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Differentiation between leaks and slips in oxidative phosphorylation

B.H. Groen, J.A. Berden and K. van Dam

E.C. Slater Institute for Biochemical Research, University of Amsterdam, Amsterdam (The Netherlands)

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We have measured the thermodynamic efficiency of oxidative phosphorylation of isolated rat-liver mitochondria during oxidation of succinate. Furthermore, we have calculated what the effect of proton leak or slip in the redox pumps should be on the efficiency of energy transduction in oxidative phosphorylation. These calculations were compared with experiments in which the efficiency was determined in the presence of induced proton leak or redox slip. The results of these experiments are in agreement with the predictions. It is concluded that it is possible to distinguish experimentally between effects of proton leak and redox slip on energy transduction.

Introduction

Oxidative phosphorylation, which for instance takes place in mitochondria, is an important process for the conversion of energy. According to the chemiosmotic model, introduced by Mitchell [1], the (free) energy liberated by oxidation of substrates is used to create a proton gradient which in its turn is used to synthesize ATP. This process functions as a linear energy converter that uses the energy yield from a downhill process – substrate oxidation – to drive an uphill process – ATP synthesis.

Although to date much is known about oxidative phosphorylation, many aspects are still unsolved. It is generally accepted that protons play an important role but the exact mechanism is still under discussion. There are several models for the mechanism by which oxidation and phosphorylation can be uncoupled.

One of the possible approaches to study quantitative aspects of the functioning of energy converters is non-equilibrium thermodynamics (NET), first introduced by Kedem and Caplan [2,3]. In NET, oxidative phosphorylation is described by two flows, the rate of oxygen-consumption (J_o) and the rate of ATP synthesis (J_p), and two forces, the oxygen potential (ΔG_o) and the phosphate potential (ΔG_p) (Eqn. 1).

$$J_p = L_{op} \cdot \Delta G_o - L_{pp} \cdot \Delta G_p \quad (1a)$$

$$J_o = L_{oo} \cdot \Delta G_o - L_{op} \cdot \Delta G_p \quad (1b)$$

The phenomenological coefficients reflect the relationship between each flow-force pair. These coefficients also determine the coupling factor, q, and the phenomenological stoichiometry, Z (Eqns. 2 and 3).

$$q = L_{op} / \sqrt{L_{oo} \cdot L_{pp}} \quad (2)$$

$$Z = \sqrt{L_{pp} / L_{oo}} \quad (3)$$

Efficiency (η) is defined as the quotient of the output and input power (Eqn. 4), and the maximum efficiency is determined solely by q (Eqn. 5).

$$\eta = -J_p \cdot \Delta G_p / J_o \cdot \Delta G_o \quad (4)$$

$$\eta_{max} = q^2 / \left(1 + \sqrt{(1 - q^2)}\right)^2 \quad (5)$$

NET has been further developed by Stucki [4,5], who proposed that an energy converter may be optimized for efficiency as well as for one out of four different output functions. Each combination corresponds to a specific value for the coupling coefficient q. NET does not take into account the mechanism of energy transduction because it considers the energy converter as a black box.

Abbreviations: TMPD, tetramethyl-*p*-phenylenediamine; η, efficiency; n_o and n_p, number of protons translocated by redox pumps and ATPsynthase; L_{oo}, L_{op}, L_{pp}, L_o and L_p, phenomenological coefficients; L_o^s, L_p^s and L_H^l, factors for redox slip, ATPsynthase slip and proton leak; ΔG_o and ΔG_p, oxygen potential and phosphate potential; $\Delta\tilde{\mu}_{H^+}$, protonmotive force; NET, non-equilibrium thermodynamics.

Correspondence: K. van Dam, E.C. Slater Institute for Biochemical Research, University of Amsterdam, Plantage Muidergracht 12, 1018 TV Amsterdam, The Netherlands.

