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Protein and DNA Reactions Stimulated by Electromagnetic Fields

MARTIN BLANK

Department of Physiology and Cellular Biophysics, Columbia University, New York, New York, USA

The stimulation of protein and DNA by electromagnetic fields (EMF) has been problematic because the fields do not appear to have sufficient energy to directly affect such large molecules. Studies with electric and magnetic fields in the extremely low-frequency range have shown that weak fields can cause charge movement. It has also been known for some time that redistribution of charges in large molecules can trigger conformational changes that are driven by large hydration energies. This review considers examples of direct effects of electric and magnetic fields on charge transfer, and structural changes driven by such changes. Conformational changes that arise from alterations in charge distribution play a key role in membrane transport proteins, including ion channels, and probably account for DNA stimulation to initiate protein synthesis. It appears likely that weak EMF can control and amplify biological processes through their effects on charge distribution.

Keywords Electric fields; Magnetic fields; Charge transfer; Protein; Hydration energy; DNA.

The Problem

Mark Twain once defined common sense as the sense that tells you the earth is flat. For most people, that line generally evokes a guilty smile. We know the earth is not flat even as our senses deceive us into believing that it is. In the study of biological effects of electromagnetic fields (EMF), we know that we do not usually perceive effects of these fields. However, we also know that biochemical and physiological measurements show profound effects of EMF on living cells. As scientists, we try to let science guide our common sense.

To put EMF in perspective, we know that of the four fundamental physical interaction forces, EM forces are those that mainly affect living systems. One would expect that biological responses to EM forces evolved over time in optimizing the ability of cells to survive. However, it appears that biological systems are unusually sensitive to EMF in frequency ranges that are unlikely to have been experienced by

Address correspondence to Martin Blank, Department of Physiology, Columbia University, 630 West 168 Street, New York, NY 10032; E-mail: mb32@columbia.edu

living systems before the advent of modern technology. Obviously, EMF must affect the same systems and reactions as were affected by other factors that played a role in the adaptation of living systems.

One of the other factors is easy to pinpoint, an ability to influence molecular interactions with water. Water is an essential component of living systems, so much so, that the search for life beyond Earth is essentially a search for the water needed to sustain it. Water has many unusual properties, among which is an ability to interact with and dissolve ions and many biopolymers. Because water hydrates molecules and forms solutions, chemical forces play a major role in biological systems. Of course, hydration forces are ultimately electromagnetic, e.g., water dipoles interacting with ions and the charged groups on proteins, but their effects are easier to describe in chemical terms and using thermodynamic properties. Natural biopolymers such as proteins and nucleic acids in solution are hydrated, and changes in charge distribution can lead to changes in molecular conformation. Such structural changes are generally accompanied by changes in hydration and very large changes in heat and entropy.

EMF interact with molecules to cause changes in charge distribution, but when considering biological mechanisms, we must also focus on the cell as the functional unit and on the ultra-thin ($\sim 10\,nm$) cell membrane that surrounds the cell and controls traffic in and out of the cell. The cell is sustained by biochemical reactions, many of which involve electron transfer, while cell functions are generally carried out by membrane components and involve ions. In this review, we shall consider electron and ion transport processes in solution and across membranes. We shall also discuss the effects of EMF on two major classes of biopolymers, proteins involved in transport across membranes, and the DNA in the cell nucleus that can be stimulated to initiate protein synthesis. Charge transfer due to EMF is a likely triggering mechanism in both biopolymers. The overall effect occurs in a two-step process, in which EMF move charges within the biopolymers, and the perturbations cause the biopolymers to change their conformation to accommodate the changes in charge distribution. Many of the biological examples discussed, e.g., the multi-subunit proteins, hemoglobin and Na,K-ATPase, and the DNA that initiates stress protein synthesis, are from studies carried out in this laboratory. Recent reviews describe EMF mechanisms in Na,K-ATPase (Blank, 2005) and in DNA (Blank and Goodman, 2007).

Electron Transfer in Chemical Reactions

Electric and magnetic fields exert a force on static and moving charges, and accelerate them. The largest effects of the fields are on electrons, the unit negative charges, because of their high charge to mass ratio. At the sub-atomic level, the Born-Oppenheimer Approximation assumes that electrons respond instantaneously compared to protons and heavier atomic nuclei. Even weak EMF in the low-frequency range can affect the rates of electron transfer reactions between molecules. A $10\,\mu T$ magnetic field exerts a very small force of only $\sim 10^{-20}$ newtons on a unit charge, but this force can move an isolated electron more than a bond length, $\sim 1\,nm$, in ~ 1 nanosecond.

Effects on electrons in chemical reactions were detected indirectly in studies of the effects of electric and magnetic fields on the Na,K-ATPase (Blank, 2005). Each field, studied separately, accelerated the reaction when the enzyme was relatively

inactive. By assuming that the same charge was affected in the two fields, one could estimate the velocity (v) and determine the nature of the charge (q) that was critical in the action of this enzyme. If both fields exerted the same force at the threshold, we can equate the electric (E) and the magnetic (B) forces:

$$F = qE = qvB. \tag{1}$$

From this $v = E/B$, the ratio of the threshold fields, and by substituting the measured thresholds (Blank and Soo, 1992, 1996), $E = 5 \times 10^{-4}$ volts/m and $B = 5 \times 10^{-7}$ T (0.5 μT), we obtain $v = 10^3$ m/s. This very rapid velocity, similar to that of electrons in DNA (Wan et al., 1999), indicated that electrons were probably involved in the ATP splitting reaction and the ion transport mechanism of the Na,K-ATPase (Blank, 2005). An electron moving at a velocity of 10^3 m/s crosses the enzyme ($\sim 10^{-8}$ m) before the 60 Hz field has had a chance to change. This means that even though a low-frequency sine wave signal was used, the effective stimulus was actually a repeated DC pulse. This is true in all low-frequency studies that involve effects on fast moving electrons.

The magnitudes of the threshold fields that affect the Na,K-ATPase are in the very low range of mV/m electric field and μT magnetic field. The very small force of $\sim 10^{-20}$ newtons on an electron and the very small dimensions and short times, calculated above, are relevant at the molecular level for the proteins and DNA that we consider in later sections. The small magnitudes also suggest boundary conditions on the responses that can be expected from weak fields. In essence, they question the possibility of direct effects of such weak fields on much more massive ions and molecules. There just is not sufficient EMF energy to cause significant movement of ions, especially if they are hydrated. Ions are affected by the much larger DC electric fields involved in physiological membrane processes, a subject treated below.

