On the lateral mobility of proteins in prokaryotic membranes

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The eubacterial plasma membrane, in common with other biological membranes, is now thought generally to be organized as a 'fluid mosaic' of proteins and protein complexes, dispersed in, on and through a 'sea' of phospholipid molecules arranged as a bilayer. Our attention is therefore directed to more quantitative questions, of the form "How fluid?" and "What sort of a mosaic?". In other words, how fast are the hydrodynamically constrained lateral mobilities of prokaryotic membrane proteins, and to what extent is their 'instantaneous' distribution random (Kell, 1984*a*)?

As a first approach, and from a biophysical standpoint, it is simplest to consider a 'model' system (Saffman & Delbrück, 1975; Kell & Harris, 1985*a*,*b*) consisting of a spherical shell (radius r) of phospholipid bilayer, of thickness h and 'average' viscosity η, containing cylindrical membrane protein complexes of radius a, the whole separating aqueous phases of viscosity η'. If we treat the proteins as 'hard' cylinders (i.e. ignoring 'boundary' lipids and long-range intercomplex forces) which take up a negligible area fraction of the membrane, we may relate the membrane protein translational diffusion coefficient D to the vesicle radius and to the (exponential) relaxation time τ that a protein (complex) inserted at a given position takes to adopt a 'random' position on the vesicle surface, according to the equation (Huang, 1973; Sowers & Hackenbrock, 1981):

$$\tau = r^2/2D \qquad (1)$$

It may be noted that this equation differs from the usual Einstein–Smoluchowski equation for two-dimensional 'random-walk' diffusion by a factor of 2 and by the fact that r represents the vesicle radius and not the distance diffused. Because of the squared dependence, for a given value of D, of τ upon r, values of τ to be expected are significantly smaller for prokaryotes than for eukaryotes. 'Viscosities' for typical biomembranes (above the gel-to-liquid phase transition) are in the range 1–10 P (0.1–1 Pa · s) (e.g. Cherry & Godfrey, 1981), and thus the hydrodynamically restricted value of D for a typical membrane protein of radius 5 nm and at high lipid/protein ratio is expected and found to be some 10^{-9} cm^2/s (e.g. Webb *et al.*, 1981; Vaz *et al.*, 1982). For a (spherical) micro-organism of radius 0.5 μm this implies τ = 1.25s.

It is useful first to make explicit the distinction between the mobility on a scale of time (and hence distance) that is either short or long relative to the enzymic turnover time of the protein in question, since such a distinction relates in particular to current discussions concerning pool behaviour in electron-transport chains (Rich, 1984; Ragan & Cottingham, 1985; Kaprelyants, 1985) and membranous free energy transfer (Kell & Harris, 1985*a*; Kell & Westerhoff, 1985; Slater *et al.*, 1985). We shall here be concerned mainly with the question of a long-range long-time mobility. However, a small calculation regarding the former is in order.

If a bacterial cell is modelled as a sphere of radius 0.5 μm, its membrane has an area equivalent to a square of radius 1.73 μm. If each cell contains 1000 molecules each of enzymes of type and A and B arranged in sequence as a square lattice, their centres would be 27 nm apart. If $D = 10^{-9}$ cm^2/s, the time taken to 'visit' one target molecule $\simeq$ 20 μs whilst that taken to visit 10 target molecules, a number giving kinetics indistinguishable from pool behaviour (Rich, 1984), would be some 2 ms. Given typical enzymic turnover times (1–10 ms) it is evident that in the absence of 'specific' interactions a random disposition of membrane proteins is to be expected *a priori*, certainly on a 'long' time-scale and most probably on a 'short' one. What types of factor additional to those in the simple model might therefore be invoked if values significantly lower than 10^{-9} cm^2/s are found?

