Biotransformation of Chloromethane to Methanethiol

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Monochloromethane (MeCl) was biotransformed by a mixed culture of anaerobic bacteria which had been enriched to utilize dichloromethane as the sole source of carbon and energy. Cells grown in dichloromethane-salts medium catalyzed the transformation, whereas sucrose-grown cells were inactive. MeCl disappeared almost totally, and the methyl group was recovered in a yield of 80–90% as methanethiol and traces of dimethyl sulfide, whose identities were confirmed by GC-MS. The methyl transfer was heat-labile, and free aquocobalamin did not promote the reaction, which was apparently enzymically catalyzed.

Introduction

Most known anaerobic dechlorinations of chlorinated aliphatic hydrocarbons (CHC) are biotransformations which are independent of energy generation or a significant production of amphibolic intermediates (1–6). The biotransformations studied in more detail are usually catalyzed by cofactors, be they free or enzyme-bound (7–13), though in at least one case enzymatic dechlorinative transformation occurs (14, 15). Much work with biotransformation resulted in incomplete mass balances for carbon (cf. ref 13), but the use of radioactive CHC allowed most or all products to be located (e.g., ref 3). As an example for a C-1 CHC, $^{14}\text{CCl}_4$ has been examined, and the products are some or all of the reduced compounds (CHCl_3 , CH_2Cl_2 , CH_3Cl), CO and CO_2 , and water-soluble materials (10, 13, 16, 17). The latter were presumed to represent quenched chloromethyl radicals (13). Some CS_2 has been tentatively identified (16), but no sulfur-containing compound has been conclusively identified as a transformation product.

Recent work shows some CHC to be utilized by anaerobic bacteria as sole carbon and energy sources (18–20) or as electron acceptors (21) for growth. The reaction products from the metabolic transformation of CHC in the different systems vary. When a CHC is used for growth, stoichiometric recovery of both the organochlorine (as chloride) and of the carbon atoms (as cell material and metabolic products or *cis*-1,2-dichloroethene from perchloroethene) is obtained (18–21).

We now observe that monochloromethane (MeCl) is transformed in high yield to methanethiol (MeSH) by whole cells of a mixed culture which was enriched (19) to utilize dichloromethane as a sole source of carbon and energy for growth.

Experimental Section

Materials and Apparatus. Chloromethane (MeCl), dimethyl sulfide (Me_2S), and dichloromethane (DCM) were obtained from Fluka (Buchs, Switzerland), and methanethiol (MeSH) was from Pan Gas (Luzern, Switzerland). Other chemicals and the anaerobic equipment were described elsewhere (19).

A gas chromatograph (GC) (8700, Perkin-Elmer, Beaconsfield, Bucks, U.K.) equipped with a flame ionization

detector (FID) was used routinely. The gas chromatograph was coupled to a mass spectrometer (GC-MS) (5890-5870, Hewlett-Packard, Avondale, PA).

Analytical Methods. Samples (0.3 mL) for MeCl, MeSH, and Me_2S were taken from the headspace of thermostatted reaction vessels. MeCl was determined by GC-FID after separation on Poropak P in a Teflon-coated stainless steel column (1.8 m by 2 mm i.d., mesh 80–100; Supelco Inc., Bellfonte) at 120 °C. MeSH and Me_2S were determined routinely by GC-FID after separation on Chromosyl 330 in a Teflon column (1.8 m by 2 mm i.d., mesh 80–100; Supelco) at 40 °C. GC-MS involved separation on a dimethyl polysiloxane (100%) HP-1 column (10 m, 0.53 mm i.d., 2.65 μm film thickness; Hewlett-Packard) at 30 °C and electron impact ionization at 70 eV. MeCl, MeSH, or Me_2S was distributed between the aqueous and the gas phases, so no concentration in the aqueous phase was calculated. Instead, the amount present in the reaction vessel was interpolated from a standard curve prepared with authentic material in sterile growth medium under conditions identical with the appropriate experiment. Protein in whole cells was measured in a Lowry-type reaction (22).

Organisms and Growth Conditions. The DCM-utilizing mixed culture used, culture DM, was enriched by Stromeyer et al (19); with time, the culture has been simplified to about three dominant organisms, as judged by phase-contrast microscopy. The culture was acetogenic: $2\text{CH}_2\text{Cl}_2 + 2\text{H}_2\text{O} \Rightarrow \text{CH}_3\text{COOH} + 4\text{HCl}$. The medium (pH 7.2) used was modified from that described previously (19), in that the yeast extract was eliminated, the phosphate concentration was reduced to 10 mM, and the gas phase was set at 1.5 bar. *Acetobacterium woodii* DSM 1030 was obtained from the culture collection (Deutsche Sammlung von Mikroorganismen, Braunschweig, FRG) and grown autotrophically under an atmosphere of H_2 and CO_2 (80:20, v/v) (13, 23).

