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On the dielectrically observable consequences of the diffusional motions of lipids and proteins in membranes

1. Theory and overview

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Abstract. *1.* A system consisting of an array of cylindrical, polytopic membrane proteins (or protein complexes) possessed of a permanent dipole moment and immersed in a closed, spherical phospholipid bilayer sheet is considered. It is assumed that rotation of the protein (complex) in a plane normal to the membrane, if occurring, is restricted by viscous drag alone. Lateral diffusion is assumed either to be free and random or to be partially constrained by barriers of an unspecified nature.

2. The dielectric relaxation times calculated for membrane protein rotation in a suspension of vesicles of the above type are much longer than those observed with globular proteins in aqueous solution, and fall in the mid-to-high audio-frequency range.

3. If the long range lateral diffusion of (charged) membrane protein complexes is essentially unrestricted, as in the "fluid mosaic" membrane model, dielectric relaxation times for lateral motions will lie, except in the case of the very smallest vesicles, in the sub-audio (ELF) range.

4. If, in contrast, the lateral diffusion of membrane protein complexes is partially restricted by "barriers" or "long-range" interactions (of unspecified nature), significant dielectric dispersions may be expected in both audio- *and radio*-frequency ranges, the critical (characteristic) frequencies depending upon the average distance moved before a barrier is encountered.

5. Similar analyses are given for rotational and translational motions of phospholipids.

6. At very low frequencies, a dispersion due to vesicle orientation might in principle also be observed; the dielectrically observable extent of this rotation will depend, *inter alia,* upon the charge mobility and disposition of the membrane protein complexes, as well as, of course, on the viscosity of the aqueous phase.

7. The role of electroosmotic interactions between double layer ions (and water dipoles) and proteins raised above the membrane surface is considered. In some cases, it seems likely that such interactions serve to raise the dielectric increment, relative to that which might otherwise have been expected, of dispersions due to protein motions in membranes. Depending upon the tortuosity of the ion-relaxation pathways, such a relaxation mechanism might lead to almost any characteristic frequency, and, even in the absence of protein/lipid motions, would cause dielectric spectra to be much broader than one might expect from a simple, macroscopic treatment.

Key words: Dielectric spectroscopy, fluid mosaic, membrane, lateral electrophoresis, protein diffusion

Introduction

Two major mechanisms are thought to account for the dielectric behaviour of charged, membrane-bounded cell or vesicle suspensions in the audio- and radio-frequency ranges. The β-dispersion (Schwan 1957), occurring in the radio-frequency range, is thought to be dominated by a Maxwell-Wagner effect occurring at the interface between the cytoplasm and the poorly conducting plasma (cytoplasmic) membrane, whilst the α-dispersion, centred in the audio-frequency range, is in general ascribed to the relaxation, tangential to the charged membrane surface, of the ion cloud surrounding the cell and constituting the diffuse double layer (see e.g. Schwan 1957, 1963, 1977, 1981a, b, 1983a, b; Falk and Fatt 1968; Schwan et al. 1970; Cole 1972; Hasted 1973; Carstensen and Marquis 1975; Schanne and Ceretti 1978; Grant et al. 1978; Pethig 1979; Asami et al. 1980; Pilla 1980; Schwan and Foster 1980; Adey 1981; Stoy et al. 1982; Zimmermann

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1982; Harris et al. 1984; Pethig 1984). Other mechanisms are believed to operate at higher frequencies, but will not here concern us further (see e.g. Foster and Schepps 1981; Illinger 1981; Foster et al. 1982; Clegg et al. 1982; Gabriel et al. 1982).

The Maxwell-Wagner effect has been widely reviewed in this context; given the relative consistency of biological membrane capacitances, its relaxation time is then a function of the vesicle radius and the internal and external conductivities alone (e.g. Schwan 1957; Asami et al. 1980; Schwan and Foster 1980; Stoy et al. 1982; Zimmermann 1982; Epstein and Foster 1983; Harris and Kell 1983). The ion cloud polarisation mechanism is believed to exhibit an inverse square dependence upon the vesicle radius; little is known with certainty concerning its exact dependence upon the valency and conductivity of the ions in the diffuse double layer (see e.g. Schwarz 1962; Einolf and Carstensen 1971, 1973; Dukhin and Shilov 1974; Schwan 1983b; Kell 1983 and references therein). Thus, neither of these two types of mechanism considers a potential contribution of the rotational or translational mobilities of the lipids and proteins contained in the membrane to the dielectric properties of the vesicle suspension.

In recent experiments (Harris and Kell 1983; Harris et al. 1984; Kell 1983, 1984b), we have studied the passive electrical properties of a number of prokaryotic and eukaryotic microorganisms in the frequency range 100 Hz to 13 MHz. It was observed in particular (Harris et al. 1984) that the *breadth* of the β-dispersion of suspensions of *Paracoccus denitrificans* protoplasts was significantly greater than could be accounted for by invoking a distribution of cell radii, whilst assuming that a Maxwell-Wagner type of mechanism constituted the sole means of dielectric relaxation in this frequency range. Further, in bacterial chromatophores, Kell (1983, 1984b) recently observed a novel dispersion, the μ-dispersion, which, due to its sensitivity to the cross-linking reagent glutaraldehyde, he ascribed to some motional characteristics of the lipids and protein complexes of the chromatophore membrane. The very small size of bacterial chromatophores ensured that this dispersion was not obscured by the classical type of α- and β-dispersions, since their relaxation times were shifted to frequencies higher than that (those) of the μ-dispersion.

Since substantial rotational and diffusional mobility are now thought to be properties of the great majority of membrane-located proteins (at temperatures above the gel-to-liquid phospholipid phase transition), it is to be expected (i) that such motions should be observable by dielectric spectroscopic means, and (ii) that dielectric spectroscopy might therefore serve as a powerful tool in the characterisation of such motions in biomembrane vesicles generally. Nevertheless, we are not aware of any systematic discussion to date of the dielectric relaxation times or dielectric increments of such dispersions which might be expected on the basis of these properties of biomembranes and their constituent lipids and proteins. The purpose of the present article is thus to provide such a discussion, which might act as a framework for the refinement of experimental work in this area. An extension of our own experimental work, in the light of the present discussions, forms the subject of an accompanying paper (Harris and Kell 1985).

The "fluid mosaic" model system considered

The system considered is diagrammed in Fig. 1. This consists of a phospholipid bilayer membrane of thickness h containing an ensemble of cylindrical, polytopic integral membrane protein complexes of radius a "randomly" dispersed in the vesicle membrane. The viscosities of the membranous and aqueous phases are given respectively by η and η'. Only one protein complex is shown in Fig. 1 for clarity, but, by definition, the concept of a complex implies a lack

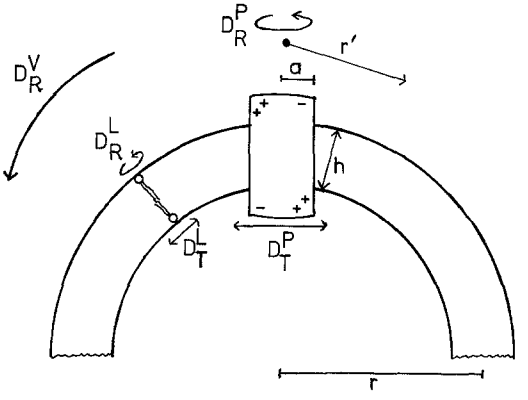

Fig. 1. The model membrane system considered. We treat the system as consisting of an ensemble of spherical bilayer membranes of thickness h, arrayed in which are cylindrical membrane proteins of radius a, which extend beyond the bilayer surfaces. Rotation of lipids and proteins is possible with rotational diffusion coefficients D_R^L and D_R^P, respectively. The vesicle radius is r. Translational diffusion coefficients for lipids and proteins are D_T^L and D_T^P respectively. If translational diffusion is not limited by hydrodynamic forces alone, the average lateral distance possibly diffusible is r'. The membrane "viscosity" is η, whilst that of the aqueous phases (taken to be the same in both intra- and extra-vesicular spaces) is given by η'. No other proteins are shown, but it is assumed that collectively they take up areas of as much as 30%–50% in vivo. Orientation of a vesicle with a rotational diffusion coefficient D_R^V is also permitted, as indicated. For further details see text

of long-range interactions with other, separate "complexes" (see Saraste 1983). Our model thus exemplifies, and follows from, "a prediction of the fluid mosaic model ... that the two-dimensional long-range distribution of integral proteins in the plane of the membrane is essentially random" (Singer and Nicolson 1972).

