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Universal energy principle of biological systems and the unity of bioenergetics

(pairing principle/electronic energy release/enzymes as energy machines/thermodynamics of bond rupture)

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ABSTRACT **Electronic energy (chemical bond energy) is the exclusive source of utilizable energy in biological systems. The release of this energy is mediated enzymically. The energy required to rupture a single covalent or ionic bond is prohibitively high under physiological conditions [in the range of 80–200 kcal/mol (1 kcal = 4.18 kJ)]. By the technique of the pairing of bond rupture (two juxtaposed bonds ruptured simultaneously) and the pairing of bond formation, enzymes can bypass the huge thermodynamic barrier to chemical change inherent in rupture of a single bond and operate within thermal limits. Enzymes accordingly can be conceived of as the energy machines that translate this principle. The principle of this transduction is that the energy required for forming a new covalent bond can fall within thermal limits when the original charged atom partner to the bond is displaced by a substitute charged atom under conditions in which the charge field of the bond remains constant during the substitution. In the transition from classical enzymology to energy coupling, muscular contraction, template-dependent replication, etc., new dimensions and possibilities are added to the basic enzymatic machinery. Specialized molecular devices (membranes, filaments, channels, templates, etc.) have to be introduced to make possible these extensions and permutations of enzymology. But it is demonstrable that the basic pairing principle is fully preserved during any of these modifications or extensions. Long range movement—of an ion, a filament, or a template—is the most important property introduced into classical enzymology in the transition to energy coupling systems.**

The question we are posing in the present communication is whether there is a unique principle applicable to all of bioenergetics that underlies the release of electronic energy just as there is a unique principle applicable to the entire domain of heredity that underlies the replication of DNA, RNA, and proteins (1). Is there, in short, only one way of "bleeding" electronic energy from metabolite molecules to do biological work? Parenthetically, it should be emphasized that electronic energy is the only energy source available in living systems; and even in photosynthesis, in which light energy mediates the formation of chemical bonds, it is the electronic energy thus trapped that is released to do work. The study of energy coupling has traditionally focused on this very problem of the mechanism of release and utilization of electronic energy, but the dividends from such studies were marginal as long as the mechanisms proposed were in terms of paraphysical principles such as the high-energy intermediate (2) or its latter day replacement, the protonmotive force (3). It was only when the direct coupling pattern in the mitochondrial energy coupling system was first recognized (4) that attention could be directed to the device by which electronic energy is liberated—namely, the enzyme. If direct coupling is the hallmark of both energy coupling and enzymic catalysis, then coupling has to be enzyme-mediated—a conclusion that has been documented and developed elsewhere (5).

Implicit in the technique of direct coupling, such as the coupling of electron transfer to ion transport by electron transfer complexes or the coupling of ATP pyrophosphorolysis to phosphorylation of glucose by hexokinase, is the notion of pairing. The separation of electron and proton in electron transfer complexes is paired to the separation of cation and anion, to mention only the first of three pairing maneuvers. The rupture of the P—O—P bond of ATP is paired to the rupture of the C—O—H bond in glucose in the hexokinase reaction, and the formation of the P—O—H bond in ADP is paired to the formation of the C—O—P bond in glucose. Direct coupling thus means that bond rupture and bond formation are paired processes.

Why should the pairing principle be an invariant attribute of enzymic catalysis? The rupture of a single covalent or electrostatic bond into the component charged atoms (separated to infinite dilution) requires 80–200 kcal/mol (1 kcal = 4.18 kJ) depending on the nature of the bond as shown by the data of Table 1 drawn from standard sources (6–8). There clearly is no way by which an enzyme can bring about catalysis by rupturing one bond at a time. The energy required for one-at-a-time bond rupture would be prohibitively high physiologically. It is important to recognize that forming a new bond requires not only rupturing the original bond but providing the partners for the new bond. One without the other would be useless. The perturbed system would merely return to the original bonded state without any change being effected in absence of the availability of partners for reaction. The pairing technique by which bonds are ruptured and formed in pairs gets around both the formidable thermodynamic barrier (9) and the dilemma of virtual change. The essence of the pairing technique is that *net* charge separation is avoided because the rupture of an original bond is synchronous with the formation of a new bond. The separation of a positively charged atom from its negatively charged partner is synchronized with the proximation of another positively charged atom and vice versa.*

