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An Enzymological Approach to Mitochondrial Energy Transduction

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Abstract

An approach to the problem of mitochondrial energy transduction is outlined. The approach is based on the fundamental assumption that there is an intimate relation between the mechanisms of enzyme catalysis and energy transduction. The implications of this assumption for the coupling of two chemical reactions and the coupling of a chemical reaction to an ion flux are discussed.

The basic physical problem of the mitochondrion is the mechanism of energy transduction. Examples of mitochondrial transductions are the coupling of an oxido-reduction to a bond rearrangement reaction (oxidative phosphorylation) and the coupling of either type of chemical reaction to an ion flux. The apparent requirement of a common intermediate for coupling these different physical and chemical processes is both restrictive and suggestive. Among the various models of mitochondrial function that have been proposed to date the conformational model[1–4] has for me the greatest intuitive appeal. Unfortunately, since none of these models can yet claim a solid experimental foundation, one is forced at this stage to rely on his intuition, however unreliable that might be.

One of the fundamental concepts in the conformational model is the coupling of a chemical reaction and a conformational transition. It is very possible, and perhaps even likely that enzyme catalysis may generally involve conformational changes on the part of the enzyme as an intrinsic feature of enzyme catalysis.[5–7] If this should prove to be the case, then the mere demonstration of conformational changes in the enzymes associated with oxidative phosphorylation would not provide conclusive evidence for a conformational model of oxidative phosphorylation. Consequently, a conformational model would be very difficult to directly verify experimentally. Nonetheless, the very

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likelihood of conformational changes being an intrinsic feature of enzyme catalysis suggests an "enzymological" approach to the problem of energy transduction. This merely means that one tries to construct a model of energy transduction drawing only upon the familiar properties of enzymes. This may seem like a rather trivial statement, but, as I will attempt to demonstrate, it has decidedly nontrivial consequences.

Formulation of Problem

The mitochondrion has the capacity to reversibly couple a number of different processes. It is instructive to briefly review from a purely phenomenological point of view what the requirements are that acceptable models of mitochondrial function must meet.

The principal coupled process is oxidative phosphorylation. This is essentially the coupling of two chemical reactions, substrate oxidation through the respiratory chain and ATP synthesis. The thermodynamic driving force for two coupled chemical reactions is the difference in driving forces of the two partial reactions.[8, 8a] Thus if the driving force for substrate oxidation is greater than for ATP hydrolysis, oxidative phosphorylation ensues. Conversely, if the driving force for ATP hydrolysis is greater than for substrate oxidation, reversed electron transfer ensues, at least through the first two coupling sites. If the driving forces for the two partial reactions are equal, then a tightly coupled system is in equilibrium despite the fact that neither of the two subsystems considered separately is in equilibrium. Under these conditions (State 4) both respiration and ATPase activity are severely inhibited in well coupled mitochondria.[9, 10]

Another coupled process in mitochondria is active transport. Both respiration and ATP hydrolysis can be coupled to a flux of ions across the inner mitochondrial membrane. I will consider some of the mechanistic details later, but here I merely wish to note that the ion gradients established across the inner membrane constitute a form of free energy. There are a number of reports in the literature on the coupling of potassium efflux and proton influx to ATP synthesis so that this coupled process too appears to be reversible.[11–14] This is as one would expect from the principle of microscopic reversibility.

Still another system is the transhydrogenation reaction. The reduction of $NADP^+$ by NADH can be coupled to either respiration or ATP hydrolysis.[15] Conversely, van de Stadt *et al.* have recently shown that the oxidation of NADPH by NAD^+ can drive ATP synthesis.[16] Grinius *et al.* have also shown that the oxidation of NADPH by NAD^+ can drive ion translocation.[17]

The overall picture which seems to emerge therefore is that of a number of different free energy forms (redox, bond energy, ion

gradients) which can all be reversibly transformed—one into another. Each of these free energy forms might be considered to constitute a thermodynamic subsystem. In a truly tightly coupled system, which may be only an idealization, the total system may be at equilibrium despite the fact that each of the subsystems taken separately is far from equilibrium. This is the case when the thermodynamic driving forces of the subsystems are all nonzero but equal to one another. If the total system is perturbed from equilibrium by changing the driving force of one of the subsystems then the total system relaxes to a new equilibrium state. Thus, for example, if ADP is added to a system in State 4, a certain number of moles of substrate are oxidized and a stoichiometrically related number of moles of ATP are synthesized until a new equilibrium state is attained.

