0014-4754/93/121059-05$1.50 + 0.20

Is ATP synthesized by a vacuolar-ATPase in the extremely halophilic bacteria?

L. I. Hochstein* and D. Lawson

Ames Research Center, Moffett Field (California 94035-1000, USA)

Abstract. The proton-dependent synthesis of ATP was demonstrated in representative members of the genera *Halobacterium*, *Haloarcula*, and *Haloferax*. In all cases, synthesis was not inhibited by nitrate or N-ethylmaleimide, inhibitors of the vacuolar-like ATPases found in Archaea, but was affected by azide, an inhibitor of F_0F_1-ATP synthases. These observations extend the earlier observations with *Halobacterium saccharovorum* and suggest that ATP synthesis in these organisms is brought about by an F_0F_1-ATP synthase.
Key words. Halobacterial ATPase; archaeal ATPases; ATP synthesis; *Halobacterium*; *Haloarcula*; *Haloferax*.

Introduction

Membranes prepared from *Halobacterium saccharovorum* contain an ATPase whose properties make unlikely that it is an F_0F_1-ATP synthase[11,20]. ATP hydrolysis is stimulated by various detergents including Triton X-100 (ref. 19). This is due, in part, to making ATP available to that fraction of the enzyme located within the closed vesicles which constitute about 90% of the vesicle population. Triton also activates the enzyme since following purification little if any ATP hydrolysis is detected without the detergent[21]. Similar ATPase activities occur in membranes from *Hb. salinarium*, *Haloferax mediterranei*, *Hf. denitrificans*, *Haloarcula vallismortis*[21], and *Natronobacterium magdii* (L. I. Hochstein, unpublished data). The subunit structure and patterns of inhibitor sensitivity of the ATPase from *Hb. saccharovorum* suggest that it is related to the vacuolar ATPases that occur in the Eucarya[29]. Another vacuolar-like ATPase is also present in *Hb. salinarium* (formerly *Hb. halobium*)[27]. This enzyme differs from the *Hb. saccharovorum* ATPase with respect to detergent activation, salt activation, and pH optimum[30]. These differences, as well as the differences in the membrane-bound ATPases from *Hf. mediterranei*, *Hf. volcanii*[1], *Sulfolobus acidocaldarius*[18,22] and *S. solfataricus*[12] suggest that the Archaea possess a variety of vacuolar-like ATPases whose function is yet to be unequivocally defined.

The amino acid sequences of the two largest ATPase subunits from several Archaea [2,3,14,16] and vacuolar-ATPases suggest that while they are closely related, they are but distantly related to F_0F_1-ATP synthases, and that the three types arose from an ancestral proton-translocating vacuolar-type of ATP synthase[7]. A consequence of this notion is that the vacuolar-like archaeal ATPases are ATP synthases. Although the vacuolar-like enzyme from *Hb. salinarium* has been suggested to be ATP synthase[13,25], two recent observations suggest that the Archaea may synthesize ATP using an uncharacterized F_0F_1-ATP synthase. Recently, a DNA fragment from *Methanosarcina barkeri* was amplified using oligonucleotide primers that were homologous to highly conserved sequences of the β subunit of F_0F_1-ATP synthase[31]. In the other case, ATP synthesis in *Hb. saccharovorum* is not inhibited by N-ethylmaleimide (NEM) or nitrate, which are inhibitors of vacuolar ATPase activity, but is affected by azide[10], an inhibitor of F_0F_1-ATP synthases but not vacuolar ATPases[6]. To determine if the patterns of inhibition observed in *Hb. saccharovorum* occurred in other extreme halophiles, we examined ATP synthesis in several species. It was of particular interest to examine ATP synthesis in *Hb. salinarium* since ATP synthesis in vesicles from this organism is not inhibited by azide, but is affected by NEM and nitrate[26]. The results that we report indicate that while differences exist among the extreme halophiles with respect to the conditions required to demonstrate ATP synthesis, in every case ATP synthesis is inconsistent with the operation of vacuolar-type ATPase.

