VOLTAGE SENSITIVITY OF THE PROTON-TRANSLOCATING ADENOSINE 5'-TRIPHOSPHATASE IN *STREPTOCOCCUS LACTIS*

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1. Introduction

The reversible, membrane-bound ATPase of bacteria couples the movements of H^+ to the synthesis of ATP [1–4]. Because this reaction is associated with the transfer of charge across a membrane, the bacterial ATPase catalyzes ATP formation when either electrical or chemical (pH) gradients are imposed [1,2,5–7], as does the ATPase of mitochondria and chloroplasts [8–11]. In principle, kinetic responses to these two different driving forces might depend on a variety of factors. Thus, in attempting to decide between many possibilities, it seems important to characterize the synthetic reaction with regard to its voltage and pH sensitivity. Work with submitochondrial particles has shown that imposed electrical or pH gradients can initiate ATP formation at rates comparable to those found during oxidative phosphorylation [11]. However, the rapid decay of these gradients made it difficult to compare their relative efficiencies in driving the reaction. Similarly, the relevant studies with chloroplasts have been performed under conditions where membrane capacitance was continually charged and discharged [12], or where only the pH gradient was measureable, and effects of membrane potential could not be examined [13,14]. Here it has been possible to explore this topic using the anaerobic bacterium, *Streptococcus lactis*. The results indicate that membrane potentials and pH gradients of equal thermodynamic value elicit identical rates of ATP formation. This suggests that inward moving protons can interact with the ATPase in a productive manner only after the full profile of the electric field has been crossed. Moreover, these results appear to exclude limiting versions of models for energy coupling in which membrane potential and pH gradient might have different mechanistic targets [15,16].

2. Materials and methods

Cells of *Streptococcus lactis* (ATCC 7962) were washed with and then placed in 0.1 M potassium phosphate (pH 8) at 13–15 mg dry wt/ml (20–25 μl cell water/ml [1]). To impose a pH gradient cells were diluted 20-fold with 0.1 M potassium phosphate (pH 8) and treated (3–5 min) with 10 μM valinomycin before adding 2 N sulfuric acid to lower external pH to $\leqslant$5.5. When only the membrane potential was varied, cells were first treated with the ionophore and then diluted 20-fold into buffer containing choline in partial replacement of potassium; 10 s after dilution, acid was added to bring outside pH to ~5.1. Vigorous mixing was ensured by suspending the vial containing diluted cells over a magnetic stirrer; external pH was measured at the end of the experiment. All work was done at 25–27°C. ATP synthesis was monitored during the first 30 s after addition of acid by removing aliquots at 6 s intervals and placing them in an equal vol. iced 0.4 N perchloric acid containing 0.08 M potassium thiocyanate. ATP content of neutralized extracts was estimated with the luciferin–luciferase reaction, essentially as in [1,6]; the mean of 4–5 trials was used to establish ATP content in each sample.

The thermodynamic contribution made by the pH gradient was calculated as $2.3RT/F$ times the difference between known initial internal pH (pH 7.6 [6]; confirmed for this work) and variable external pH. Membrane potential was estimated by applying the Nernst relationship to the ratio of constant

internal potassium (380 mM, as in [1]) and variable external potassium. It is likely that these imposed gradients were relatively stable over the time needed to assay changes of cellular ATP. At a total driving force of 200 mV, measurements of internal pH (in preparation) suggest acidification of only 0.4 pH units after 30 s; an acidification of 0.6 pH units is calculatable using known internal buffering power [17] and passive membrane conductance to H^+ [17], since at this driving force passive and coupled proton entry occur at comparable rates [1]. The overall exchange of H^+ and K^+ would not significantly alter the ratio of internal and external potassium. Other data ([1,6] and unpublished experiments) indicate that endogenous pools of ADP and phosphate were not significantly depleted under these conditions.

3. Results

Figure 1 describes results from the first and last in a series of 7 expt. In such work it was possible to reliably estimate increases of ATP as the phosphate potential was driven towards a new equilibrium position after manipulation of either the pH gradient or membrane potential. Increases of ATP were linear with time for 24–30 s when net driving forces of $\lesssim$220 mV were imposed; extrapolation to zero time indicated a common basal level of ATP. At higher driving force ($\geqslant$240 mV) linearity beyond 12–18 s was not observed. The results shown in fig.1 suggest two conclusions.

(1) There is no necessary correlation between the rate of ATP formation and the external concentration of protons, even though H^+ might be considered a participant in the overall reaction (compare open and closed triangles).

(2) Over a range in which rates of ATP formation change by ~25-fold (driving forces of 180–230 mV), rate accelerations caused by increasing the thermodynamic contribution of the pH gradient are the same as those caused by increasing the membrane potential (compare ○ and ▲).

