On the role of the membrane proton conductance in the relationship between rate of respiration and protonmotive force

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(Received 9 April/30 May 1985; accepted 6 June 1985)

The rate of respiration in mitochondria is not a unique function of the protonmotive force, depending on whether the protonmotive force is varied by addition of ADP or uncouplers. This result has been generally considered to contradict the chemiosmotic theory. Recently, O'Shea & Chappell [*Biochem. J.* (1984) **219**, 401–404] claimed that this observation can be reconciled with the chemiosmotic theory, provided only that the proton conductance of the membrane is different in the presence of ADP or uncouplers. This hypothesis is shown here to be necessary but not sufficient to account for the experimental data and the reason for the contradiction between this recent interpretation and earlier interpretations is pointed out.

The general principle of chemiosmotic coupling, as first formulated by Mitchell (1961), has now been widely accepted; it is generally agreed that energy conservation in mitochondria, chloroplasts and bacteria is accompanied by the formation of a proton electrochemical potential difference (or protonmotive force, Δp), which is in turn utilized to drive ATP synthesis (Boyer *et al.*, 1977). Nevertheless, a certain number of results (see, for reviews, Westerhoff *et al.*, 1984; Ferguson, 1985) do not seem to be in accordance with the standard delocalized chemiosmotic hypothesis. This led to the proposal of several modifications of the theory (Westerhoff *et al.*, 1984).

One of the most significant observations that seems to argue against the original formulation of chemiosmosis is the fact that when ADP or varying amounts of protonophores are added to mitochondria, the measured respiratory rate is much higher with ADP than with the amount of protonophore which lowers Δp to the same extent (Padan & Rottenberg, 1973; Azzone *et al.*, 1978; Zoratti *et al.*, 1981). This observation has been considered to indicate that chemiosmosis does not give a full description of oxidative phosphorylation (Padan & Rottenberg, 1973; Zoratti *et al.*, 1981; Westerhoff *et al.*, 1984), unless it is envisaged the possibility of 'allosteric' interactions (i.e. not

energy-transducing) between ADP (or uncouplers) and the respiratory chain (Westerhoff *et al.*, 1984).

In contrast, very recently, O'Shea & Chappell (1984) claimed that these results can be reconciled with the chemiosmotic theory, provided only that the rate of proton conductance through the membrane is not the same when it is catalysed by the reversible ATPase or by uncouplers. The reason for the contradiction between their conclusion and earlier interpretations of the chemiosmotic theory is not straightforward. In view of the confusion that contradictory interpretations of the data may bring, a detailed discussion of the hypothesis of O'Shea & Chappell (1984) appears necessary. The object of this paper is not to reiterate previous arguments, but rather to express fully the proposal of O'Shea & Chappell (1984) within the framework of the standard chemiosmotic theory, in order to determine the reason for this contradiction. In order to do this, the hypothesis of O'Shea & Chappell (1984), which was only stated qualitatively, is expressed quantitatively, and the experiments of ' Δp titration' with ADP or uncouplers are simulated with a simple electrical circuit, analogous to the protonic circuit across the membrane, which reflects the basic assumptions of the standard delocalized chemiosmotic theory. It is shown that the possibility of different conductivities of the membrane, in the presence of ADP or uncouplers, is not sufficient to account for these observations within the logic of the chemiosmotic hypothesis.

Abbreviation used: FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone.

Membrane conductance and the chemiosmotic proton circuit

The experimental results under consideration, as obtained by several workers (Padan & Rottenberg, 1973; Azzone *et al.*, 1978; Zoratti *et al.*, 1981; O'Shea & Chappell, 1984), are summarized in Tables 1 and 2 of O'Shea & Chappell (1984). When Δp is decreased from 190 mV to 180 mV by controlled addition of ADP or of the protonophore FCCP, the rate of respiration increases from some 11.6 nmol of O_2 /min per mg of protein to 89 or 12.4 nmol of O_2 /min per mg of protein respectively. Alternatively, when respiration is increased from 12 to 100 nmol of O_2 /min per mg of protein by adequate addition of ADP or FCCP, Δp is decreased to 150 mV in the one case (ADP) and to 0 mV in the other case (FCCP). Thus, to the same rate of respiration correspond different Δp values and to the same Δp value correspond different rates of respiration, depending upon the agent used to increase respiration and decrease Δp .