Materials and methods

Cells were prepared as was the case with *Hb. saccharovorum*[10]. The cells were suspended in 50 mM Tris. HCl/4M NaCl/10 mM $MgCl_2$ pH 8 (TNM) buffer, incubated for 20 h at 25 °C on a gyrorotary shaker operated at 150 RPM and designated as 'starved cells'. ATP synthesis was also determined as previously described[10] except that synthesis was initiated by the addition of 20 µl of 250 mM pH 3 sodium citrate buffer (which resulted in a final pH of 4.1). To avoid complications arising from photophosphorylation[8], the assays were carried out in subdued light. In those cases when cells were to be preincubated in potassium-containing buffers, they were first incubated in such buffers for 15 min at room temperature. The amount of ATP synthesized was corrected for the ATP found in cells that were not acidified. The arginine-dependent synthesis of ATP was carried in the same manner except that

10 µmols of arginine was added to start the reaction and the incubation time was increased to 30 min. There was no difference in the rate of ATP synthesis when either the proton or arginine-dependent synthesis was carried out in a nitrogen atmosphere. Proteins were determined as previously described[10]. *Hb. salinarium* (strain R_1), *Ha. hispanicum*, *Hf. mediterranei* (strain R_4), and *Hf. volcanii*, were obtained from J. Lanyi, R. Vreeland, B. Tindall, and W. F. Doolittle, respectively. *Hb. saccharovorum* (strain M6) was the culture maintained in this laboratory since isolation[32].

Results

The synthesis of ATP

All of the organisms synthesized ATP when the pH of the medium in which they were suspended was acidified. The figure describes the process as it occurred in *Hb. salinarium* after the cells were 'starved' for 24 h. ATP synthesis was relatively rapid upon acidification with pH 3 sodium citrate buffer and attained an apparent steady state between 1 and 2 min. There was no change in the intracellular ATP concentration in cells maintained at pH 8 during this time. While the kinetics of ATP synthesis was similar in all organisms used in this study, the conditions resulting in ATP synthesis were not always the same. In general, little or no ATP synthesis was observed when the intracellular ATP concentration was high, and conditions that depleted cells of ATP resulted in cells that were able to synthesize ATP. Table 1 summarizes the intracellular ATP concentrations and the rates of ATP synthesis in the organisms used in this study. *Hb. salinarium* contained 6 nmols ATP/ mg cell protein shortly after harvesting. The intracellular ATP concentration decreased to about 5% of its initial value when the cells were 'starved' overnight in the dark at 24 °C in TNM buffer. Such cells synthesized 15 nmols of ATP/ min/ mg cell protein when acidified by the addition of citrate buffer. The ability to synthesize ATP was rapidly lost when cells were incubated in the dark at 24 °C with approximately half of the activity disappearing after about 3 days (fig. inset). However, the age of the cells did not change how various inhibitors affected ATP synthesis although the kinetics of ATP synthesis became more nonlinear (concave downward).

The intracellular ATP concentration in *Ha. hispanicum* following harvesting was 24 nmols ATP/ mg cell protein. No synthesis of ATP was observed in *Ha. hispanicum* assayed after harvesting or in cells that were 'starved' for 24 h. Addition of citrate had the effect of inducing a loss of ATP. The high intracellular concentration of ATP suggested that the absence of ATP synthesis was due to the lack of sufficient quantities of ADP. If this were so, reducing the intracellular ATP to increase the concentration of ADP should result in ATP synthesis. 'Starvation' had a minimal effect on the concentration of intracellular ATP in *Ha. hispanicum*. When *Ha. hispanicum* was suspended in potassium-containing 25 mM Tris/10 mM $MgCl_2$ pH 8 buffer, the intracellular ATP concentration decreased whenever the KCl concentration was 2 M or more and the NaCl concentration was less than 2 M. Higher concentrations of NaCl (with correspondingly lower KCl concentrations) inhibited ATP depletion. ATP was most rapidly lost in pH 8 buffer that was 2.7 M and 800 mM with respect to KCl and NaCl, respectively. The addition of citrate to *Ha. hispanicum* suspended in 25 mM Tris.HCl/2.7 M KCl/800 mM NaCl/10 mM $MgCl_2$ pH 8 buffer resulted in the synthesis of 37 nmols ATP/ min/ mg cell protein (table 1).

