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Harmaline-resistant mutant of *Methanothermobacter thermautotrophicus* with a lesion in Na^+/H^+ antiport

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Abstract. A spontaneous mutant of *Methanothermobacter thermautotrophicus* resistant to the Na^+/H^+ antiporter inhibitor harmaline was isolated. The Na^+/H^+ exchange activity in the mutant cells was remarkably decreased in comparison with wild-type cells. Na^+/H^+ antiport activity of wild-type cells grown in the high Na^+ concentration (125 mmol/l) was significantly increased as compared to the cells grown under low Na^+ concentration (6.25 mmol/l) conditions. In contrast, harmaline resistant mutant showed almost the same Na^+/H^+ antiport activity under both these conditions. While harmaline profoundly inhibited methanogenesis in the wild-type, increased methanogenesis was observed both in the presence and absence of harmaline in the mutant strain. ATP synthesis driven by methanogenic electron transport was significantly enhanced in the mutant cells. The experimental data revealed the differential expression of A flavoprotein and molybdenum-containing formylmethanofuran dehydrogenase 1 subunit C in harmaline-resistant mutant. The overexpression of these proteins might contribute to harmaline resistance. Taken together the results indicate that harmaline resistance in this mutant has arisen as a consequence of mutation(s) in antiporter gene(s) or protein(s) linked to antiporter activity. Moreover this work provides the evidence that Na^+/H^+ exchanger deficiency in harmaline-resistant mutant can induce overexpression of several proteins participating in methanogenesis.

Key words: Methanogens — Harmaline resistance — Sodium/proton exchange

Introduction

Methanogens as members of the *Archaea*, belong to a group of microorganisms containing both H^+- and Na^+-dependent energetic cycle and the Na^+/H^+ antiporter(s). Despite numerous studies, the physiological and bioenergetic roles of these two bioenergetic cycles and of the Na^+/H^+ antiporter have not been satisfactorily explained so far. Kaesler and Schönheit (1989) have suggested that primarily generated electrochemical sodium gradient ($\Delta\tilde{\mu}_{Na+}$) can be converted in *Methanothermobacter thermautotrophicus* by means of Na^+/H^+ antiporter activity to electrochemical proton gradient ($\Delta\tilde{\mu}_{H+}$). The resulting $\Delta\tilde{\mu}_{H+}$ can be utilized *via* the A_1A_0 ATP synthase for ATP production (Kaesler and Schönheit 1989; Schäfer et al. 1999). In methanogens containing cytochromes, the H_2:heterodisulfide oxidoreductase system and F_{420} H_2:heterodisulfide oxidoreductase system are associated with membrane and coupled to proton translocation (Deppenmeier and Müller 2008). On the contrary it was suggested that in methanogens without cytochromes, the corresponding H_2:heterodisulfide reductase is only loosely associated with cytoplasmic membrane and is probably not coupled to proton translocation (Thauer et al. 2008). In such a case Na^+/H^+ antiporter might be an exclusive $\Delta\tilde{\mu}_{H+}$ generator. These ideas present an unusual new energetic situation in methanogens and Na^+/H^+ antiporter might acts in this complex energetic system as a basic regulatory element especially in cell without cytochromes, such as *M. thermoautotrophicus*. Inhibitory studies of Na^+/H^+ exchanger in methanoarchaea suggested that it is involved in pH and pNa homeostasis but its function in bioenergetics of methanoarchaea has not been resolved yet.

