CONFORMATIONAL BASIS OF ENERGY TRANSDUCTION IN MITOCHONDRIA

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1. Introduction

The conformational mechanism of energy transduction which we proposed in 1967 was based on an intuitive evaluation of the significance of large amplitude configurational changes which parallel energized processes in mitochondria [1–3]. By now a considerable body of information has been accumulated about the details of the configurational transitions in the mitochondrion [4–6]. Moreover, the theory of structured systems with the capability for conservation of energy by a conformational mechanism is now being developed systematically [7]. This interplay of experiment and theory has made it possible to formulate the conformational hypothesis more explicitly and precisely.

The tripartite repeating unit of the cristal membrane is the molecular unit of mitochondrial energy transduction. While in theory each repeating unit is a complete operational unit, in practice we are dealing with two dimensional arrays of repeating units within a membrane continuum and with the cooperative effects intrinsic to such arrays.

Each repeating unit contains three basic systems which collectively effectuate the conformational transduction of energy. These systems are (a) the primary energizing system (one of the complexes of the electron transfer chain), (b) the conformational frame system, and (c) the discharge-directing system (the ATPase complex is one such system). The electron transfer process within each complex of the chain leads to a conformational transition [8–10]. But since the electron transfer system is enclosed within a rigid frame (the conformational frame system [7]), the conformational expansion of the complex during electron flow is restricted. It is this restriction in the permissible extent of conformational change imposed by the frame system which is the key to the efficient transduction of redox into conformational energy. The conserved energy is stable for a sufficient period to permit its utilization for one of three alternative work performances – union of ADP and Pi to form ATP, translocation of ions, or transfer of a hydride from NADH to $NADP^+$. Stored conformational energy can be lost by a spontaneous discharge process or conserved by a directed discharge process. The work performances represent devices which are intrinsic to the repeating units and which can direct the discharge of stored conformational energy in a highly specific manner.

The unique feature of the mitochondrial as compared to the muscle "conformational" system is that of reversibility. Not only can electron transfer drive ATP synthesis but ATP hydrolysis can reverse the direction of electron transfer. The electron transfer complex can serve either as the energizing system or as the discharge-directing system. The same options are open to the ATPase complex. Thus, the conformational frame system can be energized at either end of the repeating unit – by electron transfer in the basepiece or by hydrolysis of ATP in the headpiece. The stalk may be considered a device for transmitting the energized state from basepiece to headpiece or in the reverse direction.

2. The conformational cycle

Each repeating unit can exist in two conformational states – nonenergized (NE) and energized (E).

Electron transfer or ATP hydrolysis in absence of inorganic phosphate leads to the E conformation and in presence of inorganic phosphate to the E-T conformation (E-T is the abbreviation for energized-twisted). The repeating unit in the E-T conformation can be discharged either by ADP with formation of ATP or by Ca^{2+} with formation of $Ca_3(PO_4)_2$. The conformational cycle for oxidative phosphorylation or for translocation of Ca^{2+} and Pi can be described by the sequence NE → E-T → NE; the cycle for energized transhydrogenation by the sequence NE → E → NE. It is implicit in this formulation that the level of inorganic phosphate determines whether the energized conformation will be the E or E-T form. At the present time the geometry of the repeating unit in the three conformations (NE, E, and E-T) cannot be accurately specified but the general geometric features of each conformation have been deduced from electron micrographic evidence.

3. The configurational cycle

When all the repeating units in a given crista are in the same conformation, the membrane is in the corresponding configuration (NE, E, or E-T). When, however, the conformations of the repeating units are mixed, the configuration of the membrane also has a hybrid character. There are two optional forms of the NE configuration (orthodox and aggregated) and we are now aware of a set of factors which determine the options. There are at least three optional forms of the E-T configuration (tubular, zigzag, and vesicular). Here again, some of the factors which determine the options have been uncovered.

It is obvious that a configurational change in a membrane which involves thousands of repeating units will lag behind a synchronous change in the conformation of these repeating units. Moreover, osmotic and water changes are intrinsic to the conformational changes and are crucial determinants of the extent of the configurational rearrangement of the cristal membrane. Thus, in mitochondria *in situ*, the crista in the NE (orthodox) configuration is maximally contracted (minimum intracristal space); in the E configuration, almost maximally expanded; and in the E-T configuration, expanded with a helical twist [1–3,6,11].