To overcome this, Westerhoff and Van Dam have developed the Mosaic Non-Equilibrium Thermodynamic model [6]. Using the chemiosmotic mechanism, this model describes oxidative phosphorylation by three flows, J_o, J_p and J_H (rate of proton translocation), and three forces, ΔG_o, ΔG_p, and $\Delta\tilde{\mu}_{H^+}$ (protonmotive force), as in Ref. 7. In the MNET model, however, the influence of several processes on the overall efficiency, namely proton leak, or slipping redox and ATPsynthase pumps [8,9] can be included explicitly by the factors L_o^s, L_p^s and L_H^l, respectively. To allow for the known saturation of enzymes, ΔG terms are replaced by ΔG^ξ terms in the equations for the flows (Eqn. 6). Eqn. 4 remains the same, i.e., it contains the truly thermodynamic parameter, ΔG. If a ΔG^ξ is introduced in Eqn. 6a (or 6b), the values for L_o (and L_p) change. Since we may not expect that the change in L_o (or L_p) will be equal for ΔG_o^ξ (or ΔG_p^ξ) and $\Delta\tilde{\mu}_{H^+}$, we allow for a difference in change by introducing an 'asymmetry coefficient' γ (γ_o in Eqn. 6a, and γ_p in Eqn. 6c) [6].

$$J_o = (L_o + L_o^s)\cdot\Delta G_o^\xi + n_o\cdot\gamma_o\cdot L_o\cdot\Delta\tilde{\mu}_{H^+} \quad (6a)$$

$$J_p = (L_p + L_p^s)\cdot\Delta G_p^\xi + n_p\cdot\gamma_p\cdot L_p\cdot\Delta\tilde{\mu}_{H^+} \quad (6b)$$

$$J_H = n_o\cdot L_o\cdot\Delta G_o + (n_o^2\cdot L_o + n_p^2\cdot L_p + L_H^l)\cdot\Delta\tilde{\mu}_{H^+} + n_p\cdot L_p\cdot\Delta G_p \quad (6c)$$

In the steady state, when $J_H = 0$, we can eliminate $\Delta\tilde{\mu}_{H^+}$ and express the phenomenological coefficients of the two-flow-force system in the parameters of the three flow-force system. This reveals that slip of redox and ATPsynthase pumps and H^+ leak have different effects on L_{oo}, L_{pp} and L_{op} (Eqn. 7).

$$L_{oo} = L_o^s + L_o\cdot(L_p\cdot\gamma_p\cdot n_p^2 + L_H^l)/L_t \quad (7a)$$

$$L_{pp} = L_p^s + L_p\cdot(L_o\cdot\gamma_o\cdot n_o^2 + L_H^l)/L_t \quad (7b)$$

$$L_{op} = n_o\cdot\gamma_o\cdot n_p\cdot L_o\cdot L_p/L_t \quad (7c)$$

$$L_{po} = n_o\cdot\gamma_p\cdot n_p\cdot L_o\cdot L_p/L_t \quad (7d)$$

$$L_t = n_o^2\cdot\gamma_o\cdot L_o + n_p^2\cdot\gamma_p\cdot L_p + L_H^l \quad (7e)$$

The introduction of the asymmetry coefficients has two important consequences. First, the cross-coefficients L_{op} and L_{po} are no longer identical: Onsager symmetry is only valid near equilibrium. Therefore, the coefficient L_{op} in Eqn. 1b should be replaced by L_{po}. Furthermore, L_{oo} and L_{pp} are changed. As a consequence, at complete coupling (q is equal to 1.00) the phenomenological coefficient Z is no longer equal to the theoretical stoichiometry n_o/n_p, but to $(n_o/n_p)\cdot\sqrt{\gamma_o/\gamma_p}$.

The value for Z gives information about which of the three possible processes, namely H^+ leak, redox slip and/or ATPsynthase slip, have contributed to a less than complete coupling.

TABLE I

Qualitative effect of redox slip, ATPsynthase slip and H^+ leak on the phenomenological coefficients, Z and $\Delta G_{p(state\,4)}$

Process that decreases q	Effect on				
	L_{oo}	L_{op}	L_{pp}	Z	$\Delta G_{p(state\,4)}$
Redox slip	+	=	=	−	=
ATPsynthase slip	=	=	+	+	−
H^+ leak	+	=	+	−	−

Table I and Fig. 1 summarize the predicted effects of slip and H^+ leak on the phenomenological coefficients, Z and $\Delta G_{p(state\,4)}$.

We measured the efficiency of succinate driven oxidative phosphorylation in rat-liver mitochondria. Furthermore, we studied experimentally the effects of H^+ leak and redox slip on this efficiency by adding gramicidin, 2,4-dinitrophenol or TMPD, respectively. We conclude that it is possible to establish whether an unknown compound induces redox slip or H^+ leak.

