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Relation between enzymic catalysis and energy coupling

(catalytic unit/coupling unit/polarization and coupling/electron transfer chain/cation transfer chain)

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ABSTRACT The principles that underlie enzyme catalysis also apply to energy coupling processes. A comparison is made between a kinase system that mediates the phosphorylation of glucose by ATP (hexokinase), as the prototype for enzymic catalysis, and the mitochondrial electron-transfer complexes, as the prototypes for energy coupling systems. Induced polarization of chemical bonds and charge separation and elimination are common component events of both enzyme catalysis and energy coupling. Thus, definite limits can be imposed on models of energy coupling; they must comply with the basic principles of enzymic catalysis.

The study of energy coupling in biological systems goes back more than 40 years—to the discovery of oxidative phosphorylation (1, 2)—but the nature of energy coupling has proved to be one of the more elusive problems of bioenergetics. The failure to recognize the basic mechanisms of energy coupling stems largely from an insistence on applying principles that are supposedly peculiar to such a process. In this paper, we have emphasized the similarities between enzymic catalysis and energy coupling and discussed the common principles that must apply to both. Indeed, we have shown that enzymic catalysis and energy coupling are two sides of the same coin and that any model of energy coupling must conform to these principles.

We will first describe the basic mechanisms of enzymic catalysis, with particular reference to the hexokinase system, and then extend this rationale to energy coupling systems.

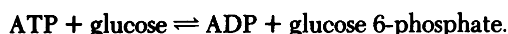
Basic principles of enzymic catalysis

In enzymic catalysis, the substrate molecule(s) undergoes chemical rearrangement. It is not inexact to describe an enzyme as a molecular device for inducing charge separation in one or more pairs of atoms in an otherwise inert molecule. In such a model, catalysis begins with charge separation and ends with charge elimination. Charge separation in the substrate is directly coupled to charge separation in the enzyme; thus, coupling is, in fact, the induction of paired charge separations in two interacting molecules. Coupling involves a coulombic interaction between two separated charged pairs and leads to covalent bond formation between oppositely charged species.

There is growing evidence that enzymes generally generate a charge-pair combination during catalysis (3–5). Thus, we may conclude that coupling in enzymic catalysis is coulombic in nature and, equally importantly, that coupling is direct. The enzyme does not generate a potential or store conformational energy as the result of catalysis.

Coupling in hexokinase

Hexokinase, an enzyme that is widely distributed in biological systems, consists of only one protein (6) and catalyzes the following reaction:



$$\text{ATP} + \text{glucose} \rightleftharpoons \text{ADP} + \text{glucose 6-phosphate}.$$

The pyrophosphorolysis of ATP does not proceed in the absence of glucose and thus may be considered to be coupled to the phosphorylation of glucose. The pyrophosphorolysis of ATP to ADP and P_i is an exergonic reaction ($\Delta G = -7.3$ kcal; 1 cal = 4.184 J), and the phosphorylation of glucose to glucose 6-phosphate is an endergonic reaction ($\Delta G = +3$ kcal) (7). Thus, the phosphorolysis of ATP mediated by the kinase (the driving reaction) drives the phosphorylation of glucose (the driven reaction).

Although hexokinase is a simple protein, there is ample precedent to postulate two catalytic centers in the enzyme—one for pyrophosphorolysis of ATP (catalytic unit 1) and one for phosphorylation of glucose (catalytic unit 2). The atomic distance between the two centers is small enough to allow transfer of a molecular species from one center to the other. At catalytic unit 1, the terminal pyrophosphate bond of ATP is ruptured and ADP is released and, at catalytic unit 2, glucose is phosphorylated and a proton is released. Bond rupture at catalytic unit 1 is directly coupled to bond formation at catalytic unit 2 (Fig. 1).

We can think of the domain of catalytic unit 1 as the polarizing center of hexokinase and of the domain of catalytic unit 2 as the center in which polarization is induced. The polarization of ATP is stabilized only when paired to the polarization of glucose, whereby a tetrad of charged species is formed. Thus, in the overall process, there are two coupled events: the coupling of charge separation in catalytic unit 1 to charge separation in catalytic unit 2 and the coupling of charge elimination in catalytic unit 1 to charge elimination in catalytic unit 2. Coulombic forces are paramount in these coupled processes, inducing both polarization with bond rupture and bond formation. [The concept of an enzyme as a thermally activated inducer of bond polarization has been promoted by Fröhlich (8, 9) and discussed further by Green (10).] The conclusions to be drawn from this analysis are that coupling is an intrinsic part of enzymic catalysis and that coupling by its very nature is rooted in the pairing principle—pairing of catalytic units, pairing of polarization, pairing of charge separation and elimination, and pairing of oppositely charged species.

