterized in detail with respect to its physiology and biochemistry.

Introduction

Tetrachloroethene (PCE) is a volatile chlorinated hydrocarbon, which is a frequent pollutant of ground water or soil due to ist extensive use during the last 50 years in e. g. fat extraction, dry cleaning of textiles, and machine scouring. Because of its toxic effects on biological systems on the one hand and its effect on the ozone layer of the atmosphere on the other hand, the application of PCE is no longer desirable. Efforts have been undertaken to substitute for this compound and to decontaminate polluted environments. During the last few years, the application of bioremediation has been discussed (see e. g. Vogel and McCarty, 1985; Freedman and Gossett, 1989). A prerequisite for bioremediation of PCE polluted environments is the availability of bacteria capable of PCE dechlorination.

Due to the four chlorine substituents, the carbon backbone of PCE is highly oxidized. Therefore, it cannot be easily attacked by oxygen, resulting in persistence of PCE under aerobic conditions. Biotransformation of PCE obviously occurs exclusively under anaerobic conditions involving reductive dechlorination of the compound.

So far, complete reductive dechlorination of PCE to ethene (or ethane) has only been observed in mixed cultures (Freedman and Gossett, 1989; DiStefano *et al.*, 1991; DeBruin *et al.*, 1992), which converted PCE *via* trichloroethene (TCE), dichloroethene (DCE), and vinyl chloride (VC) to ethene (or ethane).

Recently, efforts were undertaken to isolate pure cultures of anaerobic bacteria able to dechlorinate PCE. Pure cultures of '*Dehalobacter restrictus*'

(Holliger and Schumacher 1994) and of *Dehalospirillum multivorans* (Neumann *et al.*, 1994; Scholz-Muramatsu *et al.*, 1995) have been described to reductively dechlorinate PCE to *cis*-1,2-dichloroethene (DCE) in their energy metabolism and to couple this reaction to energy conservation (tetrachloroethene respiration) (Scholz-Muramatsu *et al.*, 1995; Schumacher and Holliger, 1996). Both organisms are able to grow at the expense of H_2 and PCE as sole energy sources. The process of tetrachloroethene respiration upon growth on these substrates is schematically summarized in Fig. 1.

A coupling of ATP synthesis to reductive dechlorination is only feasible if thermodynamically favourable. The $\Delta G^{\circ\prime}$ value for PCE reduction with H_2 according to the equation

$$PCE + 2H_2 \rightarrow \textit{cis}\text{-1,2-DCE} + 2H^+ + 2Cl^-$$

is about -376 kJ/mol. Hence, PCE reduction with H_2 is a highly exergonic reaction, which is due to the positive standard redox potential at pH 7, $E^{\circ\prime}$, of the couples PCE/TCE ($+580$ mV) and TCE/DCE ($+540$ mV). For microorganisms, PCE is — besides several transition metal ions — the most positive-potential electron acceptor under anoxic conditions. PCE reduction was studied in detail with *D. multivorans*. In this communication, the physiology, energetics, and biochemistry of PCE reduction in this organism will be described.

Dehalospirillum multivorans

D. multivorans is a gram-negative strict anaerobe, which clusters with the ε-subgroup of the *Proteobacteria*. In contrast to '*D. restrictus*', which is restricted to

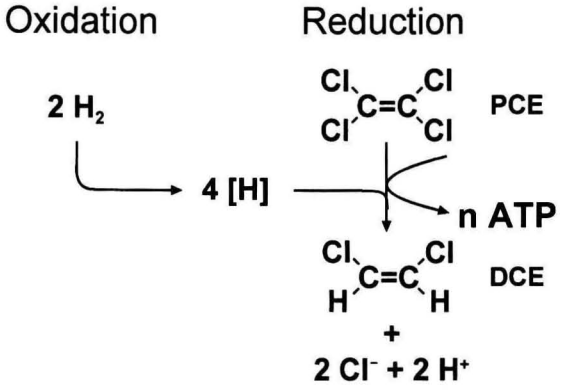

Fig. 1. Simplified scheme of tetrachloroethene respiration. ATP is formed exclusively in the reductive part of the energy metabolism via electron transport phosphorylation.

H_2 and PCE as energy source, *D. multivorans* is an organism with a relatively wide substrate spectrum. Besides H_2, the organism utilizes formate, pyruvate, glycerol, lactate, and ethanol as electron donors for catabolic reduction reactions. Alternative electron acceptors in energy metabolism are fumarate, nitrate, and possibly also S° (Scholz-Muramatsu *et al.*, 1995). The only substrate utilized in the absence of an electron acceptor is pyruvate.