Materials and Methods

Rat-liver mitochondria were isolated from male Wistar rats by the method of Hoogeboom [10] as described by Myers and Slater [11], using 275 mM mannitol, 2 mM Mops (pH 7.5) as the isolation medium. Mitochondria were incubated at 25 °C in a vessel equipped with a stirring device. The medium contained 5 mM $MgCl_2$, 3 mM Na_2HPO_4, 2 mM EGTA, 10 mM Mops, 10 mM succinate, 1 mM malate, 10 mM glucose, 3 μg per mg protein rotenone (pH 7.5).

The medium also contained mannitol to a final osmolarity of 290 mosM. Various amounts of ATP and hexokinase were added as an ADP-regenerating system to vary ΔG_p. In some experiments 2,4-dinitrophenol, gramicidin or TMPD was added. Oxygen consumption was measured with a Clark oxygen electrode (Yellow Springs Instruments, Yellow Springs, OH). We used two different methods to measure the rate of ATP synthesis. In the first method three samples were taken from the incubation (volume 1.6 ml, 1.0 mg protein per ml) at different points of time. Reactions were terminated by adding an equal volume of 7% (w/v) cold $HClO_4$, and put on ice. Within 15 min samples were neutralized with cold 2 M KOH/0.2 M Mops to pH 6–8 and immediately frozen. ATP synthesis was determined afterwards from the glucose 6-phosphate content of these samples. In the second method the oxygraph vessel (volume 3.15 ml, 0.2 mg protein per ml) was placed in the cuvette holder of an Aminco DW2 dual wavelength spectrophotometer. Glucose-6-phosphate dehydrogenase and $NADP^+$ were added to the incubation buffer. ATP synthesis was measured as the increase in absorption at $A_{340\,nm} - A_{365\,nm}$ (or $A_{365\,nm}$ −

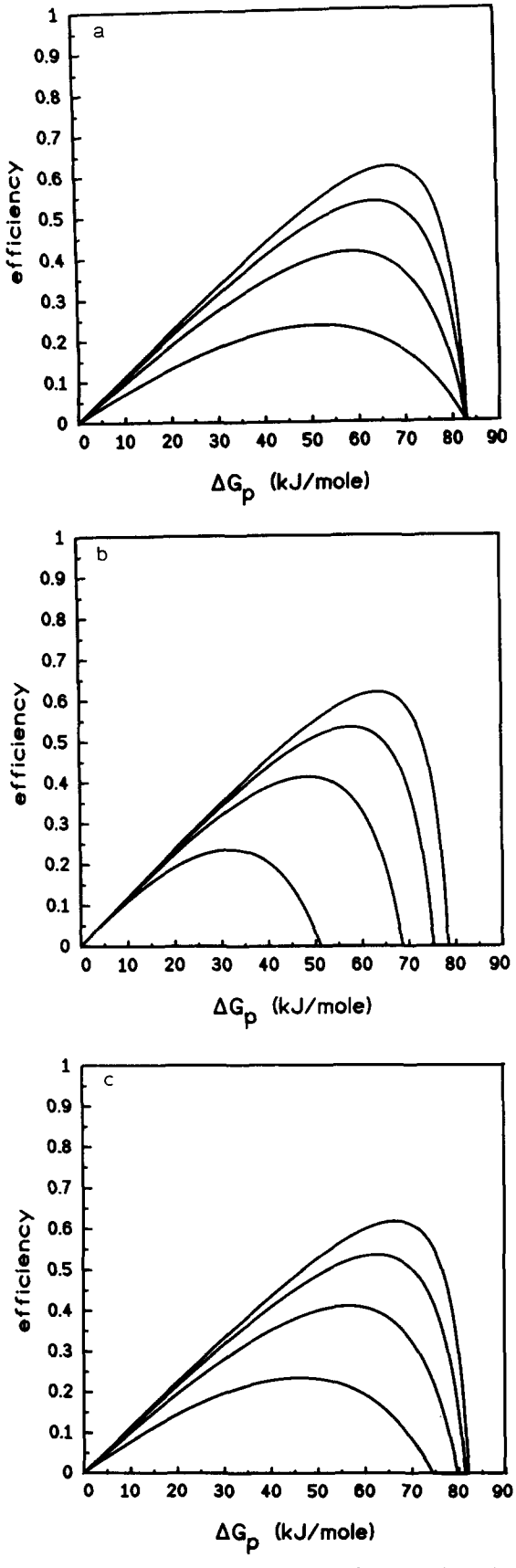

Fig. 1. The effects of redox slip (a), ATPsynthase slip (b) and proton leak (c) on the efficiency of oxidative phosphorylation. Each graph shows the curves for the following values for q: 0.972, 0.953, 0.910, 0.786.