Because the entire maneuver can be consummated in the absence of any energy source other than thermal energy, it would appear that the pairing technique requires that the enzyme act as an oscillator. Oscillatory fluctuations of the two

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* The thermodynamic barrier exists not only to the rupture of heteropolar bonds, as in the overwhelming majority of enzymic reactions, but also to the rupture of homopolar bonds as in cobalamine-dependent enzymic reactions. Although the species separated in the latter case are not charged, the energy barrier to the separation of these species is still considerable and well above thermal limits. The necessity for the pairing maneuver in enzymic catalysis applies, therefore, with equal force to the rupture of homopolar bonds even though the cohesive forces that hold the bond atoms together may be smaller in homopolar catalysis than in heteropolar catalysis.

Table 1. Bond energies of covalent and ionic bonds

Bond	Atomization energy,* kcal/mol		Bond energy,† kcal/mol
	Calc.	Exp.	
Covalent:			
C—H	98.5	99.4	
N—H	93.4	93.4	
O—H	111.8	110.8	
S—H	87.9	87.8	
C—C	85.2	83.2	
C=C	143.5	144	144
C—O′	81	—	
C=O″	192.3	192.1	256.7
Ionic:			
NaCl	105.8	99	97.5
KCl	101.9	101.6	
LiCl	122.6	114.3	111.9
CsCl	97.9	101	

* Atomization energy for gaseous state at 25°C; data from Sanderson (7), table 10-6 for the covalent bonds and table 7-1 for the ionic bonds.
† Atomization energy for gaseous state at 25°C; data from *Handbook of Chemistry and Physics* (8), pp. F158–F166.

bonds (exactly positioned) would, in principle, bring about the conditions requisite for the execution of the pairing maneuver. Only the beginnings have been made in developing the theory of the enzymically mediated pairing maneuver and the role of the enzyme as an oscillator in effecting this maneuver. It is outside the scope of the present communication to do more than indicate the outlines of this development. The enzyme molecule can be visualized as a unit that in its active form can undergo thermally activated oscillations. The enzyme is, in fact, structured to undergo a precision type of oscillation. Modification of the structure can thus lead to loss of oscillatory capability and parallel loss of enzymic activity. The oscillation provides the physical means by which new bonds can replace old bonds by atom displacement under conditions in which the charged fields of the partner atoms remain constant during the substitution. Peticolas and his group (10) have reported evidence for oscillatory properties of chymotrypsin and pepsin when observed by laser beam spectroscopy in the Raman low-frequency range (29 cm^{-1}), and they have shown that inactivation of the enzyme can be correlated with loss of this capability. This may be only one dimension of the problem. The oscillatory properties of enzyme molecules that are relevant to enzymic catalysis may be related to, but not identical to, the oscillations in enzyme proteins of the kind reported in chymotrypsin and pepsin.

The phenomenon of membrane fusion appears to involve the same component elements that underlie enzymic catalysis—namely, oscillatory capability of the reacting membranes and the inverse relationship between the field of the displaced charged group and the field of the displacing charged group. In membrane fusion, multiple electrostatic bonds are ruptured and a like number of new bonds are formed. The original set of bonds are at right angles to the new set of bonds. The bilayer type structure of membranes is, of course, ideally suited for oscillations, both in the plane of the membrane and at right angles to the plane of the membrane. We could think of proteins as structured like a bilayer membrane in that the exterior is polar on both sides and the interior is hydrophobic. This type of structure is ideally suited for rhythmic oscillations by virtue of the foldability of the hydrophobic sector.

Although the enzymologists have rarely come to grips with the issue of direct versus indirect coupling (paired versus unpaired charge separation), a large body of experimental evidence is available on this issue. In all ATP-energized kinase reactions there is no recorded case of ATP being pyrophosphorylyzed in the absence of the phosphate acceptor (11). Unpaired charge separation would require such a result. There is also no evidence that, in transfer reactions in which some group is transferred from a donor to an acceptor molecule, the donor molecule will dissociate the transferable group in absence of the acceptor molecule (12). In the reaction mechanisms that have been proposed as general models for enzyme reactions, such as concerted reaction mechanisms (13) or the ping-pong mechanism of Cleland (14), pairing is an essential ingredient. Thus we may say that, although the pairing technique and direct coupling have not been explicitly singled out by enzymologists as a *sine qua non* for catalyzed release of electronic energy, nonetheless, mechanisms that violate this pairing technique have never been proposed.

