Localised chemiosmosis and active transport of lactose in *Escherichia coli*

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Received 28 March 1984

The phosphorylation potential (ΔG_p) in mitochondria has been found to be relatively insensitive to changes in the protonmotive force (Δp). Similar observations have been made for lactose transport in *Escherichia coli*. Localised chemiosmosis has been invoked to explain these data. Here a postulate of localised chemiosmosis was put to experimental test and found to be incorrect. Alternative explanations for the Δp-dependence of lactose transport are presented.

Localised chemiosmosis *Protonmotive force* *Lactose transport* *Stoichiometry*
Escherichia coli

1. INTRODUCTION

The chemiosmotic hypothesis places the osmotically active H^+ in a central position in membrane bioenergetics. The electron transport chain is seen to transduce the chemical energy available in compounds such as NADH and succinate into the translocation of H^+ across membranes. Since biological membranes are generally impermeable to ions an electrochemical gradient of H^+ ($\Delta\tilde{\mu}_{H^+}$) is generated. The $\Delta\tilde{\mu}_{H^+}$ can then be used to drive endergonic membrane-linked reactions (e.g., active transport, ATP-synthesis and chemotaxis). $\Delta\tilde{\mu}_{H^+}$ is composed of two parameters, the membrane potential ($\Delta\psi$) and the pH gradient (ΔpH):

$$\Delta\tilde{\mu}_{H^+} = F\Delta\psi - RT\ln\Delta\mathrm{pH} \quad (1)$$

where R is the gas constant and T the absolute temperature. $\Delta\tilde{\mu}_{H^+}$ can be related to the protonmotive force through the Faraday constant.

$$\Delta p = \Delta\tilde{\mu}_{H^+}/F \quad (2)$$

The above concepts of chemiosmotic coupling are now well established [1,2]. However, there is still much controversy with regard to the route the H^+ takes from the Δp-generating reaction to the Δp-consuming reaction. In other words, does the H^+ cycle occur mainly through localised membranous pathways or mainly through the bulk aqueous phases?

One of the main observations that lends support to the hypothesis of localised chemiosmosis is that the phosphorylation potential (ΔG_p) has been found to be relatively insensitive to changes in Δp. Thus at low Δp values high (>3) H^+/ATP stoichiometries would have to be invoked to explain the data [3–5]. We have observed a similar phenomenon with lactose transport in *E. coli*. Δp_{lac} (the electrochemical gradient of lactose expressed in mV) was also found to be relatively insensitive to changes in Δp [6]. The results in [3–5] cannot be accommodated within the chemiosmotic coupling hypothesis. To explain the Δp-dependence of ΔG_p authors in [5] have postulated the existence of energy transducing supercomplexes. That is, the respiratory chain and the ATP-synthase (or other Δp-consuming proteins) are closer to each other than to sites in the membrane through which H^+-leakage may occur (fig.1). In addition, the H^+-current that connects

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00145793/84/$3.00

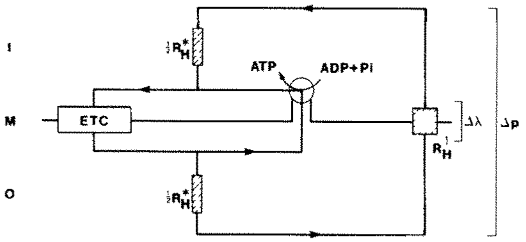

Fig.1. The Van Dam formulation of localised chemiosmosis (after [7]). In the Van Dam formulation of localised chemiosmosis it is envisaged that the electron transport chain (ETC) and the ATP-synthase exist as a supercomplex. H^+ leakage ($R^{\mathrm{I}}_{\mathrm{H}}$) is thought to be, on average, farther away from the electron transport chain than the ATP-synthase. $R^{\mathrm{I}}_{\mathrm{H}}$ and R^{*}_{H} represent the resistance met by H^+ trying to enter the membrane phase (M) and bulk phases (O and I), respectively. $\Delta\lambda$ is the localised protonmotive force and Δp the bulk phase protonmotive force. Δp is out of equilibrium with $\Delta\lambda$ by a factor $(1 + R^{*}_{\mathrm{H}}/R^{\mathrm{I}}_{\mathrm{H}})^{-1}$.

