Arch Microbiol (1993) 159: 244–249

Archives of
Microbiology

On a reversible molybdenum-containing aldehyde oxidoreductase from *Clostridium formicoaceticum*

Hiltrud White, Claudia Huber, Richard Feicht, Helmut Simon

Lehrstuhl für Organische Chemie und Biochemie, Technische Universität München, Lichtenbergstrasse 4, W-8046 Garching, Germany

Received: 29 July 1992/Accepted: 9 October 1992

Abstract. *Clostridium formicoaceticum* grown in the presence of 1 mM molybdate and about 1.5×10^{-5} mM tungsten (present in the 5 g yeast extract/l of the growth medium) forms two reversible aldehyde oxidoreductases in an activity ratio of about 45:55. The fraction of 45% does not bind to the octyl-Sepharose column, whereas the 55% aldehyde oxidoreductase binds to this column. From cells grown on a synthetic medium without the addition of tungstate only about 2% of the aldehyde oxidoreductase of the crude extract binds to octyl-Sepharose. The enzyme not binding to octyl-Sepharose has been purified as judged by electrophoresis. It is pure after about 50 fold enrichment. According to SDS gel electrophoresis the enzyme consists of identical 100 kD subunits. Based on gel chromatography it seems to be a trimer. Per subunit 0.6 molybdenum, 7 iron, 6.6 acid labile sulphur, about 0.1 pterin-6-carboxylic and <0.05 tungsten have been found. The first 13 amino acids from the amino end show no similarity with the W-containing aldehyde oxidoreductase from the same bacterium. With reduced tetramethylviologen ($E_0 = -550$ mV) the new molybdenum containing enzyme can reduce various aliphatic and aromatic acids to aldehydes. The pH optimum is at 6.0. For the dehydrogenation of butyraldehyde a rather broad pH region from pH 6 to 10 shows almost no variation of rate. From 15 different aldehydes acetaldehyde exhibits the highest rate. The K_m value for butanal is 0.002 and for propionate 7.0 mM. Compared with the tungsten enzyme the molybdenum enzyme is only moderately oxygen-sensitive.

Key words: Aldehyde oxidoreductase – *Clostridium formicoaceticum* – Molybdenum containing – Substrate specificity – Tungsten-containing

We previously described a tungsten-containing aldehyde oxidoreductase from *Clostridium thermoaceticum* (White et al. 1989; Strobl et al. 1992) and a clearly different one found in *C. formicoaceticum* (White et al. 1991). Whereas these enzymes effectively reduce nonactivated carboxylic acids, for the also tungsten-containing aldehyde-ferredoxin-oxidoreductase of *Pyrococcus furiosus* only the oxidation of aldehydes is reported (Mukund and Adams 1991). We had already observed, when *C. formicoaceticum* was grown in the presence of 1 mM molybdate without added tungstate in the complex growth medium, a specific activity of only 0.3 U/mg protein for the aldehyde dehydrogenase reaction resulted. In the presence of both trace elements in 10 µM concentrations or 1 mM tungstate alone, up to 2 U/mg protein aldehyde dehydrogenase activity were present in crude extracts (White et al. 1991). Because of the yeast extract there are always low concentrations of about 7×10^{-8} M molybdenum and 1.5×10^{-8} M tungsten, even without an extra addition of molybdate or tungstate in the medium of *C. formicoaceticum* (White and Simon 1992). When crude extracts of cells grown with additional tungstate were applied to an octyl-Sepharose column, all of the aldehyde dehydrogenase activity bound to the column and was then purified as tungsten-containing aldehyde oxidoreductase (White et al. 1991). When crude extracts of molybdate-grown cells were applied to the same column, about 50% of the activity bound to octyl-Sepharose and could then be purified (data not shown). Now we proceeded to purify the enzyme species formed in the presence of 1 mM molybdate in the complex medium, which did not bind to the octyl-Sepharose column to compare its properties with the aforementioned tungsten enzyme.

