STATISTICAL MECHANICS OF LIPID MEMBRANES

Protein Correlation Functions and Lipid Ordering

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ABSTRACT An expression is derived for the lipid-mediated intermolecular interaction between protein molecules embedded in a lipid bilayer. It is assumed that protein particles are accommodated by the bilayer, but they distort the lipids in some manner from their equilibrium protein-free configuration. We treat this situation by expanding the free energy density in the plane of the membrane as a Taylor series in some arbitrary parameter and its gradient. Minimization of the total membrane energy for a given particle configuration yields the interparticle interaction energy for that configuration. A test of the model is provided by measurement of the protein-protein pair distribution function from freeze-fracture micrographs of partially aggregated membranes. The measured functions can be simulated by adjustment of two parameters (*a*) a lipid correlation length that characterizes the distance over which a distortion of the bilayers is transmitted laterally through the bilayer, and (*b*) a term quantifying the energy of the protein-lipid interaction at the protein-lipid boundary. Correlation lengths obtained by fitting the calculated particle distribution functions to the data are found to be several nanometers. Protein-lipid interaction energies are of the order of a few kT.

INTRODUCTION

Many studies have shown that membrane proteins affect the behavior of neighboring lipids (Wallach et al., 1979; Mateu et al., 1978; Moore et al., 1978; Watts et al., 1979; Davoust et al., 1979; Chapman et al., 1977). A related issue is the arrangement of protein particles among their neighbors, especially their tendency to cluster, even in the absence of cytoskeletal interactions (Pearson et al., 1979; Copps et al., 1976; Melhorn and Packer, 1976; Finegold, 1976). It has been proposed that the interaction between proteins is caused by their influence on lipids (Schröder, 1977; Owicki and McConnell, 1979; Owicki et al., 1978; Marčelja, 1976). Evidence for a protein-protein interaction, which is dependent on the lipid environment, is seen in freeze-fracture studies of Chen and Hubbell (1973) and Lewis and Engelman (1983), in which both bacteriorhodopsin and dark-adapted rhodopsin are seen to cluster when reconstituted into relatively thick phosphatidyl choline membranes.

Here we present a model for the interaction of protein molecules in a lipid bilayer. We assume that a protein molecule is able to fit into the bilayer and thereby match its hydrophobic and hydrophilic regions with those of the surrounding lipids, but that this fitting causes some distortion of the protein-free equilibrium configuration of the bilayer and hence raises its energy relative to that equilibrium state. We derive an expression for the intermolecular potential energy arising from the perturbation of the lipid bilayer by the protein molecule.

A convenient test of the model is provided by measurement of the protein-protein pair distribution function from freeze-fracture micrographs of membrane systems that contain particles in a state of partial aggregation (Pearson et al., 1979; Gershon et al., 1979; Pearson et al., 1983; Donnell and Finegold, 1981). The pair distribution function, $w(r, \Delta r)$, is given by the ratio of the density of particles in an annulus between r and $(r + \Delta r)$ nm from an average particle in the micrograph, to the overall mean particle density. As aggregation standards, we have measured the particle pair distribution functions from pictures of *Acholeplasma laidlawii* membranes previously reported by James and Branton (1973). In a recent paper we have shown that pair distribution functions derived from particles seen in freeze-fracture micrographs can be modeled using the relationship between the pair distribution function and the radial correlation function, $g(r)$, of a system of particles, namely

$$w(r, \Delta r) = \frac{2\int_r^{r+\Delta r} g(r)\, r\, dr}{\Delta r(2r + \Delta r)}. \quad (1)$$

The radial correlation function can be calculated from the interparticle potential energy using published algorithms

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(Lado, 1968). The expression derived here for a lipid-mediated potential energy between particles can thus be used to model these measured functions. Our results suggest that aggregation state of particles in a membrane may be described by two parameters, which are properties of the lipid phase and protein-lipid interaction, respectively. It is possible that changes in these properties can be invoked to explain rearrangement of membrane proteins during many biological processes, for example, gap and tight-junction formation (Decker and Friend, 1974), thylakoid stacking (Staehlin, 1976), the acrosome reaction (Friend and Rudolf, 1974), and the discocyte-to-echinocyte transformation of red blood cells (Chevalier, 1974).

THEORY

Thermodynamics of Lipid Ordering

The most fundamental approach to protein-lipid interactions is via statistical mechanics. Although necessary to completely understand their interactions, it is inconvenient when analyzing their macroscopic behavior.

Here we resort to a thermodynamic model of the lipids. This approach allows us to study protein statistical mechanics via an approximation of the lipid-mediated force by its mean value. We assume that the lipids have a degree of freedom, an order parameter, $\phi(\underset{\sim}{r})$, which is a scalar function of position $\underset{\sim}{r}$ on the membrane surface and characterizes the lipid state at that point. The deviation of $\phi(\underset{\sim}{r})$ from ϕ_0, its value at unconstrained equilibrium, measures the extent of perturbation. We then express $f(\underset{\sim}{r})$, the two-dimensional free energy density, as a function of $\phi(\underset{\sim}{r})$ and $\underset{\sim}{\nabla}\phi(\underset{\sim}{r})$

$$f(\underset{\sim}{r}) = f(\phi_0) + \epsilon(\phi, \underset{\sim}{\nabla}\phi), \tag{2}$$

where $\epsilon = 0$ when $\phi(\underset{\sim}{r}) = \phi_0$ everywhere. We expand ϵ as a Taylor series around ϕ_0.