Thus, we may crudely characterise the possible motions of the proteins by a rotational diffusion coefficient D_R^P in a plane normal to the membrane and a lateral, translational diffusion coefficient D_T^P. Similar diffusion coefficients for lipids are D_R^L and D_T^L. Rotation (i.e. orientation) of the entire vesicle with a rotational diffusion coefficient D_R^V is also permitted. The vesicle is supposed to be representative of an ensemble of such vesicles randomly disposed between two planar electrodes parallel to the left- and right-hand sides of the page. The protein is drawn so as to indicate that although it has a variety of charged groups on its aqueous surfaces, they are so arrayed (in this case) that the protein has no net permanent dipole moment in a plane normal to the membrane. Nevertheless, as we shall see, the fact that it is confined in a membrane has a significant effect upon its dielectrically observable properties. However, to explore this idea, it is first necessary briefly to summarise the dielectric properties of *aqueous* solutions of globular proteins in the audio- and radio-frequency ranges. For completeness, we begin by reminding readers of the Debye (1929) theory of dielectric dispersion.

Dielectric dispersion

As discussed in extenso in many excellent texts and monographs (e.g. Schwan 1957, 1963; Fröhlich 1958; Daniel 1967; McCrum et al. 1967; Hasted 1973; Hedvig 1977; Grant et al. 1978; Schanne and Ceretti 1978; Pethig 1979), the passive electrical properties of all types of condensed matter may vary as a function of frequency, or, in other words, they may exhibit the property of dielectric dispersion. For systems in thermal equilibrium, and which do not store energy between the cycles of a sinusoidally varying electrical field, this manifests as a frequency dependence (dispersion) of the conductance and capacitance of a sample of the material placed between two electrodes. Allowing for the electrode geometry (i.e. the cell constant), we obtain the familiar Debye equation for the complex permittivity (ε^*):

$$\varepsilon^* = \varepsilon_\infty + \frac{\varepsilon_S - \varepsilon_\infty}{1 + i\,\omega\,\tau}, \tag{1}$$

where ε_S and ε_∞ are respectively the permittivities at frequencies which are very low and very high relative to that of the relaxation, τ is the relaxation time, $\omega\,(=2\,\pi f)$ the frequency in radians per second and $i = \sqrt{-1}$. Separating the real and imaginary parts, we obtain:

$$\varepsilon' = \varepsilon_\infty + \frac{\varepsilon_S - \varepsilon_\infty}{1 + \omega^2\,\tau^2} \tag{2a}$$

$$\varepsilon'' = (\varepsilon_S - \varepsilon_\infty)\,\frac{\omega\,\tau}{1 + \omega^2\,\tau^2}. \tag{2b}$$

The quantity $(\varepsilon_S - \varepsilon_\infty)$ is known as the dielectric increment $\Delta\varepsilon$. ε'', the dielectric loss, is also related to the change in conductivity as the frequency range of interest is scanned, according to the relation:

$$\varepsilon'' = \frac{\sigma' - \sigma'_L}{2\,\pi f\,\varepsilon_0} \tag{3}$$

where σ' is the (real part of the complex) conductivity, σ'_L, the limiting value of the conductivity at low frequencies and ε_0 is the permittivity of free space $(= 8.854 \times 10^{-14}$ F/cm).

In practice, real systems exhibit a spectrum of relaxation times, and relaxation times and their dielectric behaviour may be described by an empirical modification of the Debye equation introduced by Cole and Cole (1941):

$$\varepsilon^* = \varepsilon_\infty + \frac{\varepsilon_S - \varepsilon_\infty}{1 + (i\,\omega\,\tau)^{1-\alpha}}, \tag{4}$$

where $0 \leqq \alpha < 1$. One important mathematical feature of this analysis is that a plot of ε'' against ε' takes the form of a semi-circle depressed below the abscissa, such that a line drawn between this centre and the point $(\varepsilon_S, 0)$ or $(\varepsilon_\infty, 0)$ makes an angle $(\alpha\,\pi/2)$ radians with the abscissa. The actual distribution of relaxation times corresponding strictly to this behaviour is rather complicated (see e.g. Cole and Cole 1941; Schwan 1957; Hasted 1973), but, as discussed by Schwan (1957) and Salter (1981), inter alia, a variety of fairly simple relaxation time distributions (e.g. normal, rectangular) exhibit a behaviour that is for practical purposes indistinguishable from that of the "true" Cole-Cole behaviour. Although other modifications of the Debye equation have been proposed (see, for a survey, Boyd 1980), none has yet achieved widespread usage in work with biological systems, and we do not therefore discuss them here. Normalised plots of the Cole-Cole equation for the permittivity and loss, using different values for α, are given in Fig. 2. The so-called critical (characteristic) frequency f_c is that frequency at which the permittivity takes the value $(\varepsilon_S - \varepsilon_\infty)/2$, or at which the dielectric loss is maximum. The critical frequencies determined by the two methods are not necessarily equal, but for values of α not too much greater than 0, the relaxation time $\tau\,(= 1/2\,\pi f_c)$ is given approximately by:

$$\tau = \frac{(\varepsilon_S - \varepsilon_\infty)\,\varepsilon_0}{\sigma'_\infty - \sigma'_L}. \tag{5}$$

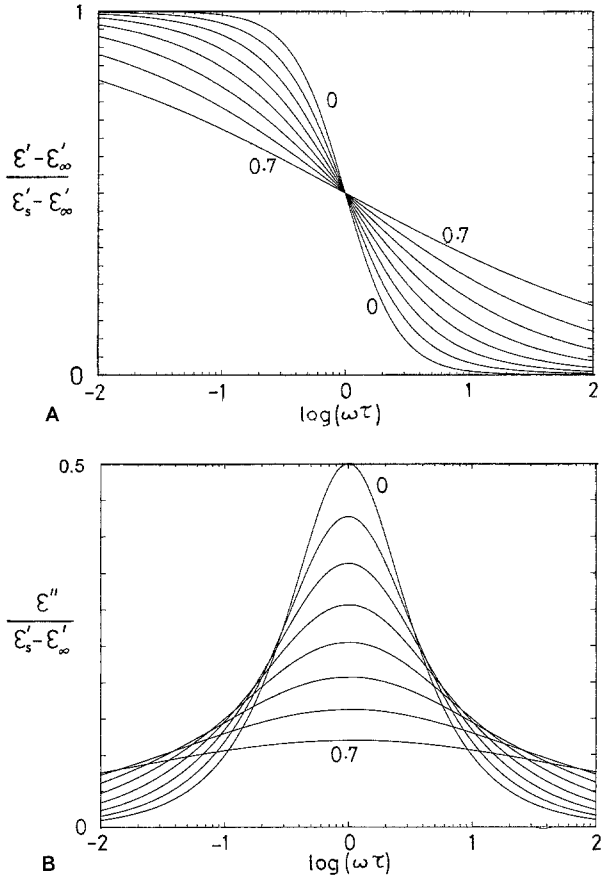

Fig. 2. The normalised Cole-Cole functions. The normalised real (**A**) and imaginary (**B**) parts of the permittivity, as a function of the normalised frequency, for various values of the Cole-Cole α. Values used for α are 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 and 0.7. The permittivity at the characteristic frequency is independent of α; this, and the sharpness of a dispersion when $\alpha = 0$, shows that, given the superposition theorem, a "broad" dispersion is equivalent to a sum of "narrow" dispersions with appropriately changed characteristic frequencies

Although these equations were derived for describing the rotation of rigid molecules with a permanent dipole moment, behaviour conforming to such equations is formally (and experimentally) indistinguishable from that of any movement (e.g. hopping) of charge in response to an alternating field (e.g. Jonscher 1975; Lewis 1977; Gascoyne et al. 1981). Thus we may characterise the properties of a (linear) dielectric dispersion by the variables f_c, $\Delta\varepsilon$ and α. In this vein, we may now consider the dielectric properties of aqueous solutions of globular proteins.

