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Electromagnetic Acceleration of Electron Transfer Reactions

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Abstract The Moving Charge Interaction (MCI) model proposes that low frequency electromagnetic (EM) fields affect biochemical reactions through interaction with moving electrons. Thus, EM field activation of genes, and the synthesis of stress proteins, are initiated through EM field interaction with moving electrons in DNA. This idea is supported by studies showing that EM fields increase electron transfer rates in cytochrome oxidase. Also, in studies of the Na,K-ATPase reaction, estimates of the speed of the charges accelerated by EM fields suggest that they too are electrons. To demonstrate EM field effects on electron transfer in a simpler system, we have studied the classic oscillating Belousov–Zhabotinski (BZ) reaction. Under conditions where the BZ reaction oscillates at about 0.03 cycles/sec, a 60 Hz, 28 μT (280 mG) field accelerates the overall reaction. As observed in earlier studies, an increase in temperature *accelerates* the reaction and *decreases* the effect of EM fields on electron transfer. In all three reactions studied, EM fields accelerate electron transfer, and appear to compete with the intrinsic chemical forces driving the reactions. The MCI model provides a reasonable explanation of these observations. J. Cell. Biochem. 81:278–283, 2001.

Key words: electromagnetic field; electron transfer reactions; Belousov–Zhabotinski reaction

Weak low frequency electromagnetic (EM) fields activate important biological processes in cells [Goodman et al., 1993; Hong, 1995; Blank, 1995a], including biosynthesis of stress proteins [Goodman and Blank, 1998]. Since the reactions occur at very low field strengths, the initiating forces are very weak. EM fields interact with moving charges, and electrons (with their high charge/mass ratio) may be especially influenced and cause changes in the reaction rate. The moving charge interaction (MCI) model suggests that stress protein synthesis is initiated by interaction of EM fields with moving electrons in DNA [Blank and Goodman, 1997, 1999, 2000]. EM fields accelerate electron transfer in cytochrome oxidase [Blank and Soo, 1998a], and the calculated speed of the moving charges in the Na,K-ATPase suggests that they are electrons. The magnitudes of forces that affect the enzyme reactions compare to those that act on electrons in DNA, so that the MCI model could be the basis of EM activation of DNA [Blank and Goodman, 2000].

Additional insights into the mechanism come from the observation that EM fields increase charge transfer rates inversely with the reaction rate in both Na,K-ATPase [Blank, 1992; Blank and Soo, 1992, 1996] and cytochrome oxidase [Blank and Soo, 1998a, 1998b]. This implies competition of the EM field force with the chemical driving force. Another observation is that the frequency optima of both enzyme reactions are close to reaction turnover numbers, suggesting a "tuning" mechanism. The ability of EM field forces to interact with chemical driving forces and affect electron transfer rates may be the basis for *electrically* stimulated changes in muscle protein synthesis (that involve activation of DNA and) where the frequency dependence is related to the particular proteins synthesized [Blank, 1995b].

Demonstration of the proposed EM field mechanisms with a simpler electron transfer reaction would provide an additional test of the

Abbreviations used: EM, electromagnetic; Hz, Hertz; mG, milligauss; μT, microtesla; MCI, Moving Charge Interaction model; BZ, Belousov–Zhabotinski reaction.

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MCI model under simpler conditions. For this reason, we have chosen to study the classic Belousov–Zhabotinski (BZ) reaction [Field et al., 1972], an oscillating redox reaction system involving malonic acid, Br^-, BrO_3^-, and a redox catalyst, usually Ce^{3+}/Ce^{4+}. Ten separate reactions have been identified, and the electron transfer kinetics that lead to oscillations in the redox potential set by Ce^{3+}/Ce^{4+} have been analyzed [Field et al., 1972].