Figure 2 summarizes these and the other experiments. The data support the earlier conclusion regarding the rate equivalence of chemical and electrical gradients in driving ATP synthesis. It is also apparent that there is an exponential relationship between total driving force and the rate of ATP formation. Within 175–230 mV this relationship suggests 1.8 H^+ translocated/molecule ATP formed, provided that the driving force does not change the velocity constant or state of activity of the ATPase, in agreement with other tests of stoichiometry (2 H^+/ATP) made in bacterial system [6,18]. Technical limitations may become important at the higher driving forces, where measured rates are still considerably less than estimated maximal velocity [1,17]. Thus:

(i) The assumed driving force may be overestimated because of rapid net proton entry accompanying accelerated ATP synthesis;

(ii) Rates of ATP appearance may be underestimated (compared to estimates at lower driving forces) by the manual sampling procedure used.

Nevertheless, even at high net driving forces there is an equivalent kinetic response to imposed electrical and chemical gradients of comparable thermodynamic

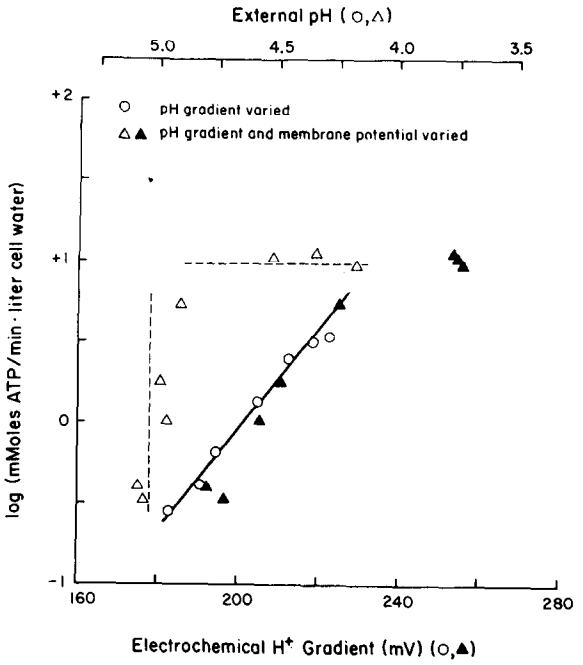

Fig.1. Dependence of the rate of ATP appearance on external pH and the electrochemical proton gradient. Symbols: (○) variable pH gradient, constant membrane potential; (△,▲) variable pH gradient and membrane potential. Two separate experiments are shown (circles and triangles). The upper abscissa is used to indicate rate as a function of external pH; the lower abscissa is used to express rate as a function of the total electrochemical proton gradient. The solid line gives the correlation established by these and other experiments (see fig.2).

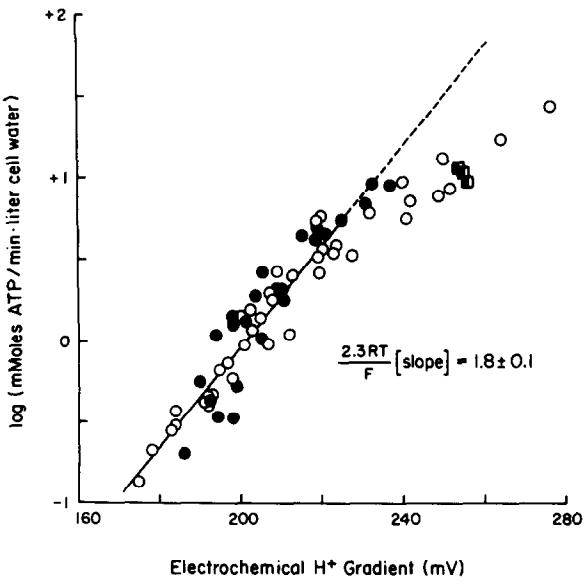

Fig.2. Rate of ATP appearance as determined by the electrochemical proton gradient. Results from 7 separate expt are presented. Symbols: (○) variable pH gradient, constant (~16 mV) membrane potential; (▲) constant pH gradient (~170 mV), variable membrane potential; (▣) variable pH gradient and membrane potential at constant (~255 mV) electrochemical proton gradient (see fig.1). At the two highest driving forces, rate was estimated from the increase over basal ATP after 6 s. The 95% confidence interval for $2.3RT/F$ times the slope of the regression line is 1.63–2.05. In any single experiment results indicating positive slope for ATP content versus time were accepted only if a positive slope was found also for the next higher driving force. This selection was necessary, since a zero or negative slope could be found for driving forces centering about the reversal potential of the ATPase. Since hydrolysis of ATP could be observed as this reversal potential was approached from below (not shown), it has been assumed that on the time scale of these experiments there is rapid release of any regulatory processes controlling ATP hydrolysis by the ATPase.

weight. Since internal pH was the same in all experiments, these results show that 10-fold increases in external $[H^+]$ yield the same rate enhancement as a 60 mV elevation of membrane potential.

4. Discussion

Here the general plan was to impose a pH gradient (~170 mV) so that the ATPase reaction would be nearly at equilibrium with the phosphate potential of the intact cell (~370 mV [unpublished]). Subsequently, increases of either the pH gradient or membrane potential served to drive ATP synthesis. It is clear that both membrane potential and pH gradient have profound effects on the rate at which the ATPase catalyzes ATP formation. In addition, as rate determining elements for the reaction, there is a simple quantitative relationship between these thermodynamic parameters. Two general classes of explanations might account for observations that ATP formation is driven by either electrical or chemical gradients [1,2,5–11].

