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*Discussion Letter*

# Electrostatic coupling between membrane proteins

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Charges on membrane proteins are argued to produce very large electric fields within the membrane which may be felt by neighbouring membrane proteins. The activity of many membrane proteins may be sensitive to the electric field in the membrane; thus one membrane protein may affect the activity of another via the local electric field without any contact between the two. More specific electrostatic interactions are possible with binding between the two proteins. The possible roles of such interactions in bioenergetics, neurophysiology and signal transduction are discussed.

Membrane protein; Electric field, intramembrane; Bioenergetics; Signal transduction; Ion channel

## 1. INTRODUCTION

The hydrophobic cores of phospholipid bilayers and proteins normally have very low dielectric constants and conductivities [1] relative to aqueous solutions. This means that charges held within biological membranes have very large electric fields surrounding them relative to charges in solution [2]. All membrane proteins carry charges or dipoles either on their surface or core, which produce electric fields in the membrane, and these fields may be felt by neighbouring proteins. I will call any protein that produces a significant electric field within the membrane a 'field producing protein'. Most membrane proteins appear free to diffuse randomly in the plane of the membrane (although there are many exceptions) [3], but maintain a fixed orientation relative to the plane of the membrane. Thus a protein diffusing randomly in the membrane will experience changes in the size and direction of the local electric field, as it approaches and recedes from other membrane proteins.

The biological activity of some membrane proteins, for example ion pumps and channels, are known to be sensitive to the transmembrane electric field ($\Delta\Psi$), and these activities should also be sensitive to the intramembrane electric field. I will call any protein whose functional activity is significantly dependent on the intramembrane field a 'field sensitive protein'. The activity of electric field sensitive membrane proteins may therefore depend on their proximity to other field producing membrane proteins. Collision or near collision between a field producing protein and a field sensitive protein may result in a change in the activity of the field sensitive protein (see fig.1). Whether there will in fact be a significant change in activity depends on (i) whether the electric field sensitive element of the field sensitive protein experiences a significant change (relative to its field sensitivity) in the electric field due to the field producing protein as the two proteins approach and collide, and (ii) whether the change in field is over a time scale which can significantly affect the activity of the field sensitive protein.

The electric field produced by a membrane protein may change due to for example (i) the uptake, loss or redistribution of electrons, protons or other inorganic ions, (ii) the binding or release of charged metabolites (substrates or agonists) or other proteins, (iii) phosphorylation (or methylation of charged residues), or (iv) conformational change. Such changes in the field of membrane proteins might result in changes in the activity of field sensitive membrane proteins. Such a possibility presents both a problem (of separating activities) and a potential benefit (for regulation).

Skulachev [4] and Tsong and Astumian [5] have proposed related hypotheses suggesting that the local electric field may be important in bioenergetic coupling. I want to assess here whether the intramembrane field produced by membrane proteins is large enough and experienced for long enough to affect significantly the activity of field sensitive proteins.

## 2. THE FIELD DUE TO A MEMBRANE DIPOLE AND ITS SIGNIFICANCE

The electric field produced in the membrane by a protein is the sum of all the coulomb forces due to the

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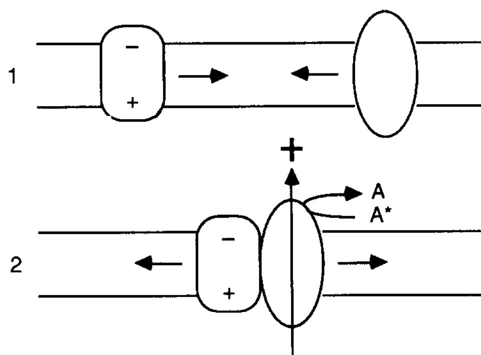


Fig.1. Schematic illustration of the kinetic stimulation of a pump, channel or other field sensitive activity (oval shape) by the local electric field of a dipole on another integral membrane protein (rectangular shape). The areas between the parallel lines represent the membrane, the areas above and below this are the aqueous phases. The transmembrane $\Delta\Psi$ is assumed to be just below threshold for activation of the field sensitive proteins, which are therefore inhibited when the proteins are far apart (stage 1). But during collision or near collision of the proteins the field sensitive protein experiences an altered electric field in the membrane sufficient kinetically to stimulate turnover (stage 2) represented by the reaction A* to A and/or the transfer of charge. If the dipole is modifiable or varies during turnover of the field producing protein then the relationship between $\Delta\Psi$ and the activity of the field sensitive protein may be modifiable or vary with the metabolic state of the field producing protein. Field sensitive proteins may also be field producing proteins.