$A_{400\,nm}$ in the presence of TMPD) due to NADPH formation. One sample was taken per incubation and treated as above to determine ΔG_p and ΔG_o. Both methods gave essentially the same results except at extreme ATP/ADP ratios [12].

Protein content was determined by the biuret method [13] using bovine serum albumin as a standard, ADP, ATP, phosphate, glucose 6-phosphate and malate were determined fluorometrically or spectrophotometrically (in the neutralized samples) according to standard procedures [14–17]. Succinate was determined with HPLC chromatography using a Biorad Aminex ion-exclusion HPX 87H column, a refractometer (Knauer, Berlin) and a SpectraPhysics SP4270 integrator (CA, USA). The phosphate potential was calculated using a computer program written by Lemasters [18] based on the method of Rosing and Slater [19]. The enzymes and other biochemicals were obtained from Boehringer (Mannheim, F.R.G.).

Results and Discussion

Efficiency of oxidative phosphorylation

We measured the efficiency of oxidative phosphorylation of rat-liver mitochondria as a function of the phosphate potential at constant ΔG_o with several batches of mitochondria. The substrate was succinate, rotenone was added to inhibit complex I. Fig. 2 shows the results of two typical, independent experiments. With non-linear regression analysis, based on the method of Newton-Raphson [20], we ascertained which values for q and Z gave the best fitting curve (Eqn. 4).

This analysis produces the ratio of the phenomenological coefficients. Fitting the data to Eqns. 1a and 1b yields the absolute values of the phenomenological coefficients. The calculated curve fits reasonably well to the experimental data. The values of q and Z are approx. 0.91 and 3.0, respectively. However, if the coupling coefficient is close to 1.0, then Z should be approximately equal to the P/O ratio. A value for Z of 3.0, however, is much higher than any proposed P/O ratio for succinate [21,22]. Therefore, this approximation appears to be unsatisfactory.

The simple proportional approximation used to derive these values probably fails partly because it does not take into account saturation effects of the succinate oxidation pathway. Such effects can be incorporated in the MNET model. The ΔG_o for succinate oxidation is relatively high, namely approx. 166 kJ/mol. When the phosphate potential has a value of 40 to 60 kJ/mol, then oxidation of succinate theoretically delivers more free energy than necessary to synthesize 1.5 to 2.0 molecules of ATP, which is the generally accepted value for the P/O ratio [21–23]. Furthermore, ΔG_o is so high that it is beyond the domain were J_o depends proportionally on ΔG_o. Thus, part of the ΔG_o cannot be used

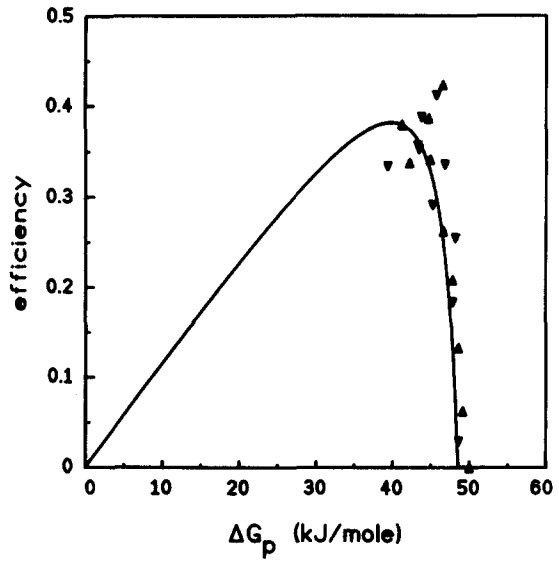

Fig. 2. The efficiency of oxidative phosphorylation of isolated rat-liver mitochondria measured as a function of ΔG_p. Values for q and Z were determined with the NET-model and the MNET-model.

	Net-model	MNET-model
q	0.91	0.975
Z	3.00	1.996

to create a proton gradient across the inner-mitochondrial membrane.

To account for this, in MNET the equation for oxygen consumption (J_o) is changed: ΔG_o is replaced by $\Delta G_o^{\pounds}$, and the asymmetry coefficient γ_o is added. We assumed that $\Delta G_p^{\#}$ is 0, so γ_p is equal to 1.0. $\Delta G_o^{\pounds}$ is equal to the amount of free energy released by substrate oxidation (ΔG_o) from which a certain amount of free energy ($\Delta G_o^{\#}$) is subtracted. The value of $\Delta G_o^{\#}$ depends on the region of the flow-force relationship [6].