$$\epsilon(\phi, \underset{\sim}{\nabla}\phi) = \epsilon = k_1(\underset{\sim}{\nabla}\phi)^2 + k_2(\phi - \phi_0)^2 + \ldots. \tag{3}$$

Linear terms would merely shift the equilibrium state, while higher-order terms preclude analytic solutions. The first term in Eq. 3 produces the cooperativity, an energetic cost for spatial variation of ϕ. The second term represents the restoring force tending to keep ϕ at its equilibrium value, ϕ_0. Models based on the same idea have been developed by Marčelja (1976), Schröder (1977), Owicki et al. (1978), and Owicki and McConnell (1979). Eq. 3 has also been used to describe protein effects on spin label partitioning (Kleeman and McConnell, 1976). We shall refer further to these works in the discussion.

Minimizing the total free energy yields the field equation

$$\left(\frac{\nabla^2}{\eta^2} - 1\right)\phi(\underset{\sim}{r}) + \phi_0 = 0, \tag{4}$$

where $\eta^2 = k_2/k_1$. η^{-1} is a correlation length that always appears in combination with a spatial variable. Eq. 4 can be solved for $\phi(\underset{\sim}{r})$ using the appropriate boundary conditions corresponding to a given particle configuration. Substitution of $\phi(\underset{\sim}{r})$ into Eq. 3 then yields an expression for the energy density $\epsilon(\phi, \underset{\sim}{\nabla}\phi)$ that can be integrated over the membrane area to obtain the energy of the particle configuration for which Eq. 4 was originally solved. Implicit in our approach is the assumption that η^{-1} for the lipids is a slowly varying function of the protein concentration. This should be a good approximation for the concentrations of proteins seen in the natural membranes studied here. However, it will be seen later that in general ϕ_0, the equilibrium order parameter, needs to be replaced by a bulk order parameter averaged over the membrane and $\langle\phi\rangle$ is a function of lateral protein density.

We proceed to study the order parameter when there are one and two particles in the membrane. In both cases, the procedure is to solve Eq. 4, substitute $\phi(\underset{\sim}{r})$ into Eq. 3, and integrate over the membrane to find the total energy. Its dependence on the arrangement of the particles reveals their interaction.

Single Particle Membrane Energy

Let ϕ be constrained to be $\bar{\phi}$ at the edge of a protein particle, which we assume to be circular with radius r_0. The solution of Eq. 4 satisfying this and the requirement that $\phi(\underset{\sim}{r}) = \phi_0$ at $r = \infty$ is

$$\phi(\underset{\sim}{r}) = \phi_0 + c_1 K_0(\eta r), \tag{5}$$

where $c_1 = (\bar{\phi} - \phi_0)/K_0(\eta r_0)$, K_n is a nth order modified Bessel function of the second kind (Gray, 1966) and r is the distance of $\underset{\sim}{r}$ to the particle center.

The self energy, E_1, is the difference in order parameter (OP) energy between a membrane with one particle and one with none. The OP energy is zero in the absence of protein so E_1 is found by substituting $\phi(r)$ into Eq. 3 and integrating over the membrane

$$E_1 = c_1^2 k_1 \int_0^{2\pi}\int_{r_0}^{\infty} \{[\underset{\sim}{\nabla} K_0(\sigma)]^2 + K_0(\sigma)^2\}\,\sigma\, d\sigma\, d\theta \tag{6}$$

$$= 2\pi(\bar{\phi} - \phi_0)^2 k_1 \sigma_0 K_1(\sigma_0)/K_0(\sigma_0), \tag{7}$$

where $\sigma = \eta r$, $\sigma_0 = \eta r_0$, and $\underset{\sim}{\nabla}$ now refers to σ rather than r.

E_1 appears repeatedly in our results. Its most obvious role follows from its definition. The change in OP energy when a particle moves from one lipid phase to another is the difference in E_1 between them. This is related to the standard free energy of transfer when the OP-independent interactions are the same in both phases. E_1 can thus control protein distribution between coexisting lipid phases. Fig. 1 shows the dependence of E_1 on σ_0.

It has been assumed that the OP equals some constant $\bar{\phi}$ adjacent to the protein and that the direct constraining interactions do not extend away from the protein surface. In addition, the protein has been assumed to exert its influence along the perimeter of a circle. Although the latter assumption cannot be exact, we are concerned with mean behavior in regions larger than lipid molecules. Any shape irregularity would thus be smeared out. The model makes no assumption about the depth of the

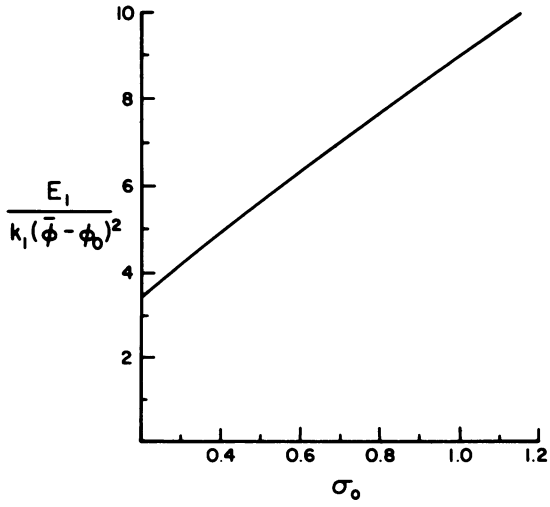

FIGURE 1 The dependence of the particle self-energy E_1 (expressed in units of $k_1(\bar{\phi} - \phi_0)^2$ on the reduced particle size σ_0 ($\equiv \eta r_0$).

