Piezoelectric Theory of Enzymic Catalysis as Inferred from the Electromechanochemical Principles of Bioenergetics

(energy transduction/molecular machines/protein pulsation/biological semiconductivity)

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ABSTRACT Starting from the electromechanochemical principles of bioenergetics formulated by Green and Ji, a theory is proposed which describes enzymic catalysis in terms of piezoelectricity in semiconductors. The choice of this particular physical effect for describing catalytic processes is founded on the following experimental observations: most of the amino-acid residues of enzymes, as well as a large number of other biologically important molecules, exhibit piezoelectric resonances; besides, enzymes can behave like semiconductors. In the proposed theory the catalysis is assumed to be accomplished by means of three fundamental processes: (a) the lowering of the substrate-product energy barrier; (b) the electron-induced selective amplification of the low-frequency vibrational waves present in thermal background in the enzyme structure; and (c) the channeling into the substrate of the energy associated with the amplified waves and utilization of this energy for generating electrical or mechanical fields inside a susceptible region of the substrate. A mathematical description of the theory is outlined, and a rough estimate of some quantities involved in the process of wave amplification is also reported.

The search for a unitary principle underlying enzymic catalysis is one of the most intriguing problems of contemporary biology (1, 2). Many models have been proposed for explaining enzyme catalytic power and enzyme specificity, but, as Reiner properly observed (3), the proposed theories are partial in that none of them embraces all the known facts in the framework of a unified treatment.

The electromechanochemical (EMC) theory of bioenergetic processes, recently formulated by Green and Ji (4-7), is a well-ordered set of principles which, when applied to the enzymic catalysis, provide a powerful tool for the overcoming of any partial view about the catalytic processes.

Limitations of space prevent us from discussing those principles extensively; therefore, we shall only mention three fundamental concepts upon which the EMC theory is based. First, enzymes are visualized as macromolecular devices that episodically and impermanently convert thermal energy of the environment into EMC potential energy of the enzymic system. Second, the EMC energy is directed to the polarization of a susceptible bond of the substrate and is used for this purpose within the lifetime of the energized state. Third, enzymes possess a high informational content by means of

which all the maneuvers enabling the substrate-product conversion are controlled; this informational content is expressed by the concept of negative entropy, which measures the non-statistical order of living systems.

The intrinsic physical nature of the EMC theory prompted us to explore the possibility of rationalizing enzymic catalysis in terms of some solid-state physics phenomena with the main purpose of suggesting some crucial experiments.

The basic, and by far the most important phenomenon that we refer to is piezoelectricity. The piezoelectric (PE) effect establishes a close correspondence between electrical and mechanical processes so that any variation of electrical quantities (fields and displacements) causes a reversible variation of mechanical quantities (stresses and strains) and vice versa (8). The interaction between electrical and mechanical quantities occurs in an asymmetric structural matrix in which the electrical polarization of the medium is the determinant factor of the interaction itself. This polarization may be caused by ions or charged species that result, for example, from a chemical reaction and that may be trapped at some particular sites of the material. Thus, in a PE body, electrical, mechanical, and chemical energies are interconvertible.

The basic parameter that measures the energy conversion efficiency is the PE coupling coefficient, K^2 (9). For the common PE materials, K^2 values range from 0.1 to 0.7.

Normally, the PE effect largely concerns insulators, but some interesting phenomena can occur if the body is a semiconductor.

Piezoelectricity and semiconduction have been widely recognized in an impressive number of organic and biological molecules (10). In particular, we emphasize the fact that almost all the amino-acid residues that compose enzyme molecules exhibit piezoelectricity (11) and that many proteins behave like semiconductors (12).

These findings entitle us to visualize enzymes as composite PE semiconductors (Fig. 1) that manipulate energy in such a way that the following functions are accomplished:

(a) The energy barrier, which has to be overcome in order to achieve the substrate-product conversion, is lowered by the action of some boundary agents that tie a susceptible region of the substrate to proper electrical and mechanical constraints.

(b) The low-frequency vibrational waves, which are present in thermal background throughout the enzyme structure, are selectively amplified by an electron current according to a process that is very usual in PE semiconductors.

Abbreviations: EMC, electromechanochemical; PE, piezoelectric.

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(c) The energy associated with such amplified waves is channelled into the labilized substrate, giving rise to mechanical or electrical fields by means of which the substrate-product conversion is accomplished.

All enzymes, with the exception of transferases, can be represented by the model we are proposing. The description of transferase catalysis requires supplementary concepts; therefore, it will be the object of a future paper, although some ideas have been already put forward in a previous report (13).

This paper will be primarily concerned with the statement of some basic concepts. Therefore, the mathematical formulation of the problem will be handled in a simplified form. Moreover, the results of the numerical calculations performed on the basis of the above theory must be considered as a rough estimate of the hypothesized processes. More precise calculations must be referred to each specific enzyme.

THE THEORY

Lowering of the substrate-product energy barrier

The energy necessary to convert a substrate into the product (hereafter referred to as the conversion energy) may manifest itself as mechanical or electrical work to be done on the substrate according to whether a deformational field or an electric field has to be induced in a susceptible region of the substrate for achieving the conversion.

The lowering of the conversion energy is one of the most important functions of enzymes. This process may be explained in terms of PE effect if the substrate, considered as a PE body, is assumed to be held in the catalytic cavity in such a way that a susceptible region of the substrate is under the influence of proper electrical or mechanical boundary states (for a detailed treatment of the effects of boundary states on the performance of a PE body, see ref. 8, pp. 260-283).

Let us first consider the case in which mechanical energy is required for substrate-product conversion; this means that the susceptible region must be strained. The energy per unit volume to be expended mechanically to produce a strain S in the susceptible region, when the substrate is far from enzyme and insulated from any source of electrical fields, is $cS^2/2$, where c is the elastic modulus of the material (the coefficient relating stress to strain according to Hooke's law). If in the catalytic cavity the susceptible region is connected to a charge-supplying agency that allows the compensation of any electrical state inside the region (short-circuit condition), the elastic modulus is reduced by a factor $(1 - K^2_r)$. Here K^2_r is the PE coupling coefficient of the susceptible region supposed different from the PE coupling coefficient K^2_s of the other regions of the substrate. As a consequence, the mechanical conversion energy also is reduced by the same factor.

We assume that the agents that accomplish the function of such electrical compensation are the electrophilic and nucleophilic groups present in the catalytic cavity.

Let us now consider the case in which the conversion energy is of electrical form. This energy can be reduced if the susceptible region of the substrate is prevented from deforming (clamped condition). In this case, in fact, the dielectric constant of the region would be $(1 - K^2_r)$ times the value relevant to a nonclamped susceptible region. Since the electrical energy is proportional to the dielectric constant, it follows that the energy to be expended electrically to produce an electric field inside the clamped susceptible region is $(1 - K^2_r)$ times the

energy necessary to generate the same field when the region is free to deform.

The well-integrated structure of the catalytic cavity favors the assumption that in many enzymes some portions of the substrate may be rigidly clamped to particular sites of the cavity.

From the above one can deduce that, if the proper boundary conditions are fulfilled, the energy to be channelled from the enzyme to the substrate for achieving the conversion is given by

$$W = (1 - K^2_r)W_s, \quad [1]$$

where W_s is the conversion energy without assistance from enzyme.

Note that W may become quite small as K^2_r approaches unity. The value of K^2_r depends strongly on the static electric polarization of the susceptible region. The protonation of the substrate may be a potent agent in producing, stabilizing, or modifying such an electrical polarization.

Selective amplification of low-frequency vibrational waves

Low-frequency acoustical modes, which involve all or large portions of the protein molecule, have been observed in the vibrational spectra of certain proteins (14).

Many investigators (15-17) realized the importance of acoustic or vibrational waves for energy transfer at the molecular or submolecular level, but the great potentialities of these motions for enzymic catalysis do not appear to have been fully explored. The thermal-EMC principle of Green's theory is the only exception to this state of affairs.

As a matter of fact, the energy associated with such vibrations is fairly low, at least 100 times as low as the energy necessary for enzymic reactions. In consequence, unless an amplification mechanism is invoked, the above-mentioned motions seem to be of little or no value for catalysis.

There are, however, good reasons to believe that an amplification mechanism of such motions does exist and that it results from the simultaneous presence of piezoelectricity and semiconductivity in the enzymic structure. We will show later that an amplification mechanism based on such physical phenomena can account for the high power of enzymes and for their specificity as well.

It is well known that in a PE semiconductor, with or without the presence of inhomogeneities, a small portion of the acoustic band of thermal lattice vibrations can be selectively amplified by mobile charge carriers (electrons or holes) drifting, under an impressed electric field, with a drift velocity greater than the sound velocity (18, 19). The amplification is made possible by transfer of energy and momentum from charge carriers to the acoustic waves. This type of interaction arises because in a PE material the acoustic waves generate variable electric fields that exchange energy with charge carriers drifting under the impressed electric field.

The situation in the amplifying region may be described as follows. Before the application of the electric field a uniform distribution of acoustic energy, W_0 , is spread throughout the structure. Upon the application of the right potential difference across the amplifying region, charge carriers (hereafter considered to be electrons) are forced to flow from the cathode toward the anode with a velocity greater than the sound velocity. A narrow band of acoustic waves, originating at any

point along the structure, grows in energy as it propagates in the direction of the current. In this way a distribution of mechanical energy, which increases from the cathode to the anode, replaces the initial isotropic distribution in thermal equilibrium.

For the occurrence of the amplification process the following conditions must be satisfied:

(i) The drift velocity, v_d , of the electrons must exceed the velocity, v_s , of the acoustic waves propagating in the direction of the current. Here, $v_d = \mu E$, where μ is the electron mobility and E is the electric field applied across the amplifying region.

(ii) For waves propagating at an angle θ from the electron flow axis the amplification occurs when

$$v_d \cos \theta > v_s. \quad [2]$$

(iii) Among the acoustic waves present in the thermal background, only those having frequencies within a restricted band can be amplified. This band is centered around an optimal frequency, f_m , which, in a first approximation, is given by §

$$f_m = (1/2\pi)(\sigma e v_s / \epsilon_a \mu k T)^{1/2}, \quad [3]$$

where σ is the electrical conductivity of the material, e is the electronic charge, ϵ_a is the absolute dielectric constant, k is the Boltzmann's constant, and T is the absolute temperature.

(iv) At the frequency, f_m , the amplification is maximum when the applied electric field is equal to

$$E_m = (v_s/\mu)[1 + 2(\sigma \mu k T / \epsilon_a e v_s^2)^{1/2}]. \quad [4]$$

The amplification is thus a highly selective process, and this selectivity depends strongly on the material parameters.

Through the amplifying region the energy grows nearly exponentially from the cathode to the anode, and, under favorable conditions, the amplified energy may be even ten orders of magnitude greater than the value at thermal equilibrium.

At the anode, the excess energy ΔW_a over the thermal equilibrium value W_0 is given by (neglecting the intrinsic absorption of the lattice)

$$\Delta W_a = W_0[v_d/(v_d - v_s)][(\exp \alpha L) - 1], \quad [5]$$

where L is the length of the amplifying region and α is the gain coefficient. The latter quantity depends on the material parameters already mentioned and is strongly influenced by: local inhomogeneities, which alter the resistivity and mobility profile (18); multiple reflections of the waves from the ends of the amplifying region, which give rise to the enhancement of the wave intensity in the region (20); feedback between the electron current and the waves with a possible super-exponential growth of the energy (21); and the presence of electromagnetic fields coming from the localized vibrations of dipoles (22). Representing all these special effects by means of a function ϕ , we put

$$\alpha = K_a^2 (f_m/v_s)^2 \phi, \quad [6]$$

where K_a^2 is the PE coupling coefficient of the amplifying region.

A great variety of electrical and mechanical effects is associated with the amplification of acoustic energy. In partic-

§ We use the classical treatment of acoustic amplification, neglecting the quantum-mechanical formulation or other more general theories only for the sake of simplicity.

FIG. 1. Schematic picture of piezoelectric catalysis.

ular, fractures, dislocations, and high field domains have been observed in the region near the end electrode as a consequence of single or multiple passages of the amplified acoustic energy (23).

Can this type of energy amplification be operative in enzymes? To answer this question, at least in a primitive form, we present the results of some simple calculations performed on the basis of the only data at present available.

To begin with, we consider the enzyme to be somewhat like a three-dimensional crystal.

Moreover, Cope (24) successfully explained the kinetics of many enzyme reactions by considering the possibility that a redox potential difference arises between two different reaction sites of the enzymic particle. This redox potential difference acts as a battery which causes an electron current to flow across the enzymic structure.

Starting from these arguments, we assume that, in consequence of the binding of the substrate to the enzyme, an electric field arises along the direction of the two principal reaction sites of the enzyme, namely, the active and the allosteric sites, with the substrate and cofactors acting as massive electrodes (Fig. 1). These two electrodes delimit a region (hereafter referred to as the active region) which is considered some hundreds of Ångströms long and is equivalent to the amplifying region of a PE semiconductor. An electron current is assumed to flow in the active region towards the substrate.