Given the premise that the enzyme is invariably the molecular instrument for the release of electronic energy, then two important conclusions can be drawn: first, that the enzyme is in the true sense of the phrase an energy machine; and second, that the universal energy principle of biological systems executed uniquely by enzymes is the pairing principle. A machine is a structured device for converting energy from one form to another. The enzyme machine converts electronic energy to the coulombic energy of charged species (15). In classical enzymic catalysis this transduction is the prelude to the formation of new bonds with a net decrease in free energy. But in energy coupling systems this transduction is the prelude to doing biological work driven by the free energy decrease in the transition from the original to the final set of bonds. The question is How can classical enzymic catalysis be modified so that biological work can become a by-product of this catalysis? Let us consider one such modification that bridges the gap between enzyme catalysis and energy coupling.

To visualize more easily the nature of the modifications introduced into the basic enzymic pairing maneuver, let us represent this maneuver in the form of the tetrad diagram shown in Fig. 1*A* in which the original bonds AB and CD are lined up vertically and the new bonds AC and BD are lined up horizontally. In Fig. 1*B* the same maneuver leading to the coupling of electron transfer to transport of cations is represented. The tetrad relationship of paired bonds disappearing and paired bonds appearing is fully preserved. But clearly there have been important innovations in the details of the basic technique though not in the principle. First, one of the starting bonds and the final bonds are ionic in nature—AC being long range and AB, CD, and BD being short range (atomic). The separation of electron and proton is mediated by an electron transfer chain to capture the electron and by an aqueous phase at the membrane interface into which the proton is extruded. The separation of cation and anion is mediated by an ion transport channel to capture the cation and by the same aqueous phase at the membrane interface into which the anion is extruded. By virtue of compartmentation (separate devices for capturing electron and cation, respectively, and separate but juxtaposed domains for the initial paired charge separation), the coupled sequences are kept physically apart and can interact with one another only via long range coulombic attractive forces.

As the result of this set of modifications, translational movement has been introduced into enzymology (active transmembrane transport of a cation) as well as coupling flexibility (depending upon the nature of the positively charged ion transported, a whole set of coupling options are open). In addition, these modifications result in the stabilization of the charged species—the species that are required to do biological work. We

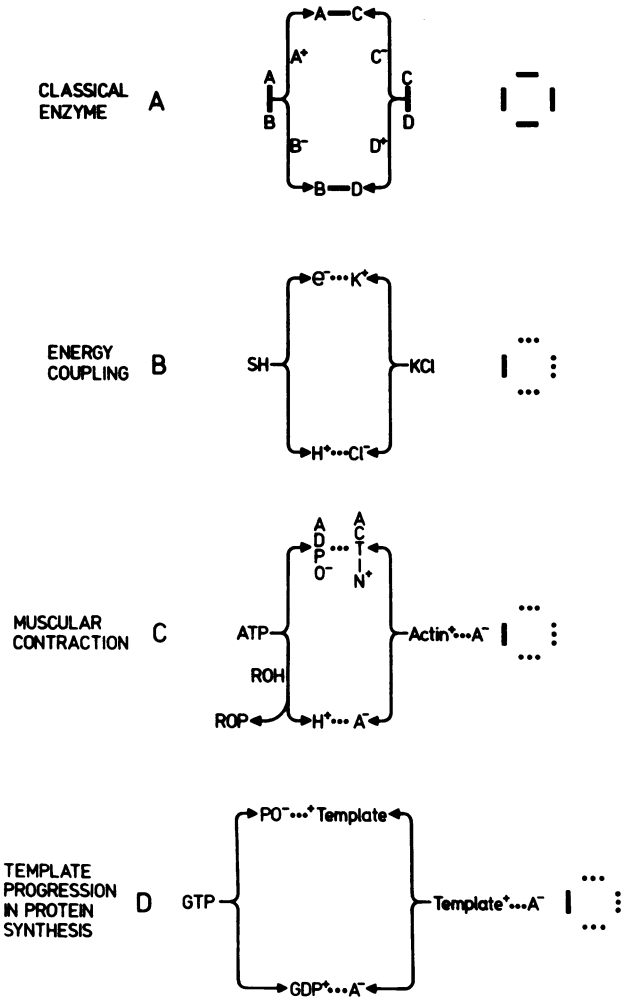

FIG. 1. Paired charge separation and recombination: variations of the enzymic pairing maneuver. In *B*, SH represents an oxidizable substrate of an electron transfer complex (NADH, QH_2, ferrocytochrome *c*). In *C*, ROH corresponds to the residue in myosin that is phosphorylated during ATP pyrophosphorolysis. In turn, enzyme hydrolysis of ROP initiates a second movement of actin. In *D*, the template corresponds to RNA. A^- represents an anion charge paired to actin $^+$ or template $^+$.

