Biochem. J. (1987) 243, 499–505 (Printed in Great Britain)

The control of electron flux through cytochrome oxidase

Michael P. MURPHY and Martin D. BRAND
Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge CB2 1QW, U.K.

1. The electron flux through cytochrome oxidase is a linear function of the net thermodynamic force across the complex over a limited range of conditions. 2. Over a wide range of conditions the electron flux is a complicated function of the percentage reduction of the cytochrome *c* pool and of $\Delta\psi$ (at low values of ΔpH). 3. We have estimated the elasticities of electron flux through cytochrome oxidase to ΔE_h of the redox reaction catalysed by cytochrome oxidase (or to cyt c^{2+}/cyt c^{3+}) and to $\Delta\psi$. The elasticities varied depending on the values of $\Delta\psi$ and of the percentage reduction of the cytochrome *c* pool. 4. At intermediate rates (which may correspond to those *in vivo*) the electron flux through cytochrome oxidase is controlled to about the same extent by $\Delta\psi$ and by ΔE_h.

INTRODUCTION

Studies of the control of flux in metabolic pathways have been transformed by the development of a quantitative theory of metabolic control (Kacser & Burns, 1973, 1979; Heinrich & Rapoport, 1974; Westerhoff *et al.*, 1984). In this context, to say A controls B means that alteration in the concentration or activity of A causes an alteration in the concentration or activity of B. This theory has been applied to the control of mitochondrial respiration by Groen *et al.* (1982) and Tager *et al.* (1983). These workers used isolated mitochondria respiring on succinate with an extramitochondrial ADP-regenerating system of hexokinase/glucose. They showed that in state 4 respiration was controlled by the proton leak of the inner mitochondrial membrane, whereas in state 3 control of respiration was shared by cytochrome oxidase, the dicarboxylate carrier, the adenine nucleotide carrier and hexokinase.

This description of the control of respiration emphasizes that control may be shared between several steps and allows for different distributions of control under different metabolic conditions. It also reconciles previous descriptions which postulated that the major controls on respiration were ADP concentration (Chance & Williams, 1955), the extramitochondrial ΔG_P (Klingenberg, 1964), the extramitochondrial ΔG_P and the redox state of the cytochrome *c* pool (Erecinska & Wilson, 1982) or the extramitochondrial ATP/ADP ratio (Davis & Davis-Van Thienen, 1984). The approach leads to a very useful, but incomplete, description of the control of respiration. It indicates which components of the system control the rate of respiration, but it does not describe the mechanisms by which changes in rate are achieved. Ultimately this will occur by the kinetic effects on cytochrome oxidase of the redox state of electron transport chain components and the magnitude of Δp (Hansford, 1980, 1985; Ferguson & Sorgato, 1982).

In the present paper we analyse the dependence of electron flux through cytochrome oxidase on its effectors, Δp and the redox state of the cytochrome *c* pool. We define an effector as any system component, such as a substrate, which on altering in magnitude changes the activity of a system parameter, such as an enzyme. The analysis has similarities to that of Erecinska & Wilson (1982) but incorporates different assumptions and is carried out within the framework of metabolic control theory and the chemiosmotic theory of oxidative phosphorylation. Part of this work has been published in preliminary form (Murphy & Brand, 1986).

EXPERIMENTAL

Preparation of mitochondria

Rat liver mitochondria were prepared by differential centrifugation (Chappell & Hansford, 1972) in a medium containing 250 mM-sucrose, 5 mM-Tris and 1 mM-EGTA adjusted to pH 7.4 with HCl at 25 °C. Mitochondrial protein was assayed by the biuret method using bovine serum albumin as a standard (Gornall *et al.*, 1949).

Measurement of respiration rates

The rate of oxygen consumption (J_O) was measured polarographically using a Clark electrode with a thermostatted water jacket. The electrode was calibrated by the addition of known amounts of NADH to medium containing phenazine methosulphate and catalase (Robinson & Cooper, 1970).

Measurement of matrix volume

Mitochondrial matrix volume was measured by using [^{14}C]sucrose (20 nCi/ml) as an extramitochondrial marker and ^3H_2O (0.5 μCi/ml) to determine the total pellet volume. Matrix volume was calculated as ^3H_2O space − [^{14}C]sucrose space. Matrix volumes were in the range 0.5–0.7 μl/mg of mitochondrial protein.

Measurement of ΔpH

ΔpH was determined from the accumulation ratio of

Abbreviations used: $\Delta\psi$, electrical potential across the mitochondrial membrane; ΔpH, pH difference across the mitochondrial inner membrane; Δp, protonmotive force across the mitochondrial inner membrane ($\Delta\psi - 61.5\Delta$pH at 37 °C); ΔE_h, difference in redox potential; ΔG_P, phosphorylation potential; J_O, rate of oxygen consumption; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; TPMP, methyltriphenylphosphonium; cyt, cytochrome.

[^{3}H]acetate (2 μCi/ml). The accumulation ratio was calculated as:

([^{3}H]acetate space − [^{14}C]sucrose space)/matrix volume

ΔpH (in mV) is $-61.5 \cdot \log$(acetate accumulation ratio) at 37 °C. Under all conditions reported in this paper values of ΔpH were scattered between 10 mV and −10 mV and were ignored.