Material and methods

Chemicals

Methylviologen dichloride and 4,4'-bipyridyl for the synthesis of 1,1'-carbamoylmethylviologen (NH_2CO-MV) (Günther et al. 1987) were from Aldrich Chemie, Steinheim, FRG). For the synthesis of tetramethylviologen (TMV) 2-methylpyridine was condensed by metallic sodium in principle as described (Heuser and Stoehr 1890) to 2,2'-dimethyl-4,4'-bipyridyl. This was two-fold N-methylated to TMV (Lal and Petrow 1949). The product showed the expected

Abbreviations: AOR, aldehyde oxidoreductase; BV, benzylviologen; MV, methylviologen; NH_2CO-MV, 1,1'-carbamoylmethylviologen; TMV, 1,1',2,2'-tetramethylviologen

Correspondence to: H. Simon

^{1}H-NMR and elemental analysis. The viologens were reduced in an electrochemical cell (Thanos et al. 1987). The dimeric D,L-glyceraldehyde (Aldrich-Chemie) was heated in anaerobic H_2O to 70 °C for 90 min. According to enzymatic analysis (Goedde and Langenbeck 1984) 30–40% of the monomer had formed.

Cell material

Clostridium formicoaceticum DSM 92 was grown on complex medium as described (White et al. 1991) except that Na_2WO_4 was omitted and the concentration of Na_2MoO_4 was increased to 1 mM. The organism was also grown on defined minimal medium according to Leonhardt and Andreesen (1977) with some modifications. The ingredients were per litre: 1.0 g NH_4Cl, instead of $(NH_4)_2SO_4$, $(NH_4)_2Fe(SO_4)_2 \cdot 6\,H_2O$, substituted for Fe($NH_4$)-citrate; $MnCl_2 \times 4\,H_2O$, 2.0 mg; Na_2WO_4, omitted; sodiumthioglycolate, omitted; $Na_2MoO_4 \cdot 2\,H_2O$, 2.4 mg; $ZnSO_4 \cdot 1\,H_2O$, 1 mg; sodium dithionite, 50 mg.

Anaerobic conditions

Because of the oxygen sensitivity of the enzymes and the assay systems oxygen was excluded at all times. Anaerobic procedures were carried out as described (White et al. 1989).

Enzyme assays and analytical procedures

AOR activity was tested as aldehyde dehydrogenase activity with NH_2CO-$MV^{+\cdot}$ according to White et al. (1989) and as carboxylic acid reductase with $TMV^{+\cdot}$ according to White et al. (1991). Substrate concentrations were varied as indicated. Inactivation due to oxygen was determined by incubating 0.5 ml purified enzyme solution under an atmosphere of air with intermittent shaking at 4 °C. The pH optimum was determined by conducting the described assay in the various buffers and at the pH values indicated. Protein was determined using the modified method of Bradford (Read and Northcote 1981). Analyses of metal-and prosthetic group content were performed according to the following references: iron (Brumby and Massey 1967), acid-labile sulphur (Chen and Mortenson 1977), tungsten and molybdenum (Cardenas and Mortenson 1974), pterin (Forrest and Mitchell 1954; Johnson and Rajagopalan 1982) with the HPLC-analysis (White et al. 1991) and flavin (Spencer et al. 1976). In order to prepare pterin-6-carboxylic acid from the enzyme it was boiled only for 15 min with permanganate. The isoelectric point was determined with the PhastSystem according to the recommendation of the manufacturer.

Enzyme purification

The basic buffer containing 0.05 M Tris/HCl pH 7.0, about 5 µM NH_2CO-MV and 0.25 mM dithionite was used for the equilibration of all columns, unless stated otherwise. The crude extract was prepared as described (White et al. 1991).

Octyl-Sepharose CL-4B (Pharmacia, Freiburg, FRG). The column (2.5 × 17.5 cm) was equilibrated with 0.5 M ammonium sulphate in basic buffer before the crude extract was applied. The molybdate-containing AOR did not bind to the column and was contained in about 30 ml of the equilibration buffer. Residual protein, including the tungsten-containing AOR, was eluted with basic buffer.

