

## Involvement of a Quinoprotein Alcohol Dehydrogenase and an NAD-dependent Aldehyde Dehydrogenase in 2-Chloroethanol Metabolism in *Xanthobacter autotrophicus* GJ10

By DICK B. JANSSEN,\* SYTZE KEUNING AND BERNARD WITHOLT  
Groningen Biotechnology Centre, University of Groningen, Nijenborgh 16, 9747 AG Groningen,  
The Netherlands

(Received 1 June 1986; revised 25 July 1986)

---

An inducible methanol dehydrogenase showing high activity with 2-chloroethanol was purified from 2-chloroethanol-grown cells of the 1,2-dichloroethane utilizing bacterium *Xanthobacter autotrophicus* GJ10. The enzyme consisted of a 60 kDa polypeptide that was associated with a 10 kDa polypeptide and contained pyrrolo-quinoline quinone (PQQ) as a prosthetic group. Chloroethanol-grown cells of strain GJ10 also contained an inducible NAD-dependent chloroacetaldehyde dehydrogenase. Its involvement in the metabolism of 2-chloroethanol was inferred from its absence in a 2-chloroethanol non-utilizing mutant. Three different isolates of *X. autotrophicus* that do not utilize 2-chloroethanol for growth produced chloroethanol dehydrogenase and chloroacetaldehyde dehydrogenase activities at similar levels as strain GJ10. It is concluded that both dehydrogenases are involved in the metabolism of natural compounds and due to their broad substrate specificity fortuitously also play a role in the metabolism of the xenobiotic compounds 1,2-dichloroethane and 2-chloroethanol.

---

### INTRODUCTION

*Xanthobacter autotrophicus* GJ10 is a nitrogen-fixing hydrogen bacterium that is able to utilize 1,2-dichloroethane or 2-chloroethanol as a sole carbon and energy source (Janssen *et al.*, 1984, 1985). The organism constitutively produces two dehalogenases, one specific for 1,2-dichloroethane and related haloalkanes (Keuning *et al.*, 1985), and one specific for halogenated carboxylic acids. It was proposed that 1,2-dichloroethane is degraded via 2-chloroethanol, chloroacetaldehyde and chloroacetic acid to glycolate. During growth on 1,2-dichloroethane or methanol, *X. autotrophicus* GJ10 produced both methanol dehydrogenase and 2-chloroethanol dehydrogenase activities. This simultaneous induction raised the supposition that the activities are related to a single protein (Janssen *et al.*, 1985). Conversion of 2-chloroethanol by purified methanol dehydrogenase was observed by Anthony & Zatman (1967) for the enzyme of *Pseudomonas* M27 and by Beardsmore-Gray *et al.* (1983) for the enzyme of *Methylophilus methylotrophus*.

Further metabolism by the above route would require the presence of chloroacetaldehyde dehydrogenase. This activity has not yet been demonstrated in *X. autotrophicus* GJ10, but an NAD-dependent chloroacetaldehyde dehydrogenase was described in an unidentified 1,2-dichloroethane utilizing bacterium (Stucki *et al.*, 1983).

In this paper, we describe the regulation and properties of a quinoprotein alcohol dehydrogenase that is also involved in the metabolism of methanol and 2-chloroethanol. Furthermore, the characteristics of a 2-chloroethanol non-utilizing mutant demonstrate the involvement of an inducible NAD-dependent aldehyde dehydrogenase in ethanol and 2-chloroethanol metabolism.

---

*Abbreviations:* PQQ, pyrrolo-quinoline quinone; MMV, mineral salts-vitamins medium; PMS, phenazine methosulphate; PPM, pyrophosphate-methanol buffer.

## METHODS

*Organism and growth conditions.* All organisms used in this study were strains of *Xanthobacter autotrophicus* (Wiegel *et al.*, 1978). The characteristics of strain GJ10 were described by Janssen *et al.* (1984, 1985). Strain GJ10M41 is a 1,2-dichloroethane non-utilizing mutant that was isolated as described below. Strain GZ29 and strain 7C (the type strain of this species; Wiegel *et al.*, 1978; Tunail & Schlegel, 1974) were kindly donated by Professor H. G. Schlegel. Strain XD was isolated as a methanol utilizing organism and was a gift from Dr L. Dijkhuizen.