Measurement of $\Delta\psi$

$\Delta\psi$ was calculated from the distribution of the lipophilic cation TPMP$^+$. A correction for intramitochondrial binding of TPMP$^+$ was made by comparing the distribution of TPMP$^+$ with that of Rb$^+$ over a range of values of $\Delta\psi$ induced by valinomycin and different external potassium concentrations (Brown & Brand, 1985). Rb$^+$ was assumed not to bind and to have an activity coefficient of 1. A graph of the TPMP$^+$ accumulation ratio against that for Rb$^+$ was linear with a slope of 1/0.44 and an intercept on the TPMP$^+$ axis of 32. It covered membrane potentials up to 176 mV so it was necessary to extrapolate the calibration to correct for intramitochondrial binding of TPMP$^+$ at membrane potentials higher than this. The membrane potential (in mV) is given by the Nernst equation:

$$\Delta\psi = 61.5 \cdot \log[0.44(\text{TPMP accumulation ratio}) - 32]$$

The extramitochondrial TPMP$^+$ concentration was usually determined using a TPMP$^+$-sensitive electrode (Brown & Brand, 1985). The TPMP$^+$ accumulation ratio was calculated using the matrix volume determined in parallel.

At low values of $\Delta\psi$ (below about 100 mV) the changes in extramitochondrial TPMP$^+$ concentration were too small to be measured accurately by the electrode, so TPMP$^+$ accumulation was measured using [^{3}H]TPMP$^+$ (50 nCi/ml).

Measurement of cytochrome *c* redox state

The cytochrome *c* redox state was measured from $A_{550} - A_{540}$ using a Perkin–Elmer 557 double-beam/dual-wavelength spectrophotometer. The E_h of cytochrome *c* was calculated using a midpoint potential for cytochrome *c* of 235 mV (Dutton *et al.*, 1970) and that of the oxygen/water couple was calculated using a midpoint potential of 820 mV at pH 7 (George, 1965). The E_h of the oxygen/water couple is 792 mV in 50% air-saturated medium at 37 °C and pH 7.2. It does not alter greatly as the air saturation of the medium changes, going from 796 mV at 90% saturation to 782 mV at 10% saturation. All experiments were carried out in this range, so ΔE_h for the reduction of oxygen to water with the simultaneous oxidation of cytochrome c^{2+} was calculated using a constant E_h value of 792 mV for the oxygen/water couple. The extramitochondrial pH of 7.2 was used in calculating ΔE_h (Ferguson & Sorgato, 1982; Murphy *et al.*, 1985). ΔE_h (in mV) is given by:

$$\Delta E_h = 792 - [235 + 61.5 \cdot \log(\text{cyt } c^{3+}/\text{cyt } c^{2+})]$$

Experimental protocol

All experiments were done at 37 °C in medium containing 2 mg of mitochondrial protein/ml, 120 mM-KCl, 5 mM-potassium phosphate, 5 mM-potassium Hepes, 1 mM-potassium EGTA, 5 mM-glucose, 100 μM-potassium acetate, 2 mM-$MgCl_2$, 8.3 μM-rotenone, 0.13 μg of nigericin/ml, 5 μM-TPMP bromide, 10 mM-potassium succinate at pH 7.2. All other additions are mentioned in the Figure legends.

TPMP$^+$ accumulation ratio and J_O were determined simultaneously with a TPMP$^+$-sensitive electrode and a Clark electrode in a 3 ml system. Medium, mitochondria and inhibitor (or uncoupler) were added, the system was closed and the magnetic stirrer switched on. The TPMP$^+$-sensitive electrode was calibrated by addition of small amounts of TPMP$^+$ up to a final concentration of 5 μM. When a steady baseline had been reached, succinate was added. After new steady state values of TPMP$^+$ accumulation ratio and oxygen consumption rate were reached (2–5 min), the system was allowed to become anaerobic (or uncoupler was added) to cause the TPMP$^+$-sensitive electrode to regain its baseline. Small corrections were made for the effect of baseline drift and the direct effect of the addition of substrate on the electrode. For titrations the inhibitor (or uncoupler) was added prior to the substrate. Each titration point represents the mean of triplicate experiments ± S.D.

The redox state of the cytochrome *c* pool was measured by placing 3 ml of medium, mitochondria and inhibitor (or uncoupler) in a cuvette stirred by an air-driven paddle. This was placed in the spectrophotometer and a steady baseline obtained. This was taken as the absorption of the fully oxidized cytochrome *c* pool. Succinate was then added and the absorption was measured; 100% reduction of the cytochrome *c* pool was then achieved by adding KCN (10 mM). Incubations took 2–5 min. Each titration point represents the mean of triplicate experiments ± S.D.

Experiments with radioisotopes were done in triplicate as follows. Medium (1 ml) containing mitochondria and succinate was added to plastic centrifuge tubes and the mitochondria were incubated for 3 min. They were then spun down with an Eppendorf bench centrifuge and 500 μl of the supernatant was removed and placed in a scintillation vial for counting. The remaining supernatant was decanted and the residue was carefully removed from the pellet with a tissue. The pellet was then resuspended by vortex-mixing in 40 μl of Triton X-100 (20%, v/v). The bottom of the centrifuge tube, containing the resuspended pellet, was cut off into a scintillation vial and 350 μl of water was added to equalize quenching in the supernatant and pellet vials. 3.8 ml of scintillant (Beckman Ready-Solv EP) was then added. Radioactivity in the samples was assayed by dual channel counting in a LKB Wallac 1217 Rack Beta liquid-scintillation counter with corrections for quench and crossover. An approx. 10-fold excess of ^{3}H over ^{14}C or ^{86}Rb was used to lessen the amount of crossover. The data were fed directly from the counter to a BBC microcomputer for on-line calculation of apparent spaces using the formula:

$$\text{apparent space } (\mu\text{l}) = \frac{\text{radioactivity in total pellet}}{\text{radioactivity per } \mu\text{l of supernatant}}$$

Matrix volumes and accumulation ratios were calculated from apparent spaces as described above.

