# **Charged Pair Current Networks in Bioenergetics**

(enzymes/energy transduction/diagrammatic rules)

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ABSTRACT The operation of bioenergetic systems is postulated to be based on charged pair networks. The basic elements of the networks are pairs of opposite charges accompanied by conformational changes of the protein medium. The elementary events are the separation and recombination of charged pairs and partner exchange between two such pairs. The last event makes the construction of networks possible and provides for the flexibility of coupling modes in mitochondria. Bioenergetic systems appear to use electricity in a hitherto unsuspected way. The networks are constructed entirely on electrostatic principles. The possibility of generalization to include mechanical elements is discussed.

### I. Introduction

The electromechanochemical model for energy coupling in bioenergetics has emerged from the work of Green and collaborators (1) in recent years. The principal task of bioenergetic systems is the conservation and transduction of free energy. It has been shown that the processes can involve thermal energy without violating the second law of thermodynamics (2). Transduction usually begins and ends with stable chemical bonds with electrical and mechanical energy forms in between. The participating units at the electrical and mechanical level are microscopic charges (electrons, protons, and ions) and semimacroscopic units (protein molecules and macromolecular assemblies). For these units the electrical and mechanical aspects are not separable because mechanical distortion (conformational change) accompanies the electrical charges.

The task of a bioenergetic system can be performed only if three minimal and somewhat contradictory requirements are satisfied. The structure must be stable and therefore must be constructed of molecules (3), i.e., some strong bonds must exist. It must be macroscopic enough to yield sufficiently definite laws of motion, so its activity will be adequately defined and not completely random (4). Finally, it must not be too large and bound so strongly as to prevent thermal energy from activating the motions in question. Thus, there are limitations not only on the proper mix of the various bond strengths, but also on size. Bioenergetic and other biological systems must be carefully perched at the borderline of the microscopic and the macroscopic.

It is clear then that the bioenergetic machinery consists of macromolecules and macromolecular assemblies not by accident, and the matter that it handles must be smaller than itself. It, therefore, manipulates chemical bonds, electrons, protons, and ions.

#### II. Structures with free energy

The transduction of chemical to electromechanical energy is performed by an enzyme removing a positive and a negative charge from a substrate (1). There are numerous known examples of such positive and negative charges moving in pairs. One can rationalize why this should be so. The enzyme facilitates the separation of the two charges by lowering the free energy required for this process by enthalpic and entropic factors. Once the two charges are separated sufficiently so that the protein medium sees two separate charges, there is no reason for further separation to confer additional entropic advantages (except for mixing entropy) and the Coulomb attraction of the two charges will resist larger separations. Thus, there is a distance of separation with a second minimum in the free energy, the first one corresponding to the chemical bond. The free energies in the two stable states ought to be equal under optimal circumstances (Fig. 1).

A conventional view of a catalyzed reaction leads from a set of chemical bonds in the reactants to a different set in the products. The role of the catalyst is to lower the activation free energy of the reaction. The activated state may have separated charges, but their concentration is negligibly low. In some enzyme-catalyzed reactions chemical bonds are broken not to be replaced by others, but to be replaced by separated charges. The enzyme not only changes reaction rates but changes the nature of the products. Thus, two enzymatic reactions of this type are necessary to create as many stable charges as an ordinary catalyzed reaction would produce for the activated state (Fig. 2). Enzymes may catalyze also the reverse reaction and may also facilitate reactions between separated charged pairs.

The ability of enzymes to separate charges to definite distances is of the utmost importance for bioenergetics, because it opens up possibilities not available for ordinary chemical reactions. An ordinary chemical reaction goes from reactants to products and the process is finished. Separated charged pairs allow the building of elaborate networks of reactions and thus provide for the multiplicity of mitochondrial functions. Networks must be built out of the interactions of charged pairs.

It is obviously possible to store free energy in chemical bonds by making reactions leading to stronger bonds inaccessible. The situation is not so clear when free energy is stored in the electromechanical form. In this case free energy is distributed over a relatively large volume around separated charges and there is a clear danger that control over it can be lost. It appears to be very difficult to concentrate free energy again at a single point (5). This difficulty can be overcome by a correctly designed medium. The problem of dissipation in ordinary chemical reactions and in reactions involving separated charges are not as different as one would believe at first sight. Chemical reactions involve charge rearrangements in bonds and, if carefully chosen, will not dissipate free energy.

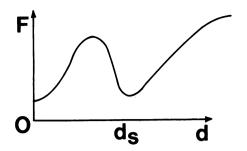


FIG. 1. Free energy as a function of the separation of a positive and negative charge. d = 0 corresponds to a chemical bond and  $d_s$  to the separated charges.