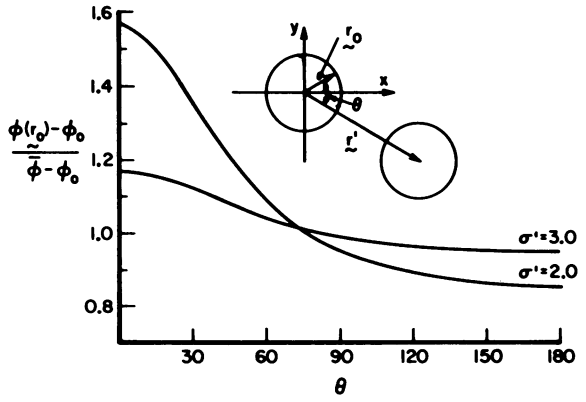

FIGURE 2 In solving Eq. 4 for the two particle problem, we invoke the circular boundary condition $\phi = \bar{\phi}$ at both particles. The solution given by Eqs. 8 and 9 is therefore inexact. We illustrate here the departure from circular symmetry by comparing $\bar{\phi}$ with the exact $\phi(\underset{\sim}{r}_0)$. The dependence of $[\phi(\underset{\sim}{r}_0) - \phi_0]/(\bar{\phi} - \phi_0)$ against θ is shown, where $\underset{\sim}{r}_0$ is some point on the boundary of one particle, and θ is defined as in the diagram. The reduced particle size, σ_0, is taken to be 1.0, and σ', the interparticle separation, is 2.0 and 3.0. The circular boundary condition corresponds to $[\phi(r_0) - \phi_0]/(\bar{\phi} - \phi_0) = 1.0$.

protein in the membrane since only two-dimensional structure is important.

Interaction Between Two Particles

The interaction between two protein particles in a membrane can be found by solving Eq. 4 when $\phi(\underset{\sim}{r})$ is constrained to $\bar{\phi}$ at the boundaries of two particles. Consider a particle centered at the origin and one at $\underset{\sim}{r} = \underset{\sim}{r}'$. Outside the particles $\phi(\underset{\sim}{r})$ is roughly

$$\phi(\underset{\sim}{r}) = \phi_0 + c_2 [K_0(\eta r) + K_0(\eta|\underset{\sim}{r}' - \underset{\sim}{r}|)], \tag{8}$$

where

$$c_2 = (\bar{\phi} - \phi_0)/[K_0(\sigma') + K_0(\sigma)]. \tag{9}$$

This is inexact because $\phi(\underset{\sim}{r})$ is not consistent on the protein boundaries. Fig. 2 shows the variation of $[\phi(\underset{\sim}{r}_0) - \bar{\phi}]/(\phi_0 - \bar{\phi})$ with θ, the angular coordinate of $\underset{\sim}{r}_0$ on a protein perimeter. The distortion from circular symmetry is small so we neglect it here.

The two particle OP energy, E_2, is found by combining Eqs. 3, 8, and 9

$$E_2 = c_2^2 k_1 \int\int \{[\underset{\sim}{\nabla} K_0(\sigma)]^2 + [\underset{\sim}{\nabla} K_0(|\underset{\sim}{\varrho} - \underset{\sim}{\varrho}'|)]^2 + 2\underset{\sim}{\nabla} K_0(\sigma) \cdot \underset{\sim}{\nabla} K_0(|\underset{\sim}{\varrho} - \underset{\sim}{\varrho}'|) + K_0(\sigma)^2 + K_0(|\underset{\sim}{\varrho} - \underset{\sim}{\varrho}'|)^2 + 2K_0(\sigma) K_0(|\sigma - \sigma'|)\} \alpha\underset{\sim}{\varrho}, \tag{10}$$

which may be condensed into the less formidable result.

$$= 2k_1 c_2^2 [M(0) + M(\sigma')] \tag{11}$$

using the definition

$$M(\sigma') = \int_0^{2\pi}\int_{\sigma_0}^{\infty} A\sigma \, d\sigma \, d\theta - \int_0^{2\pi}\int_0^{\sigma_0} A\,\sigma \, d\sigma \, d\theta \tag{12}$$

and

$$A = \underset{\sim}{\nabla} K_0(\sigma) \cdot \underset{\sim}{\nabla} K_0(|\underset{\sim}{\varrho} - \underset{\sim}{\varrho}'|) + K_0(\sigma) K_0(|\underset{\sim}{\varrho} - \underset{\sim}{\varrho}'|). \tag{13}$$

The second term in M corrects for integrating the first over the interior of the particle at σ'. Eq. 12 can be simplified with the following addition theorem (Gray and MacRobert, 1952)

$$K_0(|\underset{\sim}{\varrho} - \underset{\sim}{\varrho}'|) = I_0(\sigma) K_0(\sigma') + 2\sum_{p=1}^{\infty} I_p(\sigma) K_p(\sigma') \cos p\gamma \tag{14}$$

valid when $\sigma < \sigma'$. The region $\sigma > \sigma'$ is handled by interchanging σ and σ'. I_p is an order p modified Bessel function of the first type, and γ is the angle between σ and σ'. The summation drops out because the cosine integrates to zero. Carrying out the integration, we find

$$M(\sigma') = 2\pi K_0(\sigma')[2\sigma_0 I_0(\sigma_0) K_1(\sigma_0) - 1]. \tag{15}$$