Dielectric properties of aqueous solutions of globular proteins

The overwhelming majority of globular proteins so far studied seem to possess a permanent dipole moment of several hundreds of Debyes (where unit charges separated by a distance of 1 Å, or 1 charge-Å, have a dipole moment of 4.8 *D*) (see. e.g. Oncley 1943; Takashima 1962, 1969; Grant and South 1972; South and Grant 1972; Gerber et al. 1972; Takashima and Minakata 1975; Petersen and Cone 1975; Wada 1976; Hasted 1973; Grant et al. 1978; Pethig 1979; Eden et al. 1980; Hasted et al. 1983; Kell and Hitchens 1983). Studies on the temperature and viscosity dependence of the relaxation time(s) of the "anomalous dispersion" of such proteins indicate rather unequivocally that their radio-frequency dielectric properties are dominated by the rotation (around the two axes of the equivalent ellipsoid of revolution) of this permanent dipole (e.g. Takashima 1969; Takashima and Minakata 1975). The magnitude of the dielectric increments observed are equivalent to the positional exchange of only a small number of charges of opposite sign separated by a molecular diameter, a number far less than the total number of charged groups present on (in) the protein. Other, more or less viscosity-independent, mechanisms potentially contributing to the RF dielectric properties of aqueous solutions of globular proteins, such as the Kirkwood-Shumaker-Scheider type of proton fluctuation mechanism (Kirkwood and Shumaker 1952; Scheider 1965), are now thought to assume only a relatively minor importance. Residual inconsistencies between calculated and experimental dielectric increments might reasonably be explained by the *cooperative* motions of the protein side-chains (Takashima 1969; Kell and Hitchens 1983). The total dielectric increments of such proteins lie in the range 1–15 relative permittivity units per g/100 ml), corresponding to dipole moments per molecular weight unit of roughly 5–20 *D* per kilodalton (Oncley 1943; Gerber et al. 1972; Petersen and Cone 1975; Takashima 1969; Schwan 1981 a).

The characteristic frequency (in Hz) for the rotational relaxation of aqueous solutions of (spherical) globular proteins is given by:

$$f_c = 1/2\,\pi\,\tau = kT/8\,\pi^2\,\eta'\,a^3, \qquad (6)$$

where the symbols have the same meaning as those in Fig. 1. As the protein of interest becomes more elongated (ellipsoidal), more than one relaxation may be observed, leading to a broadening of the dispersion (Oncley 1943; Takashima 1969; Takashima and Minakata 1975; Grant et al. 1978). Nevertheless, given typical protein dimensions, and the viscosities of aqueous physiological solutions, (approx. 0.01–0.015 *P*, where 1 *P* = 0.1 Pa s), it is to be expected, and is observed, that the characteristic frequencies for the relaxation times of aqueous solutions of globular proteins generally fall within a

decade of 1 MHz. As Schwan and Foster (1980) have put it, "this RF dispersion is quite noticeable in pure protein solutions, but in tissues and cell suspensions it only contributes slightly to the large β-dispersion found in these materials." What then are the mechanisms dominating the dielectric behaviour of tissues and cell suspensions in the audio- and radio-frequency ranges?

Dielectric behaviour of tissue and cell suspensions

As mentioned in the introduction, most workers ascribe the dielectric dispersion(s) (measured with extracellular electrodes) of cell suspensions in the audio-frequency range to counterion motions tangential to charged membrane surfaces. Schwan (1983b) gives a recent discussion of various other proposals. Whilst there will of course be additional tissue-dependent mechanisms contributing to dielectric relaxations in the audio-frequency range, in the interests of generality we will confine our discussion to the simple cell model considered in Fig. 1. Thus, for instance, we will not here consider the very large contributions to the low-frequency dielectric properties of intact bacterial cells caused by their possession of a rigid and highly charged cell envelope (see Einolf and Carstensen 1973; Harris et al. 1984 and references therein). Therefore, for measurements carried out on cell suspensions with extracellular electrodes, only the counterion polarisation type of mechanism, of those discussed by Schwan (1983b), is even in principle applicable. Such a mechanism, however, would not alone seem to be able to account, for instance, for the fact that erythrocyte ghosts possess an α-dispersion whilst their parent red blood cells do not (Schwan and Carstensen 1957). Certain other difficulties with the counterion polarisation mechanism are alluded to by Kell (1983). Thus other, as yet uncertain, mechanisms must be invoked to account for the low-frequency dielectric behaviour of cells conforming to the model in Fig. 1. Although not explicitly considered in Fig. 1, we must also mention the possibility that carrier-mediated ion-gating or ion-transfer reactions might conceivably serve to make some contribution in some cases.

The radio-frequency dielectric properties of membrane-bounded cells are thought to be dominated by the capacitive charging of the relatively ion-impermeable cell membrane itself. In this regard, it is of great historical significance that Fricke (1925) in fact used measurements of the large RF dielectric dispersion to determine for the first time that biological membranes must indeed be of molecular thickness (3–10 nm). However, the view seems widely to have evolved that this (Maxwell-Wagner) effect is the only mechanism contributing significantly to the RF-dielectric properties of membrane-bounded cells and vesicles, properties that are described, within this framework, by the so-called suspension equations (see e.g. Schwan 1957; Pauly and Schwan 1959; Pauly et al. 1960; Hanai et al. 1975; Schanne and Ceretti 1978; Asami et al. 1980 a, b; Schwan and Foster 1980; Asami and Irimajiri 1984; Harris and Kell 1985). However, as discussed for instance by Pauly et al. (1960), by Pauly (1963) and by Cole (1970), the ostensible distribution of relaxation times observed, and reflected in the Cole-Cole α, significantly exceeds any conceivable size- or internal conductivity-distribution possessed by the vesicles. A more extensive discussion of this is given in the accompanying paper (Harris and Kell 1985) and elsewhere (Kell and Harris 1985). Thus, other mechanisms of dielectric relaxation must surely also be invoked to explain the RF dielectric properties of protein-containing-biomembrane-bounded cells and vesicles. In particular, since it is now widely recognised that integral membrane proteins and lipids may exhibit both rotational and translational motions, it must be construed that such motions might also contribute to the dielectric relaxational behaviour of such systems. The purpose of the rest of the present article is to give an overview of the characteristic frequencies and dielectric increments to be expected if such behaviour is indeed occurring.

Rotational relaxation times of membrane proteins

As is done in Fig. 1, it is simplest, and fairly accurate, to treat the rotational motions of a protein (complex) in a biomembrane as those of a hard cylinder of radius a rotating in a plane normal to the membrane, which is possessed of a "bulk" viscosity η. Yet, recognising the anisotropic, heterogeneous and granular nature of biomembranes, most workers have employed the term "microviscosity", a term derived from fluorescence polarisation measurements (see Edidin 1974; Azzi 1975; Shinitzky and Barenholz 1978; Cherry 1979), to relate the rotational relaxation time to the rotational diffusion coefficient by means of the Stokes-Einstein equation. Viscosities (microviscosities) so derived lie in the range $1-10\,P$ (0.1–1 Pa s) when the lipids are not in the "gel" phase (Edidin 1974; Evans and Hochmuth 1978; Cherry 1979; Cherry and Godfrey 1981; Webb et al. 1981; Vaz et al. 1982; Hoffman and Restall 1983; but cf. Hughes et al. 1982). Viscosities lower even that $1\,P$ (which corresponds roughly to that of olive oil at room temperature) are obtained if one considers possible restrictions to

motion in a "wobbling-in-a-cone" model used in certain fluorescence depolarisation experiments (Kawato et al. 1977, 1981; Kinosita et al. 1977; Ikegami et al. 1982). The increase in fluidity as the membrane core is approached (Hauser and Phillips 1979; Israelachvili et al. 1980; Davis 1983) surely indicates that the "viscosity" or "fluidity" does not possess a sharp value, and, in particular, estimates of "surface viscosity" give values significantly greater than those quoted here (Evans and Hochmuth 1978). Nevertheless, we shall assume, in common with other workers, that we may smear out these effects such that the lipid matrix surrounding the membrane proteins of interest may indeed be characterised by "a" single viscosity η.

From the Stokes-Einstein equation, therefore, we have, for the characteristic frequency (f_c) for rotation of a membrane protein:

$$f_c = kT/8\,\pi^2 a^2 h\,\eta, \tag{7}$$

or

$$D_R = 1/\varphi = kT/4\,\pi\,\eta\,a^2 h \tag{8}$$

(see above and e.g. Oncley 1943; Cherry and Godfrey 1981), where a is the protein radius, h the membrane thickness, k Boltzmann's constant, T the absolute temperature, D_R the rotational diffusion coefficient and φ the rotational correlation time.

