

# A MOLECULAR MECHANISM OF THE ENERGETIC COUPLING OF A SEQUENCE OF ELECTRON TRANSFER REACTIONS TO ENDERGONIC REACTIONS

BO CARTLING, *Department of Biophysics, Arrhenius Laboratory, Stockholm University, S-106 91 Stockholm, Sweden, and the Department of Theoretical Physics, Royal Institute of Technology, S-100 44 Stockholm 70, Sweden and*

ANDERS EHRENBERG, *Department of Biophysics, Arrhenius Laboratory, Stockholm University, S-106 91 Stockholm, Sweden*

**ABSTRACT** A molecular mechanism of the energetic coupling of a sequence of electron transfer reactions to endergonic reactions is proposed and discussed from a physical point of view. The scheme represents a synthesis of concepts of electron transfer by tunneling and the conformational and chemiosmotic aspects of energy coupling processes. Its relation to existing experimental information and theoretical models is discussed, and further experimental tests are suggested.

## INTRODUCTION

Different mechanisms of energy transfer between energy-transducing units, such as electron transfer complexes, ATPase, and ion translocators, have been proposed. They differ with respect to the form in which energy is primarily stored, e.g. between its generation by electron transfer reactions and its utilization for phosphorylations or ion translocations. The chemiosmotic hypothesis by Mitchell (1, 2) works with a proton electrochemical potential across the coupling membrane composed of a proton gradient and a membrane potential. Slater's chemical (3) and Boyer's conformational (4) hypotheses are based on high-energy intermediates, chemically modified and conformationally excited energy-transducing units, respectively. The verification of either of these hypotheses has been the objective of intense research activities. The chemical hypothesis has been abandoned because of unsuccessful attempts to identify the high-energy chemical intermediates. Throughout the years, the chemiosmotic and conformational hypotheses have been modified and developed with respect to more detailed mechanisms. The present situation might be summarized as follows (5).

It appears that the chemiosmotic and conformational hypotheses are not necessarily mutually exclusive, but may represent different aspects of a common underlying mechanism. A proton electrochemical potential across the coupling membrane can be reversibly generated by energy-transducing units. Several energy-transducing units undergo energy-linked conformational changes, reversibly related to the generation of

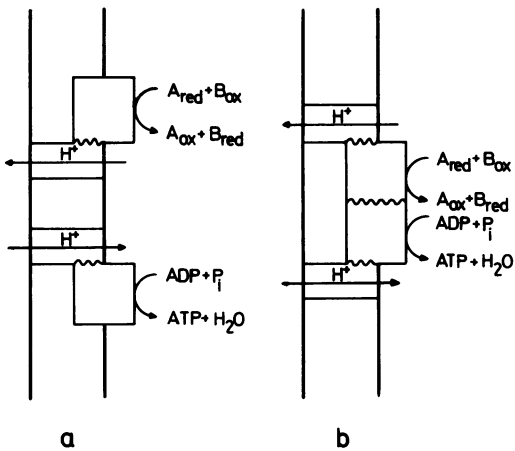


FIGURE 1

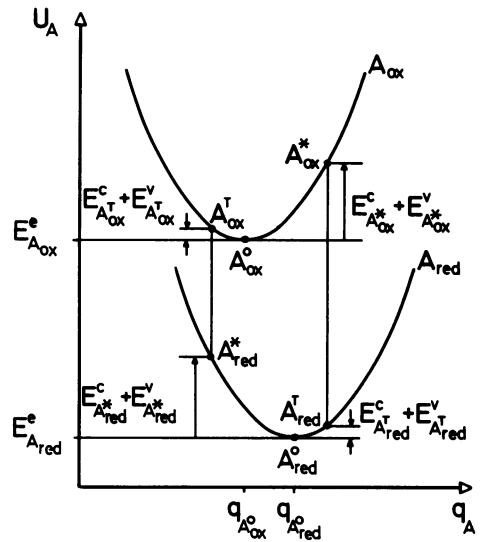


FIGURE 2

FIGURE 1 Possible mechanisms of energy transfer between energy-transducing units: (a) via a proton electrochemical potential; (b) through direct conformational interactions in addition to the mechanism in a. A section of a membrane is shown in which the square boxes are energy-transducing units, exemplified by an electron transfer complex and an ATPase, and the rectangular boxes represent conformationally driven proton pumps. The wavy lines indicate conformational interactions. Based on reference 5.

