Proceedings of the National Academy of Sciences
Vol. 67, No. 2, pp. 544–549, October 1970

The Conformational Basis of Energy Transductions in Biological Systems

Chairman's Introductory Remarks

D. E. Green

INSTITUTE FOR ENZYME RESEARCH, UNIVERSITY OF WISCONSIN, MADISON, WISCONSIN 53706

Read before the Academy April 29, 1970

The theme of this symposium is the conformational basis of energy transductions in biological systems. The conformational model shown in Fig. 1 expresses fairly well the essence of this theme. I do not intend to speak to the correlational studies[1–5] which provide the experimental foundation for the model nor to the studies presented in the first paper by J. H. Young *et al.*[6] which rationalize active transport in the mitochondrion in terms of the conformational model. I have in mind the broader perspective in which I believe the conformational model may have to be placed. I would like to develop two points for your consideration—first, that the conformational model appears to be in line with well-established chemical precedence and experience—and second, that the conformational model can explain the structure–function relation in energy-transducing systems as no other model has been able to do.

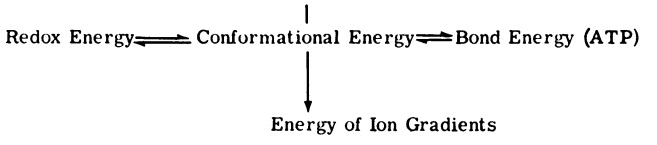


FIG. 1. Conformational model of mitochondrial energy transduction.

The energy cycle of the transducing unit of the mitochondrion (the tripartite repeating unit) may be described in terms of the following characteristics. The unit can exist in nonenergized and an energized conformation (we shall ignore the fact that there are several alternative energized conformations). The energized conformation is metastable and its lifetime is long enough for the conserved energy to be utilized in a directed manner.[7] When the appropriate reagents are present, the unit in the energized state will relax, carrying out synthesis of ATP; if these reagents are not present, the energized unit will relax spontaneously, with thermal dissipation of the energy. Not all workers in the field are prepared to equate the energized state of the mitochondrion with an energized conformation. But I believe it is correct to say there is general agreement about the fact of there being two states (nonenergized and energized), about the metastability of the energized state, and about the options for disposition of the conserved energy whatever the form of this energy.

With this catalogue in mind of the properties of the mitochondrial transducing unit, let us consider a simple molecular transducing system with which you are all undoubtedly familiar (see Fig. 2). The absorption of a photon will "kick" a molecule such as benzophenone first into an excited singlet and then nonradiatively into an excited triplet state which is metastable (that is, the excited state is relatively long-lived). There are two options for the disposition of the energy and for the return to the ground state—either by radiative emission in the form of phosphorescence or by nonradiative decay with liberation of thermal energy.

Now let us return to the mitochondrial energy-transducing unit and consider the energy cycle in terms of events analogous to those invoked for the excitation of benzophenone by light energy (Fig. 3). We shall equate the ground state with

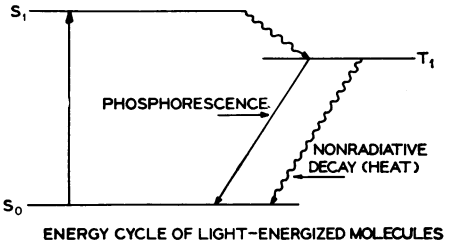


FIG. 2. Energy diagram showing how the absorption of a photon "kicks" the energy level of a molecule from the ground state (S_0) first to the singlet state (S_1) and thence by nonradiative decay to the triplet state (T_1), which is the metastable state. The two options for the decay of the excited molecule in the triplet state to the ground state are either phosphorescence or nonradiative decay.

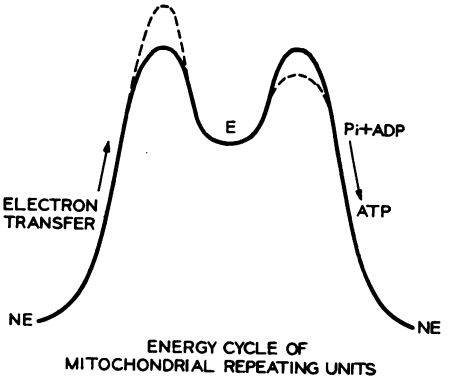
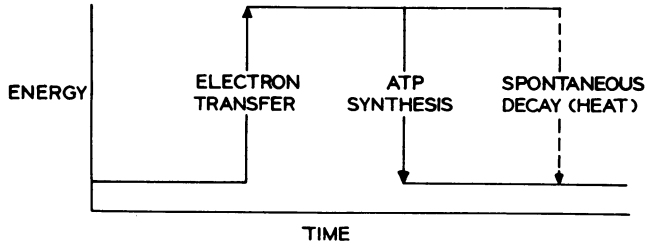