When calculating $M(0)$, we make the approximation, justified later, of ignoring the second integral in Eq. 12 for $\sigma' = 0$ and obtain

$$M(0) = 2\pi\sigma_0 K_0(\sigma_0) K_1(\sigma_0) = E_1/k_1 c_1^2. \tag{16}$$

The lipid-mediated potential between the two particles is then

$$V(\sigma') = E_2 - 2E_1 = 2E_1 \left\{ \frac{1 + M(\sigma')/M(0)}{[1 + K_0(\sigma')/K_0(\sigma_0)]^2} - 1 \right\}. \tag{17}$$

Fig. 3 shows a plot of $V(\sigma')E_1$ vs. σ', for various particle radii σ_0 or ηr_0. Finally, the integration leading to Eq. 16 includes the inside of the particle not centered on the coordinate origin. To estimate the error we evaluate the integral (see Fig. 4)

$$I^* = \int_{-\alpha}^{\alpha}\int_{\sigma'-\sigma_0}^{\sigma'+\sigma_0} [K_0^2(\sigma) + K_1^2(\sigma)]\,\sigma \, d\sigma \, d\gamma \tag{18}$$

$$= \frac{2\sigma_0}{\sigma'}[(\sigma' - \sigma_0) K_0(\sigma' - \sigma_0) K_1(\sigma' - \sigma_0) - (\sigma' + \sigma_0) K_0(\sigma' + \sigma_0) \cdot K_1(\sigma' + \sigma_0)]. \tag{19}$$

Table I shows values of σ' below with I* exceeds 5% of $M(0)$ for values of $\sigma_0 = 0.2 - 2.0$. Since the particles do not interpenetrate, $\sigma' > 2\sigma_0$. σ_0 is typically ~1. The correction to $M(0)$ can thus be neglected.

Many-Particle Effects on the Order Parameter

The presence of other particles near a pair of interacting particles will have an effect on the magnitude of their interaction, because the presence

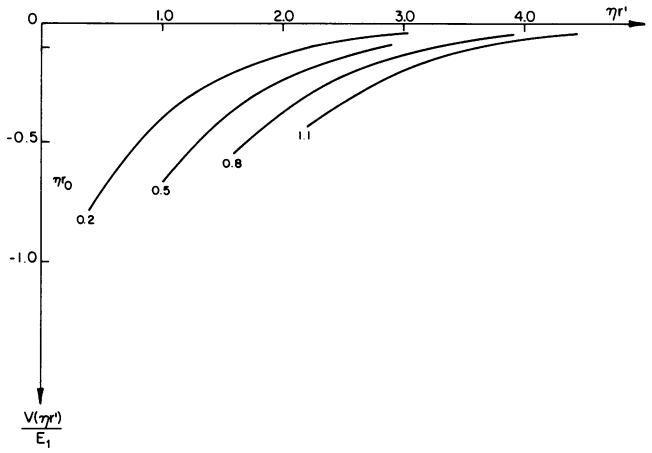

FIGURE 3 Calculated two-particle potential energies (expressed in units of E_1) for various particle sizes ($\eta r_0 = 0.2, 0.5, 0.8$, and 1.1).

TABLE I
VALUES OF $\eta r'$ BELOW WHICH I^* EXCEEDS 5% OF $M(0)$

ηr_0	$\eta r'$
0.2	0.52
0.5	1.36
0.8	2.00
1.1	2.64
1.4	3.26
1.7	3.70
2.0	4.60

of particles in the membrane will alter the bulk value of ϕ from ϕ_0 and will change the measure of the lipid-protein interaction. We quantify this effect by invoking the super-position approximation, i.e.,

$$\phi(\underline{r}) = \phi_0 + \sum_i c_N^i K_0(\eta|\underline{r}_i - \underline{r}|). \tag{20}$$

When the particles are all identical, the boundary condition at particle j is roughly

$$\bar{\phi} - \phi_0 = c_N^i K_0(\eta r_0) + \sum_{i\neq j} c_N^i K_0(\eta|\underline{r}_j - \underline{r}_i|). \tag{21}$$

To find c, we assume that the membrane is homogeneous, so the neighbors of each particle are similarly arranged. The c's are thus all about the same, and we assume them to be equal. Spatial variation of concentration is thereby ignored. Setting the c's equal to c_N in Eq. 21, we solve for c_N

$$c_N = \frac{\bar{\phi} - \phi_0}{K_0(\eta r_0) + \sum_{i\neq j} K_0(\eta|\underline{r}_j - \underline{r}_i|)}. \tag{22}$$

We now replace $K_0(\eta|\underline{r}_i - \underline{r}_j|)$ by its mean value

$$<K_0(\eta|\underline{r}_i - \underline{r}_j|)> \equiv \frac{2\pi}{N-1}\int_0^\infty g(r)\,K_0(\eta r)\,r\,dr,$$

which may be readily integrated if we made the approximation $g(r) \approx 1.0$.