The ability to dechlorinate PCE appears to be constitutive, at least in the strain investigated. This facilitated the investigations on tetrachloroethene reduction considerably, since with pyruvate plus fumarate and in the presence of yeast extract growth is very fast ($t_d \approx 1$–2 h) and results in high cell yields. When the bacterium was grown on a defined medium with H_2 and PCE as sole energy sources and acetate as carbon source (the organism is not capable of autotrophic CO_2 fixation), the organism grew much slower ($t_d \approx 20$ h) at a specific growth yield of at most 2.8 g cells (dry weight) per mol Cl^- released. The low growth yields with PCE are surprising with respect to the favourable thermodynamics of PCE reductive dechlorination (see above). A possible explanation for this observation will be discussed below.

Purification and properties of tetrachloroethene reductive dehalogenase

The tetrachloroethene reductive dehalogenase (PCE dehalogenase) catalyzes in vitro the reduction of PCE to TCE and of TCE to DCE with reduced methyl viologen as artificial electron donor (Neumann *et al.*, 1994, 1995). Methyl viologen is blue in its reduced state and colourless in its oxidized state. PCE reduction can easily be measured spectrophotometrically by the decrease in absorbance at 578 nm. Although in crude extracts the enzyme is not oxygen sensitive, the assay has to be performed under anoxic conditions. The reduction of PCE *via* TCE to DCE is shown in Fig. 2.

The enzyme exhibits catabolic activities in crude extracts. Incubation of extracts in the presence of low concentrations of propyl iodide led to a rapid inactivation of the enzyme; this was only observed under reducing conditions, i.e.

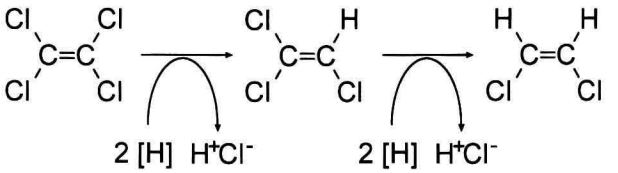

Fig. 2. Tetrachloroethene reductive dechlorination to dichloroethene catalyzed by tetrachloroethene reductive dehalogenase.

in the presence of titanium(III) citrate. In the absence of an electron donor, the enzyme remained unaffected by the alkyl halide. The inactivation could be reversed by illumination. These findings pointed to the involvement of a corrinoid in reductive PCE dechlorination (Neumann *et al.*, 1995). From the data, the reaction mechanism depicted in Fig. 3 may be derived.

It is known for 'free' and protein-bound corrinoids that the standard redox potential at pH 7, depending on the corrinoid type, of the couple cob(II)/cob(I)alamin is lower than -0.5 V, whereas that of cob(III)/cob(II)alamin is far more positive. According to the scheme depicted in Fig. 3, one low-potential electron and one electron at a more positive potential would be required for the reductive dechlorination of PCE or TCE.

The involvement of a corrinoid in reductive dechlorination is not surprising, since it was known from earlier studies, that corrinoids mediate the abiotic dehalogenation of halogenated methanes in the presence of an electron donor (Krone *et al.*, 1989, 1991). However, no significant abiotic dechlorination of PCE occurred in the presence of corrinoids and Ti(III) citrate (unpublished results). Therefore, PCE dehalogenation has to be considered as a biotic rather than an abiotic process.

The PCE dehalogenase was purified approximately 100-fold to apparent homogeneity from pyruvate/fumarate-grown cells (Neumann *et al.*, 1996). The purified enzyme mediated PCE dechlorination at a rate of near 2600 nkat.mg^{-1}. It consisted of one single subunit of an apparent molecular mass of 57 kDa; gel filtration revealed an apparent molecular mass of 58 kDa. Per 1 mol monomeric enzyme 1 mol corrinoid (or 1 mol cobalt), about 8 mol Fe, and 8 mol acid-labile sulfur were detected.

In the meantime, the gene encoding PCE dehalogenase has been cloned in *Escherichia coli*. Sequencing of the gene is currently performed in our laboratory.