Ammonium sulphate precipitation. To the enzyme containing fraction three volumes of a cold saturated ammonium sulphate solution in H_2O were added, the solution was slightly stirred for 10 min and then centrifuged 5 min at 42000 × g. The sediment was dissolved in 2 ml basic buffer and desalted by gel filtration with an Econopac DG 10 column (BioRad). After desalting the enzyme fraction was diluted with an equal volume of basic buffer. *FPLC Hema-IEC BIO 1000Q* (Alltech, Unterhaching, FRG). The desalted enzyme fraction after ammonium sulphate precipitation was applied to a Hema-IEC Bio 1000Q. Bound enzyme was eluted with a 0–0.5 M KCl gradient (30 ml) at 0.2 M KCl.

FPLC Mono Q (Pharmacia HR 5/5). The enzyme was bound to the ion exchange column and eluted with a linear KCl gradient from 0–0.5 M KCl (30 ml) at 0.30–0.34 M KCl.

FPLC Superformance hydroxylapatite 75–5 (Merck, Darmstadt, FRG). After the enzyme was bound to the column it was eluted with a linear gradient of 0–50 mM potassium phosphate buffer pH 7.0 (15 ml) at 12–25 mM potassium phosphate.

FPLC Superose 6 (Pharmacia). For the determination of molecular weight by gel filtration, pure enzyme was applied to a Superose 6 column equilibrated and eluted with basic buffer containing 0.1 M KCl. Molecular weight standards were ferritin (440000), catalase (240000), aldolase (158000), bovine serum albumin (67000) and cytochrome *c* (12500).

Polyacrylamide-gel electrophoresis was carried out as described (White et al. 1991).

Results

Purification of molybdenum-containing aldehyde oxidoreductase

As already mentioned complex medium to which no extra tungstate was added contained about 1.5×10^{-8} M tungsten (White and Simon 1992). The molybdenum-containing aldehyde oxidoreductase of *Clostridium formicoaceticum* was purified in four column-chromatographic steps and was homogeneous after only about 50-fold purification as judged by gel electrophoresis (Fig. 1, *lane A*). A summary of the purification procedure is given in Table 1. The octyl-Sepharose chromatography was needed to separate the two aldehyde oxidoreductases in *C. formicoaceticum*. When crude extracts of cells grown on complex medium which contained equal amounts of molybdate and tungstate, or even a 100-fold excess of molybdate, were applied to an octyl-Sepharose column all of the enzyme activity applied bound to the column (data not shown). When the cells were grown in complex medium without added tungstate and 1 mM molybdate i.e. molybdate was in about 7×10^4-fold excess over the tungstate present due to the yeast extract, about 50% of the activity was binding and behaved like the tungsten containing AOR when purified to homogeneity. Applying crude extracts of cells grown on defined synthetic medium to which also no tungstate was added, resulted in only 2% binding of AOR-activity.

The Mo-AOR eluted from the octyl-Sepharose column in a volume of about 30 ml. It was concentrated by ammonium-sulphate precipitation, desalted by gel filtration on a Econo-Pac DG 10 column and applied successively to two ion-exchange columns and a hydroxylapatite column for purification to homogeneity. The pure enzyme was in a volume of 2 ml with a protein concentration of 0.5 mg/ml. The specific activity of this enzyme, e.g. for butanal dehydrogenation was with 6.7 U/mg protein rather low, when compared with the tungsten-containing AOR from the same organism, which is about 8 times higher (~54 U/mg protein) (White et al. 1991) or the corresponding enzyme of *C. thermoaceticum*, which is about 2 orders of magnitude higher (~500 U/mg protein; Strobl et al. 1992) when purified. The ratio of aldehyde dehydrogenase and carboxylic acid reductase

Table 1. Purification of molybdenum-containing AOR from molybdate grown *Clostridium formicoaceticum*. The purification was from 9 g wet packed cells of *C. formicoaceticum* grown in the presence of 1 mM molybdate, without added tungstate. The enzyme activity was determined for the aldehyde dehydrogenase reaction, which is about 5–10 times as fast as the carboxylic acid reductase activity, when both reactions are tested as described