FIG. 3. Energy diagram for the mitochondrial repeating units. Electron transfer "kicks" the repeating unit into an excited state at a higher energy level. The excited repeating unit in the presence of inorganic phosphate (P_i) and ADP will relax to the ground state with simultaneous synthesis of ATP. The dotted lines represent the change in activation energy induced by ADP and P_i—a change which introduces a bias in the direction of the conformational transition.

the nonenergized conformation, and the energized state with the energized conformation, bearing in mind that it is not light energy but the redox energy of electron transfer which "kicks" the mitochondrial unit into the energized conformation.[7] You will note that the valley in the energy diagram is a token of the activation barrier to the spontaneous relaxation of the energized transducing unit to the ground state, and this valley accounts for the metastability of the energized conformation. When ADP and P_i are added to the mitochondrial system, ATP is synthesized, and the repeating unit simultaneously relaxes to the nonenergized conformation. ATP synthesis stands in the same relation to the mitochondrial system as does phosphorescence to the benzophenone light-activated system. Both processes represent the consummation of a transductive event—in one case the transduction of conformational energy to the bond energy

of ATP, in the other the transduction of the electronic excitation energy of a molecule into light energy. Another point of similarity between the two transducing systems is emphasized in Figure 4, namely the two options for decay of the mitochondrial system—directed discharge and spontaneous decay.



ENERGY COUPLING VERSUS SPONTANEOUS DECAY
THE METASTABLE ENERGIZED STATE

FIG. 4. The two options for decay of the mitochondrial repeating unit in the energized conformation, and the metastable character of the energized conformation.

It might be useful to clarify one key point about conformational change before we proceed with the argument. The transition of a protein from conformational state A to conformational state B of a higher energy level may be accomplished merely by changing conditions which lower the energy level of B. Then the conformational transition proceeds spontaneously. We are not concerned with this kind of transition. The problem is how to "kick" a protein into a high energy conformation without changing the external conditions. Electron transfer or ATP hydrolysis has to propel the protein into a higher-energy conformation without change in the external conditions which will diminish the energy differential between the two conformations. We have to think of the role of electron transfer in the mitochondrial system as equivalent to that of a photon in the benzophenone system.

The postulate of energized conformations in protein systems such as the mitochrondrial transducing unit is by no means pure speculation. Harold Scheraga and his colleagues at Cornell University have been engaged in a fundamental series of theoretical studies[8,9] with the objective of deducing the lowest-energy, stable conformation of polypeptides such as gramicidin. They have demonstrated by rigorous argument that these polypeptides have a large number of possible conformational states which can differ one from another by amounts of energy ranging from a fraction of a kilocalorie to tens of kilocalories. These different conformational states are described as energy minima and have a metastable character. These studies of Scheraga provide powerful support for the possibility of high-energy, metastable conformational states in protein protein systems.

The notion of high-energy conformations is not rooted in the tertiary structure of a protein. It can be extended to the quaternary structure of a multiprotein system; indeed the relatively large-amplitude conformational changes of the

repeating units observed by high-resolution electron microscopy would strongly support the view that quaternary changes are predominant in the transition of the mitochondrial repeating unit from the nonenergized to the energized conformation.[7]

According to the conformational model there is a 1:1 correspondence between the conformation of the transducing unit and the energy state.[3] There is much evidence to support this correlation. When this correlation is joined to the possibility of high-energy conformational states in protein systems, the plausibility of the conformational model is enormously enhanced.

If these considerations on the excited conformational states of protein systems have validity, an obvious prediction may be made. Just as photochemistry opened the door to a new realm of chemical phenomena (the properties of molecules in excited states), so energy transduction in the mitochondrion may open the door to the realm of the excited states of proteins.

Perhaps there is merit in considering the question of high-energy intermediates in terms of this new perspective. There are intermediates both in ground-state and in excited-state chemistry. The intermediate in ground-state chemistry can be isolated and characterized. The intermediate in excited-state chemistry cannot be isolated though its reality is unquestioned.

Finally let us return to the fundamental structure–function relation in the mitochondrion and other transducing systems. If function involves the manipulation of conformational states, then since the conformation and structure of a transducing system are two sides of the same coin, obviously the function of the transducing system will be determined by its structure. Thus, the conformational model of energy transduction provides a profound rationale for the long-postulated relation between structure and function.