While the possibility of an experimental artifact (such as an artifactual collapse of $\Delta\psi$ in the presence of FCCP during the anoxic period associated with the separation of the mitochondria from the incubation medium) cannot be completely ruled out, it will not be considered here.

Let us first summarize the reasoning of O'Shea & Chappell (1984). The outward flux of protons across the respiratory chain, $J_{\text{gen.}}$, which generates Δp , is given by

$$J_{\text{gen.}} = J_{\text{ox.}} \cdot n$$

where $J_{\text{ox.}}$ is the rate of oxygen consumption and n the number of protons translocated per oxygen atom consumed. It is assumed here that n is constant. At steady state, $J_{\text{gen.}}$ is equal to $J_{\text{diss.}}$, the inward flux of protons, which dissipates Δp and which is a function of Δp through L , the proton conductance. Thus:

$$J_{\text{ox.}} \cdot n = J_{\text{gen.}} = J_{\text{diss.}} = L \cdot \Delta p \quad (1)$$

In fact, L is not independent of Δp , since the dependence of $J_{\text{diss.}}$ on Δp is highly non-linear (Nicholls, 1974, 1977; Clark *et al.*, 1983; Krishnamoorthy & Hinkle, 1984). It would be certainly better to write

$$J_{\text{diss.}} = f(\Delta p)$$

instead of eqn. (1), which can be misleading by implying linearity. Keeping this in mind, the above equation will nevertheless be used as in O'Shea & Chappell (1984).

Addition of ADP or FCCP to the mitochondrial suspension leads to an increase in L , and, thus, to a

decrease in Δp and to a concomitant increase in $J_{\text{ox.}}$.

O'Shea & Chappell (1984) propose that the rate of proton conductance is not the same if it is catalysed by the reversible ATPase or by the proton ionophore FCCP. They claim that the experimental results summarized above can be explained if it is assumed that: "under the conditions of complete uncoupling by carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone, the proton conductance catalysed by the uncoupler is higher than that catalysed by the ATPases, and thus the steady-state Δp is much smaller. Alternatively, under conditions whereby the Δp is titrated with the two reagents but the Δp is maintained at a relatively high value ... the proton conductance due to the ATPase is much higher than that due to the uncoupling with carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone. Thus the ADP-stimulated rate of respiration is also much higher than that of the uncoupler-stimulated mitochondria."

It is further stated that: "only if the same proton conductance were induced fortuitously at the same Δp would the same rate of respiration result."

The hypothesis of O'Shea & Chappell (1984) implies that the curve $J_{\text{diss.}}$ versus Δp is different for a mitochondrial suspension in the presence of a given concentration of ADP, or of FCCP. Fig. 1 illustrates the proposal of O'Shea & Chappell (1984) by a possible example of the relationship between $J_{\text{diss.}}$ and Δp for two different concentrations of ADP or FCCP. Here, for the sake of simplicity, L_{FCCP} (the conductance of the membrane for a given concentration of FCCP) is taken to be constant (see Krishnammoorthy & Hinkle, 1984), while the relationship $J_{\text{diss.}}$ versus Δp , in the presence of ADP, is non-linear, and thus L_{ADP} (the conductance of the membrane in presence of a given concentration of ADP) varies with Δp (see Clark *et al.*, 1983). Apparently, as stated by O'Shea & Chappell (1984), a high Δp value corresponds to different $J_{\text{diss.}}$ values in the case of mitochondria incubated in the presence of ADP or FCCP, while at low Δp values, one value of $J_{\text{diss.}}$ corresponds to different values of Δp . Nevertheless, the consideration of a single relationship between $J_{\text{diss.}}$ and Δp does not allow determination of the actual values of $J_{\text{diss.}}$ and Δp ; this is only because the mitochondrial membrane, envisaged as a resistor of conductance L_{ADP} or L_{FCCP} , is considered independently of the whole proton circuit in which it is inserted.