Cells were grown aerobically at 30 °C in a mineral salts medium (MMV) containing ( $l^{-1}$ ) 5.37 g  $Na_2H-PO_4 \cdot 12H_2O$ , 1.36 g  $KH_2PO_4$ , 0.5 g  $(NH_4)_2SO_4$ , 0.2 g  $MgSO_4 \cdot 7H_2O$ , 5 ml salts solution and 1 ml vitamins solution (Janssen *et al.*, 1984). Carbon sources were added as indicated at 10 mM. For the isolation of 2-chloroethanol dehydrogenase, cells were grown on 20 mM-2-chloroethanol in a 10 l fermenter (Braun Biostat E) at pH 7.0 and 70% oxygen saturation.

*Preparation of crude extracts.* Cells were collected by centrifugation, washed with 10 mM-Tris/ $H_2SO_4$  buffer, pH 7.5 (Tris buffer), and resuspended in Tris buffer [ $2 \text{ ml (g wet wt)}^{-1}$ ]. The cells were disrupted by sonication [ $10 \text{ s (ml suspension)}^{-1}$  at 60 W output in an Ultrasonics W-375 sonicator]. After centrifugation (30 min, 45000 g), a crude extract was obtained and used for enzyme assays within 6 h.

*Enzyme assays.* Alcohol dehydrogenase activities were determined at 30 °C in a biological oxygen monitor equipped with a Clark-type oxygen electrode. Incubation mixtures contained (in a final volume of 6 ml) 60 mM-sodium pyrophosphate buffer (pH 9.0), 23 mM- $NH_4Cl$ , 4 mM-substrate and a suitable amount of enzyme in 1 ml Tris buffer. The reaction was started by the addition of 0.2 ml 30 mM-phenazine methosulphate (PMS), and oxygen consumption was followed. One unit of activity (U) is defined as that catalysing an oxygen consumption rate of  $1 \mu\text{mol min}^{-1}$ . Values were corrected for oxygen uptake in the absence of substrate.

Chloroacetaldehyde dehydrogenase activities were assayed spectrophotometrically at 30 °C by following the reduction of NAD at 334 nm. Reaction mixtures contained (in a final volume of 1 ml) 100 mM-sodium pyrophosphate, pH 8.75, 1 mM-NAD, 1 mM dithiothreitol, 10 mM- $\beta$ -mercaptoethanol, 5 mM-chloroacetaldehyde, and a suitable amount of enzyme in 0.1 ml Tris buffer containing 1 mM-dithiothreitol and 10 mM- $\beta$ -mercaptoethanol. The latter two compounds were added to the crude extracts immediately after preparation, since this was found to stabilize enzyme activity. The reaction was started by the addition of substrate. One unit is defined as the activity catalysing the formation of  $1 \mu\text{mol NADH min}^{-1}$ .

1,2-Dichloroethane dehalogenase and chloroacetic acid dehalogenase were assayed by following chloride production as described by Janssen *et al.* (1985) and Keuning *et al.* (1985). Protein concentrations were determined by the Lowry method using bovine serum albumin as the standard.

*Purification of 2-chloroethanol dehydrogenase.* The enzyme was purified at 4 °C from 2-chloroethanol-grown cells (13 g dry wt) principally according to the procedure described by Schär *et al.* (1985) for the isolation of methanol dehydrogenase from *Hyphomicrobium* MS223. Briefly, crude extract was prepared in 10 mM-sodium pyrophosphate buffer, pH 9.0, containing 10 mM-methanol (PPM buffer), and subsequently fractionated by stepwise addition of solid ammonium sulphate. The 55–80% saturation precipitate was dissolved in 10 ml PPM buffer, dialysed overnight against 21 PPM buffer, and then applied to a DEAE-cellulose column (Whatman DE52,  $2 \times 30 \text{ cm}$ ) that had been previously equilibrated with PPM buffer. Activity passed through the column without retention and was concentrated by precipitation with ammonium sulphate (80% saturation). The enzyme was further purified by Sephacryl S-300 chromatography. Activity was eluted from the column ( $2 \times 100 \text{ cm}$ ) with PPM buffer, concentrated by ammonium sulphate precipitation, and applied to a Sephadex G-100 column ( $2 \times 80 \text{ cm}$ ). After elution with PPM buffer, fractions containing the highest activity were pooled and stored frozen at  $-20 \text{ °C}$  until used.