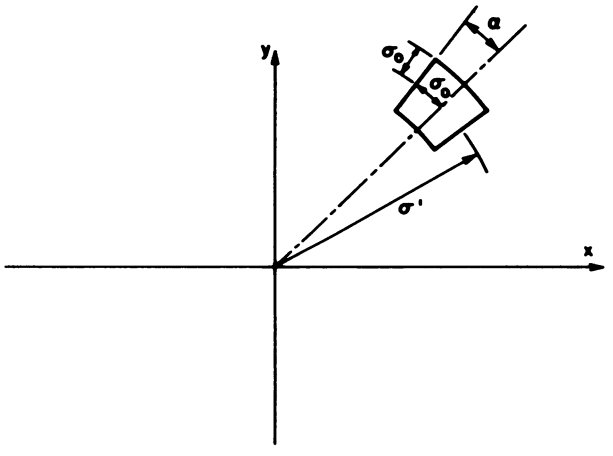

FIGURE 4 The segment representing the region of integration used in Eq. 5.

The outcome of this coarse graining is

$$c_N = \frac{\bar{\phi} - \phi_0}{K_0(\sigma_0) + 4\pi(\rho/\eta^2)\,\sigma_0 K_1(2\sigma_0)}, \tag{23}$$

where ρ denotes the particle density.

Substituting this result into Eq. 20 yields, for the average value of OP

$$\langle\phi\rangle = \phi_0 + c_N N \langle K_0(|\underline{\sigma} - \underline{\sigma}_i|)\rangle, \tag{24}$$

where σ is any point not inside any particle. At sufficiently low particle densities, $\langle\phi\rangle$ can be written as

$$\begin{aligned}\langle\phi\rangle &= \phi_0 + c_N 2\pi(\rho/\eta^2)\int_{\sigma_0}^\infty K_0(\sigma)\,\sigma\,d\sigma \\ &= \phi_0 + c_N 2\pi(\rho/\eta^2)\,\sigma_0 K_1(\sigma_0).\end{aligned} \tag{25}$$

Thus, in the many-particle problem, the interaction between any pair of particles is screened by their simultaneous interaction with all the others: (*a*) the effective bulk order parameter in the domain is increased from ϕ_0 to $\langle\phi\rangle$, and (*b*) the effect of the lipid-protein interaction on the order parameter at the boundary of any protein molecule is reduced from $\bar{\phi} - \phi_0$ to $(\bar{\phi} - \phi_0)(1 - Y)$, where

$$Y = \frac{2\pi(\rho/\eta^2)\,\sigma_0 K_1(\sigma_0)}{K_0(\sigma_0) + 4\pi(\rho/\eta^2)\,\sigma_0 K_1(2\sigma_0)}. \tag{26}$$

Since, in the two-particle problem (cf., Eq. 2) E_1 varies quadratically with $(\bar{\phi} - \phi_0)$, it follows that the intermolecular potential energy between any pair of protein molecules in the present case needs merely to be rescaled by a factor $(1 - Y)^2$ provided ρ is sufficiently low. Physical plausibility demands that $Y < 1$.

Previous Work on Lipid-mediated Interactions

We now compare our model of lipid-mediated protein-protein interactions with previous works. Earlier investigators predict, as we do, that proteins that perturb lipids tend to aggregate. Marčelja (1976) and Schröder (1977) use a mean field model to describe the behavior of a lipid order parameter, η. Marčelja assumes a hexagonal lattice for the lipids, wherein each site, j, is characterized by an order parameter η_j. Schröder assumes a lipid continuum and derives a differential equation for the change in order parameter $\delta\eta(\underline{r})$ away from the average value, η_0, in the absence of the proteins. Both Marčelja and Schröder include the influence of proteins on the surrounding lipid by including a lipid-protein interaction term in an expression for the energy of the system. In both cases, η is taken to be the average of the second-order Legendre polynomial $\eta = \langle P_2(\nu)\rangle$, where ν is an angle describing either lipid hydrocarbon chain or segment orientation. Schröder fixes the value of the order parameter at the protein-lipid boundary and solves for $\delta\eta(\underline{r})$ in the two-particle case, thereby deriving an expression for the interprotein interaction energy. Marčelja solves for the order parameter self-consistently, and hence the interprotein interaction energy, numerically. Neither author takes into account the saturation effect on the order parameter of protein particles near the two particles whose interaction is being considered.

Since we shall not attempt to give a molecular interpretation of the order parameter, introduced in the present work, further comparison with the work of Marčelja and Schröder must await further data on the nature and range of the order parameter mediating interprotein interactions.

PAIR DISTRIBUTION FUNCTIONS

Access to the two-particle interparticle potential of a system of particles, such as seen in a freeze-fracture micrograph, is provided in principle by measurement of the

pair distribution function of the set of particles embedded in the membrane. For example, in a recent paper (Pearson et al., 1983) we showed that the pair distribution function (PDF) of a random distribution of protein could be simulated by assuming the proteins to be disks with no interprotein interaction other than the hard repulsive core. The protein particles were modeled as if they were a two-dimensional fluid, and the PDF's were calculated using the Ornstein-Zernike equation

$$g(r) = 1 + c(r) + \rho \int c(|\underset{\sim}{r} - \underset{\sim}{s}|)\,[g(\underset{\sim}{s}) - 1]\,d^2s. \quad (27)$$

$c(r)$ is the direct correlation function that at the particle densities studied here may be related to $g(r)$ by the Percus-Yevick relation

$$g(r) = g(r)\,\{1 - \exp[V(r)/kT]\}. \quad (28)$$

This approach is quite general, and we wish now to test the attractive lipid-mediated potential derived here using these relations.