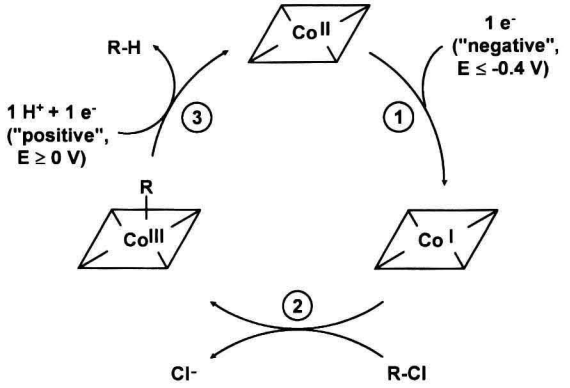

Fig. 3. Tentative scheme of the reaction mechanism of PCE dehalogenase. [Co] indicates the corrinoid in the respective oxidation state. The cobalt has to be in the $+1$ state to be alkylated by the alkyl halide (PCE or TCE = R-Cl). For the reduction of cob(II)alamin to cob(I)alamin (reaction 1) a low-potential electron is required.

Studies on energy conservation *via* tetrachloroethene respiration

It has already been mentioned that PCE reduction with H_2 is a thermodynamically favourable process due to the positive standard redox potentials $E^{\circ\prime}$ of the couples PCE/TCE (+580 mV) and TCE/DCE (+540 mV). The potential difference between H_2 ($E^{\circ\prime}$ $H^+/H_2 = -414$ mV) and PCE or TCE, respectively, is more than 0.9 V (under standard conditions at pH 7), which would theoretically account for about 9 mol electrogenic protons translocated across the cytoplasmic membrane per mol Cl^- released. Assuming that 3 protons are required for the synthesis of 1 ATP in the ATP synthase reaction (like in other organisms), the synthesis of approximately 3 mol ATP per mol Cl^- would be feasible. In this case, a specific growth yield Y_S of about 15–20 g cells (dry weight) per mol Cl^- could be obtained.

This theoretical value is far away from what has been measured with *D. multivorans* (2.8 g/mol; see above), even if the value given for standard conditions is corrected for the actual experimental conditions. This finding indicated that a proton pump is not involved in energy conservation *via* PCE respiration.

The reason for this discrepancy is probably the reaction mechanism of the enzyme, especially the involvement of a corrinoid as prosthetic group. As outlined in Fig. 3, The redox potential of the cob(II)/cob(I)alamin couple is in theory lower than or equal to −0.5 V. Therefore, it was assumed that a low-potential electron donor is required for PCE reduction. We studied the influence of the redox potential on PCE reduction as well as the enzyme activity with electron donors of similar structure (mostly viologens) and different redox potentials (Miller *et al.*, unpublished results). The enzyme activity was dependent on the redox potential with an estimated half-maximal velocity at about −0.4 V. With different artificial electron donors, a significant enzyme activity was only observed when the redox potential of the electron donor was lower than −0.36 V. These findings support the assumption that a low-potential electron donor is required for reductive dechlorination of PCE and that the redox potential of the prosthetic group is lower than or close to −0.4 V.

To elucidate the mechanism of energy conservation in tetrachloroethene respiration, it is important to know the cellular localization of the enzymes involved in the process. The hydrogenase was membrane-associated and faced the periplasm, whereas the PCE dehalogenase appeared to be a soluble, cytoplasmic enzyme. The electrons derived from H_2 oxidation have to cross the membrane from the outside to the inside of the cell. A simple model for energy conservation coupled to PCE reduction is shown in Fig. 4. This model involves an energy conservation mechanism similar to that of fumarate respiration in *Wolinella succinogenes* (Kröger *et al.*, 1992), with the exception that fumarate reductase is membrane associated.

The problem with the model depicted in Fig. 4 is the transfer of an electron ($E^{\circ\prime} = -414$ mV) derived from H_2 oxidation outside the cell to the corrinoid ($E^{\circ\prime}$ cob(II)/cob(I)alamin $\leqslant -400$ mV) of the PCE dehalogenase inside the cell (see Fig. 3, reaction 1) against an electrochemical proton potential Δp of about