*Molecular mass determinations.* The subunit size of the protein was determined by SDS-PAGE on 10% or 15% (w/v) polyacrylamide according to the method of Laemmli (1970). The following proteins were used for calibration: phosphorylase B (92 kDa), bovine serum albumin (68 kDa), ovalbumin (45 kDa), aldolase (40 kDa), chymotrypsinogen (25 kDa) and cytochrome *c* (12.4 kDa). The gels were stained with Coomassie brilliant blue.

The molecular mass of the native enzyme was estimated using a Sephadex G-100 column that was run as described above. The column was calibrated with alcohol dehydrogenase (145 kDa), bovine serum albumin, ovalbumin, trypsin inhibitor (20.1 kDa) and cytochrome *c*. All calibration proteins were obtained from Boehringer.

Cross-linking with the bifunctional reagent *N,N*-dimethylsuberimidate ( $0.5\text{--}5 \text{ mg ml}^{-1}$  in 0.2 M-triethanolamine buffer, pH 8.5) was done for 16 h at 30 °C, using an enzyme solution ( $1 \text{ mg ml}^{-1}$ ) that has been previously dialysed overnight against triethanolamine buffer.

*Isolation of 1,2-dichloroethane non-utilizing mutants.* Mutants of strain GJ10 that do not utilize 1,2-dichloroethane for growth were obtained after nitrite mutagenesis and glycine contraselection, using a procedure adopted from Wilke & Schlegel (1979). Mutants were contraselected for 20 h in MMV medium containing 2% (w/v) L-glycine and 5 mM-1,2-dichloroethane; surviving cells were grown in MMV medium containing 10 mM-glycolic acid for 20 h. After two enrichment cycles, cells were plated on MMV medium with glycolate as carbon source. Mutants were identified by replica plating, purified and stored on nutrient agar plates.

Table 1. Regulation of 2-chloroethanol dehydrogenase and chloroacetaldehyde dehydrogenase formation

Crude extracts were prepared from cells grown with different carbon sources and enzyme activities were determined as described in Methods. Values represent means from two or three experiments.

Growth substrate	Specific activity [mU (mg protein) <sup>-1</sup> ]		
	Methanol dehydrogenase	2-Chloroethanol dehydrogenase	Chloroacetaldehyde dehydrogenase
1,2-Dichloroethane	520	480	109
2-Chloroethanol	440	415	68
Citrate	< 10	< 10	< 10
Methanol	508	515	15
Ethanol	295	295	200
Glycolate	404	438	13

Table 2. Purification of 2-chloroethanol dehydrogenase

The enzyme was purified from 13 g (dry wt) of 2-chloroethanol-grown cells, according to the procedure given in Methods. After each step, methanol dehydrogenase and 2-chloroethanol dehydrogenase activities were determined in the pooled fractions.

Step	Protein (mg)	Dehydrogenase activity [U (mg protein) <sup>-1</sup> ]		Purification factor	Yield (%)
		Methanol	2-Chloroethanol		
Crude extract	6200	0.44	0.4	1	100
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> precipitation	646	2.8	2.6	6.5	69
DEAE-cellulose	101	12.9	12.0	30	52
Sephacryl S-300	40	14.4	13.0	32.5	22
Sephadex G-100	19	17.0	15.5	39	12

## RESULTS

### Identification and regulation of enzymes involved in 2-chloroethanol metabolism

Crude extracts of 2-chloroethanol-grown cells of *X. autotrophicus* GJ10 were found to contain 415 mU (mg protein)<sup>-1</sup> of PMS-linked 2-chloroethanol dehydrogenase activity. Without addition of ammonia to the assay mixture or with Tris/H<sub>2</sub>SO<sub>4</sub> buffer instead of sodium pyrophosphate buffer, a value of 240 mU (mg protein)<sup>-1</sup> was obtained. No activity could be detected when PMS was omitted from the incubation mixture or when it was replaced by 1 mM-NAD or NADP.

With 2-chloroacetaldehyde as the substrate, crude extracts of 2-chloroethanol-grown cells reduced NAD at a rate of 68 mU (mg protein)<sup>-1</sup>, under the standard assay conditions. No chloroacetaldehyde dehydrogenase activity could be detected with NADP or PMS as the electron acceptor. With Tris/H<sub>2</sub>SO<sub>4</sub> buffer instead of sodium pyrophosphate buffer in the incubation mixture, an activity of 35 mU (mg protein)<sup>-1</sup> was obtained. The enzyme was stabilized in crude extracts by addition of reducing agents, with less than 20% inactivation occurring during storage at 4 °C for 20 h. The optimum pH for activity was 8.7.