In the search for weak fields that can cause biological effects, we realized that weak DC magnetic fields are also unlikely to affect physiological processes for the same reasons. The ability of DC magnetic fields to affect lifetimes of free radical pairs (Steiner and Ulrich, 1989) only occurs at field strengths that are several orders of magnitude higher than the AC magnetic field thresholds mentioned earlier and other studies to be discussed. This review is focused on the effects of the low levels of EMF, comparable to those in the environment, that are apt to influence biological processes, so the effects of DC magnetic fields will not be considered.

Electrons are not usually invoked in the mechanism of the Na,K-ATPase, so it was necessary to demonstrate the effects of magnetic fields on electrons in known electron transfer reactions. This was done by studying electron transfer from cytochrome C to cytochrome oxidase (Blank and Soo, 1998) and in the oxidation of malonic acid (Blank and Soo, 2003), also known as the Belousov-Zhabotinsky (BZ) reaction. In both of these reactions, as well as in the Na,K-ATPase reaction, the following was true:

- Magnetic fields accelerated the rate of the reaction at very low thresholds. The experimentally determined threshold values were Na,K-ATPase (0.2–0.3 μT), cytochrome oxidase (0.5–0.6 μT), BZ reaction ($<$0.5 μT).
- In all three cases, magnetic fields were most effective when the intrinsic chemical forces were low, showing that EMF competes with the intrinsic chemical forces driving the reactions. To emphasize the fact that EMF will affect a reaction only

when the intrinsic chemical forces are weak, a recent study reported no effect of magnetic fields on the BZ reaction (Sontag, 2006) under conditions where the chemical forces swamped the magnetic forces. The magnetic fields were only applied well after the reaction was under way and the chemical forces had already set the oscillatory pattern of the reaction.

It was interesting that the two enzymes studied showed frequency optima close to the reaction turnover numbers, Na,K-ATPase, 60 Hz; cytochrome oxidase, 800 Hz, suggesting that the EMF were interacting optimally when in synchrony with the molecular kinetics (Blank and Soo, 2001). As we shall see in a later section, this is not true for magnetic field interactions with DNA, which are stimulated in both the power frequency and radio frequency ranges (Blank, 2007). EMF interactions with DNA do not appear to involve electron transfer reactions with well-defined kinetics. There are no other frequency data on enzymes to add to this list; studies on the enzyme ornithine decarboxylase (Byus et al., 1987) were done at 60 Hz only. While there are very few examples from which to generalize, it is reasonable to expect frequency optima only where electron transfer reactions have well-defined kinetics.

There are additional frequency data for DNA that should be mentioned, but the experiments are quite different from the above studies and the results cannot be compared. The studies involved stimulation of DNA in striated muscle to produce specific muscle proteins by stimulating (electrical) action potentials in the attached nerves. The stimulation of DNA will be discussed in detail in a later section, but the electric fields associated with the action potentials are likely to stimulate electron movement in DNA of the muscle nuclei (Blank, 1995). The two frequencies studied in muscle, high (100 Hz) and low (10 Hz) frequency, were chosen to correspond to the frequencies of the fast muscles and slow muscles that are characterized by different contraction rates and different proteins. In the experiments, either the fast or slow muscle proteins were synthesized at the high- or low-frequency stimulation rates corresponding to the frequency of the action potentials. This clear frequency dependence on electric fields was to be expected from the muscle physiology, but it is unlikely to have come from particular electron transfer reactions as in cytochrome oxidase. It is more probable that an entire region of DNA, coding for multiple proteins, was activated simultaneously.

Many of the biochemical charge transfer reactions that occur in living cells are oxidation-reduction reactions, but by and large, they have not been the concern of biologists interested in EMF mechanisms. It is the electrochemists who study electron transfer mechanisms at electrode surfaces driven by electric fields, and who ask such questions as the number of steps in a reaction, number of electrons transferred per step, rate of each step, etc. Those concerned with biological EMF mechanisms are oriented towards cell function and focus on physical chemical processes involving membranes and ions, the topic of the following section.

Cell Membranes and Ion Transfer

The functional unit in physiological systems, the cell, is surrounded by an ultra-thin ($\sim$10 nm) cell membrane having the basic structure of a phospholipid bilayer. The bilayer serves as a matrix in which many different functional elements (e.g., enzymes, channels, transporters) are embedded in varying amounts in different tissues. In the

red cell, a relatively inactive cell, the functional elements constitute about half of the membrane (Blank et al., 1979), while in active synaptic vesicle membranes there is twice as much protein by weight as lipid. A diagram of a synaptic vesicle membrane is on the cover of the November 17, 2006 issue of *Cell*.

Cells are sustained by biochemical reactions, many of which involve electron transfer, but the charge transport processes in many cell functions (e.g., nerve, muscle conduction) primarily involve ions and the much more energetic electric fields needed to transport them. This accounts for the focus on ions and electric fields as triggers of physiological processes. The word trigger is appropriate. Electric fields transfer relatively small amounts of charge that cause changes in the membrane, which then allow the normal ion gradients to cause much larger changes in the cell. This will become clearer when we discuss the effects of electric fields on ion gradients across membranes and on ion channels in membranes.

Ion transport differs from electron transport in many ways. Ions are much more massive, have both positive and negative charges, and are stable in solution. In ion transport studies carried out in electric fields, cations and anions move in opposite directions and at different speeds because of their different sizes and degrees of hydration. These differences lead to significant *ion concentration changes* due to ion transport across ion selective membranes.

Living cells have compositions that differ markedly from the surrounding solutions, so natural membranes normally separate solutions having very different ionic compositions and concentrations. K is the main intra-cellular cation and Na is the main extra-cellular cation, so large ionic gradients exist across cell membranes (see bold faced symbols K and N in Fig. 1). Most cell membranes are cation selective, and differences in the rates of diffusion of K and Na across membranes lead to membrane potentials of about 100 mV. Ionic leaks are compensated by 'ion pumps', such as the Na,K-ATPase to be discussed in a later section, so the steady-state potentials are known as resting potentials. When nerves or muscles are activated, the changes in membrane potential are called action potentials.