Now, since proteins can move with so-called "boundary lipid" (see Vanderkooi and Bendler 1977), which is immobile on an esr but not an nmr timescale (Chapman et al. 1979; Kang et al. 1979; Marsh 1983; Benga and Holmes 1984), there is a certain ambiguity concerning the protein radius which we should construe in the present analysis. However, the uncertainties in the "viscosity" far outweigh any uncertainties in the effective (lipo)-protein radius in intact membranes. We may then plot, as in Fig. 3, the characteristic frequency for the (putatively dielectrically observable) rotational relaxations (in a plane normal to the membrane) that we may expect for proteins or protein complexes with a radius typical of those found in biomembranes. In Fig. 3, a membrane thickness of 4.5 nm is used. However, since the "thickness" also lacks a sharp value, the choice of "thickness" is left to the reader, who may adjust the chosen value effectively by modulating the chosen membrane microviscosity, since (Eq. (7)) the two scale together. It may be observed (Fig. 3) that, for typical membrane protein complexes, the characteristic frequency for rotational relaxation should be expected to lie in the decade 2–20 kHz. Such values assume "free" rotation of non-aggregated complexes, and may be expected, all other things being equal, to be essentially independent of the radius of the cell (vesicle) under consideration.

In practice, it would seem that the percentage of "immobile" (say $\varphi > 20$ ms) protein is a function of the lipid:protein ratio, and may exceed 50% in vivo (Kawato et al. 1981, 1982; Muller et al. 1984; Dixit and Vanderkooi 1984). Whether the aggregation that this implies is "non-specific", as sometimes stated (Kawato et al. 1982), or is in fact of functional significance in vivo is presently unknown. Even if, as seems possible, there were to be in general relatively few non-hydrodynamic barriers to the rotation of both intrinsic (Cherry 1979; Hoffman and Restall 1983) and extrinsic (Froud and Ragan 1984) membrane proteins, this would not of itself tell us whether or not there may be significant barriers to the long-range *lateral* diffusion of such membrane protein complexes. The addition of polymers to aqueous solutions, for instance, causes translational diffusion to become much more restricted than rotational diffusion (Laurent and Obrink 1972). Therefore, and since it may well be that protein diffusion in the plane of the membrane is not in general wholly unrestricted (Webb et al. 1981; Weaver 1982; Almers and Stirling 1984; Jain 1983; Agutter and Suckling 1984; McCloskey and Poo 1984; Kell 1984a; Kell and Westerhoff 1985; but cf. e.g. Cadenas and Garland 1979; Hackenbrock 1981), we will retain as conceptual possibilities the ideas both of free and restricted lateral diffusion. Cognate problems relating to the structure of the aqueous cytoplasm of cells have recently been excellently reviewed by Clegg (1984).

The lateral diffusion of membrane protein complexes

Although many spectroscopic techniques such as nmr and esr have been used to demonstrate the lateral mobility of membrane components, only two approaches would seem to possess the real possibility of establishing to what extent the degree of *long-range* translational motional freedom implicit in the fluid mosaic membrane model actually exists in practice. These methods are fluorescence recovery after photobleaching (FRAP), in various embodiments (see e.g. Cherry 1979; Peters 1981; Webb et al. 1981; Vaz et al. 1982, 1984; Hoffman and Restall 1983), and "lateral electrophoresis" (e.g. Jaffe 1977; Poo 1981; Sowers and Hackenbrock 1981; and see later).

The analysis usually used to evaluate the two-dimensional lateral diffusion coefficients of membrane proteins is that expounded by Saffman and Delbrück (1975), Saffman (1976). Using the same model as that in Fig. 1, they derived the relation:

$$D_T = (kT/4\,\pi\,\eta h)\left[\ln\left(\frac{\eta\,h}{\eta'\,a}\right) - \gamma\right] \tag{9}$$

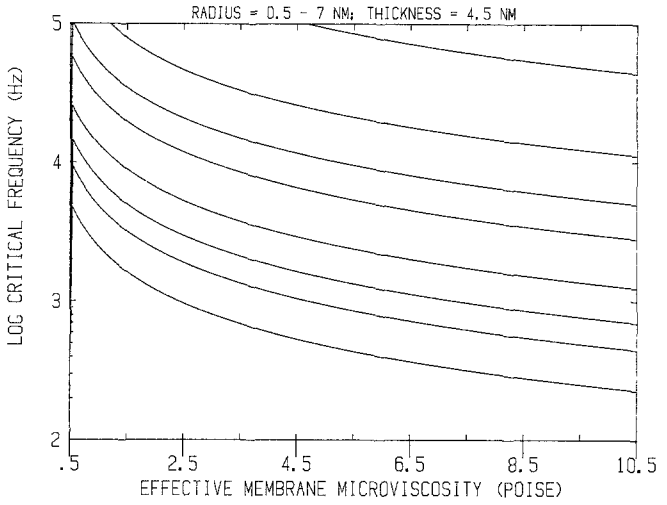

Fig. 3. Critical (characteristic) frequency for the rotational diffusion of "hard", cylindrical membrane proteins of the radius (in nm) indicated, as a function of the effective membrane microviscosity. The membrane thickness is taken to be 4.5 nm and the temperature 298 K

where $\gamma\,(=0.5772)$ is Euler's constant, and the other symbols are as in Fig. 1. If diffusion is free and unrestricted, and the area taken up by the diffusing particles is not too great, the exponential relaxation time $\tau(=1/2\,\pi f_c)$ for positional randomisation is related to the *vesicle* radius (r) by:

$$\tau = r^2/2\,D_T \qquad (10a)$$

(see e.g. Sowers and Hackenbrock 1981; Poo 1981; Zimmermann 1982).

In contrast, if, due for instance to "long-range" protein/protein interactions, to lipid crystallisation or to extramembranal constraints, a protein may move only an average distance r', then we may take it that:

$$\tau = r'^2/4\,D_T. \qquad (10b)$$

(Note the distinction between r and r' used herein.) We therefore have:

$$f_c = (kTK/4\pi^2\eta r^2 h)\left[\ln\left(\frac{\eta\, h}{\eta'\, a}\right) - \gamma\right], \qquad (11)$$

where $K=1$ and $r=r$ (the vesicle radius) in the case of free diffusion, whilst $K=2$ and $r=r'$ (average diffusion distance) in the case of restricted diffusion. The characteristic frequencies of such relaxations, for a range of membrane "viscosities", are plotted in Fig. 4. As widely recognised, the *protein* radius, and also the extramembranal "viscosity", is buried in the logarithmic term of Eq. (11); alteration of the value (4.5 nm) used for the former in Fig. 4 therefore has little substantive effect upon the curves shown. The influence of electroosmotic forces

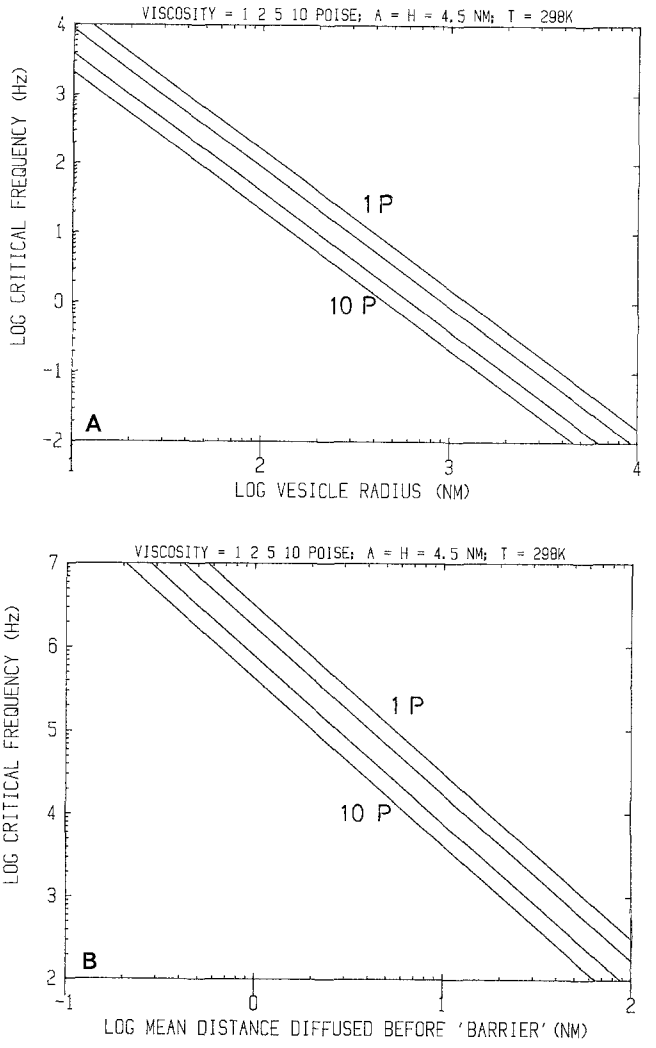

Fig. 4. Critical (characteristic) frequency for the translational diffusion of membrane proteins of radius 4.5 nm in a membrane of thickness 4.5 nm. Aqueous viscosity is 1.25 cP. Membrane viscosity = 1, 2, 5 or 10 P, as indicated. **A** Free diffusion: characteristic frequencies are a function of the vesicle radius. **B** Restricted diffusion: characteristic frequencies are a function of the distance moved before a "barrier" is encountered