Step	Protein [mg]	Enzyme [U]	Specific activity [U/mg]	Yield [%][b]	Purification factor [n-fold][b]
Crude extract	830	251 (125)[a]	0.30 (0.15)[a]	100	1
Octyl-Sepharose not binding	216	109	0.50	87	3.3
$(NH_4)_2SO_4$ precipitation	132	78	0.59	63	3.9
Alltech-IE	21	47.5	2.26	38	15
MonoQ-IE	6.5	18	2.77	14	18.5
Hydroxylapatite	0.92	6.2	6.7	5	45

[a] Assuming that both AORs are present in an activity ratio of about 1 : 1 in the crude extract
[b] Calculation based on the Mo-AOR

specific activity throughout purification was about 5:1 (data not shown).

Physical properties and composition

PhastSystem native gradient gels showed after electrophoresis of the purified enzyme one band when silver stained (Fig. 1, *lane A*). According to the procedure described (White et al. 1991) the band also showed enzymic activity (not shown). The apparent molecular mass determined with this method was about 170 kDa for the native enzyme. Gel filtration chromatography resulted in an apparent molecular mass of 300 kDa for the same enzyme preparation. SDS gel electrosphoresis showed only one band at 100 kDa (Fig. 1, *lane B*). Since the molecular mass determination by gel filtration seems more reliable than by native gel eletrophoresis, the enzyme is most likely a trimer with identical subunits of 100 kDa.

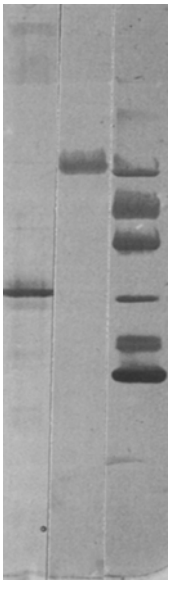

Fig. 1. PhastSystem gradient gels of purified molybdenum-containing aldehyde oxidoreductase from *Clostridium formicoaceticum*. *Lane A* silver stain of native gradient gel of purified AOR; *Lane B* silver stain of SDS gel of purified AOR: *Lane C* silver stain of M_r markers: 1) phosphorylase (97000), 2) bovine serum albumin (67000), 3) ovalbumin (43000), 4) carbonate dehydratase (30000), 5) soybean trypsin inhibitor (20100) and 6) α-lactalbumin (14400)

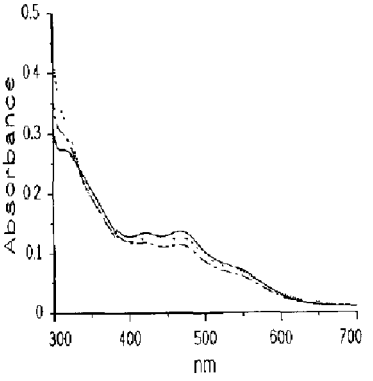

Fig. 2. UV/Vis spectrum of purified aldehyde oxidoreducatase. ····· 0.53 mg/ml aldehyde oxidoreductase as isolated; —·—·— reduced with 0.17 mM butanal, - - —— oxidised with 55 mM propionate

The absorbance spectrum of the purified enzyme is shown in Fig. 2. At $\lambda = 420$ nm the enzyme has $\varepsilon = 67\ mM^{-1}\ cm^{-1}$ after reduction with butyraldehyde and $\varepsilon = 80\ mM^{-1}\ cm^{-1}$ after oxidation with propionate, based on a molecular weight of 300 kDa. The enzyme seems to be partially oxidized after purification (Fig. 2).

The enzyme has an isoelectric point of 5.3, as determined by PhastSystem isoelectric focusing.

Metal-content and prosthetic group determinations showed, that the enzyme contained 7 iron, 6.6 acid-labile sulphur, 0.6 molybdenum and less than 0.05 tungsten atoms per 100 kDa subunit. Also, an average of 0.1 mol pterin-6-carboxylic acid per subunit could be calculated after permanganate oxidation of the enzyme after five independent determinations, of which one showed a content of 0.5 mol pterin-6-carboxylic acid per subunit. Less than 0.03 mol flavins per subunit were present.

A partial amino acid sequence showed for the first 13 positions: Met-Lys-Met-Leu-?-Lys-Lys(Gly)-Leu-Leu-Val-Asn-Gly-Ile.