individual charges and dipoles on the protein plus those induced in the surrounding membrane and aqueous medium. As an example I shall estimate the field due to a dipole of unit electric charges located in the centre of a membrane (fig.2). The size and shape of the field around the dipole can be estimated from electrostatic theory using the method of images (see fig.2). We assume that the membrane is a homogeneous slab of dielectric constant 3 (a compromise between a value of 2 for the hydrocarbon phase of the phospholipid and a value of about 4 for proteins [1]) and thickness 6 nm (a compromise between values of 4–5 nm for the bilayer and up to 10 nm for membrane proteins) surrounded by a homogeneous aqueous phase of dielectric constant 78.5 (i.e. that of the aqueous solution). The electric field (component parallel to the dipole) at the centre of the membrane is plotted in figs 3 and 4 as a function of the distance from the dipole for three different dipole separations. The field is calculated assuming that the aqueous phase has either zero conductivity (fig.3) or infinite conductivity (fig.4); the real case lies somewhere in between. A dipole of larger than unit charge would obviously produce a larger field. It is evident from these figures that the field produced by the dipole in the membrane is significant compared to the transmembrane field at distances closer than about 5 nm to the dipole and falls off very rapidly at larger distances (the field is roughly inversely proportional to the cube of the distance). The calculation is obviously crude but agrees well with a theoretical calculation made by a different method [6].

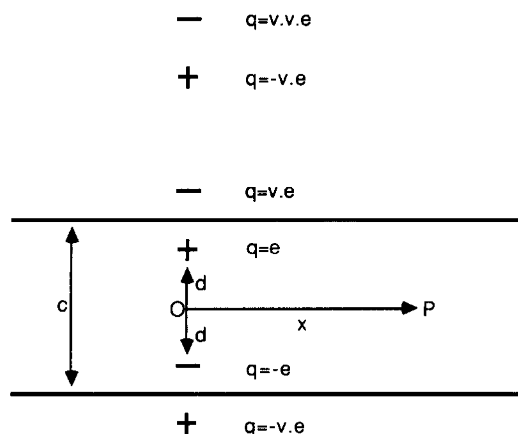


Fig.2. Model membrane with dipole and image charges. A slab of thickness $c$ and dielectric constant $\epsilon_M$ is surrounded by an aqueous medium of dielectric constant $\epsilon_A$ (taken as 78.5). A dipole of single positive and negative charges (charge $q = e$ the electronic charge) and separation $2d$ is located at 0 in the centre of the dielectric slab, equidistant from the two surfaces and with axis perpendicular to the surfaces. The dipole charges induce image charges in the surrounding medium with reduced charge $q = e \cdot v$ located symmetrically with respect to the dielectric boundary. These image charges in turn induce image charges, so that there is an infinite series, with reduced charge $q = e \cdot v^n$ where $n$ is the number of reflections. Only the first few image charges are shown here. The electric field component ($E_v$) parallel to the dipole at a point P at the centre of the membrane distance $x$ from the dipole can be derived from Coulombs law and for each charge is given by:

$$E_v = \frac{2dq}{4\pi\epsilon_O\epsilon_M(x^2 + d^2)^{3/2}}$$

where $\epsilon_O$ is the electric constant. The field at P is the sum of the contributions from the dipole charges and all the image charges. Although this generates an infinite series, the sum of the series rapidly converges and can easily be estimated by computer.

The electric field component at 5 nm from the dipole is equal to that due to a transmembrane $\Delta\Psi$ of 6–18 mV (figs 3 and 4). Are these local fields large enough to affect significantly the activity of field sensitive membrane proteins? The drop in $\Delta\Psi$ required to double the rate of state 4 respiration in mitochondria is variously reported to be 3–10 mV (see for example [7]), the drop in $\Delta\Psi$ required to halve the rate of maximal oxidative phosphorylation is variously reported to be 5–20 mV (see for example [7]), and the drop in $\Delta\Psi$ required to double ion currents through channels is about 5 mV (see for example [8]). Thus, the field produced at 5 nm from a dipole in the membrane is large relative to the field sensitivity of many membrane proteins.