Reactions involving separated charges are also charge rearrangements and within the appropriate structures need not dissipate free energy. These structures are apparently proteins. The structures participate in the reactions.

Clearly the separation of electromechanical energy into electrical and mechanical components is impossible in these situations. There are interactions of charges with each other and charge interacts with the protein causing conformational change; the conformational changes may overlap and interact with each other, and finally each charge may interact with the conformational change accompanying the other charge. The important distinction is between chemical energy on one hand and electromechanical on the other.

#### III. Construction of charged pair current networks

Section *II* implicitly contains all the principles necessary for the construction of charged pair current networks. These shall now be made explicit and diagrammatic rules will be formulated for applications. At the outset we shall restrict ourselves to electrons, protons, and monovalent ions, i.e., to singly charged entities. Generalization to divalent ions will be treated later.

The principles are as follows:

1. Charges are separated and recombined in oppositely charged pairs. These events are diagrammatically represented by Fig. 3A and imply chemical to electromechanical or reverse transduction. The *filled and empty circles* represent the initial and final chemically bonded states, respectively. The *lines with arrows represent* the direction of propagation of the separated charges. The

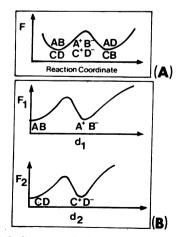


FIG. 2. (A) Ordinary catalytic reaction. (B) Enzyme catalyzed reactions terminating in the production of charged pairs.

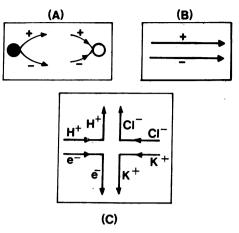


FIG. 3. (A) Charge separation and recombination. (B) Propagating pair state. (C) Partner exchange.

lines should be labeled also with the appropriate chemical symbols, if the corresponding charge carriers are known.

- 2. Opposite charges travel together in correlated states, i.e., at finite separations. The diagrammatic representation is given by Fig. 3B.
- 3. The elementary interaction between two charged pairs is an exchange of partners. This is represented by the partner exchange diagram of Fig. 3C.  $H^+$ ,  $e^-$ ,  $K^+$ , and  $Cl^-$  have been arbitrarily picked as representative examples. Note the construction of the diagram. Two oppositely charged pairs enter at opposite ends of the same arm of the cross. Members of the same pair are in juxtaposition. The charges on the second pair are inverted compared to the first one. Thus, on the outgoing arm of the cross again opposite charges are paired. The transduction is electromechanical to electromechanical.
- 4. Partner exchange diagrams can be combined into networks of unlimited size and shape. Arms can be left open or closed depending on whether the reactions initiate or terminate with chemical bonds. The diagrams need not be complete but can be left unfinished if so desired. An example is electron-Pi antiport motion in mitochondria (Fig. 4).

The geometrical properties of Fig. 4 are deceptively simple, because it is the two-dimensional representation of complicated three-dimensional pathways, but it is topologically correct. Note the presence of the closed loop indicating the cycling transport of a cation in the linkage system. Such cycling transport resolves one antiport motion into two sym-

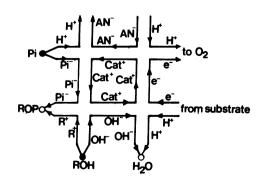


FIG. 4. Electron-Pi antiport motion in oxidative phosphorylation.

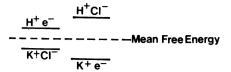


FIG. 5. Free energy conservation in partner exchange.

port motions. This is a necessary consequence of our postulated principles.

## **IV.** Network properties

The networks outlined above conduct oppositely charged pairs. They exhibit overall charge neutrality, but their charged internal structure is essential to their reactions and transport. Thus, bioenergetic systems, and therefore, by implication all biological systems, possess the capacity to conduct electricity in a novel manner. In conventional electrical conduction, opposite charges move in opposite directions (if charges of both signs are mobile). Their direction of propagation is fixed by an electrical potential gradient. Biological systems retain this capacity. The new element is that a free energy gradient is capable of moving oppositely charged pairs in the same direction.