Dechlorination of MeCl by Whole Cells. The transformation of MeCl by culture DM or by *A. woodii* was examined at 30 °C in 20 mL of medium contained in 150-mL screw-cap bottles fitted with Viton stoppers; the gas phase was a mixture of N_2 and CO_2 (80:20, v/v) or H_2 and CO_2 (80:20, v/v), respectively. The media contained 6.4 mM Na_2S . Culture DM was grown in 10 mM DCM-salts medium (final biomass: 5–8 mg of protein/L). After the DCM had disappeared, cultures were used immediately or autoclaved and used. In each case 65 μmol of MeCl was added, and its concentration was monitored for up to 3 weeks. *A. woodii* was grown autotrophically in the presence of 65 μmol of MeCl. The final biomass after 3 weeks was 12–15 mg of protein/L. Replicate (3–5) experiments were done in separately prepared medium, and each replicate involved duplicate cultures.

Cobalamin-Related Transfer of Methyl Groups. Chemical transformation of MeCl was examined at 60 °C in 20 mL of potassium phosphate buffer (50 mM, pH 7.8) contained in light-protected, 150-mL screw-cap bottles fitted with Viton stoppers; the gas phase was a mixture

Figure 1. Transformation of MeCl to MeSH and Me₂S in representative experiments with culture DM. The transformation was examined in nongrowing cells after exhaustion of the carbon and energy source, dichloromethane, as described in the Experimental Section: ●, MeCl; ○, MeCl in sterile control; □, MeSH; Δ, Me₂S.

of N₂ and CO₂ (80:20, v/v). H₂S (128 μmol), titanium(III) citrate (200 μmol), or aquocobalamin (0.72 μmol) was added separately, as indicated in the text, and the reaction was initiated by the addition of MeCl (22 μmol). Replicate experiments contained assays, all of which were done in duplicate.

Results and Discussion

Culture DM did not utilize MeCl as a growth substrate. However, MeCl was extensively transformed by culture DM which had been pregrown in DCM-salts medium (Figure 1). No methane was observed, but two products, I and II, were detected which cochromatographed (GC) with methanethiol (MeSH) and dimethyl sulfide (Me₂S), respectively. No other product was detected.

Product I was confirmed to be MeSH by GC-MS (m/z 48 [M^+ (the molecular ion)], 47 ($M^+ - H$), 45 ($M^+ - H - 2H$), 33 ($M^+ - CH_3$), 32 ($^{32}S^+$), and 15 (CH_3^+). Product II was similarly confirmed to be Me₂S (m/z 62 [M^+ (the molecular ion)], 47 ($M^+ - CH_3$), 45 ($M^+ - CH_3 - 2H$), 33 ($M^+ - CH_3 - CH_3 + H$), 32 ($^{32}S^+$), and 15 (CH_3^+). These spectra were identical with those of the appropriate authentic material.

MeCl was largely stable in sterile, anaerobic, sulfide-containing growth medium (Figure 1 and Table I). Losses of about 10% over 2 weeks were typical, a portion (6%) of which was recovered as MeSH and Me₂S (Table I). We presume that losses of MeCl through the stopper were about 2%/week and that a slow spontaneous reaction of sulfide with MeCl occurred, analogous to the reaction of the sulfide ion and monochloroacetate to give thioglycollate (see ref 24).

The mass balance for the methyl groups recovered in MeSH, Me₂S, and MeCl after 3 weeks was between 80 and 90% of the MeCl added. The same products were obtained spontaneously, but at a fraction of the reaction rate, so most of the products formed were formed biologically. We presume that few other products are formed.

When culture DM was pregrown with a nonchlorinated substrate (10 mM sucrose), no alteration in the relative proportions of the components of the culture was detected. These cells caused some unidentified loss of MeCl but negligible formation of MeSH and Me₂S (Table I).