Materials

[^{14}C]Sucrose, [^{3}H]acetate, ^3H_2O and ^{86}RbCl were from Amersham International. [^{3}H]TPMP bromide was from New England Nuclear. All other chemicals were from BDH, Fisons or Sigma.

RESULTS

Electron flux through cytochrome oxidase as a function of the net thermodynamic force across the complex

It would be convenient for a description of the control of respiration if the electron flux through cytochrome oxidase was a simple function of its effectors, Δp and the redox state of the cytochrome *c* pool. The description would be particularly straightforward if the flux was a unique (preferably linear) function of the overall thermodynamic force across the complex. There is no reason *a priori* why this should be so, but linear force/flux relationships have been found for many regions of oxidative phosphorylation (Rottenberg, 1973). Such linear relationships may lead to most efficient energy transduction at high rates (Stucki *et al.*, 1983). Linear force/flux relationships have also been shown for some individual components of oxidative phosphorylation such as the cytochrome bc_1 complex (Brown & Brand, 1985; but see Brand *et al.*, 1986), the H^+-ATPase (Van Dam *et al.*, 1981) and NADH:ubiquinol oxidoreductase (Rottenberg & Gutman, 1977). It is therefore reasonable to hope that a simple force/flux relationship may exist for cytochrome oxidase.

The thermodynamic force across the complex, $-\Delta G/F$, is given by:

$$-\Delta G/F = 2\Delta E_h - (n+2)\Delta p$$

where Δp is given by $\Delta\psi - 61.5\Delta pH$, n is the number of protons translocated across the inner mitochondrial membrane for each pair of electrons passing through cytochrome oxidase and 2 is the number of charges translocated across the inner mitochondrial membrane on passing two electrons from cytochrome *c* to the matrix side of cytochrome oxidase. ΔE_h is calculated using the extramitochondrial pH (Ferguson & Sorgato, 1982; Murphy *et al.*, 1985); had the internal pH been used to determine ΔE_h, the Δp term would have to have been expanded to $-(n+2)\Delta\psi + n\Delta pH$. The $H^+/2e^-$ stoichiometry of cytochrome oxidase is assumed to be 2. If a value of 4 were to be used the qualitative relationships we report in this paper would not be greatly affected.

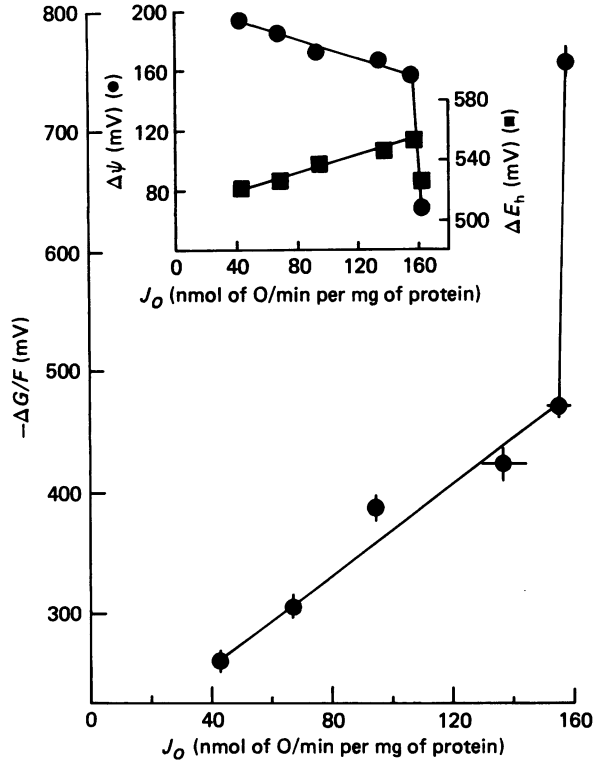

Fig. 1 Uncoupler titration of the thermodynamic driving force across cytochrome oxidase and J_O

Conditions are given in the Experimental section. J_O was increased by titration with FCCP up to 0.33 μM. The inset shows how $\Delta\psi$ and ΔE_h change during the titration.

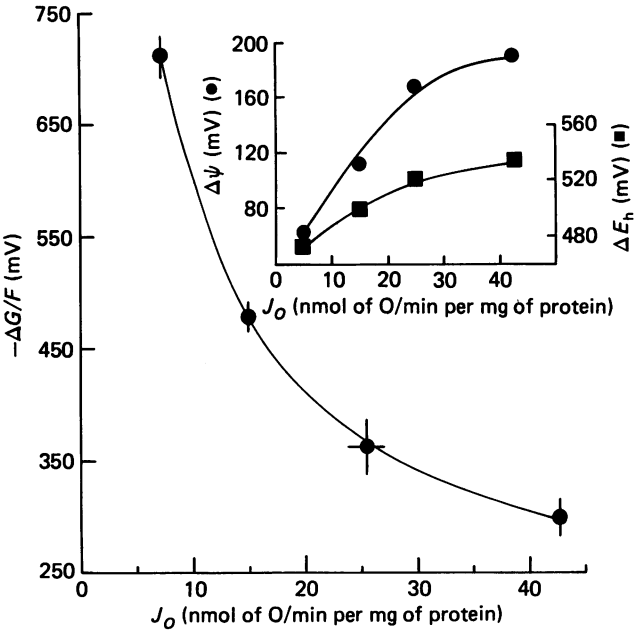

Fig. 2. Inhibitor titration of the thermodynamic driving force across cytochrome oxidase and J_O

Conditions are given in the Experimental section. J_O was decreased by titration with malonate up to 8 mM. The inset shows how $\Delta\psi$ and ΔE_h change during the titration.