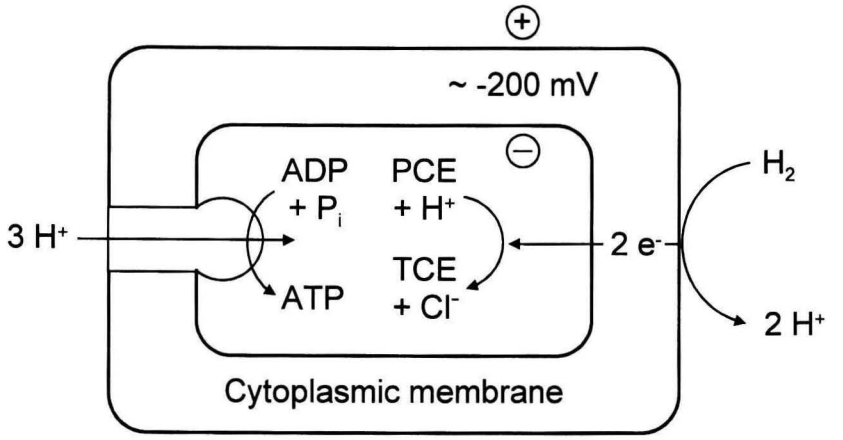

Fig. 4. Simple model of tetrachloroethene respiration in *D. multivorans*.

−200 mV (which is the average Δp found so far for all organisms). This problem can be solved assuming that the low-potential electron required for cob(II)alamin reduction has to be translocated across the cytoplasmic membrane involving a reversed electron flow according to the model depicted in Fig. 5.

The other, positive-potential electron (required for reaction 3 in Fig. 3) is driven across the membrane by the potential difference between H^+/H_2 and cob(III)/cob(II)alamin. Assuming a H^+/ATP stoichiometry of 3:1, the resulting ATP yield per mol chloride ion released would be about 1/3. This would be in accordance with the low growth yields observed for the organism (see above).

To confirm or disprove the involvement of a reversed electron flow, preliminary studies on the influence of ionophores on PCE reduction in whole

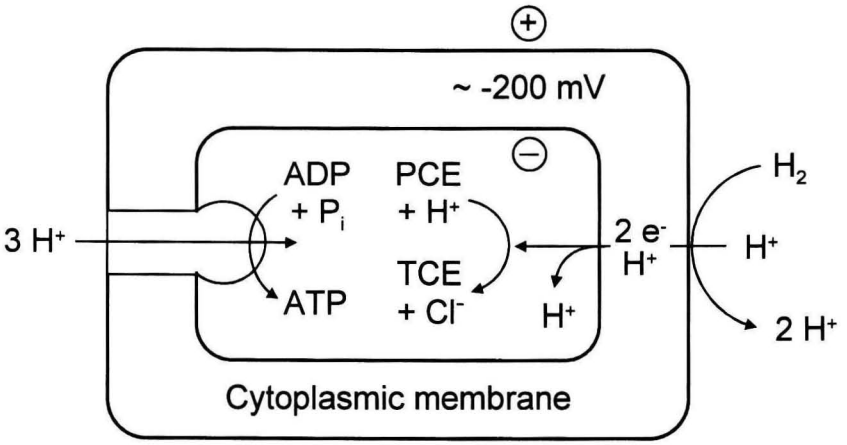

Fig. 5. Model of tetrachloroethene respiration involving a reversed electron flow. One electron is translocated along with a proton in a reversed electron flow.

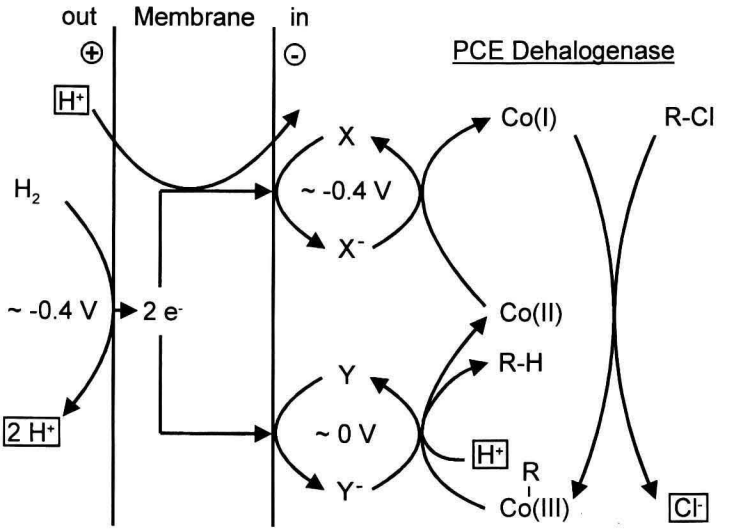

Fig. 6. Detailed tentative scheme of tetrachloroethene respiration. The low-potential electron required for the reduction of cob(II)alamin to cob(I)alamin (represented by Co(II) and Co(I)) is driven across the membrane by a reversed electron flow. X and Y are unknown secondary electron carriers.

