MINI-REVIEW

Role of Energy in Oxidative Phosphorylation

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Abstract

This article reviews the current status of information regarding the role of energy in the process of oxidative phosphorylation by mitochondria. The available data suggest that in submitochondrial particles (SMP) energy is utilized for the binding of ADP and P_i and for the release of ATP bound at the catalytic sites of F_1-ATPase. The process of ATP synthesis on the surface of F_1 from F_1-bound ADP and P_i appears to be associated with negligible free energy change. The rate of energy production by the respiratory chain modulates the kinetics of ATP synthesis between a low K_m (for ADP and P_i)–low V_{max} mode and a high K_m–high V_{max} mode. The K_m extremes for ADP are 2–3 μM and 120–150 μM, and V_{max} for ATP synthesis at high rates of energy production by bovine-heart SMP is about $440 \, s^{-1}$ (mole F_1)$^{-1}$ at 30° C, which corresponds to 11 μmol ATP (min · mg of protein)$^{-1}$. The interaction of dicyclohexylcarbodiimide (DCCD) or oligomycin at the proteolipid (subunit c) of the membrane sector (F_0) of the ATP synthase complex alters the mode of ATP binding at the catalytic sites of F_1, probably to one of lower affinity. It has been suggested that protonic energy might be conveyed to the catalytic sites of F_1 in an analogous manner, i.e., via conformation changes in the ATP synthase complex initiated by proton-induced alterations in the structure of the DCCD-binding proteolipid. Finally, the relationship between the steady-state membrane potential ($\Delta\psi$) and the rates of electron transfer and ATP synthesis has been discussed. It has been shown, in agreement with the delocalized chemiosmotic mechanism, that under appropriate conditions $\Delta\psi$ is exquisitely sensitive to changes in the rates of energy production and consumption.

Key Words: Oxidative phosphorylation; energy communication; affinity change; kinetic modalities; membrane potential.

Introduction

Through study of the mechanism of ATP hydrolysis by isolated F_1 ATPases, some of the fundamental features of the mechanism of ATP

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synthesis in oxidative and photosynthetic phosphorylation have been eluci-
dated. For example, it has been shown in agreement with the original postu-
late of Boyer *et al.* (1973) that synthesis of F_1-bound ATP from F_1-bound
ADP and P_i does not require energy (Rosing *et al.*, 1977; Choate *et al.*, 1979;
Boyer *et al.*, 1982; Russo *et al.*, 1978; Feldman and Sigman, 1982; Grubmeyer
et al., 1982; Sakamoto and Tonomura, 1983), and that the hydrolysis of ATP
(hence the reversal of this reaction) by F_1 does not involve a phosphorylated
intermediate (Webb *et al.*, 1980). The catalytic sites of F_1 have been located
on the β subunits or at the $\alpha\beta$ interfaces (Esch and Allison, 1978; Pougeois
et al., 1979; Cross, 1981; Williams and Coleman, 1982; Amzel and Pedersen,
1983; Senior and Wise, 1983; Lüben *et al.*, 1984) and a number of essential
protein residues have been identified (Esch and Allison, 1978; Pougeois *et al.*,
1979; Ferguson *et al.*, 1975; Frigeri *et al.*, 1977; Ting and Wang, 1980; Vignais
and Lunardi, 1985; Duncan *et al.*, 1986; for a recent reassessment by site-
directed mutagenesis, see Parsonage *et al.*, 1987). Other studies have demon-
strated that a single catalytic site on F_1 binds ATP very tightly and partially
hydrolyzes it to bound ADP and P_i ($K_{eq} = 0.5$) (Grubmeyer *et al.*, 1982). The
rate of the uni-site ATP hydrolysis is extremely slow, and is accelerated
10^4 to 10^6 times when additional catalytic sites on F_1 are allowed to bind
ATP (Cross *et al.*, 1982; Duncan and Senior, 1985; Noumi *et al.*, 1986).
These and other studies (Feldman and Sigman, 1982; Grubmeyer *et al.*,
1982; Kozlov and Skulachev, 1977; Gresser *et al.*, 1982) have indicated that
the catalytic sites on F_1 exhibit cooperativity in ATP hydrolysis such that
substrate occupancy of additional catalytic sites greatly enhances turnover at
the first site by accelerating the product (ADP) off rate (Grubmeyer
et al., 1982).

While these and other important recent findings regarding the mech-
anism of ATP hydrolysis by F_1 ATPases (see reviews by Cross, 1981; Senior
and Wise, 1983; Futai and Kanazawa, 1983; Hatefi, 1985, and the other
articles in this volume) have been invaluable in understanding the mech-
anism of ATP synthesis, there is a fundamental aspect of the latter pro-
cess that could not be investigated with the use of isolated F_1. That is
the role played by energy. We shall, therefore, concentrate in this article
on recent findings relevant to the role of energy in oxidative phosphory-
lation, especially since space limitation does not permit a review of wider
scope. The specific points discussed will be (1) energy-induced substrate-
product affinity changes, (2) kinetic modalities of ATP synthesis, (3) energy
communication between F_0 (the membrane sector of the ATP synthase
complex) and F_1, and (4) the mechanism of energy transfer among the
mitochondrial energy-linked reactions.

Energy-Induced Substrate/Product Affinity Changes

As was mentioned above, Boyer and coworkers proposed in 1973 that ATP synthesis on the surface of F_1 does not require energy (Boyer et al., 1973). They also suggested that the energy-requiring steps in oxidative phosphorylation are substrate (ADP, P_i) binding and product (ATP) releasing (Rosing et al., 1976). These conclusions were based on the effect of uncouplers on two types of P_i–HOH ^{18}O exchange. Thus, "intermediate" P_i–HOH oxygen exchange by submitochondrial particles (SMP) treated with ATP, ADP, and P_i was found to be uncoupler-insensitive, whereas "medium" exchange was shown to be uncoupler-sensitive. The former result was interpreted to mean that P_i formed from ATP hydrolysis was reutilized, before its release from the enzyme, to resynthesize F_1-bound ATP in a reaction that did not require energy and resulted in uncoupler-insensitive "intermediate" P_i–HOH oxygen exchange. Subsequent studies in Boyer's (Choate et al., 1979; Boyer, 1979; Boyer et al., 1982; Gresser et al., 1982) and other laboratories (Russo et al., 1978; Feldman and Sigman, 1982; Grubmeyer et al., 1982; Sakamoto and Tonomura, 1983) provided strong support for this conclusion, as already mentioned. The finding that "medium" P_i–HOH oxygen exchange was uncoupler-sensitive was interpreted to mean that the binding of medium P_i as a preliminary step for the oxygen exchange reaction was an energy-promoted process (Rosing et al., 1976). This conclusion and the predictions that ADP binding and ATP releasing were also energy-requiring steps were subsequently supported by results from other laboratories.

