Hypothesis

EFFECTS OF DISCRETE CHARGES AND DIELECTRIC PROPERTIES OF MEMBRANE–WATER INFERFACE ON ELECTRIC POTENTIALS INSIDE MEMBRANES

With reference to charge separation in photosynthesis

J. T. DUNIEC and S. W. THORNE
CSIRO, Division of Plant Industry, PO Box 1600, Canberra City, ACT 2601, Australia

Received 11 February 1981

1. Introduction

In [1,2] we presented a model for the charge separation and subsequent proton and ion movement in a photosynthetic membrane. The model assumed that one side of the membrane (e.g., intrathylakoid side in chloroplasts) would be more negatively charged than the other. This asymmetry leads to the dark equilibrium internal electric field in the membrane which, in the light, directs the light-induced charge carriers from donors located at inner side to acceptors positioned at the outer thylakoid surface.

As would be expected, the calculated magnitude of such an electric field, or, which is equivalent, the surface potential difference, depends on the assumptions which are made in the particular mathematical model. A commonly used theoretical framework is based on the Gouy-Chapman theory of the electric double layer [3]. In this theory one assumes a sharp boundary between the aqueous region where ions are diffused, and the hydrophobic part of the membrane. The boundary itself is considered to be uniformly charged with a surface charge density corresponding to the distribution of real surface ionizable groups. One then solves the Poisson-Boltzmann equation [3] in one dimension and finds the electrostatic potential in the aqueous region and on the surface of the membrane. For two surfaces the situation is somewhat more complicated, but little error is made by applying the Gouy-Chapman theory to the two surfaces separately, as if the membrane was infinitely thick. Let us denote the surface potential on one side of the membrane by Ψ_{s1} and on the other by Ψ_{s2}. Let their difference be $\Delta\Psi_s$. What is the magnitude of $\Delta\Psi_s$? The surface potentials on each side depend on the salt concentrations in the bulk solution and on the surface charge densities. Typically, for the surface charge density relevant to chloroplast membranes (2.5 μC/cm^2 [4,5]) the surface potential varies from ~−130 mV to −20 mV as the concentration of monovalent salt changes from 0.001 mol/l to 0.2 mol/l. At the same time the surface potential on the opposite side of the membrane must be of the same order. Thus it is easy to see that:

(a) $\Delta\Psi_s$ is relatively small. Typically it is ~10 mV when the surface charge densities do not differ much and the salt concentration is not too high [2].
(b) $\Delta\Psi_s$ decays for high salt concentrations as the surface potentials tend to zero.

The above features of the calculated values of the surface potential difference cannot be reconciled in our model with the following experimental findings:

(a′) The light-induced potential change is of the order of ⩾50 mV for a photosynthetic thylakoid membrane [6];
(b′) The charge separation, as monitored by the electrochromic effect, is fairly insensitive to ionic conditions [7].

Thus, we must ask the question.

> If the asymmetry of the surface charges, and hence the built-in electric field is responsible for the vectorial charge separation across the membrane [1,2], is the Gouy-Chapman theory adequate for the description of the electric fields and potentials inside the membrane?

This theory adequately predicts the average value of the electric potential at a certain distance from, or on the surface of the membrane. Thus it is a valuable tool in investigating all phenomena which depend on the average potential. Examples are: local values of pH; concentrations of ions near the membrane surface; or forces between the membranes. However, in the case of charge separation at the reaction centre of a plant or bacterial photosystem as well as in other molecular processes the local electric fields and potentials are important. Thus in a mathematical model which would predict magnitude of such local fields and potentials the discrete nature of charges at the water–membrane interface must be considered.

An additional simplification usually made in the calculations of membrane electric potentials is the neglect of the structure of the region immediately adjacent to the hydrophobic part of the membrane. Here, we investigate the effects of discrete charges as well as the dielectric properties of this region on the magnitude of local electric potentials in the membrane.

2. The discrete charge model

There are several models describing local electric potentials in membrane systems due to discrete charges at the water–membrane interface (review [8], [9–11]). Here, we have used the technique applied in [11] for calculations of electric potentials due to arbitrary distribution of surface charges. A complication in our problem is the presence of two water–membrane interfaces, hereafter called polar regions. Whilst the details of the technique which enables us to calculate the electric potential of an arbitrary distribution of discrete charges within and without the membrane will be reported elsewhere, we consider here a very simple example (fig.1). We are doing this only for the purpose of discussion of the possible magnitudes of local electric potentials, and the example considered may not relate to any specific biological system. The results given below can also be obtained by a modification (which admits the polar regions) of Brown's technique [8].