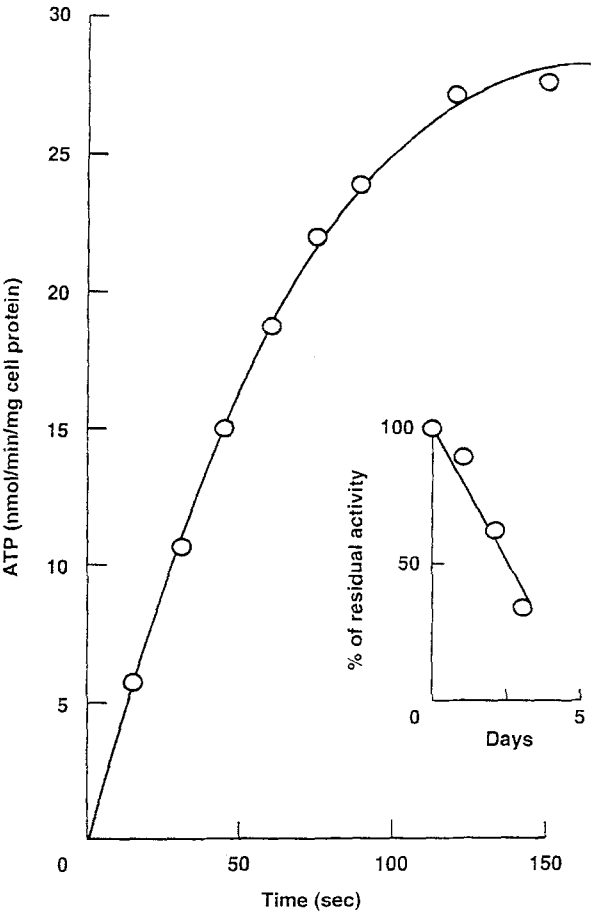

Figure. ATP synthesis on *Hb. salinarium*. Cells were suspended in 25 mM Tris.HCl/4 M NaCl/10 mM $MgCl_2$ buffer pH 8 and incubated for 1 min at 37 °C in subdued light before ATP synthesis was initated by the addition of 20 µl of 250 mM pH 3 sodium citrate buffer. Aliquots were periodically removed and assayed within 5 min for ATP as described in 'Materials and methods'. The intracellular ATP concentration of cells maintained at pH 8 did not change significantly (< 10%) during the course of the experiment.
Inset: stability of ATP synthesis. Cells suspended in 50 mM Tris.HCl/4 M NaCl/10 mM $MgCl_2$ buffer pH 8 and incubated at 25 °C in the dark while shaking at 150 RPM. Aliquots were diluted 1:10 in 0.2% Triton X-100 and assayed for intracellular ATP within 5 min.

Table 1. ATP synthesis and the intracellular ATP concentration in representative extreme halophiles

Organism	Intracellular ATP	ATP synthesis
	(nmol/ mg cell protein)	(nmol/ min/ mg cell protein)
Hb. salinarium[a]	6	19
Hb. saccharovorum[a]	16	24
Ha. hispanicum[b]	24	31
Hf. mediterranei[b]	53	37
Hf. volcanii[b]	48	15

ATP synthesis was determined in cells suspended in either 25 mM Tris/4 M NaCl/10 mM $MgCl_2$[(a)] or 25 mM Tris/2.7 M KCl/800 mM NaCl/10 mM $MgCl_2$[(b)] pH 8 buffer as described in 'Materials and methods'. The intracellular ATP was determined within 2 h after the cells were harvested.

No ATP synthesis occurred in either of the *Haloferax* species when cells, suspended in 25 mM Tris.HCl/4 M NaCl/10 mM $MgCl_2$ pH 8 buffer, was acidified with citrate buffer. As was the case with *Ha. hispanicum*, the addition of citrate led to the depletion of intracellular ATP. In the case of *Hf. mediterranei*, the initial intracellular ATP concentration was 53 nmols/ mg cell protein and it decreased slowly when the cells were 'starved' in TNM buffer. The ability of *Hf. mediterranei* to maintain a high intracellular ATP concentration probably relates to the presence of polyhydroxybutyrate, which occurs in high concentrations in this organism[5] and the utilization of this substance as an energy source. The intracellular ATP decreased rapidly when *Hf. mediterranei* was suspended in 25 mM Tris.HCl/2.7 M KCl/800 mM NaCl/ 10 mM $MgCl_2$ pH 8 buffer, and such cells synthesized 37 nmols ATP/ min/ mg cell protein when acidified by the addition of citrate. The intracellular ATP concentration of *Hf. volcanii* was also relatively high and no ATP was synthesized when the cells were suspended in buffer that was 4 M with respect to NaCl. Suspending such cells in 25 mM Tris.HCl/2.7 M KCl/800 mM NaCl/ 10 mM $MgCl_2$ pH 8 buffer led to the rapid decrease in the intracellular level of ATP, and 37 nmols ATP/ min/ mg cell protein were synthesized upon the addition of citrate buffer.