Because of the differences in steady-state concentrations across the membrane, and because the permeability of the membrane to K is normally much greater than to Na, small currents due to applied electric fields can cause large changes in the ionic concentrations at the membrane surfaces. Take the examples given in Fig. 1, of an electric field across a cation selective membrane that separates a cell from its surrounding solution. Both solutions contain the cations sodium (N) and potassium (K), shown with N higher outside and K higher inside, as normally distributed in cells and with the symbols for the steady state N and K in proportion to the concentrations. (The anions are the same concentration on both sides and assumed not to cross the cation selective membrane.)

An ion current, indicated by arrows, will be carried by both ions, but in different proportions because of the steady-state ion concentrations across the membrane. In the top panel for an outward current, the major part of the current is carried by K. In the bottom panel for an inward current, the major part of the current is carried by N. The main result of a sustained DC current flow in either direction, shown in italic symbols, is a decreased cation gradient across the membrane for each cation. This means that a depolarizing current that normally stimulates a nerve and causes sodium ion flux actually decreases the concentration gradient (i.e., the chemical driving force) of the sodium ions that start the action potential. The decreased cation gradients across the membrane also decrease the membrane potential and affect

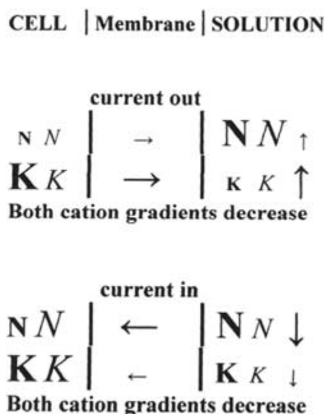


Figure 1. Changes in sodium (N) and potassium (K) concentrations at the surfaces of a cation selective membrane due to the current flow in outward and inward directions, as indicated by the arrows in the membrane. The relative sizes of the symbols N and K in the solution compartment indicate the relative concentrations in the two solutions, and the sizes of the arrows indicate the relative magnitudes of the current. The bold symbols represent the steady-state concentrations and the italic symbols show the concentrations after current flow in the two different directions. The upper diagram is for current out of the cell, when cations in the solution increase, and the lower diagram is for current into the cell, when cations in the solution decrease. Current in either direction leads to a reduction in the concentration gradients of both cations.

the distribution of charge across the membrane. As discussed in a later section, because of a direct effect on the charges as well as an indirect effect due to lowering the membrane potential, a depolarizing current opens ion channels, which are the major contributor to the increased ion fluxes. The depolarizing currents also have a direct effect through the changes in ion concentration at the membrane surfaces.

The changes in concentration at the membrane surfaces persist there, because they are dissipated slowly by diffusion into the solution. Such changes were demonstrated when the actual concentration of ions at a surface was measured by transporting surface active ions across liquid/liquid interfaces. The surface active ions carried the direct (DC) current and also indicated their presence at the interface by changes in interfacial tension (Blank and Feig, 1963). The concentration changes during current flow were significant and relatively long lived.

Intuitively, one expects that passing an alternating current (AC) through a cell might leave no net effect, because the processes during the initial half of the cycle would be canceled in the second half, when the electric field is reversed. However, it is easy to see from Fig. 1 that for cation selective cell membranes with cation gradients across them, the effects of AC on cation concentrations are additive. When considering an entire cell, the inward current directed into one side of a cell appears to be balanced by an outward current on the other side. Here again, we see from Fig. 1 that the effects on both sides of a cell are in the same direction. Cation gradients are reduced on both sides.

Because the effects on the cation concentrations are additive, even small AC electric fields lead to significant changes over time. The effects of AC currents through a simple theoretical model membrane showed that the concentrations do not increase indefinitely because of diffusion away from the surface and binding

reactions with fixed charges at the membrane surface. The effects varied with the AC frequency (Blank and Blank, 1986), depending upon the ion binding constants to fixed counter charges on the surface and ion mobilities in solution. It has been known for a long time that AC currents across nerves can reduce and block their activity. AC apparently decreases the ion gradients to the point that they can no longer drive the action potentials.

The fixed charges at a membrane surface not only can bind to the ions near the surface layer, but the change in surface charge can affect ion transport through the surface. To study the effect of the charge on a surface on the ability of ions to diffuse across the boundary, Miller and Blank (1968) used charged monolayers to show that the rate of ion transport is controlled by the charge on the surface. The effects of charged surfaces on the ability of ions to cross an interface could be explained by the expected ion concentration changes in the surface region, e.g., fixed positive charges reduce concentrations of adjacent cations and increase anion concentrations.

These studies show that ions at membrane surfaces may be important for understanding biological ion transport across membrane dimensions and in millisecond time scales. Actually, the surface concentration of ions at an axon membrane surface in the steady state is comparable to the magnitude of the ionic flux during an action potential. The number of ions stored at an axon membrane surface, having a capacitance of 10^{-6} farads/cm^2 and a resting potential of 100 mV, is about $\sim 10^{-12}$ ions/cm^2. The magnitude of the ion flows in an action potential is also about $\sim 10^{-12}$ ions/cm^2 of nerve axon membrane surface.

When discussing ion concentration changes at membrane surfaces and changes in polarization across membranes, it is important to realize that there is a major difference between the characteristic response times of chemical systems and electrical systems. In transient or non steady-state membrane processes, the two driving forces for ionic movement, the chemical potential for diffusion and the electrical potential for migration, change at very different rates. A membrane can be depolarized quite rapidly, with time constants on the order of 1–10 *micro*seconds, while chemical potentials readjust at much slower rates with time constants of about 1 *milli*second, characteristic of diffusion processes over distances on the order of cell diameters. It is therefore possible to generate unbalanced chemical gradients for short periods of time by manipulating membrane (electrical) potentials. The disparity in the response times of the two forces that drive ions across membranes can lead to unusual transient ionic fluxes.

Biological systems add an additional complication to the changes expected in physical systems, i.e., changes in ion concentration at surfaces due to depolarizing currents and due to the great disparity between the rates of change in concentration and electrical potential. In biological systems there are voltage-dependent ion channels that open when depolarized. This topic will be discussed in greater detail in a later section.