I consider here the simple proton circuit of the delocalized chemiosmotic theory, analogous to an electrical circuit in which the generator is the respiratory chain and the external resistor is the membrane. The e.m.f. and the internal resistance of the generator are easily derived.

The chemiosmotic theory states that $J_{\text{ox.}}$ is only a

Fig. 1. *Membrane conductance in mitochondria*
A possible example of the effects on the membrane conductance of two increasing concentrations of ADP (ADP_1 and ADP_2) and of two increasing concentrations of FCCP ($FCCP_1$ and $FCCP_2$), illustrating the proposal of O'Shea & Chappell (1984).

function of Δp and of the affinity of the redox reaction, A_{ox} :

$$J_{ox} = g(\Delta p, A_{ox})$$

For the sake of simplicity, a linear relationship between flows and forces will be assumed. As discussed below, this is in no way essential to the demonstration. According to a non-equilibrium thermodynamics formulation of the chemiosmotic theory (Caplan & Essig, 1969), the rate of respiration is given by:

$$J_{ox} = L_{oh} \cdot \Delta p + L_o \cdot A_{ox}$$

and thus:

$$J_{diss} = n \cdot J_{ox} = n \cdot L_{oh} \cdot \Delta p + n \cdot L_o \cdot A_{ox} \quad (2)$$

where A_{ox} can be considered to be constant during the experiment provided that the mitochondria are sufficiently energized and aerated. The basic principle of chemiosmosis, i.e. that the redox and the ATPase H^+ pumps are interconnected only by Δp , implies that J_{ox} is not related to A_p , the affinity

Fig. 2. *The proton circuit across the mitochondrial membrane*
The symbols are defined in the text.

of the phosphorylation reaction (there is no term such as $L_{op} A_p$). Moreover, in the original chemiosmotic hypothesis, which is the one considered by O'Shea & Chappell (1984), ADP and uncouplers are not supposed to interact with the respiratory chain so that L_{oh} and L_o are independent of the addition of ADP or FCCP.

Eqn. (2) can be written:

$$\Delta p = -L_o \cdot A_{ox} / L_{oh} + J_{diss} / n \cdot L_{oh}$$

The analogy with an electrical circuit shows that the respiratory chain can be considered as a generator of an e.m.f., $E = -L_o \cdot A_{ox} / L_{oh}$, and of an internal conductance $-n \cdot L_{oh}$. Fig. 2 gives the analogous electrical circuit across the membrane: the only effect of the addition of ADP or FCCP is to change the conductance of the external resistor (the membrane) from a value L to a value L_{ADP} or L_{FCCP} respectively, the other parameters of the circuit (E and the internal resistance) being independent of the presence of ADP or FCCP.

The value of the current, J_{diss} , in the circuit and of Δp across the membrane, which are determined by the values of E , $-n \cdot L_{oh}$ and L_{ADP} or L_{FCCP} , are obtained graphically by the intercept of the relevant curve J_{diss} versus Δp given by eqn. (1) (for a given concentration of ADP or FCCP) and of the line of the equation:

$$J_{diss} = n \cdot L_{oh} \cdot \Delta p + n \cdot L_o \cdot A_{ox} \quad (\text{Fig. 3}).$$

Clearly, when Δp is fixed by an adequate addition of ADP or FCCP, J_{diss} is completely determined by E and L_{oh} and can only have a single value. Alternatively, to a fixed value of J_{diss} , obtained either in the presence of ADP or FCCP, must correspond only one value of Δp . Fig. 3 shows what happens when Δp is titrated by ADP or

Fig. 3. 'Titration' of Δp at high Δp values. Titration of Δp by addition of ADP (curve A) and of three increasing concentrations of FCCP (lines B₁, B₂ and B₃), according to the logic of the chemiosmotic proton circuit.

