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Sodium dependence of methane formation in methanogenic bacteria

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1. INTRODUCTION

Methanogenic bacteria are strictly anaerobic Archaebacteria [1]. They generate methane from CO_2, formate, methanol, methyl amines or acetate, and couple these exergonic processes to the synthesis of ATP. The mechanism of coupling is still not understood.Achemiosmoticcouplingmechanismhas been indicated [2–10]. However, there is ambiguous evidence that methanogenesis is directly linked with the generation of a proton-motive force.

Growth and methane formation from CO_2 in *Methanobacterium thermoautotrophicum* is dependent on the presence of low concentrations of extracellular sodium [11]. Here, we show that dependence of methanogenesis on sodium appears to be a general phenomenon. Methane formation from CO_2 and acetate in all methanogens tested required sodium. The Na^+ ionophore monensin completely inhibited methane formation when the extracellular sodium concentration and pH were low. The findings indicate that Na^+ plays an important role in the energy metabolism of these bacteria. Possible functions of sodium are discussed.

2. MATERIALS AND METHODS

Methanobacterium thermoautotrophicum strain Marburg (DSM 2133, Deutsche Sammlung für Mikroorganismen, Göttingen) and strain ΔH DSM 1053), *Methanobacterium bryantii* (DSM 863), *Methanobrevibacter* strain AZ (DSM 744), *Methanospirillum hungatii* (DSM 864) and *Methanosarcina barkeri* strain Fusaro (DSM 804) were grown on H_2 and CO_2 [1,12–14]. *M. barkeri* was also grown on methanol or acetate [15]. The cells were harvested and suspensions prepared as in [11]. Monensin was obtained from E. Lilly Research Centre (Windlesham).

Methane formation from CO_2 and H_2 by the cell suspensions was assayed in 120 ml serum bottles closed with a rubber stopper and filled with 5 ml reaction mixture and 115 ml 80% H_2/20% CO_2 gas pressurized to 2 atm. (80% N_2/20% CO_2 where indicated). The reaction mixture contained per ml: 3 μmol K_2HPO_4, 2 μmol KH_2PO_4, 2 μmol $MgSO_4$, 5 μmol dithiothreitol; 0.2–10 μmol NaCl, 10 μmol CH_3OH (where indicated), 0.2 μmol acetate (where indicated), and 0.2 mg bacteria (dry wt). The final pH of the reaction mixture was near 6.0. The reaction was started by injection of the bacteria. The reaction was at 35°C except for *M. thermoautotrophicum*, where it was at 60°C. The gas and liquid phases were rapidly mixed by continuously shaking on a gyratory shaker (200 rev./min). Gas samples were withdrawn with a gas-tight syringe at the times indicated in fig.1 and analyzed for CH_4 by gas chromatography [11].

3. RESULTS

Methane formation from CO_2, methanol or acetate in cell suspensions of the 5 organisms investigated was found to be dependent on the presence of low concentrations of sodium in the incubation medium. KCl could not substitute for NaCl, indicating that methanogenesis was dependent on sodium rather than on chloride. The results of a characteristic experiment is given in fig.1. Under the experimental conditions the methane formation rate was constant over >60 min. The rate increased with increasing sodium concentrations to a max-

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Table 1

Sodium dependence of methane formation in cell suspensions of methanogenic bacteria at pH 6 (for assay conditions see section 2)

Organism	CH_4 formation from	app. K_s-value for sodium (mM)	V_{max} (μmol CH_4/ min mg bacteria)	μ[a] (h^{-1})
Methanobacterium				
thermoautotrophicum strain Marburg	CO_2	1.1	3.2	0.43
strain ΔH	CO_2	1.0	1.0	0.30
Methanobacterium bryantii	CO_2	0.6	0.3	0.04
Methanobrevibacter				
arboriphilus	CO_2	0.4	0.6	0.139
Methanospirillum hungatii	CO_2[b]	0.5	0.26	0.03
Methanosarcina barkeri	CO_2	1.1	0.2	0.063
	CH_3OH[c]	1.1	0,25	0.073
	CH_3COOH[c]	1.0	0.03	0.023

[a] μ is the specific growth rate of the organisms
[b] 25 mM K_2HPO_4/KH_2PO_4 rather than 5 mM K_2HPO_4/KH_2PO_4
[c] The experiment was performed under 80% N_2/20% CO_2 as gas phase