There are a number of aspects of enzymic catalysis that should be emphasized before we turn to energy coupling systems. Although it is reasonable to assume two directly coupled catalytic centers in hexokinase, one for pyrophosphorolysis of ATP and the other for phosphorylation of glucose, their separate resolution and demonstration of catalytic function is likely to be experimentally difficult or even impossible. Further, the actual charge separation and charge elimination and the coupling between each catalytic center take place in a hydrophobic cleft or channel of the molecule, shielded from the attenuating effects of the aqueous environment. The postulate of channels within enzymes is well-founded in enzymological experience

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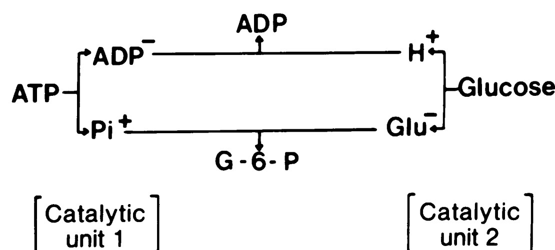

FIG. 1. ATP-coupled phosphorylation of glucose. The reaction sequence shown is a nonconcerted type; i.e., it involves the finite existence of charged species that are stabilized through enzyme–substrate interaction. [A similar concerted mechanism could be drawn that would avoid the necessity of depicting the finite existence of these highly reactive charged species. The question of which mechanism applies is debatable (for example, see ref. 14); however, we believe that it is the structural attributes of the enzyme molecule itself (provision of channels and hydrophobic domains) that make a nonconcerted mechanism feasible and likely.]

(11–13)—the equivalent to this in energy coupling systems would be the hydrophobic domain of the membrane. Also, enzymic coupling is short-range, allowing for transfer of charged groups within this hydrophobic pocket. Finally, there is no need to involve any force other than coulombic interaction to rationalize coupling in enzymic catalysis; the pairing of reactions and the direct coupling implicit in the mechanisms of enzymic catalysis effectively rule out devices such as electrical potentials or energy storage.

Energy coupling systems

The mitochondrial electron-transfer system consists of four complexes that mediate the following sequences: NADH $\rightarrow Q_{10}$ (complex I), succinate $\rightarrow Q_{10}$ (complex II), $QH_2 \rightarrow$ ferricytochrome *c* (complex III), and ferrocytochrome *c* $\rightarrow O_2$ (complex IV, cytochrome oxidase). Each electron-transfer complex has three sectors that we may define as the dehydrogenase, the coupling unit, and the hydrogenase. The dehydrogenase sector mediates the oxidation of a primary reductant (RH_2), the coupling unit sector mediates the transfer of charge, and the hydrogenase sector mediates the reduction of the terminal acceptor (A). In the dehydrogenation sector, an electron and a proton are extracted from RH_2 or RH; the electron is sucked into an acceptor chain (the electron transfer chain), and the proton is released into the aqueous phase. At the hydrogenation site, on the opposite face of the complex and membrane, an electron from the chain and a proton from the aqueous medium are combined to hydrogenate A to AH or AH to AH_2. Thus, two enzymes—the dehydrogenase and the hydrogenase—are involved in the electron-transfer sequence from RH_2 to A. The dehydrogenase may be equated with catalytic unit 1 of hexokinase (the center of charge separation) and the hydrogenase may be equated with catalytic unit 2 (the center of charge elimination).

Consider a classical enzyme that can transfer a hydrogen atom from $substrate_1$ to $substrate_2$. The enzyme can form a complex with $substrate_1$ and, by paired charge separation and elimination, transfer the H to $substrate_2$. However, if $substrate_1$ and $substrate_2$ are separated by a membrane, then it will be necessary for the elements of H to be transported by the enzyme over some considerable distance. Now this is fairly close to what actually happens in an electron-transfer complex, except that the element transported is not a hydrogen atom but an electron. The devices of charge separation (the dehydrogenase) and charge elimination (the hydrogenase) are identical in principle to those of enzyme catalysis. The special unit of energy coupling, and of the electron-transfer complexes, is the coupling unit.

In enzyme catalysis, charge transfer is a short-range phenomenon but, in energy coupling, it is relatively long-range—it is the coupling unit that has evolved to mediate this transfer. The coupling unit serves two basic functions. First, because of its oxidation-reduction groups, it mediates charge separation and electron transfer. Second, and more important, the coupling unit provides a mechanism for coupling. Given the separation of electron and proton, the movement of the electron through the electron-transfer chain of the coupling unit can be coupled to the movement of a positively charged ion. The coupling unit is thus a device by which electron-cation coupling can be consummated (Fig. 2). In this model, charge separation in the dehydrogenating unit will induce charge separation of some salt, such as KCl. Then, the electron will be sucked into the electron-transfer chain by an oxidation-reduction reaction and the K^+ will be sucked into the ion-transfer chain by coordination with ionophoric residues in the chain. The movement of the electron down the electron-transfer chain will drive the movement of K^+ down the ion-transfer chain. Finally, charge elimination in the electron-transfer process (protonation of A to AH) will drive charge elimination in the ion-transfer chain ($K^+ + OH^- \rightarrow KOH$). The net change per electron is the transport of one cation across the membrane.

