An electrogenic sodium-translocating ATPase in *Methanococcus voltae*

S.W. Carper and J.R. Lancaster, jr

Department of Chemistry and Biochemistry, Utah State University, Logan, UT 84322, USA

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A membrane potential negative inside generated by the electrogenic efflux K^+ via valinomycin was demonstrated to be a driving force for the synthesis of ATP in *Methanococcus voltae*, but only in the presence of sodium. ATP synthesis was prevented by the addition of external potassium or of the sodium ionophore monensin, but was not inhibited by the protonophore SF6847. ATP synthesis in the absence of potassium in the medium could also be driven by a chemical concentration gradient of sodium directed inwards, but only in the presence of a permeant counterion (tetraphenylborate). These results show that *M. voltae* contains an electrogenic ATP-driven ion pump which translocates sodium.

Methanogen *Bioenergetics* *Na^+ pump* *ATPase* *Ion transport*

1. INTRODUCTION

The exact role of sodium in the bioenergetics of methanogens is not yet clear. It has been shown that low concentrations of Na^+ (millimolar) stimulate methane formation in several species [1]. *Methanobacterium thermoautotrophicum* (delta-H and marburg) is dependent on Na^+ for growth and methane formation from H_2 and CO_2 [2]. Sodium is also required for methanol oxidation in cells of *Methanosarcina barkeri*, but not for CH_4 formation from H_2/CH_3OH or ATP synthesis [3]. ATP synthesis in *Mb. thermoautotrophicum* (marburg) induced by an artificially generated membrane potential is greatly stimulated by sodium [4]. Recently, the presence of an Na^+/H^+ antiporter in this species has been reported [5].

Sodium stimulates methanogenesis and is required for the growth of *Methanococcus voltae* [6], which also possesses an Na^+-dependent transport system for isoleucine [7]. We have proposed previously the existence of a membrane-bound ATPase in *M. voltae* which translocates ion(s) other than H^+ or K^+ [8]. Here, we demonstrate that the ion translocated is Na^+.

2. MATERIALS AND METHODS

M. voltae strain PS (DSM 1537) was grown in the medium described in [6]. Cells in the mid-logarithmic phase of growth were harvested by centrifugation (10000 × *g*, 10 min), washed, and resuspended in sucrose buffer (0.8 M sucrose/10 mM $MgCl_2$/10 mM Tricine NaOH, pH 8.0) to a final protein concentration of 4–5 mg/ml. 1.2 ml of the cell suspension were placed in test tubes in a 38 heating block. Ethanolic solutions of ionophores were added to the cell suspensions, to a final concentration of 10 μM (2–2.5 nmol/mg), except when indicated otherwise in the figure legends. After the addition of ionophores the cell suspensions were allowed to incubate for 25 min. NaCl and KCl were added from 4 M stock solutions. NaCl was added at zero time. KCl was added immediately after the addition of the ionophore.

All solutions were anaerobic and all manipulations were performed under strictly anaerobic conditions. ATP was determined by the luciferin-luciferase assay as in [9]. Protein was determined as in [10]. Valinomycin, monensin, and sodium

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tetraphenylborate (TPB) were purchased from Sigma (St. Louis, MO). SF6847 was a gift from P. Hinkle, Cornell University.

3. RESULTS

Fig.1 shows the effect of NaCl on cell suspensions of non-respiring *M. voltae*, pretreated with and without ionophores. In the presence of 10 μM valinomycin the addition of NaCl (final concentration 0.4 M) results in an increase in ATP levels. This increase is due to the membrane potential induced by valinomycin-catalyzed electrogenic potassium efflux since the addition of KCl to the medium prevents the effect. This result has been described previously in *Mb. thermoautotrophicum* [4], and can be attributed either to a sodium stimulation of an ion-translocating ATPase (involving some ion other than Na^+) or to the electrogenic movement of sodium through such an ion pump. When NaCl is added to cells in the absence of valinomycin there is a slight but detectable increase in the ATP level (fig.1). As demonstrated below, this increase is most probably due to Na^+ moving into the cell in response to its chemical concentration gradient.

The addition of the sodium-translocating ionophore monensin prevents the Na^+-induced increase in ATP levels (fig.1), presumably by allowing sodium to move into the cell instead of through the ATPase. This result argues against the possibility that the increase in ATP is due to the presence of sodium in the medium as opposed to transport.

Fig.2 shows that a proton gradient is not involved in ATP production since 5 μM SF6847, a potent protonophore shown previously to catalyze electrogenic proton movement in this organism [8], has little effect on sodium-induced ATP synthesis. This result argues against an obligatory role for proton(s) in this process.

Fig.3 shows that a concentration gradient of NaCl can provide the sole driving force for the synthesis of ATP. When NaCl is added to a cell suspension with no other additions a very slow increase in ATP levels is observed. However, preincubation with the membrane-permeant ion TPB^- results in an increase in ATP upon NaCl addition, in the absence of potassium. We interpret this

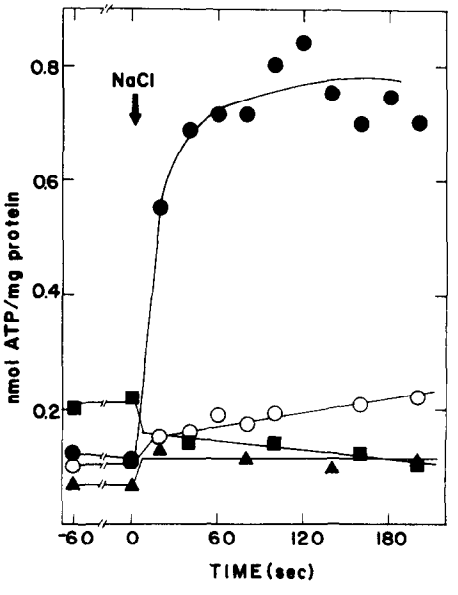

Fig.1. Dependence of membrane potential-driven ATP synthesis on sodium movement. Cells were treated as described in section 2. NaCl was added to the cell suspensions to a final concentration of 0.4 M at the solid arrow. (●) 10 μM valinomycin, (○) ethanol control, (▲) 10 μM valinomycin + 0.1 M KCl, (■) 10 μM valinomycin + 10 μM monensin.

