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Short communication

878 — DIELECTRIC SPECTROSCOPY OF PROTEIN TRANSLATIONAL DIFFUSION IN PROKARYOTIC MEMBRANES AND MEMBRANE VESICLES

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In principle, measurement of the passive electrical properties of biomembrane-bounded vesicle suspensions by dielectric spectroscopy should provide an insight into their organisation. Frequency-domain dielectric spectroscopy, in the frequency range 1 kHz–13 MHz, has been applied to the study of *Rhodopseudomonas capsulata* chromatophores [1,2].

A large (*ca.* 2000 permittivity units) dielectric dispersion is apparent, with a critical frequency around 12 kHz (which corresponds to a relaxation time of 1.25×10^{-5} s). This so-called μ-dispersion is sensitive to the action of the cross-linking reagent glutaraldehyde, and is not seen at the chromatophore isoelectric point [3] and has therefore been ascribed to the two-dimensional diffusional motions of membrane-bound proteins (we do not here discuss the potential contribution of rotational motions of proteins and the diffusional motions of lipids). The relaxation time for the *lateral electrophoresis* of proteins in a spherical shell membrane is given by:

$$\tau = \frac{r^2}{2D_T} \tag{1}$$

where r is the vesicle radius, D_T the translational diffusion coefficient and τ the relaxation time [$= 1/(2\pi f_c)$, where f_c is the critical frequency]. Thus the average diffusion coefficient may be estimated, in principle, from measurement of the relaxation time and the particle radius alone. In the case of *Rps. capsulate* chromatophores, of radius 18 nm, D_T is calculated to be 1.2×10^{-7} cm^2/s, a value much faster than the lateral diffusion coefficient generally accepted, 10^{-10}–10^{-9} cm^2/s, for protein mobility in membranes. This large apparent diffusion coefficient may then be interpreted to indicate that the distance a protein is able to diffuse before encountering a barrier is not wholly unrestricted.

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From the equation which has been suggested by Saffmann and Delbrück [4] to describe the two dimensional lateral diffusion coefficient of membrane proteins,

$$D_T = \frac{kT}{4\pi\eta l}\left[\ln\left(\frac{\eta l}{\eta' a}\right) - \gamma\right] \tag{2}$$

it can be seen that

(*i*) a change in the viscosity of the aqueous phase (η') should have a rather insignificant effect on D_T, and

(*ii*) a decrease of 20°C in the ambient temperature should alter D_T by 1 or 2% per °C, provided that the lipids remain in the *fluid* state.

In equation (2) k = Boltzmann's constant, T = absolute temperature, η = membrane viscosity, η' = viscosity of aqueous phase, a = protein radius, l = membrane thickness and γ = Euler's constant (= 0.5772).

However, in recent work [2] we have shown that the μ-dispersion is significantly more sensitive to changes in the aqueous viscosity than predicted. This suggests that protein motions within biomembranes are more strongly coupled to the ionic and dipolar motions in the double layer than was previously supposed. Another finding supporting this interpretation is that the distribution in relaxation times for the Maxwell–Wagner-type (β-) dispersion, even in the presence of glutaraldehyde, is much greater than can be accounted for by any kind of heterogeneity in a sample of bacterial protoplasts.

Typically, protein complexes of energy-coupling membranes extend significantly beyond the *surfaces* of the phospholipid bilayer, thus presenting a barrier to the tangential motion of counterions in the diffuse double layer. This protrusion of the proteins may provide a means by which the diffusional motions of proteins are tightly coupled to the movement of ions in the double layer, and may account for the great breadth of the distribution in relaxation times commonly encountered in energy coupling membrane vesicle suspensions.

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