(McLaughlin and Poo 1981) is not, therefore, considered in this treatment.

It is evident (cf. Fig. 4A and B) that the degree of freedom of lateral mobility has a most dramatic bearing upon the characteristic frequency of the potentially observable dielectric relaxations due to the lateral mobility of proteins in the membrane. In particular, if lateral diffusion is rather restricted, relaxations with values for f_c in the range 100 kHz to as much as 10 MHz might be anticipated; for accessible vesicle radii, such relaxation times are not possible if diffusion is unrestricted (Fig. 4A and B). Of course, *heterogeneity* in the degree of restriction of protein mobilities is also possible.

As discussed above, a dielectric relaxation of the Debye type is characterised not only by its relaxation time but also by its dielectric increment (i.e. magnitude) $\Delta\varepsilon'$. This in turn depends upon the concentration of dipolar particles, their molecular dipole moments and the degree of restriction on their motions. What sort of dielectric increments might we expect for mechanisms such as those presently under consideration, based upon what is known of the structure of membrane proteins?

Dielectric increments due to membrane protein rotations

It is usual to relate the observable dielectric increment $\Delta\varepsilon'$ due to the rotation of an aqueous globular protein with a permanent dipole to its molecular dipole moment μ according to the relation:

$$\mu = \sqrt{9{,}000\, kT\, \Delta\varepsilon' / 4\pi NHc} \qquad (12)$$

(e.g. Oncley 1943; Takashima 1969; Petersen and Cone 1975; Grant et al. 1978), where c is the *molar* protein concentration, N Avogadro's number and H is an empirical constant which is usually taken (from a comparison between calculation and experimental data for glycine) to have the value 5.8. In Eq. (12), μ is in Debyes, but since two unit charges of opposite sign separated by 1 Å (10^{-10} m) possess a dipole moment of 4.8 D, we may express the effective dipole moment of any potentially mobile distribution of charges in the units of charge-Ångström by dividing the right-hand side of Eq. (12) by 4.8. This allows us to obtain a more intuitive feel for the number and distance of charge movements required to account for a dielectric increment obtained experimentally. Such curves, conforming to Eq. (12), are plotted in Fig. 5.

In the case of membrane proteins, we are not aware of any dielectric measurements of freely rotating proteins of known mean structure (charge distribution). Thus, our considerations here must be only semi-quantitative. For typical membrane protein complexes of diameter 8 nm, at a total molar concentration in suspension of 20 μM, rotation of 8 charges of opposite sign around an axis normal to the plane of the membrane would give a dielectric increment of only 20 permittivity units. Thus protein *rotation* in a membrane is likely to make a much smaller contribution to the low-frequency dielectric properties than any medium- or long-range translational motion in the plane of the membrane (Kell and Westerhoff 1985). Note that in the case of the translational motion of membrane proteins, the number of charge-Å computed from Eq. (12) should

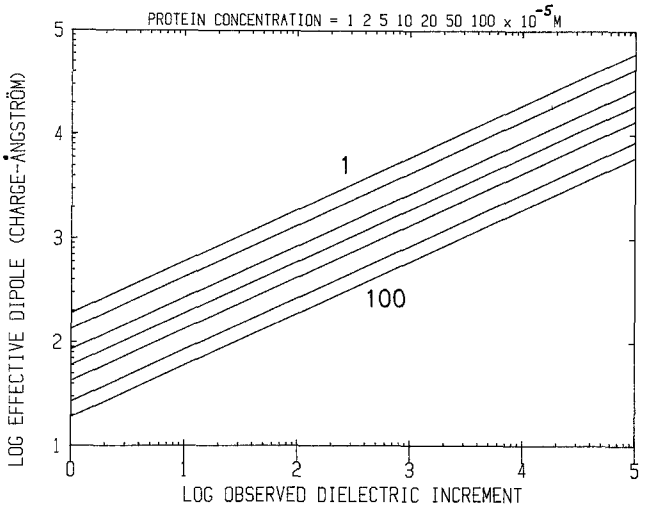

Fig. 5. Dielectric increments due to motions of proteins in membranes. The formal molecular dipole moment due to the translational motion of proteins in membranes (in charge-Å) is plotted against the observable dielectric increment, for molar protein concentrations of 1, 2, 5, 10, 20, 50 and 100×10^{-5} M, using Eq. (12). $T = 298$ K, and the empirical parameter $H = 5.8$

be divided by 2 to obtain the number of net, externally-facing charges moving in the plane of the membrane; this is because the relation 4.8 D = 1 charge-Å considers the positional exchange of two charges of opposite sign, and not the translational motion of the number of *net* charges. Equally, in the crudest model of translational motion, it is to be assumed that the net charges are positioned somewhere on the axis of rotation of the protein in a plane normal to the membrane. Where exactly should this be?

Vesicle size and transmembrane penetration of the electrical field

As drawn in Fig. 1, if the membrane is invisible to the field, the protein in question has no rotational dipole moment, whilst if the membrane acts as a barrier to the flow of current, the protein has an apparently marked dipole moment. The transition between these two types of behaviour occurs over a range of frequencies, the characteristic frequency of which is that governed by the suspension equations relating to the Maxwell-Wagner effect (as applied to spherical shell membranes) (see e.g. Schwan 1957; Schwan and Foster 1980; Zimmermann 1982). The relaxation time τ is given by:

$$\tau = r C_m \left(\frac{1}{\sigma_i} + \frac{1}{2\sigma_o} \right), \qquad (13)$$

where r is the vesicle (cell) radius, C_m the membrane capacitance per unit area and σ_i and σ_0 are respectively the internal and external conductivities. Thus the critical frequency for the transition between these behaviours is given by:

$$f_c = (\sigma_i \sigma_o)/((\pi r C_m)(\sigma_i + 2\sigma_o)). \quad (14)$$

It is evident that this effect alone can serve significantly both to broaden and to make asymmetric the dielectric dispersions observed in appropriate cases.

For completeness, it should be mentioned that the situation discussed here is even more complicated than the simple foregoing treatment would indicate, since there is an ambiguity in the values of the conductivity which should be used in describing σ_i and σ_0; due to the protein motions themselves and the imperfect separability of contributions due both to "classical" and other dispersions of the surface admittance type (see McLaughlin and Poo 1981; Rabinowitz 1982; Kell 1983; Harris and Kell 1985 and later), σ_i and σ_0 values are themselves frequency-dependent. However, these second-order complexities will not be pursued herein. It is, however, necessary to discuss the possible contributions of lateral and rotational motions of phospholipids to the biomembrane system considered.

Dielectrically observable motions of phospholipids

As before, we consider the model shown in Fig. 1. In other words, we assume that the lipids are arrayed in a bilayer configuration, and consist of both neutral (zwitterionic) and charged phospholipids. Sterols and galactolipids are not considered. It is assumed that both rotation in a plane normal to the membrane and translation in the plane of the membrane are permitted. The dielectric properties of phospholipids of biological interest have been studied in unilamellar (Schwan et al. 1970; Redwood et al. 1982), multilamellar (Kaatze et al. 1975, 1979a, b; Hence 1980; Pottel et al. 1984) and other (Shepherd and Buldt 1978, 1979) systems. The numerous studies of the passive electrical properties of *planar* bilayer membranes (BLM) are not relevant to our present considerations, and are not here considered further (see e.g. Hanai et al. 1964, 1965; Tien 1974; Fettiplace et al. 1975; Haydon et al. 1977); no substantive dispersions due to motions normal to the plane of the membrane have been found for BLM in the frequency range under discussion.