have represented only the initial events in the coupled reactions shown in Fig. 1 *B*, *C*, and *D*; the terminal events for one form of energy coupling are shown in Fig. 2. These coupled reactions represent a set of variations in the basic enzymological pairing maneuver by which the transition can be made to energy coupling, muscular contraction, template-mediated synthesis of DNA, RNA and protein, and, finally, photochemical energy coupling. Each such transition requires a special set of devices (membranes, chains, channels, filaments, templates, photochemically activated molecules, etc.). But whatever the nature of the new devices, the basic tetrad pattern of enzyme-induced chemical change that translates the pairing principle is fully preserved.

Out of these considerations emerges a perspective of enzymology that subtends all the bioenergetic fields in which the release of electronic energy is paramount (Table 2). In each such subdivision the basic enzymic maneuver is modified and modulated in a unique fashion which usually requires the introduction of some new structural device. It is now more readily understandable why it may be impossible to isolate classical enzymes from energy coupling systems. When the unit of enzyme action is inextricably linked to special devices such as chains, channels, filaments, etc., then the dissection of the enzyme (assuming this to be possible) from these essential components can be a meaningless performance.

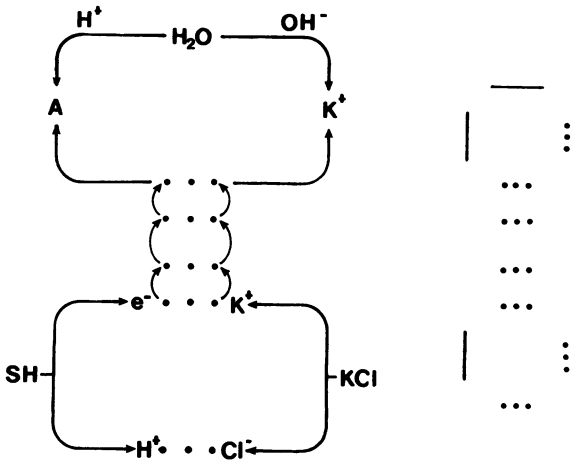

FIG. 2. Bond pattern for coupling of electron transfer to transport of cations. SH, primary hydrogen donor; A, terminal hydrogen acceptor; —, covalent bond; ···, electrostatic or ionic bond.

A crucial distinction between classical enzyme catalysis and energy coupling lies in the nature of the bonds ruptured and formed. In the former case the bonds are exclusively covalent; in the latter case, at least one of the starting bonds is electrostatic and both of the new bonds formed are electrostatic. The possibility of the movement of charged species is introduced by electrostatic bonding as shown in Fig. 2. The two partners of the bond can move together in lock step through their respective chain or channel, transferring from one acceptor to the next. There are two tetrads—one at the beginning of the energy coupling sequence and the other at the termination of the sequence.

The relationship between photochemical enzyme systems such as bacterial rhodopsin and classical enzymes needs some comment. A more meaningful comparison can be made between cytochrome oxidase and bacterial rhodopsin—both of which are energy coupling systems. Both systems can mediate the coupling of electron transfer to ion transport (Fig. 3); both systems contain an electron transfer chain and an ion transport chain (16). Hence the pairing technique is common to both cytochrome oxidase and the bacterial rhodopsin system. What is different is the way in which charge separation in the electron transfer chain is induced. In cytochrome oxidase this is achieved by thermally induced oscillatory fluctuations. In the bacterial rhodopsin system this is achieved by light acting upon a susceptible dehydrogenatable residue in the carotenoid molecule. But in both cases this charge separation in the electron transfer chain, or its equivalent in bacterial rhodopsin, is paired to charge separation in the ion transport channel. For that reason

Table 2. Domains of bioenergetics in which the pairing principle of enzymic catalysis applies