Table 2. Relative activity for various substrates of the molybdenum-containing AOR from *Clostridium formicoaceticum*. Substrate concentrations were 30 mM for the acids if not indicated otherwise and 0.12 mM for the aldehydes

Substrate	Relative activity [%]	Substrate	Relative activity [%]
Formate	5	Formaldehyde	63
Acetate	67	Acetaldehyde	265
Propionate	100	Propanal	145
Butyrate	110	Butanal	100
		Butanal[b]	70
Cinnamate	200	Cinnamaldehyde[b]	101
Cinnamate[a]	144		
Crotonate	55	Crotonaldehyde	83
Benzoate	<2	Benzaldehyde[b]	100
Caprylate	23	Octanal[b]	75
D,L-Glycerate	<1	D,L-Glyceraldehyde	8
m-Fluorobenzoate	30	Decanal[b]	45
p-Fluorobenzoate[a]	<1	Citral[b]	40
m-Hydroxybenzoate	<2	Anisaldehyde[b]	78
p-Hydroxybenzoate	<1	Salicylaldehyde[b]	13
m-Methoxybenzoate	5	5-Norbornen-2-aldehyde[b]	<1
p-Methoxybenzoate	<1		
6-Chloronicotinate	65		
D,L-Lactate	<1		

[a] 20 mM substrate concentrations
[b] Solution contained 0.6% dimethylformamide

Catalytic properties

The molybdenum-containing AOR is, like the tungsten-containing AORs from *C. formicoaceticum* (White et al. 1991) and *C. thermoaceticum* (White et al. 1989; Strobl et al. 1992) an oxidoreductase in the strict sense, i.e. it not only oxidizes aldehydes, but also reduces carboxylic acids in aqueous medium.

While pyridine nucleotides do not function as cofactors (data not shown), viologens served as artificial electron mediators. The natural electron acceptor/donor for the enzyme is not known yet. For the reduction of carboxylic acids so far, only the very negative $TMV^{+\cdot}$ ($E_0' = -550$ mV) functioned as electron donor. With $MV^{+\cdot}$ ($E_0' = -440$ mV) no reaction could be observed. For the oxidation of aldehydes various viologens can accept the electrons from the enzyme. While NH_2CO-MV (100%) and MV (86%) worked almost equally well, TMV (48%) and BV (38%) showed less than half of the initial oxidation rate.

Alcohols, like propanol or butanol, were no substrates for the enzyme. Purified enzyme reduced carboxylic acids to the corresponding aldehydes only. In a carboxylic acid reductase assay, with propionic acid as the substrate, 120 µM $TMV^{+\cdot}$ was oxidized and 50 µM propanal and <2 µM propanol were detected by GLC. A list of substrates tested with the enzyme so far is presented in Table 2. The enzyme accepted a wide range of aldehydes readily, while the corresponding acids were not always reduced as well. This was especially striking with the pair benzoate/benzaldehyde. The aldehyde here was a very good substrate, the acid was not accepted at all. Acetaldehyde showed the highest dehydrogenation rate. Glyceraldehyde was oxidized only with a rate ~3% of that of acetaldehyde. Glycerate was not reduced.

The AOR showed as aldehyde dehydrogenase a K_m for butanal of 2.0 µM and up to 200 µM substrate concentration no inhibition was observed. As carboxylic acid reductase a K_m for propionic acid of 7.0 mM was determined. The pH optimum for carboxylic acid reduction was at pH 6.0. At pH 5.5 about 85% and at pH 6.8 about 70% of the maximum activity were detected. The aldehyde oxidation shows a very broad pH optimum. From pH 6 to pH 10 there was very little difference in the oxidation activity (Fig. 3). The buffer of the assay system seems to play an important role. Phoshates were inhibitory (Fig. 3).

Compared with the tungsten containing AOR from *C. formicoaceticum* the enzyme is only moderately oxygen sensitive. For the oxidation reaction 86% activity was still present after 1 h storage under air, which decreased further to 69% activity after 23 h storage. For the reduction reaction 55% activity was still present after 1 h, which decreased further to 30% activity left after 23 h storage. Strict anaerobic storage over the same period resulted in 90% activity left for both reactions.