It is to be mentioned that there is a marked interpretative disparity between the measurements made in unilamellar liposomes and in multilamellar systems; the former workers (Schwan et al. 1970; Redwood et al. 1972) consider neither rotational nor (for charged lipids) translational motions as potential contributors to the dispersion(s) observed, whilst the latter workers discuss mainly lipid head-group rotation, and do not lay nearly so much stress upon a Maxwell-Wagner type of dispersion mechanism Kaatze et al. 1975, 1979a, b; Henze 1980; Shepherd and Buldt 1978, 1979). Evidently, both types of mechanism may in principle contribute to the dielectric relaxations observed; the rather large capacitances (up to 7.7 $\mu F/cm^2$) required to explain the data solely in terms of a Maxwell-Wagner effect (Schwan et al. 1970; Redwood et al. 1972) tend to indicate that both mechanisms do indeed contribute. As suggested before (Kell 1983), measurements with photopolymerisable phospholipids (see e.g. Hupfer et al. 1983; Johnston et al. 1983; Fuhrhop and Mathieu 1984; Juliano et al. 1984) would seem to offer a clear cut experimental opportunity to resolve this issue. We may therefore consider the potential contribution of lipid motions to the frequency-dependent dielectric properties of biological cell and vesicle suspensions.

Within the framework of the model of Fig. 1, we may very crudely treat the phospholipids, whether charged or neutral (zwitterionic) as hard cylinders rotating in a plane normal to the membrane, which is itself possessed of a bulk viscosity η. In this case, we assume that rotation of a lipid at a given position in one half of the bilayer is not specifically coupled to that in the other half of the bilayer, so that the effective membrane thickness to be construed is one half of that used in the protein case treated in Fig. 3. We may therefore plot the critical frequency for di-

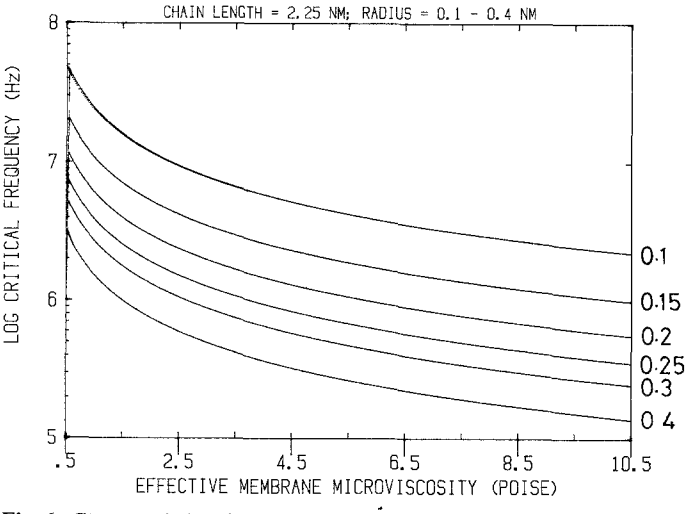

Fig. 6. Characteristic frequency for phospholipid rotation. The same equation as that used in Fig. 3 is plotted herein, using phospholipid "radii" of 0.1, 0.15, 0.2, 0.25, 0.3 and 0.4 nm

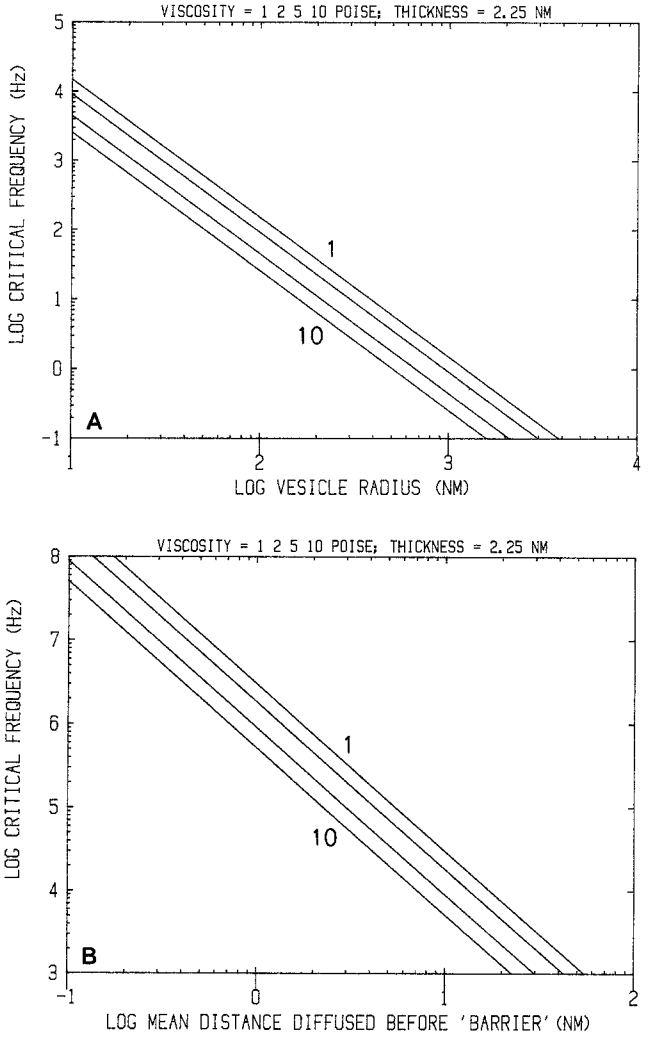

Fig. 7. Characteristic frequency for phospholipid translation if (**A**) free or (**B**) restricted. The same equation is used as in the protein case treated in Fig. 4, with a phospholipid "radius" of 0.25 nm. The membrane thickness used is 2.25 nm (half a bilayer); whilst the Saffman-Delbrück equation assumes equal access of the diffusing particles to each aqueous phase, the errors involved in ignoring this herein will be very small. Aqueous viscosity is taken to be 1.25 cP. $T = 298$ K

electric relaxation due to phospholipid rotation as a function of the "radius" of the rotating molecules and the effective membrane microviscosity (Fig. 6). It is seen (Fig. 6) that for a typical head-group "radius" of 0.2–0.25 nm, and a membrane microviscosity of 3 *P*, critical frequencies for rotation of the whole phospholipid lie in the range 1–5 MHz, much as observed (Kaatze et al. 1979a, b; but cf. Pottel et al. 1984).

Evidently, a hard cylinder is a much poorer approximation to a fluid, flexible phospholipid molecule than it is to an integral membrane protein (notwithstanding the marked and important fluctuational behaviour of the latter; see e.g. Welch et al. 1982); it is thus to be assumed that the lower "radius" and fluidity in the hydrocarbon region itself, together with a Maxwell-Wagner effect and other motions, in particular rotation of the zwitterionic or charged head-group itself, can account for the somewhat higher frequently dielectric relaxations also observed (Schwan et al. 1970; Redwood et al. 1972; Kaatze et al. 1975; Shepherd and Buldt 1978, 1979; Pottel et al. 1984), in the decade 10–100 MHz (cf. Fig. 6).

Calculations based on Eq. (12) indicate that, for a phospholipid concentration of 10 m*M* (approx. 7.5 mg/ml), the dielectric increment due to rotation is not expected to exceed 5–10 permittivity units. Thus phospholipid rotation is likely to make only a rather modest contribution to the (LF- and) RF-dielectric properties of biological cell and vesicle suspensions, and we do not consider it further. However, a somewhat different picture emerges for *lateral* motions of phospholipids.

Since only charged phospholipids immersed in a matrix of neutral or oppositely charged phospholipids can effect a dielectrically observable lateral motion, we will confine our attention to this situation. As in the case of proteins, we may consider both free (long-range) and restricted lateral diffusion, using Eq. (11). The results, for typical values of phospholipid "radius" and membrane microviscosity, are given in Fig. 7, whilst Fig. 8 gives the expected dielectric increment caused by lateral diffusion of charged phospholipids in the plane of the membrane.