The second point to be made is that the bacterial plasma membrane, like the inner mitochondrial membrane, has a typical protein/lipid ratio of 3 : 1 (although some physiologically significant variability is possible; see, e.g., Dombek & Ingram, 1984). As pointed out by Sowers & Hackenbrock (1981), however, the relative areas have a somewhat lower ratio, since membrane protein complexes are not flush with the phospholipid head groups. Now whilst bacterial respiratory and other protein complexes are known to be smaller and simpler than their eukaryotic (i.e. mitochondrial) counterparts, published electron micrographs do suggest that protein complexes take up some 30–50% of the membrane area in each case (e.g. Kleeman & McConnell, 1974; Sowers & Hackenbrock, 1981), although more systematic and definitive bacterial work seems warranted. The 'archipelago effect' (Saxton, 1982), which considers the extent to which the fact that proteins do not take up a negligible area of the membrane and thus (even though they are treated as hard cylinders) have net (long-range) diffusion coefficients lower than expected, will not therefore decrease the diffusion coefficient of a hard cylinder by more than an order of magnitude for protein areas in the range quoted (Jacobson & Vojcieszyn, 1981; Kell, 1984*b*; Pink, 1985; Sowers & Hackenbrock, 1985). Thus we do not expect values of D to be much less than 10^{-10} cm^2/s and, for bacteria of radius r = 0.5 μm, τ to exceed 12.5 s. Although few biophysical studies have sought accurately to estimate values of the long-range two-dimensional diffusion coefficients of membrane proteins in bacteria (although many more qualitative studies of membrane 'fluidity', homoeoviscous adaptation and so forth are available; McElhaney, 1982; Melchior, 1982), there do seem to be at least some cases in prokaryotes in which one is forced to invoke values of D (for long-range diffusion) much smaller than 10^{-10} cm^2/s (e.g. Kaprelyants & Ostrovsky, 1984; Kell, 1984*a*, *b*). Such findings are particularly noteworthy in prokaryotes since a substantial cytoskeleton and geometrically extensive membrane/cell wall interactions are not currently thought to be present in bacteria.

Biosynthetic studies using penicillin-induced lysis to distinguish dispersive from conservative modes of the distribution of membrane proteins between mother and daughter cells provide an approach to placing bounds on values of D. Unfortunately, such experiments have as yet given conflicting results (Cadenas & Garland 1979; Kepes & Autissier, 1972; Poole, 1981). To obtain a more striking and apparently clear-cut example of a non-random organization of membrane protein complexes we must turn our attention to differentiating prokaryotes.

Two prokaryotic systems are of special interest here: facultative diazotrophs (Post *et al.*, 1982, 1983; Payne & Socolofsky, 1984) and facultative phototrophs (Oeize, 1981; Kaufmann *et al.*, 1982, Chory *et al.*, 1984; Drews, 1985). In each case (*op. cit*) the transition from heterotrophic growth to the stated growth conditions results in the extreme lateral differentiation of the cytoplasmic membrane so that specialized areas ('intracytoplasmic membranes'), invaginated but contiguous with the cytoplasmic membrane, are formed. Such structures would require, in our simple model above, that $D \ll 10^{-12}$ cm^2/s (Kell, 1984*a*). Whilst the physical basis for such 'organization' remains quite unknown, there

is evidence that it persists in chromatophores derived from the membrane invaginations of photosynthetic bacteria (Casadio *et al.*, 1984).

Finally, we have recently initiated a novel and entirely non-destructive approach to the assessment of the mobility of charged and dipolar species in biological membranes: the use of dielectric spectroscopy (Kell, 1983; Harris & Kell, 1985; Kell & Harris, 1985*a*, *b*; Kell & Westerhoff, 1985). Since a full discussion of this method is available in the above-mentioned papers, it will suffice to state that the findings to date would suggest (i) that in the absence of strong external forces the mobility of prokaryotic membrane proteins is not restricted by hydrodynamic forces alone, and (ii) that electro-osmotic interactions between double layer ions and polytopic membrane protein complexes are both more extensive and more subtle than has perhaps heretofore been realized.

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