The understanding of the function of Na^+/H^+ exchanger and its cooperation with above mentioned bioenergetic sub-

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systems requires new experimental tools. We suggested that genetic approach to modulation or elimination of Na^+/H^+ antiport in cells of *M. thermoautotrophicus* might be suitable for these purposes. Recently we have isolated a spontaneous mutant of *M. thermautotrophicus* resistant to the Na^+/H^+ antiporter inhibitor amiloride (Šurín et al. 2007). The study of the Na^+/H^+ antiporter in the amiloride-resistant mutant has shown that this mutant exhibits very specific phenotypic characteristics like reduced Na^+/H^+ antiport and elevated production of methane and ATP. The molecular study of amiloride-resistant mutant did not allow us to assign a Na^+/H^+ antiporter activity to a product of a particular gene from the *M. thermautotrophicus* genome or to identify protein(s) participating in this complex process. Therefore we decided to isolate mutants resistant to harmaline as another inhibitor of Na^+/H^+ antiporter in methanoarchaea (Schönheit and Beimborn 1985; Šmigáň et al. 1995). Such a mutant could have alterations in Na^+/H^+ antiporter itself and could thus provide a tool to extend our knowledge on the properties and the physiological role of Na^+/H^+ antiporter and its bioenergetic function in *M. thermautotrophicus*. Moreover, the molecular study of such mutant could provide a very promising experimental approach for identification of the protein/gene for the Na^+/H^+ antiporter and could lead to new ideas on the molecular mechanism of energy coupling in methanoarchaea. This paper reports on a mutant of *M. thermautotrophicus* resistant to harmaline exhibiting a lesion in Na^+/H^+ antiporter activity accompanied by an increase in methane and ATP formation. Moreover, this study revealed the differential expression of several proteins that may contribute to harmaline resistance in the mutant strain.

Materials and Methods

Growth conditions and isolation of harmaline-resistant mutant

Growth conditions of the *M. thermautotrophicus* strain ΔH were as described earlier (Šmigáň et al. 1984). Solid media were prepared by the addition of 1% (w/v) Gelrite to the medium. All manipulations were performed under strict anaerobic conditions. For the isolation of harmaline-resistant mutant of *M. thermautotrophicus*, growth medium (Šmigáň et al. 1984) containing K_2CO_3 instead of Na_2CO_3 was supplemented with 6.25 mmol/l NaCl. To select harmaline-resistant mutants, cultures were grown for 24 h in liquid medium in the presence of 800 μmol/l harmaline. Cells were washed in growth medium and 1×10^8 cells were plated directly in an anaerobic box (Forma Scientific, model 1024) on solid medium (pH 7) containing harmaline. Plates were incubated at 60°C in a stainless-steel anaerobic jar for 14 days. Eight clones able to grow in the presence of harmaline were isolated and one of them was characterised in this study.

Measurements of Na^+/H^+ antiport activity

Na^+-induced pH changes in weakly buffered cell suspensions were monitored by Orion 91-03 semimicroelectrode connected to Orion Research Ion Analyser 901 equipped with Kipp-Zonen BD40 recorder.

Spectrofluorimetric measurements of Na^+/H^+ antiport activity were performed as described earlier (Šmigáň et al. 1995) with following modifications. Exponentially grown cells were harvested and washed in 20 mmol/l MES buffer containing 50 mmol/l NH_4Cl (pH 6), under N_2 atmosphere, resuspended in 20 mmol/l HEPES, 10 mmol/l KCl (pH 7), to final concentration of 4.5 mg of cell proteins *per* milliliter, and maintained at 7°C under N_2 atmosphere. Aliquots of cells corresponding to 45 μg of cell protein were transferred to a stirred anaerobic cuvette containing 2 ml of buffer: 50 mmol/l HEPES, 10 mmol/l KCl, 5 mmol/l $MgCl_2$ and 2.5 mmol/l acridine orange preheated to 55°C. Quenching and Na^+-induced dequenching of acridine orange were monitored under N_2 atmosphere at 55°C using Perkin-Elmer LS-50B spectrofluorimeter at excitation and emission wavelengths of 493 and 530 nm, respectively. Fluorescence before cells addition was assumed to be 100%.