It was shown by Hatefi et al. (1982) in several mitochondrial energy-linked reactions (i.e., oxidative phosphorylation, NTP–$^{32}P_i$ exchange, ATP-driven electron transfer from succinate to NAD, and transhydrogenation from NADH to NADP) that ln (V_{max}/K_m) decreased linearly with increased uncoupling of the particles by various uncouplers. Examples are shown in Figs. 1 and 2. These results suggested that the affinity of the substrates of the energy-driven reactions examined for their respective enzymes increased upon membrane energization (Hatefi et al., 1982). Further, the linear relationship between ln (V_{max}/K_m) and the degree of membrane coupling or energization suggested that the data could be used to calculate the change in the binding energy of the substrates involved in going from an uncoupled to a coupled state. This is because the increment in binding energy of a ligand in going from state 1 to state 2 of an enzyme could be estimated from $\Delta\Delta G_{2-1} = RT\ln [(k_{cat}/K_m)_2/(k_{cat}/K_m)_1]$ (Fersht, 1985, 1987). For example, in Fig. 1A, where oxidative phosphorylation activity was decreased by 90% at 200 nM carbonylcyanide m-chlorophenylhydrazone (CCCP), the binding
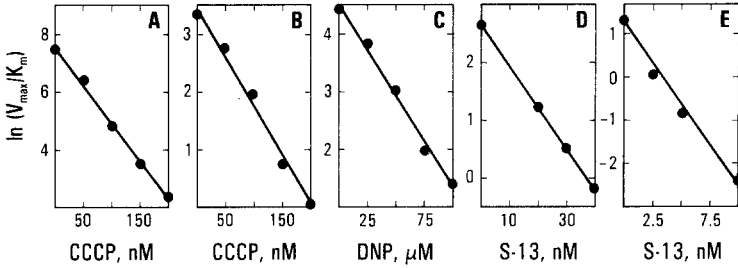
Fig. 1. Plots of ln (V_{max}/K_m) versus partially uncoupling concentrations of carbonylcyanide *m*-chlorophenylhydrazone (CCCP), 2,4-dinitrophenol (DNP), and 5-chloro-3-*t*-butyl-2'-chloro-4'-nitrosalicylanilide (S-13) as shown for ATP synthesis with P_i (0.5 to 40 mM) as the variable substrate (A), ATP synthesis with ADP (40–1000 μM) as the variable substrate (B), ITP-$^{32}P_i$ exchange with P_i (1.3–21.5 mM) as the variable substrate (C), ATP-driven succinate reduction of NAD with NAD (0.02–0.2 mM) as the variable substrate (D), and respiration-energized transhydrogenation from NADH to 3-acetylpyridine adenine dinucleotide phosphate (AcPyADP) with AcPyADP (5–200 μM) as the variable substrate (E). All apparent K_m and V_{max} values were derived from double-reciprocal plots. From Hatefi *et al.* (1982) with permission.
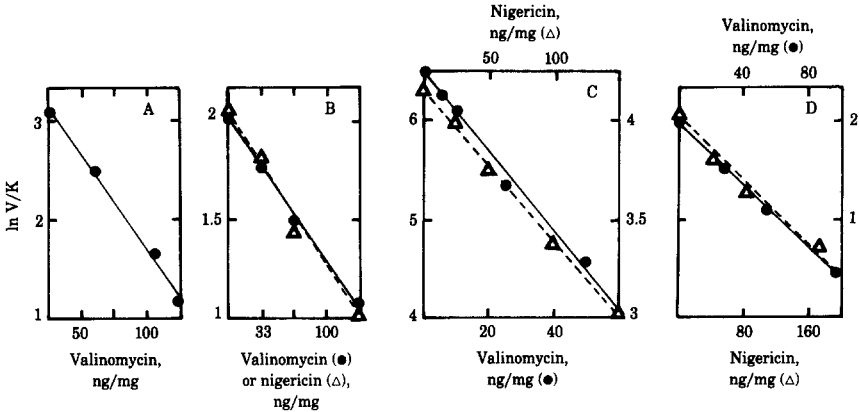

Fig. 2. Plots of ln (V_{max}/K_m) versus partially uncoupling concentrations of valinomycin plus nigericin as shown for oxidative phosphorylation with ADP (30–1000 μM) as the variable substrate (A), oxidative phosphorylation with P_i (0.5–20 mM) as the variable substrate (B), ATP-$^{32}P_i$ exchange with ATP (0.4–2.0 mM) as the variable substrate (bottom abscissa and left ordinate) and with P_i (2.5–20 mM) as the variable substrate (top abscissa and right ordinate) (C), and NADH → AcPyADP transhydrogenation with AcPyADP (5–100 μM) as the variable substrate (D). K^+ concentration varied from 33 to 50 mM. In A, the concentration of nigericin was 0.5 μg/mg of protein; that of valinomycin was as shown. In B, the concentration of valinomycin was 1.3 μg/mg of protein in the experiments marked by $\triangle$, and nigericin was as shown; the concentration of nigericin was 0.5 μg/mg of protein in the experiments marked ●, and valinomycin was as shown. In C, the concentration of the fixed ionophore (valinomycin in $\triangle$, and nigericin in ●) was 0.5 μg/mg of protein. In D, the concentration of the fixed ionophores was, per mg of protein, 0.56 μg of nigericin in ● and 1.25 μg of valinomycin in $\triangle$. All apparent K_m and V_{max} values were obtained from double-reciprocal plots. From Hatefi *et al.* (1982) with permission.

energy change for P_i between this state and the fully energized state of SMP in the absence of CCCP would calculate to about 3.2 kcal/mol. By comparison, the binding energy change for ADP was about 2 kcal/mol (Fig. 1B). It should be pointed out that the values derived from Figs. 1 and 2 may be low estimates, because SMP preparations are generally leaky, and in this experiment P/O = 1.0 with succinate as the respiratory substrate. Nevertheless, what is indicated by the data of Figs. 1 and 2 is that in oxidative phosphorylation, uphill electron transfer from succinate to NAD, and transhydrogenation from NADH to NADP, membrane energization appears to increase the binding energy of the substrates of the driven reactions.

More recently, it was demonstrated by Penefsky (1985a) that the tight binding of [γ-^{32}P]ATP added to a molar excess of SMP (uni-site binding, $K_a = 10^{12} M^{-1}$) was greatly decreased upon membrane energization by initiation of respiration with addition of NADH or succinate, and resulted in partial ($\sim 30\%$) release of the bound [γ-^{32}P]ATP. Furthermore, the NADH-induced rate of ATP release was comparable to the rate of NADH-supported ATP synthesis. These results indicated, therefore, that the release of tightly bound ATP under uni-site conditions is at least partially promoted by energy derived from respiration. Assuming that this phenomenon would also pertain to *de novo* synthesized ATP under physiological substrate concentrations, the results described above could be summarized as depicted in Fig. 3 in which reactions 1 and 3 involve little free energy change, while reactions 2 and 4 are energy-promoted processes in the direction of the heavy arrows. This figure suggests certain common features with the mechanism of ATP synthesis by cation-transport ATPases (Pedersen and Carafoli, 1987a,b). Cation binding by these so-called P-type ATPases changes the mode of P_i binding from E–P to E$\sim$P (Jencks, 1980; Pickart and Jencks, 1984). This step is formally analogous to step 2 of Fig. 3 in which protonic energy (proton binding to F_0F_1?) increases the binding energy of P_i (and ADP). The next step is also basically the same in both mechanisms, where
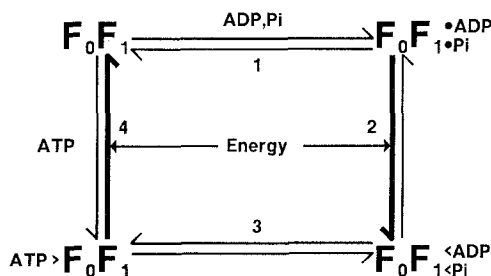

Fig. 3. The energy-promoted steps (heavy arrows) in ATP synthesis during oxidative phosphorylation.

ATP synthesis occurs in a reversible, isoenergetic manner (Rosing *et al.*, 1977; Choate *et al.*, 1979; Boyer *et al.*, 1982; Russo *et al.*, 1978; Feldman and Sigman, 1982; Grubmeyer *et al.*, 1982; Sakamoto and Tonomura, 1983; Pickart and Jencks, 1984). In addition, in both processes, the *de novo* synthesized ATP is tightly bound to its respective enzyme (Rosing *et al.*, 1977; Gresser *et al.*, 1982; Pickart and Jencks, 1984).

Kinetic Modalities of ATP Synthesis

As mentioned above, isolated and membrane-bound F_1 exhibit site–site cooperativity in ATP hydrolysis. The most dramatic effect of site–site cooperativity is observed in going from uni-site conditions, where [ATP] is substoichiometric with respect to [F_1], to multisite conditions where [ATP] is far in excess of [F_1]. Under uni-site conditions, ATP was shown to be tightly bound to F_1 ($K_a = 10^{12} M^{-1}$) and very slowly hydrolyzed ($V = 10^{-4} s^{-1}$), while under multisite conditions ATP was hydrolyzed at a $V_{max} = 600 s^{-1}$ and exhibited an apparent $K_m = 2.5 \times 10^{-4} M$ (Grubmeyer *et al.*, 1982; Cross *et al.*, 1982). When the kinetics of ATP hydrolysis by F_1 were analyzed at the ATP concentration range of 1–5000 μM, the data best fitted a triphasic curve with apparent K_m values of 10^{-6}, 1.5–2.0×10^{-4}, and $10^{-3} M$ (Table I) (Wong *et al.*, 1984). The high K_m component was not always detected, and the kinetic data could be analyzed for only two K_m values when F_1 was activated by pretreatment with ATP or by assay in the presence of bicarbonate. The kinetics of ITP hydrolysis also exhibited only two K_m values. These data

Table I. Kinetic Constants for ATP and ITP Hydrolysis by Mitochondrial and Bacterial
F_1-ATPases