We now used the MNET-model to determine which values for $\Delta G_o^{\#}$ and the phenomenological coefficients gave the best fit, for the data shown in Fig. 2. The value for $\Delta G_o^{\#}$ turned out to be 66 kJ/mol, so $\Delta G_o^{\pounds}$ has a value of 100 kJ/mol. The estimation of the value for $\Delta G_o^{\#}$ is based on the following criteria.

The maximum value is determined by q: if the value for $\Delta G_o^{\#}$ is too high then q becomes 1.0 or higher. If the value chosen for $\Delta G_o^{\#}$ is too low, then the calculated values for the efficiency are too low compared to the measured efficiencies. Secondly, $\Delta G_o^{\#}$ should have the same value for all experiments with the same value for ΔG_o. Thirdly, the best value for $\Delta G_o^{\#}$ is determined by non-linear regression analysis (χ^2). The values for L_{oo}, L_{op}, L_{po} and L_{pp} are 12.64, 24.56, 24.76 and 50.56, respectively. Thus the values for q and Z are 1.99 and 0.975, respectively. The value for the coupling coefficient is close to 1.0. This indicates that if q is equal to 1.0 then the values for the phenomenological coefficients are 12, 24, 24 and 48, respectively, thus Z is equal to 2.0. Furthermore, the fact that we found different values for L_{op} and L_{po} implies that γ_o is not equal to 1.0. The fact that γ_o is not equal to 1.0 is not unexpected. Van Dam et al. [24] investigated whether J_o is a linear function of ΔG_o at constant $\Delta\tilde{\mu}_{H^+}$ and vice versa. The conclusion was that the possibility could not be excluded that γ_o differs from 1.0 under some conditions. The value for Z is higher than the value reported by Beavis and Lehninger [25]. They reported values for q and Z of 0.95 and 1.71, respectively. However, they calculated Z from J_p/J_o and q ($J_p/J_o = q \cdot Z$ if q is nearly 1.0). This equation is no longer valid if a factor $\Delta G_o^{\#}$ is introduced to account for saturation of the succinate oxidation pathway, because in that case we cannot expect that γ_o will be equal to 1.0. According to Lemasters [18] the values for q and Z are 0.981 and 2.0, respectively (determined in oxygen-jump experiments). Z was calculated from the equation $J_p/(J_o - (J_o)_{state4}) = Z/q$. However, there is no basis for the correction of J_o for state 4 oxygen consumption: this implies that the oxygen consumption in state 4, caused by passive proton leak, is still fully present during state 3. If Z is calculated from $J_p/J_o = Zq$ then the same argument mentioned above in relation to the studies of Beavis and Lehninger [25] applies.

The value of 0.975 for the coupling coefficient indicates optimization for economic output power. This would indicate that mitochondria isolated from rat-livers are optimized for the same output function both when succinate or glutamate is added as substrate [4,5,26]. Furthermore, the value for Z provides us with information on the question by which of the three processes mentioned in the introduction q may have been adjusted. Two of these processes, namely H^+ leak and redox slip, give rise to a decrease in Z, whereas ATPsynthase slip increases Z. The calculated value for Z that corresponds to a q of 0.975, which would have been caused solely by H^+ leak or redox slip, would be between 1.94 and 1.96 (Z would be equal to 2.0 if the coupling were complete). Although it is difficult to quantify the exact contribution of redox slip, ATP synthase slip and H^+ leak to the adjustment of q to a value of 0.975, we can say that the value for Z indicates that q must at least in part be adjusted by slipping ATPsynthases.

It is not possible to use the same argument to explain the high value for Z calculated by the simple NET model, because if Z would have a value of 3.0, then there would be so much slip in the ATPsynthase that q would be only 0.647. This value of q corresponds to maximally 15% efficiency, while efficiencies of 40% were actually measured.

Although it has been shown by Papa and Guerieri [27] that addition of oligomycin to submitochondrial particles lowers the oxygen consumption in state 4,

probably by blocking the slipping ATPsynthases, until now the experimental evidence that this is also the case for isolated mitochondria is incomplete [28].