The BZ reaction is directly relevant to biochemical oxidations that occur in complex in vivo systems, and components of the Krebs cycle (e.g., malate) have been studied as substitutes for malonic acid. The BZ reaction, itself, has been invoked [Prigogine et al., 1969] as a model for biological processes that are characteristic of life, since organized structures (periodic in space and time) develop in homogeneous solutions when energy is dissipated in such reactions.

MATERIALS AND METHODS

The usual Belousov–Zhabotinski (BZ) reaction components are malonic acid, Br^-, BrO_3^-, and the redox catalyst is Ce^{3+}/Ce^{4+}. Many substitutes can be used while still maintaining the oscillatory nature of the reaction, and we have substituted Fe^{2+}/Fe^{3+} as the redox catalyst. Our experimental protocol is best described in terms of the following solutions:

Solution A: 15 g $NaBrO_3$ in 211 ml acid solution (10 ml conc H_2SO_4 in 201 ml distilled H_2O)

Solution B: 2 g NaBr dissolved in 20 ml distilled H_2O

Solution C: 2 g malonic acid in 20 ml distilled H_2O

Ferroin: a redox indicator, 0.25 mol $FeSO_4$ and 0.25 mole 1,10 phenanthroline in 10 ml distilled H_2O (the Fe^{2+} complex is red, and the Fe^{3+} complex is pale blue)

The reaction was studied in a 50 ml capacity reaction vessel having a glass jacket through which water from a thermostated bath was circulated to maintain constant temperature (usually 25°C). Solution A (12 ml) was added to the vessel, and platinum (redox electrode) and reference (calomel electrode with a $NaNO_3$ salt bridge to eliminate Cl^-) electrodes were set in place, along with a mechanical stirrer. The stirrer was attached to a motor by a ~3 ft flexible shaft to keep the motor (and its EM field) at a fair distance from the reacting solution. The redox potential was measured with a Keithley 610A Electrometer, and the output recorded.

A brown color appeared as soon as 1.0 ml of solution B was added. When 2 ml of solution C was added to the reaction mixture, the brownish color started to fade, until the solution became colorless (in about 2 min at 25°C). At this point, 1.0 ml of ferroin redox indicator solution was added and the redox potential recorded. The solution turned red, and after an initial induction period, oscillations started in both electrode potential and in the color of the solution (between red and blue). After waiting for four oscillations to insure that a regular pattern had been set, oscillations were counted for about a 10-minute period, and the exact duration noted. Under these conditions, the oscillations lasted over 20 min (see Fig. 1). The period of oscillation was calculated by dividing the duration (in seconds) by the number of full oscillations (cycles) in electrode potential. The inverse of the period is the rate (in cycles per second). The reaction rate has the same units as EM field frequency, but we shall refer to the chemical kinetic measure as rate to try to avoid confusion.

Prior to the start of the experiment, the reaction vessel was placed in Helmholtz coils in a mu metal box, and ambient fields were measured to insure a low background of about 0.1 μT (1 mG) inside the box. The mechanical stirring motor was placed at least 2 ft from the reaction vessel to keep the background reading low. The Helmholtz coils (Electric Research and Management, Pittsburgh, PA) consisted of 19-gauge wire bundles wound 164 times around a square form 13 cm long and 14 cm wide with 8 cm spacing. The applied EM fields were generated using a 3312A Hewlett-Packard Function Generator. The frequency was fixed, and the output was fed into the coils and into a 3478A Hewlett-Packard digital multimeter used to measure the voltage. The resulting field intensity was measured inside the Helmholtz coils using a Metex M3800 multimeter with an attached test coil from Electric Field Measurements (W. Stockbridge, MA). The output of the function generator was adjusted to give the desired EM field level in the coils, and this is the magnitude of field recorded.