Still on the phenomenological level one might now ask about the manner in which these various free energy forms are coupled to one another. A *necessary precondition* for coupling these various free energy forms together is that each subsystem be constrained from independently approaching equilibrium. The simplest way to achieve this constraint is to reversibly couple each of the subsystems to a common intermediate free energy form. At equilibrium the intermediate itself would be in equilibrium with each of the subsystems. Thus the various subsystems would be both constrained from independently approaching equilibrium and coupled together by one and the same mechanism.

A common intermediate free energy form is supported by the fact that a given reaction (e.g. ATP hydrolysis) can be catalyzed by just one enzyme (e.g. oligomycin-sensitive ATPase) to drive reversed electron transfer, ion translocation, and transhydrogenation. This type of inhibitor result provides strong support for the concept that each of these coupled processes share in part a common pathway, as required if there is a common intermediate.[18]

Further support for a common intermediate is provided by the fact that all of these processes can be uncoupled from one another by the so-called classical uncouplers.[4, 18] This could be readily explained if the uncouplers act so as to decrease the stability of the intermediate. If the relaxation time for spontaneous decay of the intermediate becomes much less than the relaxation times for the various coupled processes, then the intermediate relaxes in an uncoupled manner before it can relax in a coupled manner. The constraint preventing each subsystem from independently approaching equilibrium is thereby removed.

If the rate of spontaneous relaxation is not zero, then there exists no true state of equilibrium apart from the state in which each of the subsystems is at equilibrium (a state of no interest for mitochondriology). The essential point to recognize, however, is that the

constraint preventing each of the subsystems from independently approaching equilibrium need be only relative in the sense that tight coupling merely requires that *relative* to the rates of coupled relaxation the rate of spontaneous, uncoupled relaxation be slow, not zero.

The picture of the mitochondrion that emerges from this discussion is that of a variety of free energy forms all reversibly coupled to one another by being reversibly coupled to a common intermediate free energy form.[4, 18] We are now ready to ask about the physical and chemical nature of this intermediate.

Before proceeding I might note here that the manner of coupling the various subsystems to one another is actually more complex than that described here because I have ignored the concentration gradients across the inner membrane of the reactants and products of the various chemical reactions.[19] Rather than attempting to deal with the integrated mitochondrial system, I have merely tried to describe a kind of first approximation as a starting point for dealing with the problem of energy transduction.

Coupling Catalyst

In this section, I would like to outline in somewhat more detail what is meant by an enzymological approach to energy transduction. Basically it means that there must be an intimate relation between the mechanisms of enzyme catalysis and energy transduction. This may appear to be inconsistent with the conclusion of the previous section that a necessary precondition for coupling is the constraint preventing each subsystem from independently approaching equilibrium. By definition a catalyst allows a chemical reaction to reach equilibrium more rapidly. However, a moment's reflection reveals that an enzyme could be a "coupling catalyst" if two conditions are met: (1) the enzyme catalyzed reaction is *inhibited* and (2) the inhibition is relieved by the transfer of free energy from one subsystem to another.

