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Models of Localized Energy Coupling

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Abstract

It is proven that any model of localized protonmotive energy coupling that relies upon properties of a homogeneous surface phase must, when operated in the steady state, lead to bulk phase electrochemical potentials for protons that are as large as those required by the delocalized chemiosmotic theory. To obtain models consistent with experiments supporting localized energy coupling requires some kind of surface heterogeneity for the proton conducting pathways. Two general classes of heterogeneous surface models are mentioned. One class involves phase-separated lipid domains. The second class involves hydrogen-bonded chains in proteins that traverse the membrane laterally.

Key Words: Energy coupling; chemiosmotic theory; proton gradients.

Introduction

It has been amply demonstrated that bioenergetic systems can function according to the delocalized chemiosmotic theory of Mitchell (Mitchell, 1979; Boyer *et al.*, 1977; Jagendorf, 1975; Graber, 1982). Nevertheless, many studies (Ort and Melandri, 1982; Ort *et al.*, 1976; Baccarini-Melandri *et al.*, 1977; Horner and Moudrinakis, 1983) have provided evidence that some intact systems operate in a way requiring more localized protonmotive forces (Williams, 1975). Such findings are not inconsistent with the basic principles of protonmotive forces nor with those experiments on intact or reconstituted systems that behave consistently with the delocalized chemiosmotic theory. Rather, they suggest that some native systems may go to the trouble to provide special proton pathways between those proteins that pump the

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protons to higher electrochemical potential and those proteins that utilize these protons. One advantage of a special nonbulk pathway is that the time to charge up the system could be reduced compared to bulk phase delocalized energy coupling because it would not be necessary to change the pH of entire bulk phases. Also, it is conceivable that the special pathway is the main, lower-resistance pathway for proton current and that the bulk-phase pathway is the alternative, less efficient pathway that would only play a major role when the special pathway is disrupted or, in the case of reconstituted systems, not constructed. Accordingly, it seems important to consider the possibility of localized energy coupling and to formulate clearly what constraints may apply to models of localized energy coupling.

One proposed model of localized energy coupling is that the boundary water at the surface of the membranes could be the special pathway for proton transport (Kell, 1979). Another proposed model is that special lipids in the membrane could strongly buffer the protons as they emerge from the energizing protein and provide an efficient transport path along the surface (Haines, 1983; Haraux and de Kouchkovsky, 1983).

The first task of this paper is to prove that the preceding models, when operated in the steady state, lead to properties quite inconsistent with the experiments supporting localized energy coupling; namely, such models will equilibrate the bulk electrochemical potentials to be practically identical with those that would obtain from delocalized energy coupling. The proof of this result is very general and applies to any model that utilizes the properties of a homogeneous phase that covers the surface of the membrane. This result is not unknown; it has long been recognized by at least one group of researchers in devising models (Van Dam *et al.*, 1978), and it has been explicitly mentioned by Mitchell (1981) in rebuttal to Kell who acknowledges the result (Westerhoff *et al.*, 1984). However, in the authors' experience this result is still not commonly known or appreciated, perhaps because no careful proof has been given. Therefore, the first goal of this paper is to prove in the fullest generality this constraint that serves to eliminate many simple models of localized energy coupling. Nevertheless, this does not mean that all models of localized energy coupling are impossible or unlikely. Indeed, it is possible to devise models that do not utilize such homogeneous surface phases. The second goal of this paper is to discuss briefly two such classes of models. First, we turn to the proof.

Proof

Figure 1a shows a cross section of a bioenergetic membrane. We consider all models that postulate special surface phases, designated in Fig. 1a