If we make the assumption that 30%–50% of the lipids possess a (single net) negative charge, con-

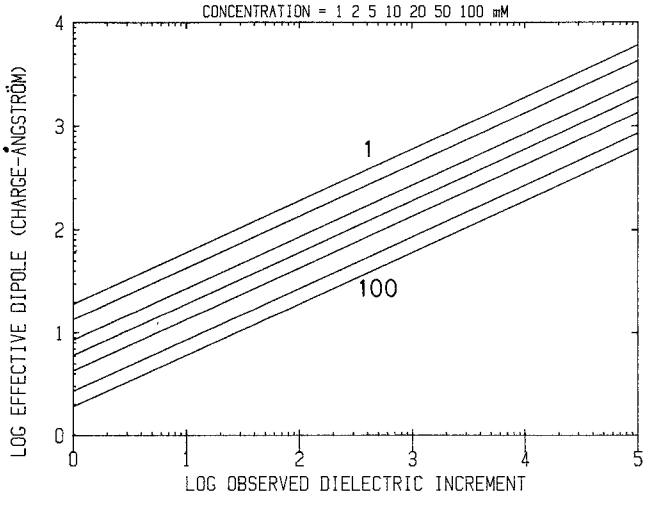

Fig. 8. Dielectric increment due to motion of phospholipids. The equation plotted, as in Fig. 5, is Eq. (12), using the value $H = 5.8$. $T = 298$ K. Concentrations of *charged* phopholipids are 1, 2, 5, 10, 20, 50 and 100 m*M*

sistent with the well-known net negative charge on biological membranes at neutral pH, a suspension of small unilamellar vesicles of diameter 35 nm, containing a total of 5 mM phospholipids, could exhibit a dielectric increment of several hundred permittivity units (Fig. 8), with a characteristic frequency (if free diffusion is occurring) in the range 1–5 kHz (Fig. 7A). If the average diffusion length is more restricted than is implicit in the fluid mosaic model, both the relaxation time and the dielectric increment will be decreased by an amount dependent upon the average distance moved before a "barrier" is reached (Figs. 7B and 8), as in the protein case discussed earlier.

Lateral electrophoresis of membrane components; the relation between visual and dielectric observability

It was first pointed out by Jaffe (1977), Jaffe and Nuccitelli (1977), and has since been amply confirmed (e.g. Poo and Robinson 1977; Poo et al. 1979; McLaughlin and Poo 1981; Poo 1981; Sowers and Hackenbrock 1981), that the application of a steady electrical field to a biomembrane-bounded cell or vesicle suspension should result in the lateral redistribution of charged membrane particles (integral membrane protein complexes). Similar effects should follow from the application of any sinusoidally varying field whose frequency is less than the characteristic frequency of the Maxwell-Wagner-type β-dispersion of the vesicles in question. Further, such field-dependent motions should *necessarily* be accompanied by a frequency dependence of the dielectric properties of such a system, i.e. they shoud be reflected as a dielectric dispersion, the dielectric increment and relaxation time of which may be used to gain important information concerning the rate and extent (randomness) of protein (and lipid) motions in the plane of the cell membrane (Kell 1983). However, the relationship between the magnitude of the visually and dielectrically observable motions of membrane proteins (if their lateral mobility is restricted only be hydrodynamic forces) is itself a function of the cell radius (Jaffe and Nuccitelli 1977). Since this fact relates to dielectric observations concerning the organisation of fluid mosaic membranes, we must consider this in some detail.

It is first necessary to state again that the rotation of a molecule with a permanent dipole moment μ Debyes is formally equivalent to the *juxtaposition* of a number (n) of unitary charges of opposite sign separated by a distance d Ångström, such that $\mu = 4.8\,nd$. The same *formal* effective dipole moment applies if $2n$ charges *move* a distance of d Ångström, their places being taken, for geometrical reasons, by neutral molecules.

Under the normal conditions of a dielectric experiment. The average angle (θ) between the (formal) dipoles and the polarising field is given by:

$$\langle \cos\theta \rangle = \mu E_1 / 3\,kT \tag{15}$$

(see Pethig 1979, p. 9), where E_1 is the local field. For low values of the applied field, in which the polarisibility is independent of the field strength, i.e. in which the linear, passive electrical properties are being investigated, Eq. (15) indicates that the extent to which the particles actually achieve their potential extrema of position or orientation is a linear function of (i.e. in proportion to) the field strength, as might be expected. The full equation, of which Eq. (15) is an approximation for low values of $x\,(=\mu E_1/kT)$, is represented by the Langevin function:

$$L(x) = \coth(x) - 1/x \tag{16}$$

(e.g. Jaffe and Nuccitelli 1977; Gabler 1978; Pethig 1979). As discussed by Pethig (1979, pp. 8–9) and by Zimmermann (1982), taking a dipole moment for the water molecule of 1.8 D, the value of x, even in a field of 1 kV/cm, is only approx. 1.5×10^{-4}. Thus the *visually*, though not the *dielectrically*, observable orientation would in this case be vanishingly small. The Langevin function is plotted in Fig. 9. It may be seen that for $\mu E_1/kT = 5$, approximately 80% (actually 80.009% at 25 °C) of all the molecules will be oriented in line with the electrical field, the field:dipole moment ratio being equal, at 298 K, to $6.159 \times 10^9\,V \cdot m^{-1} \cdot (Debye)^{-1}$. In other words, a field of only 1 V/cm will suffice *both visually and*

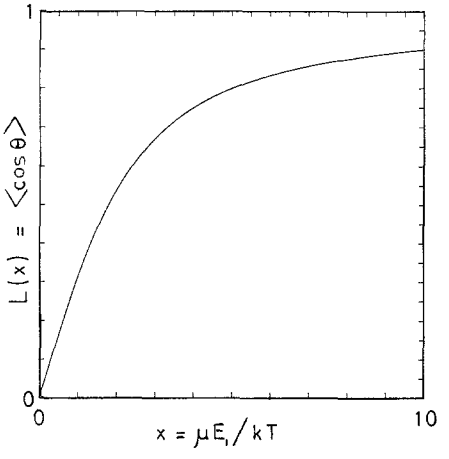

Fig. 9. The Langevin function. The mean of the cosine of the angle between the orienting field and a population of dipoles is plotted as a function of field strength in units of ($\mu E_1/kT$). For further discussion, see text

dielectrically to orient 80% of the mobile particles provided that their motion is formally equivalent to a dipole moment of 1.28×10^7 charge-Å or 1280 charge-micrometres. Juxtaposing proteins containing a net charge of 25 units of opposite sign, located at the poles of a eukaryotic cell of diameter 50 μm, will account for this behaviour, whilst if the field is 10 V/cm, such proteins need bear only 2.5 net (extracellularly facing) negative charges. Obviously such latter values lie well within the range of values presently known for, or realistically ascribable to, membrane proteins, values which, it is to be assumed, are independent of the radius of the cell in the boundary membrane of which the protein of interest happens to reside. In the former case, though, one seems bound to infer that a contribution from electroosmotic forces is indeed necessary, not only qualitatively but quantitatively to explain the data (McLaughlin and Poo 1981; Rabinowitz 1982; Kell 1983). Now, fields of 1 V/cm are by no means atypical of those used in the measurement of the dielectric properties of biological cells. How do the two types of value compare when we consider model, spherical prokaryotic cells (or similarly sized vesicles) of diameter 1 μm?

Given that we need, to move 80% of the protein complexes as far from one side of a 1 μm diameter prokaryote to the other as is possible, a total "charge-distance" equal to 1,280 charge-micrometers per (V/cm) (see above), we need, for proteins of the same net charge, a field *in direct proportion to the cell radius*, in this case of the order of 50−500 V/cm. The latter values are actually sufficient to drive ATP synthesis in vesicles of the stated size (e.g. Witt et al. 1976; Gräber 1981; Hamamoto et al. 1982; Schlodder et al. 1982), although they are lower than that (65 V/mm) used by Sowers and Hackenbrock (1981) in the only study on lateral electrophoresis of which we are aware made with vesicles of this size. Thus, although in the latter study (on mitoplasts of diameter approx. 0.9 μm), a visually observable and apparently unrestricted migration of membrane particles was induced electrically, the field per unit length, for a constant, average, interprotein distance, would have been proportionately larger, and hence capable of overcoming weak protein/protein interactions if they exist. Thus (Kell and Westerhoff 1985), it would be desirable to carry out such studies at widely differing field strengths, given the present doubt concerning the randomness (in the absence of an external field) of the mobility of the proteins of energy coupling membranes (see above). Similarly, at field strengths of 1 or 10 V/cm, the number of *visually* observable intramembrane particles electrophoresed in these experiments (Sowers and Hackenbrock 1981) would have been insignificant, whilst their *dielectric observability* would have been unimpaired. Thus, the conclusion, even in a linear system, is that for a given exciting field, the dielectric observability of lateral electrophoresis is independent of, and its visual observability strongly dependent upon, the vesicle radius, provided, of course, that there are negligible nonhydrodynamic restrictions to particle motion. One should also mention, of course, that the overlap between this type of mechanism of dielectric dispersion with that of other types of mechanism, such as counterion polarisation, will also be a function of the vesicle size.