Subcellular fractionation, analysis and identification of proteins

Soluble supernatant fractions (centrifuged at 100 000 × *g*) and membrane vesicle proteins were isolated as described previously (Majerník et al. 2003). Membrane vesicles were also used to prepare chloroform/methanol extracts according to (Ruppert et al. 2001). Soluble supernatant fractions and membrane vesicles were analysed by a native polyacrylamide gel electrophoresis (PAGE) (Laemmli 1970) without the addition of sodium dodecyl sulfate (SDS) and 2-mercaptoethanol to buffers. SDS-PAGE was performed according to (Schägger and von Jagow 1987). Protein bands stained by Coomassie blue staining were excised from the gel, digested by trypsin and analysed using a MALDI TOF/TOF 4700 Proteomic Analyser (ABI). The Mascot search engine was used to identify cognate proteins by matching MS of detected peptides to *M. thermautotrophicus* proteins. Proteins were quantified by the Lowry method (Lowry et al. 1951) using bovine serum albumin (BSA) as standard.

Measurement of methane and ATP formation

Methane formation from CO_2 and H_2 by the cell suspension was measured by an analysis of glass vial headspace gases by GC. Aliquots were measured using a CarloErba Fractovap 4200 equipped with a 2-m steel column packed with Sepharon AE 200–300 μm and a thermal conductivity detector using argon as a carrier gas (Šmigáň et al. 1984). ATP synthesis

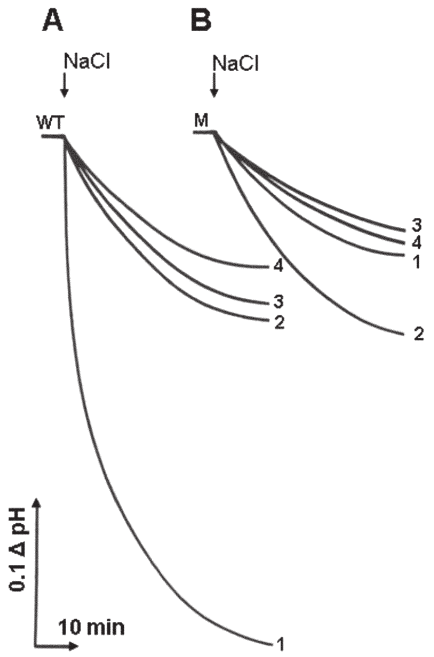

Figure 1. Changes of external pH in suspensions of *M. thermautotrophicus* wild-type (WT) cells (A) and harmaline resistant mutant (M) cells (B). Cells grown to exponential phase either in the presence of 125 mmol/l NaCl (high Na^+) or 6.5 mmol/l NaCl (low Na^+) were harvested, washed and resuspended in buffer (0.2 mol/l KCl, 2 mmol/l Tris : HCl, pH 6.8) to a concentration of 1.1 mg protein/ml, transferred to a double-necked vial and incubated at 60°C under constant stirring. **A.** High Na^+ cells: control (1), 800 μmol/l harmaline (2). Low Na^+ cells: control (3), 800 μmol/l harmaline (4). **B.** High Na^+ cells: control (1), 800 μmol/l harmaline (2). Low Na^+ cells: control (3), 800 μmol/l harmaline (4). Cells grown in the presence of 6.5 mmol/l NaCl. The arrows indicate the addition of NaCl to a final concentration of 60 mmol/l. All experiments were reproducibly repeated at least three times, and a representative data set is shown.

driven by methanogenic electron transport in cell suspensions of the wild-type and harmalin-resistant mutant was measured according to Schönheit and Perski (1983).

Results

Harmaline and amiloride have been shown to be competetive inhibitors of the Na^+/H^+ antiporter in *M. thermautotrophicus* (Schönheit and Beimborn 1985). It is important to emphasize that these two substances are chemically unrelated. Recently we have isolated a spontaneous mutant of *M. thermautotrophicus* resistant to the Na^+/H^+ antiporter inhibitor amiloride (Šurín et al. 2007).