The effects of inhibitors on ATP synthesis

The effects of 3 classes of inhibitors were examined: those that affect proton translocation; those that inhibit vacuolar ATPase; and those that inhibit F_0F_1-ATP synthases. The results with *Hb. salinarium* are summarized in table 2. Carbonyl cyanide 3-chlorophenylhydrazone (CCCP) or dicyclohexylcarbodiimide (DCCD) was added as 1% methanolic solutions. Methanol at that concentration had no significant effect on ATP synthesis. CCCP, which acts by collapsing proton gradients, inhibited ATP synthesis 67% and 100% at concentrations of 1 and 10 μM, respectively. DCCD, which inhibits ATP synthesis by blocking proton translocation, inhibited ATP synthesis in *Hb. salinarium*. The extent of synthesis depended on the concentrations of DCCD and cells, and the time cells were exposed to the inhibitor at pH 8 before initiating ATP synthesis. For example, no inhibition was observed when cells containing 340 μg cell protein were preincubated for 60 min in the presence of 10 μM DCCD. The same concentration of DCCD produced inhibitions of 65% and 87% when cells containing the equivalent of 170 μg and 85 μg of protein were used. ATP synthesis was inhibited 96% in the presence of 0.2% Triton X-100, a concentration that lysed the cells. Thus, inhibitors that either abolished or blocked the utilization of proton gradients inhibited ATP synthesis. Nitrate and N-ethylmaleimide (NEM) inhibit ATPase activities from *Hb. saccharovorum*[29] and *Hb. salinarium*[26]. Therefore, they should inhibit ATP synthesis if the vacuolar-like ATPase activities from these Archaea were ATP synthases. NEM did not inhibit ATP synthesis in *Hb. salinarium* (table 2). As with *Hb. saccharovorum*[10], the steady-state level of ATP was higher in the presence of NEM than in its absence. The addition of nitrate also resulted in slightly higher steady-state levels of ATP although the results could be considered marginal. Azide, which does not inhibit the vacuolar-like ATPases from *Hb saccharovorum*[20] and *Hb. salinarium*[27], was an effective inhibitor of ATP synthesis. The inhibition of ATP synthesis by azide is the most persuasive evidence for the presence of F_0F_1-type ATP synthase in the extreme halophiles. However, this interpretation is

Table 2. The effect of ATPase inhibitors on the ATP synthesis in *Hb. salinarium*

Addition		ATP (pmol)			% of Control
		+Cit	−Cit	+Δ	
None		165	22	143	-
Methanol	(1%)	147	5	142	99
CCCP	(1 μM)	37	14	33	22
DCCD	(50 μM)	26	20	6	4
NEM	(5 mM)	225	2	222	155
Nitrate	(80 mM)	182	2	180	126
Azide	(2.5 mM)	48	21	27	19
Triton	(0.2%)	13	7	6	4

Hb. salinarium (1.5 mg cell protein) was suspended in 25 mM Tris/4 M NaCl/10 mM $MgCl_2$ pH 8 buffer and ATP synthesis was determined as described in 'Materials and methods'.

ambiguous since azide could be acting as an uncoupler (J. Lanyi, pers. comm.) It should be possible to distinguish between azide acting as an inhibitor of the F_0F_1-ATP synthase and as an uncoupler by noting its effect on ATP synthesis during substrate level phosphorylation. Arginine supports the anaerobic growth of *Hb. salinarium*[9] where ATP is synthesized in a reaction involving the phosphorylation of ADP by carbamyl phosphate[9] which is produced from arginine by way of the arginine hydrolase pathway[4]. As shown in table 3, *Hb. salinarium* synthesized ATP in the presence of arginine, and 2.5 mM azide was without any significant effect. Interestingly, the presence of 0.2% Triton X-100 virtually depleted the cells of ATP. We interpret this to reflect the detergent activation of the vacuolar-like ATPase, which we previously showed to activate the ATPase from *Hb. salinarium*[21]. The failure to observe this phenomenon during the proton-dependent synthesis of ATP probably reflects the time–scale of the two assays. The effect of azide on the arginine-dependent synthesis of ATP by *Hb. saccharovorum* is also shown in table 3. Table 4 summarizes the action of these various inhibitors on the proton-dependent synthesis of ATP in the other extreme halophiles. The general pattern that emerged was that, although the extent of inhibition differed, azide inhibited in every case while NEM and nitrate either enhanced the intracellular level of ATP or were without any significant effect.