The two dehydrogenases were inducible enzymes (Table 1). During growth with 2-chloroethanol, 1,2-dichloroethane or ethanol, the formation of both chloroethanol dehydrogenase and chloroacetaldehyde dehydrogenase was derepressed. Growth with methanol or glycolate only derepressed 2-chloroethanol dehydrogenase, showing that the enzymes are not coordinately regulated.

### Purification and characterization of 2-chloroethanol dehydrogenase

2-Chloroethanol dehydrogenase was purified from crude extracts of cells grown with 2-chloroethanol as carbon source; the purification scheme is summarized in Table 2. The enzyme was purified 39-fold, with an overall yield of 12%, which implies that 2-chloroethanol

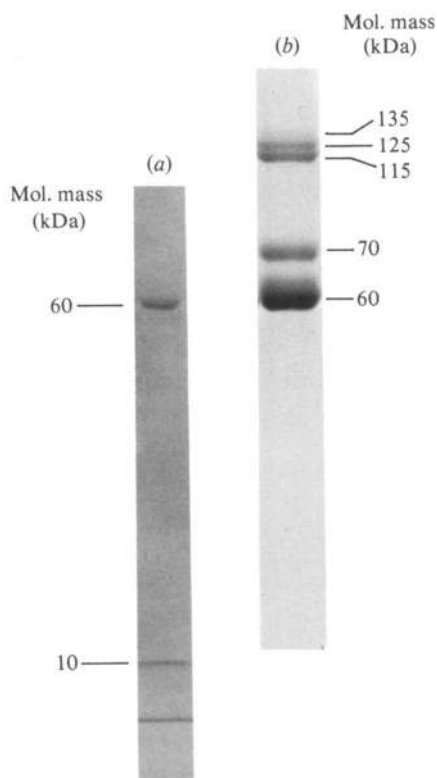


Fig. 1. SDS-PAGE of quinoprotein alcohol dehydrogenase from *X. autotrophicus* GJ10. The molecular masses of the relevant bands are indicated. (a) Electrophoresis of purified enzyme on a 15% polyacrylamide gel. (b) Electrophoresis of purified enzyme on a 10% polyacrylamide gel after cross-linking with *N,N*-dimethylsuberimidate.

dehydrogenase accounts for at least 2.5% of the total cellular protein. Methanol dehydrogenase activity copurified with 2-chloroethanol dehydrogenase: the ratio of the activities measured with 2-chloroethanol and with methanol as substrate was constant at 0.9 throughout the purification procedure.

SDS-PAGE of the purified enzyme gave two polypeptide bands. Calibration indicated that these had molecular masses of 60 kDa and 10 kDa, showing that a small polypeptide remained associated with the 60 kDa protein during the two gel filtration steps. Gel filtration on Sephadex G-100 indicated that the molecular mass of the purified native dehydrogenase was 66 kDa. However, after dialysis of the purified enzyme against 10 mM-Tris/H<sub>2</sub>SO<sub>4</sub>, pH 7.5, and rechromatography on Sephadex G-100, a molecular mass of 120 kDa was obtained. This value was also found when gel filtration was done in 50 mM-sodium phosphate buffer, pH 7.5, indicating that the subunit composition of the enzyme strongly depends on the experimental conditions.

Dimers of 2-chloroethanol dehydrogenase were also found after treatment with the bifunctional coupling reagent dimethylsuberimidate (Fig. 1). The appearance of a protein band at 70 kDa suggests that the reagent couples the 10 kDa polypeptide to the dehydrogenase. It is reasonable to assume that the bands with molecular masses of 115 kDa, 125 kDa and 135 kDa are generated, respectively, by a dehydrogenase dimer, a dimer with one 10 kDa protein, and a dimer coupled to two 10 kDa proteins.

The absorption spectrum of the purified 2-chloroethanol dehydrogenase showed a maximum at 345 nm with a shoulder at 410 nm, which is characteristic of alcohol dehydrogenases containing PQQ as a prosthetic group (Anthony, 1982; Duine & Frank, 1981*a*).

Table 3. *Enzyme activities in X. autotrophicus GJ10 and in the mutant GJ10M41*

Crude extracts were prepared from cells of strain GJ10 and the 1,2-dichloroethane non-utilizing mutant GJ10M41 after growth with different carbon sources. All values represent means from two or three experiments. NT, Not tested.