Preparation	Substrate	K_{m1}	V_{max1}[a]	K_{m2}	V_{max2}	K_{m3}	V_{max3}	Total V_{max}
MF_1[b]	ATP	$\leqslant 10^{-6}$	$\leqslant 4$	1.6×10^{-4}	47	10^{-3}	29	80
$MF_1(+HCO_3^-)$	ATP	$\leqslant 10^{-6}$	$\leqslant 2$	1.6×10^{-4}	120	—	—	122
MF_1 activated ($+HCO_3^-$)	ATP	$\leqslant 10^{-6}$	$\leqslant 8$	1.8×10^{-4}	160	—	—	168
MF_1	ITP	$\approx 10^{-7}$	0.1	9×10^{-4}	100	—	—	100
TF_1	ATP	10^{-5}	1.0	2.8×10^{-4}	16	10^{-3}	18	35
EF_1	ATP	2×10^{-5}	1.0	1.5×10^{-4}	4	—	—	5^c

[a] V_{max} values are in μmol ATP or ITP hydrolyzed (min · mg of protein)$^{-1}$ at 30° C, and K_m values are in molar ATP.
[b] MF_1, TF_1, and EF_1 refer, respectively, to the bovine mitochondrial F_1, the thermophilic bacterium F_1, and the *E. coli* F_1. The ATP and ITP concentration ranges used for MF_1 and TF_1 were 1–5000 μM in the presence of 1 mM excess $MgSO_4$, and that for EF_1 was 3 μM to 20 mM as MgATP. Where indicated, 10 mM $KHCO_3$ was added to the assay mixture.
[c] EF_1 preparations depleted of the ε subunit exhibited a third K_m of about 30 mM and an overall V_{max} of about 54 Dunn *et al.* (1987).

and kinetic constants for ATP hydrolysis by F_1 from themophilic bacterium PS3 (TF_1) (Wong *et al.*, 1984) and from *Escherichia coli* (EF_1) (Dunn *et al.*, 1987) are compiled in Table I.

Plots of the kinetics of SMP-catalyzed ATP synthesis at variable [ADP] or [P_i] were also found to be curvilinear and suggestive of cooperativity (Matsuno-Yagi and Hatefi, 1985; Stroop and Boyer, 1985). However, a closer scrutiny indicated that this curvilinearity was a more complex phenomenon, and the data could be analyzed in terms of a minimum of two interconvertible kinetic modalities (Matsuno-Yagi and Hatefi, 1986). One mode was characterized by low apparent K_m values for ADP (6–10 μM) and P_i ($\leqslant 0.25$ mM) and a limited capacity for ATP synthesis (apparent $V_{max} \sim 500$ nmol ATP · min^{-1} · mg of $protein^{-1}$). ATP synthesis occurred predominantly in this mode when the coupled activity of the respiratory chain relative to the number of functional ATP synthase complexes was low. The second kinetic mode was characterized by high apparent K_m values for ADP (50–100 μM) and Pi (~ 2 mM) and a very high capability for ATP synthesis. Recent studies have indicated that when energy production is not rate limiting, the apparent V_{max} in the high K_m mode could be as high as 11,000 nmol ATP (min · mg of SMP protein)$^{-1}$ at 30°C (see below). This mode of ATP synthesis predominates when the rate of energy production relative to the number of functional ATP synthases is high. Thus, as seen in Fig. 4 and Table II, progressive
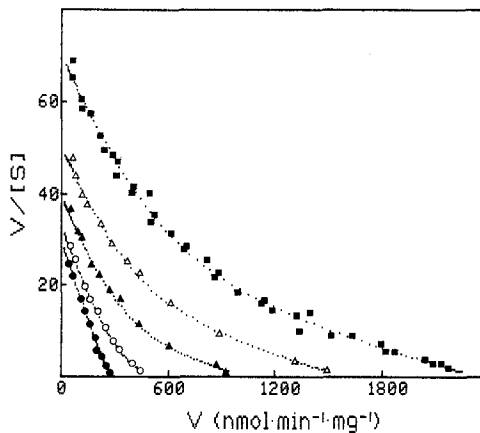

Fig. 4. Effect of attenuation of respiration rate on the kinetics of oxidative phosphorylation at variable ADP concentrations. The respiratory substrate was 0.5 mM NADH in ■, △, ▲, and ○, and 30 mM DL-β-hydroxybutyrate + 1 mM NAD in ●. The ADP concentration range was 1–1200 μM. In △, ▲, and ○, the rate of NADH oxidation was partially inhibited by addition to the assay mixture of 0.57, 1.15, and 2.0 mM Demerol, respectively. This and Fig. 5 are computer printouts. The *dots* represent the curves calculated from the K_m and V_{max} values shown in Table II. For other details, see Table II. v, nmol of ATP formed (min · mg of protein)$^{-1}$; [S], μM ADP. From Matsuno-Yagi and Hatefi (1986) with permission.

Table II. Effect of Attenuation of Respiration Rate on the Kinetics of ATP Synthesis with ADP as the Variable Substrate and NADH as the Energy Source[a]

Percent respiration	K_{m1}^{ADP}	V_{max1}	K_{m2}^{ADP}	V_{max2}	Total V_{max}
100	9.2	500	115	1840	2340
43	9.5	374	113	1219	1593
25	9.9	332	118	687	1019
18	9.3	287	95	213	500
8	8.6	255	114	34	289

[a]NADH oxidase activity was measured by a Clark-type oxygen electrode, using the same medium as was employed for assay of oxidative phosphorylation in the presence of 1200 μM ADP. One hundred percent NADH oxidase activity was 1870 ng-atom oxygen consumed (min · mg of protein)$^{-1}$. The respiratory substrate in the first four experiments was 0.5 mM NADH in the presence of 0, 0.57, 1.15, and 2.0 mM Demerol, respectively. In the last experiment, the respiratory substrate was 30 mM DL-β-hydroxybutyrate in the presence of 1 mM NAD. The K_m and V_{max} values shown were derived from the Eadie–Hofstee plots of Fig. 4. Where indicated in this and Table III, the units of K_m^{ADP} and V_{max} are, respectively, μM and nmol ATP formed (min · mg of protein)$^{-1}$. The K_m and V_{max} values given in this and Table III are assigned numbers for best fit of the computer-derived curves to the experimental data. They are not meant to imply precision to the extent shown. The accuracy of the experimental data (measured rates of ATP synthesis) should be judged from their spread in figures. From Matsuno-Yagi and Hatefi (1986) with permission.

suppression of the rate of respiration drastically diminished the contribution of the high K_m–high V_{max} mode to the overall kinetics of ATP synthesis, and as seen in Fig. 5 and Table III progressive inactivation of the ATP synthases converted the kinetics of ATP synthesis to the high K_m–high V_{max} mode. The data in Figs. 4 and 5 and Tables II and III are for ADP as the variable substrate. Similar modality changes were observed with P_i as the variable substrate (Matsuno-Yagi and Hatefi, 1986). Furthermore, as seen in Tables II and III, the low and the high K_m values for ADP remained unchanged during partial inhibition of respiration or fractional inactivation of the ATP synthase complexes.

It is clear from Figs. 4 and 5 that, in the ADP concentration range (1–1200 μM) that was used in these experiments, a principal factor that influenced the kinetics of ATP synthesis was the coupled rate of respiration relative to the number of functional ATP synthase molecules. The higher the rate of respiration and the lower the number of functional ATP synthase molecules in SMP, the greater the contribution of the high K_m–high V_{max} mode to the kinetics of ATP synthesis. Thus, it was of interest to study the kinetics of ATP synthesis at the extremes, i.e., at very low rates of respiration, and at high rates of respiration and high fractional inactivation of the ATP synthase complexes. These studies have revealed that at very low rates of respiration, the apparent K_m for ADP reaches a constant value of about 2–3 μM, and that at high rates of respiration, with $\geq$ 90% of the ATP
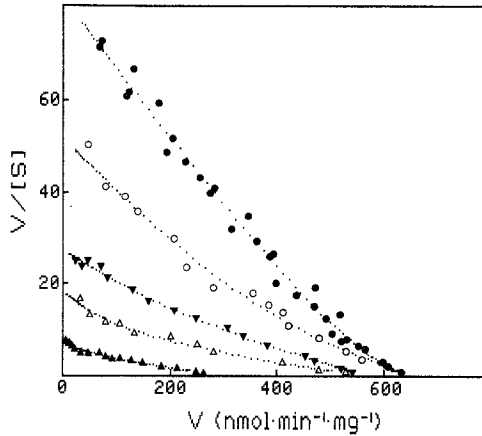
Fig. 5. Effect of partial inhibition of ATP synthase on the kinetics of ATP synthesis at variable concentrations of ADP. The respiratory substrate was 6.7 mM succinate. The ATPase activity of SMP was partially inhibited by treatment at 0°C with 5 μM DCCD for 3 h (O), 10 μM DCCD for 3 h ($\blacktriangledown$), 20 μM DCCD for 2 h ($\triangle$), or 20 μM DCCD for 3 h ($\blacktriangle$). Filled circles ($\bullet$) are for control SMP not treated with DCCD. For other details, see Table III. From Matsuno-Yagi and Hatefi (1986) with permission.