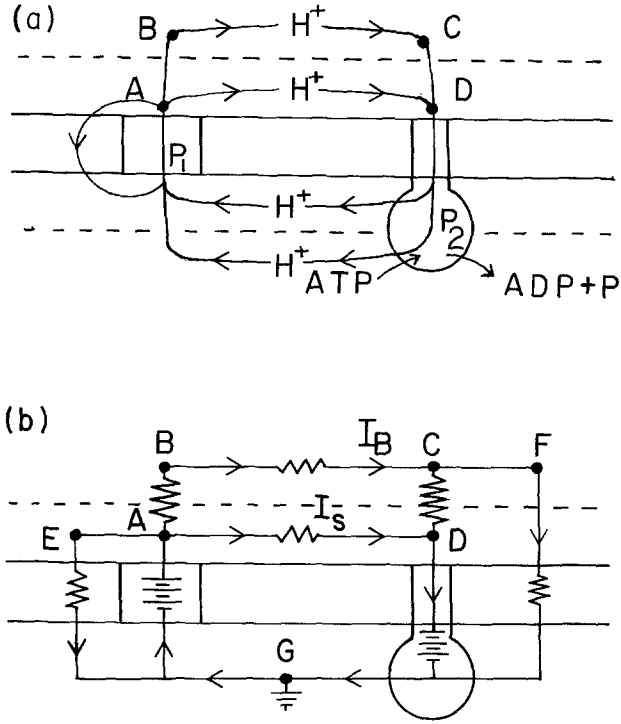

Fig. 1. (a) Schematic cross-section of a bioenergetic membrane with a protein proton pump P_1 and ATP synthase P_2. Points A and D are in the special surface phase and points B and C are in the bulk aqueous phase. (b) Equivalent electrical circuit diagram for (a) as explained in the text, with emphasis on the upper surface of the membrane. Points A–E correspond to the positions in (a). G is ground. The direct resistance from F to G is infinite (absent pathway) for homogeneous surface phases.

by the spaces between each dashed line and the membrane. Notice, however, that the dashed lines can be at a surface, as would be the case if the special phase were the head groups of lipids. Conceivably, the dashed lines could even be inside the bilayer for models not yet postulated. The models that will be considered in this section are characterized by the feature that each surface phase be laterally homogeneous in the sense that it extends over the entire surface of the membrane. Technically, each such surface phase divides space into two parts, one part on one side of the membrane which contains one bulk water phase, and the other part on the other side of the membrane which contains the other bulk water phase.

The essence of the proof is contained in the equivalent circuit diagram in Fig. 1b. Formally, this diagram only applies to the purely electrical case, but the general electrochemical case is easily disposed of once this purely

electrical case is understood. Without loss of generality we focus upon one side of the membrane only. The source of protons is represented by an electric battery in the protein P_1 that raises the potential V_A at A above the ground G level on the other side of the membrane. The ATP synthase P_2 is also represented by a battery that opposes the flow of protons from D back across the membrane. Since it is desirable that most of the work done on protons by the protein at A be consumed by the ATP synthase, V_D is nearly as large as V_A. Excess protons at point A may flow to D via the surface phase. The impedance to this flow, R_{AD}, is represented by a resistor in Fig. 1b. Similarly, the impedance between the surface phase and the bulk phase is represented by the resistors $R_{AB} = R_{CD}$. The impedance for flow through the bulk phase is R_{BC}. Finally, it is possible for protons to leak from the surface phase back across the membrane without doing any work. This is represented by the resistance between E and G. Notice, however, that there can be no leakage of protons directly from the bulk phase back across the membrane without passing through the surface phase. Therefore, the resistance embedded in the membrane between F and G in Fig. 1b must be infinite because there is no such FG pathway for the class of models being considered.

The current through the surface phase is

$$I_S = (V_A - V_D)/R_{AD} > 0 \tag{1}$$

and the current through the bulk phase is

$$I_B = (V_A - V_D)/(R_{AB} + R_{BC} + R_{CD}) > 0 \tag{2}$$

In order for the surface phase to be the favorable pathway, it is usual to postulate that

$$R_{AD} \ll (R_{AB} + R_{BC} + R_{CD}) \tag{3}$$

and recent experiments on monolayers (Teissie *et al.*, 1985) are consistent with Eq. (3), but this inequality need not be assumed for the proof. The proof follows from the fact that, in the steady state, the current from A to B must equal the current from B to C which in turn must equal the current from C to D, all of which are designated I_B in Fig. 1b. Therefore, since $I_B > 0$ from Eq. (2)

$$\begin{aligned} V_A - V_B &= I_B R_{AB} > 0 \\ V_B - V_C &= I_B R_{BC} > 0 \\ V_C - V_D &= I_B R_{CD} > 0 \end{aligned} \tag{4}$$

From Eq. (4) one has

$$V_A > V_B > V_C > V_D \tag{5}$$

which proves that, in the steady state, the potential of the bulk phase at B or C is as high as it is in the surface phase at D. Therefore, the potential measured in the bulk *must* be the same as would obtain for delocalized energy coupling.

It is instructive to state this conclusion another way. If experimental data show that the steady-state bulk-to-bulk protonmotive force is too low to account for ATP formation, then localized energy coupling cannot occur via a homogeneous proton conducting surface phase. This follows bevause V_{DG}, the critically important potential drop, must be no greater than V_{CG}, measured as insufficient. It would, of course, be possible to have localized energy coupling occur via a homogeneous proton-conducting surface phase, but in the steady state this would lead to the same bulk-to-bulk phase protonmotive force that occurs for delocalized energy coupling.