Strain	Growth medium	Specific activity [mU (mg protein) <sup>-1</sup> ]			
		1,2-Dichloroethane dehalogenase	2-Chloroethanol dehydrogenase	Chloroacetaldehyde dehydrogenase	Chloroacetate dehalogenase
GJ10	Citrate + 2-chloroethanol	185	94	90	216
GJ10M41	Citrate + 2-chloroethanol	188	<5	<10	303
GJ10M41	Methanol	NT	259	<10	NT
GJ10M41	Ethanol	120	248	<10	1740
GJ10M41	Glycolate	NT	214	<10	NT

Dehydrogenase activity with 2-chloroethanol or methanol as the substrate was maximal at pH 9.0–9.4 and a temperature of 40 °C. At temperatures of 50 °C or above, the enzyme was rapidly inactivated. The purified dehydrogenase catalysed the oxidation of a wide range of primary alcohols. 1-Propanol, 1-butanol, 1-octanol, 2-chloroethanol, 2-bromoethanol and formaldehyde were oxidized at 80–90% of the rate found with methanol. The highest rate (120%) was found with ethanol, while 1-pentanol (41%) and 3-propenol (72%) were also substrates for the enzyme. No activity was found with 2-propanol, 2-butanol, isobutylalcohol, acetaldehyde, propionaldehyde, butyraldehyde or chloroacetaldehyde. Glycolate, which induced the formation of the enzyme, was not a substrate.

#### *Characterization of a 1,2-dichloroethane non-utilizing mutant*

Enrichment for 1,2-dichloroethane non-utilizing mutants yielded strain GJ10M41 which was unable to grow with 1,2-dichloroethane or 2-chloroethanol as sole carbon source. Mutant GJ10M41 was able to grow on glycolate, methanol or citrate, but ethanol was a poor carbon source, giving a doubling time of 40 h, compared to 5.9 h for the wild type. Doubling times with methanol were 5.4 and 5.2 h for strains GJ10 and GJ10M41, respectively. In crude extracts of strain GJ10M41, prepared from cells grown on glycolate, methanol or ethanol, no chloroacetaldehyde dehydrogenase activity could be detected, while dehalogenase and 2-chloroethanol dehydrogenase levels were normal (Table 3). This suggests that the primary defect in strain GJ10M41 is a lack of chloroacetaldehyde dehydrogenase activity. The mutant was found to lack both 2-chloroethanol dehydrogenase and chloroacetaldehyde dehydrogenase when grown on a medium containing citrate and 2-chloroethanol, which may be caused by inactivation of the alcohol dehydrogenase by accumulating chloroacetaldehyde, or impairment of its synthesis by mutational or regulatory events.

The loss of 2-chloroacetaldehyde dehydrogenase activity in mutant GJ10M41 had another secondary effect: toxicity of 2-chloroethanol. In batch cultures, the doubling time of strain GJ10M41 with citrate plus 2-chloroethanol as carbon sources was 45 h, compared to 6 h for GJ10M41 with citrate alone and 6.5 h for the wild-type with both citrate and 2-chloroethanol in the medium. Addition of 2-chloroethanol to cells growing with methanol as carbon source caused a rapid inhibition of growth in strain GJ10M41 but not in strain GJ10 (Fig. 2). Thus, the inability to convert chloroacetaldehyde gives 2-chloroethanol the characteristics of a toxic substrate for this mutant.

#### *Enzymes of 1,2-dichloroethane metabolism in other strains of Xanthobacter*

The ability to produce dehydrogenases that are active with chlorinated substrates could be either specific for strain GJ10, or it could be a general characteristic of *Xanthobacter*. This was investigated by comparing strain GJ10 with three different strains that do not utilize 1,2-dichloroethane or 2-chloroethanol for growth, but can grow on glycolate. During growth on

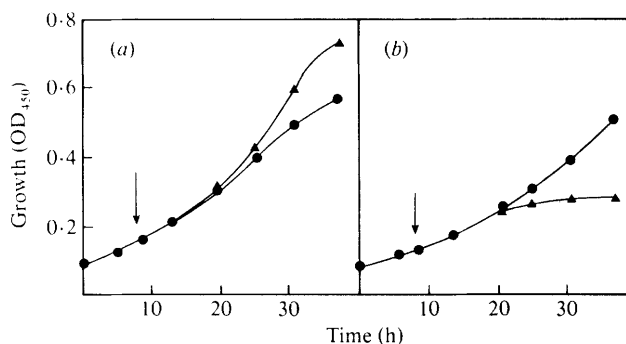


Fig. 2. Effect of 2-chloroethanol on growth of *X. autotrophicus* with methanol. Cells of strain GJ10 (a) and mutant GJ10M41 (b) were grown in batch culture with methanol as the carbon source. At the time indicated by the arrows the cultures were divided in two parts and to one of these 1 mM-2-chloroethanol was added. Growth was followed at 450 nm. ▲, 2-Chloroethanol added; ●, control.