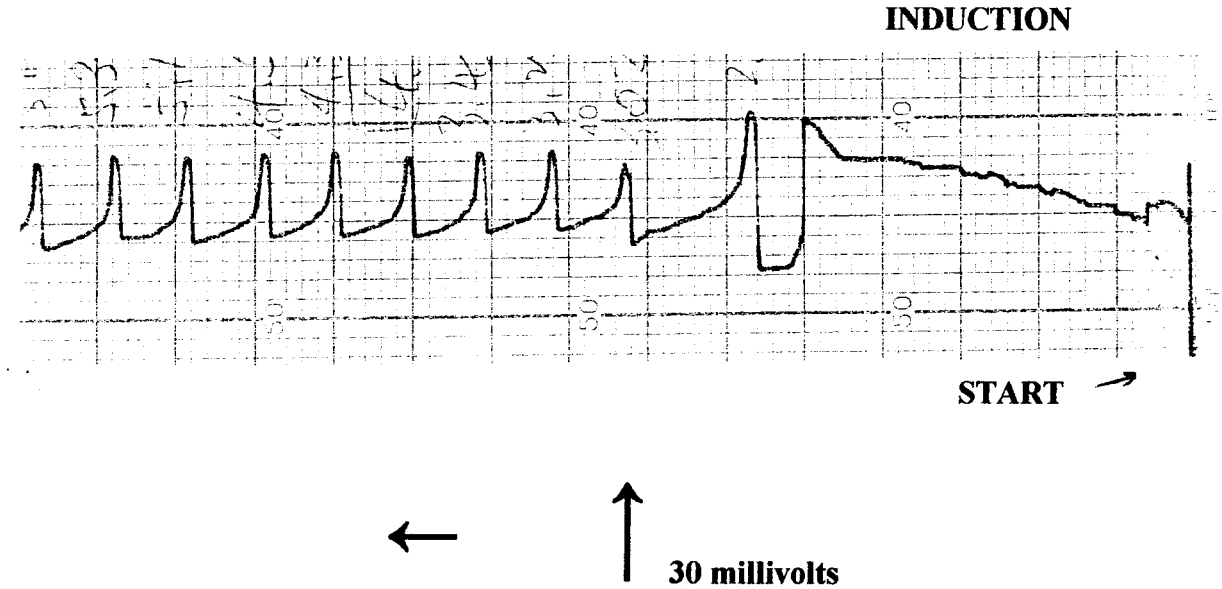

Fig. 1. A typical tracing of oscillations in Fe^{+2}/Fe^{+3} redox potential at 25°C, under control conditions (with no applied EM field). The record starts at the right, and is followed by an induction period and regular oscillations that continue for over 20 min. Electrode potential and time scales are indicated, and the BZ reaction oscillates at about 0.03 cycles/sec. Figure 1 records the measured electric potential under control conditions, and the electric field amplitudes do not change when EM fields are applied. Only the oscillation rate changes.

In order to detect a change due to the EM field, it was necessary to control the temperature of the solution in the reaction vessel. All solutions were kept at the temperature of an experiment prior to mixing, and constant temperature water was circulated through the water jacket during the course of the experiment. The circulatory pump was separated from the reaction solution to insure a low EM field background. The effect of temperature on the reaction rate can be seen in Figure 2.

RESULTS

With reagent concentrations and temperature set so that the BZ reaction oscillates at about 0.03 cycles/sec, we measured oscillations in Fe^{2+}/Fe^{3+} redox potential to obtain the reaction rate in cycles per second. Figure 1 shows the redox potential with no applied EM field. After an induction period of 2–3 min, regular oscillations begin and continued for over 20 min. When a 60 Hz, 28 μT (280 mG) EM field is applied, the amplitude of the oscillations does not change, indicating no change in the electric field. However, the EM field accelerates the reaction, causing the redox potential oscillation peaks to recur more rapidly.