Table III. Effect of Partial Inhibition of ATPase on the Kinetics of ATP Synthesis with ADP as the Variable Substrate and Succinate as the Energy Source[a]

ATPase inhibitor	Percent ATPase activity	K_{m1}^{ADP}	V_{max1}	K_{m2}^{ADP}	V_{max2}	Total V_{max}
None	100	6.5	517	56	119	636
DCCD, 5 μM (3 h)	52	7.6	340	46	315	655
DCCD, 10 μM (3 h)	25	9.1	150	39	424	574
DCCD, 20 μM (2 h)	16	6.0	63	61	489	552
DCCD, 20 μM (3 h)	6	7.1	19	60	270	289

[a]Data for oxidative phosphorylation at variable ADP concentrations (1–1200 μM) were constructed in Eadie–Hofstee plots (Fig. 5) and the plots analyzed for the K_m and V_{max} values shown. One hundred percent ATPase activity of the SMP samples untreated with DCCD was 4.3 μmol ATP hydrolyzed (min $\cdot$ mg of protein)$^{-1}$. From Matsuno-Yagi and Hatefi (1986) with permission.

synthase complexes inactivated, the apparent K_m for ADP increases to 120–150 μM as the upper limit. The above results seem to suggest that the principal factor that modulates the kinetics of ATP synthesis between the extremes described is the rate of energy production by the system. Indeed, as will be seen below, elevation of the rate of respiration and fractional inactivation of the ATP synthase complexes of SMP result in an increase in the steady-state level of the membrane potential (see Fig. 12).

A question of interest is how the rate of energy production modulates the kinetics of ATP synthesis between the extremes discussed above. The
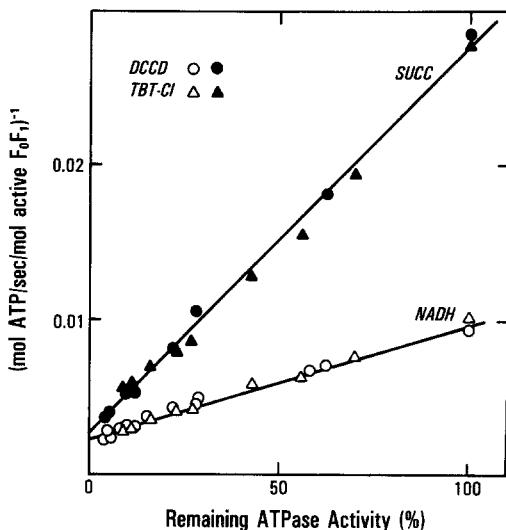
Fig. 6. Increase in the turnover rate of F_0F_1 complexes for ATP synthesis as the population of these complexes in SMP was decreased by fractional inactivation with DCCD or TBT-Cl. Respiratory substrates were NADH and succinate, as shown, and the extent of fractional inactivation of F_0F_1 complexes was monitored by the decrease in ATPase activity of uncoupled SMP as depicted on the abscissa as well as by [^{14}C]DCCD binding at F_0. The fractional inactivation of F_0F_1 complexes resulted in increase in the steady-state free energy of the system. At high inhibition of ATPase activity (e.g., $\geqslant 80\%$), the steady-state membrane potential ($\Delta\psi$) was very close to static-head $\Delta\psi$, which was about 150 mV (Matsuno-Yagi and Hatefi, 1987). From Matsuno-Yagi and Hatefi (1988) with permission.

possibility of different populations of ATP synthases with different kinetic characteristics being activated at low or high respiratory rates does not agree with experimental results, because the transition from essentially low K_m–low V_{max} to predominantly high K_m–high V_{max} kinetics is monophasic (see Fig. 6). More likely is the possibility that all ATP synthases are alike and capable of adjusting structurally and consequently in their kinetic behavior to the kinetics of energy production by the respiratory chain. In this regard, a role for the multiple copies of certain F_0 subunits (e.g., the dicyclohexylcarbodiimide-binding proteolipid) and F_1 subunits (e.g., $\alpha\beta$) can be envisaged such that, depending on the rate of energy production by the respiratory chain, greater or fewer numbers of these subunits would participate in energy transfer and ATP synthesis.

It was observed by several laboratories that fractional inhibition of F_0F_1 complexes by various chemical modifiers inhibited ATP hydrolysis more than ATP synthesis (Schäfer, 1982; Emanuel et al., 1984; Van Der Bend et al., 1985). The results were interpreted in terms of greater affinity of the inhibitor for the nonenergized versus the energized conformation of the ATP synthases, separate catalytic sites for ATP synthesis and hydrolysis, or different

ways in which ATP synthesis and hydrolysis are coupled to proton con-
duction through F_0 (Schäfer, 1982; Emanuel *et al.*, 1984; Van Der Bend
et al., 1985). As seen in Table III, a similar differential effect was observed
with N,N'-dicyclohexylcarbodiimide (DCCD) as the inhibitor, which binds
covalently and irreversibly to F_0. In fact, to the extent we have examined, all
inhibitors of F_0 (oligomycin, DCCD, organotin compounds, venturicidin)
and F_1 (efrapeptin, *p*-fluorosulfonylbenzoyl-5'-adenosine, aurovertin) behaved
essentially the same (Matsuno-Yagi and Hatefi, 1986). The reason for inhibi-
tion of ATP hydrolysis more than synthesis is that fractional modification of
$F_0 F_1$ complexes by the above reagents results in proportional loss of the
ATPase activity of SMP. However, such fractional inactivation favors the
conversion of low K_m–low V_{max} to high K_m–high V_{max} kinetics in ATP syn-
thesis. Consequently, the remaining active fraction of $F_0 F_1$ complexes syn-
thesize ATP at a faster rate. This point is clearly demonstrated in Fig. 6,
where the percent ATPase activity remaining after fractional inhibition of
$F_0 F_1$ complexes has been plotted against the reciprocal of the rate of ATP
synthesis per mole of *active* $F_0 F_1$. The inhibitors used were DCCD and
tributyltin chloride (TBT-Cl), and the respiratory substrates employed were
NADH and succinate. As seen in Fig. 6, fractional inhibition of $F_0 F_1$ com-
plexes resulted in an increase in the synthetic turnover rates of the remaining
active $F_0 F_1$ molecules with either NADH or succinate as the respiratory
substrate, and the two linear plots converged at the same point on the
ordinate. As discussed elsewhere (Matsuno-Yagi and Hatefi, 1988), the con-
vergence of the two lines on the ordinate indicates that at this point energy
production by the respiratory chain is no longer rate limiting for ATP
synthesis, and the ordinate intercept indicates that the synthetic turnover
number of the $F_0 F_1$ complexes of the SMP used was $440 \, s^{-1}$. This value is
essentially the same as the hydrolytic activity of $F_0 F_1$ complexes in SMP
$(450–500 \, s^{-1} \cdot mol \, F_1^{-1})$. It is also comparable to the highest rates recorded
for ATP synthesis by chloroplast $F_0 F_1$ $(400–420 \, s^{-1} \cdot mol \, CF_0 F_1^{-1})$ (Vinkler,
1981; Junesch and Gräber, 1985).