the two components of the supercomplex meets a resistance if the H^+ attempt to equilibrate with the external or internal bulk aqueous phases. Quantitatively the localised Δp ($\Delta\lambda$) has been postulated to be out of equilibrium with Δp by a factor $1/(1 + R^{*}_{\mathrm{H}}/R^{\mathrm{I}}_{\mathrm{H}})$;

$$\Delta p = \Delta\lambda/(1 + R^{*}_{\mathrm{H}}/R^{\mathrm{I}}_{\mathrm{H}}) \qquad (3)$$

where R^{*}_{H} and $R^{\mathrm{I}}_{\mathrm{H}}$ represent the resistance met by H^+ attempting to enter the membrane and bulk phases, respectively. In chemiosmotic terms Δp can be related to ΔG_{p} through n (the H^+/ATP stoichiometry);

$$\Delta G_{\mathrm{p}} = n\Delta p \qquad (4)$$

However, if $\Delta\lambda$ is the real intermediate of energy coupling then we have,

$$\Delta G_{\mathrm{p}} = n(1 + R^{*}_{\mathrm{H}}/R^{\mathrm{I}}_{\mathrm{H}})\Delta p \qquad (5)$$

$$= n\Delta\lambda \qquad (6)$$

The experiments mentioned above used an uncoupler-mediated increase in the H^+ permeability of the membrane to decrease Δp [3–6]. An increase in the H^+ permeability of the membrane would lead to a reduction in $R^{\mathrm{I}}_{\mathrm{H}}$. From eq.5 it can be seen that this will give rise to an ever-increasing value for n as Δp (and $R^{\mathrm{I}}_{\mathrm{H}}$) are reduced.

The prediction then is that if Δp is decreased without reducing $R^{\mathrm{I}}_{\mathrm{H}}$, the Δp-dependence of ΔG_{p} (or n) should be different. This prediction has been tested and found invalid for ATP-synthesis in mitochondria [7]. The aim of this study was to determine whether the Van Dam formulation of localised chemiosmosis [6,7] could explain the Δp-dependence of lactose accumulation in *E. coli*.

2. MATERIALS AND METHODS

2.1. *Strains and growth conditions*

E. coli K207 (*laci*$^-$*z*$^-$*y*$^+$*a*$^+$*hemA*$^-$) was supplied by Professor Dr P. Overath, Max-Planck-Institut für Biologie (Tübingen, FRG). It was stored on agar slopes of minimal medium 63 containing (g/l), maltose (40), $MgSO_4$ (0.25), casamino acids (3) and 30 μM 5-aminolevulinic acid at 4°C. For experiments, *E. coli* K207 was grown aerobically on medium 63 with the above supplements at 37°C. The culture was washed 3 times and an anaerobic culture grown to an A_{650} of 2.0. The cells were then reinoculated into 2 l of media at an A_{650} of 0.02 and grown anaerobically to A_{650} 1.0 before use in experiments. Cultures of *E. coli* K207 were routinely assayed for revertants on minimal succinate plates.

2.2. *Cell preparation*

After harvesting the cells were washed once in 120 mM Tris–HCl (pH 8.0) and treated with EDTA as in [8]. EDTA-treated cells were washed once and resuspended to 10–20 mg dry wt/ml in 5 mM Tris/5 mM Mes/150 mM choline chloride/0.2 mM KCl (pH 7.5). Valinomycin was then added to a final concentration of 4 μg/mg dry wt. For some experiments cells were then treated with DCCD (freshly prepared ethanolic solution) and the cells incubated for 1 h at 37°C. After the 1 h incubation the cells were harvested, washed twice, and resuspended to a final concentration of 1 mg dry wt/ml in the above buffer.