An analysis of the ion flows in excitable membranes, called the Surface Compartment Model (Blank, 1987), showed what happens when all of these factors occur in the layers of solution immediately adjacent to the membrane surfaces, specifically:

- the changes in ion concentration due to depolarizing currents (Fig. 1), ion flows under electrochemical forces (described by the same equations that apply to ions in solution), and any ion exchange between Na and K that occurred with fixed surface charges at the membrane surfaces due to changes in concentration;

- the disparity between the rates of change in ion concentration by diffusion and migration, and the much faster changes in electrical potential;
- the effects due to voltage-dependent ion channels that open by a charge transfer process shown in Fig. 3, to be discussed in detail later. The ion channels that had been incorporated into an empirical description of ion transport across membranes were complicated functions of time, while these were dependent on charge distribution.

The Surface Compartment Model was able to show that these factors could account for the unusual ionic fluxes seen in excitable membranes. It also showed how the apparent selectivity of channels could vary with different rates of opening. This description of ionic fluxes in excitable membranes offered insights into factors that contribute to the unusual fluxes and the apparent ion selectivity in channels.

It is obvious that the electrical activity that drives nerves and muscles utilize mechanisms that take advantage of ionic gradients that are normally present in living systems. These ionic gradients are built up by the action of membrane enzymes like the Na,K-ATPase and are fueled by the energy from the splitting of ATP. Consequently, it takes relatively little energy to trigger an action potential and take advantage of the energy stored in the ionic gradients across cell membranes. The ion fluxes that evoke an action potential are very weak stimuli by comparison. However, it does take energy to open the voltage gated ion channels and various transporters in the membrane. This source of energy, triggered by changes in charge, is the conformational energy stored in chemical structures. This is a probable explanation for the way ion channels are stimulated to open by depolarizing currents, and also for the way very weak EMF can stimulate responses in DNA, both of which require considerable energy.

Proteins and Hydration Energy–Hemoglobin Equilibria

The energetics of intermolecular interactions and interactions with water as a solvent determine membrane structure, as well as the changes that occur when perturbed by applied EMF. Among the early attempts to understand the energetics of chemical structures and their relation to chemical properties, Langmuir (1916) showed that the surface tension of a pure liquid could be derived from information about the interaction energy between molecules. Vaporizing a liquid breaks all bonds between molecules, while molecules at a liquid surface are not completely surrounded and miss interactions with the missing neighbors. It is the missing interactions that give rise to the surface tension. The unbalanced energy at a surface requires molecules to have extra energy to get to the surface, and that the liquid minimizes the energy and the surface area. Langmuir's success in relating surface tension to heat of vaporization indicated that nearest neighbor interactions account for most of the energy, and that the change in surface free energy (i.e., the surface tension) is a good approximation to the total free energy change.

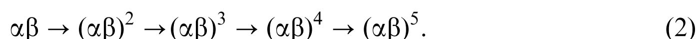
The situation in aqueous solutions is more complex, but we have estimated the total free energy change of a molecule in solution from the changes in surface area when interacting with water. In aqueous solutions, the interactions with water are quite energetic and have a profound influence on equilibria, especially those involving proteins. Lauffer (1975, 1989) characterized the aggregation of multi-subunit

protein molecules in aqueous media using the short-hand phrase "entropy driven" to summarize the energetics of the interaction. The aggregation is spontaneous (i.e., the free energy change, ΔF, is negative) and it occurs with an evolution of heat (i.e., the enthalpy change, ΔH, is positive). The negative ΔF together with the positive ΔH means that there is a large positive entropy change. Hence, "entropy driven". The large increase in entropy is due to release of many water molecules when the hydrated proteins come into contact after releasing their bound water. The increase in ΔH is another consequence of the release of water from protein surfaces and the aggregation of the protein subunits.

This description is correct but incomplete, because aggregation is very dependent on pH while hydration is not. The pH affects molecular charge, since it is well known that proteins disaggregate as the charge increases, and they aggregate as the charge decreases. Two often quoted examples are hemoglobin (Fanelli et al., 1964) and tobacco mosaic virus protein (Klug, 1979). It is possible to extend Langmuir's approach to include an effect of charge. The aggregation of multi-subunit proteins with a decrease in molecular charge can be formulated as a simple relation between molecular charge and the area of the protein molecule in contact with aqueous solvent. The basic idea is that proteins in aqueous media minimize their surface free energy by decreasing contact with the water *and* decreasing charge. For this reason, decreases in charge drive the protein toward aggregation. However, when there is an increase in charge, the two driving forces compromise and there is an increase disaggregation. The repulsive forces between charges would increase the surface free energy, and this can only be reduced by an increase in area. Disaggregation spreads the charges and lowers the repulsion between them.

This simple model using surface free energy to account for the influence of charge on subunit assembly was shown to apply quantitatively to the protein, hemoglobin (Hb) as a function of surface charge (Blank and Soo, 1987). The actual study, the disaggregation of the Hb tetramer $(\alpha\beta)^2$ into 2 dimers $(\alpha\beta)$, where α and β are protein subunits, showed that the concentration of hemoglobin dimers increased linearly with surface charge as the pH varied from the isoelectric point. As the Hb tetramers were disaggregating, the increasing charge was being spread over an increasing protein/water interface, and the surface charge density (total charge/total molecular area) remained constant.

The same surface free energy model could also account for the unusual effects of increasing concentration of Hb on the viscosity of solutions (Blank, 1984) if one assumes that the increase in viscosity with Hb concentration is due to aggregation into larger particles. The same forces that drive the aggregation of dimers to tetramers should continue because of the same loss of area upon aggregation:



$$\alpha\beta \rightarrow (\alpha\beta)^2 \rightarrow (\alpha\beta)^3 \rightarrow (\alpha\beta)^4 \rightarrow (\alpha\beta)^5. \quad (2)$$

At the point where the chain becomes long enough to close upon itself, there should be a steep change in the equilibrium. The closing of the chain means that an added $\alpha\beta$ has caused two links, with double the loss of interfacial area and double the free energy change. A closed chain would also account for the steep increase in viscosity, since a chain where the ends are joined is no longer as flexible and behaves more like a rigid rod.

