brief communication

Resonance transduction of low level periodic signals by an enzyme: an oscillatory activation barrier model

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ABSTRACT The overall rate of an enzyme catalyzed reaction is determined by the activation barrier of a rate-limiting step. If the barrier is oscillatory due to the intrinsic properties of a fluctuating enzyme, this enzymic reaction will be influenced by a low level periodic electric field through the resonance transduction between the applied field and the oscillatory activation barrier. The ATP hydrolysis activity of a highly purified, detergent solubilized Ecto-ATPase from chicken oviduct was used to test the above concept. At 37°C, this activity (1,800 μmol mg^{-1} min^{-1}) was stimulated up to 47% (to 2,650 μmol mg^{-1} min^{-1}) by an alternating electric field (AC), with a frequency window at 10 kHz. The maximal stimulation occurred at 5.0 V (peak-to-peak) cm^{-1}. The potential drop across the dimension of the enzyme was ~10 μV (micelle diameter 20 nm). The activation barrier, or the Arrhenius activation energy, of the ATP splitting was measured to be 30 kT and the maximal barrier oscillation was calculated to be ~2.5 kT according to the oscillatory activation barrier (OAB) model. With the optimal AC field, full impact of the electric stimulation could be effected in much less than a second. The OAB model is many orders of magnitude more sensitive for deciphering low level periodic signals than the electroconformational coupling (ECC) model, although the latter has the ability to actively transduce energy while the former does not. By the OAB mechanism, the detecting limit of an external electric field by the ATPase, in a cell 20 μm in diameter, would be 5 mV cm^{-1}, but could be much lower for other membrane enzymes or receptors (e.g., nV cm^{-1}). We propose that mechanisms similar to the OAB model could explain how a weak electromagnetic field or acoustic noises can exert its effects on an organism or a living cell.

INTRODUCTION

The biological effects of very low level electromagnetic fields (μV cm^{-1} to nV cm^{-1}) and sonic noises are of serious concern to the public and of interest to investigators working on biological energy and signal transduction and on environmental issues. Cells or organisms have, after millions of years of evolution, acquired the ability to sense very low level periodic signals, e.g., mechanical, acoustic, electric, or thermal signals, or the oscillation of nM to μM concentrations of certain chemical messengers or regulators (1–3). This ability of cells or organisms implies that there are mechanisms by which molecules of cells can respond to the small perturbations caused by these signals (4–6). The main difficulty for understanding the phenomenon is how a cell can recognize a signal that, on appearence, is many orders of magnitude weaker than the thermal electric noises in its immediate surroundings. Weaver and Astumian have considered Johnson/Nyquist thermal resistance noise in a membrane's electric circuit to be the limit of detection by a cell (6). The electroconformational coupling (ECC) model (7–11) was taken as the basis of the molecular response to a membrane electric potential. Their estimate places the limits of detection close to those observed experimentally although, by similar reasoning, Adair has dismissed the mechanism as a plausible one (12). Despite the fact that an externally applied electric field would be amplified by as much as $1.5\,R/d$ times in the plasma membrane of a cell, R and d being the radius of the cell and the thickness of the membrane, respectively (4), an ELF (extremely low frequency low level electromagnetic field) is still considered a few orders of magnitude too weak to influence the rate or induce a shift of chemical equilibria of most membrane reactions (12). The oscillatory activation barrier (OAB) model discussed here could resolve such difficulties.

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RESULTS AND DISCUSSION

Oscillatory activation barrier and resonance phenomenon. To explain some basic premises of the concept, we will use AC stimulation of the ATP hydrolysis reaction of the

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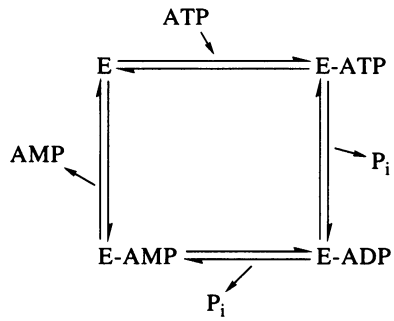
Ecto-ATPase (13,14) as an example.