Whether the field sensitive element in a field sensitive protein would approach to within 5 nm of a dipole in another membrane protein depends on (i) the radii of the proteins in the membrane, (ii) where the dipole and field sensitive element are located on the proteins relative to the protein/lipid/aqueous interphases, and (iii) other steric factors, such as the existence of supramembrane structures or repulsive forces between

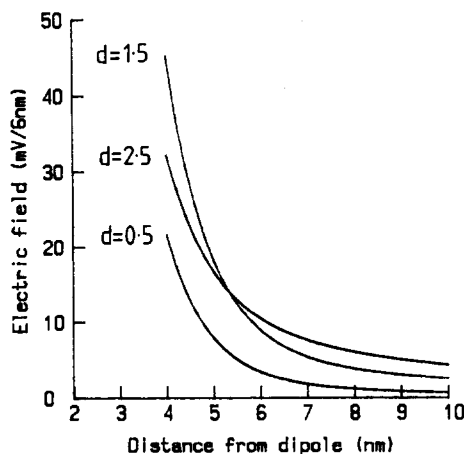


Fig.3. Electric field strength at the centre of the membrane as a function of distance from a dipole, calculated for a surrounding medium of zero conductivity. The electric field component at the centre of the membrane parallel to a dipole ($q = e$) is plotted against the distance from the dipole ($x$ in fig.2). The field was calculated for the case where the surrounding aqueous medium has zero conductivity, but includes the reaction field due to the polarization of this medium (thus $\nu$ was calculated as $\nu = (\epsilon_M - \epsilon_A)/(\epsilon_M + \epsilon_A)$). The field was calculated with membrane thickness ($c$) equal to 6 nm, the dielectric constant of the membrane ($\epsilon_M$) equal to 3, and dipole separation ($2d$) equal to either 1, 3, or 5 nm. The field has been converted to a $\Delta\Psi$ equivalent field by multiplying by the membrane thickness (taken as 6 nm). The field strengths at distances closer than 4 nm are not shown.

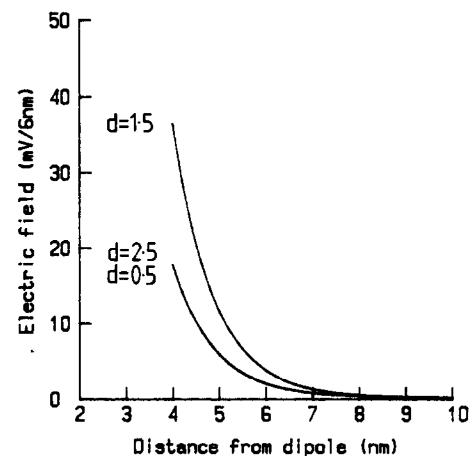


Fig.4. Electric field strength at the centre of the membrane as a function of distance from a dipole, calculated for a surrounding medium of infinite conductivity. The same as fig.3 except the field was calculated for a surrounding medium of infinite conductivity, i.e. $\nu = -1$. The calculated field strengths at the centre of the membrane were virtually identical for dipole separations ($2d$) of 1 and 5 nm.

the proteins. The radii in the membrane of the adenine nucleotide carrier, cytochrome oxidase, acetylcholine receptor and rhodopsin are estimated to be about 1.5, 2, 1.5 and 2 nm respectively [9,10]. Thus it is not unreasonable that a dipole on a membrane protein should approach to within 5 nm of the field sensitive element on another membrane protein.