Energy Communication between F_0 and F_1

In principle, the energy-promoted binding changes shown in Fig. 3
could result from H^+-induced ionization changes of ligand/protein moieties
at the catalytic sites or from conformation changes of the catalytic sites in
response to protonation or energy-induced changes elsewhere in the $F_0 F_1$
complex. Two sets of recent experiments suggest that the latter is a likely
possibility. It was shown by Penefsky (1985b) that the uni-site tight binding
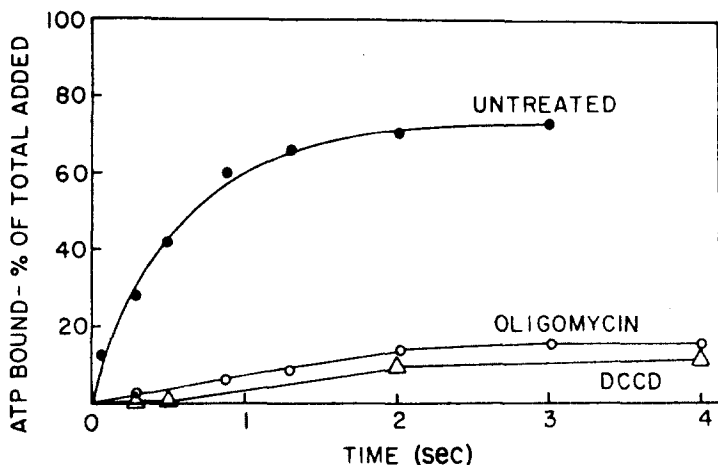of ATP to SMP was greatly inhibited when the particles were pretreated

Fig. 7. Inhibition by DCCD and oligomycin of [γ-^{32}P]ATP binding to submitochondrial particles. ●, Control particles incubated with ethanol alone; △, particles treated with 1 nmol DCCD in ethanol/mg of protein for 24 h at 5°C, which resulted in 90% inhibition of ATPase activity; ○, particles treated with 3 μg of oligomycin in ethanol/mg of protein, which resulted in 93% inhibition of ATPase activity. From Penefsky (1985b) with permission.

with oligomycin or DCCD (Fig. 7). These results suggested to Penefsky that modification of the DCCD-binding protein in F_0 altered the characteristics of the uni-site binding domain of F_1, resulting in impaired binding of ATP. Concurrently, it was shown by Matsuno-Yagi et al. (1985) that the ATP-induced (1–10 mM ATP) fluorescence change of aurovertin bound to F_1 in preparations of SMP or the ATP synthase complex (complex V), but not in preparations of isolated F_1, was inhibited when the preparation was treated with DCCD or oligomycin (Fig. 8). The degree of inhibition of the ATP-induced fluorescence change of bound aurovertin by DCCD or oligomycin correlated precisely with the degree of inhibition of ATPase activity by these reagents (Fig. 9). However, inhibition of ATPase per se was not the cause, because inhibition by organotin compounds or venturicidin had no effect on the ATP-induced fluorescence change of aurovertin. Thus, it appeared that this effect was concerned specifically with modification of the DCCD-binding protein. When 2′,3′-O-(2,4,6-trinitrophenyl)adenosine 5′-triphosphate (TNP-ATP) was used instead of ATP, the aurovertin fluorescence change was larger, and DCCD and oligomycin had no effect on the TNP-ATP-induced fluorescence change. However, at appropriate relative concentrations, prior addition of ATP completely prevented the effect of TNP-ATP in oligomycin-treated systems, and addition of ATP after TNP-ATP completely reversed the TNP-ATP-induced aurovertin fluorescence change in the oligomycin-treated particles (Fig. 10). These results indicated that, at physiological
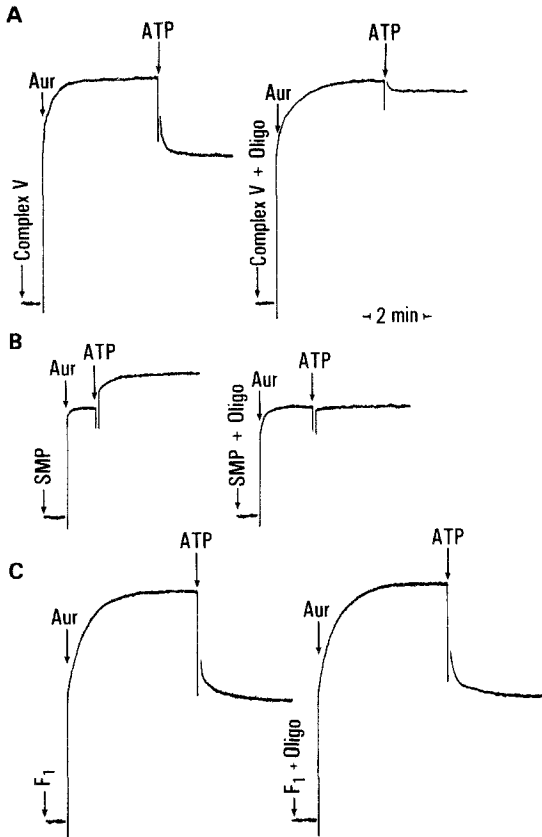
Fig. 8. Effect of oligomycin on the ATP-induced changes in the fluorescence of aurovertin (Aur) bound to complex V (A), SMP (B), and F_1-ATPase (C). The excitation and emission wavelengths were 366 and 470 nm, respectively. The concentrations of oligomycin and the degrees of inhibition of ATPase activity were 11 μg/mg of protein and 98% inhibition for complex V, 3 μg/mg of protein and 97% inhibition for SMP, and 50 μg/mg of protein and no inhibition for F_1-ATPase. Where indicated, the final concentrations of added aurovertin and ATP were, respectively, 0.63 μM and 1.25 mM in this and Figs. 9 and 10. From Matsuno-Yagi *et al.* (1985) with permission.

concentrations, ATP binding to F_1 was not inhibited by DCCD or oligo-mycin, but that its mode of binding was altered, probably to one of lower affinity. This interpretation agrees with the results of Penefsky obtained at ATP concentrations substoichiometric to F_1, and with the results obtained with the very tight-binding ATP analog, TNP-ATP. Thus, both sets of data, one set obtained under uni-site ATP-binding conditions and another under physiological ATP concentrations, indicate that in a totally inhibited enzyme ligand binding at the DCCD-binding protein of F_0 affects the catalytic sites
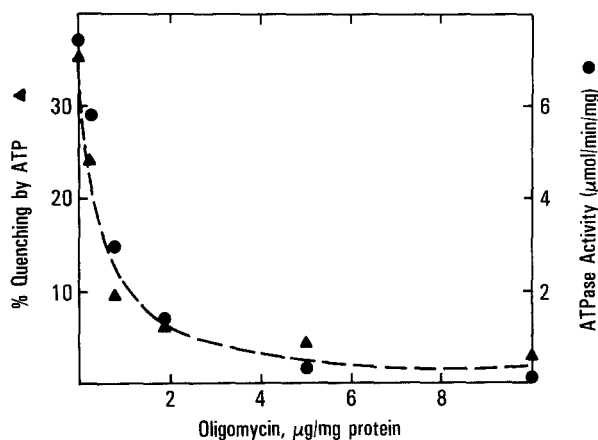
Fig. 9. Correlation between inhibitions of complex V ATPase activity and ATP-induced quenching of the fluorescence of bound aurovertin at different oligomycin concentrations. Complex V was incubated with the indicated concentrations of oligomycin and assayed for ATPase activity. ATP-induced fluorescence quenching was measured as in Fig. 8. From Matsuno-Yagi *et al.* (1985) with permission.

of F_1, resulting in a decreased affinity for ATP. It is, therefore, possible that energy-induced release of ATP from F_1 catalytic sites involves a similar mechanism. For example, protonation of the DCCD-binding subunits in F_0 could induce structural changes in the enzyme complex with a similar consequence in the affinity of F_1 catalytic sites for ATP.