To extend our knowledge on the function of this antiporter we tried to isolate a new spontaneous mutant of *M. thermautotrophicus* resistant to 800 μmol/l harmaline. Wild-type and the harmaline-resistant mutant strains showed comparable growth yields (0.245 mg and 0.24 mg of total cell protein/ml of growth medium, respectively) after 24 h of growth in standard culture media with 125 mmol/l NaCl. We hypothetized that harmaline-resistant mutant might have alterations in Na^+/H^+ antiporter itself as it was found in amiloride resistant mutant. To test the Na^+/H^+ antiporter activity of harmaline-resistant mutant and wild-type cells, the extent of Na^+-induced acidification of the external medium by cell suspension cultivated in liquid media supplemented with 6.25 mmol/l or 125 mmol/l NaCl was monitored. The Na^+/H^+ exchanger activity in the mutant cells was remarkably decreased in comparison with wild-type cells. The Na^+/H^+ antiport activity of wild-type cells grown in the high sodium ion concentration (125 mmol/l) was significantly increased as compared to the cells grown under low Na^+ (6.25 mmol/l) conditions. On the other hand, the harmaline-resistant mutant showed almost the same Na^+/H^+ antiport activity under both conditions (Figure 1). Harmaline profoundly inhibited the Na^+/H^+ antiporter activity in the wild-type while this inhibitor even stimulated Na^+/H^+ exchange in mutant cells (Figure 1). The results of these experiments demonstrate that the increasing Na^+ ion concentration in growth medium leads to a significant increase of the Na^+/H^+ antiporter activity in wild-type cells. These results also indicate that Na^+/H^+ antiporter is inductive in *M. thermautotrophicus*.

To characterize Na^+/H^+ antiporter activity in the harmaline-resistant mutant strain in more detail, a spectrofluorimetric method was used for determination of kinetic parameters for the Na^+/H^+ exchange in both wild-type and mutant cells, respectively. The apparent maximum velocity V_{max} and Michaelis constant K_M were 73.6 a.u. (arbitrary unit) and 5.5 mmol/l for the wild-type, and 47.6 a.u. and 4.4 mmol/l for the mutant. Almost two-fold decrease in V_{max} in the mutant strain indicates either a reduced amount of Na^+/H^+ antiporter or a lower catalytic efficiency rather than a change in its affinity for Na^+ ions. These results were similar to our results obtained in the study of amiloride resistant mutant.

In 1985, Schönheit and Beimborn presented the evidence for a function of the Na^+/H^+ antiporter in methane formation and intracellular pH regulation (Schonheit and Beimborn 1985). It was therefore interesting to study the effect of significantly reduced activity of the Na^+/H^+ antiporter in harmaline-resistant mutant on Na^+-dependent methane formation. As can be seen in Figure 2, harmaline-resistant mutant exhibited methanogenesis under resting conditions even in the presence of 800 μmol/l harmaline, whereas methanogenesis was strongly inhibited under the same conditions in the wild-type cells. Since Na^+/H^+ antiporter activity is supposed to be sensitive to uncoupling, we tested the effect of the uncoupler 3,30,40,5-tetrachlorosalicylanilide