In Fig. 1 $-\Delta G/F$ is plotted against J_O for an experiment in which the rate of oxygen consumption was stimulated by addition of the uncoupler, FCCP. There is a simple, linear relationship between $-\Delta G/F$ and J_O from fully coupled respiration to the kinetic limit of the electron transport chain. Possibly, this mimics the physiological stimulation of J_O which occurs when ATP turnover is increased, causing Δp to decrease. In Fig. 2 $-\Delta G/F$ is again plotted against J_O, but for an experiment in which the electron flux was inhibited with different amounts of malonate. This titration does not give as pronounced a plateau in the plot of $\Delta\psi$ against J_O as was found at 25 °C (results not shown) but the nonlinearity is still evident. The relationship between $-\Delta G/F$ and J_O obtained in Fig. 2 is very different from that found for the uncoupler titration. The inhibitor titration may be similar to the physiological decrease of J_O by inhibition of substrate supply.

We conclude that over a limited range of conditions (Fig. 1) the flux through cytochrome oxidase is a simple, linear function of the net thermodynamic force across the complex; this may be physiologically relevant. However, in Fig. 2 the relationship is very different, and over the whole range of conditions (Figs. 1 and 2) it is not possible to describe the electron flux through cytochrome

oxidase as a simple function of the net thermodynamic force across the complex. Therefore, some further kinetic control on electron flux must be operating.

Dependence of electron flux on $\Delta\psi$ and % reduction of the cytochrome *c* pool at clamped low values of ΔpH

As electron flux through cytochrome oxidase is not, in general, a simple function of $-\Delta G/F$, other descriptions of the control of flux through the complex must be considered. A first step towards such a description is to investigate the relationship between the flux through cytochrome oxidase and the magnitude of its effectors, Δp and the redox state of the cytochrome *c* pool. To simplify the analysis for all experiments in this paper we clamped ΔpH at a very low value (checked with [^{14}C]acetate) using nigericin and high extramitochondrial potassium so that Δp was expressed entirely as $\Delta\psi$. The relationship is represented in Fig. 3, where we show J_O as a function of both % reduction of the cytochrome *c* pool and $\Delta\psi$.

Due to the small variations between different mitochondrial preparations it was not possible to use absolute values of measured points in constructing Fig. 3. First, a primary data set from a single experiment was used to form a basis for the construction of Fig. 3. Then, a range of titrations with FCCP and malonate were carried out on mitochondria which were already partially uncoupled (in the case of the malonate titrations) or partially inhibited (in the case of the FCCP titrations). Thus the relationship between J_O, $\Delta\psi$ and % reduction of the cytochrome *c* pool was established over different parts of the surface shown in Fig. 3. These relationships were then normalized to fit the primary data set wherever they intersected with it and used to construct the surface shown in Fig. 3. The extent of normalization required varied, but was usually about 5–10 nmol of O/min per mg of protein. The points on Fig. 3 are intersections on the grid nearest to the experimentally determined lines. Because of its manner of construction, Fig. 3 is not a quantitative description of the dependence of J_O on $\Delta\psi$ and % reduction of the cytochrome *c* pool. However, we are confident that it is a good qualitative description of the relationship.

From Fig. 3, it can be seen that the electron flux depends strongly on the % reduction of the cytochrome *c* pool. The dependence of flux on % reduction of the cytochrome *c* pool is roughly similar over the whole range of $\Delta\psi$ and % reduction of the cytochrome *c* pool covered. In contrast, the dependence of electron flux on $\Delta\psi$ varies considerably over the same range. It is high when both $\Delta\psi$ and % reduction of the cytochrome *c* pool are high. At low values of $\Delta\psi$, there is no effect of $\Delta\psi$ on the flux; that is, the electron transport chain is kinetically limiting. These relationships are shown more graphically by the elasticities, discussed below.