1. Classical enzymes
2. Multienzyme complexes: pyruvate dehydrogenase complex, hydroxylating systems, xanthine oxidase, phospho*enol*pyruvate-energized glucose-transporting system, fatty acid synthetase
3. Energy coupling: membrane systems
4. Energy coupling: filament systems, microtube systems
5. Energy coupling: template systems
6. Photochemical energy coupling: membrane systems

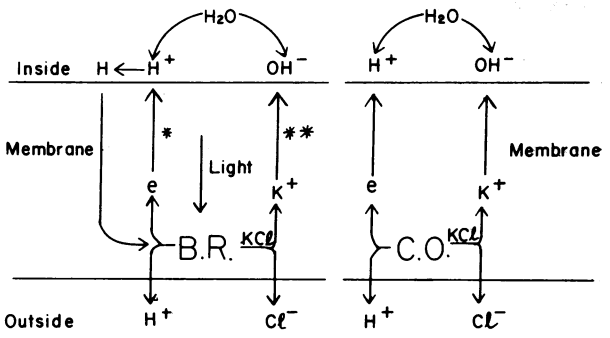

FIG. 3. Energy coupling patterns of bacterial rhodopsin (B.R.) and cytochrome oxidase (C.O.). *, Isoprenoid chain of B. R. serves as the quasi-electron-transfer chain. B. R. is regenerated to its original state after each light absorption cycle by hydrogen transfer. **, Clusters of rhodopsin molecules form a transmembrane cation-transport channel.

we may consider rhodopsin as a light-activated enzyme system which, like classical enzymes, releases electronic energy by the pairing technique.

In the long history of efforts to rationalize energy coupling, the notion of energy storage has been persistent and taken several forms—the high-energy intermediate, the membrane potential or protonmotive force, the conformational model. Energy storage *ipso facto* means indirect coupling and the absence of the pairing principle. It also means that energy coupling cannot be enzyme-mediated because enzyme catalysis is consummated only via the pairing technique. Finally, energy storage requires an initial investment of 80–200 kcal/mol. Clearly, the notion of energy storage derivative from indirect coupling is physically unsound in enzyme-mediated reaction systems (9).

To return to a point made in brief with respect to the enzyme as transducer, the thermodynamics of the conversion of electronic energy to the coulombic energy of separated, charged species as in energy coupling needs some comment. Because, as shown in Fig. 1, the separated charged species ultimately form covalent or ionic bonds, it must be assumed that the energies of the separated charge species and the energies of the bonds which these form must be comparable in magnitude. Is that in fact so? The calculations of Parsegian (15) suggest that the energy of a singly charged species in a hydrophobic medium can be as high as 50 kcal/mol, and hence the energy of a pair of such species can be comparable to that of the energy of a covalent bond. Given the reversibility of enzyme-mediated reactions, it is a reasonable inference that the conversion of electronic bond energy (E_B) to coulombic bond energy (E_C) by the enzyme-mediated pairing technique is a reversible process:

$$E_B\,(A - B) \underset{\leftarrow}{\overset{\text{enzyme}}{\rightleftharpoons}} E_C\,[A^- + B^+].$$

That must mean that this transduction can take place without significant energy loss. It is difficult at first glance to see how energy loss could be avoided in this transduction when charged species (e.g., the electron and cation) are separated by at least 15 Å in their respective chain or channel. Such separation should result in a marked diminution in total energy and that result would argue against the reversibility of transduction. But it is not excluded that, by virtue of the intense electrostatic field and the conformational flexibility of energy coupling complexes, it would be possible for an electron transfer complex to undergo a twisting constriction that would allow the distance separating electron and cation to be a magnitude at which energy loss would be minimal. Parsegian (15) invokes a local major constriction of a liposomal bilayer in response to the presence of a charged species inserted in the bilayer, and some comparable constriction could be invoked for electron transfer complexes to rationalize the reversibility of the enzyme-mediated transduction.

In nerve transmission membrane, potentials are undoubtedly generated when the units of nerve action are stimulated by appropriate signals (electric current; acetylcholine and other neurotransmitters). These membrane potentials are consequences of the transduction of the osmotic energy of salt gradients (salts of Na^+ and K^+) into the coulombic energy of uncompensated charged species (Na^+, K^+, Cl^-, etc.), and this transduction is mediated by cation-specific ionophoric channels. The point to be made is that, although unpaired transduction of electronic energy to coulombic energy is a prohibited option for enzymes, no such prohibition applies to the channel-mediated transduction of osmotic energy to coulombic energy in neural signaling systems.

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