The conformational cycle of the repeating units according to our hypothesis is an absolute requirement for coupling; however, the configurational cycle of the membrane certainly is not. Under appropriate steady state conditions the repeating units must rapidly fluctuate between energy states, but the membrane shows no signs of configurational transitions [5,12].

4. Measurement of configurational and conformational change

The configurational state of the cristal membrane can be accurately evaluated by electron microscopic examination of rapidly fixed mitochondrial suspensions. When steady state conditions are carefully avoided, and conformational synchrony of the repeating units obtains, then configuration can be taken as a direct measure of conformation [5]. Light scattering at 90° measures the NE to E-T or E to E-T configurational transitions in isolated beef heart mitochondria [4,5] and aggregated to orthodox configurational transition in adrenal cortex mitochondria [13]. These light scattering measurements of configurational changes are also accurate indices of conformational changes when conformational synchrony of repeating units is achieved by eliminating steady state conditions, i.e., conditions which lead to rapid turnover of the energized state. A more direct measure of conformational change in the repeating units of coupled particles is change in pH of the external suspending medium [14]. Conformational change in the NE to E transition leads to the emergence of buried charged groups as the polypeptide chains rearrange. A new equilibrium is established between the conformationally altered mitochondrion and the external medium. A movement of protons, cations, and anions is required for the establishment of the new equilibrium. Even particles which lack the capability for coupling electron transfer to synthesis of ATP may still show uncoupler-sensitive pH changes under energizing conditions - a token that the lesion in the coupling mechanism of the particle is not at the level of the first conformational transition. For the pH shift to serve as a reliable index of conformational change, the mitochondrial particles must be exposed to conditions (ageing or freezing) or reagents (Ca^{2+}, valinomycin) that eliminate permeability of the membranes to ions as a barrier to rapid equilibration.

5. Correlative studies

In the past two years we have carried out extensive studies to determine whether changes in the energy state of the mitochondrion or submitochondrial particle could be correlated with changes in the conformational state as measured by configurational change. Reagents or conditions which induce or suppress the generation of the energized state should induce or suppress the corresponding conformational changes. The reagents tested included uncouplers, inhibitors of electron transfer and of ATP hydrolysis, ADP, Pi, Ca^{2+} and other divalent metal ions [3–5]; the conditions included high concentrations of sucrose [15], the presence or absence of substrate or of Pi or of oxygen or of ATP, etc. By any of three methods of measuring conformational change described above, a correlation was found to obtain between the energy and the conformational state of the particle. A further critical test of the correlation was passed with the demonstration that the half-time of proton release was approximately equal to the turnover of the electron transfer components in mitochondria under coupling conditions [16].

We have been able to establish a correlation between energy and conformational state not only in isolated mitochondria from various sources but also in mitochondria *in situ* and in submitochondrial particles such as ETP_H [4,17]. On the basis of these multiple lines of evidence we consider the case for identifying the energy and conformational states of the mitochondrion as complete.

6. Modulation of the configurational cycle

The ultrastructural details of the configurational cycle vary considerably from one particle to another and from one set of conditions to another. These variations are referable to differences in the amount of matrix protein [11], in the concentration of Ca^{2+} and Mg^{2+} in the suspension medium [13], and in the osmotic pressure of the suspending medium [15], etc.

Mitochondria fall into categories in respect to their configurational patterns [11]. Those of the first category (e.g., beef heart mitochondria] show very clearly each of the three configurational states of the cristal membrane; those of the second category (e.g., rat liver mitochondria) have a condensed appearance when the cristal membrane is energized which makes it difficult to distinguish between the E and E-T configurations. Mitochondria of the first category appear to have a relative minimum of protein in the matrix space; those of the second category appear to have relatively large amounts of protein in the matrix space. Matrix protein profoundly modulates the ultrastructural expression of the configurational changes when mitochondria of the second category are energized. But we now recognize that these differences in the configurational pattern of the cristal membrane in no way violate the generality of the thesis that the basic conformational cycle of the repeating units is a universal for all types of mitochondria.

Coupled submitochondrial particles such as ETP_H clearly show configurational changes when exposed to energizing conditions in presence of inorganic phosphate. These changes can be followed either by electron microscopic examination [17] or by light scattering measurements [4]. The E-T configuration in ETP_H takes the form of an invagination of the boundary membrane into the interior space.