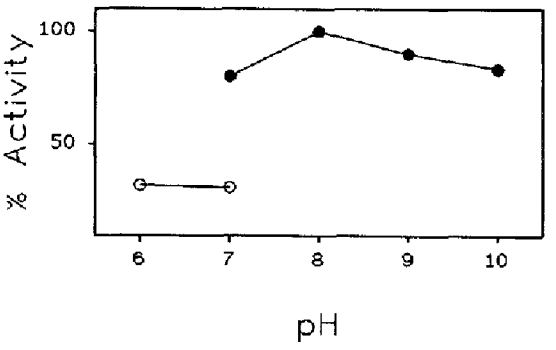

Fig. 3. pH Optimum of aldehyde dehydrogenase activity of purified molybdenum-containing aldehyde oxidoreductase from *Clostridium formicoaceticum*. The activity was determined with butanal and NH_2CO-MV in Tris-HCl (● ●) or phosphate (○—○) buffer

Discussion

Even though the reduction of carboxylic acids to the corresponding aldehydes in aqueous systems, without prior activation of the acids is surprising, enzymes catalyzing the reaction are present in diverse strictly anaerobic organisms (White and Simon 1992). The AORs purified so far from *Clostridium thermoaceticum* (White et al. 1989; Strobl et al. 1992), *C. formicoaceticum* (White et al. 1991) and *Pyrococcus furiosus* (Mukund and Adams 1991) were all tungsten-containing enzymes. Results obtained with crude extracts or cell suspension of various eubacteria seemed to indicate that for some organisms molybdenum also stimulated enzymic activity of the AOR, except for *C. thermoaceticum* where molybdenum clearly led to the formation of enzymically inactive AOR (White and Simon 1992).

This report describes for the first time a reversible AOR, which contains molybdenum and not tungsten. It is clearly different from the already described tungsten-containing AOR of *C. formicoaceticum* (White et al. 1991). It is formed when there is a great excess of molybdenum over tungsten ($7 \times 10^4 : 1$) in the growth medium. But even under these conditions an enzyme is still present in crude extracts of *C. formicoaceticum* which behaves as the already described tungsten containing enzyme. The fact that in synthetic medium to which no tungstate but molybdate was added only 2% of the octyl-Sepharose binding enzyme activity is formed could be explained by assuming that molybdenum is not incorporated into this protein. From this one must conclude that the presence of 1.5×10^{-8} M tungstate besides 1×10^{-3} M molybdate is sufficient to form the octyl-Sepharose binding W-containing AOR besides the Mo-AOR. This phenomenon will be studied separately. It could be an interesting example of a very effective differentiation of the chemically very similar anions MoO_4^{2-} and WO_4^{2-}. Since the specific activity of purified W-AOR is ten times as high as that of purified Mo-AOR the proteins based on weight are in the ratio of W-AOR : Mo-AOR of $\sim 1:10$ in the crude extract of cells grown on complex medium. For *Methanobacterium wolfei* there is a report of a new W-containing formylmethanofuran dehydrogenase. This organism also seems to contain a Mo-containing enzyme catalyzing the same reaction (Schmitz et al. 1992).

Differences in the catalytic properties of the two AORs of *C. formicoaceticum* were substrate and viologen specificity. While the W-AOR functioned with $MV^{+\cdot}$ as electron donor and benzoate was reduced fastest of the carboxylic acids tested so far (White et al. 1991) neither $MV^{+\cdot}$ was oxidized with propionate nor benzoate was reduced with $TMV^{+\cdot}$ by the Mo-AOR. Also the oxygen sensitivity is different. The W-AOR is much more oxygen labile. It loses in 5 min under air about 20% of its activity (White et al. 1991) and the Mo-AOR less than 4%. The two enzymes have different molecular weights for the native form as well as the subunits and most important while one contains tungsten, the other contains molybdenum. Also, they have a completely different amino acid sequence, when comparing the first 13 amino acids from the amino end (this report and White et al. 1991). This clearly shows that the two enzymes though contained in the same organism and catalyzing the same reactions are different proteins and no isoenzymes.