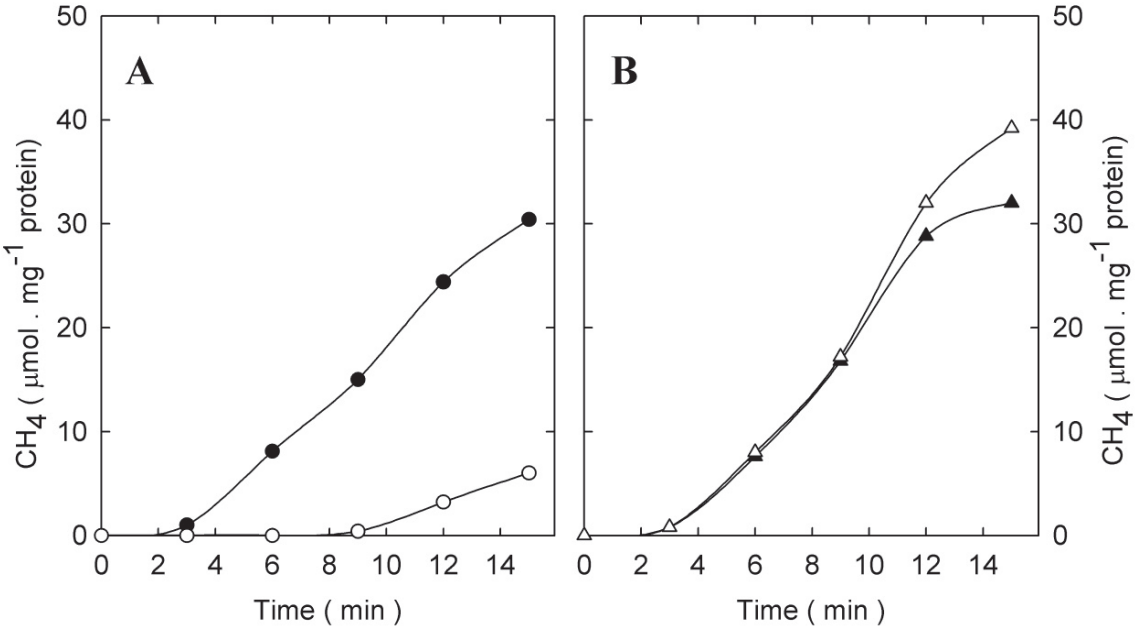

Figure 2. Time course of methane formation from H_2/CO_2 in the wild-type and harmaline-resistant mutant of *M. thermautotrophicus*. Cells were suspended in 50 mmol/l MOPS-Tris buffer (pH 6.8), 10 mmol/l NaCl and 5 mmol/l $MgCl_2$ to a concentration of 1.8 mg protein/ml. The suspensions in 22 ml flasks were pressurized with H_2/CO_2 (4 : 1) to 100 kPa and incubated at 60°C. **A.** Wild-type cells: control (●), 800 μmol/l harmaline (○) **B.** Mutant cells: control (▲), 800 μmol/l harmaline (Δ). Methane was measured at the times indicated. All experiments were reproducibly repeated at least three times and a representative data set is shown.

(TCS) on growth and methane production in the wild-type and harmaline-resistant mutant cells. Significant differences were found between the ability of mutant and wild-type cells to synthesize methane and to grow in the presence of this uncoupler (Table 1).

While harmaline and TCS strongly inhibited growth and methanogenesis in the wild-type, both parameters were insensitive to these inhibitors in the mutant strain. These findings support the idea that in wild-type cells, $\Delta\tilde{\mu}_{Na+}$ can be converted to $\Delta\tilde{\mu}_{H+}$ by Na^+/H^+ antiporter (Kaesler and Schönheit 1989) and resulting $\Delta\tilde{\mu}_{H+}$ can be then directly utilized *via* the A_1A_0 ATP synthase for ATP production. In the mutant strain with modified Na^+/H^+ antiport system, $\Delta\tilde{\mu}_{Na+}$ cannot be converted by means of the Na^+/H^+ antiporter to $\Delta\tilde{\mu}_{H+}$. Moreover, $\Delta\tilde{\mu}_{H+}$ cannot be generated in these cells by $\Delta\tilde{\mu}_{H+}$ generator – H_2: heterodisulphid reductase since this enzyme does not work in the methanoarchaea cells lacking cytochromes (Deppenmeier et al. 1996; Thauer et al. 2008). This indicates that Na^+ ions are the exclusive coupling ions in this mutant. Amiloride (800 μmol/l), another inhibitor of the Na^+/H^+ antiporter in methanoarchaea, inhibited methane formation in the harmaline-resistant mutant strain under non-growing conditions (not shown). This demonstrated the absence of crossresistance between harmaline and amiloride in the harmaline-resistant mutant and indicated different biochemical mechanisms of resistance to harmaline and amiloride in *M. thermautotrophicus*. Na^+/H^+ antiport in methanoarchaea seems to be a process that couples the circulation of H^+ and Na^+ ions across the cytoplasmic membrane.