Table 4. *Enzyme levels in different strains of Xanthobacter*

Enzyme activities were determined in extracts prepared from ethanol-grown cells.

Strain	Specific activity [mU (mg protein) <sup>-1</sup> ]			
	1,2-Dichloroethane dehalogenase	2-Chloroethanol dehydrogenase	Chloroacetaldehyde dehydrogenase	Chloroacetate dehalogenase
GJ10	232	330	174	416
GZ29	<5	930	155	<5
7C	<5	804	184	<5
XD	<5	748	171	<5

ethanol, *X. autotrophicus* strains GZ29, 7C and XD produced 2-chloroethanol and chloroacetaldehyde dehydrogenases at similar levels as strains GJ10 (Table 4). The three strains did not produce 1,2-dichloroethane dehalogenase or chloroacetate dehalogenase, indicating that only the dehydrogenases should be considered as general enzymes of *X. autotrophicus*.

#### DISCUSSION

The results presented in this paper demonstrate that the oxidation of 2-chloroethanol to chloroacetate in *X. autotrophicus* GJ10 is catalysed by two different dehydrogenases. The chloroethanol dehydrogenase appeared to be a PQQ-containing alcohol dehydrogenase that due to its broad substrate specificity was active with 2-chloroethanol. Quinoprotein alcohol dehydrogenases have been identified in several methylotrophic bacteria (Anthony, 1982; Duine & Frank, 1981a). The enzymes require ammonia or amines for activity and catalyse the oxidation of several primary alcohols. It has been shown that 2-chloroethanol can be a substrate for these enzymes (Anthony & Zatman, 1967; Beardmore-Gray *et al.*, 1983).

The quinoprotein alcohol dehydrogenase of *X. autotrophicus* GJ10 was found to be a 60 kDa polypeptide with which a 10 kDa protein was associated. A subunit molecular mass of 60 kDa has been found for several other dye-linked alcohol dehydrogenases (Anthony, 1982; Schär *et al.*, 1985). Recently, Weaver & Lidstrom (1985) observed simultaneous induction of 60 kDa and 10 kDa polypeptides in *Xanthobacter* H4-14 during growth on methanol, and they suggested that the 10 kDa polypeptide could be a cytochrome *c<sub>H</sub>*. Together with cytochrome *c<sub>L</sub>*, this protein is involved in electron transport from the dehydrogenase to cytochrome oxidase during oxidation of methanol (Beardmore-Gray *et al.*, 1983). However, from the absorption spectrum of the purified dehydrogenase, we conclude that the 10 kDa protein found here has lost its haem group or is not a cytochrome.

The molecular mass of undenatured alcohol dehydrogenase was 66 kDa or 120 kDa depending on the composition of the buffer used for gel filtration. Thus, subunit composition is a poor criterion for the classification of quinoprotein dehydrogenases. Regarding substrate specificity, the alcohol dehydrogenase of *X. autotrophicus* GJ10 strongly resembles the methanol dehydrogenase of *Methylophilus methylotrophus* (Beardmore-Gray *et al.*, 1983), *Methylomonas methanica* (Patel *et al.*, 1978) and *Methylosinus sporium* (Patel & Felix, 1976). The enzyme described here is similar to these proteins in having a high isoelectric pH, as indicated by its lack of binding to DEAE-cellulose at pH 7.5.

Formaldehyde was a good substrate for the alcohol dehydrogenase. The oxidation of this compound is assumed to be the result of hydration to the *gem*-diol (Sperl *et al.*, 1974). Acetaldehyde and higher aldehydes (up to C<sub>4</sub>) are not substrates for the enzyme, although they are also highly hydrated in water. This can be explained by assuming the presence of two different catalytic sites on the enzyme (Duine & Frank, 1981*a*; Schär *et al.*, 1985). On the other hand, there seems to be a correlation between enzyme activity with higher aldehydes and activity with secondary alcohols. The alcohol dehydrogenase from *Rhodopseudomonas acidophila* (Sahm *et al.*, 1976; Yamanaka & Minoshima, 1984), *Hyphomicrobium* MS223 (Schär *et al.*, 1985) and an unidentified Gram-negative bacterium (Duine & Frank, 1981*b*) are exceptional in that they show activity with secondary alcohols. For these enzymes, C<sub>2</sub> to C<sub>4</sub> aldehydes are also substrates. Thus, an alternative explanation is that the lack of oxidation of both higher aldehydes and of secondary alcohols is caused by steric hindrance, since unlike primary alcohols and formaldehyde, these compounds have two bulky groups bound to the same carbon atom as the hydroxyl group of the substrate.

