PROTON CONDUCTANCE OF THE THYLAKOID MEMBRANE: MODULATION BY LIGHT

Mordechay SCHÖNFELD and Joseph NEUMANN
Tel Aviv University, Department of Botany, The George S. Wise Center for Life Sciences, Tel Aviv, Israel

Received 30 November 1976

1. Introduction

According to the chemiosmotic hypothesis, efficient coupling between electron transport and ATP synthesis requires a low proton-conductance of the coupling membrane, while a high conductance should be specifically associated with the ATP synthetase [1]. By analogy to dynamic electric conductance we define dynamic proton conductance, l_H, by

$$l_H = \frac{dJ_H}{dX_H}$$

where J_H is the proton flux and X_H the protonmotive force [1]. l_H is obtained from the slope of the curve of proton flux versus proton-motive force.

We have found that the proton conductance of the thylakoid membrane, is very low at low light intensities, and increases by several orders of magnitude with increase in light intensity. The sharp increase in proton conductance occurs when X_H reaches a certain threshold value. This threshold for proton conduction is related to the critical ΔpH required for ATP synthesis; the latter requirement was documented previously [2]. The results presented indicate that the increase in conductance is associated with the coupling factor of the chloroplast (CF_1), while the proton conductance of the membrane proper is negligible.

This paper forms a part of a Ph.D. thesis to be submitted to Tel Aviv University by M.S.

2. Materials and methods

Chloroplasts from lettuce leaves were prepared essentially as previously described [3], except for the final washing which was in 0.4 M sucrose, 0.01 M NaCl, 0.01 M tricine pH 8.0. Oxygen uptake was monitored with a clark type electrode, and ATP synthesis with a glass electrode [4]. ΔpH was measured by the fluorescence changes of 9-amino acridine [5], and osmotic volumes were determined according to Rottenberg et al. [6].

3. Results and discussion

The proton conductance, l_H, was obtained from the slope of the 'flux versus force' plot, where the proton flux and proton-motive force were measured at various light intensities. The proton flux in the steady state cannot be measured directly, but it can be calculated from the rate of electron transport using the accepted stoichiometry of $2H^+/e^-$ [7]. To enable a comparison with other systems, the proton flux which is usually expressed in μeq × mg chl^{-1} × h^{-1} (i.e., specific activity) was converted to electric units, i.e., ampere × cm^{-2} by dividing the specific activity by the Faraday number, and by using a value of 4×10^{13} chlorophyll molecules × cm^{-2} thylakoid membrane [8]. The protonmotive force, is given (in volts) by $X_H = \Delta\psi + 0.06\ \Delta pH$ (at room temperature). Since the contribution of $\Delta\psi$ (the membrane potential) is negligible under the conditions of continuous illumination [6,9] X_H was calculated directly from ΔpH.

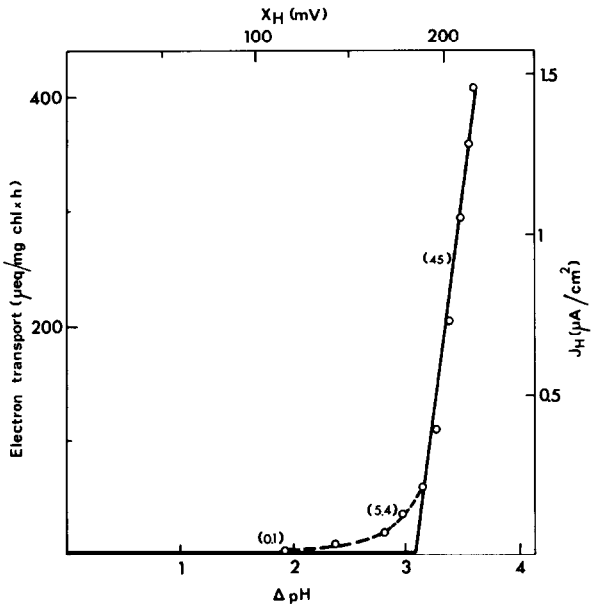

Fig.1. The dependence of electron transport on ΔpH, or alternatively a plot of J_H versus X_H. Maximal light intensity, 1.5×10^5 erg × cm^{-2} × s^{-1}. Lower light intensities were obtained by using calibrated metal screens. The reaction mixture contained 30 mM NaCl, 4 mM P_i, 2 mM $MgCl_2$, 0.2 mM methyl viologen, 1 mM NaN_3, 30 mM tricine at pH 8.1 and chloroplasts equivalent to 20 μg/ml. for ΔpH determination it contained in addition 1 μM 9-amino acridine. The solid lines present a linear approximation of the curve.