An attractive interparticle potential energy would be seen in a freeze-fracture micrograph as partial aggregation of the particles. A convenient set of micrographs of partially aggregated systems is seen in Fig. 1 of James and Branton (1973). These authors selected five systems ranging from random to completely aggregated as aggregation standards in a study of the effect of lipid composition on particle distribution in *Acholeplasma laidlawii* membranes. We present particle patterns obtained from their Fig. 1 in our Fig. 5. However, for the measured PDF to represent an intermolecular potential energy function in the context in which we present it here, it is assumed that the membrane, although partially aggregated, is still homogeneous. There must be no phase separation, either of one lipid phase from another, or of the protein from the lipid phase. Accordingly, from the work of James and Branton (1973), we limit ourselves to a consideration of Figs. 1 *a*, *b*, and *c*, in which protein particles appear throughout the membrane. Fig. 1 *e* seems to show complete separation of either a lipid phase or the protein phase from the lipid. We discuss later the problems that may be associated with using a natural membrane system for these measurements.

The PDF for each micrograph was determined by placing the micrograph on a graphic tablet (Tektronix, Inc., Beaverton, OR) and recording the coordinates of each particle in the plane of the picture. The annulus size (Δr in Eq. 1) was 10 nm. These measured PDF's were then simulated using the lipid-mediated potential energy, Eq. 17, as the interparticle potential energy in Eq. 28. The many-particle correction, Eq. 26 was included. Eqs. 27 and 28 were solved for $g(r)$ using the algorithm of Lado (1968), and the PDF, $w(r, \Delta r)$, was calculated from $g(r)$ using Eq. 1. The procedure used for simulation entailed adjusting η^{-1}, the correlation length, until the rate of decay of the calculated PDF from its peak was similar to that of the measured function, and then adjusting the protein-lipid interaction energy, $(k_1\phi_0 - \bar{\phi})^2$, until a fit was obtained as judged visually. An empirical observation, which aided the fitting was that η^{-1} for a measured PDF was approximately the distance required for the PDF to decay by one-half from its peak value.

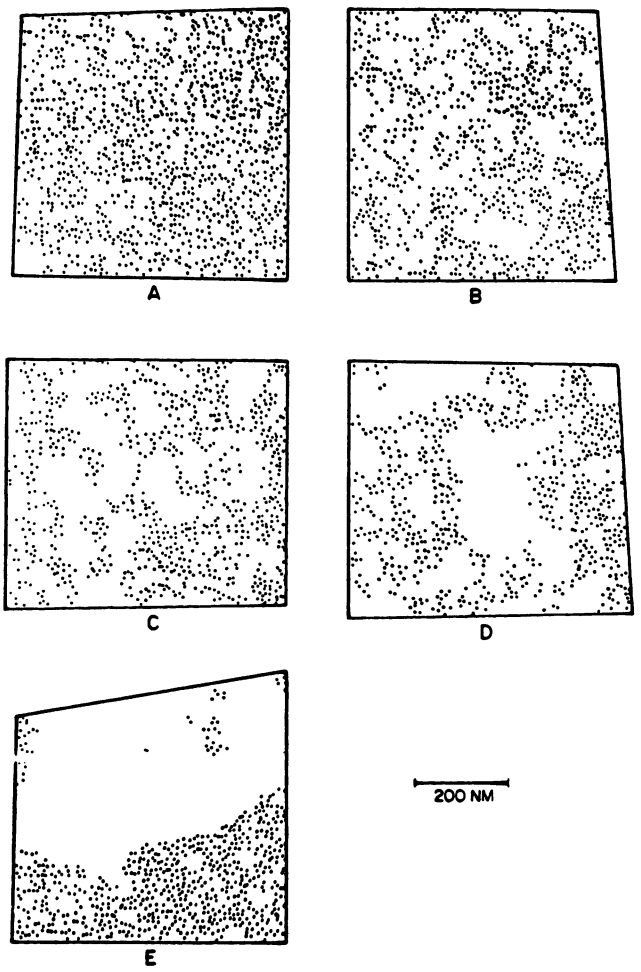

FIGURE 5 Computer-generated representation of the particle distributions in the EM pictures taken from Fig. 1 of James and Branton (1973).

In Fig. 6*a–c* we show PDF's of the systems of James and Branton (1973) under consideration here. Table II summarizes the parameters that give the best fits. The correlation lengths appear to be between 5 and 10 nm for the partially aggregated membranes. Fig. 1 *a* of James and Branton (1973) is consistent with a PDF (our Fig. 6 *a*) with the freeze-fracture particles being randomly diffusing hard disks. The increasing aggregation state upon going from Fig. 1 *a* to *c* can be modeled by increasing lipid correlation length and protein-lipid interaction energy.