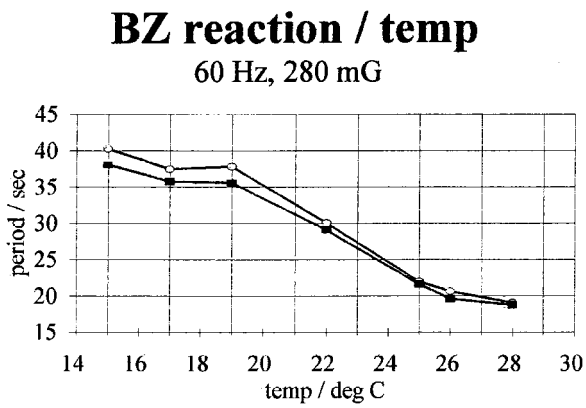

Fig. 2. The period of oscillation (in sec) of the overall BZ reaction, under control conditions (○), and with an applied 60 Hz, 28 μT (280 mG) field (■), is shown for different temperatures. Each point is the result of eight independent measurements.

$$\% \text{ acceleration} = 100\left\{\frac{\text{EM rate}}{\text{control rate}} - 1\right\}.$$

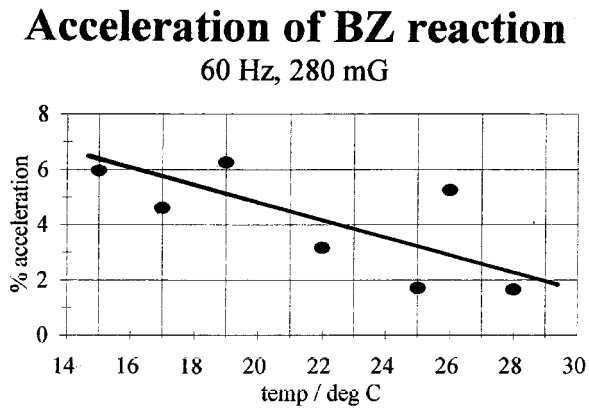

Fig. 3. The % acceleration (●) is plotted as a function of temperature, showing a decrease with increasing temperature. Each point is the result of 16 independent measurements, 8 under control conditions and 8 with an EM field.

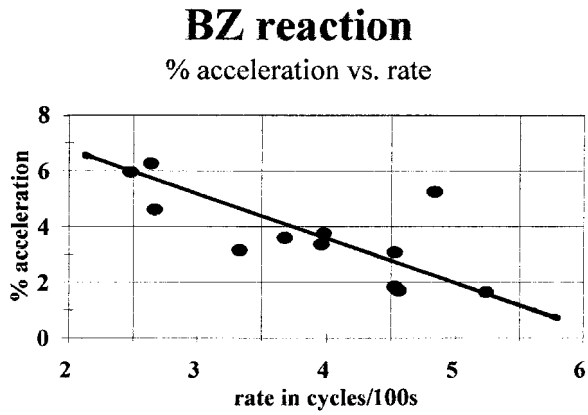

Fig. 4. The % acceleration (●) is plotted as a function of the reaction rate (in cycles / 100 sec) for experiments under a variety of conditions, i.e., where temperature, field strength, and frequency were varied. Each point is the result of 16 independent measurements, 8 control and 8 EM field. The decrease in % acceleration with increasing rate suggests a competition of the EM field forces with the intrinsic chemical driving forces.

Studies of the effects of temperature (Fig. 2) on the % acceleration show that temperature dependence of the overall BZ reaction is in the normal range for chemical reactions. A temperature coefficient of about 10%/°C means a doubling of the rate for a 10° rise, and an activation energy of about 10 kcal/mol. Figure 3 shows that EM fields accelerate the reactions by about 2–6%, the effect decreasing with an increase in temperature. However, temperature is not the independent variable. Earlier results on two enzyme reactions [Blank and Soo, 1992, 1996] suggested that the EM field effect depended on the rate. When additional data for the BZ reaction, at different field strength and frequency, are included with the temperature data, Figure 4 shows an inverse dependence on the reaction rate, as in earlier results on cytochrome oxidase (Fig. 5) and Na,K-ATPase (Fig. 6). These data support an interaction between magnetic and chemical forces.