Radiochemical labelling and experiments with spheroplasts have suggested that the PQQ-dependent alcohol dehydrogenase of methylotrophic bacteria is a periplasmic enzyme (Kasprzak & Steenkamp, 1983; Alefounder & Ferguson, 1981). The soluble 1,2-dichloroethane dehalogenase of *X. autotrophicus* GJ10 seems to be a cytoplasmic enzyme, as suggested by the presence of *N*-formylmethionine at the amino terminus (Keuning *et al.*, 1985). The above would imply that during growth with 1,2-dichloroethane the substrate is taken up into the cytoplasm to become dehalogenated to 2-chloroethanol, which is subsequently exported to the periplasm to undergo conversion to chloroacetaldehyde, followed by re-uptake and further metabolism in the cytoplasm. In accordance with this, we observed that 2-chloroethanol may transiently accumulate in the medium during growth of strain GJ10 with 1,2-dichloroethane in batch culture (up to 0.5 µM 2-chloroethanol with 5 mM substrate; details not given).

The second dehydrogenase that plays a role in 1,2-dichloroethane and 2-chloroethanol utilization appeared to be an inducible NAD-dependent chloroacetaldehyde dehydrogenase. Its involvement in 2-chloroethanol metabolism was evident from the observation that the enzyme was lacking in a mutant that could not grow with 1,2-dichloroethane or 2-chloroethanol, but that still utilized glycolate. The enzyme is also involved in the metabolism of the natural compound ethanol, as shown by the extremely slow growth of the mutant when this alcohol was provided as sole carbon source. NAD-dependent chloroacetaldehyde dehydrogenase has previously been demonstrated in a 1,2-dichloroethane utilizing bacterium that was assumed to degrade 1,2-dichloroethane directly to chloroacetaldehyde by an oxidative reaction (Stucki *et al.*, 1983).

The evolutionary development of catabolic routes for the degradation of anthropogenic compounds is an intriguing problem. Here, we have shown that the chloroethanol dehydrogenase and the chloroacetaldehyde dehydrogenase produced by *X. autotrophicus* GJ10 for the metabolism of 1,2-dichloroethane should be considered as normal proteins with a function in the metabolism of the natural compounds methanol (and other primary alcohols) and ethanol, respectively. Both enzymes seem to be generally present in *Xanthobacter*, including strains that do not utilize chlorinated substrates, and fortuitously play a role in the metabolism of xenobiotic compounds due to their broad substrate specificity. The additional ability to produce two constitutive dehalogenases allows strain GJ10 to utilize chlorinated substrates. Whether these dehalogenases are encoded by some transmissible genetic elements remains to be determined.

The authors thank Professor Duine, Technical University of Delft, for confirming the presence of PQQ in purified alcohol dehydrogenase, and Professor H. G. Schlegel, University of Göttingen as well as Dr L. Dijkhuizen for a gift of bacterial strains. Thanks are due to Ine Bont for typing the manuscript. This work was financed in part by the Netherlands Programme Commission for Biotechnology.