The four papers that have been assembled for this afternoon's program may appear to have little in common. But there is in fact a unifying thread which runs through these talks. Let me develop some features of this unity. When a major conformational change takes place in a multiprotein system, it is not only predictable from first principles[10] but directly verifiable by experiment that such a change will probably have multiple sequelae (see Table 1). There is

TABLE 1. *Sequelae of conformational change in repeating units of membrane systems.*

A. *Conformational Change within a Repeating Unit*
Change in titratable sulfhydryl groups, in fixed charge, in binding sites, in ionization of dissociable groups, in proton ejection or uptake, and in the molecular geometry of the repeating unit.

B. *Conformational Change within Arrays of Repeating Units in a Membrane*
Change in membrane configuration, in light scattering, in membrane potential, in water and ion movements, and in osmotic pressure.

really no need to expand on these sequelae—they are consequences of unfolding ionizable groups which were previously buried or vice versa. These sequelae are the obvious choices for intrinsic conformational probes—a list of which is shown in Table 2. Britton Chance has been one of the pioneers in this area of conformational probes. The use of the proton shift[11] or the change in spectrum of cytochrome b[12] as measures of conformational change are among his important

TABLE 2. *Intrinsic conformational probes of the energy cycle in the mitochondrial system.*

1. Ejection or uptake of protons
2. Increased binding or release of weak acid anions such as inorganic phosphate and of divalent cations such as Ca^{++}
3. Change in the number of the titratable sulfhydryl groups
4. Change in the absorption spectrum of appropriate light-absorbing species
5. Change in membrane configuration

contributions to our armory of intrinsic conformational probes. But in addition, Britton Chance has also pioneered in the use of extrinsic conformational probes—probes which are added to and react with the mitochondrial system. Anilino-1-naphthalenesulfonic acid is one such probe, the fluorescence of which can provide useful information about the immediate molecular environment and about changes in this environment.[13]

The design of conformational probes is now a well-developed art. Many of you are familiar with the elegant spin-label reagents of Hardin McConnell.[14] Lubert Stryer of Yale University is in the same great tradition. He will be introducing us to a new dimension in the design of reporter molecules in which there are long-range dipole–dipole interactions between energy donor and acceptor groups. In the design of versatile conformational probes of the future, the ground-rules worked out by Lubert Stryer will undoubtedly be invaluable.

Finally, we have two transducing systems represented in this symposium—the mitochondrion by John Young and Britton Chance and the muscle fiber by Manuel Morales. We look to Manuel Morales for guidance as to the applicability of the conformational model to transducing systems other than the mitochondrion.

As a final remark before opening the symposium, I should stress that the perspective which I have proposed for the conformational model is my own preference. I will leave it to the other speakers to develop their own perspective. While there is general agreement among workers in the mitochondrial field that significant conformational changes parallel energized processes, consensus about the meaning of these conformational changes has yet to be reached. By focusing attention on the conformational theme in energy transducing systems, I had hoped that this symposium would make an important contribution to progress in this field.

[1] Penniston, J. T., R. A. Harris, J. Asai, and D. E. Green, *Proc. Nat. Acad. Sci. USA*, **59**, 624 (1968).

[2] Harris, R. A., J. T. Penniston, J. Asai, and D. E. Green, *Proc. Nat. Acad. Sci. USA*, **59**, 830 (1968).

[3] Green, D. E., J. Asai, R. A. Harris, and J. T. Penniston, *Arch. Biochem. Biophys.*, **125**, 684 (1968).

[4] Harris, R. A., M. A. Asbell, J. Asai, W. W. Joly, and D. E. Green, *Arch. Biochem. Biophys.*, **132**, 545 (1969).

[5] Williams, C. H., W. J. Vail, R. A. Harris, M. Caldwell, D. E. Green, and E. Valdivia, *J. Bioenergetics*, **1**, 147 (1970).

[6] Young, J. H., G. A. Blondin, G. Vanderkooi, and D. E. Green, *Proc. Nat. Acad. Sci. USA*, **67**, 550 (1970).

[7] Green, D. E. and J. H. Young, *Amer. Sci.*, **58**, Nov–Dec (1970).

[8] Gibson, K. D. and H. A. Scheraga, *Physiol. Chem. Phys.*, **1**, 109 (1969).

[9] Mornany, F. A., G. Vanderkooi, R. W. Tuttle, and H. A. Scheraga, *Biochemistry*, **8**, 744 (1969).
[10] Unpublished studies of G. Vanderkooi.
[11] Chance, B. and L. Mela, *J. Biol. Chem.*, **241**, 4588 (1966); **242**, 830 (1967).
[12] Chance, B., *J. Biol. Chem.*, **242**, 2729 (1965).
[13] Azzi, A., B. Chance, G. K. Radda, and C. P. Lee, *Proc. Nat. Acad. Sci. USA*, **62**, 612 (1969).
[14] Hubbell, W. L. and H. M. McConnell, *Proc. Nat. Acad. Sci. USA*, **64**, 20 (1969).