In order to extend the proceding proof to the general electrochemical case, one might appeal to the theorem that linear transport does not depend upon whether the driving forces are pH gradients or electrical fields, as long as one is in the linear transport regime. While this argument might suffice for most researchers, in view of the interest in localized energy coupling, the general case will be proven explicitly now. The proof proceeds logically in a very similar way to the proof for the electrical case. Again, the proton flux from A to B must be equal to the proton flux from B to C which must be equal to the proton flux from C to D; otherwise, the proton concentrations at B and/or C will change with time and one would not have a steady state. In order that protons flow passively from A to B, the electrochemical potential μ_B for protons at B must be less than the electrochemical potential μ_A at A. Incidentally, it may be mentioned that ATP synthesis, being a reversible reaction, may be run as slowly as desired; therefore, one may guarantee being in the linear transport regime and that local electrochemical potentials exist. Similarly, it follows that

$$\begin{aligned} \mu_B &> \mu_C \\ \mu_C &> \mu_D \\ \mu_A &> \mu_B \end{aligned} \tag{6}$$

Therefore, as in the purely electrical case, the total electrochemical potential in the bulk phase at B and C must be intermediate between the electrochemical potential at A and D and therefore must be much higher than at G. This completes the proof for the general case.

The only possible way that a model with a homogeneous surface phase could be consistent with experiments supporting localized energy coupling is that the experiments never achieve the steady state because of very long equilibration times. Since ATP synthesis begins in 5–8 msec after initiation of

pumping (Ort *et al.*, 1976) and since experiments may proceed for many minutes, this would require inequality (3) to hold by a factor of much more than 1000 since the equilibration times for diffusion from the surface to the bulk would be required to be much longer than the duration of the experiment in order that it be impossible to attain the steady state. Such slow rates of diffusion are inconsistent with the rapid equilibration times measured by Nachliel and Gutman (1984).

Consistent Localized Models

In order to obtain a true steady state in which V_A and V_D are high and V_B and V_C are low, one only needs a leak from the bulk phase back across the membrane *without* crossing the special surface phase. It is a simple exercise for the reader to show that V_B and V_C may be made as close to the ground voltage, V_G, as desired by making the resistance R_{FG} of the leak much smaller than the resistance $R_{AB} = R_{CD}$ in Fig. 1b [Westerhoff *et al.* (1981) give the formula.] As was noted before, such a leak from B, C, or F to G is a topological impossibility if the surface phase is assumed to be homogeneous. (It may be noted that homogeneity in this context does not require a single type of lipid in the membrane or the absence of either integral or peripheral protein.) Therefore, we now consider two classes of models which have heterogeneous surface phases.

The first class of models that we shall consider postulates that there are at least two coexisting phases of lipids in the membrane. These phases would not necessarily, nor even likely, be coexisting phases of gel and liquid crystal states. Rather, both phases could be fluid, liquid crystal phases. A phase diagram of this type was reported (Wu and McConnell, 1975) some years ago. This phase diagram consists of fluid phase coexisting ending in a critical point at high temperatures, a normal occurrence in phase behavior of fluid mixtures. Given the diversity of lipids in natural membranes, such phase diagrams should not be considered unlikely. One of the fluid phases is richer in some lipids than the other. In general such phase separation would be advantageous in that the lipid requirements of one protein could be such that it would partition into one of the fluid phases and a different protein would partition into a different fluid phase (Nagle and Scott, 1978). For the particular purposes in hand of providing a model of localized energy coupling, it would be required that both the proton-pumping protein and the ATP synthase partition into the same lipid phase (call it phase I) and that phase I has the property of providing the special, low-resistance pathway for localized proton transport. As shown in Fig. 2a, the lateral topology could be such that one can circumnavigate the cell membrane without leaving