Effect of artificial slip and H^+ leak on the efficiency of oxidative phosphorylation

To test the predictions summarized in Table I further, we studied the effects of induced redox slip and H^+ leak on the efficiency of oxidative phosphorylation. H^+ leak was induced by adding one of the uncouplers gramicidin or 2,4-dinitrophenol: both cause H^+ leak by increasing proton permeability of the inner mitochondrial membrane, thereby dissipating $\Delta\tilde{\mu}_{H^+}$. Gramicidin induces H^+ leak in the presence of a high concentration of potassium ions, as was demonstrated by Luvisetto and Azzone [29], although Rottenberg and Koeppe [30] claim that gramicidin always also has a 'decoupling' effect.

Redox slip was induced by adding TMPD; TMPD causes apparent redox slip by shuttling electrons from complex II to cytochrome *c*, so that the intervening complex III cannot translocate protons.

First the effect of 2,4-dinitrophenol and TMPD on the rate of oxygen-consumption and the phosphate potential of mitochondria respiring in state 4 was determined. Both redox slip and H^+ leak increase the state 4 oxygen consumpion. In state 4 J_p is zero, so ΔG_p must be equal to $L_{op} \cdot \Delta G_o^{\pounds}/L_{pp}$ (Eqn. 1a). According to Eqn. 7 an increased leakiness of the membranes – which means an increased L_H^l – changes L_{op} as well as L_{pp}, and the net effect is that ΔG_p decreases. On the other hand, an increased redox slip – which gives rise to an increase of L_{oo} by an increase of L_o^s – does not change L_{op} and L_{pp}, so ΔG_p also should not change.

The effect of 2,4-dinitrophenol was determined in three independent experiments (Fig. 3). The results show a linear relation between the increase of the oxygen consumption and the decrease in the phosphate potential, in line with the prediction: if the concentration of 2,4-dinitrophenol was increased from 0 to 150 μM, J_o increased roughly 6-fold, and ΔG_p decreased approx. 2 kJ/mol. Similar results were obtained with gramicidin (not shown). The effect of TMPD on J_o and ΔG_p is also shown in Fig. 3. Addition of 0–1 mM TMPD increased the oxygen consumption from 45 to 110 μM/min per mg protein, but ΔG_p remained constant, at approx. 60.5 kJ/mol.

Redox slip and H^+ leak should have a different effect upon the relation between J_p and J_o (Eqns. 1 and 7). As stated above, both increase the oxygen consumption in state 4. If we eliminate ΔG_p from Eqn. 1, and substitute the phenomenological coefficients by Eqn. 7a, 7b and 7c, we obtain the following relationship between J_p and J_o (the factor γ_o is left out).

$$J_p = \alpha \cdot J_o + \beta \cdot \Delta G_o^{\pounds} \tag{8a}$$

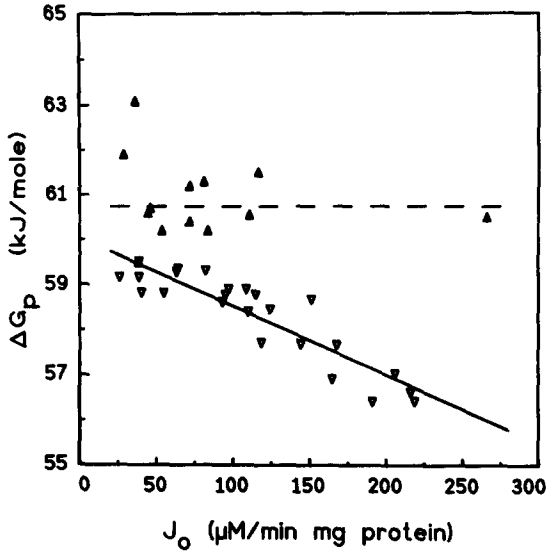

Fig. 3. Effect of leak, caused by 0–150 μM DNP, and redox slip, caused by 0–1 mM TMPD, on the oxygen consumption (J_o) and phosphate potential (ΔG_p) of rat-liver mitochondria respiring in state 4. ———, DNP; - - - - - -, TMPD.

$$\alpha = (n_o/n_p) + (L_H^l \cdot L_p + L_p^s L_t)/(n_o n_p L_o L_p) \tag{8b}$$

$$\beta = (L_H^l \cdot (L_o + L_o^s) \cdot (L_p + L_p^s) + n_p^2 \cdot L_p \cdot L_p^s \cdot (L_o + L_o^s) + n_o^2 \cdot L_o \cdot L_o^s \cdot (L_p + L_p^s))/(n_o n_p L_o L_p) \tag{8c}$$

In a graph of J_p as a function of J_o, α corresponds with the slope, and β corresponds with the intercept on the *x*-axis, if $\Delta G_o^{\#}$ is experimentally kept constant. In case of increasing leak, both the slope and the intercept should increase. This causes lines determined with different uncoupler concentrations to intersect.