The special design requirement of bioenergetic circuits is that degradation of free energy to heat should be minimal. This poses some restrictions on charged pair pathways. First the propagation of a charged pair should not diminish its free energy. This means that all pairs of sites that a given pair can occupy should have the same free energy except for activated configurations in which the free energy has fluctuated to a temporarily higher level subject to the second law of thermodynamics (2). We should keep in mind that we are talking not just about the pair of charges but also about the properties of the protein as medium which are part and parcel of the entire phenomenon. The second requirement is that partner exchange should occur without loss of free energy. This means that the pathways allowed for the pairs before and after exchange should be carefully matched in free energy (Fig. 5). This is just like requiring a chemical reaction which does not dissipate free energy and should be possible by careful choice. Nature has apparently solved this problem by using proteins. It is immediately obvious that such careful control is a lot easier if the motion of each pair is along one-dimensional pathways. Allowing charges to move individually and in more than one dimension would make control more difficult.

#### V. The coupling mechanism

One of the long-standing problems of bioenergetics is the coupling mechanism between the electron transfer chain and ATP formation. The chemiosmotic hypothesis has recently been disproved, the high energy intermediate has eluded detection, and conformational energy transfer had the qualities of the smile of the Cheshire cat (1). With the present picture, a definite suggestion as to the nature of the linkage system can be made. Part of this system, namely that coupling the electron transfer chain moves across the membrane in symport with a monovalent positive ion and this ion returns in symport with the phosphate. In this cyclic turnover, the cation participates in four charged pairs. The free energy of the (Cat<sup>+</sup>, e<sup>-</sup>) pair is parcelled out among the (H<sup>+</sup>, e<sup>-</sup>) and (Cat<sup>+</sup>, An<sup>-</sup>) pairs, and some may be used to separate (H<sup>+</sup>,

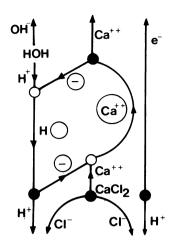


FIG. 6. The motion of Ca<sup>++</sup> and ionophore across membrane.

An<sup>-</sup>). If the (H<sup>+</sup>, An<sup>-</sup>) pair recombines at the next junction, this free energy is returned to the coupling system. Some free energy is used for creating (H<sup>+</sup>, Pi<sup>-</sup>) and (R<sup>+</sup>, OH<sup>-</sup>), which is then channelled into ROP and also to (Cat<sup>+</sup>, OH<sup>-</sup>). (H<sup>+</sup>, OH<sup>-</sup>) recombines and this free energy is injected into the next cycle. The net result is that the free energy originating from the entering (H<sup>+</sup>, e<sup>-</sup>) pair is partially passed to the exiting (H<sup>+</sup>, e<sup>-</sup>) and partially deposited at ROP. A similar diagram for ADP would produce the ingredients needed for ATP production.

Elements of both the high energy intermediate and the conformational hypothesis are present in this mechanism. The pathway available for  $(Cat^+, An^-)$  going from the electron to phosphate must be of high energy. Thus the  $(Cat^+, An^-)$  pair is the high energy intermediate. It has both high and low energy forms. In the low energy form  $An^-$  is  $OH^-$ . The high energy is not located in a conventional bond but in the complex interaction of two charges with the proteins and this interaction has conformational characteristics. The only theory that does not contribute to this picture is the chemiosmotic hypothesis.

Divalent Ions. Divalent ions such as  $Ca^{++}$  and  $Mg^{++}$  fit into the diagrammatic scheme with minor modifications. The motion of  $Ca^{++}$  with an ionophore across the membrane is shown in Figure 6. Divalent ions combined with an ionophore are equivalent to a monovalent ion in a partner exchange diagram.

#### **VI.** Implications

The charged pair network hypothesis makes it possible to systematically examine bioenergetic processes from a unified point of view. It is possible to represent bioenergetic processes using network diagrams. Missing elements of the reactions may be found in this manner. The site of action of uncouplers and inhibitors may be identified. Parts of complicated networks can be unplugged and other parts can be plugged in instead. This provides for great flexibility of coupling options.

It is also necessary to examine redox potential measurements from this point of view. Present theories of variable midpoint potential (6, 7) have to be reexamined. Contact of the electron transfer chain with the electrodes, via redox mediators, implies that charge carrier pairs are perturbed by the removal of one charge or by the addition of one. Since this

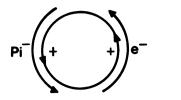


FIG. 7. Antiport motion of two negative charges coupled by a cogwheel consisting of a protein molecule and two positive charges.

radically alters the separated pair structure, the meaning of redox potential measurements is obscure at the moment.

Finally, the relationship of coupling options, structural features, and charge flow may be elucidated. It may turn out that the electrostatic features of the present model will not suffice by themselves. It is conceivable that the antiport motion of the electron to Pi<sup>-</sup> should be accomplished by a mechanical device, such as a protein molecule to which two

cations are rigidly attached. The protein would act as a cogwheel coupling the two negative charges in synchronized antiport flow (Fig. 7).

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