FIGURE 2 Potential energy surfaces for nuclear motion, as given by the total electronic energy as a function of a generalized nuclear coordinate, of one electron transfer enzyme A in its oxidized and reduced forms,  $A_{ox}$  and  $A_{red}$ .  $A^0$  refers to the conformational equilibrium and vibrational ground state of A,  $A^T$  to a thermally excited state, and  $A^*$  to a general excited state.  $E_A^e$ ,  $E_A^c$ , and  $E_A^v$  denote the minimum, conformational, and vibrational energies, respectively;  $q_A$  stands for the set of conformational and vibrational coordinates  $\{q_A^c, q_A^v\}$ .

a proton electrochemical potential. The generation of a proton electrochemical potential probably involves conformationally driven proton pumps coupled to the energy-transducing units (6). Energy transfer between energy-transducing units can take place via a proton electrochemical potential, but may involve direct conformational interactions in addition (see Fig. 1).

In this paper, a molecular mechanism of the energetic coupling of a sequence of electron transfer reactions to endergonic reactions, possibly underlying the chemiosmotic and conformational aspects, is proposed and discussed from a physical point of view. Its relation to existing experimental information and theoretical models is discussed, and further experimental tests are suggested.

### THE COUPLING MECHANISM

The coupling mechanism is conveniently described by means of potential energy surfaces of the electron transfer enzymes. These energy surfaces represent the potential en-

ergy for nuclear motion as given by the total electronic energy including the nuclear repulsion energy as a function of nuclear coordinates. The nuclear degrees of freedom of an electron transfer enzyme are divided into global conformational modes and local vibrational modes, characterized by different dynamic behavior. Corresponding to this, the total potential energy is considered as a superposition of three contributions,

$$U_A(q_A) = E_A^e + U_A^c(q_A^c) + U_A^v(q_A^v). \quad (1)$$

A denotes an electron transfer enzyme in either its oxidized or reduced form,  $A_{\text{ox}}$  or  $A_{\text{red}}$ , and  $q_A$  stands for the set of conformational and vibrational coordinates  $\{q_A^c, q_A^v\}$ . The first term on the right-hand side of Eq. 1,  $E_A^e$ , is independent of the nuclear coordinates, but varies with the redox state, i.e. the electron occupation number, indicated by the superscript "e." The conformational coordinates,  $q_A^c$ , are of a collective type, each being composed of several cooperatively acting nuclear coordinates. A conformational transition is a relaxation type process during which kinetic energy is dissipated to the surroundings. A detailed description of the conformational dynamics requires the fine structure of the conformational part of the potential energy,  $U_A^c(q_A^c)$ , corresponding to barriers between local energy minima. The vibrational modes exhibit ordinary oscillator behavior determined by the vibrational part of the potential energy,  $U_A^v(q_A^v)$ . In either of the oxidized or reduced forms of an electron transfer enzyme, three states are distinguished.  $A^\circ$  refers to the conformational equilibrium and vibrational ground state of A,  $A^T$  to a thermally excited state, and  $A^*$  to a general excited state. Schematic energy surfaces of an electron transfer enzyme are shown in Fig. 2. Any fine structure of the conformational part of the potential energy,  $U_A^c(q_A^c)$ , is left out.