2.3. *Measurement of $\Delta\psi$, ΔpH and Δp_{lac}*

$\Delta\psi$, ΔpH and Δp_{lac} were measured using centrifugation-based assays. Cells at 1 mg dry wt/ml were incubated at 25°C in a stirred glass vessel for 5 min. Radioactive probes were then added; $^{86}Rb^+$ (5 μM; 0.2 μCi/ml) for $\Delta\psi$, [^{14}C]benzoate (2 μM; 24.4 Ci/mol) for ΔpH and

[^{14}C]lactose (5 μM; 57.7 Ci/mol) for Δp_{lac} along with catalase (250 μg/ml) and ^3H_2O (1–2 μCi/ml). After approx. 20 min incubation with probes, 0.5 ml samples were removed and transferred into Eppendorf centrifuge tubes (1.6 ml capacity) containing 10 μl of 2-volume H_2O_2. The tubes were then centrifuged at 12000 × g for 20 s. The radioactivity in the pellets and supernatants was assayed and $\Delta\psi$, ΔpH and Δp_{lac} calculated as in [12]. The measurements for all 3 parameters were made in triplicate and represent averages for 3–4 experiments.

2.4. *Cytoplasmic volumes*

The cytoplasmic volume has been shown to be a significant experimental variable [9]. Here the cytoplasmic volume was measured using [^{14}C]sucrose and ^3H_2O as in [10,12]. The cytoplasmic volume was not affected by variations in pH$_o$ or DCCD concentration and was found to be 1.1 μl/mg dry wt.

2.5. *Materials*

Materials were obtained as in [6,10].

3. RESULTS

To reduce Δp without reducing R_H^l a mutant strain (*E. coli* K207) derived from *E. coli* ML308-225 (*E. coli* ML308-225 was used in [6] to ascertain how reductions in Δp affected Δp_{lac}) was used. *E. coli* K207 lacks the ability to synthesize cytochromes when grown in the absence of 5-aminolevulinic acid [11]. Therefore it can only generate a protonmotive force by ATP-hydrolysis. Two methods were used to reduce Δp: firstly, by variation of pH$_o$ (external pH) and secondly, by using DCCD. Increasing pH$_o$ from 5.5 to 7.5 has been shown to reduce ΔpH to near zero and increase $\Delta\psi$ by some 50–60 mV [6]. Similar results were obtained with *E. coli* K207 (not shown). However, the absolute magnitude of Δp was lower by some 80–100 mV. In addition, Δp was found to be relatively independent of pH$_o$. Δp_{lac} was also found to be independent of pH$_o$ and the calculated stoichiometry was near one (fig.2). These results confirm earlier findings that Δp_{lac} is not dependent on pH$_o$ or the composition of Δp but is dependent on the absolute magnitude of Δp [12]. Addition of DCCD (10–100 μM) blocks the extrusion of H$^+$ via the ATPase leading to a reduction in Δp (from 130 to 20 mV). Measurement of Δp_{lac} allowed calculation of the H$^+$/lactose stoichiometry. As can be seen from fig.2 the Δp-dependent of n ($\Delta p_{lac}/\Delta p$) is independent of the way in which Δp is reduced (cf. dotted line to open circles).

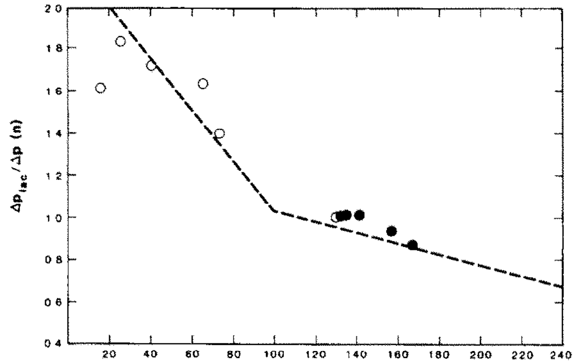

Fig.2. Δp-dependence of Δp_{lac} in *E. coli* K207. $\Delta\psi$, ΔpH and Δp_{lac} were measured as described in section 2.3. The dotted line represents $\Delta p_{lac}/\Delta p$ (n) values as a function of Δp obtained by increasing the H$^+$ permeability of the membrane (reducing R_H^l). (●) Δp reduced by increasing pH$_o$. (○) Δp reduced by using DCCD.