A schematic representation of an inhibited ATPase is given in Fig. 1. The reaction takes place in four steps: (1) the attractive enzyme-substrate interactions stabilize both the enzyme and the reactant in higher energy states such that the perturbed reactant lies energetically closer to the appropriate transition state, (2) the enzyme-bound reactant is transformed to the enzyme-bound products, (3) the products dissociate from the enzyme leaving it in the higher energy conformation, and (4) the enzyme spontaneously relaxes from $\mathscr{E}$ to E so that the cycle can begin again. If conformation $\mathscr{E}$ is a long lived or metastable conformation, then the rate limiting step in the reaction is the slow spontaneous decay of conformation $\mathscr{E}$. Thus the dissociation

of products from an enzyme in a metastable conformation provides a mechanism of inhibiting an enzyme catalyzed reaction.

Although the enzyme represented in Fig. 1 would have a low ATPase activity it would tend to have a high $^{32}P_i \rightleftharpoons$ ATP and ^{14}C-ADP $\rightleftharpoons$ ATP exchange activity if the standard free energy difference between conformations $\mathscr{E}$ and E is sufficiently large. Under these conditions the enzyme would act as a free energy reservoir and thereby couple the exergonic hydrolysis of unlabelled ATP to the endergonic synthesis of labelled ATP. If the labelled and unlabelled species are considered as separate subsystems, then the addition of $^{32}P_i$ or ^{14}C-ADP to a medium containing ATP, P_i or ADP, and the enzyme of Fig. 1 leads to a transfer of free energy from the unlabelled to the labelled subsystem until equilibrium is attained between the two.

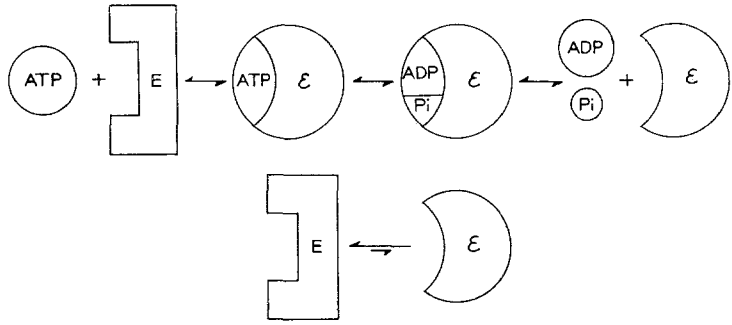

Figure 1. Schematic representation of inhibited ATPase. Perturbations of both enzyme and substrates are represented by geometric distortions. The spontaneous relaxation from conformation ϵ to conformation E is assumed to be slow, i.e., ϵ is a metastable conformation.

The enzyme of Fig. 1 therefore satisfies the two requirements of a coupling catalyst: (1) the enzyme catalyzed reaction is inhibited, and (2) the inhibition is relieved by the transfer of free energy from one subsystem to another. The two subsystems are constrained from independently approaching equilibrium by the very mechanism that couples them together. This is the essence of a coupling catalyst. (This concept will be developed more fully elsewhere.)[20]

This model for coupling together two *chemically equivalent* reactions (i.e. differing only by isotopic substitution) can be readily generalized to coupling two *chemically different* reactions if one postulates the existence of a coupling catalyst with *two* active sites, one active site to catalyze one partial reaction and another active site to catalyze the other partial reaction. Thus, for example, if electron transfer could also be reversibly coupled to the transition $E \rightleftharpoons \mathscr{E}$, respiration would be inhibited unless a mode of rapid relaxation is available for the

metastable conformation $\mathscr{E}$. In the presence of sufficient levels of ADP and P_i, ATP synthesis would provide such a mode of relaxation, as illustrated in Fig. 1. Thus the release of respiratory control would be achieved by the same mechanism that couples oxidation to phosphorylation. In such a model tight coupling for oxidative phosphorylation would require a high activity for the ATP-linked isotopic exchange reactions discussed above.[21]

However, to postulate a coupling catalyst with two active sites provides only a formal, not a mechanistic solution to the problem of oxidative phosphorylation. Such an approach does not address itself to the fundamental question of the mechanism by which the respiratory and ATPase enzyme complexes are coupled together. To get at this question one might try to characterize the physical nature of the hypothesized conformational transitions and then ask what it is about these transitions that lends itself to the coupling of different enzyme complexes.