DISCUSSION

EM Fields Accelerate Electron Transfer

It is usually stated that the biological mechanisms of EM field effects are not well understood, but the MCI model of interaction with moving electrons appears to account for observations in the well-defined systems discussed here. The low thresholds of EM field effects, where measured, indicate that the weak forces are apt to have their greatest effect on particles with lowest mass, i.e., electrons. Electron transfer is affected by EM fields in both the BZ and cytochrome oxidase reactions. As discussed in the next paragraph, the charges in Na,K-ATPase are probably electrons as well.

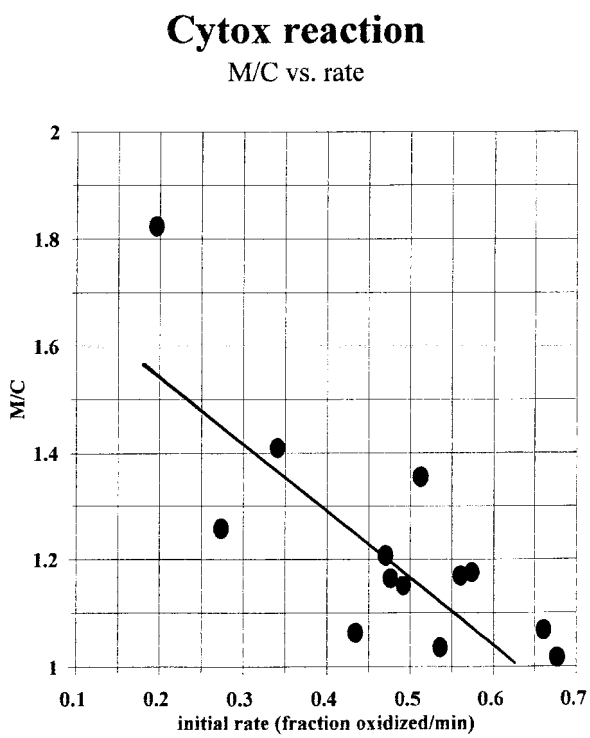

Fig. 5. M/C (●), the rate of the cytochrome oxidase reaction in a 60 Hz, 2–10 μT (20–100 mG) EM field divided by the control rate (in the absence of an EM field), is plotted as a function of the initial reaction rate (in fraction oxidized / min). These data, replotted from Blank and Soo [1998a] show that the EM field effect decreases as the reaction rate increases.

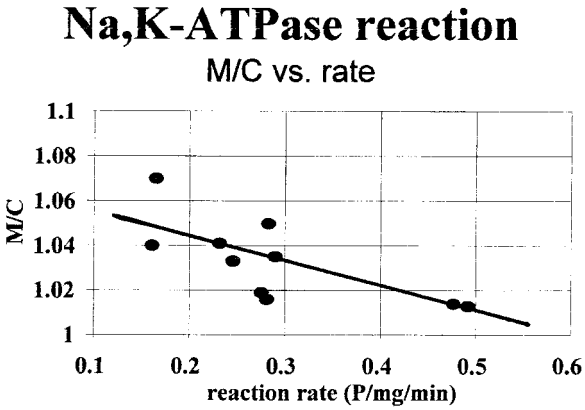

Fig. 6. M/C (●), the rate of the Na,K-ATPase reaction in a 60 Hz, 0.5–10 μT (50–100 mG) EM field divided by the control rate (in the absence of an EM field), is plotted as a function of the steady-state reaction rate (in mmol phosphate/ mg protein/ min). These data, replotted from Blank and Soo [1996], show that the EM field effect decreases as the reaction rate increases.

Data from Na,K-ATPase studies enable estimating of the speed of the moving charges. The magnetic force (newtons), $F = qvB$, where a unit charge, $q = 1.6 \times 10^{-19}$ C, v = velocity (in m/s) and B = 10 μT (100 mG). The electric force (newtons), $F = qE$, where E is in V/m. If we assume that the forces at the electric and magnetic thresholds [Blank and Soo, 1992, 1996] are equal, $F = qE = qvB$, then $E = vB$ and $v = E/B$. The measured thresholds, 5×10^{-4} V/m and 5×10^{-7} T (5 mG) for E and B [Blank and Soo, 1992, 1996], lead to $v = 10^3$ m/s. The large magnitude of v, comparable to electron speeds in DNA [Wan et al., 1999], suggests that the moving charge in the Na, K-ATPase is an electron. (The Na,K-ATPase also catalyzes phosphorylation, the reverse reaction normally coupled to electron transport in mitochondria.) Electron movement in the Na,K-ATPase is probably associated with the early charge shift measured by Hilgemann [1994].

The calculated velocity of the electron, 10^3 m/s, enables us to better characterize the EM field that interacts with the electron. The electron moves through the Na,K-ATPase from the catalytic site (where ATP is split) across the full thickness of the enzyme to the other side of the membrane, about 10 nm, and the whole process is complete in 10^{-11} sec. Since a 60 Hz sine wave lasts 1/60 sec, the moving electron must see a constant field, i.e., a DC field of either polarity and of any amplitude up to the maximum.

The BZ and enzyme reactions are quite similar. In all the three experiments:

- The result of EM field interaction is an acceleration of the reaction.
  The effect of an *induced electric field* is reversed in the second half of the sine wave. Charges accelerated by the field in the + phase of a cycle should be accelerated in the opposite direction in the − phase of a cycle. A *magnetic field* generates a force orthogonal to the direction of movement of the electron, and in the second half of the sine wave the force is orthogonal in the opposite direction. But in both halves of the magnetic sine wave, the electron has a component in the original direction. Interaction with a magnetic field provides a *ratchet mechanism* that allows a process to proceed essentially in one direction only.
- The effect of the EM field varies inversely with the rate of the reaction, indicating competition of the magnetic driving force with the intrinsic chemical driving forces.
  Electrical and chemical forces drive reactions of charged chemicals through the combined electrochemical potential. In these studies, magnetic forces combine with chemical driving forces, but the effects are not additive in the same way. There appears to be a maximum reaction rate that the magnetic force cannot raise.
- EM fields accelerate all linked redox reactions.
  EM fields accelerate both forward and backward reactions in the equilibrating reactions of cytochrome oxidase. We now see this for all 10 BZ reactions.

Effect of EM Fields on Electrons in DNA

Returning to the problem of EM field-initiated biosynthesis, we can summarize recent supporting evidence for direct EM field interactions with electrons in DNA:

- Interactions occur in model systems at electron velocities similar to those found in DNA. The magnetic force due to a 10 μT (100 mG) field on an electron, 10^{-22} N, causes an acceleration of 10^8 m/s^2 or 10^7 times gravity, comparable to the force produced by an electric field of 10^{-2} V/m. This may be sufficient to change electron transfer in the DNA at sites where electric fields are weak. EM fields can accelerate electrons being

conducted in DNA along the stacked bases of the double helix [Arkin et al., 1996; Dandliker et al., 1997; Wan et al., 1999]. The charge velocity in Na,K-ATPase, 10^3 m/s, is similar to the electron transfer rate in DNA of 400 m/s [Wan, et al., 1999], so the forces that affect enzyme reactions at low field strengths may also cause changes in DNA when electrons move at comparable velocities.

- A defined DNA segment has been associated with a response to EM fields. Lin et al. [1999] have identified a specific DNA segment associated with the response to EM fields. This suggests that EM field interaction may be optimized by particular groupings of bases in DNA. Removal of an EM-associated segment eliminates the response, and transfection into a promoter construct renders it EM field responsive.

Use of Magnetic Stirrers in Kinetic Studies

Many kinetic studies are done using magnetic stirrers, with relatively large AC fields at the bottom of the beaker in contact with the stirrer motor. There probably is good reason to check the studies of redox reactions to see if the kinetic constants have not been overestimated as a result of acceleration by the fields.

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