Energy Communication Among the Mitochondrial Energy-Linked Enzyme Systems

In recent years, increasing numbers of investigators (Kell, 1979; Sorgato *et al.*, 1980; Zoratti *et al.*, 1982; Westerhoff *et al.*, 1984; Herweiger *et al.*, 1985; Rottenberg, 1985; Slater, 1987; Dilley *et al.*, 1987) have questioned the validity of the chemiosmotic premise (Mitchell, 1961, 1966) that in mitochondria, chloroplasts, and bacteria, protonic energy propagates in a delocalized manner by way of the transmembrane electrochemical potential of protons ($\Delta\tilde{\mu}_{H+}$). A major problem in considering $\Delta\tilde{\mu}_{H+}$ as the principal mode of energy transfer is that, as originally demonstrated by Sorgato *et al.* (1980) in oxidative phosphorylation catalyzed by SMP, attenuation of the rate of respiration results in a parallel decrease in the rate of ATP synthesis with little or no change in $\Delta\tilde{\mu}_{H+}$ (Sorgato *et al.*, 1980; Zoratti *et al.*, 1982; Yagi *et al.*, 1984). An example of this phenomenon, demonstrated in our laboratory (Matsuno-Yagi and Hatefi, 1987) with the use of highly active SMP, is shown
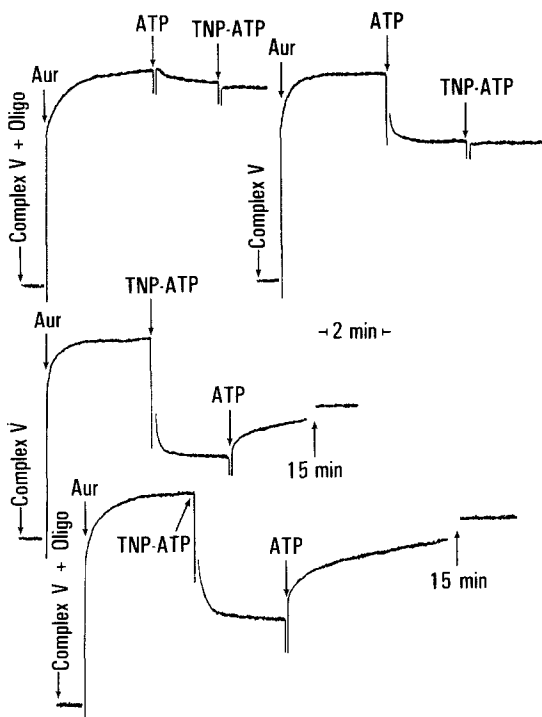
Fig. 10. Competition between ATP and TNP-ATP in quenching the fluorescence of complex V-bound aurovertin (Aur). Where indicated, complex V was pretreated with $5\,\mu g$ of oligomycin (Oligo) per mg of protein, which resulted in 98% inhibition of ATPase activity. The small decrease in fluorescence upon addition of TNP-ATP ($0.42\,\mu M$) after ATP ($1.25\,mM$) was due to the filter effect of TNP-ATP. From Matsuno-Yagi et al. (1985) with permission.

in Fig. 11. The respiratory substrates used were NADH, succinate, and β-hydroxybutyrate + NAD. It is seen that by attenuating respiration the rate of ATP synthesis was decreased from 2800 nmol $(min \cdot mg\ of\ protein)^{-1}$ to half that value with no detectable change in steady-state membrane potential ($\Delta\psi$). Furthermore, when steady-state $\Delta\psi$ did fall at low rates of ATP synthesis, so did static-head $\Delta\psi$, which suggested that the principal reason for the $\Delta\psi$ fall at these low rates of energy input was energy drain due to membrane leakiness. In other words, at high variable rates of energy input, when membrane leakiness was not an overwhelming factor, the rate of ATP synthesis was determined by the rate of substrate oxidation, but not by the magnitude of steady-state $\Delta\psi$ which remained unchanged. Similar results were obtained with ATP-driven reverse electron transfer from succinate to NAD (Matsuno-Yagi and Hatefi, 1987). Attenuation of the rate of energy input with the use of suboptimal concentrations of ATP decreased the rate
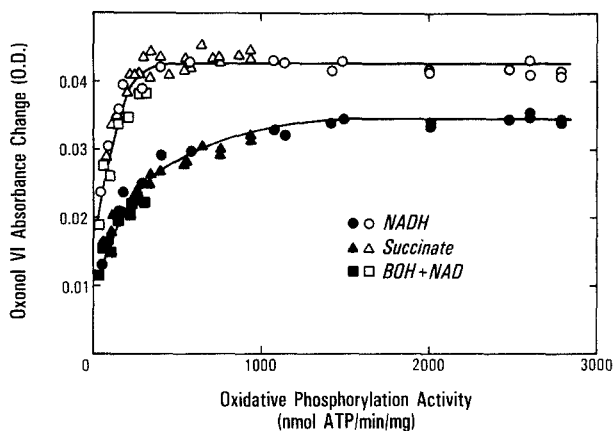
Fig. 11. Effect of attenuation of respiration rate on membrane potential and oxidative phosphorylation activity of SMP. Membrane potential was monitored by the absorbance change of oxonol VI (2 μM) at 630 minus 603 nm under static head (open symbols) and steady-state (filled symbols) conditions. ADP concentration was 1.2 mM, and the concentrations of respiratory substrates in this and Figs. 12–14 were 0.5 mM NADH, 5 mM potassium succinate, and 30 mM sodium DL-β-hydroxybutyrate plus 2 mM NAD. The rate of respiration was attenuated to various extents by addition to the reaction mixtures of 0.1–1.5 mM Seconal when the respiratory substrate was NADH (O, ●) or β-hydroxybutyrate (BOH) plus NAD (□, ■) or by addition of 0.02–0.25 mM potassium malonate when the respiratory substrate was succinate (△, ▲). The oxidation rates of these substrates in the absence of inhibitors and presence of 1.2 mM ADP were, respectively, 1770, 177, and 800 nmol (min · mg of protein)$^{-1}$ in this and Figs. 12–14. From Matsuno-Yagi and Hatefi (1987) with permission.

of NAD reduction from about 300 to about 100 nmol (min · mg of protein)$^{-1}$ with no detectable change in steady-state $\Delta\psi$. Furthermore, only when static-head $\Delta\psi$ began to fall at very low rates of ATP hydrolysis did steady-state $\Delta\psi$ also begin to diminish.

At first glance, these results suggest that steady-state $\Delta\psi$ is insensitive to changes in the rates of energy input and outflow. However, as seen in Fig. 12, this is not the case. The magnitude of steady-state $\Delta\psi$ varied with the rate of energy input as it was set at high, intermediate, and low levels with the use, respectively, of NADH, succinate, and β-hydroxybutyrate + NAD as respiratory substrates. It was also changed when the rate of energy utilization for ATP synthesis was progressively diminished by fractional inactivation of F_0F_1 complexes with DCCD. Similar results are shown in Figs. 13 and 14. In the former, the respiratory substrates were again NADH, succinate, and β-hydroxybutyrate + NAD. The rate of ATP synthesis was attenuated with the use of suboptimal ADP concentrations, and steady-state and static-head $\Delta\psi$ were measured by the absorbance change of oxonol VI in one set of experiments and by the fluorescence change of ANS in a second set. With each respiratory substrate, the data points at the lowest $\Delta\psi$ on the ordinate
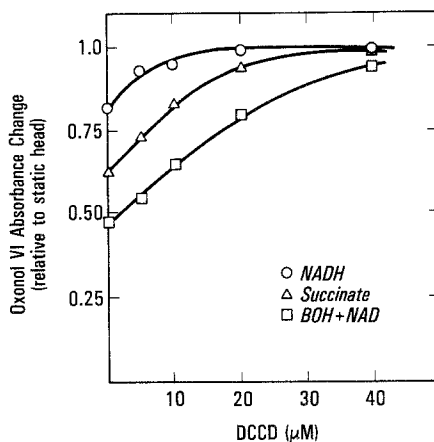
Fig.12. Effects of respiration rate and partial inhibition of ATP synthase by DCCD on the steady-state level of membrane potential during oxidative phosphorylation. SMP at 10 mg/ml in 0.25 M sucrose and 50 mM Tris acetate, pH 7.5, were preincubated for ≥ 3 h on ice with the indicated amounts of DCCD. Oxonol VI absorbance changes were monitored as described in Fig. 11. In this and in Figs. 13 and 14, static-head absorbance changes (OD) were 0.033 with NADH and succinate as respiratory substrates, and 0.031 with β-hydroxybutyrate plus NAD as respiratory substrate. The concentrations of the respiratory substrates in this and in Figs. 13 and 14 were the same as in Fig. 11.