Characterization of Conformational Transition

One can partially characterize the hypothesized conformational transitions by drawing upon the reasonably well characterized passive transport properties of the inner membrane and then simply seeking a self-consistent model of active transport. We recently adopted this approach in developing a model that effectively incorporates basic features of the chemiosmotic model[22] into the conformational model.[23, 24] To introduce this model I will try and place the reversible coupling of a chemical reaction and an ion flux within the context of the reversible coupling of two thermodynamic subsystems previously discussed.

As previously mentioned, a necessary precondition for coupling various subsystems together is that each subsystem be constrained from independently approaching equilibrium. If the subsystem is a concentration gradient across a membrane, then it must be a concentration gradient of a relatively *impermeant* species in order to be stable against spontaneous decay. The requirement for an impermeant species is analogous to the requirement for an *inhibited* enzyme catalyzed chemical reaction discussed in the previous section.

One can distinguish two categories of active *salt* transport in mitochondria, one facilitated by the neutral ionophores and one facilitated by the monocarboxylate ionophores. A common denominator of both types of active salt transport appears to be that *one and only one* of the ions of the salt crosses the membrane via an electrogenic mechanism while the other ion crosses via an electrically neutral mechanism either with a proton or in exchange for a proton. Given these passive transport properties, a necessary and sufficient condition

for such a salt to be relatively impermeant is that the proton and hydroxyl conductances of the membrane be small. That is, the protons and hydroxyl ions must be constrained from being passively transported across the membrane via an *electrogenic* mechanism. Although both ions of the salt are readily exchangeable, the stability of the proton gradient in such a system insures the stability of the salt gradient.

Let us now consider the mechanistic problem of coupling a flux of an impermeant salt, as defined here, to a conformational cycle which is itself coupled to a chemical reaction as discussed in the previous section. If one accepts that the passive transport properties outlined here and discussed more fully elsewhere[24] are a valid first approximation to those of the inner mitochondrial membrane, then self-consistency requires that the conformational cycle involves an effective transmembrane transport of protons. This is the only way to obtain a *transmembrane* flux of an *impermeant* salt.

We have recently presented a model that satisfies this requirement by invoking: (1) pK changes of ionizable groups on opposite sides of the membrane (asymmetric membrane Bohr effect) and (2) a mechanism of facilitated proton conductance to allow for rapid equilibration between these two sets of ionizable groups. The conformational cycle thereby incorporates the proton generated membrane potential and pH gradient of the chemiosmotic model as schematically represented in Fig. 2. The mechanistic details of these hypothesized maneuvers are admittedly hazy at this point, but something along these lines is required simply for self-consistency.

Excitation in Fig. 2 corresponds to the conformational transition $E \rightarrow \mathscr{E}$. It is important to recognize, however, that although relaxation must correspond in a *net* sense to the conformational transition $\mathscr{E} \rightarrow E$, the enzyme is relaxing via a *different* pathway than that by which it was excited. In this model relaxation releases the inhibition of respiratory or ATPase activity, and as discussed previously this release must be the consequence of transferring free energy from the chemical subsystem to the concentration gradient subsystem. This transfer takes place in two steps in the present model. First, there is the electrogenic transport of ions in response to the proton generated membrane potential. Then, subsequent to the electrogenic transport of ions, there is the generation of the pH gradient and the subsequent transport of those species that cross the membrane either with a proton or in exchange for a proton. Only upon completion of the proton cycle is relaxation complete.

The fact that in salt transport the enzyme relaxes via a pathway different than that by which it was excited has important implications for characterizing the conformation $\mathscr{E}$ as an intermediate in any of the

coupled chemical reactions of the mitochondrion. Electrogenic transport of ions, which initiates relaxation in active salt transport, is presumably *not* on the pathway of any of the coupled chemical reactions. Thus in the present model the pH gradient, which occurs only subsequent to the electrogenic transport of ions, would also *not* be on the pathway of any of the coupled chemical reactions. A membrane potential, however, would be on the pathway of all the coupled chemical reactions in this model. Thus a partial characterization of the hypothesized conformational transition has been achieved.