Fig.1 illustrates a single, cylindrical particle embedded in a neutral membrane (e.g., a bacteriorhodopsin molecule in a lecithin vesicle). To the far left and far right (fig.1) is the aqueous region characterized by a dielectric constant ϵ_w of ~80. The middle region is the hydrophobic part of the membrane whose dielectric constant can be assumed $\epsilon_m = 2.4$ [12]. The polar regions which separate the membrane proper from the aqueous regions also have different dielectric properties. Each region consists of a few layers of water molecules bound to the head-groups of lipids and hydrophylic groups of proteins. There may also be adsorbed ions and other molecules such as sucrose. The dielectric constant of the polar region must be lower than that of the bulk water for two reasons:

(a) The bound water molecules cannot form associations of larger dipole moments, as they do in the bulk region [3];
(b) The presence of other than water material affects a composite dielectric constant [13].

Thus, although the measured dielectric constant of the water–metal interface is ~15 [3], in the case of biological membranes it may even be lower. To cover different possibilities we have considered three values for the dielectric constant of the polar region: a low $\epsilon_p = 6$, medium $\epsilon_p = 20$ and high $\epsilon_p = 50$.

In the example considered we have assumed that univalent charges were located on the edges of the particle in the polar regions, 2 Å from the hydrophobic

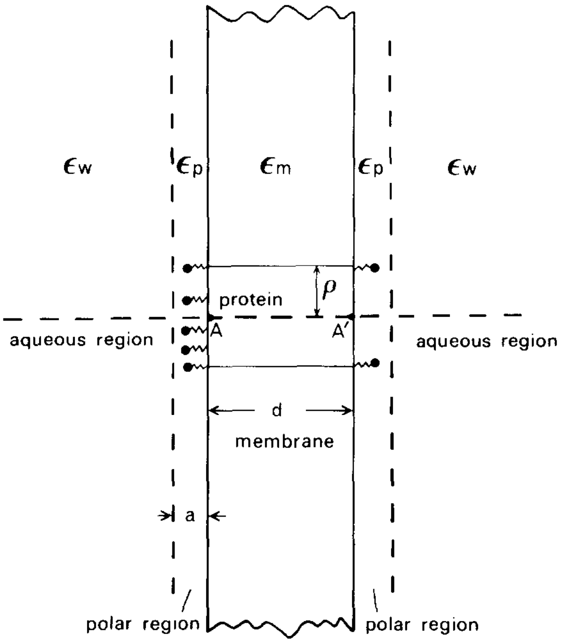

Fig.1. A cylindrical protein particle of radius ρ is embedded in the membrane whose hydrophobic core has thickness d. The polar headgroups of lipids (not shown) and the protein are located in the adjacent polar regions of thickness a. The dielectric permittivities of the aqueous regions, polar regions and the interior of the membrane (including the protein) are characterized by ϵ_w, ϵ_p and ϵ_m, respectively. The protein carries surface charges located at the polar regions distance ρ from the symmetry axis AA' of the particle.

part of the membrane and the distance ρ of either 10 Å or 12 Å from the symmetry axis of the particle. The thickness of the membrane was assumed 40 Å and the thickness of the polar regions 12 Å [14]. We have calculated the electric potential profiles along the symmetry axis of the particle for two sets of salt concentrations in the bulk solution:

(a′) High salt, with 0.1 mol/l of mono- and 0.01 mol/l of di-valent cation concentration;

(b′) Low salt, with 0.01 mol/l of mono- and 0.001 mol/l of di-valent cation concentration.

In fig.2 we plot the electric potential profile for the case of 5 positive charges at the left-hand side (l.h.s.) and 2 positive charges at the right-hand side (r.h.s.) polar region. High salt conditions were assumed and other parameters as specified in the caption. It can be seen that the values of the dielectric constant of the polar regions have a large effect on the calculated potential profiles. For $\epsilon_p = 6$ the maximum of the potential is 692 mV (relative to zero in the bulk) whilst for $\epsilon_p = 50$ it is only 118 mV.