As mentioned earlier, Na^+/H^+ antiporter could modulate the driving force for ATP synthesis under physiological conditions (Kaesler and Schönheit 1989; Šmigáň et al. 1995). Therefore, we studied the potential role of the Na^+/H^+ antiporter in total ATP synthesis in more detail. Comparison of wild-type and mutant ATP synthesis driven by methanogenic electron transport has shown that total ATP synthesis is

Table 1. Effect of TCS and harmaline on methane production of wild-type and harmaline-resistant mutant[a]

Treatment	CH_4/24 h (mmol)	
	Wild-type	Mutant
Control	1.3	1.2
TCS	0.26	1.2
Harmaline	0.0	1.16

[a] Wild-type and harmaline-resistant mutant were cultivated in medium supplemented with 125 mmol/l NaCl in the presence of 3 μmol/l TCS or 800 μmol/l harmaline. Methane was determined after 24 h by gas chromatography.

higher in the mutant strain (Figure 3). We have also determined the effect of significantly reduced Na^+/H^+ antiporter activity in the mutant strain on ATP synthesis driven by an electrogenic potassium efflux in the presence of sodium ions. The results clearly showed significantly enhanced ATP synthesis in the harmaline-resistant mutant (Figure 4). It should be noted in this context that native PAGE combined with MALDI TOF/TOF mass spectrometry did not reveal increased levels of A_1 and A_0 subcomplexes of the A_1A_0-type synthase in the mutant strain (not shown).

These findings indicate that the Na^+/H^+ antiporter may compete with the Na^+-translocating ATP synthase for sodium ions during ATP synthesis, as it was suggested previously (Šmigáň et al. 1995). Similarly, Becher and Müller (1994) have found that inhibition of the Na^+/H^+ antiporter in *M. mazei* led to a stimulation of ATP synthesis driven by a methyl transfer reaction generating $\Delta\mu_{Na}{}^+$, as well as by artificially created ΔpNa.

To identify molecular basis of the harmaline resistance, mass spectrometry analysis of the protein composition of membrane vesicles and supernatant fraction derived from wild-type and harmaline-resistant cells was performed. The preliminary results of MS analysis of the protein composition of membrane vesicles and supernatant fractions revealed that mutant cells contained significantly higher amounts of type A flavoprotein and molybdenum-containing formylmethanofuran dehydrogenase. It has been suggested recently that these proteins play a direct role in flavin-based electron bifurcation in methanoarchaea lacking cytochromes (Thauer et al. 2008).

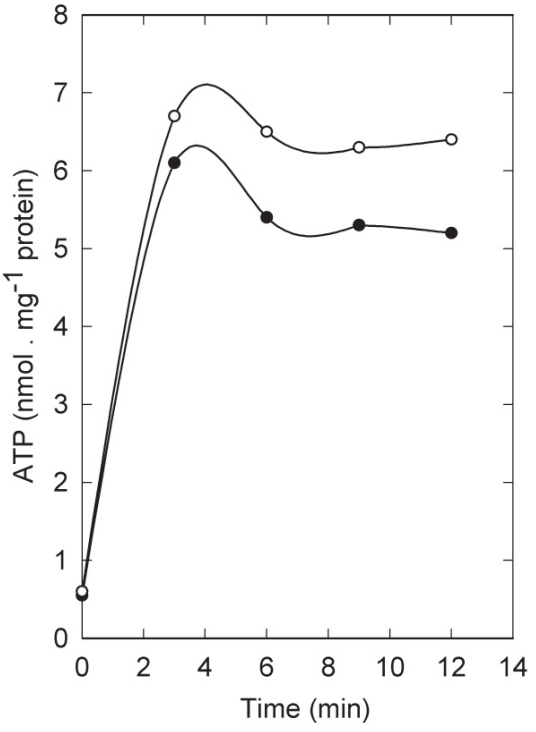

Figure 3. Methanogenesis-driven ATP synthesis in the wild-type and harmaline-resistant mutant of *M. thermautotrophicus*. Cells were suspended in 50 mmol/l MOPS-Tris buffer (pH 6.8), 10 mmol/l NaCl and 5 mmol/l $MgCl_2$ to a concentration of 1.1 mg protein/ml. Gas phase was 80% H_2/20% CO_2. ● wild-type cells, ○ mutant cells. All experiments were reproducibly repeated at least three times and a representative data set is shown.