1. There may be both voltage-sensitive (but pH-independent) and pH-responsive (but voltage-insensitive) steps, each of which might be rate limiting under appropriate conditions [15,16]. Here one must suppose that for unexplained reasons the responses of these separate events to voltage and pH are equivalent within the range explored.
2. More simply, there may be one rate limiting step, responsive to the electric field as well as external pH, and that both voltage and pH are converted into a common signal. Here, but not necessarily in 1., one could expect a regular and quantitative relationship between membrane potential and external pH as they alter the rate at which the reaction approaches equilibrium.

Biochemical studies of the proton-translocating ATPase have shown that the enzyme is divisible into two subdomains, termed F_0 and F_1 [3,4]. Based on work suggesting that the catalytic activity of F_0 represented a facilitation of transmembrane H^+ movements, Mitchell proposed [19] that in the intact enzyme this sector might behave as a 'proton well'. This idea assigns a permissive rather than instructive role to F_0, for it requires diffusion of H^+ within F_0 prior to energy coupling. In this way, H^+ diffusion within F_0 could convert a portion or all of the electrical driving force on protons into a pH differential [15,19,20]. Evidence in support of this idea is so far restricted to studies verifying that the F_0 sector, or one of its components, shows ionophoretic activity towards H^+ [21–24]. Support of a different kind is provided by the work summarized here. Thus, these results are most simply interpreted as showing that H^+ must diffuse across the entire electric field prior to energy coupling. One plausible structural corollary is that energy coupling occurs at the inner surface of

the (bacterial) membrane. This is in contrast to several other transmembrane systems, where H^+-reactive sites are only partially sensitive to the bulk electric field and may lie within the membrane [25,26].

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References

[1] Maloney, P. C. (1977) J. Bacteriol. 132, 564–575.
[2] Maloney, P. C. (1978) Biochem. Biophys. Res. Commun. 83, 1496–1501.
[3] Harold, F. M. (1977) Curr. Top. Bioenerget. 6, 83–149.
[4] Kagawa, Y. (1978) Biochim. Biophys. Acta 505, 45–93.
[5] Maloney, P. C., Kashket, E. R. and Wilson, T. H. (1974) Proc. Natl. Acad. Sci. USA 71, 3896–3900.
[6] Maloney, P. C. and Wilson, T. H. (1975) J. Membr. Biol. 25, 285–310.
[7] Wilson, D. M., Alderete, J. F., Maloney, P. C. and Wilson, T. H. (1976) J. Bacteriol. 126, 327–337.
[8] Reid, R. A. (1974) Biochem. Soc. Spec. Publ. 4, 113–129.
[9] Jagendorf, A. T. and Uribe, E. (1966) Proc. Natl. Acad. Sci. USA 55, 170–177.
[10] Witt, H. T., Schlodder, E. and Gräber, P. (1976) FEBS Lett. 69, 272–276.
[11] Thayer, W. S. and Hinkle, P. C. (1975) J. Biol. Chem. 250, 5336–5342.
[12] Gräber, P. and Witt, H. T. (1976) Biochim. Biophys. Acta 423, 141–163.
[13] McCarty, R. E. and Portis, A. R., Jr. (1976) Biochemistry 15, 5110–5114.
[14] Portis, A. R., Jr. and McCarty, R. E. (1976) J. Biol. Chem. 251, 1610–1617.
[15] Mitchell, P. and Moyle, J. (1974) Biochem. Soc. Spec. Publ. 4, 91–111.
[16] Kozlov, I. A. and Skulachev, V. P. (1977) Biochim. Biophys. Acta 463, 29–89.
[17] Maloney, P. C. (1979) J. Bacteriol. 140, 197–205.
[18] Petty, K. M. and Jackson, J. B. (1979) FEBS Lett. 97, 367–372.
[19] Mitchell, P. (1968) Chemiosmotic Coupling and Energy Transduction, Glynn Res. Ltd, Bodmin.
[20] Mitchell, P. (1974) FEBS Lett. 43, 189–194.
[21] Okamoto, H., Sone, N., Hirata, H., Yoshida, M. and Kagawa, Y. (1977) J. Biol. Chem. 252, 6125–6131.
[22] Sone, N., Yoshida, M., Hirata, H. and Kagawa, Y. (1978) Proc. Natl. Acad. Sci. USA 75, 4219–4223.
[23] Nelson, N., Eytan, E., Notsani, B.-E., Sigrist, H., Sigrist-Nelson, K. and Bitler, C. (1977) Proc. Natl. Acad. Sci. USA 74, 2375–2378.
[24] Fillingame, R. H., Knoebel, K. and Wopat, A. E. (1978) J. Bacteriol. 136, 570–581.
[25] Schwab, W. G. W. and Komor, E. (1978) FEBS Lett. 87, 157–160.
[26] Woodhull, A. M. (1973) J. Gen. Physiol. 61, 687–708.