SOME UNREALISTIC ASSUMPTIONS IN THE THEORY OF CHEMI-OSMOSIS AND THEIR CONSEQUENCES

R J P WILLIAMS
Inorganic Chemistry Laboratory, South Parks Road, Oxford OX1 3QR, England

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1. Introduction

One way of setting out the alternative views as to the way in which energy is transduced in biology using energised protons is as follows

(a) Input → equilibrium trans-membrane energy → ATP

(b) Input → kinetically-controlled membrane energy → ATP

Consider a vesicular system of two aqueous phases and a membrane An equilibrium trans-membrane energy is generated by a system in which each of the aqueous phases is in full equilibrium and no energy is stored in the membrane A kinetically-controlled membrane energy is a system of three phases (note however that a parallel case of two phases can now be developed) in which the energy is restricted to a local membrane region and before it is used it does not diffuse out and equilibrate with the aqueous phases with which it is in direct contact In (a) the membrane must be impermeable to protons except through the ATPase channel, in (b) the membrane may leak in regions away from those where protons are generated and rapidly utilised 'Kinetically-controlled' throughout this article then describes a diffusion-restricted series of movements The most favoured version of (a), chemi-osmosis, suggests that in the first step energy is confined in concentration and field gradients associated with protons, especially, in two equilibrium aqueous phases isolated by an impermeable, rigid, insulating, membrane No energy is stored in the membrane We deliberately neglect, partly for ease of discussion, the movement of all other cations and anions which could occur cooperatively It is known that there are many other ion movements across membranes, e g , magnesium and chloride fluxes across chloroplast membranes on energisation The version of the first step of (b) which requires close parallel scrutiny is that charges are generated locally within the confines of the membrane, and are generated concomitantly with conformational changes The second step of both (a) and (b) will be treated later as it differs in (a) and (b) through the different steady states set up by the first steps

In this paper I wish to examine the basic assumptions of version (a) while making only a few asides about (b) since I consider that the assumptions in (a) must make it an unrealistic model for the understanding of energy transduction In fact using realistic assumptions will make it clear that a kinetically-controlled path (b) is almost unavoidable in biological systems

2. Basic assumptions in chemi-osmosis steady states

Chemi-osmosis uses the general equation that the energy stored in a cation gradient across an inert membrane is, with conventional symbols

$$\Delta G = \Delta\psi + Z\Delta\log M_{in}/M_{out} \qquad (1)$$

This equation is valid for the model systems it describes but I wish to show that in any real biological system it is incorrect The way in which it is incorrect is of extreme importance to the understanding of biological membranes We consider the case of $M = H^+$ The derivation of eq (1) has explicit and implicit assumptions

2.1. *Assumption: No force acts on the membrane since otherwise it stores energy*

Let us suppose that a steady state is set up following chemi-osmotic theory, that is a vesicle restricted by a membrane contains an excess of charge of one kind in an inner aqueous phase and outside this vesicle there is excess of the opposite charge in a second aqueous phase. It is reasonable to suppose that in all real biological systems which resemble this model vesicle the opposed membrane surfaces will also be charged whether that charge arises from the binding of charges from the aqueous phases or from the fixed charges present in the membrane, proteins and lipids. Such is the nature of the chemicals which are the membrane. It follows that a force, F, is exerted on the membrane proportional to the membrane-bound charge distribution and the potential, $\Delta\psi$, sensed by electrodes. The above assumption cannot be correct.

2.2. *Assumption: The membrane is rigid since otherwise it stores energy*

Now the membrane is again not, as chemi-osmosis supposes it to be, a rigid barrier. It is observed to deform on energisation in both mitochondria and chloroplasts and to relax on de-energisation [1]. In other words it is an elastic medium which experiences a force, F, through the potential, $\Delta\psi$. Such a medium changes its energy by a term proportional to the force, F, times any displacement in the field, $\delta 1$, where:

$$F\delta 1 = \delta\Delta G' = \delta\Delta U' - T\delta\Delta S' \qquad (2)$$

$\delta\Delta G'$ is now one energy stored in the membrane which has come about from the generation of the potential, $\Delta\psi$, across it. We analyse the integrated (per unit mass) $\Delta G'$ in terms of $\Delta U'$ and $\Delta S'$ below. (The membrane is in no way an inert medium and responds to chemical and mechanical forces as well as to electrical forces.) $\Delta G'$ has some interesting features. Unless the membrane is equally charged everywhere, like a metal plate, its charge distribution cannot be evenly delocalised along the membrane. In fact energisation of biological membranes is known to cause a variation in deformation differentially along their length [1]. There must also be more elastic and less elastic regions as the membrane is not a uniform material. Thus energy storage varies from point to point in the membrane and conformational changes also vary. We might well ask, what would a spectroscopic probe see in such a membrane when it is energised? Clearly it depends upon the response of the region in which the probe is placed. (In other words the probe is susceptible to many changes which must occur and there is no simple relationship between probe response and ψ). The assumption cannot be correct.

2.3. *Elasticity*

The analysis of $\Delta U'$ and $\Delta S'$ for elastic materials, such as rubber, shows that $\Delta U'$, which can be looked upon as bond bending and stretching here, is small compared with the configuration entropy change, $T\Delta S'$ [2]. Thus $\Delta G'$, via $T\Delta S'$, is at least in part a conformation change in the membrane under energisation and is inevitable even if not yet of known function. General knowledge of biological systems would suggest however that all such effects have evolved functionally. The simplest functions here would be that the elastic properties become part of the energy conservation especially since they could provide a control feature, i.e., rearrangement in the membrane alters the activities of membrane component enzymes, quite absent from chemi-osmosis. The inevitability of the conformation change demands a much deeper questioning of chemi-osmosis.

2.4. *The movement of membrane charges*

The total energisation is so far:

$$\Delta G = \delta\Delta G' + \Delta\psi + Z\Delta\mathrm{pH} \qquad (3)$$

However this expression is not yet correct since it is inevitable that the conformation change in the membrane alters the relative disposition of its fixed charges so that there are two new terms involving separately the internal membrane potential and chemical potential energies. The full equation is therefore now:

$$\Delta G = \delta\Delta G^{\circ} + \Delta\psi' + Z\Delta\mathrm{pH} \qquad (4)$$

where $\delta\Delta G^{\circ}$ includes $\delta\Delta G'$, and $\Delta\psi'$ includes $\Delta\psi$. We see immediately that just as measurements using probes in membranes cannot be simply related to ψ of eq. (1) so attempts to measure ψ by permeant

ions, which in aqueous phases would apparently discharge ψ but in fact will discharge $\delta\Delta G^\circ + \psi'$, do not relate to ψ alone On the other hand measurement of the uptake of bases to replace $Z\Delta$pH will apparently measure this term only We shall show next that the real situation is much more complex for the charge distribution in the membrane must alter in a further way (Note in no way at this stage does this discussion relate to the debate between protagonists about local and delocalised effects We are analyzing what would happen when biological systems move charge across membranes – giving effects very different from those chemi-osmotic theory supposes)

2 5 *Reorganisation of charges in membranes*

Given that a membrane contains a set of acid/base centres, then, when a potential is applied, we must consider the effect on the equilibrium distribution of charge in the membrane for two types of base

$$B + H^+ \leftrightarrows HB^+ \text{ (e g , imidazole)} \quad (5)$$

base

$$B^- + H^+ \leftrightarrows HB \text{ (e g , carboxylate)} \quad (6)$$

The reactions can be written for all cations and anions other than B and H^+, i e , metal ions and, e g , chloride. Here we fix attention on the proton Let us assume that there is no effect on neutral species B and HB The effect of a field on the free energy of HB^+, B^- and H^+ depends on their charge and where they are in the field Without quantitative analysis there is bound to be a change in ion distribution within the membrane This will give rise to two new energy storage modes one chemical and one purely electrical, i.e., a $\delta\Delta G''$ and a $\Delta\psi''$ term Of course, if the field is produced by a movement of a specific chemical species then the free energy of that species is adjusted and will drive the above equilibria one way or another This paragraph asserts again that the energy stored by generating a field across any real biological membrane cannot be written in terms of ψ and $Z\Delta$pH Due to charging of the membrane and movements of charges there is a term in $\delta\Delta G^\circ$ and electric potentials in the membrane This is in addition to changes due to the elastic properties of membranes The meaning of $Z\Delta$pH, analysed by permeant bases, is not simple