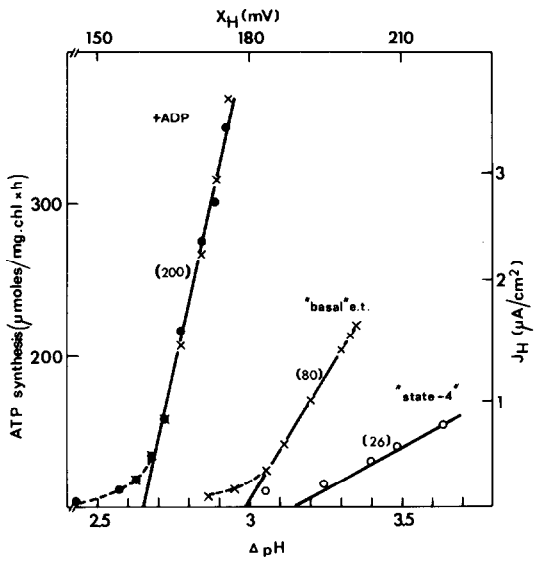

Fig.2. The effect of ATP synthesis on the proton 'flux versus force' plot. Conditions as in fig.1 except that 0.6 mM ADP were added where indicated. Under 'State-4' conditions readings were taken at the different light intensities after 25 μM ADP had been phosphorylated to completion at full light intensity and electron transport declined to the controlled steady rate. Full dots along the (+ADP) line are parallel readings of rates of ATP-synthesis in this experiment.

In fig.1 electron transport is plotted versus ΔpH; electron transport and ΔpH were measured in parallel experiments at various light intensities. The values of J_H and X_H are presented also in electric units. The numbers in parentheses in the body of the figure, denote the proton conductance in μmho × cm^{-2}, as calculated from the slope of the curve at selected points. The curve obtained is not linear, and l_H is therefore not constant. At low light intensities, large increments in X_H are associated with small increments of J_H and the proton conductance is thus very low. The conductance is several orders of magnitude higher at high light intensities, where small increments in X_H are associated with large increments of J_H. A sharp increase in l_H, is seen at ΔpH≈3 (compare with [10]).

Despite the complex dependence of J_H on X_H (compare with [11]), the curve can be closely approximated by two straight lines as shown by the solid lines in fig.1. Using this approximation it can be concluded that the proton conductance below a 'ΔpH-treshold' of approximately 3 units is negligible, whereas above the threshold the conductance is high.

Figure 2 shows the effect of phosphorylation on the 'flux versus force' plot. The addition of ADP which initiated phosphorylation, increased markedly the proton conductance and also lowered the ΔpH-threshold for the increase in proton conduction. On the other hand, when ATP synthesis stops due to ADP exhaustion, i.e., under 'State-4' conditions [12], the proton conductance is markedly lowered, and the threshold is shifted to a higher ΔpH.

In this experiment we measured also the rates of ATP synthesis, which were plotted ás a function of ΔpH (fig.2). The curve obtained is not linear (supporting previous results [11,13]). However, as

seen it renders itself to a linear approximation similar to that shown in fig.1. The 'phosphorylation versus ΔpH' curve, coincides with the 'J_H versus ΔpH' curve down to a common ΔpH-threshold, strongly indicating that the threshold for proton conduction, and the ΔpH-threshold reported for ATP synthesis [2], are two facets of the same phenomenon.

We found that DCCD and Dio-9, which are known to interact with CF_1 also reduced the proton conductance about 6- to 7-fold.

Taken together these results indicate that the coupling factor which catalyzes ATP synthesis is responsible for the high proton conductance (above ΔpH of 3). The coupling factor was previously implicated in proton conduction both by theoretical consideration [1], and experimental evidence [14].

Since the high proton conductance mediated by CF_1 is obtained only above the ΔpH-threshold, it is possible to calculate a maximal value for the proton conductance of the membrane proper when ΔpH is maintained below this threshold (fig.1). The conductance below the threshold, is less than 0.1 μmho × cm^{-2}, which is about three orders of magnitude lower than the conductance above the threshold. This value of proton conductance of the thylakoid membrane is of the same order of magnitude as that of the proton conductance of the inner membrane of rat liver mitochondria (0.45 μmho × cm^{-2}) as measured by Mitchell and Moyle by a different method [15].