The physiological role of the AORs in clostridia is still unclear. Mukund and Adams (1991) proposed a new pyroglycolytic pathway of glucose fermentation for the archaeon *P. furiosus* as it has been shown for thermoacidophilic archaebacteria like *Sulfolobus solfatarius* and *Thermoplasma acidophilum* (Danson 1988). This pathway has been confirmed largely by Schäfer and Schönheit (1992). The role of the aldehyde ferredoxin oxidoreductase of *P. furiosus* in this pathway is the oxidation of glyceraldehyde to glycerate under evolution of hydrogen. This reaction is barely catalysed by the clostridial enzymes (White et al. 1991; Strobl et al. 1992). *C. formicoaceticum* ferments fructose via the Embden Meyerhof pathway (O'Brian and Ljungdahl 1972). Fermentation products are acetate and formate. Both acids are converted to the corresponding aldehydes by the here described enzyme (Table 2), which could then be further reduced to the alcohols by an alcohol dehydrogenase also present in *C. formicoaceticum*. Analysis of fermentation broths showed no methanol and only a trace of ethanol (< 5 mM) compared to about 50 mM acetate in the late stationary phase (data not shown). Judged by the purification factor necessary to obtain pure enzyme fractions about 3–4% of the total protein in tungstate-grown cells seems to be W-AOR (White et al. 1991) and about 2% of the protein in molybdate grown cells seems to be Mo-AOR. Proteins synthesised in such large amounts should play an important physiological role. It may be in the oxidation of acetaldehyde to acetate. This reaction has a redox potential $E'_0 = -581$ mV (Loach 1976). The arising electrons, which are negative enough to reduce carboxylates, could be used in the formation of acetyl-CoA by the carbon monoxide dehydrogenase for the initial reduction of CO_2 to CO. The standard redox potential of CO/CO_2 is $E'_0 = -560$ mV (Diekert et al. 1985). This would be an additional or alternative way for the CO formation, by the reduction of CO_2 with reduced ferredoxin formed by the oxidative decarboxylation of pyruvate (Fuchs 1986). The E'_0 of this ferredoxin is only about -360 mV (Elliott and Ljungdahl 1982). However, for the formation of acetaldehyde from pyruvate one would have to assume a pyruvate decarboxylase in addition to the pyruvate dehydrogenase.

Acknowledgements: We are especially grateful to Dr. W. Seilmeier for the determination of the partial amino acid sequence. We thank E. Krywald for skillful technical assistance. The work was financially supported by Deutsche Forschungsgemeinschaft (SFB 145), as well as Fonds der Chemischen Industrie.

References

Brumby PE, Massey V (1967) Determination of nonheme iron, total iron, and copper. Methods Enzymol 10: 463–474

Cardenas J, Mortenson LE (1974) Determination of molybdenum and tungsten in biological materials. Anal Biochem 60: 372–381

Chen JS, Mortenson LE (1977) Inhibition of methylene blue formation during determination of the acid-labile sulfide of

iron-sulfur protein samples containing dithionite. Anal Biochem 79: 157–165

Danson MJ (1988) Archaebacteria: the comparative enzymology of their central metabolic pathways. Adv Microb Physiol 29: 165–231

Diekert G, Fuchs G, Thauer RK (1985) Properties and function of carbon monoxide dehydrogenase from anaerobic bacteria. In: Poole RK, Dow CS (eds) Microbial gas metabolism. Academic Press, London Orlando San Diego, pp 115–130

Elliott JI, Ljungdahl LG (1982) Isolation and characterization of an Fe_8-S_8 ferredoxin (Ferredoxin II) from *Clostridium thermoaceticum*. J Bacteriol 151: 328–333

Forrest HS, Mitchell HK (1954) Pteridines from *Drosophila*. II. Structure of the yellow pigment. J Am Chem Soc 76: 5658–5662

Fuchs G (1986) CO_2 fixation in acetogenic bacteria: variations on a theme. FEMS Microbiol Rev 39: 181–213