and the highest rate of ATP synthesis on the abscissa represent experiments at saturating [ADP]. It is seen that as the ADP concentration was decreased and the rate of ATP synthesis was thereby attenuated, there was an upward adjustment of steady-state $\Delta\psi$. The lower the rate of energy input, the greater the upward adjustment of $\Delta\psi$ for the same relative decrease in the rate of ATP synthesis. In Fig. 14, the respiratory substrates were succinate and β-hydroxybutyrate + NAD. The rate of ATP synthesis was attenuated by the use of suboptimal ADP concentrations as well as by fractional inactivation of F_0F_1 complexes, once with DCCD and another time with TBT-Cl. Furthermore, in one set of experiments the rate of energy input by succinate oxidation was also partially inhibited with the use of malonate. These results are essentially the same as in Fig. 13, demonstrating again the response of steady-state $\Delta\psi$ to attenuation of the rates of energy input and outflow. Similar results were obtained for GTP-driven uphill electron transfer from succinate to NAD and transhydrogenation from NADH to NADP (Matsuno-Yagi and Hatefi, 1987). In both cases, suppression of the rate of GTP hydrolysis lowered steady-state $\Delta\psi$, and the use of suboptimal conditions for the driven reactions raised it. Thus, unlike the data of Fig. 11, these results indicate that steady-state $\Delta\psi$ is exquisitely sensitive to changes in the rates of energy production and consumption. The question is how these two seemingly discordant sets of results can be reconciled.
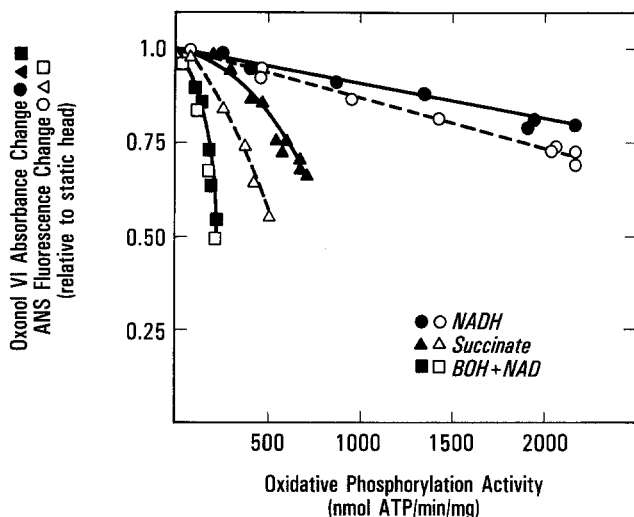
Fig. 13. Relationship between steady-state membrane potential and the rates of respiration and ATP synthesis at variable ADP concentrations. The ADP concentration range used was 1.2–1200 μM. Membrane potential was monitored using the absorbance change of oxonol VI as in Fig. 11, or the flurorescence change of 1-anilinonaphthalene-8-sulfonate (ANS, 2 μM) with excitation and emission wavelengths at 390 and 480 nm, respectively. The respiratory substrates were NADH, succinate, and β-hydroxybutyrate + NAD, as indicated. The data points at the lowest $\Delta\psi$ on the ordinate were obtained in the presence of 1200 μM ADP, and those at higher $\Delta\psi$ with decreasing concentrations of ADP down to 1.2 μM. From Matsuno-Yagi and Hatefi (1987) with permission.

Our current working hypothesis is that $\Delta\psi$ is indeed in direct communication with the energy input and outflow systems of mitochondria, as shown by the data of Figs. 12–14. However, $\Delta\psi$ does not reflect the rate of energy flow through the system. This is clear from the plateau region of static-head $\Delta\psi$ in Fig. 11. From the beginning to the end of this plateau, the rate of NADH oxidation increases at least tenfold, while $\Delta\psi$ remains at a constant level, which probably represents the capacitance of the membrane. In other words, there is no correlation between static-head $\Delta\psi$ and the increasing rate of energy dissipation through the system as the rate of NADH oxidation increases from about 100 nmol (min · mg of protein)$^{-1}$ at the start of the plateau to about 1100 nmol (min · mg of protein)$^{-1}$ at the end of the plateau. The same reasoning applies to the steady-state $\Delta\psi$ which, in addition to the static-head energy leak, involves energy drain via the functioning ATP synthase complexes. Under these conditions, the $\Delta\psi$ plateau is at a lower level (decreased membrane capacitance), but again the magnitude of $\Delta\psi$ does not reflect the increasing rates of energy drain through membrane leakage and ATP production as the rate of energy input is increased. Fractional inactivation of the ATP synthase complexes raises the steady-state level of $\Delta\psi$ toward
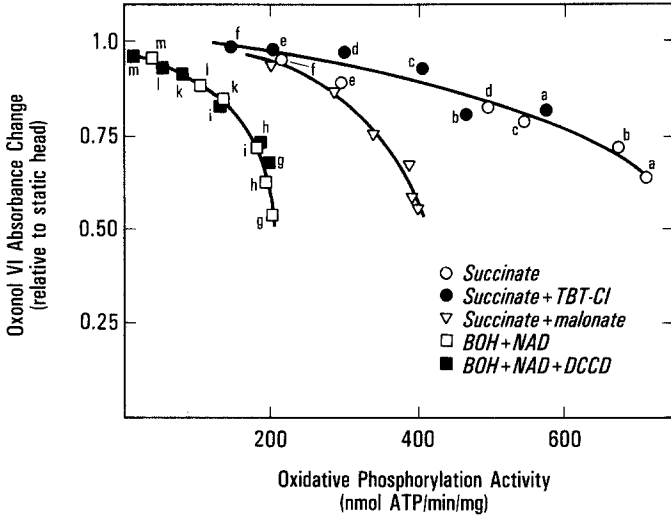
Fig. 14. Effects of partial inhibition of ATP synthase or respiration on steady-state membrane potential and oxidative phorphorylation activity at variable ADP concentrations. SMP at 10 mg/ml in 0.25 M sucrose and 50 mM Tris acetate, pH 7.5, were preincubated on ice for ⩾30 min with 10 μM tributyltin chloride (TBT-Cl) (●) or for ⩾3 h with 10 μM DCCD (■). Respiratory substrates used were succinate (○, ●, ∇) and β-hydroxybutyrate (BOH) plus NAD (□, ■). In ∇, 50 μM malonate was present in the reaction mixtures. ADP concentrations (μM) were: a, 1200; b, 500; c, 120; d, 50; e, 12; f, 5; g, 1800; h, 500; i, 120; k, 30; l, 8; m, 2. From Matsuno-Yagi and Hatefi (1987) with permission.

the static-head level as the limit (see Figs. 12–14), but at any given steady-state plateau the rate of energy flow through the system can increase without much altering $\Delta\psi$. Additional experiments in support of this concept are currently being completed and will be presented elsewhere.

Conclusions

The studies reviewed in this article have indicated that oxidative energy influences several parameters in the process of ATP synthesis by the mito-chondrial $F_0 F_1$ complexes. Energy increases the affinity of F_1 for ADP and P_i, and decreases the affinity of F_1 for tightly-bound ATP at the catalytic sites. Also, the rate of energy production modulates the kinetics of ATP synthesis between a high K_m (for ADP and P_i)–high V_{max} (for ATP synthesis) mode and a low K_m–low V_{max} mode. In this kinetic modality change, the apparent K_m for ADP varies from about 120–150 μM at high rates of energy production by the respiratory chain to about 2–3 μM at low rates of energy production. When energy production is not rate limiting, the apparent V_{max}

for ATP synthesis can be as high as $440 \, s^{-1}$ (per mol of F_0F_1 complex) at 30°C, which corresponds to $11 \, \mu mol$ ATP synthesized (min · mg of SMP protein)$^{-1}$. In addition to the above, membrane energization is considered to cause the dissociation of the ATPase inhibitor protein from the β-subunits of F_1, thereby allowing an uninhibited steady-state rate of ATP synthesis (Vignais and Satre, 1984; Schwerzmann and Pedersen, 1986).

An important point with regard to the above energy-promoted processes is that they all involve the catalytic subunits of F_1. Furthermore, they could all be accomplished by appropriate changes in the conformation of these subunits. Thus, it is quite conceivable that a mechanism analogous to that shown in Fig. 3 might underlie the energy-induced conformation change of the catalytic subunits of F_1. In other words, energy-induced structural changes at specific F_0 loci might be transferred within the ATP synthase complex to the catalytic subunits, resulting in the binding affinity and the kinetic modality changes discussed above. In what manner the energy liberated from substrate oxidation is transmitted to the ATP synthase complexes is a matter of debate at present. However, our results are in no way inconsistent with $\Delta\psi$ representing the membrane potential associated with delocalized protonic energy transfer in mitochondria.