## REFERENCES

- ALEFOUNDER, P. R. & FERGUSON, S. J. (1981). A periplasmic location for methanol dehydrogenase from *Paracoccus denitrificans*: implications for proton pumping by cytochrome *aa<sub>3</sub>*. *Biochemical and Biophysical Research Communications* **98**, 778–784.
- ANTHONY, C. (1982). *The Biochemistry of Methylo-trophs*. London: Academic Press.
- ANTHONY, C. & ZATMAN, L. J. (1967). The microbial oxidation of methanol. Purification and properties of the alcohol dehydrogenase of *Pseudomonas* sp. M27. *Biochemical Journal* **104**, 953–959.
- BEARDSMORE-GRAY, M., O'KEEFE, D. T. & ANTHONY, C. (1983). The methanol:cytochrome *c* oxidoreductase of methylotrophs. *Journal of General Microbiology* **129**, 923–933.
- DUINE, J. A. & FRANK, J. (1981*a*). Methanol dehydrogenase: a quinoprotein. In *Microbial Growth in C<sub>1</sub> Compounds*, pp. 31–41. Edited by H. Dalton. London: Heyden.
- DUINE, J. A. & FRANK, J. (1981*b*). Quinoprotein alcohol dehydrogenase from a non-methylotroph, *Acinetobacter calcoaceticus*. *Journal of General Microbiology* **122**, 201–209.
- JANSSEN, D. B., SCHEPER, A. & WITHOLT, B. (1984). Biodegradation of 2-chloroethanol and 1,2-dichloroethane by pure bacterial cultures. In *Innovations in Biotechnology*, pp. 169–178. Edited by E. H. Houwink & R. R. van der Meer. Amsterdam: Elsevier.
- JANSSEN, D. B., SCHEPER, A., DIJKHUIZEN, L. & WITHOLT, B. (1985). Degradation of halogenated aliphatic compounds by *Xanthobacter autotrophicus* GJ10. *Applied and Environmental Microbiology* **49**, 673–677.
- KASPRZAK, A. A. & STEENKAMP, D. J. (1983). Localization of the major dehydrogenases in two methylotrophs by radiochemical labeling. *Journal of Bacteriology* **156**, 348–353.
- KEUNING, S., JANSSEN, D. B. & WITHOLT, B. (1985). Purification and characterization of hydrolytic haloalkane dehalogenase from *Xanthobacter autotrophicus* GJ10. *Journal of Bacteriology* **163**, 635–639.
- LAEMMLI, U. K. (1970). Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature, London* **227**, 680–685.
- PATEL, R. N. & FELIX, A. (1976). Microbial oxidation of methane and methanol: crystallization and properties of methanol dehydrogenase from *Methylo-sinus sporium*. *Journal of Bacteriology* **128**, 413–424.
- PATEL, R. N., HOU, C. T. & FELIX, A. (1978). Microbial oxidation of methane and methanol: crystallization of methanol dehydrogenase and properties of holo- and apo-methanol dehydrogenase from *Methylomonas methanica*. *Journal of Bacteriology* **133**, 641–649.
- SAHM, H., COX, R. B. & QUAYLE, J. R. (1976). Metabolism of methanol by *Rhodospseudomonas acidophila*. *Journal of General Microbiology* **94**, 313–322.
- SCHÄR, H.-P., CHEMLA, P. & GHISALBA, O. (1985). Methanol dehydrogenase from *Hyphomicrobium* MS223. *FEMS Microbiology Letters* **26**, 117–122.
- SPEERL, G. T., FORREST, H. S. & GIBSON, D. T. (1974). Substrate specificity of the purified primary alcohol dehydrogenases from methanol-oxidizing bacteria. *Journal of Bacteriology* **18**, 541–550.
- STUCKI, G., KREBSER, U. & LEISINGER, T. (1983). Bacterial growth on 1,2-dichloroethane. *Experientia* **39**, 1271–1273.
- TUNAIL, N. & SCHLEGEL, H. G. (1974). A new coryneform hydrogen bacterium: *Corynebacterium autotrophicum* strain 7C. I. Characterization of the wild type strain. *Archives of Microbiology* **100**, 341–350.
- WEAVER, C. A. & LIDSTROM, M. E. (1985). Methanol dissimilation in *Xanthobacter* H4-14: activities, induction and comparison to *Pseudomonas* AM1 and *Paracoccus denitrificans*. *Journal of General Microbiology* **131**, 2183–2197.
- WIEGEL, J., WILKE, D., BAUMGARTEN, J., OPITZ, R. & SCHLEGEL, H. G. (1978). Transfer of the nitrogen-fixing hydrogen bacterium *Corynebacterium autotrophicum* Baumgarten *et al.* to *Xanthobacter* *gen. nov.* *International Journal of Systematic Bacteriology* **28**, 573–581.
- WILKE, D. & SCHLEGEL, H. G. (1979). A defective generalized transducing bacteriophage in *Xanthobacter autotrophicus* GZ29. *Journal of General Microbiology* **115**, 403–410.
- YAMANAKA, K. & MINOSHIMA, R. (1984). Comparison of two dye-linked alcohol dehydrogenases of *Rhodospseudomonas acidophila*: their substrate specificity and behavior toward oxygen. *Agricultural and Biological Chemistry* **48**, 171–179.