Table I. Transformation of MeCl by Culture DM and by *A. woodii*

biological material	comps recovered from 65 μmol of MeCl after 15 day (μmol/assay) ^a				recovery of methyl group (%)
	MeCl	MeSH	Me ₂ S	methane	
sterile medium	58	3.5	0.2	<0.001	95
culture DM, native, induced ^b	8	42.1	2.4	<0.001	84
culture DM, autoclaved ^b	44	3.9	0.7	<0.001	76
culture DM, native, noninduced ^c	39	0.5	0.4	0.003	62
<i>A. woodii</i> , native	22	4.1	0.03	<0.001	40

^a The values are the average of duplicate determinations; individual measurements were reproducible ($\pm 5\%$). ^b Culture pregrown with DCM, after which the experiment was started by the addition of MeCl. ^c Culture pregrown with sucrose, after which the experiment was started by the addition of MeCl.

Table II. Involvement of Aquocobalamin and H₂S in Transformation of MeCl

assay component present	products formed from 22 μmol of MeCl in 150 min (μmol/assay) ^a				recovery of methyl group (%)
	MeCl	MeSH	Me ₂ S	methane	
none	21.8	<0.01	<0.01	<0.01	99
titanium(III) citrate	17.6	<0.01	<0.01	0.02	80
aquocobalamin + titanium(III) citrate	10.0	<0.01	<0.01	0.31	47
H ₂ S	19.2	1.7	0.7	<0.01	101
aquocobalamin + H ₂ S	19.6	1.4	0.7	<0.01	102
titanium(III) citrate + H ₂ S	15.9	0.6	0.5	<0.01	80
aquocobalamin, titanium(III) citrate + H ₂ S	8.0	0.4	0.3	0.70	42

^a The values are the average of duplicate determinations; individual measurements were reproducible ($\pm 5\%$).

Similarly, when DCM-grown culture DM was autoclaved, we observed some decrease of MeCl but only negligible formation of MeSH and Me₂S (Table I). Heat-stable factors, thus, do not play an important role in the reaction catalyzed by culture DM, and induced cells are required. We presume that an inducible methyl transferase is synthesized in culture DM.

Disappearance of MeCl in the presence of *A. woodii* was detected in an earlier study (10) where the carbon balance was incomplete. Some disappearance of MeCl was also observed in this study (Table I), but only trace amounts of MeSH and Me₂S were formed. The fate in *A. woodii* of the methyl group from MeCl is still unclear.

Cobalamin-mediated transfer of alkyl groups to thiols is well-established (25, 26), so we explored the possibility that reduced aquocobalamin (B_{12b}) would catalyze the methyl transfer from MeCl to sulfide (Table II). When MeCl was incubated in buffer alone, a negligible loss of MeCl occurred. In the presence of the reductant titanium(III) citrate, a significant disappearance of MeCl occurred, which was increased in the presence of aquocobalamin, but no thiols and only traces of methane were formed. The major fate of the methyl group is unknown. In the presence of sulfide alone, a portion of the MeCl reacted, and the methyl groups were fully recovered in thiols. The additional presence of aquocobalamin did not alter the extent of the reaction or the recovery of the methyl groups. The addition of titanium(III) citrate to sulfide

and aquocobalamin caused a marked increase in the transformation of MeCl but a suppression of the formation of thiols and some release of methane. There was thus no aquocobalamin-dependent formation of methanethiol. Similar data, including the poor mass balance for the methyl group, have been reported elsewhere (8). The unidentified product(s) from titanium(III) citrate-reduced aquocobalamin (Table II) may be identical with or overlap those from *A. woodii* (Table I), which contains vitamin B₁₂ (27) that is active in dechlorination (13).

Methyl transferases other than cobalamin-mediated reactions are known under both aerobic and anaerobic conditions (28–31). Whereas inorganic sulfide is recognized as a methyl acceptor (28, 31), the donor is usually a complex organic compound such as *S*-adenosylmethionine (29) or methoxylated aromatic compounds (31). MeCl has been identified as a methyl donor for a range of organic molecules in fungi (32, 33). Our work extends the range of organisms using this donor to anaerobes.

MeCl and its biotransformation products, MeSH and Me₂S, are well-known natural products, and each is readily degraded aerobically as well as anaerobically (20, 30, 34, 35). The physiological significance of the anaerobic transfer of the methyl group from MeCl to H₂S is as yet unknown. Organisms may be able to couple generation of high-energy bonds to the highly exergonic reaction

(Gibb's free energy was calculated from data in ref 36.) Alternatively, the biotransformation may be a fortuitous side reaction of an enzyme of totally different function. In the environment, the reaction could result in detoxification of MeCl released in an aqueous phase because, once in the atmosphere, MeSH and Me₂S are more rapidly transformed in the atmospheric sulfur cycle (residence time for Me₂S about one day (30)) than MeCl in the halocarbon cycle (residence times for MeCl about one year (37)).

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