DISCUSSION

Lewis and Engelman (1983) have studied the effect of lipid hydrocarbon chain length on the state of aggregation of

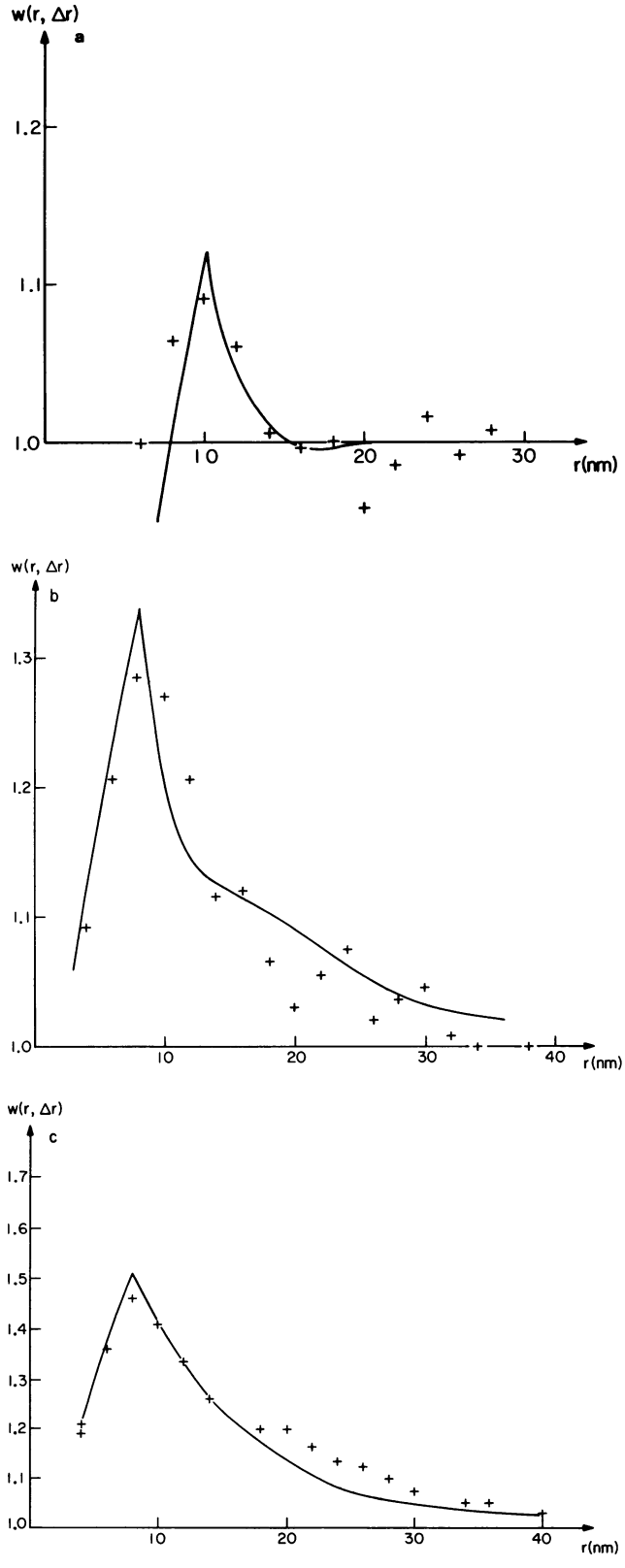

FIGURE 6 Measured (+) and simulated (−) PDF's for the particle (*a*) $\rho = 0.0032$ nm^{-2}, $r_0 = 5.0$; (*b*) $\rho = 0.0025$ nm, $r_0 = 4.0$ nm; (*c*) $\rho =$ distributions in Fig. 1 *a–c* of James and Branton (1973). $\Delta r = 10$ nm. 0.0025 nm^{-2}, $r_0 = 4.0$ nm.

TABLE II
PARAMETERS USED IN SIMULATING PARTICLE DISTRIBUTION FUNCTIONS

Electron micrograph	k_1D^2	E_1	η^{-1}	r_0
James and Branton (1973)	*kT*	*kT*	*nm*	*nm*
Fig. 1 *a*	0.0	—	—	5.0
Fig. 1 *b*	0.65	4.5	6	4.0
Fig. 1 *c*	1.30	6.4	10	4.0

bacteriorhodopsin reconstituted into phosphatidyl choline of various hydrocarbon chain lengths. If bacteriorhodopsin is reconstituted into thin phosphatidylcholine bilayers such that the boundary layer of lipids is capable of stretching to accommodate the protein, then the protein adopts a random arrangement in the plane of the membrane (Pearson et al., 1983). In a situation where lipids and proteins in a bilayer are unable to match their hydrophilic and hydrophobic surfaces, separation of the protein phase from the lipid phase occurs. This separation is manifested in freeze-fracture micrographs as aggregation of the protein particles.

In cases where the lipid hydrophobic surface is thicker than that of the protein, both random and aggregated protein states are seen. Perturbation of the lipid by the protein can be short range or extend further into the bilayer. If the lipid adjusts its length by changing the number of gauche bonds per hydrocarbon chain, then the perturbation would be expected to be short range (Pearson et al., 1983). An alternative mechanism by which a thick bilayer may accommodate the protein is lipid tilt. Tilt distortions would be expected to be of longer range, by analogy with similar distortions seen in liquid crystals (de Gennes, 1974). Tilting of the lipid molecules may, therefore, produce an interaction between protein molecules (Cornell et al., 1982). Partial or total aggregation of the protein molecules would then be a consequence of propagation laterally through the bilayer of tilt distortion at the protein-lipid boundary.