Discussion

Hb. salinarium (and representative members of *Haloferax* and *Haloarcula*) synthesized ATP when the bulk medium was acidified by the addition of citrate buffer. The inhibition of ATP synthesis by CCCP, DCCD, and Triton X-100 was consistent with the supposition that ATP synthesis was the consequence of a proton-dependent process. In no case was synthesis inhibited by nitrate or NEM agents that inhibit the vacuolar-like ATPases of extreme halophiles. When these putative inhibitors had an effect, it was to enhance the steady-state levels of ATP. These elevated steady-state levels of ATP suggest the presence of two activities: a hydrolytic ATPase (inhibited by NEM and possibly nitrate) and a synthetic enzyme (unaffected by these agents).

ATP synthesis was inhibited by azide, an inhibitor of F_0F_1-ATP synthases but none of the vacuolar-like archaeal ATPases[15, 17, 18, 20, 22, 26, 28] or vacuolar ATPases[6]. Thus, the results reported earlier with *Hb. saccharovorum*[10] were not unique to that organism. What is puzzling in these observations is the discrepancy in the behavior between cells, reported here, and membrane vesicles of *Hb. salinarium*[26]. In vesicles, ATP synthesis is inhibited by NEM and nitrate (although relatively high concentrations of both are required) and is unaffected by 6.3 mM azide. The differences would not appear to be related to the organisms used in each study since the one used to demonstrate ATP synthesis in vesicles is a bacteriorhodopsin-less mutant derived from the same strain of *Hb. salinarium* employed in this study[13].

The conditions required to demonstrate ATP synthesis varied among the various genera. The two *Halobacterium* species synthesized ATP when assayed in buffer containing 4 M NaCl; the *Haloarcula* and *Haloferax* species did not. In fact, the addition of citrate resulted in the depletion of ATP. What the *Haloarcula* and *Haloferax* had in common was the relatively high intracellular concentration of ATP and an intracellular ATP pool that was rapidly depleted when cells were incubated in pH 8 buffer containing relatively high concentrations of KCl. These results are consistent with what others have observed: that the intracellular concentration of potassium ion is rapidly lost when *Hf. volcanii* is incubated in buffer lacking potassium[23]; that the depletion of intracellular potassium in *Hb. salinarium* is accompanied by the an increase in the ATP pool[24]; and in *Hf. volcanii*, potassium transport is an active process that requires ATP[23]. Therefore, we visualize that when *Haloarcula* and *Haloferax* are suspended in TNM buffer, an efflux of potassium takes place that is accompanied by the synthesis of ATP. This results in potassium-poor cells and levels of ADP too low to support

Table 3. The effect of azide on the arginine-dependent ATP synthesis in *Hb. salinarium* and *Hb. saccharovorum*

Organism	ATP (pmol/min/mg protein)		
	Control	+Azide	Triton
Hb. salinarium	377	367	11
Hb. saccharovorum	60	63	-

Hb. salinarium (1.3 mg cell protein) and *Hb. saccharovorum* (1.2 mg cell protein) were incubated for 1 h at room temperature in 25 mM Tris/4 M NaCl/10 mM $MgCl_2$ pH 8 buffer. Where indicated, 1.25 µmols of sodium azide or 0.2% Triton X-100 were also present. After incubating for 1 min at 37 °C, ATP synthesis was initiated by the addition of 10 µmols of arginine and terminated after 30 min by diluting an aliquot 1:10 in 0.2% Triton X-100. ATP was determined as described in 'Materials and methods'.

Table 4. Effect of ATPase inhibitors on ATP synthesis in *Hf. mediterranei*, *Hf. volcanii*, *Ha. hispanicum* and *Hb. saccharovorum*

Inhibitor		ATP synthesis (% of control)			
		Med.[a]	Volc[b]	Hisp[c]	Sac[d]
Methanol	(1%)	111	112	97	95
CCCP	(10 µM)	0	33	19	0
DCCD	(10 µM)	17	6	1	33
NEM	(2.5 µM)	93	114	97	168
Azide	(250 µM)	0	3	60	45

ATP synthesis was determined in 25 mM Tris/2.7 M KCl/800 mM KCl/10 mM $MgCl_2$ pH 8 buffer (*Hf. mediterranei*[a], *Hf. volcanii*[b], and *Ha hispanicum*[c]) or 25 mM Tris/4 M NaCl/10 mM $MgCl_2$ pH 8 buffer (*Hb. saccharovorum*[d]) as described in 'Materials and methods'.