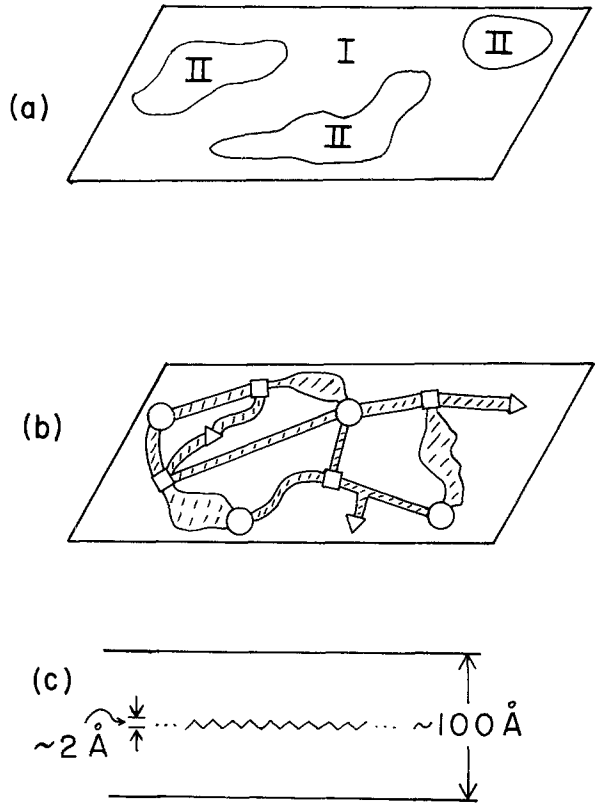

Fig. 2. (a) One possible lipid domain structure for fluid I and fluid II phases viewed from above the membrane. (b) Shaded areas are possible projections onto the plane of the membrane of proteins (integral, peripheral, or cytoplasmic) containing hydrogen-bonded chains. The proton pump proteins are shown as squares, the ATP synthase as circles, and other proton-consuming proteins as triangles. (c) An enlarged sketch of a slice of the protein containing a hydrogen-bonded chain involving amino acid side groups or bound waters.

phase I; this would be a reasonable arrangement since then each proton-pumping protein could supply protons via the efficient surface phase I to every ATP synthase. However, provided that the domains of phase II in Fig. 2a were large enough to contain at least one protein of both types, another case is that the proton-pumping protein and the ATPase would partition into phase II in Fig. 2a which would then, in this case, be the surface phase with low resistance to lateral proton transport. This latter case would lead to the small system stochastic kinetics analyzed recently (Westerhoff and Chen, 1985). In either case, the other lipid phase(s) would provide the pathway for the direct leak, FG, from the bulk to the other side of the membrane. As has been emphasized by Deamer and Nichols (1983),

the permeability of lipid bilayers to protons is rather high, with equilibrium times of order 20 min even for well-buffered systems. However, the measurements of Nachliel and Gutman (1984) indicate, for at least a few lipid membranes, that the equilibration time between surface and bulk is considerably faster. In steady state the actual deviation from delocalized energy coupling of the bulk-to-bulk protonmotive force will depend primarily upon the ratio of the latter equilibration time to the former one; these equilibration times, in turn, are related to the effective resistances, R_{AB} and R_{FG}, in Fig. 1b. At first sight it might also be thought that the preceding model is unduly contrived to explain the experiments and that any well-tuned cell would not have a second, leaky lipid phase but would only have the special, efficient proton-transporting phase because that would be more efficient. However, notice that the special, efficient proton-transporting phase must also be leaky to protons and that R_{EG} in Fig. 1b should not be supposed to have a much different value than R_{FG}. Therefore, this class of models with two lipid phases is no less efficient for ATP synthesis than models with only one homogeneous phase.

A second possible class of models for localized energy coupling utilizes proteins rather than lipids for lateral proton conduction. In this class of models the special pathways for lateral proton translocation may be hydrogen-bonded chains (Nagle and Tristram-Nagle, 1983) (proton wires) embedded in either (a) integral membrane proteins, (b) peripheral membrane proteins, or (c) proteins in the cytoskeleton. In any of these three cases, (a)–(c), the projection looking down on the membrane could be schematized as shown in Fig. 2b. The large resistance, R_{AB}, in Fig. 1b would be the resistance to flow of energized protons off the designated hydrogen-bonded chains embedded in the proteins, through a hydrophobic protein insulation, into bulk water, as shown in Fig. 2c, or into the lipid. Such hydrogen-bonded chains (which would not literally be required to be singly connected chains but might consist of more extended hydrogen-bonded networks) could provide the low resistance R_{AD} in Fig. 1b. The leak, R_{FG}, goes from the bulk phase, around the special proteins, through the membrane.

Case (a) of integral membrane proteins has been suggested by Dilley and colleagues (Laszlo *et al.*, 1984; Dilley *et al.*, 1982) from chemical modification studies. This permitted identification of eight or nine thylakoid polypeptides having amine groups apparently in sequestered domains (Laszlo *et al.*, 1984), although exact determination of the location of the buffering (hydrogen-bonding) groups has not yet been achieved. Case (c) of cytoskeletal proteins has been suggested by Berry (1981), not only for localized energy coupling but also to explain high-efficiency transport of protons between different membranes and organelles in the cell without changing the pH of the cytoplasm. The "protoneural network" model of Kell *et al.* (1981)

can be made consistent with case (b) when the requirement is added that the proton-conducting pathways contained in the peripheral proteins cannot cover the entire surface. All three cases are consistent with systems reconstituted without these proteins obeying delocalized energy coupling. Cases (b) and (c) are especially consistent with disturbed or harshly treated systems obeying delocalized energy coupling because the putative special pathways would be easily removed from the membrane. This is relevant to the situation wherein spinach thylakoid ATP formation has been reported as responding as though energized by localized proton gradients by some workers (Ort *et al.*, 1976; Graan *et al.*, 1981), but as though energized in a delocalized mode by others (Vinkler *et al.*, 1980; Davenport and McCarty, 1980), all using rather similar techniques.

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