The effects of different number and valence of charges are illustrated in fig.3 where the potential profiles are plotted for the following combinations of surface charges: (a) 5,2; (b) 3,2 and 3,−1; where the first number is the sum of charges of the l.h.s. polar region and the second represents the same for the r.h.s. polar region (cf. fig.1). Other parameters as specified in the caption in fig.2. Two conclusions can be drawn from this example:

1. The electric potential profile in the aqueous region on either side of the membrane is essentially determined by the charges at the adjacent polar region.
2. Inside the membrane, the electric field, which is the measure of the slope of the electric potential, cannot be considered constant.

Table 1 gives the values of the calculated potential difference for the points A and A' (see fig.1) which are positioned on the symmetry axis of the particle at the opposite boundaries between the polar regions and the hydrophobic part of the membrane. It can be seen that the variation in salt concentrations of one order of magnitude has a small effect compared with the effects of variations of the dielectric constant of the polar regions, or changes in the distance between the charges and the axis AA' for which the potentials are calculated.

3. Conclusions

The local electric fields and potentials inside the membrane depend significantly on:

1. A particular distribution of surface and membrane charges;

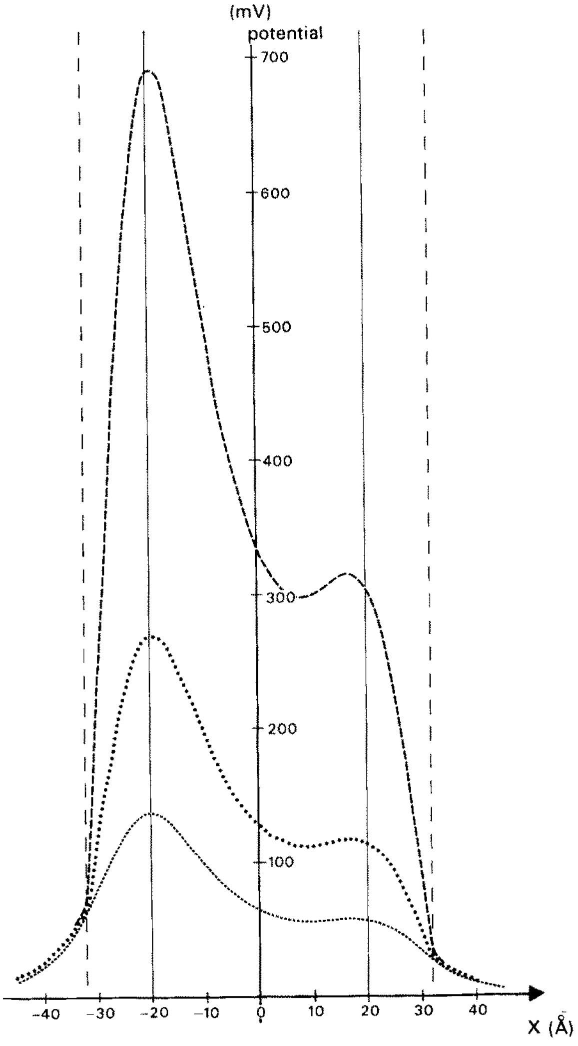

Fig.2. The electric potential profiles due to 5 positive charges at the l.h.s. and 2 positive charges on the r.h.s. of the membrane calculated along the symmetry axis (AA') of the protein. X denotes the position with respect to the centre of the membrane; the boundaries of different regions are indicated. The calculations were performed assuming the following values of the parameters: a = 12 Å; d = 40 Å; ρ = 12 Å; the concentration of mono-valent cations 0.1 mol/l and the concentration of divalent cations 0.01 mol/l. The top curve was calculated for $\epsilon_p = 6$, the middle curve for $\epsilon_p = 20$ and the bottom curve for $\epsilon_p = 50$.