The relation between changes in interfacial area and the free energy change enabled a semi-quantitative estimate of the energy change due to the changes in molecular shape when Hb is oxygenated. The conformational changes in Hb, documented by X-ray crystallography, enabled estimation of the interfacial area. The charge on the Hb at different pH's could be determined from titration studies, such as those in the study of disaggregation. The data enabled calculation of the acid and alkaline Bohr effects, the names given to the variation of the oxygenation equilibrium constant with pH and ionic strength (Blank, 1975).

The success of the surface free energy model in calculating the acid and alkaline Bohr effects demonstrated the predictive value of the relation between changes in surface free energy and the equilibrium constant. This idea also led to understanding the physical meaning of the empirical Hill coefficient that is widely used as a measure of cooperativity. By using the surface free energy model to estimate conformational changes (e.g., Hb), it was possible to show that the changes in free energy that affect the equilibrium constant are simply related to the Gibbs surface excess, a fundamental property in surface chemistry (Blank, 1989). According to the surface free energy model, the Hill coefficient is not empirical and is not constant. It varies with the degree of reaction, has a maximum value at the half way point, and is definitely equal to unity at both extremes. The approach to unity has been observed in the reaction between Hb and oxygen (Paul and Roughton, 1951).

The surface free energy model is a way to estimate the energy changes due to the hydration of nascent hydrophilic surfaces of biopolymers, such as proteins and nucleic acids, in terms of the surface free energies of newly formed surfaces. To make calculations, one needs estimates of surface areas and surface charge, so it has been relatively easy to apply these ideas to many properties of Hb, a well-characterized molecule. The model has also related the conformational changes of voltage-gated channel proteins (Blank, 1987, 1989) to the depolarizing currents that transfer charge across a channel, and the conformational changes of the Na,K-ATPase (Blank, 2005) and other membrane transporters to the charge movement when ATP splits. The same effects of EMF on charge movement may account for the ability of EMF to cause DNA to initiate protein synthesis (Blank and Goodman, 2007). These are examples of biological amplification that are related through the ability of small charge movements to stimulate large structural changes utilizing the energy stored in biopolymer conformation. The following three sections are devoted to Na,K-ATPase, ion channel proteins, and DNA.

Membrane Transport Proteins–Na,K-ATPase

Many of the biological transport systems embedded in membranes are multi-subunit proteins that can open to both sides of a membrane in alternate conformations. This process enables the binding of substances to one side of the protein and subsequent release to the other side after a conformation change. The opening of a transporter creates new protein water interfaces and involves changes in binding of the subunits with each other, the water and the bilayer lipids. Similar reactions occur when the protein opens on the other side. If the two open states of the protein on opposite sides of the membrane were of approximately the same energy, it would minimize the energy required for the transport. In transport, the conformation change is usually triggered by the energy released from th ATP splitting reaction.

This type of transport mechanism has been documented for many different substances. A short list of recent articles includes studies on β-galactosidase and glucose-6-phosphate (Locher et al., 2003), various drugs (Dong et al., 2005; Yin et al., 2006; Reyes and Chang, 2005), zinc (Lu and Fu, 2007), metal chelates (Pickett et al., 2007), and vitamin B12 (Hvorup et al., 2007). The mechanism is best known from its association with the Na,K-ATPase, the enzyme that "pumps" Na and K ions against their gradients across cell membranes.

The Na,K-ATPase is probably the best studied of this class of transporters, known as ABC (ATP Binding Cassette) transporters, and as such it offers insights into how ATP driven conformation changes can occur in bilayer structures. The lipid bilayer membrane is stable because of hydration forces, and the term hydrophobic interactions used to describe these forces indicates that the lipid molecules interact with each other and avoid contact with water molecules. Exposing bilayer lipid molecules to water is energetically unfavorable, so membrane transport mechanisms utilize multi-subunit proteins in the bilayer that have hydrophobic areas that can interact with lipid molecules in the bilayer and hydrophilic areas that can interact with water at the surfaces. Because of their compositions, transporters can flip their conformations from inner-face-open-to-water to outer-face-open-to-water to enable the transfer of molecules by expanding the hydrophilic areas and contracting the hydrophobic and vice versa. In the Na,K-ATPase the different conformations are determined by the binding of Na, K and ATP.

The Na,K-ATPase is composed of two polypeptide chains (α and β) that extend through the bilayer in the form of a tetramer ($\alpha_2\beta_2$). The ATPase activity resides in the α chain and is directly influenced by the ion concentrations in contact with the two sides of the enzyme (Skou, 1957; Tonomura, 1986; Lauger, 1991; Jorgensen et al., 2003). The Na,K-ATPase is activated when sodium ions bind on the inside surface and potassium ions on the outside surface. In a complete cycle, the catalytic unit splits ATP on the inside surface, and for each ATP molecule split, 3 Na^+ ions move from inside out and 2 K^+ ions from outside in.

The enzyme complex has two conformations, E_1 when Na^+ ions (and ATP) are bound on the inside, and E_2 when K^+ ions are bound on the outside. The ion binding sites are not fully accessible to ion exchange with the surrounding solutions in the two conformations (Rephaeli et al., 1986; Glynn and Karlish, 1990). Potential sensitive dyes show charge shifts at specific points in the ATP-splitting cycle (Buhler et al., 1991). A release of Na^+ ions accompanied a rapid movement of charge when binding sites open to the outer surface in the presence of Na^+ ions (Hilgemann, 1994). These data suggest that conformational changes of the Na,K-ATPase and charge shifts within the protein are involved in the mechanism. The effects of applied low frequency electric and magnetic fields on Na,K-ATPase function, presented earlier, provide additional evidence of rapid charge movement that contributes to the conformation change after the enzyme has reacted.