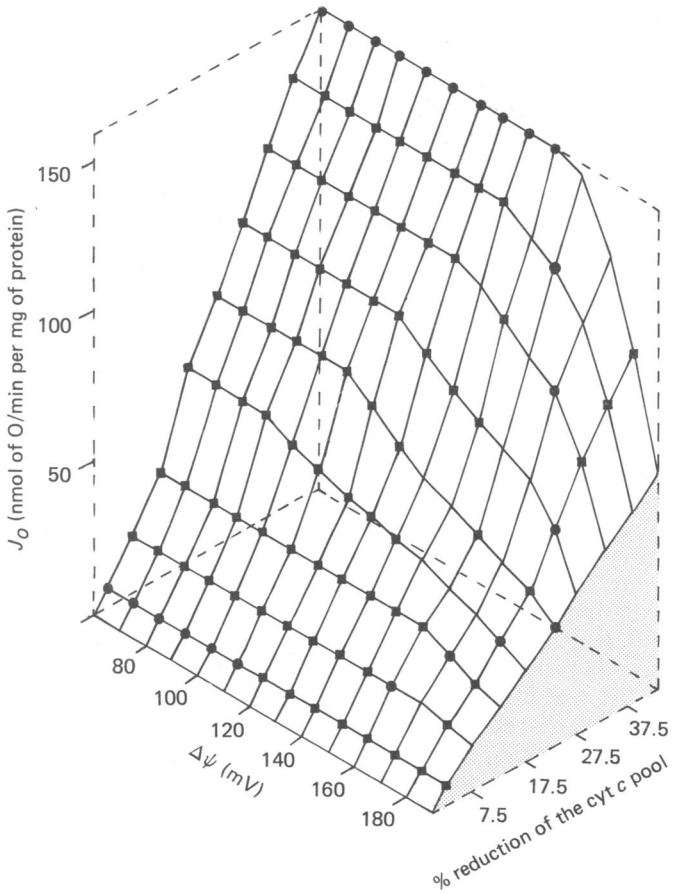

Fig. 3. J_O as a function of $\Delta\psi$ and % reduction of the cytochrome *c* pool

Circles represent the primary data set and squares are experimentally verified regions normalized to fit that set. See the text for details.

Localized chemiosmotic coupling

The stimulation of respiration rate by ADP sometimes gives a different relationship between J_O and Δp from that obtained when uncouplers are used to stimulate oxygen consumption (Padan & Rottenberg, 1973; for a review see Ferguson, 1985). A variety of localized

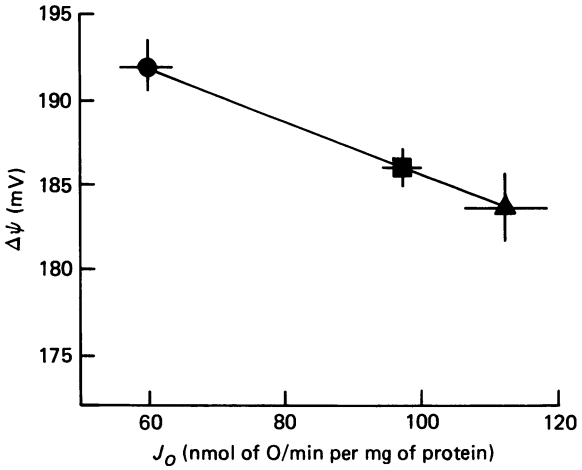

Fig. 4. $\Delta\psi$ versus J_O: J_O stimulated by ADP (▲) or by uncoupler (■)

Conditions are given in the Experimental section. No further additions were made for one point (●). This point was unchanged by the presence of oligomycin/ADP/hexokinase. For the other points ADP (0.130 mM) and excess hexokinase were present with no further additions (▲) or with oligomycin (1.6 μg/ml) and FCCP (33.3 nM) (■). The % reduction of the cytochrome *c* pool was also measured under each condition. For the experiment with ADP it was 41(±8)% and for the experiment with FCCP it was 38(±6)%.

chemiosmotic coupling schemes have been proposed to explain these discrepancies (Ferguson, 1985). If our description of electron flux through cytochrome oxidase is to have utility, the relationship between J_O, $\Delta\psi$ and % reduction of cytochrome *c* must be unique and independent of how we alter these parameters.

Fig. 4 shows the result of an experiment carried out under conditions similar to those of Figs. 1–3 in which respiration was stimulated either by uncoupler or by ADP. $\Delta\psi$, J_O and % reduction of the cytochrome *c* pool were then measured. This experiment gives a respiratory control ratio of 2 because of the presence of nigericin. In the absence of nigericin, the respiratory control ratio is about 5. In agreement with Nicholls & Bernson (1977), titration with uncoupler gave a linear relationship between $\Delta\psi$ and J_O (results not shown). The experimental points in Fig. 4 lie on one line; thus the lack of a unique relationship between J_O and $\Delta\psi$ is not apparent under the conditions we have used. The % reduction of the cytochrome *c* pool was approximately the same whether the respiration rate was stimulated by ADP or to the same extent by uncoupler. The experimental conditions with ADP and FCCP were as similar as possible. The only difference was the presence of FCCP and oligomycin in the experiment with uncoupler. We conclude that under the conditions we have used there is no evidence for ambiguous relationships between $\Delta\psi$ and J_O. However, other results from our laboratory (Brown, 1986), under conditions different from those in the present paper, showed a discrepancy between ADP and FCCP titrations of $\Delta\psi$ and J_O. This discrepancy varied from day to day and was not due to differences in the redox state of the cytochrome *c* pool.

Elasticity of electron flux through cytochrome oxidase to $\Delta\psi$

The experiments described above show qualitatively that the control of electron flux through cytochrome oxidase by $\Delta\psi$ and by the % reduction of the cytochrome *c* pool varies with the conditions. The description can be made more quantitative by calculating the elasticities (Westerhoff *et al.*, 1984). The elasticity of a system parameter (such as an enzyme activity) describes its dependence on its physiological effectors (such as its substrates). It is the fractional change in flux through the enzyme divided by the fractional change in the effector. It is defined for infinitesimal changes and is dimensionless. It is possible to consider a thermodynamic quantity as an effector (Westerhoff *et al.*, 1983). We define the elasticity (ϵ) of electron flux (v) through cytochrome oxidase to $\Delta\psi$ as:

$$\epsilon_{\Delta\psi} = \frac{RT}{F}\cdot\frac{1}{v}\left(\frac{\partial v}{\partial\Delta\psi}\right)$$

We determined the slope of J_O versus $\Delta\psi$ for each line segment in Fig. 3 and calculated the elasticity of electron flux through cytochrome oxidase to $\Delta\psi$ at different values of $\Delta\psi$ and the % reduction of the cytochrome *c* pool. This is shown in Fig. 5. Note that the elasticity is negative, because increasing $\Delta\psi$ lowers flux.