We measured the flows in the absence and presence of 2,4-dinitrophenol (results not shown). The results were the same as in Westerhoff and Van Dam [6] and Van Dam et al. [31]. From the intersection point of the lines determined with and without 2,4-dinitrophenol we concluded that the n_o/n_p ratio (the theoretical P/O ratio) is approx. 1.4. From the values for the theoretical stoichiometry and the phenomenological coefficient Z we may estimate that the value for γ_o is approx. 1.8 (if γ_p is 1.0 then $n_o/n_p = Z \cdot \sqrt{\gamma_o}$). In case of redox slip the intercept in the plot of J_p as a function of J_o should change, but the slope should remain the same (α-contains no L_o^s, Eqns. 8).

We measured the flows again, but now in the absence and presence of TMPD. In both experiments we found a linear relation between J_p and J_o (Fig. 4). The point of intersection with the *x*-axis (the oxygen consumption in state 4) increased with increasing redox slip. The slope of the line did not change by the addition 350 μM TMPD, as was predicted by the model calculation.

The molecular basis of these phenomena may be described as follows. Redox slip interferes with the

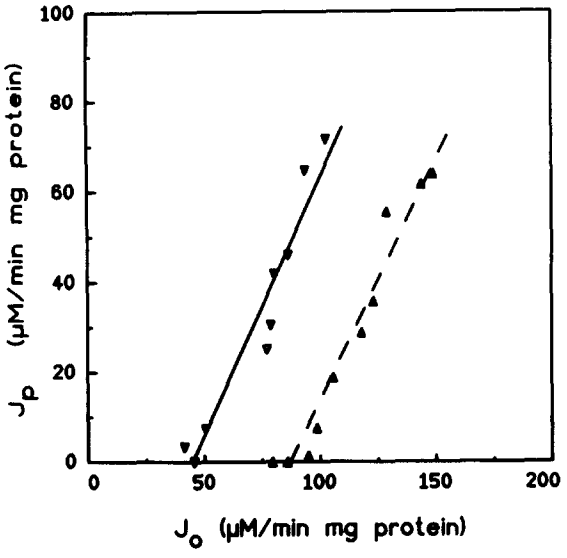

Fig. 4. Effect of redox slip, caused by 350 μM TMPD, on the oxygen consumption (J_o) and ATP production (J_p) of isolated rat-liver mitochondria. ———, control; - - - - - -, 350 μM TMPD.

generation of the protonmotive force, but this effect can be compensated by increasing the state 4 oxygen consumption. As long as the respiratory chain does not function at maximal velocity the effect of redox slip, namely a decrease in H^+/O, can be compensated by increasing the state 4 oxygen consumption. Apart from the increase in state 4 oxygen consumption the oxidative phosphorylation functions as if there is no redox slip: the protonmotive force, the phosphate potential and the dJ_p/dJ_o are the same as in the absence of TMPD.

In the case of H^+ leak, the oxygen consumption also increases. When the oxygen consumption increases, as the mitochondria proceed from state 4 to state 3, the $\Delta\tilde{\mu}_{H^+}$ decreases. This results in a diminished H^+ loss caused by proton leak. Therefore the amount of oxygen consumption induced by proton leak decreases, which causes the P/O ratio to rise.

We calculated the efficiency of oxidative phosphorylation in the presence of artificial redox slip and H^+ leak.

The experiment shown in Fig. 2 was repeated in the presence of 350 μM TMPD. The best fitting curve corresponds to a coupling coefficient of 0.930, and a Z of 1.89. For comparison, Fig. 5b also shows the control curve: TMPD clearly changes the curve. The maximal efficiency is lowered to approximately 20%, but $\Delta G_{p(state4)}$ has not changed. The values for L_{oo}, L_{op}, L_{po} and L_{pp} are 14.15, 24.53, 25.20 and 50.56, respectively: L_{oo} is increased, compared to the values for the control curve (presumably because of an increase of L_o^s) and this indicates redox slip. Redox slip causes a decrease of q as well as Z (Table I). An analogous experiment was performed with gramicidin (1.9 pmol/mg protein). Fig. 5a shows the curve in the presence of gramicidin together with the control curve. Gramicidin clearly changes the efficiency of oxidative phosphorylation. Values for q and Z are now 0.91 and 1.948. At this concentration of gramicidin the stimulation of the oxygen consumption by ATP synthesis was small, so it was not possible to determine the absolute values of the phenomenological coefficients, using Eqns. 1a and 1b. However, as the values for the phenomenological coefficients have changed in such a way that L_{oo} and L_{pp} are increased with respect to L_{op}, we may conclude that gramicidin has caused an increased H^+ leak.