(1)

This enzyme hydrolyses both ATP and ADP to produce AMP and P_i. The hydrolysis of ATP and ADP under ordinary assay conditions (e.g., in 4 mM ATP or ADP, with no added AMP and P_i) is spontaneous and strongly endergonic. In the OAB model, an electric field does not influence chemical equilibrium of a reaction. Instead, it will alter the rate by interacting with the activation barrier. For the development of the concept and simplification of the analysis, Scheme 1 will be represented by a rate-limiting step (with rate constants k_{12} and k_{21} and a faster step that includes all other steps (with overall rate and constants r_{12} and r_{21}).

$$\text{Substrate} + ① \underset{k_{21}}{\overset{k_{12}}{\rightleftharpoons}} ② \underset{r_{12}}{\overset{r_{21}}{\rightleftharpoons}} ① + \text{Product} \qquad (2)$$

1 and 2 denote two different states of the enzyme. Scheme 2 is a two-step process, where $k_{12}, k_{21} \ll r_{12}, r_{21}$. The ratios of these rate constants give the equilibrium constants, $K_{12} = k_{21}/k_{12}$ and $R_{12} = r_{21}/r_{12}$. When [S] $\gg$ K_m (the Michaelis-Menten constant), the overall rate of Scheme 2 can be described by

$$dP_1/dt = -(k_{12} + r_{12} + k_{21} + r_{21})P_1 + k_{21} + r_{21}, \qquad (3)$$

where P_1 is the probability of the enzyme in state 1. Note that $P_1 + P_2 = 1$. The overall rate of the substrate-to-product conversion is (15,16)

$$A_0 = \frac{k_{12}r_{21} - k_{21}r_{12}}{k_{12} + k_{21} + r_{12} + r_{21}}. \qquad (4)$$

In Eqs. 3 and 4, $k_{12}, k_{21} \ll r_{12}, r_{21}$.

The activation barrier for the rate limiting step on the substrate side is assumed to be oscillatory, with the height of the barrier XkT and a characteristic frequency $f_0 = \omega_0/2\pi$. Thermal noise will cause the barrier to oscillate by $\delta X_{\text{thermal}}$, but its effects would be independent of the applied field. If an applied AC field $E_{AC} = E^0_{AC} \sin(\omega_{AC}t)$ has a frequency $f_{AC} = \omega_{AC}/2\pi$ close to f_0, there will be a resonance between E_{AC} and the enzyme oscillator. After a period of time τ, the oscillation of the barrier will reach a steady-state amplitude. The net effect of an applied field on the two relevant rate constants is

$$k_{12} = k^0_{12} \exp(-\delta X_{AC}/kT) \quad \text{and}$$

$$k_{21} = k^0_{21} \exp(-\delta X_{AC}/kT), \qquad (5)$$

k^0_{12} and k^0_{21} are the rates in the absence of an electric field, and $\delta X_{AC} = \delta X^0_{AC} \cos(\omega_{AC}t + \alpha)$, where α is a phase shift.

The amplitude of the electric response δX^0_{AC} as a function of ω_{AC} would depend on model of an enzyme oscillator (17). In the absence of any structural information, we will tentatively accept a simple symmetrical form.

$$\delta X^0_{AC} = g\omega_{AC}E^0_{AC}/[(\omega_0 - \omega_{AC})^2 + \beta^2]. \qquad (6)$$

Here, g and β are a proportionality constant and a damping constant, e.g., friction, respectively. Fig. 1 shows the dependence of δX^0_{AC} on ω_{Ac} and on E^0_{AC} for this simple case.

Enzyme activity in an oscillating field. How could the overall rate, Eq. 4, be affected by an applied periodic field? The oscillation of the activation barrier implies that k_{12} and k_{21} will also oscillate (Eq. 5). The overall rate has to be calculated from the probabilities of the enzyme in states 1 and 2, i.e., P_1 and P_2, respectively (15,16). If we make the assumption below, then P_1 would not change much in one period. Integration of Eq. 3 would give the same Eq. 4 for the overall rate. However, here k_{12} and k_{21} are replaced with mean values of the two rate constants over time, i.e., $\langle k_{12}\rangle$ and $\langle k_{21}\rangle$, respectively. The assumption is: $f_{AC} \gg k_{12}$ or k_{21}. This condition greatly simplifies mathematical procedure. The effect of barrier oscillation on rate arises from the nonlinearity of the response and its net effect on the two limiting rate constants could be expressed as a function only of E^0_{AC} and ω_{AC}.

$$\langle k_{12}\rangle = k^0_{12}F(E^0_{AC}, \omega_{AC}); \qquad \langle k_{21}\rangle = k^0_{21}F(E^0_{AC}, \omega_{AC}), \qquad (7)$$

where

$$F(E^0_{AC}, \omega_{AC}) = f_{AC}\int \exp(-\delta X^0_{AC} \cos \omega_{AC}t/kT)\, dt. \qquad (8)$$

The integration is over one half a period. The integrated form is

$$F(E^0_{AC}, \omega_{AC}) = 1 + (\delta X^0_{AC}/kT)^2/4 + 2^{3/2}\pi^{1/2} \cdot [\sinh(\delta X^0_{AC}/kT)/(\delta X^0_{AC}/kT)^{1/2} - (\delta X^0_{AC}/kT)^{1/2}]. \qquad (9)$$

For a small δX^0_{AC}, Eq. 8 is approximated by $1 + (\delta X^0_{AC}/kT)^2/4$ and for a large δX^0_{AC}, it is approximated by $(2\pi kT/\delta X^0_{AC})^{1/2} \exp(\delta X^0_{AC}/kT)$. The mean overall rate in an AC field is

$$\langle A_{AC}\rangle = \frac{r_{21}(1 - K_{12}R_{21})}{1 + K_{12} + r_{21}(1 + R_{21})/[k^0_{12}F(E^0_{AC}, \omega_{AC})]}. \qquad (10)$$

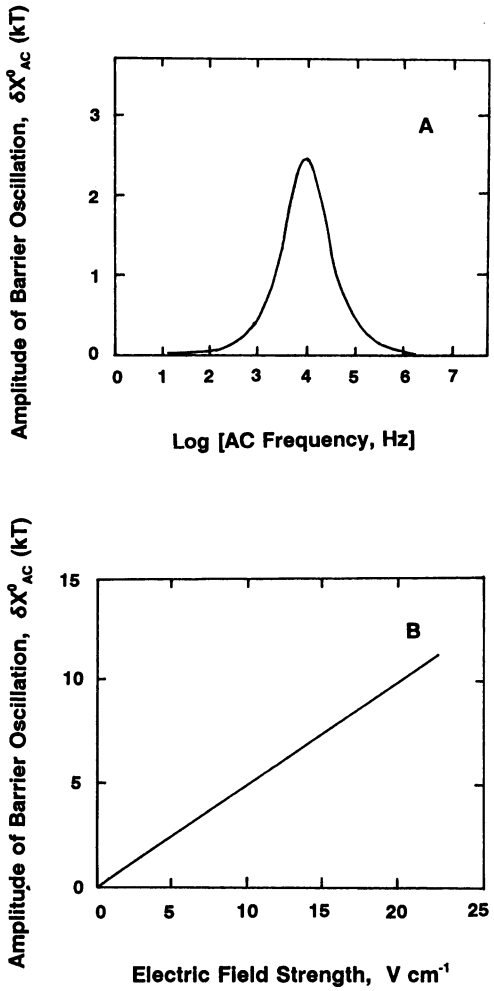


FIGURE 1 (*A*) Frequency dependence of the amplitude of the electric field induced oscillation δX^0_{AC} calculated according to Eq. 6, with $g = 3.8 \times 10^4$ cm s V^{-1}; $\omega_0 = 6.28 \times 10^3$ s^{-1}; $E^0_{AC} = 5$ V cm^{-1}; $\beta = 6.28 \times 10^4$ s^{-1}. (*B*) Dependence on field strength of the AC field E^0_{AC} according to Eq. 6, with $\omega_{AC} = 6.28 \times 10^4$ s^{-1} and other parameters used in *A*. k_{12} and k_{21} depended on AC field according to Eq. 7.

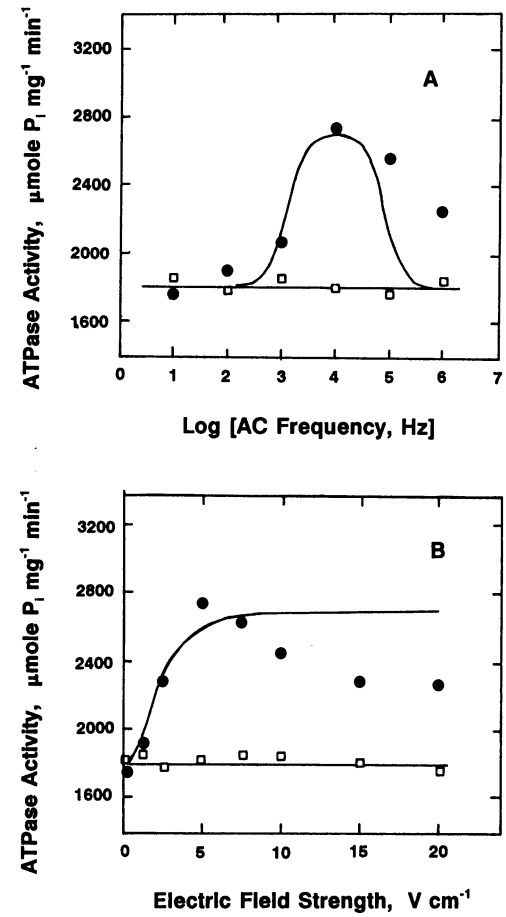


FIGURE 2 (*A*) Frequency dependence of the ATP hydrolysis activity of ECTO-ATPase. Data in squares are control (no AC stimulation) done with each sample and those in filled circles are samples stimulated by an AC of 5 V (peak-to-peak) cm^{-1}. Experiment was done at 37°C. Each data point was an average of 3 to 5 determinations. The uncertainty of ATPase activity assay was ±50 µmol mg^{-1} min^{-1}. K_m for ATP hydrolysis was determined to be 0.60 mM. The ATP concentration in the assay medium was 4 mM. The solid curve is the theoretical curve calculated according to Eq. 10 multiplied by a scaling factor *N*. The following parameters were used: $Nr_{21} = 54{,}000$ µmol mg^{-1} min^{-1}; $K_{12} = 19$; $R_{21} = 1 \times 10^{-11}$; $r_{21}/k^0_{12} = 10$; $r_{21} = 76{,}000$ s^{-1}; $g = 3.8 \times 10^4$ cm s V^{-1}; $\omega_0 = 6{,}280$ s^{-1}; $E^0_{AC} = 5$ V cm^{-1}; and $\beta = 6.28 \times 10^4$ s^{-1}. (*B*) Dependence of the AC stimulated activity on the field strength (AC of 10 kHz). The squares are for the controls and the filled circles are for the AC stimulated samples. Experimental conditions were the same as in *A*. In calculation, $\omega_{AC} = 6.28 \times 10^4$ s^{-1}. Other parameters used to simulate the data are the same as in *A*.

Eq. 10 was used to interpret experimental data on the AC stimulation of Ecto-ATPase (Fig. 2)

Electric stimulation of Ecto-ATPase activity. Ecto-ATPase, purified from chicken oviduct by a monoclonal antibody affinity column, was used to test the above concept. The enzyme is a single peptide chain of 80 kD and was solubilized in a 30-mM histidine buffer containing 0.1% non-ionic detergent NP40 (Sigma Chemical Co. catalog No. N3516; St. Louis, MO) and 4 mM $MgCl_2$, at pH 7.4. ATP hydrolysis activity was initiated by the addition of 4 mM Na_2ATP and monitored by the release of P_i according to the method of Leloir and Cardini (18). Electric stimulation was performed in a 4-mm path spectrophotometric cell, with two platinized platinum electrodes, connected to a Wavetek model 148A AM/FM/PM generator for the AC stimulation in the range 10 Hz to 1 MHz and up to 20 V cm^{-1}. The temperature was maintained at 37°C by a water circulator. Sample temperature was monitored by a microthermistor probe which has a time constant of less than 100 ms. Joule heating was less than 2°C in all measurements. Electric

stimulation was continued for 10 min, with or without 10 mM vanadate, and was stopped by the addition of trichloroacetic acid.