2 6 *The total energy*

If we now sum the effects of elasto-mechanical, chemical and electrical changes in the membrane we have the general free energy equation

$$\Delta G = \delta(\Delta G^\circ) + \Delta\psi' + Z\Delta\text{pH} + \delta\Delta G'' + \Delta\psi'' \quad (7)$$

In so far as there is a ΔpH as well as a change of potential across the membrane not only the equilibria (5) and (6) will be affected but also by back reaction the conformation of the membrane and thence (ΔG°) and ψ' We see that the simple postulates of chemi-osmosis cannot be realistically related to a biological system This is one reason for the failure of the analysis of $Z\Delta$pH and $\Delta\psi$ by probe and ion distribution methods to show quantitatively the relationship with the phosphate potential which the membranes can generate

2 7 *Summary membrane states*

To summarise the first step of (a), even if a biological membrane were to be subject to a potential and a concentration gradient at equilibrium in both aqueous phases (chemi-osmotic postulate) the elastic and charge-carrying nature of the membrane (not described by chemi-osmotic theory) mean that energy storage cannot be understood from osmotic principles. Instead membrane charge and conformational changes are inevitably interlocked with aqueous phase changes The problem is comparable with the effect of charging the plates of a condenser which results in energy storage in the dielectric There is no escape from the involvement of membrane energy storage in chemi-osmosis when realistically formulated Importantly, this account reveals how the movement of charge will be related to a great number of other changes which then permit control of membrane processes, i e , alteration of membrane charge acts like an allosteric feedback on an enzyme

It should be observed that the whole of the above discussion relates to thermodynamic control of the phases, i.e., a bulk steady state between three phases It is a proper and realistic description As I have stated many times I believe that this is not the situation in biological systems and that these systems will retain local kinetic control under energisation, see (b) To see this we turn to the flux which makes ATP

3. Basic assumptions: fluxes and membrane channels

We may pursue the analysis of chemi-osmosis further. At the moment we have only set up the general charging of the membrane, the first step of (a). We know from experiment that in agreement with the above analysis membrane charge and membrane conformation are changed on energisation [1,3,4]. However none of these energies are yet connected to ATP formation. We need to describe the actual flux in the energised steady state which links this state with the chemical reaction ADP + P_i → ATP. The two approaches both require that the membrane is modified to make a pathway for protons.

(a) Two protons flow from the aqueous phase through a channel to the ADP + P_i site and without changes in the membrane ATP is produced directly.

This is chemi-osmosis. Note that in energy calculations it is assumed that the membrane including the channel has a uniform dielectric constant.

(b) An unknown number of protons, but greater than two by experiment [5] flow from a store within the confines of the membrane through a channel, linked to the ADP + P_i → ATP site, and transduction proceeds through relaxation of electric fields, due to bound charges, and relaxation of conformational constraints.

In the light of the experimentally demonstrated [3,5] nature of the charging process we can analyse the second step of (a). As both (a) and (b) require more than one proton to move cooperatively in the making of ATP it is necessary that two or more protons come together in the same channel. The simplest suggestion would be that they are bound locally and cooperatively as the alternative is to wait for a chance fluctuation which produces at least two protons in the channel. In any event the protons represent charges in the membrane and their very presence generates an interacting electric field in the membrane which must cause other charge and conformational fluxes. Moreover such events can only occur locally where the channels or binding sites for the protons are. This means that the sites of proton flow in the ATPase are locally energised on any theory just because the proton can enter the membrane at these sites but nowhere else. We could now analyse the flux, of charges and elasticity, in exactly the same way as we have analysed the setting up of the steady energised state, and we would reach the conclusion that the general form of the energy available for interconversion between energised protons and ATP cannot be expressed by the chemi-osmotic eq. (1).