The protein-protein PDF, therefore, contains information about the extent of the protein-lipid interaction and its propagation through the bilayer. In the present work, we present a theory that allows us to gain some insights into these issues from PDF's measured from freeze-fracture electron micrographs. Our results show, for example, that it is possible to simulate very closely the PDF's from partially aggregated *Acholeplasma laidlawii* membranes using a model with essentially two adjustable parameters: (*a*) the correlation length, η^{-1}, which is a property of the lipid phase and describes the distance over which the order parameter affecting protein distribution is propagated in that phase; and (*b*) the protein-lipid interaction energy, $k_1(\bar{\phi} - \phi_0)^2$, which provides a measure of the protein-lipid interaction at the protein-lipid boundary. Other parameters in the interprotein potential, namely, r_0 and ρ, can be

deduced directly from a measured PDF and the freeze-fracture micrograph, respectively.

Given the assumptions that we have made so far, and despite the crudity of our model, the observed PDF's of *Acholeplasma laidlawii* membranes are well simulated by the potential energy function of Eq. 17, taking into account the correction to Eq. 17 described by Eq. 26. Such a simulation is obviously not unique, and there may be other potential energy functions that provide equally close fits to the measured functions. However, it is difficult to see what other mechanisms would provide such a long-range attraction, in particular since the attraction has to be a function of lipid composition.

The principal assumption that we make in calculating the pair distribution function from the particle coordinates taken from a freeze-fracture picture is that the protein-lipid system is a single phase. This assumption implies that the protein does not separate from the lipid phase and that one lipid phase does not separate from another. This is a hard assumption to justify on the basis of a freeze-fracture picture since the freeze-fracture process is taking, as it were, a still picture of a dynamic system. Further work is necessary to ascertain unambiguously whether partially aggregated systems such as those used in the study by James and Branton (1973) and referenced here do, in fact, consist of a single homogeneous phase.

The possibility of artifacts due of the *Acholeplasma laidlawii* membranes being a mixture of different lipid and protein species must be considered. The fact that a natural membrane consists of a mixture of lipids does not necessarily invalidate our approach. We have used a mean field theory to model protein membrane interactions. We assume a single correlation length is an average property of the lipid phase irrespective of whether the membrane lipids are one pure chemical species or a mixture. This assumption seems to be justified by the large magnitude of the correlation lengths obtained relative to the molecular size of the lipids in the plane of the membrane.

The possibility that the particles are not all of one protein species is a more serious objection to the use of a natural membrane system in the context of measurement of PDF's. Such inhomogeneity may be manifested as variations among the particles in r_0 and $(\bar{\phi} - \phi_0)$. Inhomogeneity in r_0 would cause broadening in the PDF. Comparison of the calculated PDF using a random hard-disk model and that measured from Fig. 1 *a* of James and Branton (1973) shows that, in fact, there is little broadening in the PDF except for some flattening of the top of the peak. The values of $r_0 = 5.0$ nm and $\rho = 0.0032$ nm^{-2} are entirely consistent with a model in which all the protein particles in that picture are randomly diffusing hard disks.

Particle inhomogeneity would not cause the increase in peak size as seen in Fig. 1 *a* to *c*. This, we propose, must be a lipid-mediated effect. If this is the case, the peak size is largely governed by the magnitude of the protein lipid interaction and in the case of a natural membrane will be simply an average of that interaction over all the protein particles.

In cases where there is a hard-disk repulsion between particles and no other type of repulsive interaction, the position of the peak in the radial correlation function, and hence the pair distribution function, is a measure of $2r_0$. The measured values of r_0 consistent with peak position for particles in the *Acholeplasma laidlawii* membranes of James and Branton (1973) are 4.0 nm in the partially aggregated membranes and 5.0 nm for particles in the random distribution of Fig. 1 *a*. The reduction of r_0 as seen in Fig. 1 *a* to *b* and *c* is consistent with the appearance of the particles in the photographs. Particles appear to be somewhat smaller in Fig. 1 *b* than in *a*. It is not possible to tell at this point whether this represents a genuine conformational change in the membrane proteins or is an artifact in the freeze-fracture process caused by the change in lipid composition. However, in all the pictures we have analyzed so far, the position of the peak in the PDF is consistent with the radius of particles estimated from the pictures.

One parameter of particular importance is the self energy, E_1, of the protein. E_1 is defined by Eq. 6 and represents the energy cost of placing a protein particle into the bilayer. We have so far assumed the lipids to be uniform. When they separate into two phases, preferential incorporation of protein into one of the phases would resemble precipitation. For instance, Cherry et al. (1980) have shown that bacteriorhodopsin in lecithin precipitates when cholesterol is added. The phase behavior of lecithin and cholesterol is only beginning to be understood. However, they form a two-phase system under many conditions (Lentz et al., 1980). In terms of our model, a large difference of E_1 between the two phases would appear as precipitation. Alternatively, cholesterol could raise E_1 or the lipid correlation length, thus causing protein precipitation. This latter mechanism could also account for the bacteriorhodopsin aggregation in ordered lipid phases (Cherry et al., 1978) and in nature (Henderson, 1977; Casadio and Stoeckenius, 1980). The value of E_1 depends on $(\bar{\phi} - \phi_0)^2$. Adjusting $(\bar{\phi} - \phi_0)$ may therefore be a mechanism for controlling the state of aggregation of membrane-bound proteins and hence their interaction. One way of changing $(\bar{\phi} - \phi_0)$ is via the protein. This could account for aggregation induced by proteolysis (Branton, 1971; Speth et al., 1972; ligand binding (Gunn and Kirk, 1976; Kahn, 1976), or rhodopsin dark adaptation (Chen and Hubbell, 1973). