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Electromagnetic acceleration of the Belousov–Zhabotinski reaction

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Abstract

Acceleration of the Belousov–Zhabotinski (BZ) reaction, in stirred homogeneous solutions, by low frequency electromagnetic (EM) fields has provided new insights into EM interaction mechanisms. The acceleration varies inversely with the basal reaction rate, indicating that the applied magnetic field and the intrinsic chemical driving forces affect the same electron transfer reaction. The amplitude and frequency dependence of the EM field interactions are also consistent with interaction during electron transfer. A mechanism based on interaction with moving electrons offers a way of explaining the ability of EM fields to stimulate gene expression, in particular the stress response, since electrons have been shown to move in DNA.

Keywords: Electromagnetic acceleration; Belousov–Zhabotinski reaction; Homogeneous solution

1. Introduction

The Belousov–Zhabotinski (BZ) reaction [1], the oxidation of malonic acid in the presence of bromide, bromate, and an inorganic redox catalyst such as the Fe^{+2}/Fe^{+3} couple, is more frequently studied as a reaction-diffusion system where unusual spiral waves propagate in thin films of solution [2,3]. In stirred systems, the complex feedback patterns between individual steps of the reaction lead to regular oscillations in reactant concentrations and provide a measure of the overall rate. When phenanthroline is added, the colored Fe complex alternates between the red (reduced) and blue (oxidized) forms. Oscillating chemical reactions in homogeneous solutions are models for anabolic biochemical processes, because of the ordered 'dissipative structures' in space and time that develop as energy is degraded [4].

The BZ reaction also offers certain advantages for studying effects of electromagnetic (EM) fields on electron transport. Our research on the Na,K-ATPase from rabbit kidneys [5,6,7] and cytochrome oxidase from rat liver mitochondria [8,9], suggested that low frequency EM fields accelerated electron transfer, but the unknown components present in biological preparations always raise questions about the results. By contrast, the BZ reaction is a much simpler and cleaner system. Its components can be prepared from reagent grade chemicals, and the regular oscillations in redox potential enable continuous measurement of the rate.

Our initial studies of the BZ reaction [10] showed that EM fields accelerated electron transfer, as found with the enzymes. The effects of the applied EM field and intrinsic chemical driving forces were additive, and the EM field was less effective as the chemical forces increased. In this paper, we show that the effects of EM field strength and frequency on the reaction rate are also consistent with effects on electron transfer.

2. Experimental

The usual Belousov–Zhabotinski (BZ) reaction components are malonic acid, Br-, BrO_3- and the redox catalyst Ce^{+3}/Ce^{+4}. We substituted Fe^{+2}/Fe^{+3} as the redox catalyst in a protocol using the following solutions:

Solution A: 15g $NaBrO_3$ in 211 ml solution (10 ml conc. H_2SO_4 in 201 ml H_2O)
Solution B: 2 g NaBr dissolved in 20 ml H_2O
Solution C: 2 g malonic acid in 20 ml H_2O
Ferroin: a redox indicator, 0.25 mol $FeSO_4$ and 0.25 mol 1,10 phenanthroline in 10 ml H_2O (the Fe^{2+} complex is red and the Fe^{3+} complex is pale blue).

The reaction was studied in a 50-ml reaction vessel with a glass jacket through which water from a thermostated bath was circulated to maintain constant temperature. A 12-ml

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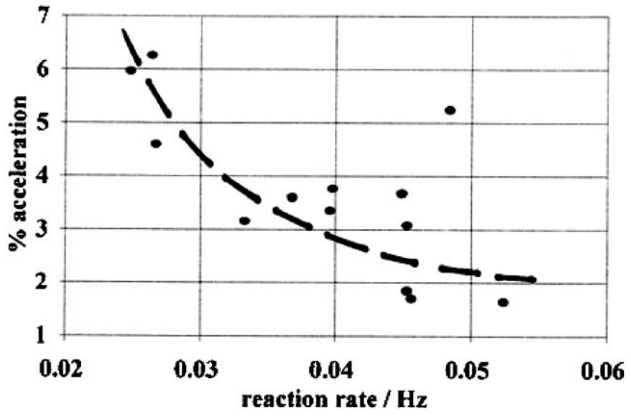

Fig. 1. The % acceleration due to the applied EM field of 280–300 mG (0.28–0.3 μT) as a function of the intrinsic reaction rate (in Hz). Each point is the result of at least 10 independent measurements, with standard errors of the mean on the order of 1%. The inverse dependence of % acceleration on rate reflects a competition between the applied EM driving force and the intrinsic chemical driving forces.

solution A 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 attached to a motor by a flexible shaft. The redox potential was measured and recorded with a Metrohm Potentiograph E536 (Brinkmann Instruments, Westbury, NY).

The brown color that appeared when 1.0ml solution B was added, started to fade when 2 ml solution C were added, and became colorless in about 2 min (at 25 °C). 1.0 ml ferroin solution was added and the redox potential recorded. The solution turned red, and after a 2–3 min induction period, oscillations started in both electrode potential and solution color. After waiting four oscillations to insure that there was a regular pattern, oscillations were counted for a 10-min period, and the rate (cycles/s) calculated. The concentrations of the reactants change during an experiment, but these changes are small enough during the short duration of a run that they do not change the BZ rates with and without field.

The reaction vessel was placed in Helmholtz coils in a mu metal box, with low background fields of about 1 mG (0.1 μT). The mechanical stirring motor was at least 2 ft from the reaction vessel to keep the background low. The Helmholtz coil (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 (Paramus, NJ). The frequency was set, and the output fed into the coils and into a 3478A Hewlett-Packard digital multimeter to measure the voltage. The resulting field intensity was measured inside the Helmholtz coils using a Metex M3800 multimeter with an attached test coil (Electric Field Measurements, W. Stockbridge, MA). The output of the Function Generator was adjusted to give the desired EM field level in the coils. The test coil for measuring field strength was calibrated for 60 Hz, and readings were corrected for different frequencies.

Temperature control is essential for detecting a change in reaction rate due to the EM field. Solutions were at the temperature of an experiment prior to mixing, and kept at constant temperature (± 0.1 °C) with water circulated through the water jacket. The circulatory pump was away from the reaction solution to insure a low EM field background. The Δperiod/Δtemp is about −2s °C [10]. With temperature control at better than ± 0.1 °C in the thermostat and reaction vessel during a run, the temperature could not account for more than ± 0.2 s variation in the period of an oscillation, and less than 1% in the acceleration of the BZ reaction as defined in the next paragraph.

Under the above conditions and with no applied EM field, the BZ reaction oscillated at about 0.03 cycles/s for well over 20 min. When an EM field was applied, the amplitude of the oscillations did not change, indicating no change in the redox potential. However, the EM field accelerated the overall BZ reaction. The rate was determined from the number of cycles per second, and the % acceleration was calculated from the following expression.

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

Experiments at each set of conditions were repeated at least 10 times, and standard errors of the mean were on the order of 1%.

3. Results

Fig. 1 shows that the % acceleration of the BZ reaction due to the applied EM field varies inversely with the

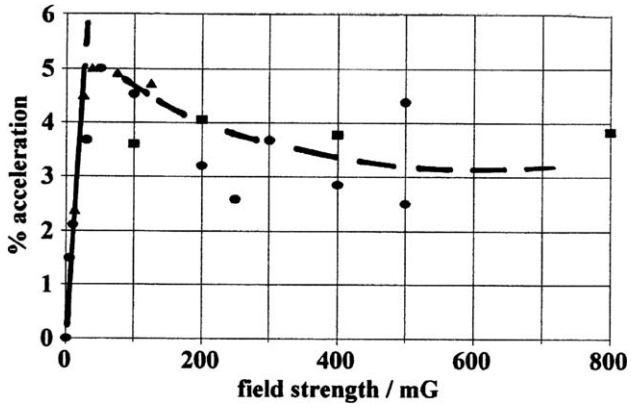

Fig. 2. The % acceleration due to the applied EM field as a function of the field strength (in mG). Data obtained at three frequencies, 30 Hz (■), 60 Hz (●) and 120 Hz (▲), are essentially part of the same curve, suggesting that the effect of field strength is independent of frequency in this range. A least squares fit of the points on the rising phase of the curve to a straight line gives a y-intercept of 1.09, slightly above the origin. The threshold appears to be near the origin and well below 5 mG. The steep rise at the onset is followed by a shallower decline at higher amplitudes.

intrinsic reaction rate. The magnitude of the experimental effect is small, but the difference between the rates with and without field is reproducible and significant. The results are also qualitatively similar to earlier studies of effects of EM fields on enzyme reaction rates. The apparent competition between EM fields and BZ reaction, also found with the two enzyme reactions [5,8], suggests that applied EM forces and intrinsic chemical forces act on the same electrons.

To determine the field strength dependence, we studied the BZ reaction over a range of amplitudes. Fig. 2 shows a steep rise in % acceleration at low amplitude, with a 'peak' below 50 mG (5 μT), and a shallower decline at higher amplitudes. This is similar to the behavior of the Na,K-ATPase [7], another reaction that goes to completion, but unlike the behavior of the cytochrome oxidase [8], where acceleration of both forward and backward reactions to an equilibrium may account for the different response. In the cytochrome oxidase study, the rate constants increased continuously when measured up to 100 mG (10 μT), and the absence of a falling phase could be due to the limited range. The data for the BZ reaction, obtained at three different frequencies, appear to lie on the same curve, indicating no variation with frequency between 30 and 120 Hz.

The low amplitude points that define the 'up' slope of Fig. 2 can be used to estimate a threshold. A least squares fit to a straight line gives a y-intercept of 1.09, slightly above the origin. Extrapolation to a point near the origin, along with an acceleration greater than 1% at 5 mG (0.5 μT), suggest that the threshold is very low. Previously measured thresholds of 2–3 mG (0.2–0.3 μT) for Na, K-ATPase [7] and 5–6 mG (0.5–0.6 μT) for cytochrome oxidase [8] reactions are of comparable low magnitudes.

Fig. 3 presents the % acceleration of the BZ reaction as a function of the EM field frequency. There appears to be a broad maximum around 250Hz, similar in shape to the maxima observed at 800Hz for cytochrome oxidase and 60Hz for Na,K-ATPase [11].

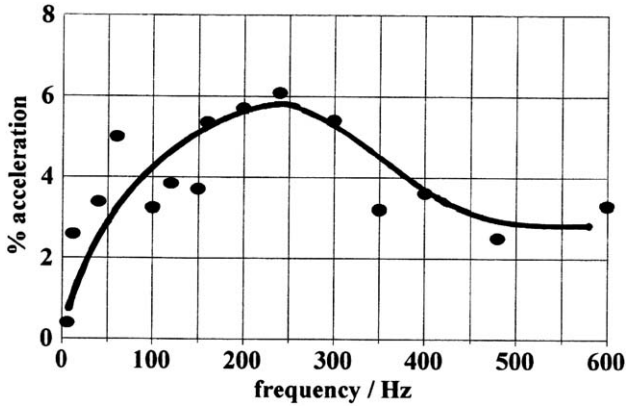

Fig. 3. The % acceleration of the BZ reaction by an applied EM field of 50 mG (5 μT) as a function of the EM field frequency (in Hz). The broad maximum around 250 Hz is similar to earlier observed maxima at ~800 Hz for cytochrome oxidase and ~60 Hz for Na,K-ATPase.

4. Discussion

4.1. Effect of EM fields on electron transfer

Our experiments have shown that low frequency EM fields accelerate the BZ reaction. The simple interpretation is that there is a direct effect of the magnetic field on the process, but one must rule out other possible interpretations, primarily that the effects are due to induced currents. Induced electric fields are certainly present, but they are generally very small (because of the small cross-sectional area of the reaction vessel), and our data show that they are not effective in our experiments. The magnitude of an induced electric field is directly proportional to the rate of change of the magnetic field, and should therefore vary with the frequency. In Fig. 2, we see that the force of interaction does not depend upon the frequency between 30 and 120 Hz. One would expect a four-fold increase in the effect between 30 Hz and 120 Hz, if the force of interaction were due to induced electric fields. By contrast, the magnetic force of interaction does not depend upon the frequency, in line with observations.

Another possible mechanism that is frequently mentioned is the effect of DC magnetic fields on free radical reactions. All our experiments, with and without AC magnetic fields, were done in the earth's DC field of 0.4G (40 μT), which is below the level at which free radical reactions are affected. By contrast, AC magnetic fields as low as 5 mG affected the BZ rate, in the presence of the earth's DC field.

Additional reasons for interpreting the effects of low frequency EM fields on the BZ reaction as being due to an interaction of the EM field with electron transfer, are:

- Additivity of magnetic and intrinsic chemical forces and the apparent competition between them when the chemical forces are very strong suggest that EM fields interact with the same electrons during the reaction.
- Similar results in three very different reactions, electron transfer in cytochrome oxidase, ATP hydrolysis by the Na,K-ATPase, and the catalyzed oxidation of malonic acid reported here (the BZ reaction), indicate that our observations are probably characteristic of EM field interaction during electron transfer rather than any specific reaction.

It should also be noted that the BZ reaction involves no cells, membranes or calcium ions—components that are frequently mentioned as essential in EM field interaction mechanisms.

The three EM field studies mentioned above were done in aqueous suspensions of membrane fragments (Na,K-ATPase, cytochrome oxidase) or homogeneous solution (BZ reaction), so the substrates have random orientations. Therefore, an EM field will react optimally only with the fraction of substrate that is properly oriented (i.e., field orthogonal to the moving electrons) and 'in synch' (electrons must be moving when the field is on).

It is difficult to think of an appropriate model for the (wave/particle) electron being transferred in solution during a chemical reaction while interacting with an EM field. It may be helpful to obtain some physical markers by assuming that EM fields interact with electrons in the same way as EM fields would affect electrons moving in a wire. The force (in newtons) of a magnetic field on a moving electron in a wire,

$$F = qvB, \quad (1)$$

where $q = 1.6 \times 10^{-19}$ C, v = velocity (in m/s) and the magnetic flux density, B, is approximately 30 μT (300 mG) for the data in Fig. 1. According to Eq. (1), at constant B, the force, F, depends only upon electron velocity. Assuming $v = 10^3$ m/s, as calculated from the Na,K-ATPase experiments [12], and probably associated with early charge shifts within the enzyme [13], $F \sim 5 \times 10^{-20}$ N. This is a very weak force, but for an electron of mass 9.1×10^{-31} kg, there would be an appreciable acceleration of about 5×10^{12} m/s^2. Even at a velocity of 1 m/s, the acceleration would be ~5×10^9 m/s^2, or ~5×10^8 times the acceleration of gravity. It should be emphasized that these order of magnitude calculations based on the classic moving charge interaction (MCI) mechanism are not supposed to be appropriate for the process being studied, but they do suggest a physical rationale for the observations. The linear dependence at low field strengths in Fig. 2 is in line with the MCI mechanism, and the very low threshold and steep dependence of the % acceleration on field strength are compatible with interactions involving high charge/mass ratio particles. The classical approach may work here because of the large number of events involved in a macroscopic solution. It is our hope that someone will propose a rigorous (quantum mechanical) model to account for the observed phenomena reported here.