Will two integral membrane proteins diffusing randomly in the plane of the membrane be in each other's vicinity long enough for the local electric field of one to affect the activity of the other? Einstein's equation for 2-D diffusion states that the mean distance diffused ($d$) in time ($t$) is $d = (4Dt)^{1/2}$, where $D$ is the diffusion coefficient of the protein. $D$ has been estimated to be $4 \times 10^{-10}$ cm$^2$ s$^{-1}$ for mitochondrial cytochrome oxidase and $bc_1$ complex and $8.4 \times 10^{-10}$ cm$^2$ s$^{-1}$ for the ATP synthase [11], $40 \times 10^{-10}$ for rhodopsin [3] and between 0 and $10 \times 10^{-10}$ cm$^2$ s$^{-1}$ for the $Na^+$ channel [3]. Thus, according to these values the mean distance diffused by a cytochrome oxidase complex in 10 $\mu$s would be about 1 nm. However, it should be noted that the estimates of $D$ above are for long range diffusion and may be underestimates for the more local diffusion considered here. The ATP synthase requires a field to be applied for a minimum time of about 10 $\mu$s before turnover can occur [5]. Thus, if cytochrome oxidase had a dipole with a field similar to that depicted in fig.3 or 4, cytochrome oxidase and the ATP synthase could be in each other's vicinity during collision for a time just sufficient to stimulate a single turnover of the ATP synthase. This time would be increased if the interacting proteins were 'sticky' (i.e. if there were short range attractive forces).

These model calculations are rough and many simplifying assumptions need to be made, but they show that if dipoles are present on integral membrane proteins they have the potential to affect significantly the activity of field sensitive membrane proteins. I will now consider the implications of these effects for three particular membrane systems.

## 3. BIOENERGETIC COUPLING

The bioenergetic membranes of animals, plants and bacteria contain redox or light driven primary pumps which transport protons against a transmembrane electric and concentration gradient, plus proton or charge driven secondary pumps and carriers which are coupled to ATP synthase or metabolite transport. The activities of most primary and secondary pumps and carriers are known to be very sensitive to the transmembrane electric field (see for example [7]) and thus probably to the intramembrane field. Due to the fact that these pumps and carriers move charges and charged metabolites around within them they will have electric dipole moments and associated local fields which vary with the reaction/transport cycle. Thus there is the potential for kinetic interaction between primary and secondary pumps via their local electric fields, for example charge separation in photosynthetic reaction centres or cytochrome oxidase might stimulate nearby ATP synthases to turn over via the local electric field (as hypothesized by Skulachev [4]) or vice versa, or the different states of the adenine nucleotide carrier might affect the kinetics of the $Ca^{2+}$ uptake carrier via the local

field (as suggested by Rottenberg and Marbach [12] to explain experimental kinetic interactions). A large number of kinetic anomalies in a wide range of bioenergetic systems have been found suggesting cross-talk between primary and secondary pumps and carriers other than through the transmembrane proton motive force [11,13]. Some of these anomalies might find explanation in terms of local intramembrane fields (as suggested by Skulachev [4]). If the local field stimulated turnover of pumps is to be significant in relation to the total flux, the frequency of collision or near collision of primary and secondary pumps must be approximately equal to or greater than the coupled flux. This has been estimated to be true for oxidative phosphorylation [11].

I am suggesting here that the potential exists for kinetic interaction between the primary and secondary bioenergetic pumps and carriers via the local electric field in the membrane due to the functional charge movements associated with turnover of these proteins. This is a generalisation of the hypothesis of Skulachev [4]. Tsong and Astumian [5] have hypothesized that significant amounts of energy may be transferred between primary and secondary bioenergetic pumps via the local (transmembrane) electric field. The calculations of the intramembrane field made here (represented in figs 3 and 4) suggest that significant energy transduction (relative to that via $\Delta\Psi$) could only occur with very closely apposed charge transferring elements.