The key to the conformation change is the rapid shift of charge across the enzyme. Figure 2 illustrates changes in a protein channel that starts with an asymmetric charge distribution. The outside surface is normally negatively charged, and the charged groups interact with water. This expanded area of contact with water is open to the outside. A significant shift in charge causes the channel to shift from an inside facing channel to an outside facing channel. If the charges crossing the enzyme are electrons, they cross very rapidly to the opposite side of the enzyme, and the ratio of charged hydrated area and uncharged unhydrated area remains virtually unchanged. With

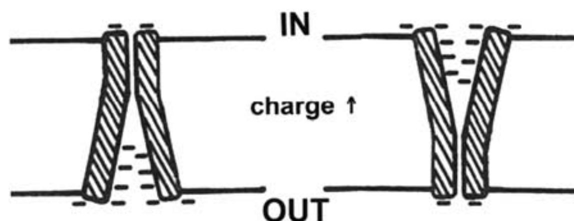


Figure 2. Changes in a protein channel that starts with an asymmetric charge distribution, and there is a large and rapid shift in charge as indicated by the arrow. The outside surface is initially negatively charged, and an expanded area of contact with water faces the outside. A large shift in charge causes the channel to change from an inside facing channel to an outside facing channel. If the charges crossing the enzyme are electrons, they cross very rapidly to the opposite side of the enzyme, and the ratio of charged hydrated area and uncharged unhydrated area remain virtually unchanged and with virtually no net change in energy.

virtually no net exothermic aggregation or endothermic disaggregation, the conformation change probably occurs with a minimum of energy change.

It is not generally accepted that ATP splitting and the accompanying ion transport involve electron transfer. However, it is quite clear from EMF measurements discussed earlier that there is a rapid flow of charge through the enzyme, resulting from the enzyme reaction. This flow of charge could trigger the sequence of conformation changes that are part of the cation transport mechanism (Blank, 2005). The effective concentrations of non-specific cation inhibitors of the Na,K-ATPase were related to the redox potentials (Britten and Blank, 1973), suggesting involvement of an electron transfer step. Many observations associate electrons with the ATPase reaction. In mitochondrial function, the ATP synthase catalyzes the same reaction and is directly coupled with electron transport. In the ATP synthase, it is possible to stop the flow of electrons in the electron transport chain with inhibitors, or to reverse the flow of electrons by changing the concentration of substrates. The electron transport chain can also be made to go in reverse when ATP is hydrolyzed and electrons are fed into the chain.

In line with the known reversibility of ATPases in mitochondria, Garrahan and Glynn (1967) were able to reverse the Na,K-ATPase reaction in red cells to generate ATP. They did this by creating a supernormal K ion gradient, thus hyperpolarizing the membrane. When the membrane potential changed from $-15\,\mathrm{mV}$ to $-85\,\mathrm{mV}$, they were able to approximately double the ATP concentration from ADP and phosphate normally present. The increase in membrane potential makes the region near the catalytic portion of the Na,K-ATPase on the inner surface of the membrane more negative. The increase in H ion concentration near the enzyme would be expected to drive the reaction toward making more ATP. In any case, the experiment clearly shows the tight coupling between the ATPase reaction and ion flow across the membrane. It also shows their similar reversibility to charge flow, the Na,K-ATPase to ion flow and the mitochondrial ATP synthase to electron flow.

Charge Transfer and Ion Channel Function

In the section on ion transfer, the transient ion flows in excitable membranes were described in terms of concentration changes in the layers of solution immediately

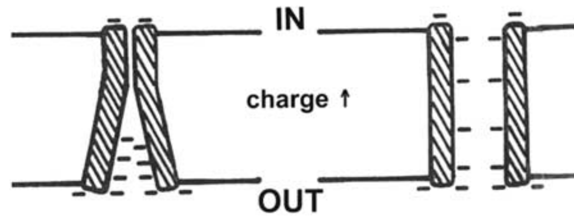


Figure 3. Changes in a protein channel that starts with an asymmetric charge distribution, and a large portion of the charge shifts rapidly, as indicated by the arrow, to spread across the length of the protein in the bilayer. If the charges crossing the enzyme are electrons, they can spread out very rapidly. The shift in charge is sufficient to open a hydrophilic channel and create a conduit for ions from inside to outside solutions. This implies that the charged parts of the protein that interact strongly with water create a continuous aqueous path. Because there is a change in the ratio of charged hydrated area and uncharged unhydrated area, this process must result in a significant change in energy. The distribution of charge depends on the membrane polarization, and if the charge movement is reversed by repolarization, the channel closes.

adjacent to the membrane surfaces. These thin regions were referred to as surface compartments and the equations describing the processes as the surface compartment model (Blank, 1987). The main processes were variations in Na and K ion concentrations due to depolarizing currents, ion exchange between ions in solution and those bound to fixed surface charges at the membrane surfaces, and the very different rates of ion concentration changes by diffusion and changes in electrical potential. Voltage-dependent ion channels that open and close depending on changes in charge distribution were included in the description, but a fuller discussion was deferred until after the section on transport mechanisms in the lipid bilayer.

The discussion of voltage dependent ion channels is easier to understand following the section on multi-subunit protein transporters that flip from inner-face-open-to-water to outer-face-open-to-water. Proteins like the Na,K-ATPase can apparently negotiate these changes with a minimum change in hydration energy by keeping the ratio of hydrated and unhydrated protein surfaces relatively constant during the charge transfer. However, this does not appear to be possible with the opening of an ion channel, where the whole length of a hydrophilic pathway through the bilayer must be open to enable the continuous flow of ions. Figure 3 shows a protein channel that starts with an asymmetric charge distribution, and where a large portion of the charge spreads across the length of the protein in the bilayer. If the charges are electrons, they can spread very rapidly. The change in the ratio of charged hydrated area and uncharged unhydrated area must result in a significant change in energy, and the energy change must be reversed when channel returns to its resting state, i.e., closes.

The surface free energy model can relate the opening of voltage gated channel proteins (Blank, 1987, 1989) to charge transfer due to the depolarizing currents, and it also provides a way to evaluate the energy changes that occur. The process shown in Fig. 3 assumes that the gating currents in excitable membranes transfer charge across the protein, and this changes the energetics of the channel protein to favor opening a channel. Since disaggregation is endothermic and aggregation exothermic, the model predicts an initial cooling as protein contacts water on channel opening, followed by heating on channel closing. The thermal changes should be quite large because of the

nature of hydration interactions and the protein surface areas involved. As described below, thermal changes occur, but not quite as predicted by Fig. 3.

Thermal measurements are generally difficult, especially when the changes are rapid and the systems small, as with nerves. It is always difficult to get an accurate measurement of temperature change when the action potentials in nerves are faster than the response time of the thermal sensors. Also, action potentials involve the opening and closing of two sets of channels at different rates. There are Na channels that enable the initial rapid depolarization, and K channels that account for the slower repolarization phase but that may open at the same time. The effect of an overlap of opening and closing on the temperature sensor further complicates the analysis. To add to the difficulties, even the easiest nerves to study contain many axons that conduct action potentials at different rates, so there is some interference because of slow and fast conducting axons. Analyzing these data is an unenviable challenge.