3.1. *Dielectric constants of real membranes and their channels*

A closer analysis of the idea of a channel gives a quite new impression of the membrane. We must return first to the analysis of the steady state. A membrane potential, $\Delta\psi$, can only be related to a known capacitance if it has a uniform dielectric constant. A membrane which has alternating regions of low dielectric constant (the impermeable lipid) and high dielectric constant (a proton channel must be like this) is to a first approximation a series of potential energy wells of alternating heights, fig.1. Bound membrane charges of opposite sign will flow into the region of minimum free energy along the membrane surface. This region is where the dielectric constant is highest, since a free ion is stabilised in a region of high dielectric. Protons in a field will then accumulate in the regions of the membrane channels. The exact description of where the energy is stored is now made very difficult due to the elastic nature of the membrane. (To use an analogy, consider a flat rubber square with a weight applied centrally; although energy is distributed generally to some extent, it is largely held locally where the weight is applied.) This analysis says that any attempt to set up a uniform potential around a membrane with channels will lead to accumulation of protons at the mouths of the channels. The importance of the channels would be slight if the charges generated were in vast excess over the channels, e.g., the Na^+/K^+ gradients of nerve [6], but here they are not as there are vast numbers of ATPase units in energy transducting membranes [7,8].

A system of alternating potential wells of the kind described by fig.1 must act as follows. Generation of a charge separated pair associated with the membrane at point A will lead to the diffusion of the pair down the energy gradient to points B where they are effectively trapped in a more stable energy region. Given that biological membranes are constructed with an almost one to one ratio of generating systems and transducing ATPases which contain the proton channels there is little possibility of generating general

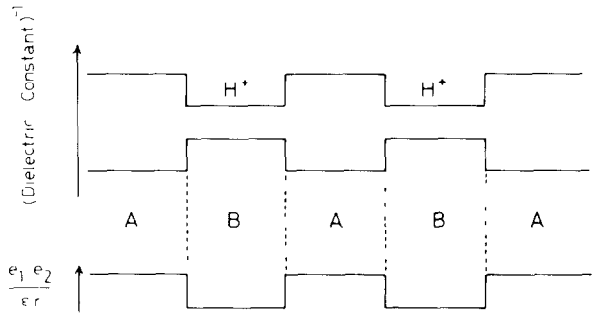

Fig 1 The diagram shows the variation of the reciprocal of the dielectric constant with distance along the membrane (Upper diagram) The proton is placed in the region of the high dielectric channel Its energisation is plotted (below) showing how it drops into traps placed regularly along the membrane The traps prevent fast lateral diffusion The proton in this diagram is not assumed to be fully 'hydrated' except in the channel region

potentials as opposed to localized effects The kinetic restriction here will be upon diffusion of a proton, away from the site of its production, further than the nearest ATPase well Obviously this is not in accordance with chemi-osmosis and we must also consider what will happen using the assumptions of that theory but an appropriate model, fig 1, for the membrane

Alternatively and in close accord with chemi-osmosis let us suppose that the ions which are released into the two aqueous phases on either side of the membrane are not membrane restricted but are hydrated, then we may suppose that the individual ions no longer differ in their individual ion potential energies no matter whether they are near or far from the surface regions A or B However, the pair-wise interaction is now greater across B Completely hydrated ions then accumulate away from the aqueous channels but can be trapped while they accumulate alongside the nearby hydrophobic parts of the membrane. The location of the ions is then the opposite of that shown in fig 1 and we must now ask if ion-pair energies are powerful enough to effectively restrict diffusion away from the membrane into the bulk before they can reach the ATPase channels (I thank Professor J Albery, Imperial College, London, for pointing out this case to me)

Both of the above cases could be made more complicated if we were to consider a variety of fixed charges in the membrane or to allow variations in the thickness of the membrane but these would only provide alternative ways of localising charges Note that in all these cases if the ATPase channels are blocked attempts to measure the build up of ψ will in fact reflect these local charge accumulations However the whole of the above physical approach to fluxes is likely to be overshadowed by chemical structure in the real biological membrane

3.2 *Chemical nature and binding capability of channels*

Recently, knowledge of the nature of the F_o protein channels has greatly increased [9] A discussion of diffusion control of protons in membranes in terms of alamethicin-like channels [10] now becomes more meaningful The important point here however is that these channels are associated with proton binding groups of pK_a around 6 0 and we have previously compared such centres to the binding groups of the active site of lysozyme [10] The pK_a and thence the bioenergetics of such a group in a conducting channel will depend upon the potential acting along the channel, see eq (5) and eq (6) above The potential then traps the proton on the binding group in the channel assuming that the negative potential is toward the bottom of the well in fig 1 Alternatively, and still following chemi-osmosis in outline, if the pH is decreased above the membrane, in the sense of fig 1, then the channel binding groups will pick up protons If there are several such binding groups a considerable charge change occurs locally within the channel At the bottom end of the channel we must presume that there is the ATPase and this must also undergo a very considerable charge change since it binds both inorganic phosphate and ADP, i e , a total negative charge of five. Thus in the membrane channel we make a large localised charge gradient across the gap between protons and ATPase As we have stressed, as the potential builds so the elastic factors will also generate conformational stress here in the channel Thus the binding of protons in the ATPase channel builds up a triggering energy which will finally generate ATP on firing but it does not require a fixed number of protons A fixed product, locally, of $\Delta G\ nH^+$ only is essential, i e , the energy generated by a flow of current (nH^+) is the product of the current and the