ATP synthesis. When such cells are incubated in potassium-containing buffer, an ATP-dependent potassium influx occurs resulting in the production of sufficient ADP to support ATP synthesis. *Hb. salinarium* and *Hb. saccharovorum* are able to reduce their intracellular ATP pool by 'starvation' which may point to a different regulatory mechanism.

In conclusion, these studies demonstrate the proton-dependent synthesis of ATP in extreme halophiles from diverse genera, and that this synthesis is inhibited by azide but not NEM. These results would be expected if synthesis were caused by F_0F_1-ATP synthases but not the vacuolar-like ATPases so far isolated from the extreme halophiles. Therefore, we propose that ATP synthesis in the extreme halophile is brought about by an as yet to be characterized F_0F_1-ATP synthase. If so, this raises questions as to the function of the archaeal vacuolar-like ATPases absent their role as ATP synthases, and if ATP is synthesized by F_0F_1-ATP synthase in other archaeae.

Acknowledgements. This research was supported by the National Aeronautics and Space Administration's Exobiology Program on the Early Evolution of Life.

*To whom correspondance should be addressed.

1 Dane, M., Steinert, K., Esser, K., Bickelsandkotter, S., and Rodriguez-Valera, F., Properties of the plasma membrane ATPase of the halophilic archaebacteria *Haloferax mediterranei* and *Haloferax volcanii*. Z. Naturf. C47 (1992) 835–844.

2 Denda, K., Konishi, J., Oshima, T., Date, T., and Yoshida, M., Molecular cloning of the β-subunit of a possible non-F_0F_1-type ATP synthase from the acidothermophilic archaebacterium, *Sulfolobus acidocaldarius*, J. biol. Chem. *263* (1988) 17251–17254.

3 Denda, K., Konishi, J., Oshima, T., Date, T., and Yoshida, M., The membrane-associated ATPase from *Sulfolobus acidocaldarius* is distantly related to F_1-ATPase as assessed from the primary structure of its α-subunit. J. biol. Chem. *263* (1988) 6012–6015.

4 Dundas, I. E. D., and Halovorson, H. O., Arginine metabolism in *Halobacterium salinarium*, an obligatory halophilic bacterium. J. Bact. *91* (1966) 113–119.

5 Fernandez-Castillo, R., Rodriguez-Valera, F., Gonzalez-Ramos, J., and Ruiz-Berraquero, F., Accumulation of poly(β-hydroxybutyrate) by halobacteria. Appl. environ. Microbiol. *51* (1986) 214–216.

6 Forgac, M., Structure and function of vacuolar class of ATP-driven proton pumps. Physiol. Rev. *69* (1989) 765796.

7 Gogarten, J. P., and Taiz, L., Evolution of proton pumping ATPases: rooting the tree of life. Photosynth. Res. *33* (1992) 137–146.

8 Hartmann, R., and Oesterhelt, D., Bacteriorhodopsin-mediated photophorylation in *Halobacterium halobium*. Eur. J. Biochem. *77* (1977) 325–335.

9 Hartmann, R., Sickinger, H.-D., and Oesterhelt, D., Anaerobic growth of halobacteria. Proc. natl Acad. Sci. USA *77* (1980) 3821–3825.

10 Hochstein, L. I., ATP synthesis in *Halobacterium saccharovorum*: evidence that synthesis may be catalyzed by an F_0F_1-ATP synthase. FEMS Microbiol. Lett. *97* (1992) 155–160.

11 Hochstein, L. I., Kristjannson, H., and Altekar, W., The purification and subunit structure of a membrane-bound ATPase from the archaebacterium *Halobacterium saccharovorum*. Biochem. biophys. Res. Commun. *147* (1985) 295–300.

12 Hochstein, L. I., and Stan-Lotter, H., Purification and properties of an ATPase from *Sulfolobus solfataricus*. Archs Biochem. Biophys. *295* (1992) 153–160.

13 Ihara, K., Abe, T., Sugimura, K.-I., and Mukohata, Y., Halobacterial A-ATP synthase in relation to V-Atpase, J. exp. Biol. *172* (1992) 475–485.