Despite the difficulties, thermal measurements have been made and analyzed, and there is agreement about the observations. In excitable membranes, the heat associated with excitation of nerve (Howarth et al., 1968) or electric organ (Keynes and Aubert, 1964) shows three distinct phases during an action potential. There is an initial, short-lived warming phase followed by a longer cooling phase of comparable amplitude and a still longer warming phase having the largest amplitude and most probably associated with recovery mechanisms. The net heat evolved is actually small in comparison with the initial heating and cooling, suggesting that the net heat is a measure of the dissipation due to the flow of ions down electrochemical gradients, and the chemical bond energy used to restore the ionic gradients.

It is difficult to interpret the measurements in terms of channel protein interactions, because there are multiple sources of thermal changes. These include current flow during the action potential, discharging and recharging the membrane capacitor, ion pumping during recovery, etc. The major changes of heat appear to be due to reversible processes, and the discharging and recharging of the membrane capacitor can account for about half of the reversible heat change observed. The changes in hydration energy during channel opening and closing are another source that may account for the reversible changes. It would be hard to find another source for the large negative heat, which is a major unexplained aspect of the process.

We can estimate the energy changes from channel opening and closing, assuming that the number of sodium channels per unit area of membrane is the same as in unmyelinated C fibers of rabbit vagus nerve (Howarth et al., 1968) of 110 nmol/kg wet weight. C fiber diameters range from 0.4–1.2 μm, so assuming an average diameter and a density of Na channels comparable to the squid axon (Levinson and Meves, 1975), it is possible to estimate the measured heats per gram from the estimated positive heat of 25 μcal/g and the negative heat of 22 μcal/g. If all of the ΔH were due to the reactions of the proteins in the channels, the negative heat is a better measure of the largely reversible ΔH for channel opening and closing. In that case, the reversible channel process involves a ΔH of about 6 kcal/mole of channel protein (molecular weight 270 kD), or about .02 cal/g of channel. The ΔH for the aggregation of tobacco mosaic virus protein (Klug, 1979) is about 0.7 cal/g. This implies that only about 3% of the protein surface is involved in the reactions affecting channel opening and closing. Since the discharging and recharging of the capacitor can account for about half of the reversible heat change observed, only $\sim$1% of the protein surface can account for the unexplained heat.

The measured heats appear of reasonable magnitude, but the sequence is at odds with what would be expected if the simple model depicted in Fig. 3 were the only source of heat exchanged. One expects the positive ΔH for channel closing to coincide with the falling phase of the action potential, and channel opening should be associated with a negative ΔH or heat absorption. The channel is certainly more complicated than the model in Fig. 3, and so are the thermal changes. The heat evolved during the discharging of the membrane capacitor is simultaneous with the heat absorbed during channel opening. The two are also opposed during repolarization and channel closing. Furthermore, the discharging is much faster than the recharging. Undoubtedly, the thermal measurements are missing a large part of the heat exchanged, and the heat changes associated with channel opening and closing are therefore much greater than we have estimated and involve a much larger fraction of the protein surface.

In the absence of an all-inclusive and accurate analysis of all the thermal contributions to the measurements, it is nevertheless clear that an action potential is accompanied by:

- a net heat evolution as one would expect in a dissipative process;
- a reversible heat due to discharging and recharging the membrane capacitor; and
- a reversible heat of channel opening and closing due to the hydration energy associated with a small part of the protein surface.

A recent article accounts for the unexplained heat changes during an action potential by suggesting the possibility of soliton propagation in the membrane lipids as the source (Jackson, 2005; Heimburg and Jackson, 2006). The authors point out that this idea can also account for the well-known Meyer-Overton correlation between the effective concentrations of a wide range of anesthetics and their oil/water partition coefficients. The Meyer-Overton correlation is not a particularly good test, because many theories predict that correlation. In a review on anesthesia, Vandam (1966) referred to two then popular new theories of anesthesia—Pauling's clathrate formation theory and Miller's dissociation pressure of hydrates—and pointed out that any theory based on weak interactions between anesthetics and other molecules is bound to correlate with the Meyer-Overton data.

A better counter argument to the soliton proposal is probably invoking Ockham's razor rather than a detailed discussion and evaluation. Simply stated, voltage-gated ion channels are acknowledged by all to be clearly associated with the action potential, and the properties of these essential proteins may be able to account for the thermal observations without the need to turn to the properties of the matrix in which the channels are embedded. It could be that some of the optical properties ascribed to the lipids by Heimburg and Jackson are also associated with the much larger structures that appear to be parts of channels, such as the cytoplasmic components of the K channel (Long et al., 2005). Certainly, the observed changes in the thermodynamic properties are to be expected from the protein channels.

Electromagnetic Field Stimulation of DNA

One of the earliest biological effects of EMF to be described was the ability to stimulate biosynthesis (Goodman et al., 1983; Goodman and Henderson, 1988). Since those early experiments, it has been shown that EMF in both extremely low frequency

(ELF) and radio frequency (RF) ranges stimulate protein synthesis. This means that even the weak EMF in the ELF range have made DNA come apart to initiate protein synthesis. So it is no surprise that EMF can cause dose dependent, single and double strand breaks in DNA at higher field strengths and higher frequencies (Lai and Singh, 1997; REFLEX Report, 2005; Ivancsits et al., 2005; Winker et al., 2005).

The data suggest that weak EMF produce strains in DNA that can cause the chains to separate, and if the strains are large enough, cause the chains to break. Since DNA is held together by H-bonds, and since EMF are most likely to act on electrons, EMF probably act on electrons in the H-bonds to weaken the bonds. Electrons could also be affected in the H-bonds that flicker in water at a frequency $\sim 10^{15}$ Hz, and that would be expected to do so in aqueous solutions as well (Fecko et al., 2003; McGuire and Shen, 2006). This would create many transient protons and electrons in and around the DNA solution that can be accelerated by EMF.