voltage. No fixed stoichiometry is demanded and even integral numbers of protons, n, only arise if the triggering of the reaction of protons occurs by binding of integral numbers of protons in the channel. In any other situation, statistical accumulation of protons in the field of the channel, gives a non-integral value to n. This removes an assumption which is often employed in the description of chemi-osmosis, and also gives a direct chemical picture of the involvement of membrane energy.

The densely packed distribution of proton ATPase channels in the energy capture devices must represent a severe restriction on diffusion. The density of Na^+/K^+ ATPase channels in nerve is quite different [6] and undoubtedly the conventional steady state equation:

$$\Delta G = \Delta\psi + Z.\log[M]_{in}/[M]_{out}$$

applies. Moreover Na^+ and K^+ bind very weakly to all protein side-chains [11] though they can still be accumulated in the regions of high dielectric of the membrane channel. Calcium behaves much more like the proton in its binding capability (but differently in its selectivity of binding). The dense packing of calcium ATPase in the sarcoplasmic reticulum may provide a diffusion limitation upon the movement of the calcium ion. It appears from this analysis that chemi-osmosis is likely to be applicable to Na^+/K^+ ATPases where it has been applied for a long time.

4. Summary

Overall this analysis stresses the inevitable build-up of localised charges in membranes if any charging of aqueous phases surrounding a real biological membrane is attempted. The second model (b) was designed to meet these points and it has the great advantage of making membrane events part of energy transduction. Elsewhere the analysis of control advantages is made [10]. A membrane which senses energisation can, through feedback, control how it will respond. This is impossible if phosphate potential and protons equilibrate.

I shall be surprised indeed if biological membranes have not evolved from the more sophisticated treatment of membrane equilibria I have given here to a kinetic utilisation of the localised conformational and charge distribution which I have shown is inevitable; see [12]. The advantages of kinetic control are considerable. In work to be published I shall show how the properties of proteins attached to membranes have evolved in specific ways so as to react to outside electrical (mechanical or chemical) gradients to generate the very special features of localised effects in biological membranes [13].

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Appendix

Estimation of energies due to elasticity

If an aqueous system within and without a charged membrane has a field imposed across its membrane then that membrane will either contract inwards or expand. In the extreme the maximum expansion is to a sphere, to give the maximum internal volume, while the maximum contraction is to a lamella disc with no internal volume, compare chloroplasts and mitochondria. The internal

volume changes with potential applied can be monitored by the uptake or expulsion of water An estimate of the energy required to take up or pump out the water can be found by considering the osmotic pressure changes of all components trapped in the vesicle At the same time it will be assumed that the external volume is large Let the concentration change be from c to c' then the energy change of the contents is $RT\ln c/c'$, integrated over all components The change of osmotic pressure is now calculated from the change in the concentration terms and this change in osmotic pressure (a force) is the force which is acting on the membrane The energy change of the membrane is equal to the integration of the force multiplied by the distance it has moved If the potential applied across the membrane is known the charge can be calculated from the equalisation of forces If the concentration or expansion of the membrane is observed then the distance moved by the charges can be found The energy storage in both the membrane and the osmotic change can then be estimated These changes are totally within the concept of equilibrium aqueous phase systems but the bioenergetics could now be quite different from that in chemi-osmosis as membrane relaxation processes could generate the ATP using membrane energies The whole process is quite independent from a consideration of local versus delocalised proton gradients

A detailed account of the study of membrane energies has been published since this article was submitted Evans, E A and Hochmuth, R M (1978) in Current Topics in Membranes and Transport (Bronner, F and Kleinzeller, A eds) vol 10, pp 1–64, Academic Press, New York