Thus, the predictions of the MNET model concerning the effects of redox slip and H^+ leak on the ef-

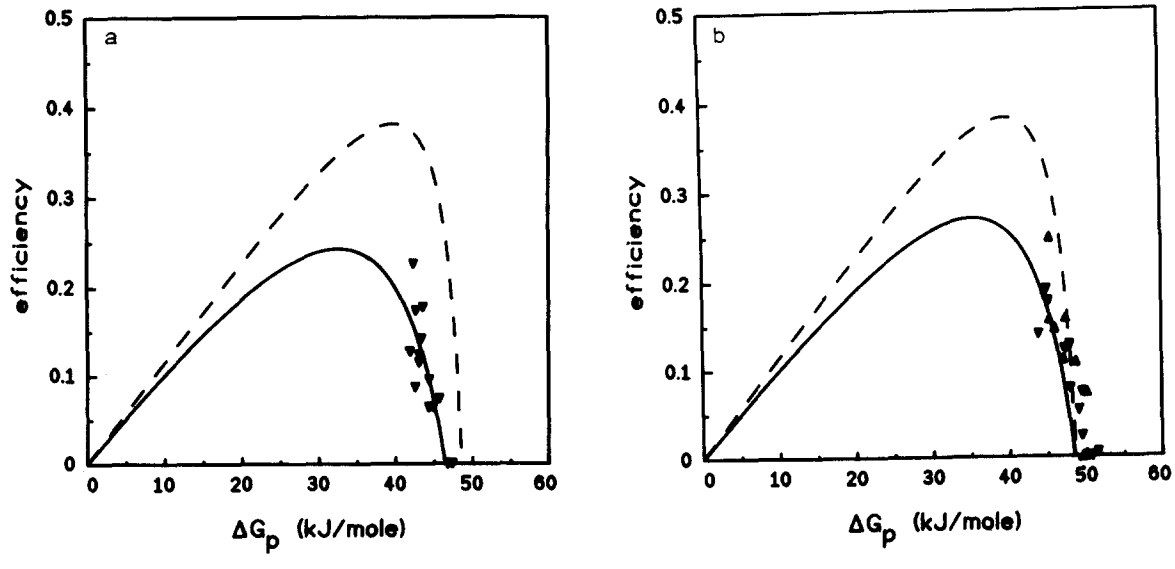

Fig. 5. Effect of proton leak, caused by 1.9 pmol/mg protein gramicidin (a), and redox slip, caused by 350 μM TMPD (b), on the efficiency of oxidative phosphorylation of isolated rat-liver mitochondria. a: - - - - - -, control; ———, gramicidin. b: - - - - - -, control; ———, TMPD.

ficiency of oxidative phosphorylation are experimentally confirmed.

Future prospects

Now that we can distinguish between redox slip and H^+ leak, it will be possible to compare the effects of different uncouplers, e.g., 2,4-dinitrophenol, gramicidin and FCCP, on the efficiency of oxidative phosphorylation. Do they act as classical uncouplers and just increase the proton permeability of the inner membrane, or is the effect more complicated, as has been reported for gramicidin under conditions of low potassium concentration [29]? Do fatty acids act as uncouplers or as decouplers, which means that they cause redox slip? We expect that mitochondria from different organs or organisms are optimized for different output functions. Results presented here indicate that rat-liver mitochondria from fed rats are optimized for economic output power: we found a value of 0.972 for the coupling coefficient. This result is in accordance with Soboll and Stucki [26]. Soboll has determined that rat-liver mitochondria have a q of 0.953 after starvation, and this indicates optimization for economic output flow: mitochondria from rat heart have – at rest – a coupling coefficient of 0.999 (Soboll, S., reported at the 19th FEBS Meeting, Rome, 1989). From measurements of the efficiency of oxidative phosphorylation in mitochondria we can determine the coupling coefficient, the output function for which these mitochondria are optimized, and possibly whether the coupling coefficient is caused by slips or leaks.

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