In research focused on the stimulation of a specific stress protein, hsp70 (Goodman and Blank, 1998; Blank and Goodman, 2002, 2004), it has been possible to identify specific DNA sequences in the promoter of this protein that are needed for the EMF response (Lin et al., 1999, 2001). This was clearly demonstrated when the EMF responsive DNA sequences were transfected into the promoter of a reporter gene, and the reporter gene responded to EMF (Lin et al., 2001). The EMF responsive DNA sequences on the promoter contain sites with bases CTCT that appear to be essential. CTCT bases have low electron affinities, so electrons would be more easily displaced. Also, the CTCT are pyrimidines, and when the H-bonds split between CTCT and the GAGA (purines) bases on the complementary chain, there is a smaller smoother area that would make it easier to disaggregate.

When electrons are displaced by EMF, it can be shown that there is a favorable energy balance in the DNA disaggregation that enables the process to proceed. Strong reactions between the newly exposed DNA surfaces and water contribute to the energetics of the process. Blank and Goodman (2007) estimated the energies associated with the changes, and showed that the aggregated and disaggregated DNA structures can have equivalent energies. A simple model of disaggregation due to an increase in charge at a local site shows that an increase in area lowers the increased charge density, and that DNA cleavage would be optimal for short segments and low initial charge. The essential CTCT sites identified on the promoter may be sites of DNA cleavage or sites from which electrons have been displaced. In DNA, the initial charge can fluctuate, since electrons in DNA are not localized and are able to move as a result of the random fluctuations in H-bonded networks. This would mean that the area of DNA exposed to water molecules also fluctuates, on a slower time scale, and that some fluctuations may produce large temporary increases in local charge density. At that point, the two DNA chains would come apart to create more surface in contact with water.

The method to estimate the energy change at the DNA site associated with the response to EMF uses the same criterion as in the disaggregation of multi-subunit proteins due to charging. In proteins, where Q is the initial charge and A the area of protein exposed to water, we found that the surface charge density, Q/A, remained constant while both Q and A increased (Blank and Soo, 1987). In DNA, Q is the initial charge due to partially ionized phosphate groups and A the initial area of a DNA segment exposed to water. We assumed the surface charge density, Q/A, remained constant while both Q and A increased. This way the tendency to minimize

the surface and to spread the charge over the maximum surface (thereby minimizing the repulsion between charges) was balanced. The separation of the DNA chains enables initiation of transcription.

If ΔA is the extra area that opens up to water when 1 charge is added to a segment having an initial charge, Q, we can set the charge density before equal to the charge density after a split

$$\frac{Q}{A} = \frac{Q+1}{A+\Delta A}. \tag{3}$$

From this,

$$\Delta A = \frac{A}{Q} = \frac{1}{\text{charge density}}. \tag{4}$$

This means it is easier to open up a larger ΔA if one starts with a larger A, but not so large as to minimize the effect of adding one charge. Also, the fractional increase in open area will be greater as the charge density decreases. In any case, the opening must be large enough to allow access to the transcription enzymes. The optimal segment size may be the four base pair CTCT that was found to be associated with the EMF response.

The stimulation of DNA by magnetic fields is related to the physiological mechanism in striated muscle, where electric fields (not EM fields) associated with action potentials stimulate the DNA in muscle nuclei to synthesize muscle proteins *in vivo* (Blank, 1995). The effect is due to the electric field stimulus, since there is a clear relation between the muscle proteins synthesized and the frequency of the action potentials. Under normal physiological conditions, an action potential along a muscle membrane creates an electric field estimated at $\sim 10\,V/m$ (Blank and Goodman, 2004). In striated muscle, this electric field drives the currents across the DNA in nuclei that are normally adjacent to the membrane carrying the action potential, and the DNA is stimulated to synthesize different muscle proteins in response to the frequency of the action potentials. The magnitude of electric field provides a large safety margin in muscle, since fields as low as $3\,mV/m$ stimulate HL60 cells, and the threshold electric stimulus for the Na,K-ATPase is even lower, at $\sim 0.5\,mV/m$ (Blank and Soo, 1992).

This model based on an ability to displace charges in DNA can account for observations on activation of DNA by either electric or magnetic fields. The same effects should be stimulated by a wide range of frequencies. ELF and RF frequencies have been shown to stimulate stress protein synthesis (Blank, 2007) and because of the relation to H-bond fluctuation frequencies described earlier, there is reason to believe that frequencies up to $\sim 10^{15}\,Hz$ would be effective (Blank and Goodman, 2007).

The Proposed Mechanism in Perspective

EMF do not have sufficient energy to directly affect large protein and DNA molecules, but even weak electric and magnetic fields can cause changes in charge distribution that trigger large structural changes in proteins. Electric and magnetic fields can move both ions and electrons, but they require very different energies

because of the different masses of the charged particles. The electric fields that normally affect ions in physiological systems are orders of magnitude stronger than the magnetic fields that affect electrons. Yet, both initial reactions cause changes in charge that couple with chemical forces and provide sufficient energy to trigger physiological processes. Much of the energy in biopolymer conformations is in the form of hydration energy, and this energy can drive many of the physiological processes stimulated by EMF. The similar effects on DNA when stimulated at high or low frequencies suggests that the biological mechanisms utilize the hydration energy stored in molecular conformations, even when strong EMF forces are available.

Biological systems tend to be energy efficient even when large energy stores are available to drive these processes. The chemical changes in biopolymers triggered by charge movements frequently involve conformational changes between structures of approximately equal energy. Also, biological systems appear to use a wide range of frequencies to drive these processes. The few biochemical reactions that show a frequency dependence (Blank and Soo, 1998b) suggest synchronization of the signal with the kinetics of the reaction. On the other hand, EMF stimulation of stress protein synthesis occurs in many cells with a wide range of frequencies (Blank, 2007).

The purpose of this review has been to develop an understanding of possible biological mechanisms of EMF based on experimental results. However, it is important that the proposals should also be considered in the context of a more general discussion in the EMF literature. In the past, a frequent criticism of experimental EMF studies describing biological changes has been the absence of a mechanism to account for the effects of weak EMF. The absence of a theoretical framework was often presented as an indication that the results were not possible. Despite the clear experimental evidence of repeatable biological effects, this point of view was made to sound plausible by the relatively large energy demands of the biological phenomena ascribed to stimulation by weak EMF. The present proposal indicates a huge energy source that can account for many biological phenomena, including those stimulated by EMF.

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