Marcus theory [14], which has been successful in calculating rate constants of electron transfer kinetics, provides additional insights. The theory relates rate constants for electron transfer to the free energy change driving the reaction, and our results suggest that the energy contributed by an externally applied magnetic field adds to the energy of the chemical change. Weak magnetic fields accelerate the rate, but magnetic fields above ~50 mG (B~5 μT) in Fig. 2, lead to the decline in acceleration characteristic of the Marcus inverted region. If this interpretation is correct, EM fields could be useful experimental tools for studying electron transfer kinetics.

According to Eq. (1), the force of interaction does not depend upon the frequency. The data of Fig. 2 are not affected by frequency between 30 and 120 Hz, but the % acceleration does vary with frequency over a wider range (Fig. 3). The wider range frequency dependence, which is different for the three reactions studied, appears to be related to the characteristics of the reactions. The optima for the two enzyme reactions are at values close to enzyme turnover numbers [11]. It is reasonable for the effect of an EM field on the overall electron transfer rate to be 'in synch' with the rate at which electrons become available in specific reactions. Therefore, the optimal frequency for the BZ reaction could represent a characteristic time of 4×10^{-3} s for the rate limiting step in the reactions [1]. Studies of the frequency of *electric* stimulation on protein synthesis in muscle [15] indicate that broad frequency maxima are probably related to reactions with specific segments of DNA that code for particular proteins.

Studies of EM field interactions have from time to time described 'windows', that is, restricted ranges within which interactions occur. Neither the amplitude dependence of Fig. 2, nor the broad maximum in the frequency dependence of Fig. 3, have the sharpness one associates with a 'window', although the shape of the curve can change depending on the scale of the frequency range being studied. In any case, increases followed by decreases in rate (i.e., apparent amplitude windows) would be expected in the Marcus inverted region.

4.2. Effect of EM fields on gene expression

EM fields affect a wide range of biological processes from simple enzyme reactions to the far more complex gene induction and protein synthesis, as in the stress response [12,16,17,18]. It is possible that the same mechanism applies in all these processes. EM fields could initiate transcription by interacting with electrons that have been shown to move within DNA [19]. The velocity of charge movement in Na,K-ATPase is similar to values for electron transfer in DNA [20], so the forces that affect enzyme reactions may be large enough to cause changes in DNA. DNA is apparently also activated by the weak *electric* (eddy) currents due to action potentials in muscle [15], and the observed frequency-specific protein synthesis suggests that different segments of DNA are affected at different rates of stimulation.

There is evidence for direct interaction of EM fields with DNA. A specific 900 base pair segment in the hsp70 promoter appears to be needed for stimulation of the stress response by EM fields [21]. Removal of the segment eliminates the response, and transfection into a promoter construct causes the construct to become EM field responsive [22]. Assuming that EM fields initiate transcription by generating repulsive forces that cause DNA chain separation, repulsion appears to be greatest near the sequences needed for stimulation by EM fields (Blank and Goodman, 2001) [23].

4.3. Implications for the health/safety issue

Research on EM fields has generally been linked to effects of environmental EM fields on human health/safety [24]. Based on our recent studies:

- the low thresholds for interaction with electron transfer reactions suggest that many interactions with weak fields in the environment are possible, although the geometric

requirements for interaction may limit the actual extent of reaction.

- the finding that EM fields induce synthesis of stress proteins, the cellular response to such harmful factors as high temperature and toxic metal ions, indicates that, despite the geometric constraints on interaction, cells do react to EM fields as potentially harmful.

In addition to the cellular studies, two recent meta analyses [25,26] of pooled data from 15 and 9 major studies, respectively, have shown a statistically significant doubling of the risk of childhood leukemia when exposures to low frequency EM fields exceed 3–4 mG (0.3–0.4 μT). The increase in the incidence of childhood leukemia also correlates with the spread of electrification in the US since 1920 [27]. The new evidence agrees with the earlier report [23] in indicating a need for further study of biological EM field interaction mechanisms.

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