Consider three successive members A, B, and C of a chain of electron transfer enzymes. Assuming, to a first approximation, the units to be independent of each other, the corresponding potential energy surfaces are shown in Fig. 3. An electron transfer from  $A_{\text{red}}$  to  $B_{\text{ox}}$  implies an energy-requiring electron removal from  $A_{\text{red}}$  and an energy-yielding electron addition to  $B_{\text{ox}}$ . This gives rise to a potential barrier for the electron, which might be penetrated by tunneling provided energy conservation is fulfilled, i.e.,

$$E_{A_{\text{ox}}}^* - E_{A_{\text{red}}}^T = E_{B_{\text{ox}}}^T - E_{B_{\text{red}}}^*, \quad (2)$$

where  $E_A$  denotes the total energy of A in the state indicated, including nuclear kinetic energy. By analogously denoting the total energies of the conformational and vibrational contributions to  $E_A$  by  $E_A^c$  and  $E_A^v$  and using Eq. 1, Eq. 2 can be written

$$\begin{aligned} E_{A_{\text{ox}}}^e + E_{A_{\text{ox}}}^c + E_{A_{\text{ox}}}^v - (E_{A_{\text{red}}}^e + E_{A_{\text{red}}}^c + E_{A_{\text{red}}}^v) \\ = E_{B_{\text{ox}}}^e + E_{B_{\text{ox}}}^c + E_{B_{\text{ox}}}^v - (E_{B_{\text{red}}}^e + E_{B_{\text{red}}}^c + E_{B_{\text{red}}}^v). \end{aligned} \quad (3)$$

It is the thermally excited energy that provides the final adjustment required to satisfy the resonance condition. A Franck-Condon principle applies to the electron transfer process because the electron transfer time is short compared to characteristic