4. DISCUSSION

The data presented here show that the Van Dam formulation of localised chemiosmosis [5] cannot be invoked to explain the Δp-dependence of n and Δp_{lac}. These data represent the first experimental evidence against a postulate of localised chemiosmosis for secondary active transport processes. Similar results to those presented here have been obtained in [7] for ATP-synthesis in mitochondria. In [7] Δp was reduced by inhibiting respiration while we have used inhibition of H$^+$-extrusion in an *E. coli* strain (K207) which lacks the ability to synthesize cytochromes when grown in the absence of 5-aminolevulinic acid.

The main problem in distinguishing whether Δp or $\Delta\lambda$ is the true intermediate in energy coupling is an experimental one. To the author's knowledge there is no direct method which can be used to distinguish between the two possible intermediates. An indirect method recently used for this purpose is that of double titration [13]. Using this approach, authors in [13–16] found that partial inhibition of the ATP-synthase increased the potency of uncouplers to inhibit ATP-synthesis. This was interpreted as evidence for $\Delta\lambda$ as the intermediate in energy coupling. More recently authors in [17] have shown that the results presented in [13–16] were most probably artifacts due to the experimental protocol used to measure the effects of uncoupler on ATP-synthesis. Using a corrected procedure it was found that partial inhibition of the ATP-synthase did not alter the potency of uncouplers in inhibiting ATP-synthesis [17].

In conclusion, the results of this study do not provide support for the hypothesis of localised chemiosmosis. Alternative explanations for the Δp-dependence of n and Δp_{lac} could be:

(i) That factors other than the lac permease contribute to the measured Δp_{lac}. For instance, it could be argued that the lactose uptake occurs via systems other than the lac permease. However, genetic results indicate the product of the *Y* gene to be the only route for lactose uptake in the concentration range studied [18]. There is a possibility that the [^{14}C]lactose used is contaminated by [^{14}C]glucose leading to an artificially high Δp_{lac}. To explain a stoichiometry of 2, the [^{14}C]glucose contamination would have to be as high as 50%. This is very unlikely for two reasons. Firstly, all the accumulated label can be released by dissipation of the protonmotive force. Secondly, the [^{14}C]lactose can be used to measure the cytoplasmic volume of strains lacking the ability to transport lactose. Measurement of cytoplasmic volumes is very sensitive to contamination. Indeed, commercially available [^{14}C]sucrose cannot be used to measure cytoplasmic volumes without prior purification. Here the [^{14}C]glucose contamination is probably no more than 5–10%.

(ii) Methods for measuring $\Delta\psi$ and ΔpH may be erroneous especially at low values of Δp such that they significantly underestimate Δp. Thus, the Δp-dependence of Δp_{lac} may just be a reflection of the increasing difficulty in measuring Δp as Δp is reduced. The indirect methods used here to measure ΔpH and $\Delta\psi$ have been corroborated by the use of ^{31}P-NMR [19] and microelectrodes in giant *E. coli* cells [20]. In addition, a wide variety of controls have been carried out to validate the use of lipophilic cations and weak acids in *E. coli* [12]. Nevertheless, it is still possible that at low values of Δp (where n rises to 2), that the assumptions inherent in the indirect measurements are invalid. For example, the A^- form of the weak acid may become permeable at low Δp. Or there may be a significant amount of Rb^+ bound to cellular components. However, this phenomenon would lead to an overestimation of Δp, leading to an even larger disparity between Δp and Δp_{lac}.

(iii) The stoichiometry of lactose transport may be variable. The apparent increase in stoichiometry to 2 as Δp is reduced from 270 to 20 mV could be due to: (a) a Δp-dependent change in stoichiometry with a small degree of slip (transport of solute without H^+); or (b) an invariant stoichiometry of 2 with a greater degree of slip. A detailed discussion of the stoichiometry of secondary active transport is really outside the scope of this paper and will be presented elsewhere (in preparation). However, at the moment this possibility seems the most likely.

ACKNOWLEDGEMENTS

The author would like to thank Drs Ian Booth and Keith Wright for helpful advice. Thanks are also due to SERC (grant GR/A.99299) and EMBO for financial support.

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