Goedde HW, Langenbeck U (1984) Glycolaldehyde. In: Bergmeyer HU (ed) Methods of enzymatic analysis, vol 6, 3rd edn. Verlag Chemie, Weinheim, pp 614–618

Günther H, Neumann S, Simon H (1987) 2-Oxocarboxylate reductase from *Proteus* species and its use for the preparation of (2R)-hydroxy acids. J Biotechnol 5: 53-65

Heuser J, Stoehr C (1890) Ueber methylirte Dipyridyle. J Prakt Chem 42 (NF): 429–436

Johnson JL, Rajagopalan KV (1982) Structural and metabolic relationship between the molybdenum cofactor and urothione. Proc Natl Acad Sci USA 79: 6856–6860

Lal AB, Petrow V (1949) Cyanine dyes from 2,2'dimethyl-4,4'-dipyridyl. J Chem Soc [Suppl] 1: 115–120

Leonhardt U, Andreesen JR (1977) Some properties of formate dehydrogenase, accumulation and incorporation of ^{185}W-tungsten into proteins of *Clostridium formicoaceticum*. Arch Microbiol 115: 277–284

Loach PA (1976) Oxidation-Reduction potentials, absorbance bands and molar absorbance of compounds used in biochemical studies. In: Fasman GD (ed) Handbook of biochemistry and molecular biology, 3rd edn. Physical and chemical data, vol. 1. CRC Press, Cleveland, Ohio, pp 122–130

Mukund S, Adams MWW (1991) The novel tungsten-iron-sulfur protein of the hyperthermophilic archaebacterium, *Pyrococcus furiosus*, is an aldehyde ferredoxin oxidoreductase. J Biol Chem 266: 14208–14216

O'Brien WE, Ljungdahl LG (1972) Fermentation of fructose and synthesis of acetate from carbon dioxide by *Clostridium formicoaceticum*. J Bacteriol 109: 626–632

Read SM, Northcote DH (1981) Minimization of variation in the response to different proteins of the Coomassie blue G dye-binding assay for protein. Anal Biochem 116: 53–64

Schäfer T, Schönheit P (1992) Maltose fermentation to acetate, CO_2 and H_2 in the anaerobic hyperthermophilic archaeon *Pyrococcus furiosus*: evidence for the operation of a novel sugar fermentation pathway. Arch Microbiol 158: 188–202

Schmitz RA, Richter M, Linder D, Thauer RK (1992) A tungsten-containing active formylmethanofuran dehydrogenase in the thermophilic archaeon *Methanobacterium wolfei*. Eur J Biochem 207: 559–565

Spencer R, Fisher J, Walsh C (1976) Preparation, characterization, and chemical properties of the flavin coenzyme analogues 5-deazariboflavin, 5-deazariboflavin 5'-phosphate, and 5-deazariboflavin 5'-diphosphate, 5' → 5'-adenosine ester. Biochemistry 15: 1043–1053

Strobl G, Feicht R, White H, Lottspeich F, Simon H (1992) The tungsten-containing aldehyde oxidoreductase from *Clostridium thermoaceticum* and its complex with a viologen-accepting NADPH oxidoreductase. Biol Chem Hoppe Seyler 373: 123–132

Thanos I, Bader J, Günther H, Neumann S, Krauss F, Simon H (1987) Electroenzymatic and electromicrobial reduction: preparation of chiral compounds. Methods Enzymol 136: 302–317

White H, Simon H (1992) The role of tungstate and/or molybdate in the formation of aldehyde oxidoreductase in *Clostridium thermoaceticum* and other acetogens; immunological distances of such enzymes. Arch Microbiol 158: 81–84

White H, Strobl G, Feicht R, Simon H (1989) Carboxylic acid reductase, a new tungsten enzyme catalyses the reduction of non-activated carboxylic acids to aldehydes. Eur J Biochem 184: 89–96

White H, Feicht R, Huber C, Lottspeich F, Simon H (1991), Purification and some properties of the tungsten-containing carboxylic acid reductase from *Clostridium formicoaceticum*. Biol Chem Hoppe Seyler 372: 999–1005