It is reasonable to assume, as a first approximation, that the potential difference between the two electrodes reaches some tens of millivolts, as is usual in biological processes. This means that an electric field ranging between 10^4 and 10^5 V·cm⁻¹ is applied across the active region (compare these values of electric fields with those reported in ref. 25). Since the electron mobility of proteins may be as high as 20–50 cm² (V·sec)⁻¹ (25), the electron drift velocity, $v_d = \mu E$, may reach values higher than the sound velocity, v_s , which in biological materials is of the order of 10^5 cm·sec⁻¹ (16).

It is highly likely, therefore, that the main condition for the acoustic wave amplification in enzymes is fulfilled.

Let us now estimate some quantities involved in the process. For this purpose we use the numerical data relevant to proteic materials reported in ref. 25. We got $\sigma = 3 \times 10^{-4}$ (Ω cm)⁻¹; $\epsilon = 2.5$; and $\mu = 20$ cm² (V sec)⁻¹. By substituting these values into Eq. 3, recalling that the vacuum dielectric constant is 8.85×10^{-14} F·cm⁻¹ and assuming $T = 300^\circ$ K, the frequency of the acoustic wave corresponding to the maximum gain is about 10^9 sec⁻¹, which is in the range of frequencies experimentally detected in proteins (14).

The optimal value of the electric field, calculated from Eq. 4, is 7.5×10^8 V·cm⁻¹. For active regions varying in

length between 100 and 1000 Å, this field corresponds to potential differences across those regions ranging from about 7 to 70 mV.

Moreover, the drift velocity results equal to 1.5×10^6 cm·sec⁻¹, and the amplification cone of the propagating acoustic energy (Eq. 2) is about 48° wide.

At present, data for calculating α and ΔW_a according to Eqs. 6 and 5 are lacking. Nevertheless, it can be said that, for an enzymic reaction to occur, the thermal background energy associated with acoustical modes needs to be amplified not more than 1000, at the worst. Amplifications far higher than the latter value can be achieved in a PE semiconductor, as already mentioned.

Utilization of the amplified energy

Let us now proceed to describe the way in which the amplified energy may be used for the substrate-product conversion.

We have assumed that the substrate is attached to the enzyme in a colinear position with the direction along which the amplification process occurs. Then the substrate is struck by the excess mechanical energy, ΔW_a , of the amplified acoustic wave. Since the substrate has been supposed to be piezoelectrically active, with a PE coupling coefficient K^2 , the portion $W_e = K^2 \Delta W_a$ of the above energy will appear in electrical form, while the remaining portion $W_m = (1 - K^2) \Delta W_a$ will be present as mechanical energy. We have already stated, in a previous section, that an amount of electrical or mechanical energy expressed by Eq. 1 is required to accomplish the substrate-product conversion. It follows that the conversion will occur when the amplification process in the enzyme is such that W_e or W_m is equal to W .

CONCLUDING REMARKS

The model we have sketched in this paper is based, first, on the lowering of the substrate-product energy barrier. This process is viewed as a result of some combined tactical maneuvers in which we include the protonation of the substrate, and the electrical or mechanical actions of the electrophilic and nucleophilic groups, as well as those of some other bonds between substrate and enzyme.

Moreover, the model emphasizes the role that a selected portion of the low-frequency oscillations of enzymes could play in catalysis. Since the thermal background energy associated with such oscillations is less than the conversion energy, the intensity of the above motions needs to be amplified. This is possible provided that enzymes are considered as PE semiconductors and the substrates are electrically able to energize the corresponding enzymes. There are noticeable experimental observations that favor these assumptions. Further experiments, performed according to the techniques commonly used in solid-state physics (18-21), could provide conclusive answers to the question about the validity of the proposed theory.

Note Added in Proof. Many investigators have dealt with the PE effect in biology. It is impossible to cite the very large number of papers so far published on this topic. However, special mention must be made of a review paper of Bassett (26), mainly focused on the PE effect in bone, in which the author hinted that the piezoelectricity could be involved also in enzyme activity. Moreover, the interaction between charge carriers and acoustic waves postulated in the present paper might mesh with some disease modelings proposed by De Ment (personal communication).

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