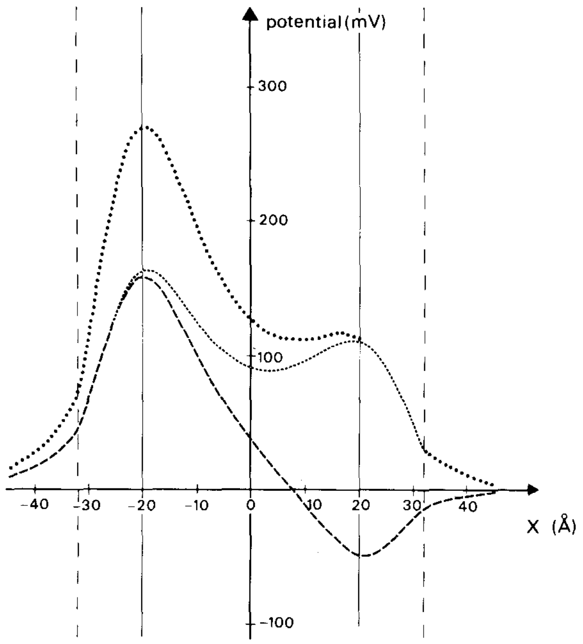

Fig.3. The electric potential profiles along the symmetry axis of the particle due to the following distribution of surface charges (all positive and univalent, except when specifically indicated): (a) 5 on the l.h.s., 2 on the r.h.s. (top curve); (b) 3 on the l.h.s., 2 on the r.h.s. (middle curve); (c) 3 on the l.h.s., 1 negative on the r.h.s. (bottom curve). The assumed dielectric constant of the polar region was $\epsilon_p = 20$.

2. Dielectric properties of the water–membrane interface.

By comparison, salt conditions in the bulk solution have only moderate effects.

Since local electric fields play an important role in the processes at the molecular level, it follows that in order to gain a better understanding of specific mechanisms, such as the charge separation in photosynthesis, one needs to know the positions of charged species (including bound ions) at the membrane–water interface and inside the membrane.

The magnitude of local potentials is significantly different from the average values predicted by the Gouy-Chapman theory. It is feasible that the built-in local potential gradients are large enough to account for the measured light-induced potentials in photosynthetic bacteria or plants; thus the theory of charge separation based on the postulated asymmetry of surface charges [1,2] is not in contradiction with experimental data.

Table 1
The electrostatic potential difference $\Delta\Psi_{AA'}$ (in mV) between the points A and A' at the opposite boundaries between the polar regions and the membrane (see fig.1)

ϵ_p value	$\rho = 12$ Å High salt	$\rho = 12$ Å Low salt	$\rho = 10$ Å High salt	$\rho = 10$ Å Low salt
50	81.7	96.2	106.8	121.6
20	156.9	172.7	213.1	229.2
6	385.3	398.8	532.3	576.0

The calculations were performed for two sets of ionic conditions: (a') 'high salt' with 0.1 mol/l of mono- and 0.01 mol/l of di-valent cations; and (b') 'low salt' where the concentrations were 10-times smaller. The distance ρ between the charges (5 at the l.h.s. and 2 at the r.h.s. polar region) and the axis AA' was either 12 Å or 10 Å. The assumed values of the dielectric permittivity of the polar regions were 6, 20 and 50. Other parameters as in the text and fig.1

References

[1] Duniec, J. T. and Thorne, S. W. (1979) FEBS Lett. 105, 1–4.
[2] Duniec, J. T. and Thorne, S. W. (1980) J. Theor. Biol. 85, 691–711.
[3] Barlow, C. A. (1970) in: Physical Chemistry. IXA Electrochemistry (Eyring, H. ed) pp. 167–246, Academic Press, New York.
[4] Barber, J. and Searle, G. F. W. (1979) FEBS Lett. 103, 241–245.
[5] Barber, J., Mills, J. and Love, A. (1977) FEBS Lett. 103, 174–181.
[6] Junge, W. (1977) Annu. Rev. Plant Physiol. 28, 503–536.
[7] Junge, W. (1980) private communication.
[8] Brown, R. H. (1974) Prog. Biophys. Mol. Biol. 28, 341–370.
[9] Nelson, A. P. and McQuarrie, D. A. (1975) J. Theor. Biol. 55, 13–27.
[10] Forsyth, P. A. jr, Marčelja, S., Mitchell, D. J. and Ninham, B. W. (1977) Biochim. Biophys. Acta 469, 335–344.
[11] Sauve, R. and Ohki, S. (1979) J. Theor. Biol. 81, 157–179.
[12] Pethig, R. (1977) in: Dielectric and Related Molecular Processes, Spec. Period. Rep. vol. 3, pp. 219–251, The Chemical Society, London.
[13] Harris, F. E. and O'Konski, C. T. (1957) J. Phys. Chem. 61, 310.
[14] Hanser, H. and Phillips, M. C. (1979) Prog. Surf. Membr. Sci. 13, 297–413.