## 4. PLASMA MEMBRANE ION CHANNELS AND PUMPS

Most plasma membrane ion channels are known to be gated by the transmembrane electric field, and the gating process (i.e. the structural rearrangement opening/closing or inactivation of the channel) is thought to be associated with a substantial change in dipole moment of the channel (the equivalent of 4–6 charges moving across the membrane for the $Na^+$ channel [14]). Thus ion channels should be both sensitive to the intramembrane field and producers of changes in the intramembrane field dependent on their functional state. Several factors reduce this potential for interaction between pumps via the local field. Some types of channels have restricted diffusion, for example the acetylcholine gated channel anchored to the cytoskeleton at the postsynaptic membrane and $Na^+$ channels on the axon hillock or at the nodes of Ranvier [3]. Where channels are not restricted their density in the membrane is low compared to other membrane proteins. Perhaps more importantly the gating process is slow, activation and inactivation occurring in the millisecond time scale [8,14]. Thus the intramembrane field produced by a freely diffusing membrane protein would not be in the vicinity of a channel long enough to affect gating, unless there were some association between the two proteins. However, given some association (and this association might itself be due to the intramembrane field) clearly membrane proteins with a large dipole moment sticking to channels could affect activation or inactivation via the intramembrane field. The binding of a protein or other molecule with a large dipole moment should change the gating potential of a channel, just as charged alkaloids or scorpion toxins do [14]. Non-channel proteins (such as receptors, G-proteins or unrelated proteins) might in this way activate channel proteins, or channel proteins activate other types of channel protein, or channel proteins activate non-channel proteins such as pumps. This might provide a simple mechanism to explain the findings that (i) the membrane bound G-protein subunits ($\beta$ and $\gamma$) may stimulate $K^+$ channels directly [15], and (ii) the cGMP phosphodiesterase of retinal rod membranes can directly (i.e. in the absence of cGMP) activate the cGMP-dependent cation channel, and this activation is itself dependent on activation of the phosphodiesterase [16]. The $Na^+/K^+$ ATPase is sensitive to the transmembrane field [17] and thus might conceivably be affected differentially by the local intramembrane field of open and closed channels, or by the binding of regulatory proteins or other molecules. Such regulation might also occur with the pumps and channels of other intracellular membranes.

## 5. SIGNAL TRANSDUCTION

Signal transduction obviously involves some degree of specificity between signal, receptor, transducer and effector molecules, so that the gross model of dipole interaction depicted in fig.1 is unlikely to be helpful in understanding signal transduction. However, if some degree of steric specificity of interaction between membrane proteins is added then the model may provide some insight into the mechanism of signal transduction.

The rhodopsin class of receptors (rhodopsin, $\beta$-adrenergic, and muscarinic cholinergic) are homologous and predicted to have 7 transmembrane $\alpha$ helices [18]. The signal, either binding of agonist or absorption of light plus the associated uptake of a proton, is thought to generate a positive charge within the bilayer region of the receptors, and also leads to multiple phosphorylation of the cytoplasmic side of the receptors [18]. These changes will change the local electric field around the receptor, particularly within the membrane, and these field changes could affect field sensitive elements in other plasma membrane proteins. The field change could also change the distribution of the receptors relative to each other or relative to other membrane proteins.

In many cases receptor activation causes the receptors to bind to a G-protein, which is followed by the

displacement of GDP by GTP on the G-protein and (whole or partial) dissociation of the $\alpha$ from the $\beta$ plus $\gamma$ subunits of the G-protein [19]. The GTP bound G-protein is then able to activate or inactivate the various membrane bound effector proteins [19]. A possible mechanism for the activation of the G-protein by the receptor or activation of the effectors by G-protein is some form of electrostatic coupling. However, this would have to involve a specific binding interaction between the proteins, and the relevant electrostatic field may be at the surface of the membrane rather than within the membrane, as the G-proteins are thought to be peripheral membrane proteins.

## 6. CONCLUSION

Charged membrane proteins may produce electric fields within the membrane, which are of significant size compared to the known sensitivity of several pumps and channels to electric field. This possibility produces several problems and opportunities to evolving biological systems. It presents problems because it implies that a membrane protein experiences considerable electrical noise from nearby proteins, and this presents problems of control, information transfer and separation of functions. However, this electrical noise may be insignificant if either the field sensitive element is sterically insulated from other membrane proteins (e.g. it is located towards the centre of a large protein or surface domains of the protein prevent close approach of the other proteins) or the field sensitive element requires the application of a field for a long time relative to that experienced from neighbouring membrane proteins. Thus steric factors allow a specificity of electrical interaction between proteins. By binding particular membrane proteins, the field generating and field sensitive elements on interacting proteins may be brought close together and for a relatively long time. Such a simple mechanism of information transfer between membrane proteins might provide a basic mechanism which has been modified differentially to suit the needs of many different systems.

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