Fig. 5 shows more graphically what we have already concluded from Fig. 3, that the flux through cytochrome oxidase is strongly dependent on $\Delta\psi$ only at high values of $\Delta\psi$ and % reduction of the cytochrome *c* pool. Note that because of the manner of construction of Fig. 3,

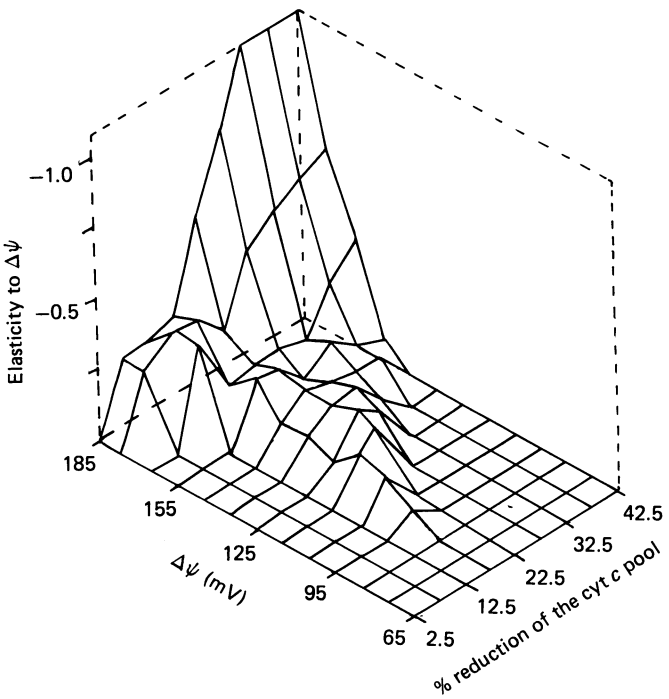

Fig. 5. Elasticity of cytochrome oxidase to $\Delta\psi$ as a function of $\Delta\psi$ and % reduction of the cytochrome *c* pool

Calculated from Fig. 3.

Figs. 5 and 6 are also only semiquantitative. At lower values of $\Delta\psi$ and % reduction of the cytochrome *c* pool, $\Delta\psi$ has no effect on electron flux through cytochrome oxidase.

Elasticity of electron flux through cytochrome oxidase to ΔE_h

The elasticity of electron flux through cytochrome oxidase to ΔE_h between cyt c^{2+}/cyt c^{3+} and oxygen/water can be defined as:

$$\epsilon_{\Delta E_h} = \frac{RT}{F}\cdot\frac{1}{v}\left(\frac{\partial v}{\partial\Delta E_h}\right)$$

The elasticity to ΔE_h is the same as the elasticity to the E_h of the cytochrome *c* pool because ΔE_h is calculated using a constant E_h for the oxygen/water couple. The ΔE_h for each % reduction in Fig. 3 was calculated and then the slopes of J_O versus ΔE_h were determined. The elasticity of electron flux through cytochrome oxidase to ΔE_h is plotted against $\Delta\psi$ and % reduction of the cytochrome *c* pool in Fig. 6.

The elasticity to ΔE_h was considered because this is a thermodynamic quantity and is therefore more directly comparable with $\Delta\psi$. The other Figures show respiration rate as a function of % reduction of the cytochrome *c* pool rather than of ΔE_h. This is because % reduction of the cytochrome *c* pool is easier to relate to physiological conditions. Alternatively, we can consider the elasticity of electron flux through cytochrome oxidase to the cyt c^{2+}/cyt c^{3+} ratio. We take the elasticity to the ratio because cyt c^{2+} and cyt c^{3+} comprise a moiety-conserved cycle (Hofmeyr *et al.*, 1986). A plot of the elasticity to this ratio against $\Delta\psi$ and the % reduction of the cytochrome *c* pool (not shown) is qualitatively similar to Fig. 6. However, this similarity may be fortuitous, because for