Fig. 2 shows that the ATP hydrolysis activity of the enzyme was stimulated by an optimal AC field (5.0 V cm^{-1}, 10 kHz) by 47%. The AC stimulated activity showed a frequency window at 10 kHz when the field intensity was 5.0 V cm^{-1} (Fig. 2 *A*). When a 10-kHz AC field was used, the AC enhancement of activity reached the maximal value at 5.0 V cm^{-1} (Fig. 2 *B*). Both the AC dependent and the AC independent ATP hydrolysis activities were completely inhibited by 10 mM vanadate. Fitting the data of Fig. 2 according to Eq. 10 gave the amplitude of the maximal barrier oscillation δX^0_{AC} at 2.5 *kT*. Lumry (19) and Gavish (20) in their theories of fluctuating enzymes estimate the time ranges of conformational fluctuations to be in milliseconds to nanoseconds, depending on conformational subclasses associated with the process. Our results (legend to Fig. 2) are in the same range predicted by these authors.

The field strength of the AC fields employed here is a few V cm^{-1}. An enzyme molecule in a micelle will experience ~10 μV of potential drop (micelle diameter ~20 nm). We may consider the barrier oscillation of the enzyme to reflect the breathing mode of the substrate binding site. If the interaction of the enzyme with the AC field were to involve movement of a charge, the interaction energy would be 10 μeV, which is equivalent to 4×10^{-4} kT, ~6,000 times smaller than the barrier oscillation of 2.5 *kT*. However, the ATP hydrolysis reaction has a large driving force (ΔG of ATP hydrolysis of ~ −15 kT) and the reaction does not require a supply of energy. The AC field was simply to enhance the barrier oscillation through the induction of a resonance between the enzyme oscillator and the AC field. If the oscillator has small inertia or friction, the barrier oscillation can be induced rapidly. If the inertia is large and energy transfer from the AC field to the barrier has a medium efficiency of 50%, 12,000 cycles of AC would be sufficient to induce a steady-state oscillation with the amplitude δX^0_{AC} of 2.5 *kT*. This means that it would take ~1 s to reach the maximal stimulation when f_{AC} was 10 kHz. New experimental design would be required to monitor directly the activity change in the one second time range. τ would be different with AC fields of different frequencies. Note that in Fig. 2 *A*, the fit in the high frequency region was not good. In Fig. 2 *B*, the effect of AC field began to decline above 7 V cm^{-1}. Apparently Eq. 6 is too simple to account for all the observed properties. By choosing an appropriate form for Eq. 6, the OAB model may reproduce complex dependence of field effects including inhibition of enzyme or receptor activity.

For an enzyme embedded in the plasma membrane of a cell of 20-μm diameter to experience a 10 μV potential drop, the cell need be exposed to an external field of only 5 mV cm^{-1} (4, 8). E^0_{AC} of 5 mV cm^{-1} is in the range of weak fields normally discussed in terms of their effects on health and the environment. The field experienced by the enzyme, 10 μV, is ~1/1,000 of that required for enzyme activation by the electroconformational coupling mechanisms (21–23). As yet, this value is not the limit of detection by the OAB model. The limit of detection of the OAB model, which depends on the time it takes to induce a resonance at a steady-state level (τ), and τ in turn depends on the δX^0_{AC} of a reaction and f_{AC}. A detection limit as low as 1 nV cm^{-1} can be easily accommodated by the OAB model.

It should mention that Benzi et al. (24) and Zhou et al. (25) have studied the phenomenon of stochastic resonance when a dynamic chemical system is modulated with a periodic driving force. A similar concept has also been discussed by Astumian et al. (26, 27) based on the ECC model. Another property of the electric stimulation is that an oscillating field may cause the concentration of a charged ligand near the enzyme to oscillate. As a consequence, the overall catalytic rate may be changed. Di Cera (28), Chen (29), Markin et al. (30), and Astumian et al. (31) have investigated such and other related chemical rate processes. How these different effects of an AC field may contribute to the observed stimulation of the ATP hydrolysis activity by the Ecto-ATPase remains to be investigated. One should emphasize again that the OAB model uses the driving force of a spontaneous chemical reaction. An AC field is to influence the overall rate of a catalytic reaction by inducing the oscillation of the activation barrier of the rate-limiting process. Our analysis suggests that the OAB model is inherently more sensitive for sensing low level electric signals than the ECC model (see also reference 30).

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