cells were performed. Assuming that an eletrochemical proton potential is required to drive the low-potential electron across the membrane, ionophores should prevent reductive dechlorination of PCE. This was indeed observed for the ionophores tetrachlorosalicylanilide, FCCP, nigericin and/or valinomycin (plus K^+) (data not shown). These results were interpreted to support the hypothesis that a reversed electron flow is involved in PCE reductive dechlorination in *D. multivorans*. Whether this is a general feature of dehalorespiration (i.e. reductive dehalogenation coupled to energy conservation via a chemiosmotic mechanism), will have to await further studies on other bacteria mediating reductive dehalogenation in their energy metabolism. These studies are currently underway in our laboratory. Fig. 6 summarizes a more detailed model of PCE reductive dechlorination in *D. multivorans* involving a reversed electron flow.

Acknowledgements

This work was supported by the BMBF, the Deutsche Forschungsgemeinschaft, the EC, and the Fonds der Chemischen Industrie.

References

DeBruin, W.P., M.J.J. Kottermann, M.A. Posthumus, G. Schraa and A.J.B. Zehnder, 1992. Complete biological reductive transformation of tetrachloroethene to ethane. *Appl. Environ. Microbiol.* **58**, 1996–2000.

DiStefano, T.D., J.M. Gossett and S.H. Zinder, 1992. Hydrogen as an electron donor for dechlorination of tetrachloroethene by an anaerobic mixed culture. *Appl. Environ. Microbiol.* **58**, 3622–3629.

Freedman, D.L., and J.M Gossett, 1989. Biological reductive dechlorination of tetrachloroethylene and trichloroethylene to ethylene under methanogenic conditions. *Appl. Environ. Microbiol.* **55**, 2144–2151.

Holliger, C., and W. Schumacher, 1994. Reductive dehalogenation as a respiratory process. *Antonie Van Leeuwenhoek* **66**, 239–246.

Kröger, A., V. Geisler, E. Lemma, F. Theis and R. Lenger, 1992. Bacterial fumarate respiration. *Arch. Microbiol.* **158**, 311–314.

Krone, U.E., R.K. Thauer and H.P.C. Hogenkamp, 1989. Reductive dehalogenation of chlorinated C_1-hydrocarbons mediated by corrinoids. *Biochemistry* **28**, 4908–4914.

Krone, U.E., R.K. Thauer, H.P.C. Hogenkamp and K. Steinbach, 1991. Reductive formation of carbon monoxide from CCl_4 and FREONs 11, 12, and 13 catalyzed by corrinoids. *Biochemistry* **30**, 2713–2719.

Neumann, A., H. Scholz-Muramatsu and G. Diekert, 1994. Tetrachloroethene metabolism of *Dehalospirillum multivorans*. *Arch. Microbiol.* **162**, 295–301.

Neumann, A., G. Wohlfarth and G. Diekert, 1995. Properties of tetrachloroethene and trichloroethene dehalogenase of *Dehalospirillum multivorans*. *Arch. Microbiol.* **163**, 276–281.

Neumann, A., G. Wohlfarth and G. Diekert, 1996. Purification and characterization of tetrachloroethene reductive dehalogenase from *Dehalospirillum multivorans*. *J. Biol. Chem.* **271**, 16515–16519.

Scholz-Muramatsu, H., A. Neumann, M. Meßmer, E. Moore and G. Diekert, 1995. Isolation and characterization of *Dehalospirillum multivorans* gen. nov., sp. nov., a tetrachlroethene-utilizing, strictly anaerobic bacterium. *Arch. Microbiol.* **163**, 48–56.

Schumacher, W., and C. Holliger, 1996. The proton eletron ration of the menaquinone-dependent electron transport from dihydrogen to tetrachloroethene in '*Dehalobacter restrictus*'. *J. Bacteriol.* **178**, 2328–2333.

Vogel, T.M., and P.L. McCarty, 1985. Biotransformation of tetrachloroethylene to trichloroethylene, dichloroethylene, vinyl chloride, and carbon dioxide under methanogenic conditions. *Appl. Environ. Microbiol.* **49**, 1080–1083.

Gabriele Diekert, Dept. of Microbiology, University of Stuttgart, Allmandring 31, D-70550 Stuttgart, Germany