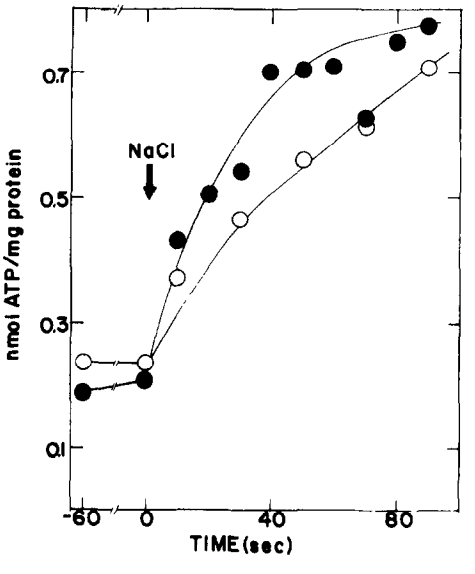

Fig.2. Uncoupler effects on membrane potential-driven, sodium-dependent ATP synthesis. Experimental conditions as in fig.1. (●) 10 μM valinomycin, (○) 10 μM valinomycin + 5 μM SF6847.

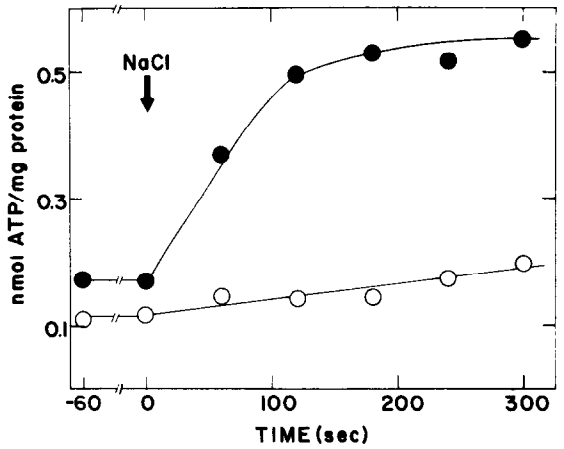

Fig.3. ΔpNa^+-driven ATP synthesis. Experimental conditions as in fig.1, but without valinomycin or added potassium (the Tricine buffer pH was adjusted with NaOH). Where indicated, sodium TPB^- was added to the cell suspension to a final concentration of 1 mM 20 min prior to the addition of NaCl. (●) 1 mM TPB^-, (○) ethanol control.

result as follows: In the absence of a permeant counterion the initial electrogenic movement inwards of sodium driven by the chemical concentration gradient will rapidly set up a reverse membrane potential and inhibit further movement, thus quickly preventing observable ATP synthesis. The inclusion of TPB^- provides a charge compensation, an inhibitory membrane potential does not develop, and ATP synthesis occurs. The total amount of ATP synthesized is approx. 50% of that induced by a membrane potential (fig.1). The slower rate of ATP synthesis could be due to rate-limiting diffusion of the TPB^- counterion.

4. DISCUSSION

The presence of an electrogenic sodium translocating ATPase is demonstrated by the following observations:

(i) Membrane potential-driven ATP synthesis requires the movement of sodium across the membrane, rather than simply its presence.

(ii) A concentration gradient of sodium (ΔpNa^+) in the absence of added potassium and presence of protonophore will drive ATP synthesis.

(iii) A counterion must be present to obtain ΔpNa^+-driven ATP synthesis, showing that the effect involves transmembrane sodium movement and that this movement is electrogenic.

As far as we are aware, this is the first direct demonstration of an electrogenic sodium-translocating bacterial ATPase. A role for a sodium gradient in ATP synthesis coupled to decarboxylation in certain bacteria has been demonstrated [11], but no data have been reported regarding a role of a transmembrane electrical field in this process. Although a sodium-translocating respiratory chain has been reported for several species of bacteria [12,13], no data have appeared regarding a sodium-translocating ATPase.

The specificity of valinomycin for potassium over sodium, while high (approx. 1000-fold [14]), is not absolute. Especially in the case of high sodium concentrations such as that in fig.1 (0.4 M) where the binding of K^+ and of Na^+ to the ionophore could be saturating, ATP synthesis might not be observed due to valinomycin-catalyzed sodium influx. This could explain why ATP synthesis occurs with TPB^- as counterion (fig.3) but not with valinomycin plus potassium (fig.1).

Based on the results presented here and in [8] we propose that the bioenergetic role of sodium in *M. voltae* is as a coupling ion for ion and solute transport, driven by coupling to the hydrolysis of ATP by a membrane-bound ATPase. ATP is synthesized coupled to methanogenesis by a direct mechanism. Evidence for a role in methanogens for sodium in transport phenomena has been reported for proton(s) [5], coenzyme M [15], amino acids [7] and nickel [16].

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