FCCP, at high Δp values. Let us suppose that a given concentration of ADP results in a Δp of 180 mV and a $J_{diss.}$ of 100 (arbitrary units) corresponding to a value of 89 nmol of O₂/min per mg of protein for $J_{ox.}$ (curve A). Addition of increasing concentrations of FCCP to similar suspensions of mitochondria results in decreasing values of Δp (curves B₁ and B₂). When a value of 180 mV for Δp is obtained for a given concentration of FCCP, it should correspond to a value of 100 for $J_{diss.}$ or to a value of 89 nmol of O₂/min per mg of protein for $J_{ox.}$ (curve B₃); it may be seen that an identical value for Δp corresponds, necessarily, to the same proton conductance, at same Δp , induced by ADP and FCCP, and thus results in the same value of $J_{diss.}$. The equality between L_{ADP} and L_{FCCP} is not fortuitous: it is a consequence of the assumptions of the theory. A similar reasoning shows that, at low Δp values, different values of Δp for a single value of $J_{diss.}$ cannot be obtained.

The contradiction between theory and experiment is, of course, directly apparent from eqn. (2), which summarizes the basic principle of the delocalized chemiosmotic theory. It is on this ground that the fact that different values of the respiration rate are obtained at the same Δp , in the presence of ADP or uncoupler, was considered by

several authors to contradict the chemiostic hypothesis. The reason for the discrepancy between the theory and the experimental results is evident: the line of the equation:

$$J_{diss.} = n \cdot L_{oh} \cdot \Delta p + n \cdot L_o \cdot A_{ox.}$$

is not the same in the presence of ADP or FCCP. This is, in fact, precisely the experimental result to be explained: $J_{ox.}$ (and thus $J_{diss.}$) is not a unique function of Δp ! If, contrary to what has been assumed here according to the chemiosmotic theory, there exist two lines for the equation:

$$J_{diss.} = n \cdot L_{oh} \cdot \Delta p + n \cdot L_o \cdot A_{ox.}$$

one in the case of the addition of ADP and another for that of FCCP, then one gets two points of intersection for a single Δp value and thus two values of $J_{ox.}$. It is worth remarking that this would not be the case if the conductance of the membrane was the same in the presence of ADP or of FCCP. Thus the hypothesis of O'Shea & Chappell (1984) is necessary but it is not sufficient to account for the experimental data.

A linear relationship has been assumed here between $J_{ox.}$ and Δp and $A_{ox.}$. If such a relationship does not hold, similar reasoning can be made by substituting for the line of the equation:

$$J_{diss.} = n \cdot L_{oh} \cdot \Delta p + n \cdot L_o \cdot A_{ox.}$$

the curve of the equation:

$$J_{diss.} = n \cdot g(\Delta p, A_{ox.}) = g'(\Delta p).$$

The fact that $J_{ox.}$ is not a unique function of Δp could mean either that the chemiosmotic theory is not an adequate description of the phenomenon or that ADP or/and FCCP interacts(s) with the respiratory chain (a possibility that has retained little attention from experimenters, but see Laties, 1973). Once again, this has already been lucidly stated by several authors (Padan & Rottenberg, 1973; Zoratti *et al.*, 1981; Westerhoff *et al.*, 1984). The analogous electrical circuit has only been introduced here for heuristic purposes, in order to follow the logic of O'Shea & Chappell (1984) and point out where it fails.

It can be seen that the paradoxical contradiction between their conclusion and a straightforward interpretation of the chemiosmotic hypothesis comes from the fact that they consider only the relationship between the protonic current through the membrane resistor and the protonmotive force, thus leaving a degree of freedom to the system, while the whole proton circuit, including the generator, should have been considered.

In conclusion, it is probable that the rate of proton conductance across the mitochondrial membrane is not the same if it is catalysed by the reversible ATPase or a proton ionophore such as

FCCP; nevertheless, this is not sufficient to explain the fact that the relationship between the rate of respiration and the Δp is different in mitochondria in the presence of ADP or of FCCP.

I thank I. R. Booth, J. B. Jackson, F. Haraux, L. Letellier, J. P. Mazat and E. Shechter for helpful discussions and comments and L. Aggerbeck for correction of the manuscript.

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