The linearized 'flux versus force' plot (figs.1, 2) can be described by a simple equation:

$$J_H = L_H (X_H - X_H^{th}) \quad \text{for } J_H > 0$$

where X_H^{th} is the threshold for proton conduction, and L_H is the conductance above the threshold. An equivalent electric circuit, (which is characterized by a similar 'current versus voltage' plot and a similar equation) is given in fig.3. The electric circuit is superimposed in this figure on a model of the thylakoid membrane. The proton pump is represented by an electric cell, and CF_1 by a resistor in series with a reverse biased voltage source. As evident from fig.3 and the above equation the threshold for proton conduction could be due to the presence of a reverse-biased proton-motive force, which would act as a potential barrier for outgoing protons in the CF_1-

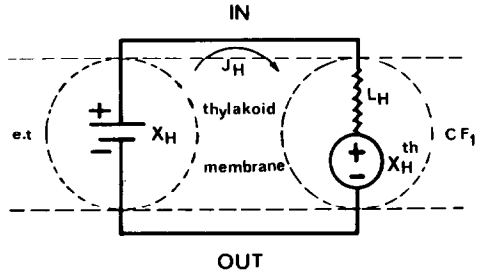

Fig.3. An equivalent electric circuit for the proton flux in chloroplasts (superimposed on a rough scheme of the membrane). See text for details. A rectifying device should have been added to the electric circuit, but was omitted for simplification.

controlled proton channel. Proton conduction through this channel will take place only when X_H is increased above this potential barrier. An opposing force of such a kind, is in fact expected from the non-equilibrium thermodynamic description of the coupling between proton transport and ATP synthesis [16]. The opposing force according to this theory should be proportional to the 'phosphate-potential' of the phosphorylating reaction. It was indeed qualitatively seen in fig.2, that addition of ADP which lowers the phosphate potential, lowered the threshold for proton conduction while the transition to State-4 in which the phosphate potential is increased, was accompanied by an increase in the threshold.

The flux–force characteristics (figs.1,2) indicate a stabilization of X_H above the threshold, very similar to the voltage stabilization by a 'zener-diode'. The zener-diode owes its stabilizing properties to the potential barrier at the PN junction which permits only low conductance below the threshold voltage, and a very high conductance above it. One can rationalize the physiological significance of a similar stabilzing mechanism for X_H. The low proton conductance of the membrane, allows the formation of a high X_H (necessary for ATP synthesis [2], even at low light intensities, when the proton flux is low. On the other hand, the high conductance above the threshold, allows a large increase in J_H with increasing light intensities, without much increase in ΔpH, thus preventing an intolerable decrease of the internal pH and an inhibition of electron transport [17].

References

[1] Mitchell, P. (1966) Biol. Rev. Cambridge Phil. Soc. 41, 445–502.
[2] Pick, U., Rottenberg, H. and Avron, M. (1974) FEBS Lett 48, 32–36.
[3] Nelson, N., Drechsler, Z. and Neumann, J. (1970) J. Biol. Chem. 245, 143–151.
[4] Chance, B. and Nishimura, M. (1967) in: Methods in Enzymology (Estabrook, R. W. and Pullman, M. E. eds), Vol. 10 pp. 641–650, Academic Press, N.Y.
[5] Schuldiner, S., Rottenberg, H. and Avron, M. (1972) Eur. J. Biochem. 25, 64–70.
[6] Rottenberg, H., Grunwald, T. and Avron, M. (1972) Eur. J. Biochem. 25, 54–63.
[7] Trebst, A. (1974) Ann. Rev. Plant Physiol. 25, 423–458.
[8] Wolken, J. J. (1963) in: Photosynthetic mechanisms in green plants pp. 575–586, PNAS/NRC, Washington DC.
[9] Stricharz, G. R. and Chance, B. (1972) Biochim. Biophys. Acta 256, 71–84.
[10] Portis, A. R., Magnusson, R. P. and McCarty, R. E. (1975) Biochem. Biophys. Res. Commun. 64, 877–884.
[11] Graber, P. and Witt, H. T. (1976) Biochim. Biophys. Acta 423, 141–163.
[12] West, K. R. and Wiskich, J. T. (1968) Biochem. J. 109, 527–532.
[13] Portis, A. R. and McCarty, R. E. (1974) J. Biol. Chem. 249, 6250–6254.
[14] Jagendorf, A. T. (1975) in: Bioenergetics of Photosynthesis (Govindjee, ed) pp. 413–492, Academic Press, NY.
[15] Mitchell, P. and Moyle, J. (1967) Biochem. J. 104, 588–600.
[16] Rottenberg, H. (1976) in: Progress in Surface and Membrane Science (Danielli, J. F., Cadenhead, A. and Rosenberg, M. D. eds) Academic Press, NY., in press.
[17] Bamberger, E. S., Rottenberg, H, and Avron, M. (1973) Eur. J. Biochem. 34, 557–563.