7. Uncouplers and inhibitors of configurational transitions

It can hardly be a coincidence that the classical uncouplers (2,4-dinitrophenol, *m*-chlorocarbonyl cyanide phenylhydrazone) suppress the first conformational transition and that reagents which selectively suppress subsequent conformational transition also uncouple oxidative phosphorylation. These results can be rationalized in terms of the hypothesis that the classical uncouplers modify the coupling mechanism so that the transduction of redox energy (or the energy of hydrolysis of ATP) to conformational energy is abortive whereas reagents such as fluorescein mercuric acetate (inhibitor of the E to E-T conformational transition [18]) prevent the selective discharge mechanism from operating and thereby compel dissipation of the energy by spontaneous discharge. Thus, it is predictable that any reagent which uncouples or inhibits oxidative phosphorylation or energized translocation of ions will suppress at least one of the transitions in the conformational cycle. A high concentration of sucrose in the external medium prevents oxidative phosphorylation

and suppresses the E to E-T conformational transition [15].

A body of evidence has accumulated that sulfhydryl groups play a determinant role in conformational transitions and that reagents which uncouple energized processes interact with specific sulfhydryl groups [19]. The action of all uncoupling agents yet tested (with the exception of 2,4-dinitrophenol) has been found to be significantly reversed by addition of dithiothreitol to the mitochondrial system [19].

8. Orthodox to aggregated configurational transition

There are three key ultrastructural events intrinsic to configurational changes in the cristal membrane of mitochondria *in situ* [6,11]: (a) expansion of the intracristal space of the cristae; (b) engagement of apposed cristae; (c) disengagement of apposed cristae and tubularization or zigzagging of the cristae. The last two of the three ultrastructural changes are believed to be referable to and to be sequellae of corresponding conformational changes in the repeating units. The first change – the expansion of the crista (orthodox to aggregated configurational transition) – may be referable to changes induced in another mitochondrial system which provisionally we may identify as a structured system probably localized between the two boundary membranes and in the intracristal space [20]. There is some evidence to suggest that expansion and contraction of this intracristal system can be effectuated by reagents such as Ca^{2+} and Mg^{2+} even when electron transfer or hydrolysis of ATP is completely prevented [13].

Whenever the expansion of the crista is blocked by appropriate means and the crista is forced to remain fixed in the orthodox configuration, the mitochondrion loses the capability for oxidative phosphorylation or energized translocation of ions [21,22]. Thus, it appears that the expansion of the crista (collapse of the matrix space) is a *sine qua non* for these two energized processes. In some way engagement of cristae is an indispensable intermediate step in the directed discharge process leading to ATP synthesis or ion translocation. A variety of reagents are known which stabilize the mitochondrion in the orthodox configuration and thereby uncouple oxidative phosphorylation. These reagents include Ca^{2+} [21], endotoxin of Bordetella [22], and silicomolybdate [23]. The orthodox to aggregated configurational transition probably plays an important control function in the mitochondrion. Isolated mitochondria almost invariably are in the aggregated configuration, and, thus, the fact as well as the significance of the transition between the two nonenergized configurations were missed until isolated mitochondria could be compared with mitochondria *in situ*.

9. Summarizing comment

The thesis that conformational change and the conformational cycle are intrinsic to the energy cycle of mitochondria is now supported by a large body of evidence. Nonetheless, direct proof is still lacking that the conformational changes involve the amounts of energy required for oxidative phosphorylation. (This gap in the evidence applies as much to the actomyosin system as to the mitochondrial system). How it would be possible by physical methods to establish the fact of the conservation of conformational energy in the repeating unit poses a formidable challenge to investigators. The conformational hypothesis accounts for the key events in coupling, rationalizes many facts hitherto incomprehensible, and had led to a large number of testable predictions. It is this synthesis and integration of a large body of experimental information which provides the inferential evidence that the conformational changes are more than virtual.

The key problems that lie ahead are three in number: (a) the definition of the conformational frame system; (b) the delineation of the mechanism for the transfer of energy from the electron transfer chain of the ATPase system to the frame system; and (c) the delineation of the mechanisms for selective discharge of the repeating unit in the energized conformation.

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