Discussion

The coexistence of two primary ion gradients $\Delta\tilde{\mu}_{H+}$ and $\Delta\tilde{\mu}_{Na+}$ in methanoarchaea representing a completely unprecedented energetic situation in prokaryotic cells could have lead to the evolution of specific transport system(s). This system might be a basic regulatory element responsible for the homeostasis of Na^+- and H^+-cycles in methanoarchaea. We suggested Na^+/H^+ antiporter as one of candidates for this role. To test this hypothesis, we applied genetic approach to prepare a model system in which modulation and/or elimination of this antiporter would be viable.

Three years ago we isolated a spontaneous *M. thermautotrophicus* mutant resistant to the Na^+/H^+antiporter inhibitor amiloride (Šurín et al. 2007). Biochemical and proteomic study of this mutant did not definitely clarify neither the predicted function of this antiporter in balancing the energy of $\Delta\tilde{\mu}_{H+}$ and $\Delta\tilde{\mu}_{Na+}$ nor the protein components responsible for Na^+/H^+ exchange in the bioenergetics of *M. thermautotrophicus*. As an extension of these studies we isolated harmaline-resistant mutant of *M thermautotrophicus*. The finding that growth in the presence of high sodium concentration is associated with an elevated Na^+/H^+ antiporter activity in wild-type cells suports the idea that the expression of this exchanger is regulated by sodium ions. It also indicates that sodium dependence of methanoarchaea is mediated not only by N-5-methyltetrahydromethanopterin: coenzyme M methyltransferase (EC 2.1.1.86) as generator of $\Delta\tilde{\mu}_{Na+}$ (a primary sodium pump) (Becher et al. 1992) but also by the Na^+/H^+ exchanger. Recently we observed a significant increase in Na^+/H^+ antiporter activity when cells progressed into the stationary growth phase. We can assume that *M. thermautotrophicus* has either one Na^+/H^+ antiporter differently regulated by Na^+ concentration or that there are at least two independent Na^+/H^+ antiporter systems each controlled by different mechanisms. Results obtained from kinetic measurements indicate that Na^+/H^+ antiporter in the mutant strain is either present in reduced amounts or it exhibits lower catalytic efficiency rather than it would have modified affinity for Na^+ ions. Similar results were achieved also in the study of amiloride resistant mutant. As mentioned above, *M. thermautotrophicus* belongs to a group of methanoarchaea without cytochromes, and its

H_2:heterodisulphid reductase is a cytoplasmic enzyme and is not coupled to transmembrane proton translocation. If this assumption is valid, Na^+/H^+ antiporter is the only possible generator of $\Delta\tilde{\mu}_{H+}$.

The results described above have raised the question if harmaline-resistant mutant cells can grow when $\Delta\tilde{\mu}_{H+}$ is dissipated in the presence of protonophore TCS. To answer this question, we studied the inhibitory effect of TCS on the methane formation in growing wild-type and harmaline-resistant mutant cells (Table 1). Our results indicate that $\Delta\tilde{\mu}_{H+}$ is a driving force for at least some endergonic processes in the wilde-type cells grown in the presence of high NaCl (125.0 mmol/l NaCl) and that Na^+/H^+ exchange activity sensitive to harmaline is responsible for generation of $\Delta\tilde{\mu}_{H+}$. On the other hand, mutant cells can grow under the same conditions in the presence of protonophore TCS and harmaline. This supports the idea that harmaline-resistant mutant with modified Na^+/H^+ antiporter can grow in the absence of $\Delta\tilde{\mu}_{H+}$ that should be dissipated in the presence of uncoupler. This finding also indicates that only