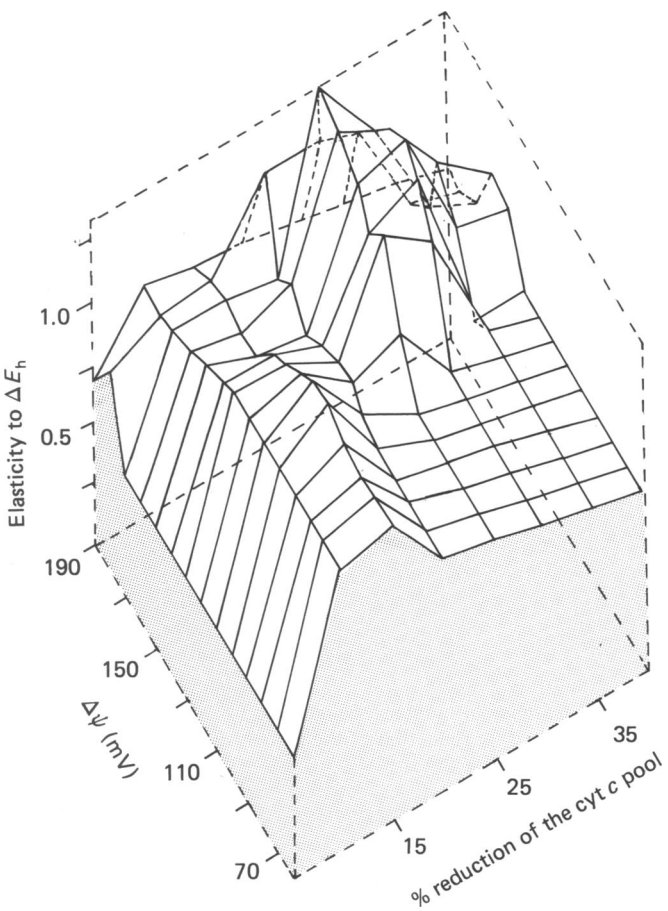

Fig. 6. Elasticity of cytochrome oxidase to ΔE_h as a function of $\Delta\psi$ and % reduction of the cytochrome *c* pool

Calculated from Fig. 3.

most of the region we consider the ratio cyt c^{2+}/cyt c^{3+} is approximately linearly related to ΔE_h: this is because of the properties of the function $\log(x/1-x)$ when plotted against $x/1-x$.

The relationship described by Fig. 6 is very different from that in Fig. 5 because J_O is strongly dependent on ΔE_h over a wide range of conditions. However, when both $\Delta\psi$ and % reduction of the cytochrome *c* pool are substantial, Figs. 5 and 6 show that the flux has approximately the same elasticity to $\Delta\psi$ as it does to ΔE_h. The % reduction of the cytochrome *c* pool in hepatocytes is about 20–25% depending on substrate (Wilson *et al.*, 1974*a*) and in perfused rat liver it is about 30% (Wilson *et al.*, 1974*b*). The mitochondrial membrane potential of hepatocytes respiring on octanoate is about 150–160 mV (C. D. Nobes & M. D. Brand, unpublished work). Therefore the elasticities to ΔE_h and to $\Delta\psi$ are of the same order of magnitude under conditions which are probably similar to those occurring *in vivo*.

DISCUSSION

The electron flux through cytochrome oxidase is a linear function of the thermodynamic force across it over a limited range of conditions. This range may be physiologically relevant. However, it is not possible to describe the electron flux through cytochrome oxidase as a simple function of the thermodynamic force over a wider range of conditions.

The relationship between the flux and its effectors is shown qualitatively in Fig. 3 and more graphically in Fig. 5 and Fig. 6. The relationship is complex. At high $\Delta\psi$ and more reduced cytochrome *c* pool the flux depends strongly on $\Delta\psi$ and the % reduction of the cytochrome *c* pool. The electron flux might therefore respond strongly to changes in both parameters *in vivo* and the high elasticities to $\Delta\psi$ and ΔE_h will tend to reduce variations in Δp (Duszynski *et al.*, 1984).

Other possible effectors of the flux through cytochrome oxidase that we have not examined include ΔpH and adenine nucleotides. As the extramitochondrial pH is buffered, ΔpH will have its effect on electron flux by altering the internal pH. Effects of the internal pH on electron flux have been reported by several groups. Increased matrix pH (or high ΔpH) lowers the rate of electron flux through cytochrome oxidase (Wilson *et al.*, 1977; Shaughnessy & Nicholls, 1985; Moroney *et al.*, 1984). Wilson *et al.* (1977) found that the relationship between electron flux and % reduction of the cytochrome *c* pool at fixed values of extramitochondrial ΔG_P (which is related to Δp) was changed quantitatively when the experiments were done at different values of pH but that the relationship was qualitatively the same.

The control of respiration by direct allosteric effects of adenine nucleotides on cytochrome oxidase has been proposed (Kadenbach, 1986). The non-catalytic subunits of the complex in multicellular organisms are considered to mediate such interactions and changes in these subunits between tissues within the same organism are proposed to represent the different requirements for control in the various tissues (Kadenbach, 1986). Photoaffinity analogues of ATP bind to a non-catalytic subunit of bovine heart cytochrome oxidase (Montecucco *et al.*, 1986) but the evidence for allosteric control of electron flux through cytochrome oxidase is as yet poor. If such effects do occur, our description of the control of electron flux through cytochrome oxidase would have to be expanded.

The effect of oxygen concentration on flux was not considered in this analysis because the apparent K_m for cytochrome oxidase to oxygen is less than 1 μM and the electron flux is therefore independent of oxygen concentration under most conditions (Erecinska & Wilson, 1982).

The application of metabolic control theory to the control of respiration has enabled us to quantify the effects on electron flux through cytochrome oxidase of $\Delta\psi$ and ΔE_h of the redox reaction catalysed by the complex. We have shown that when both are high they have similar effects on electron flux. Further work should indicate how the controls on respiration outlined by Tager's group (Tager *et al.*, 1983; Groen *et al.*, 1982) have their effects on respiration at the level of the other enzymes involved in oxidative phosphorylation. This should lead to a complete description of the control of respiration. Such a description would show, at one level, how alterations in the activities of system components such as the adenine nucleotide carrier or the matrix dehydrogenases affect respiration by changing Δp and the redox state of electron transport chain components. It would also describe, at a lower level, the dependence of electron flux through the electron transport chain on Δp

and the redox state of electron transport chain components.