Another extremely interesting feature of lateral electrophoresis arises in connection with the phenomenon of electric field-mediated cell fusion (Zimmermann 1982; Zimmermann et al. 1981; Zimmermann and Vienken 1982, 1984), a topic which forms the subject of the next section.

Lateral electrophoresis, dielectrophoresis and the dielectric behaviour of cell suspensions

As discussed in the beautifully done reviews of Zimmermann and colleagues (e.g. Zimmermann et al. 1981; Zimmermann 1982; Zimmermann and Vienken 1982, 1984; Zimmermann et al. 1984), it is now, and particularly through the efforts of these workers, possible electrically to fuse cells under controlled conditions, by means of a two-stage process. In the first stage, the cells, suspended in a medium of relatively low specific conductivity, are aligned dielectrophoretically, by the application of a sinusoidal electrical field, the frequency of which is determined empirically. This causes the cells to align with each other, and to form contact zones. Fusion is effected by means of a short (μs) electric pulse of high field strength, sufficient to cause a *reversible* dielectric breakdown of the membrane.

Now the optimal frequency for dielectrophoresis is equal to the characteristic frequency of the Maxwell-Wagner-type β-dispersion; it is thus evident that dielectric measurements (at low field strengths) just prior to, or during, the dielectrophoretic induction phase, might be used automatically to ascertain the optimal dielectrophoresis frequency. Such a strategy would of course be far more rapid and efficient than the manual assessment currently employed. Further, the dependence on the dielectrophoretic induction time and field strength of the *subsequent* field strength required for dielectric breakdown (Pilwat et al. 1981) has led to the suggestion (Zimmermann and Vienken 1982) that part of the role of the dielectrophoretic induction phase lies in segregating membrane proteins by

lateral electrophoresis so as to produce protein-free lipid domains in the areas of membrane contact. As mentioned elsewhere (Zimmermann et al. 1984; Kell 1984b), dielectric measurements during the entire electrofusion process should shed important light on the role of lateral electrophoresis during cell electrofusion. Further, as discussed in the previous section, dielectric measurements are likely to be far more sensitive than visual ones, although we note that the non-linearities implicit in the imperfectly reversible segregation (Zimmermann and Vienken 1982) of the particles by the field proscribe the use of the phrase "passive electrical properties", sensu stricto in this context.

Vesicle orientation and dielectric dispersion

An asymmetry in the charge distribution of the membrane vesicle of interest, whether native or induced by lateral electrophoresis of membrane component of ions and dipoles of their double layer, will drive the orientation of the vesicle in an electrical field. Such effects will be more marked in the case of non-spherical cells. The extent to which vesicle orientation is actually observable dielectrically (or, conversely, the extent to which it can account for a dielectric dispersion) is obviously a function of whether or not the former mechanisms (lateral motions of membrane and double layer components) can take place more rapidly than can vesicle orientation. The rate of the latter process is governed by hydrodynamic factors, exactly according to Eq. (6), modified so as to incorporate the radius of the vesicle:

$$f_c = 1/2\pi\tau = kT/8\pi^2\eta' r^3. \quad (17)$$

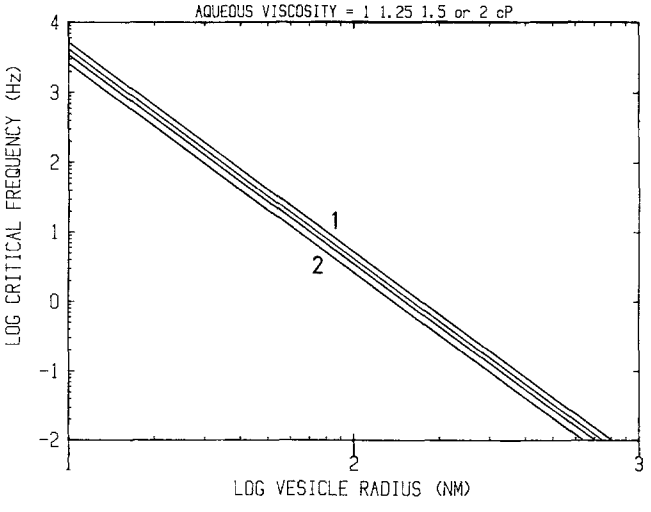

Fig. 10. Characteristic frequency for vesicle orientation. Equation (17) is plotted using values for the aqueous viscosity of 1, 1.25, 1.5 and 2 cP

This equation is plotted, for common values of the viscosity of aqueous solutions used in biochemical experiments, in Fig. 10. We are inclined to conclude therefore that vesicle orientation per se probably does not generally make a substantial contribution to the dielectrical dispersions observed in the range 10^0-10^7 Hz, since, except for the very smallest vesicles, the critical frequency values calculated are significantly lower than those ascribable to (the mechanims underlying) either the α- or the μ-dispersions (for spherical vesicles).

Electroosmotic forces and dielectric dispersion

The "classical" explanation of the α-dispersion assumes an essentially unrestricted motion of the ions of the double layer in a plane tangential to the notionally planar, charged membrane surface. However, if the zeta potential (potential at the hydrodynamic plane of shear) of (the extracellular face of) an integral membrane protein is less negative than that of the membrane "surface", rather complex behaviour can result, so that the motion of the integral membrane protein may even be in a direction opposite to that expected on the basis of simple electrostatic considerations of the sign of the DC or low-frequency field and the protein's net charge (McLaughlin and Poo 1981; Rabinowitz 1982). The relaxation time for the motions of such ions will be longer than that of the classical α-dispersion, since it is limited now not only by viscous forces in the double layer but also by the viscous drag exerted *on the membrane protein*. The dielectric increment due to this type of mechanism will be greater than that calculated on the basis of the number of electrostatic and dipolar charges on the hydrated proteins and lipids alone. Both of these factors are consistent with the data observed for the μ-dispersion in bacterial chromatophores (Kell 1983, and see Harris and Kell 1985), although an exact, quantitative calculation does not seem possible at the present time. Therefore the dielectric increments given in Figs. 5 and 8 should be construed only as the minimum possible due to the lateral electrophoretic type of mechanism of membrane component motions discussed herein. Field-driven cell *rotation*, in a direction *counter* to the rotating field (see Arnold and Zimmermann 1983), might also be explained by invoking electroosmotic interactions of the type discussed by McLaughlin and Poo (1981). Finally, the fact that protein complexes do indeed protrude beyond the membrane surface serves to complicate the simple explanation of *genuinely* tangential counterion relaxation as the cause of the α-dispersion, since such proteins will serve to impede the tangential relaxation of double

layer ions and dipoles. This point is discussed in more detail in the accompanying paper (Harris and Kell 1985).

Concluding remarks

The foregoing survey has indicated both that the motions of membrane lipids and proteins should be observable by dielectric means, and that such motions can provide an explanation for the dielectric properties of charged membrane vesicle suspensions additional to those implicit in current explanations of the classical α- and β-dispersions. In particular, it is to be expected that cross-linking reagents which do not change the surface charge density should obviate dispersions due to the present types of mechanism, but have no such effect upon the classical α- and β-dispersions. An experimental assessment of this prediction forms the main subject of an accompanying paper (Harris and Kell 1985).

Acknowledgements. We thank the Science and Engineering Research Council, UK, and ICI Agricultural Division for financial support.

Note added in proof: Uhlendorf (1984) has recently suggested that diffusional notions of fatty acids contaminating liposomes made from neutral lipids may contribute to the dielectric properties in the range 5 kHz to 10 MHz, in harmony with the present suggestions.

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