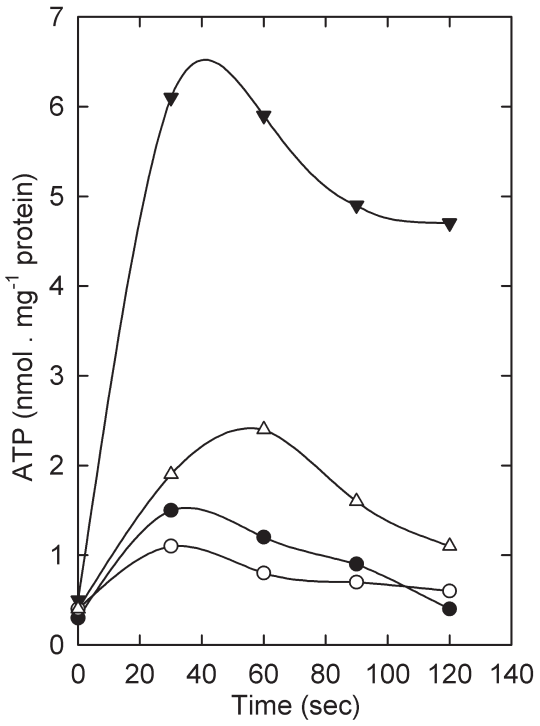

Figure 4. Potassium diffusion potential-driven ATP synthesis in the wild-type and harmaline-resistant mutant of *M. thermautotrophicus*. Cells were suspended in 50 mmol/l MOPS-Tris buffer (pH 6.8) to a concentration of 3.0 mg protein/ml. After 5 min of preincubation at 60°C, ATP synthesis was induced by the addition of valinomycin (f.c. 15 µmol/l). At t = 5 min, NaCl was added to f.c. 50 mmol/l. Wild-type cells: control (●), 3 µmol/l TCS (○). Mutant cells: control (▲), 3 µmol/l TCS (Δ).

$\Delta\tilde{\mu}_{Na+}$ generated by N-5-methyltetrahydromethanopterin: coenzyme M methyltransferase (EC 2.1.1.86) drives ATP synthesis and that Na^+ ion is the exclusive coupling ion in this mutant. This means also that $\Delta\tilde{\mu}_{H+}$ generator H_2: heterodisulphid reductase, generating $\Delta\tilde{\mu}_{H+}$ by redox potential-driven proton translocation (Deppenmeier et al. 1996), does not work in cells lacking cytochromes as suggested by Thauer et al. (2008). These authors suggested recently that in methanoarchaea group with cytochromes the first and last steps in methanogenesis from H_2 and CO_2 are coupled chemiosmotically (Thauer et al. 2008). *M. thermautotrophicus* belong to a group lacking cytochromes. Gunsalus and Wolfe (1977) observed in *M. thermautotrophicus* a stimulation of CO_2 reduction to methane by methylcoenzyme M. It was suggested that the first and last steps of methanogenesis in these methanoarchaea are energetically coupled by a cytoplasmic enzyme complex that mediates flavin-based electron bifurcation (Thauer et al. 2008). The study of harmaline-resistant mutant opened the posibility to study the role of Na^+/H^+ antiporter in these complex bioenergetic systems. Therefore we attempted to define on the protein level the molecular basis of the harmaline resistance and of accompanying phenotypic characteristics of the mutant strain. We compared the level of protein expression in different cellular fractions. The preliminary results of mass spectrometry analysis of membrane proteins in wild-type and harmaline-resistant cells revealed that the mutant strain contained significantly higher amounts of type A flavoprotein and molybdenum-containing formylmethanofuran dehydrogenase 1 subunit C. Low concentrations or absence of the Na^+/H^+ antiporter in harmaline-resistant mutant might be compensated for by elevated amounts of type A flavoprotein and molybdenum-containing formylmethanofuran dehydrogenase participating in flavin-based electron bifurcation. All these findigs support the idea that Na^+/H^+exchanger might be a clue to the adaptative bioenergetic behaviour of *M. thermautotrophicus* under the different physiological conditions. Moreover this work provides the evidence that Na^+/H^+exchanger deficiency in harmaline-resistant mutant can induce changes at the expression level of several genes participating in methanogenesis. The results imply that harmaline resistance is a consequence of decreased Na^+/H^+ antiporter expression.

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