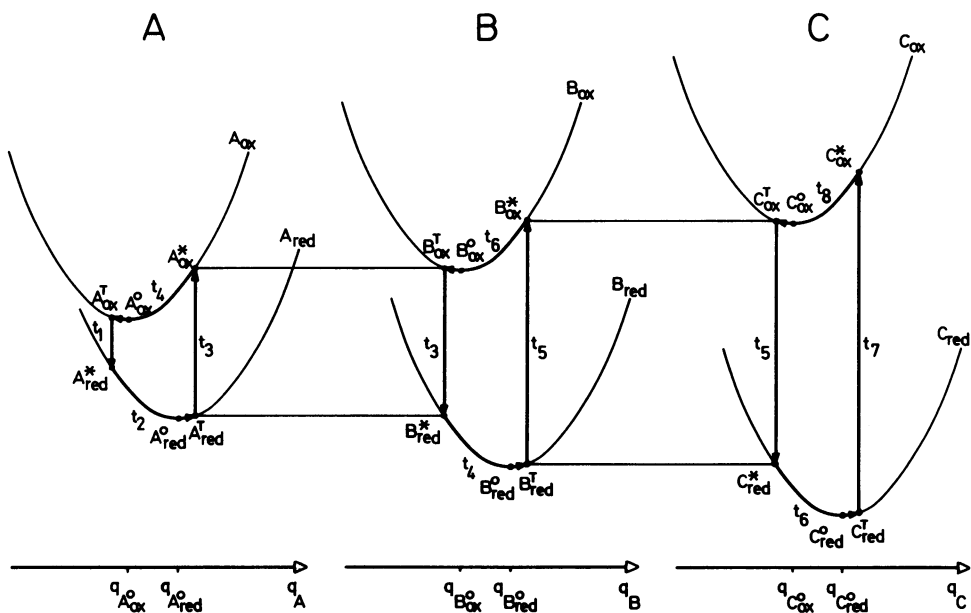


FIGURE 3

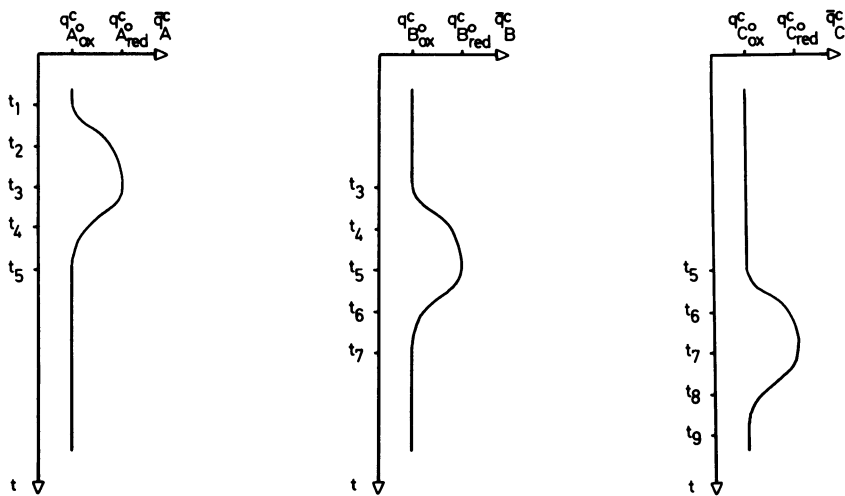


FIGURE 4

FIGURE 3 A sequence of electron transfer reactions. Potential energy surfaces of three successive enzymes, A, B and C, of an electron transfer chain are shown as in Fig. 2. For an explanation of the processes, see The Coupling Mechanism.  $t$  is a time coordinate. FIGURE 4 The conformational dynamics of the three enzymes, A, B, and C during the processes illustrated in Fig. 3 and described in The Coupling Mechanism.  $q_A^c$  represents the thermal averages of the conformational coordinates of enzyme A, and  $t$  is a time coordinate.

times of nuclear rearrangements. The electron transfer then corresponds to vertical transitions in the potential energy diagrams of the individual reactants. Thus, enzyme A is brought into the state  $A_{ox}^*$  far from thermal equilibrium. The conformational energy stored in this state is released upon relaxation toward the equilibrium conformation of  $A_{ox}$  and is available to perform work. A corresponding process simultaneously takes place at enzyme B, where the relaxation of  $B_{red}^*$  toward the equilibrium conformation of  $B_{red}$  also liberates energy. Fig. 4 shows the conformational dynamics, i.e. the time dependence of the conformation of the enzymes. Available energy for work, released upon electron transfer from A to B, is

$$W_{AB} = E_{A_{ox}}^c - E_{A_{ox}}^e + E_{B_{red}}^e - E_{B_{red}}^c. \quad (4)$$

The net heat dissipated is given by

$$Q_{AB} = E_{A_{ox}}^c + E_{A_{ox}}^v - E_{A_{red}}^c - E_{A_{red}}^v + E_{B_{red}}^c + E_{B_{red}}^v - E_{B_{ox}}^c - E_{B_{ox}}^v. \quad (5)$$

Assuming thermal excitation energies of conformations and vibrations in the oxidized and reduced forms to be similar,  $Q_{AB}$  is approximately

$$Q_{AB} \simeq E_{A_{ox}}^v - E_{A_{ox}}^e + E_{B_{red}}^e - E_{B_{red}}^v. \quad (6)$$

The free energy released upon the redox reaction is

$$\Delta E_{AB} = E_{A_{red}}^e - E_{A_{ox}}^e + E_{B_{ox}}^e - E_{B_{red}}^e, \quad (7)$$

which equals the difference of the redox potentials of A and B, as will be dealt with in the Discussion. The energy conservation criterion, Eq. 3, is equivalent to

$$W_{AB} = \Delta E_{AB} - Q_{AB}. \quad (8)$$

In a total energy diagram, an electron transfer process is represented by the intersection point between the energy surfaces of reactants and products, corresponding to con-

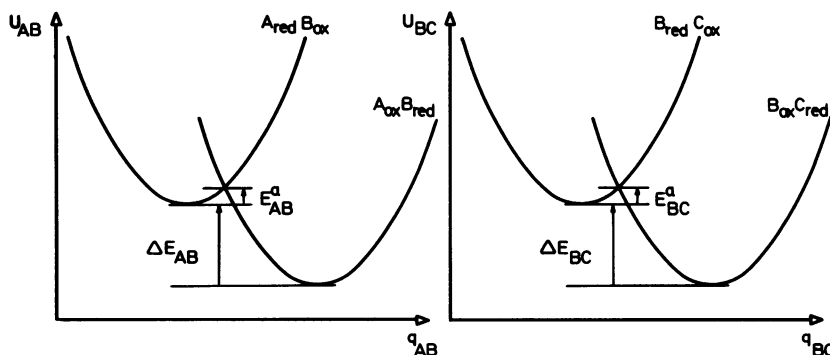


FIGURE 5 Total energy diagrams of the redox reactions illustrated in Fig. 3 and described in The Coupling Mechanism.  $U_{AB} = U_A + U_B$  and  $q_{AB} = \{q_A, q_B\}$ , where  $U_A$  is the total potential energy of enzyme A and  $q_A$  represents its nuclear coordinates. The activation energy  $E_{AB}^a$  is given by Eq. 9, and  $\Delta E_{AB}$  is defined in Eq. 7.

served total energy and nuclear coordinates (see Fig. 5). The activation energy for the redox reaction is given by

$$E_{AB}^a = E_{A_{red}}^c + E_{A_{red}}^v + E_{B_{ox}}^c + E_{B_{ox}}^v. \quad (9)$$

A subsequent transfer of the electron from B to C takes place in an analogous manner, provides a corresponding energy conservation criterion is fulfilled, a condition assumed to be satisfied when  $B_{red}$  approaches its equilibrium conformation. This requirement implies an increasing separation between the energy surfaces of the oxidized and reduced enzyme forms successively along a chain of electron transfer enzymes.

The energy released upon the conformational relaxation of an electron transfer enzyme might be transferred to another unit through direct conformational coupling. The other unit would be an ATPase or a proton pump according to the conformational and chemiosmotic hypotheses, respectively, and in general performs an endergonic reaction. The conformationally coupled system composed of an electron transfer enzyme A and another unit X might be characterized by the condition

$$q_A^c = q_X^c. \quad (10)$$

The conformational dynamics of the combined system *are* determined by the total potential energy  $U_A^c + U_X^c$ . Unit X is assumed to have a stationary potential energy for both the initial and final conformations. The model potential energies shown in Fig. 6 correspond to a system that spontaneously will perform one stroke from the initial non-

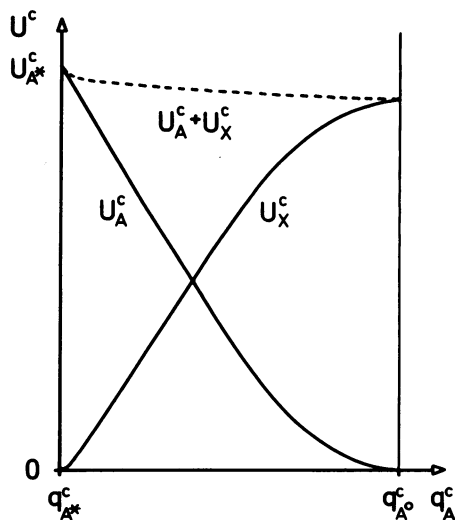


FIGURE 6 Conformational energy surfaces of an electron transfer enzyme A, another unit X, and the conformationally coupled system of A and X characterized by the condition  $q_A^c = q_X^c$ , where  $U^c$  and  $q^c$  denote conformational energy and coordinates of the unit as indicated by subscripts.  $A^*$  and  $A^o$  denote a conformationally excited and a conformational equilibrium state of A, respectively.

equilibrium state to the final equilibrium state. The established efficiency of energy transduction in biological systems, i.e. that the loss of energy is small, implies that the system operates close to reversibility. In a situation when enzyme A is uncoupled, the stored conformational energy will be dissipated to the surroundings during relaxation.

From the illustration in Fig. 3 of the operation of the enzymes, their character of cyclic machines is clear, because after a completed electron transfer process an enzyme returns to its initial state, prepared to catalyze a new electron transfer.

The mechanism appears to be an example of how nature prefers to perform hard work in accordance with the principle of a lever. Tunneling of the light electron over a long distance, in the case of a phosphorylation, eventually causes the covalent binding of a heavy phosphate group, involving its translocation over a short distance against a substantial barrier.

## DISCUSSION

The energy difference  $E_{\text{A,red}}^{\epsilon} - E_{\text{A,ox}}^{\epsilon}$  corresponds to the redoxpotential of an electron transfer enzyme A apart from an additive constant, independent of A, required for consistency with the standard reference. The incorporation of an electron transfer enzyme into the present type of system, of course, makes its actual redoxpotential different from its standard redoxpotential. It is to be noted also that the energy conservation criterion for electron transfer is given by Eq. 3 and is thus not determined by the redoxpotentials only. If some conformational restraints on an electron transfer enzyme prevent conformational equilibrium to be established, the apparent redoxpotential will be affected.

Reduction or oxidation of an electron transfer enzyme, i.e. addition or removal of an electron to its active center, is assumed to induce a global conformational change. Most electron transfer enzymes contain transition metal complexes in some form as active center, like the heme group of cytochromes or the Fe-S clusters of Fe-S proteins. The global nuclear rearrangement might originate from a local strain caused by a Jahn-Teller type of local distortion. The Jahn-Teller theorem predicts any complex in an electronically degenerate state to be unstable against a structural distortion that lifts the degeneracy. The change of electron occupation number of an active center might bring the system into a degenerate state momentarily. The point group symmetry of an active center is of course reduced in an enzyme by the protein. In case the split of the energy levels due to this fact is smaller than the Jahn-Teller energy, the description in terms of a Jahn-Teller effect might be useful.

The electron-conformation coupling has been discussed previously in the context of energy coupling processes (7) and is underlying the concept of the conformon (8,9) that may prove useful. Macromolecular conformational relaxation as a general basis of enzyme functioning has been proposed (10).

Considered separately from the global conformational changes are the local vibrational modes. By thermal excitation of vibrations of both reactants in the initial state before an electron transfer reaction, the spectrum corresponding to ionization of the

donor center as well as the spectrum corresponding to addition of an electron to the acceptor center are broadened, which increases the overlap of these spectra in case the resonance condition is not perfectly satisfied. This gives rise to a temperature-dependent transition probability for the tunneling process. Another type of vibrational excitation takes place upon the electron transfer. Within the system composed of both reactants, the electron transfer process could be viewed as an intramolecular energy transfer of electronic energy to vibrational energy upon the transition between the different bound electronic states corresponding to the reactants and the products.

Descriptions of electron transfer between ions in solution, based on quantum mechanical principles, were developed by Levich (11). In particular, the nuclear vibrations were taken into account. The contribution of the phonon modes of the polar medium was included by polaron theory. The quantum mechanical treatment has been extended by Jortner (12-16), who considers the electron transfer reaction as the decay of the initial state, which is metastable due to its coupling to the quasi-continuum of final states that the vibronic states of the products constitute. Utilizing Kubo's formalism of generating functions, the theory of this type of intramolecular energy transfer is developed (12, 13) and applied to electron transfer reactions between ions in solution (14, 15) as well as between biological molecules (16). This formalism allows the inclusion of the contribution from high-frequency vibrational modes of the first coordination layers around the ions in addition to the low-frequency phonon modes of the medium. The essential outcome of the quantum mechanical treatments is that the transition probability assumes the form of an activated rate expression of the Arrhenius type. Electron transfer reactions are unique among chemical reactions in that respect because this result is obtained without invoking the concept of an activated complex. At low temperatures, the activation energy is zero, i.e. the transition probability is temperature independent, corresponding to the physical situation of nuclear tunneling. At higher temperatures, the activation energy assumes the value expected from the intersection of the energy surfaces of reactants and products. Interpretation of the data from the classic experiment on laser pulse-induced electron transfer from cytochrome to chlorophyll in the photosynthetic bacterium *Chromatium* (17) in terms of this formalism yields the result that coupling to high-frequency vibrational modes of the first coordination layer around the active centra is more important than coupling to low-frequency phonon modes of the medium. This result is in contrast to the situation of electron transfer between ions in solution, which is the reverse. The importance of nuclear vibrations in the context of electron tunneling in biological systems has been observed by other authors (18-20), who have given simplified theoretical descriptions. It is to be noted that all the vibrational energy excited upon an electron transfer is dissipated to the surroundings with which the system of vibrational oscillators is in thermal equilibrium. The electron-vibrational coupling alone is therefore not sufficient as an underlying mechanism of energy-coupling processes.

The principles of operation outlined under The Coupling Mechanism might be underlying energy-converting systems, the properties of which, as inferred from experi-



ments, are those summarized in the Introduction. The scheme represents a synthesis of concepts of the conformational hypothesis and electron transfer by tunneling. The conformational hypothesis in turn is compatible with a chemiosmotic hypothesis based on conformationally operating proton pumps. The scheme is compatible with either of the two possible mechanisms of energy transfer between energy-transducing units illustrated in Fig. 1. These are, of course, the most important conclusions to be drawn concerning the relation of the mechanism to existing experimental information. A few further comments are of interest in this context.

The consequence of the model discussed under The Coupling Mechanism, that the separation between the energy surfaces of the oxidized and reduced enzyme forms, i.e. the redoxpotential of the enzymes, in an electron transfer chain must successively increase, is a well-known characteristic of the electron transfer chains in the inner membrane of mitochondria and the thylakoid membrane of chloroplasts. Along the respiratory chain in mitochondria, the redoxpotential difference between successive electron transfer enzymes assumes a large enough value to be useful for energy transduction at three sites. The restrictions on electron transfer between subsequent enzymes as imposed by the energy conservation criterion, expressed in Eq. 3, will provide the required specificity.

The conformational transitions of redoxenzymes between their oxidized and reduced forms have been studied experimentally by various techniques.

Hydrated electrons produced by pulse radiolysis can reduce a metalloprotein, and the subsequent changes of spectroscopic properties can be followed. For cytochrome *c*, fast and slow stages have been identified, the latter being interpreted as associated with the conformational relaxation upon reduction (21, 22).

In another type of experiment, the protein conformation of a metalloprotein is frozen, e.g. in a water-ethylene-glycol solution at liquid nitrogen temperature. By application of ionizing radiation, like  $\gamma$ -radiation, the enzyme can be reduced. The reduced active center is then bound to an apoprotein frozen in its oxidized conformation. It is possible to study the optical absorption of a hemoprotein or the electron paramagnetic resonance absorption of a Fe-S protein in this form and to distinguish such spectra from those obtained on chemically reduced enzymes, the discrepancy being ascribed to the different conformational states of the protein (23–26).

Recent experimental findings supporting the proposed mechanism are provided by Wikström (27, 28), who concludes that cytochrome *c* oxidase includes or is coupled to a conformationally driven proton pump. The proton translocation might be associated with a conformational relaxation of cytochrome *c* oxidase from a conformationally excited state, which is generated upon electron transfer from cytochrome *c*.

The vibronic coupling theory of electron tunneling has recently been experimentally tested utilizing a weak charge transfer optical absorption, which is predicted directly from the model describing vibronically coupled tunneling (29–31). Applied to an actual energy-transducing electron transfer system, this technique might also be useful in studying components of the kinetics of energy conversion processes corresponding to conformational relaxations.

A coupling of the conformation of an energy-transducing unit to an energy-transducing unit to an external electric field due to charged groups on the macromolecules would allow an external control of conformations. Recently ATP synthesis has been induced by an external static electric field (32,33). By application of an external oscillating field and varying its frequency, new information on the kinetics of energy conversion processes would be obtained.

This work was presented in part at the International Symposium on Membrane Bioenergetics, Island of Spetsai, Greece, 10-15 July 1977.

This work was supported by the Swedish Natural Science Research Council.

Received for publication 2 September 1977 and in revised form 3 April 1978.

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