14 Ihara, K., and Mukohata, Y., The ATP synthase of *Halobacterium salinarium (halobium)* is an archaebacterial type as revealed from the amino acid sequences of its two major subunits. Archs Biochem. Biophys. *286* (1991) 111–116.

15 Inatomi, K.-I., Characterization and purification of the membrane-bound ATPase of the archaebacterium *Methanosarcina barkeri*. J. Bact. *167* (1986) 837–841.

16 Inatomi, K.-I., Eya, S., Maeda, M., and Futai, M., Amino acid sequence of the α and β subunits of *Methanosarcina barkeri* ATPase deduced from cloned genes. J. biol. Chem *264* (1989) 10954–10959.

17 Inatomi, K.-I., Kamagata, Y., and Nakamura, K., Membrane ATPase from the aceticlastic methanogen *Methanothrix thermophila*. J. Bact. *175* (1993) 80–84.

18 Konishi, J., Wakagi, T., Oshima, T., and Yoshida, M., Purification and properties of the ATPase solubilized from membranes of an acidophilic Archaebacterium *Sulfolobus acidocaldarius*. J. Biochem., Tokyo *102* (1987) 1379–1387.

19 Kristjansson, H., Partial purification and characterization of an ATPase in the archaebacterium, *Halobacterium saccharovorum*. Dissertation (1983) University of Maryland.

20 Kristjansson, H., and Hochstein, L. I., Dicyclohexylcarbodiimide-sensitive ATPase in *Halobacterium saccharovorum*. Archs Biochem. Biophys. *241* (1985) 590–595.

21 Kristjansson, H., Sadler, M., and Hochstein, L., Halobacterial adenosine triphosphatases and the adenosine triphosphatase from *Halobacterium saccharovorum*. FEMS Microbiol. Rev. *39* (1986) 151–157.

22 Lübben, M., Lünsdorf, H., and Schäfer, G., The plasma membrane ATPase of the thermoacidophilic archaebacterium *Sulfolobus acidocaldarius*. Purification and immunological relationships to F_1-ATPases. Eur. J. Biochem. *167* (1987) 211–219.

23 Meury, J., and Kohiyama, M., ATP is required for K^+ active transport in the archaebacterium *Haloferax volcanii*. Archs Microbiol. *151* (1989) 530–536.

24 Michel, H., and Oesterhelt, D., Electrochemical proton gradient across the cell membrane of *Halobacterium halobium*: effect of N,N′-dicyclohexylcarbodiimide, relation to intracellular adenosine triphosphate, adenosine diphosphate, and phosphate concentration, and influence of the potassium gradient. Biochemistry *19* (1980) 4607–4614.

25 Mukohata, Y., Isoyama, M., and Fuke, A., ATP synthesis in cell envelope vesicles of *Halobacterium halobium* driven by membrane potential and/or base-acid transition. J. Biochem. *99* (1986) 1–8.

26 Mukohata, Y., and Yoshida, M., Activation and inhibition of ATP synthesis in cell envelope vesicles of *Halobacterium halobium*. J. Biochem. *101* (1987) 311–318.

27 Nanba, T., and Mukohata, Y., A membrane-bound ATPase from *Halobacterium halobium*: purification and characterization, J. Biochem. *102* (1987) 591–598.

28 Scheel, E., and Schäfer, G., Chemiosmotic energy conversion and the membrane ATPase of *Methanolobus tindarius*. Eur. J. Biochem. *187* (1990) 727–735.

29 Stan-Lotter, H., Bowman, E. J., and Hochstein, L. I., Relationship of the membrane ATPase from *Halobacterium saccharovorum* to vacuolar ATPase. Archs Biochem. Biophys. *284* (1991) 116–119.

30 Stan-Lotter, H., and Hochstein, L. I., A comparison of an ATPase from the archaebacterium *Halobacterium saccharovorum* with the F_1 moeity from the *Escherichia coli* ATP synthase. Eur. J. Biochem. *179* (1989) 155–160.

31 Sumi, M., Sato, M. H., Denda, K., Date, T., and Yoshida, M., A DNA fragment homologous to F_1-ATPase β subunit was amplified from genomic DNA of *Methanosarcina barkeri*. FEBS Lett. *314* (1992) 207–210.

32 Tomlinson, G. A., and Hochstein, L. I., *Halobacterium saccharovorum* sp. nov., a carbohydrate-metabolizing extremely halophilic bacterium, Can. J. Microbiol. *22* (1976) 587–591.