M. P. M. is grateful to Peterhouse, Cambridge, for the award of a research studentship.

REFERENCES

Brand, M. D., Price, B. D. & Brown, G. C. (1986) in Biomedical and Clinical Aspects of Coenzyme Q (Folkers, K. & Yamamura, Y., eds.), vol. 5, pp. 39–49, Elsevier, Amsterdam
Brown, G. C. (1986) Ph.D. Thesis, University of Cambridge
Brown, G. C. & Brand, M. D. (1985) Biochem. J. **225**, 399–405
Chance, B. & Williams, G. R. (1955) J. Biol. Chem. **217**, 383–393
Chappell, J. B. & Hansford, R. G. (1972) in Subcellular Components: Preparation and Fractionation (Birnie, G. D., ed.), 2nd edn., pp. 77–91, Butterworths, London
Davis, E. J. & Davis-Van Thienen, W. I. A. (1984) Arch. Biochem. Biophys. **233**, 573–581
Duszynski, J., Bogucka, K. & Wojtczak, L. (1984) Biochim. Biophys. Acta **767**, 540–547
Dutton, P. L., Wilson, D. F. & Lee, C. P. (1970) Biochemistry **9**, 5077–5082
Erecinska, M. & Wilson, D. F. (1982) J. Membr. Biol. **70**, 1–14
Ferguson, S. J. (1985) Biochim. Biophys. Acta **811**, 47–95
Ferguson, S. J. & Sorgato, M. C. (1982) Annu. Rev. Biochem. **51**, 185–217
George, P. (1965) in Oxidases and Related Redox Systems, vol. 1 (King, T., ed.), pp. 3–33, Wiley, New York
Groen, A. K., Wanders, R. J. A., Westerhoff, H. V., van der Meer, R. & Tager, J. M. (1982) J. Biol. Chem. **257**, 2754–2757
Gornall, A. G., Bardawill, C. J. & David, M. M. (1949) J. Biol. Chem. **177**, 751–766
Hansford, R. G. (1980) Curr. Top. Bioenerg. **10**, 217–278
Hansford, R. G. (1985) Rev. Physiol. Biochem. Pharmacol. **102**, 1–72
Heinrich, R. & Rapoport, T. A. (1974) Eur. J. Biochem. **42**, 89–95
Hofmeyr, J. S., Kacser, H. & van der Merwe, K. J. (1986) Eur. J. Biochem. **155**, 631–641
Kacser, H. & Burns, J. A. (1973) Symp. Soc. Exp. Biol. **32**, 65–104
Kacser, H. & Burns, J. A. (1979) Biochem. Soc. Trans. **7**, 1149–1160
Kadenbach, B. (1986) J. Bioenerg. Biomembr. **18**, 39–54
Klingenberg, M. (1964) Angew. Chem. Int. Ed. **3**, 54–61
Montecucco, C., Schiavo, G. & Bisson, R. (1986) Biochem. J. **234**, 241–243
Moroney, P. M., Scholes, T. A. & Hinkle, P. C. (1984) Biochemistry **23**, 4991–4997
Murphy, M. P. & Brand, M. D. (1986) Biochem. Soc. Trans. **14**, 887–888
Murphy, M. P., Brown, G. C. & Brand, M. D. (1985) FEBS Lett. **187**, 16–20
Nicholls, D. G. & Bernson, V. S. M. (1977) Eur. J. Biochem. **75**, 601–612
Padan, E. & Rottenberg, H. (1973) Eur. J. Biochem. **40**, 431–437
Robinson, J. & Cooper, J. M. (1970) Anal. Biochem. **33**, 390–399
Rottenberg, H. (1973) Biophys. J. **13**, 503–511
Rottenberg, H. & Gutman, M. (1977) Biochemistry **16**, 3220–3227
Shaughnessy, S. & Nicholls, P. (1985) Biochem. Biophys. Res. Commun. **128**, 1025–1030
Stucki, J. W., Compiani, M. & Caplan, S. R. (1983) Biophys. Chem. **18**, 101–109
Tager, J. M., Wanders, R. J. A., Groen, A. K., Kunz, W., Bohnensack, R., Kuster, U., Letko, G., Bohme, G., Duszynski, J. & Wojtczak, L. (1983) FEBS Lett. **151**, 1–9
Van Dam, K., Westerhoff, H. V., Rutgers, M., Bode, J. A., De Jonge, P. C., Bos, M. M. & Van Den Berg, G. (1981) in Vectorial Reactions in Electron and Ion Transport in Mitochondria and Bacteria (Palmieri, F., Quagliariello, E., Siliprandi, N. & Slater, E. C., eds.), pp. 389–397, Elsevier/North-Holland, Amsterdam
Westerhoff, H. V., Groen, A. K. & Wanders, R. J. A. (1983) Biochem. Soc. Trans. **11**, 90–91
Westerhoff, H. V., Groen, A. K. & Wanders, R. J. A. (1984) Biosci. Rep. **4**, 1–22
Wilson, D. F., Stubbs, M., Veech, R. L., Erecinska, M. & Krebs, H. A. (1974*a*) Biochem. J. **140**, 57–64
Wilson, D. F., Stubbs, M., Oshino, N. & Erecinska, M. (1974*b*) Biochemistry **13**, 5305–5311
Wilson, D. F., Owen, C. S. & Holian, A. (1977) Arch. Biochem. Biophys. **182**, 749–762

Received 12 September 1986/12 November 1986; accepted 19 December 1986