

Ferredoxin-dependent methane formation from acetate in cell extracts of *Methanosarcina barkeri* (strain MS)

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Received 3 July 1990

Cell extracts of *Methanosarcina barkeri* grown on acetate catalyzed the conversion of acetyl-CoA to CO₂ and CH₄ at a specific rate of 50 nmol·min⁻¹·mg⁻¹. When ferredoxin was removed from the extracts by DEAE-Sephacel anion exchange chromatography, the extracts were inactive but full activity was restored upon addition of purified ferredoxin from *M. barkeri* or from *Clostridium pasteurianum*. The apparent K_m for ferredoxin from *M. barkeri* was determined to be 2.5 μM. A ferredoxin dependence was also found for the formation of CO₂, H₂, and methyl-coenzyme M from acetyl-CoA, when methane formation was inhibited by bromoethanesulfonate. Reduction of methyl-coenzyme M with H₂ did not require ferredoxin. These and other data indicate that ferredoxin is involved as electron carrier in methanogenesis from acetate. Methanogenesis from acetyl-CoA in cell extracts was not dependent on the membrane fraction, which contains the cytochromes.

Methanogenesis from acetate; Acetyl-CoA; Ferredoxin; Coenzyme M; Carbon monoxide dehydrogenase; *Methanosarcina barkeri*

1. INTRODUCTION

M. barkeri is an anaerobic archaeobacterium that ferments acetate to CH₄ and CO₂ ($\Delta G^{\circ} = -36$ kJ/mol) [1]. The methyl-group of acetate is converted to CH₄ and the carboxyl-group to CO₂. Acetyl phosphate, acetyl-CoA, methyl-tetrahydromethanopterin (CH₃-H₄MPT), methyl-coenzyme M (CH₃-S-CoM), and a bound carbon monoxide ([CO]) have been identified as intermediates [2–6] (Fig. 1).

Methane formation from the methyl-group of acetyl-CoA is coupled to the oxidation of the carbonyl-group of acetyl-CoA via an electron transport chain (Fig. 1). During electron transport energy is conserved via a chemiosmotic mechanism [7,8]. 7-Mercaptoheptanoyl-threonine phosphate (H-S-HTP) has been identified as one of the electron carriers. H-S-HTP is the immediate electron donor for the reduction of CH₃-S-CoM to CH₄ yielding the heterodisulfide of H-S-CoM and H-S-HTP (CoM-S-S-HTP) as oxidation product [9]. Two other electron carriers believed to participate are cytochromes *b* and *c* [10,11] and ferredoxin [12–14].

The involvement of cytochromes in the electron transport chain is mainly indicated by the finding that only methanogens that can metabolize acetate contain these heme-proteins [11]. The reduction of the cytochromes by CO and their reoxidation by CH₃-S-CoM in cell extracts of *M. barkeri* have been demonstrated [15].

The involvement of ferredoxin in the electron transport chain is suggested by the finding that all methanogens that can metabolize acetate also contain this iron-sulfur protein.

Ferredoxin has been purified from *M. barkeri* (strain MS) [13], from *M. barkeri* (strain Fusaro) [12] and from *M. thermophila* [14]. Ferredoxin of *M. barkeri* (strain MS) is an iron-sulfur protein composed of 59 amino acids with a calculated molecular mass of 6 kDa of which the sequence has been determined [16]. It shows 41% sequence homology with ferredoxin of *C. pasteurianum* which is a 2 [4Fe-4S] protein. Eight cysteines are involved in cluster formation which are completely conserved in ferredoxin of *M. barkeri*. Despite this fact ferredoxin of *M. barkeri* (strain MS) was found to contain only a [3Fe-3S] cluster [13].

Ferredoxin of *M. barkeri* (strain Fusaro) was calculated to have a molecular mass of 6.1 kDa on the base of the amino acid composition. Indirect evidence is available that this protein contains 2 [4Fe-4S] clusters [12].

Ferredoxin of *M. thermophila* is smaller than the ferredoxin of *M. barkeri*. The molecular mass calculated from the amino acid composition is 4.8 kDa. It was found to contain only 5 cysteines [14]. Terlesky et al. [17] demonstrated that carbon monoxide dehydrogenase of *M. thermophila* uses ferredoxin as electron acceptor.

In this communication evidence is presented that ferredoxin is involved in the electron transport chain connecting the oxidation of the carbonyl-group of acetyl-CoA to CO₂ with the reduction of CH₃-S-CoM to CH₄ in cell extracts of *M. barkeri* (strain MS).

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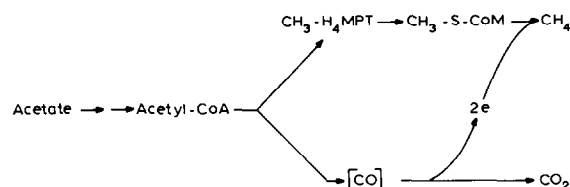


Fig. 1. Pathway of acetate conversion to CH₄ and CO₂ in *M. barkeri*. The oxidation of the carbonyl-group of acetyl-CoA to CO₂ is coupled to the reduction of the methyl-group to CH₄ via an electron transport chain associated with energy conservation. (CH₃-H₄MPT, methyl-tetrahydromethanopterin; CH₃-S-CoM, methyl-coenzyme M; [CO], bound CO).

2. MATERIALS AND METHODS

DEAE-Sephacel (fast flow), Mono Q (HR 10/10) and Superose 12 were from Pharmacia (Freiburg, FRG). Coenzyme M (H-S-CoM = 2-mercaptoethanesulfonate) and metronidazole were from Sigma (Deisenhofen, FRG) and bromoethanesulfonate was from Fluka (Buchs, Switzerland). 5,5'-Dithiobis (2-nitrobenzoid acid) (Ellman's reagent) was from Serva (Heidelberg, FRG). Methyl-coenzyme M, 7-mercaptoheptanoylthreonine phosphate (H-S-HTP) and the heterodisulfide (CoM-S-S-HTP) were synthesized as described [9]. Tetrahydromethanopterin (H₄MPT) was isolated from *Methanobacterium thermoautotrophicum* [18].

M. barkeri (DSM 800) was from the Deutsche Sammlung von Mikroorganismen (Braunschweig, FRG). The organism was grown on acetate at 37°C in a 42 liter fermenter (Braun, Melsungen, FRG) containing 30 liter medium [19]. The cells were harvested anaerobically with a continuous flow centrifuge (Heraeus Sepatech Biofuge 17 RS, Osterode, FRG) and cell extracts (100 000 × g supernatant) were prepared under strictly anaerobic conditions as described [4,5].

2.1. Preparation of ferredoxin-free extracts and of ferredoxin

Cell extract (2 ml; 80 mg protein) was loaded on a DEAE-Sephacel column (1 × 8 cm) previously equilibrated with 50 mM MOPS/KOH buffer pH 7 (10 mM MgCl₂; 0.5 mM dithiothreitol). Enzymes were eluted with the same buffer containing 300 mM NaCl. The eluate is designated as ferredoxin-free extract. Ferredoxin was eluted with the same buffer containing 500 mM NaCl. Ferredoxin fractions from about 10 ml cell extract were pooled and further purified by chromatography on Mono Q and Superose 12 [14]. Purified ferredoxin showed a relative molecular mass of 24 kDa determined by gel-filtration on Superose 12. The UV/Visible spectrum at pH 7 of oxidized ferredoxin exhibited maxima at 280 nm and at 390 nm with a shoulder at 312 nm. The ratio of ΔA₃₉₀ to ΔA₂₈₀ was 0.76. For the calculation of the ferredoxin concentration a molar extinction coefficient at 390 nm of 12,800 M⁻¹·cm⁻¹ was used [14]. Ferredoxin from *C. pasteurianum* was purified as described (ε₃₉₀ = 30000 M⁻¹·cm⁻¹) [20].

Protein was determined by the method of Bradford [21] using the Bio-Rad microassay [22] with ovalbumin as standard.

2.2. Assay conditions for ferredoxin-dependent reactions

All assays were performed in sealed 8 ml serum vials. The reactions were started by addition of cell extract and by transfer from 4°C to 37°C. Incubation was in a shaking water bath at 280 rpm. CH₄, CO₂, and H₂ were quantified after separation by gas chromatography [23].

2.2.1. CH₄- and CO₂-formation from acetyl-CoA

The 0.2 ml assay mixtures contained: 50 mM MOPS/KOH buffer pH 7; 10 mM MgCl₂; 30 mM acetyl phosphate; 1 mM coenzyme A; 1 mM coenzyme M; 0.1 mM H₄MPT; 5 mM ATP; 0.3 mM HTP-S-S-HTP (the homodisulfide of H-S-HTP); 0.5 mM dithiothreitol; 0.5–2 mg ferredoxin-free extra protein; and ferredoxin as indicated. The gas phase was H₂ at 2 · 10⁵ Pa.

2.2.2. CO₂- and H₂-formation from acetyl-CoA

The 0.2 ml assay mixtures contained: 50 mM MOPS/KOH buffer pH 7; 10 mM MgCl₂; 30 mM acetyl phosphate; 1 mM coenzyme A; 10 mM coenzyme M; 0.1 mM H₄MPT; 1 mM bromoethanesulfonate; 0.5 mM dithiothreitol; 1–2 mg ferredoxin-free extract protein; and ferredoxin as indicated. The gas phase was H₂ at 2 · 10⁵ Pa.

2.2.3. CO₂- and H₂-formation from CO

The 0.2 ml assay mixtures contained: 50 mM MOPS/KOH buffer pH 7; 10 mM MgCl₂; 1 mM bromoethanesulfonate; 0.5 mM dithiothreitol; 0.5–1 mg ferredoxin-free extract protein; and ferredoxin as indicated. The gas phase was N₂ with 5% CO at 2 · 10⁵ Pa or concentrations indicated in Fig. 3.

2.2.4. CH₄ from CH₃-S-CoM and H₂

The 0.2 ml assay mixtures contained: 50 mM MOPS/KOH buffer pH 7; 10 mM MgCl₂; 10 mM CH₃-S-CoM; 0.3 mM HTP-S-S-HTP; 5 mM ATP; 0.5 mM dithiothreitol; and 0.3 mg ferredoxin-free extract; and ferredoxin as indicated. The gas phase was H₂ at 2 · 10⁵ Pa.

2.2.5. CoM-S-S-HTP reduction by CO or by H₂

The 0.2 ml assay mixtures contained: 50 mM MOPS/KOH buffer pH 7; 10 mM MgCl₂; 5 mM CoM-S-S-HTP; 1 mM bromoethanesulfonate; 0.01–0.015 ml ferredoxin of *M. barkeri* or of *C. pasteurianum*; and 0.2–0.5 mg ferredoxin-free extract protein. The gas phase was CO or H₂ at 1.5 · 10⁵ Pa. The reaction was followed by analyzing the formation of free SH-groups (H-S-HTP and H-S-CoM) from CoM-S-S-HTP with Ellman's reagent.

Samples (0.025 ml) were withdrawn in 5 min intervals with a syringe and transferred into 0.05 ml 1 M trichloroacetic acid (4°C). After 30 min the precipitated protein was removed by centrifugation. A sample of 0.05 ml of the supernatant was neutralized with 0.05 ml NaOH (0.66 M) and then 0.1 ml Tris/HCl (1 M; pH 8.1), 0.1 ml Ellman's reagent (1 mM; solution in 50 mM sodium acetate, pH 5), and 0.7 ml H₂O were added successively. The absorbance difference against water was measured at 412 nm and corrected for the absorbance difference obtained for the samples withdrawn at *t* = 0. The method was calibrated with coenzyme M and coenzyme A as standards.

2.2.6. Metronidazole-reduction by CO

The assays were performed in sealed 1 ml-cuvettes. The 0.8 ml assay mixtures contained: 50 mM MOPS/KOH buffer pH 7; 10 mM MgCl₂; 0.005 mM ferredoxin; 0.1 mM metronidazole; and 0.02 mg ferredoxin-free extract protein. The gas phase was CO at 1.5 · 10⁵ Pa. The reaction was started by the addition of extract protein. The reduction of metronidazole by reduced ferredoxin, which is a spontaneous reaction, was followed at 320 nm (ε₃₂₀ = 9300 M⁻¹·cm⁻¹).

3. RESULTS AND DISCUSSION

3.1. Ferredoxin-dependent CH₄- and CO₂-formation from acetyl-CoA

Cell extracts (100 000 × g supernatant) of *M. barkeri* catalyzed the formation of CH₄ and CO₂ from acetyl-CoA with specific activities of 40–50 nmol·min⁻¹·mg⁻¹. When the extracts were deprived from ferredoxin by adsorption of the iron-sulfur protein to DEAE-Sephacel the activity was less than 1 nmol·min⁻¹·mg⁻¹. Upon re-addition of ferredoxin, full activity was restored. The apparent *K_m* for ferredoxin was determined to be 2.5 μM (Fig. 2, Table I). Ferredoxin isolated from *C. pasteurianum* could substitute for ferredoxin from *M. barkeri* (app. *K_m* = 0.5 μM). Viologen dyes could not replace ferredoxin; they even proved to be in-

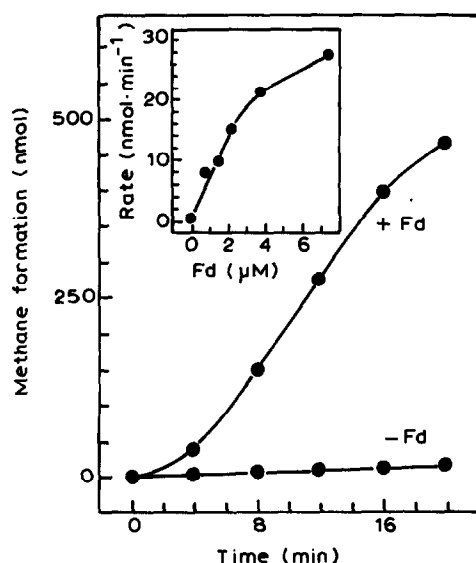
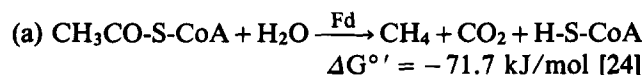
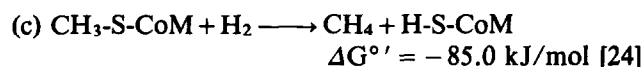
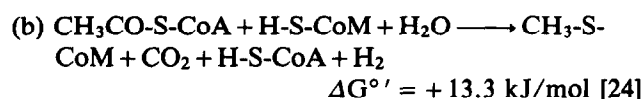


Fig. 2. Methane formation from acetyl-CoA catalyzed by ferredoxin-free extracts of *M. barkeri*. Time course in the absence and presence of ferredoxin (Fd) from *M. barkeri*. The inset shows the rate dependence on the ferredoxin concentration. The assays contained 0.58 mg protein of ferredoxin-free extract and 7.5 μ M ferredoxin or the ferredoxin concentrations indicated. For assay conditions see Materials and Methods.

hibitory. Also, Ti(III)citrate (25 mM) [6] could not substitute for ferredoxin. The results indicate that methanogenesis from acetyl-CoA in cell extract is dependent on ferredoxin.



Methanogenesis from acetyl-CoA in cell extracts of *M. barkeri* was dependent on the presence of H_2 [2]. An explanation for the H_2 requirement was recently provided by the finding that in cell extracts the reaction is composed of two partial reactions which are electrically coupled via molecular hydrogen [6].



We therefore determined which of the two partial reactions was dependent on ferredoxin.

3.2. Ferredoxin-dependent CO_2 - and H_2 -formation from acetyl-CoA under conditions of inhibited CH_4 -formation

CH_4 -formation from acetyl-CoA was inhibited by bromoethanesulfonate, which inhibits $\text{CH}_3\text{-S-CoM}$ reduction to CH_4 [1]. Under these conditions CO_2 and H_2 were formed in stoichiometric amounts and $\text{CH}_3\text{-S-CoM}$ accumulated [6,25]. The specific activity in cell extracts of *M. barkeri* was 20–30 $\text{nmol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$. In ferredoxin-free extracts the activity was less than 1 $\text{nmol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ but full activity could be restored upon addition of ferredoxin (Table I). Methylviologen (0.05 mM) rather than Ti(III) citrate (25 mM) [6] could substitute for ferredoxin. These results indicate that ferredoxin is required for CO_2 - and H_2 -production in partial reaction b.

Indirect evidence is available that partial reaction b is catalyzed by carbon monoxide dehydrogenase [26–28] which also is involved in the conversion of CO plus H_2O to CO_2 plus H_2 . Cell extracts catalyzed the latter reaction at a specific rate of 100–150 $\text{nmol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$. Ferredoxin-free extracts were only active after supplementation with the iron-sulfur-protein (Table I). For ferredoxin of *M. barkeri* and ferredoxin of *C. pasteurianum* apparent K_m values of 10 μ M and 2.5 μ M, respectively, were determined. Thus H_2 - and CO_2 -formation from CO plus H_2O showed the same ferredoxin-dependence as CO_2 - and H_2 -formation from acetyl-CoA.

Cell extracts of *M. barkeri* catalyzed the reduction of ferredoxin by CO at a specific rate of 1–2 $\mu\text{mol} \cdot$

Table I
Ferredoxin-dependent and ferredoxin-independent reactions catalyzed by cell extracts of *M. barkeri*

Reaction	Ferredoxin dependent	app. K_m (μ M)	Specific rate ($\text{nmol/min} \cdot \text{mg}$)
CH_4 and CO_2 from acetyl-CoA	+	2.5	40–50
CO_2 and H_2 from acetyl-CoA	+	2.5	20–30
CO_2 and H_2 from CO	+	10.0	100–150
CH_4 from $\text{CH}_3\text{-S-CoM}$ and H_2	–	–	100–120
CoM-S-S-HTP reduction by H_2	–	–	50–80
CoM-S-S-HTP reduction by CO	+	n.d.	200–300
Metronidazole reduction by CO	+	n.d.	1000–2000

The experiments were performed with ferredoxin-free extracts supplemented with ferredoxin of *M. barkeri*. For assay conditions see Materials and Methods
n.d. = not determined

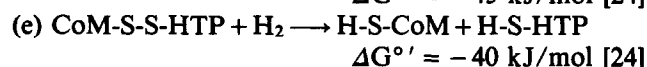
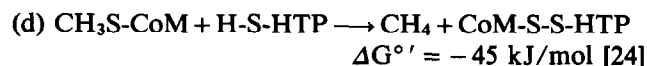
$\text{min}^{-1} \cdot \text{mg}^{-1}$ as deduced from the rate of ferredoxin-dependent metronidazole reduction by CO. Purified carbon monoxide dehydrogenase from *M. barkeri* catalyzed the reaction at a specific rate of 40–60 $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$ (unpublished results). These and the above findings suggest that ferredoxin is the direct electron acceptor in the oxidation of the carbonyl group of acetyl-CoA to CO_2 .

The rate of H_2 - and CO_2 -formation from CO was highest at a CO concentration in the gas phase of 5% (Fig. 3). Higher CO concentrations inhibited the reaction probably by negatively affecting the hydrogenase activity, which is known to be inhibited by CO [29].

3.3. Ferredoxin-independent reduction of $\text{CH}_3\text{-S-CoM}$ to CH_4 by H_2

Ferredoxin-free cell extract was found to catalyze the reduction of $\text{CH}_3\text{-S-CoM}$ to CH_4 with H_2 at a specific rate of 80–100 $\text{nmol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$. Upon addition of ferredoxin the specific rate increased by only 10–20%. Ferredoxin could be substituted by Ti(III)citrate (5 mM) [6] in this stimulatory effect. These findings indicate that ferredoxin does not directly participate in partial reaction c.

It has recently been shown that the reduction of $\text{CH}_3\text{-S-CoM}$ with H_2 (reaction c) proceeds in two steps: the reduction of $\text{CH}_3\text{-S-CoM}$ with 7-mercaptoheptanoylthreonine phosphate (H-S-HTP) to methane and CoM-S-S-HTP [9] (reaction d); and the reduction of the heterodisulfide with H_2 [30] (reaction e).



Both reactions were catalyzed by ferredoxin-free extracts, the activities (approximately 100 $\text{nmol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$) being only slightly stimulated by the addition of ferredoxin as to be expected from the finding that $\text{CH}_3\text{-S-CoM}$ reduction with H_2 was ferredoxin-independent.

When CO rather than H_2 was used as electron donor, the reduction of CoM-S-S-HTP required ferredoxin (Table I) in agreement with the property of carbon monoxide dehydrogenase to use ferredoxin as electron acceptor (see above). CoM-S-S-HTP reduction by CO proceeded at a specific rate of 200–300 $\text{nmol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$. The dependence of the rate on the CO concentration is given in Fig. 3.

3.4. On the role of cytochromes

All the experiments described above were performed in cell extracts which had been centrifuged for one hour at 100 000 $\times g$ to remove the membrane fraction, in which the cytochromes are located [10,11,15]. The membrane fraction was not required for activity: With

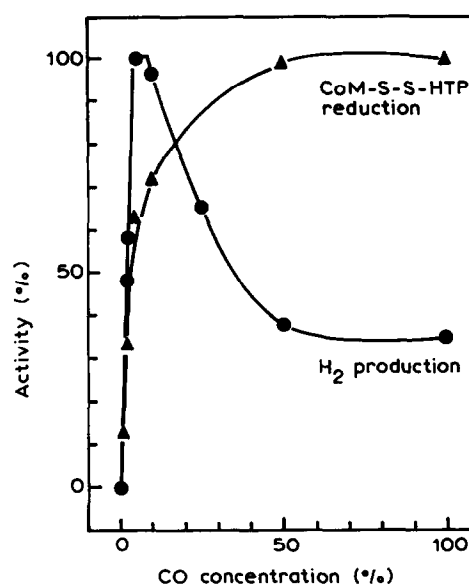


Fig. 3. H_2 production from CO and CoM-S-S-HTP reduction by CO in cell extracts of *M. barkeri*. The assays contained 15 μM ferredoxin of *C. pasteurianum*. 100% activity was 90 $\text{nmol} \cdot \text{min}^{-1}$ (0.8 mg cell extract protein) for H_2 production from CO at 5% CO in the gas phase and 110 $\text{nmol} \cdot \text{min}^{-1}$ (0.4 mg cell extract protein) for CoM-S-S-HTP reduction by CO at 100% CO in the gas phase.

cell extract centrifuged at 27 000 $\times g$ essentially the same results as with the 100 000 $\times g$ supernatant were obtained. Also the activities were not enhanced when the membrane fraction was re-added to the 100 000 $\times g$ supernatant. These findings indicate that probably cytochromes were not required for methane formation from acetate in cell extracts of *M. barkeri*.

Methane formation from acetate in intact cells is coupled with energy conservation [8], is sodium ion dependent [31], is inhibited by sodium ionophores [8], and is inhibited by H_2 [6,27]. The in vivo reaction thus differs considerably from the in vitro reaction which is not coupled with energy conservation, which is not affected by sodium ionophores [5], and which requires H_2 to proceed [2,6]. The finding that cytochromes are not involved in methanogenesis from acetyl-CoA in cell extracts thus does not exclude a function of the heme-iron proteins in methanogenesis from acetate in intact cells.

Acknowledgements: This work was supported by the Deutsche Forschungsgemeinschaft and by the Fonds der Chemischen Industrie. We thank S. Scholz, G. Börner and T. Rösser for the preparation and synthesis of coenzymes and J. Kunow for culturing and harvesting cells of *M. barkeri*.

REFERENCES

- [1] Rouvière, P.E. and Wolfe, R.S. (1988) J. Biol. Chem. 263, 7913–7916.
- [2] Krzycki, J.A. and Zeikus, J.G. (1984) FEMS Microbiol. Letters 25, 27–32.

- [3] Nelson, M.J.K. and Ferry, J.G. (1984) *J. Bacteriol.* 160, 526-532.
- [4] Fischer, R. and Thauer, R.K. (1988) *FEBS Letters* 228, 249-253.
- [5] Fischer, R. and Thauer, R.K. (1989) *Arch. Microbiol.* 151, 459-465.
- [6] Fischer, R. and Thauer, R.K. (1990) *Arch. Microbiol.* 153, 156-162.
- [7] Blaut, M. and Gottschalk, G. (1985) *Trends in Biochem. Sci.* 10, 486-489.
- [8] Peinemann, S., Müller, V., Blaut, M. and Gottschalk, G. (1988) *J. Bacteriol.* 170, 1369-1372.
- [9] Ellermann, J., Hedderich, R., Böcher, R. and Thauer, R.K. (1988) *Eur. J. Biochem.* 172, 669-677.
- [10] Kühn, W. and Gottschalk, G. (1983) *Eur. J. Biochem.* 135, 89-94.
- [11] Kühn, W., Fiebig, K., Hippe, H., Mah, R.A., Huser, B.A. and Gottschalk, G. (1983) *FEMS Microbiol. Lett.* 20, 407-410.
- [12] Hatchikian, E.C., Bruschi, M., Forget, N. and Scandellari, M. (1982) *Biochem. Biophys. Res. Commun.* 109, 1316-1323.
- [13] Moura, I., Moura, J.J.G., Huynh, B., Santos, H., LeGall, J. and Xavier, V. (1982) *Eur. J. Biochem.* 126, 95-98.
- [14] Terlesky, K.C. and Ferry, J.G. (1988) *J. Biol. Chem.* 263, 4080-4082.
- [15] Kemner, J.M., Krzycki, J.A., Prince, R.C. and Zeikus, J.G. (1987) *FEMS Microbiol. Lett.* 48, 267-272.
- [16] Hausinger, R.P., Moura, I., Moura, J.J.G., Xavier, A.V., Santos, M.H., LeGall, J. and Howard, J.B. (1982) *J. Biol. Chem.* 257, 14192-14197.
- [17] Terlesky, K.C., Ferry, J.G. (1988) *J. Biol. Chem.* 263, 4075-4079.
- [18] Börner, G. (1988) Diploma thesis, Marburg.
- [19] Karrasch, M., Bott, M. and Thauer, R.K. (1989) *Arch. Microbiol.* 151, 137-142.
- [20] Schönheit, P., Wäschler, C. and Thauer, R.K. (1978) *FEBS Lett.* 89, 219-222.
- [21] Bradford, M.M. (1976) *Anal. Biochem.* 76, 248-254.
- [22] Bio-Rad Laboratories (1981) Instruction manual.
- [23] Schönheit, P., Moll, J. and Thauer, R.K. (1980) *Arch. Microbiol.* 127, 59-65.
- [24] Thauer, R.K. (1990) *Biochim. Biophys. Acta*, in press.
- [25] Grahame, D.A. and Stadtman, T.C. (1987) *Biochem. Biophys. Res. Commun.* 147, 254-258.
- [26] Krzycki, J.A., Lehman, L.J. and Zeikus, J.G. (1985) *J. Bacteriol.* 163, 1000-1006.
- [27] Eikmanns, B. and Thauer, R.K. (1984) *Arch. Microbiol.* 138, 365-370.
- [28] Terlesky, K.C., Barber, M.J., Aceti, D.J. and Ferry, J.G. (1987) *J. Biol. Chem.* 262, 15392-15395.
- [29] Adams, M.W.W., Mortenson, L.E. and Chen, J.S. (1981) *Biochim. Biophys. Acta* 594, 105-176.
- [30] Hedderich, R. and Thauer, R.K. (1988) *FEBS Lett.* 234, 223-227.
- [31] Perski, H.J., Schönheit, P. and Thauer, R.K. (1982) *FEBS Lett.* 143, 323-326.