We could say that the work performed in charge transfer in enzymic catalysis is internal, whereas the work performed in electron transfer is external—i.e., the transmembrane transport of a cation. Coupling in enzymic catalysis is structured to consummate bond formation, whereas coupling in the electron-transfer complexes is structured to consummate transport of a cation. Thus, in this formulation of energy coupling, the principle basic to enzymic catalysis (i.e., paired-charge coupling) is strictly adhered to through the mediation of the coupling unit.

The coupling units of the electron-transfer complexes are structured to span the inner mitochondrial membrane and contain those groups responsible for the transmembrane movement of electron and cation. Identification of the components of the electron-transfer chain of each complex is easy—they are the prosthetic groups such as nonheme iron, cytochromes *b* and *a*, and copper. I.entification of the cation-transfer chain components is a more difficult task that up until now had not even been considered. Our recent studies of this problem have strongly suggested that, in the cation-transfer chain of cytochrome oxidase, tightly bound cardiolipin is the essential component (15). Initial findings with complexes I and III suggest a similar pattern of cardiolipin involvement (unpublished data).

Generally, the electron-transfer prosthetic groups, together with their apoproteins, are only a small (usually <50) percentage of the total protein of each electron-transfer complex. The remainder of the protein is concerned with providing the

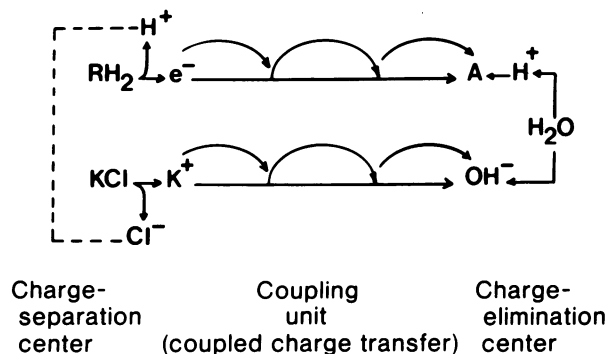

FIG. 2. Coupled electron transfer.

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elements of the ion-transfer chain and the dehydrogenase and hydrogenase components. As in the case of hexokinase, the resolution of these components is an extremely difficult task; however, some are readily recognizable. Taking cytochrome oxidase as a first example, we might equate the dehydrogenase to that subunit(s) responsible for binding of ferrocytochrome *c* and transference of an electron to heme *a* and copper, which are components of the coupling unit electron-transfer chain. The hydrogenase would consist of that subunit(s) responsible for binding of heme a_3 and copper and reduction of dioxygen, and the cation-transfer-chain components would be that subunit(s) of the coupling unit responsible for binding of cardiolipin, probably subunit I (16). In complex I, the dehydrogenase (catalytic unit 1) is a complicated array of proteins consisting of an NADH-dehydrogenase, flavin and nonheme iron, that is responsible for initial charge separation of an electron and proton and delivery of the electron to the coupling unit. In complex III, the hydrogenase (catalytic unit 2) may be composed of cytochrome *c* and nonheme iron that can accept electrons from the coupling unit (two membrane-oriented copies of cytochrome *b*).

Although the identification of the key components of each electron-transfer complex may be an experimentally arduous task, the structural arrangement of each complex is, in essence, quite simple and readily visualized. Each complex is a membrane-spanning structure consisting of a charge-separating device at one membrane face (the dehydrogenase or catalytic unit 1) and a charge-eliminating device at the opposite membrane face (the hydrogenase or catalytic unit 2) and the two are connected via the transmembranous coupling unit. Thus, an electron-transfer complex is no more than an elaborate extension of a classical enzyme, and this is true in both a structural and a functional sense—a structural extension because of the coupling unit and a functional extension because of the coupled paired charge transfer mediated by the coupling unit.

In summary then, we can consider coupling—whether in a classical enzyme, such as hexokinase, or in an electron-transfer complex—as a variation on the theme of induced charge polarization involving two catalytic centers. In energy coupling systems, a coupling unit is introduced as an extension of each of these two centers; with this extension, the door to paired charge movement is opened. But in terms of principle, coupling takes exactly the same form in hexokinase as in the electron-transfer complexes. The unit of coupling in both cases is an enzyme that has two coupled centers. In the final analysis, energy coupling has taught us that coupling is the primary process in enzymic catalysis and, conversely, enzymic catalysis is the primary process in energy coupling.

There are many additional facets of energy coupling that have not been considered. For example, intrinsic vs. extrinsic coupling (where movement of the electron is coupled to an extrinsic "cation chain"—e.g., valinomycin—rather than to its own intrinsic cation chain), driving chains in which the electron is replaced by another negatively charged species, and driven chains in which the cation is replaced by another positively charged species, such as in coupled ATP synthesis. We suggest that these alternative forms of energy coupling are variations of the same basic coupling theme discussed above and that, as such, no modification of the coupling principles would be expected.

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