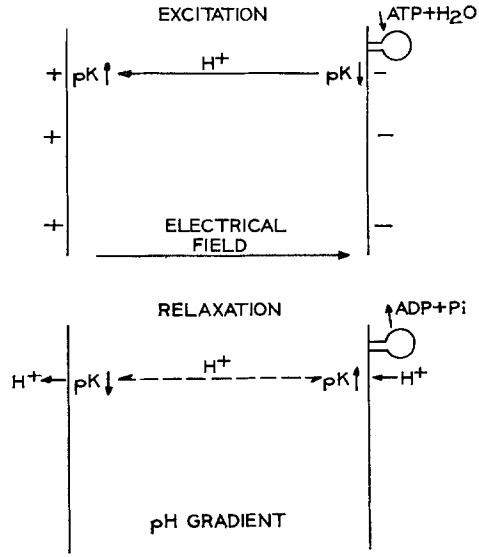

Figure 2. Conformationally induced proton translocation as the basis for the generation of the membrane potential and pH differential. The solid line connecting the two sets of ionizable groups in the excitation phase indicates high proton conductivity whereas the dotted line in the relaxation phase indicates low proton conductivity. The coupling of the conformational transition with a chemical reaction is represented by the adsorption of ATP and H_2O in the excitation phase and the desorption of ADP and inorganic phosphate (P_i) in the relaxation phase. (Reprinted by permission of Gordon and Breach, Science Publishers Inc.)

Although the present model incorporates both the membrane potential and the pH gradient of the chemiosmotic model in accounting for mitochondrial active transport, it incorporates only the membrane potential and not the pH gradient as an intermediate in the various coupled chemical reactions. This is a fundamental difference between the two models.

Conclusion

At the present stage of development the model outlined here provides only an approach, not a solution to the problem of mito-

chondrial function. This model is still vague and poorly defined concerning the central question of the mechanism of oxidative phosphorylation. Nonetheless, the approach of seeking in the properties of the enzymes the mechanisms of energy transduction as well as enzyme catalysis appears to be a promising one. To seek the mechanisms of energy transduction in the properties of the enzymes rather than in the properties of the reactions they catalyze is the fundamental distinction between this approach and the approach of both the chemical and the chemiosmotic models. The present approach is essentially just an extension of a view of enzyme catalysis in which the enzyme itself can play the role of a temporary free energy reservoir.[3,5,6]

The conclusion that a proton generated membrane potential is an intrinsic component of the common intermediate implies that in significant measure the form of the intermediate free energy is electrostatic. We might now attempt to formulate somewhat more precisely the fundamental question raised earlier: How could electrostatic free energy lend itself to coupling different enzyme systems? If this approach is on the right path, then this question is undoubtedly related to another fundamental question: Why does oxidative phosphorylation occur in a membrane system? As is well known, biological membranes have the character of parallel plate capacitors and are therefore well suited for the storage of electrostatic free energy. However, not only the storage but also the transfer of electrostatic free energy appears to be involved. As Liberman and Skulachev have already observed, if electrostatic free energy could be transmitted along the membrane, then the individual enzyme complexes of the inner mitochondrial membrane could be effectively united into a common system.[25] This observation does not in itself provide answers to the fundamental questions raised above, but it does identify what appears to be a promising area for seeking the answers to these questions.

Obviously, one cannot develop this model further without making some assumptions about the structure and arrangement of the various enzyme complexes within the inner membrane. However, an excursion into membrane structure and its relation to the present model is beyond the scope of the present paper. Hopefully, an understanding of the structure-function relation in this membrane system will soon be forthcoming.

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