Acknowledgments

References

Amzel, L. M., and Pedersen, P. L. (1983). *Annu. Rev. Biochem.* **52**, 801–824.
Boyer, P. D. (1979). In *Membrane Bioenergetics* (Lee, C. P., Schatz, G., and Ernster, L. eds.) Addison-Wesley, Reading, Massachusetts, pp. 461–479.
Boyer, P. D., Cross, R. L., and Momsen, W. (1973). *Proc. Natl. Acad. Sci. USA* **70**, 2837–2839.
Boyer, P. D., Kohlbrenner, W. E., McIntosh, D. B., Smith, T. L., and O'Neal, C. C. (1982). *Ann. N. Y. Acad Sci.* **402**, 65–83.
Choate, G. L., Hutton, R. L., and Boyer, P. D. (1979). *J. Biol. Chem.* **254**, 286–290.
Cross, R. L. (1981). *Annu. Rev. Biochem.* **50**, 681–714.
Cross, R. L., Grubmeyer, C., and Penefsky, H. S. (1982). *J. Biol. Chem.* **257**, 12101–12105.
Dilley, R. A., Theg, S. M., and Beard, W. A. (1987). *Annu. Rev. Plant Physiol.* **38**, 347–389.
Duncan, T. M., and Senior, A. E. (1985). *J. Biol. Chem.* **260**, 4901–4907.
Duncan, T. M., Parsonage, D., and Senior, A. E. (1986). *FEBS Lett.* **208**, 1–6.
Dunn, S. D., Zadorozny, V. D., Tozer, R. G., and Orr, L. E. (1987). *Biochemistry* **26**, 4488–4493.
Emanuel, E. L., Carver, M. A., Solani, G. C. and Griffiths, D. E. (1984). *Biochim. Biophys. Acta* **766**, 209–214.
Esch, F. S., and Allison, W. S. (1978). *J. Biol. Chem.* **253**, 6100–6106.

Feldman, R. I., and Sigman, D. S. (1982). *J. Biol. Chem.* **257**, 1676–1683.
Ferguson, S. J., Lloyd, W. J., Lyons, M. H., and Radda, G. K. (1975). *Eur. J. Biochem.* **54**, 117–126.
Fersht, A. R. (1985). *Enzyme Structure and Mechanism*, W. H. Freeman, New York.
Fersht, A. R. (1987). *Trends Biochem. Sci.* **12**, 301–304.
Frigeri, L., Galante, Y. M., Hanstein, W. G., and Hatefi, Y. (1977). *J. Biol. Chem.* **252**, 3147–3152.
Futai, M. and Kanazawa, H. (1983). *Microbiol. Rev.* **47**, 285–312.
Gresser, M. J., Myers, J. A., and Boyer, P. D. (1982). *J. Biol. Chem.* **257**, 12030–12038.
Grubmeyer, C., Cross, R. L., and Penefsky, H. S. (1982). *J. Biol. Chem.* **257**, 12092–12100.
Hatefi, Y. (1985). *Annu. Rev. Biochem.* **54**, 1015–1069.
Hatefi, Y., Yagi, T., Phelps, D., Wong, S.-Y., Vik, S. B., and Galante, Y. M. (1982). *Proc. Natl. Acad. Sci. USA* **79**, 1756–1760.
Herweiger, M. A., Berden, J. A., Kemp, A., and Slater, E. C. (1985). *Biochim. Biophys. Acta* **809**, 81–89.
Jencks, W. P. (1980). *Adv. Enzymol.* **51**, 75–106.
Junesch, V., and Gräber, P. (1985). *Biochim. Biophys. Acta* **809**, 429–434.
Kell, D. B. (1979). *Biochim. Biophys. Acta* **549**, 55–59.
Kozlov, I. A., and Skulachev, V. P. (1977). *Biochim. Biophys. Acta* **463**, 29–89.
Lüben, M., Lücken, U., Weber, J., and Schäfer, G. (1984). *Eur. J. Biochem.* **143**, 483–490.
Matsuno-Yagi, A., and Hatefi, Y. (1985). *J. Biol. Chem.* **260**, 14424–14427.
Matsuno-Yagi, A., and Hatefi, Y. (1986). *J. Biol. Chem.* **261**, 14031–14038.
Matsuno-Yagi, A., and Hatefi, Y. (1987). *J. Biol. Chem.* **262**, 14158–14163.
Matsuno-Yagi, A., and Hatefi, Y. (1988). *Biochemistry* **27**, 335–340.
Matsuno-Yagi, A., Yagi, T., and Hatefi, Y. (1985). *Proc. Natl. Acad. Sci. USA* **82**, 7550–7554.
Mitchell, P. (1961). *Nature (London)* **191**, 144–148.
Mitchell, P. (1966). In *Chemiosmotic Coupling in Oxidative and Photosynthetic Phosphorylation*, Glynn Research, Bodmin, England.
Noumi, T., Taniai, M., Kanazawa, H., and Futai, M. (1986). *J. Biol. Chem.* **261**, 9196–9201.
Parsonage, D., Wilke-Mounts, S., and Senior, A. E. (1987). *J. Biol. Chem.* **262**, 8022–8026.
Pedersen, P. L., and Carafoli, E. (1987a). *Trends Biochem. Sci.* **12**, 146–150.
Pedersen, P. L., and Carafoli, E. (1987b). *Trends Biochem. Sci.* **12**, 186–189.
Penefsky, H. S. (1985a). *J. Biol. Chem.* **260**, 13728–13734.
Penefsky, H. S. (1985b). *Proc. Natl. Acad. Sci. USA* **82**, 1589–1593.
Pickart, C. M., and Jencks, W. P. (1984). *J. Biol. Chem.* **259**, 1629–1643.
Pougeois, R., Satre, M., and Vignais, P. V. (1979). *Biochemistry* **18**, 1408–1413.
Rosing, J., Kayalar, C., and Boyer, P. D. (1976). In *The Structural Basis of Membrane Function* (Hatefi, Y., and Djavadi-Ohaniance, L., eds.) Academic Press, New York, pp. 189–204.
Rosing, J., Kayalar, C., and Boyer, P. D. (1977). *J. Biol. Chem.* **252**, 2478–2485.
Rottenberg, H. (1985). *Modern Cell Biol.* **4**, 47–83.
Russo, J. A., Lamos, C. M., and Mitchell, R. A. (1978). *Biochemistry* **17**, 473–480.
Sakamoto, J., and Tonomura, Y. (1983). *J. Biochem.* **93**, 1601–1614.
Schäfer, G. (1982). *FEBS Lett.* **139**, 271–275.
Schwerzmann, K., and Pedersen, P. L. (1986). *Arch. Biochem. Biophys.* **250**, 1–18.
Senior, A. E., and Wise, J. G. (1983). *J. Membr. Biol.* **73**, 105–124.
Slater, E. C. (1987). *Eur. J. Biochem.* **166**, 489–504.
Sorgato, M. C., Branca, D., and Ferguson, S. J. (1980). *Biochem. J.* **188**, 945–948.
Stroop, S. D., and Boyer, P. D. (1985). *Biochemistry* **24**, 2304–2310.
Ting, L. P., and Wang, J. H. (1980). *Biochemistry* **19**, 5665–5670.
Van Der Bend, R. L., Duetz, W., Colen, A.-M. A. F., Van Dam, K., and Berden, J. A. (1985). *Arch. Biochem. Biophys.* **241**, 461–471.
Vignais, P. V., and Lunardi, J. (1985). *Annu. Rev. Biochem.* **54**, 977–1014.
Vignais, P. V., and Satre, M. (1984). *Mol. Cell. Biochem.* **60**, 33–70.
Vinkler, C. (1981). *Biochem. Biophys. Res. Commun.* **99**, 1095–1100.
Webb, M. R., Grubmeyer, C., Penefsky, H. S., and Trentham, D. R. (1980). *J. Biol. Chem.* **255**, 11637–11639.

Westerhoff, H. V., Melandri, B. A., Venturoli, G., Azzone, G. F., and Kell, D. B. (1984). *FEBS Lett.* **165**, 1–5.
Williams, N., and Coleman, P. S. (1982). *J. Biol. Chem.* **257**, 2834–2841.
Wong, S.-Y., Matsuno-Yagi, A., and Hatefi, Y. (1984). *Biochemistry* **23**, 5004–5009.
Yagi, T., Matsuno-Yagi, A., Vik, S. B., and Hatefi, Y. (1984). *Biochemistry* **23**, 1029–1036.
Zoratti, M., Pietrobon, D., and Azzone, G. F. (1982). *Eur. J. Biochem.* **126**, 443–451.