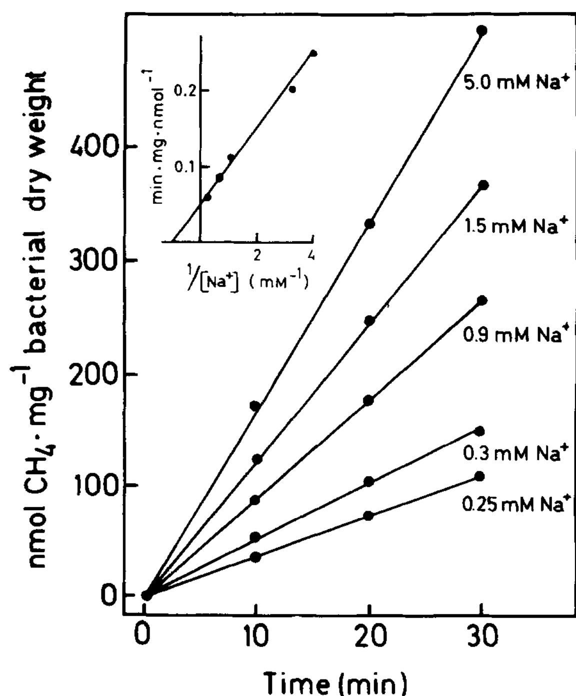

Fig.1. Sodium dependence for methanol reduction to methane in cell suspensions of *Methansarcina barkeri*. Rate of methane formation at different [Na^+]. The inset shows a double-reciprocal plot of the rate of methane formation *vs* [Na^+]. The experiment was performed with 80% N_2/20% CO_2 as gas phase. The sodium concentration was measured by atomic absorption spectroscopy [11].

imum value. Reciprocal plots of the rates *vs* the sodium concentration were linear. From the intercept on the abscissa the app. K_s for sodium was calculated. The K_s-values thus obtained are summarized in table 1. The V_{max} determined were similar to the rates of methane formation in growing cultures indicating that most of the cells in the suspensions were metabolically fully active.

The K_s-values for sodium given in table 1 were determined at pH 6.0. With *M. thermoautotrophicum* the effect of the pH on the sodium requirement was studied. The K_{Na^+} and V_{max} were found to be

Table 2

Effect of monensin on the rate of methane formation in cell suspensions of *M. thermoautotrophicum* at different pH values

Monensin (nmol/mg cells)	pH value	Extracellular Na^+ (mM)	μmol CH_4/min . mg bacteria
–	6.0	1	2.2
30		1	0.0
–		100	3.2
30		100	0.3
–	6.7	1	2.2
30		1	0.8
–		100	3.2
30		100	2.0
–	7.5	1	2.2
30		1	2.2
–		100	3.2
30		100	3.2
500		1	0.0
500		100	3.2

For assay conditions see section 2. The same reaction mixture was used except that the potassium phosphate/bicarbonate buffer was replaced by a Tris/bicarbonate buffer. The Tris levels were 2.5 mM for pH 6.0, 12.5 mM for pH 6.7, and 80 mM for pH 7.5

almost independent of the pH between 6.0–7.5.

Monensin is an ionophore mediating a Na^+/H^+ antiport [16,17]. This antibiotic (30 nmol/mg dry wt cells) completely inhibited methane formation in cell suspensions of *M. thermoautotrophicum* and *M. arboriphilus* when extracellular [Na^+] was 1 mM and the pH was 6.0. No inhibition was observed, however, when extracellular Na^+ was 100 mM and pH 7.5 (table 2).

4. DISCUSSION

The 5 methanogens investigated are representatives of distantly related orders, with which most of the biochemical and bioenergetic studies published have been performed [1]. The natural habitats of these Archaebacteria are either fresh water sediments and/or the sludge of anaerobic treatment plants where [Na^+] was only at mM levels. Prokaryotes living in such habitats generally do not require sodium for metabolic functions [18,19]. The finding that methane formation from CO_2, methanol and acetate is dependent on sodium was therefore unexpected.

Different roles of sodium in methane formation can be envisaged in transport of solutes into or out of the cells, in intracellular enzyme catalysis, and/or in the mechanism of energy conservation.

The cytoplasmic membrane of bacteria is generally considered to be freely permeable for CO_2, H_2, CH_4 and CH_3COOH. The rate of diffusion of these compounds into or out of the cells is probably not rate-limiting. A transport function of sodium therefore appears unlikely in the process of methane formation.

The intracellular sodium levels in *M. thermoautotrophicum* and in *M. arboriphilus* were found to be higher than the extracellular levels, when the medium [Na^+] was <50 mM [20,21]. This is an unusual feature, since most bacteria maintain intra-

cellular]Na$^+$] well below the outside medium [18,19]. The finding suggests that there may be a specific intracellular requirement for sodium in the methanogens, e.g., one of the enzymes involved in methanogenesis could be dependent on sodium for activity. Preliminary attempts to demonstrate this in a cell-free system failed, however [14].

Dimroth [22,23] demonstrated that oxaloacetate decarboxylation in *Klebsiella aerogenes* is directly coupled with the transport of sodium out of the cells and evidence is available for a light-driven sodium pump in *Halobacterium halobium* [24,25]. The sodium motive force thus generated in these bacteria is transformed into a proton-motive force via a sodium/proton-antiport mechanism [18,19]. It is therefore tempting to speculate that methane formation could also be primarily coupled with an electrogenic extrusion of a Na$^+$ which in turn would re-enter the cell in exchange with a proton. Protonophors (e.g., carbonyl cyanide *m*-chlorophenyl hydrazone) effectively inhibit methane formation and ATP synthesis in methanogenic bacteria [21,26], indicating that one of the steps in methane formation requires the activation by the proton-motive force and/or ATP. Taking this into account the dependence of methane formation on sodium could be explained by the involvement of sodium in the generation of the proton-motive force and/or ATP. Such an involvement would also be compatible with the finding that the sodium/proton-antiporting ionophore monensin inhibited methane formation only when the extracellular [Na$^+$] pH were low.

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REFERENCES

[1] Balch, W.E., Fox, G.E., Magrum, L.J., Woese, C.R. and Wolfe, R.S. (1979) Microbiol. Rev. 43, 260–296.

[2] Doddema, H.J., Hutten, T.J., Van der Drift, C. and Vogels, G.D. (1978) J. Bacteriol. 136, 19–23.

[3] Doddema, H.J., Van der Drift, C., Vogels, G.D. and Veenhuis, M. (1979) J. Bacteriol. 140, 1081–1089.

[4] Doddema, H.J., Claesen, C.A., Kell, D.B., Van der Drift, C. and Vogels, G.D. (1980) Biochem. Biophys. Res. Commun. 95, 1288–1293.

[5] Mountfort, D.O. (1978) Biochem. Biophys. Res. Commun. 85, 1346–1351.

[6] Sauer, F.D., Erfle, J.D. and Mahadevan, S. (1980) Biochem. J. 190, 177–182.

[7] Sauer, F.D., Mahadevan, S. and Erfle, J.D. (1980) Biochem. Biophys. Res. Commun. 95, 715–721.

[8] Sauer, F.D., Erfle, J.D. and Mahadevan, S. (1981) J. Biol. Chem. 256, 9843–9848.

[9] Jarell, K.F. and Sprott, G.D. (1981) Can. J. Microbiol. 27, 720–728.

[10] Kell, D.B., Doddema, H.J., Morris, J.G. and Vogels, G.D. (1981) in: Proc. 3rd Int. Symp. Microbial Growth on C-1 Compounds (Dalton, H. ed) pp. 159–170, Heyden, London.

[11] Perski, H.-J., Moll, J. and Thauer, R.K. (1981) Arch. Microbiol. 130, 319–321.

[12] Schönheit, P., Moll, J. and Thauer, R.K. (1979) Arch. Microbiol. 123, 105–107.

[13] Diekert G., Konheiser, U., Piechulla, K. and Thauer, R.K. (1981) J. Bacteriol. 148, 459–464.

[14] Kristjansson, J.K., Schönheit, P. and Thauer, R.K. (1982) Arch. Microbiol. in press.

[15] Scherer, P. and Sahm, H. (1981) Eur. J. Appl. Microbiol. Biotechnol. 12, 28–35.

[16] Harold, F.M. (1972) Bacteriol. Rev. 36, 172–230.

[17] Pressmann, B.C. (1976) Annu. Rev. Biochem. 45, 501–530.

[18] Silver, S. (1978) in: Bacterial Transport (Rosen, B.P. ed) pp. 221–324, Marcel Dekker, Basel, New York.

[19] Lanyi, J.K. (1979) Biochim. Biophys. Acta 559, 377–397.

[20] Sprott, G.D. and Jarell, K.F. (1981) Can. J. Microbiol. 27, 444–451.

[21] Perski, H.-J. (1982) PhD Thesis, University of Marburg.

[22] Dimroth, P. (1980) FEBS Lett. 122, 234–236.

[23] Dimroth, P. (1982) Eur. J. Biochem. 121, 443–449.

[24] Lindley, E.V. and MacDonald, R.E. (1979) Biochem. Biophys. Res. Commun. 88, 491–499.

[25] Greene, R.V. and Lanyi, J.K. (1979) J. Biol. Chem. 254, 10986–10994.

[26] Roberton, A.M. and Wolfe, R.S. (1970) J. Bacteriol. 102, 43–51.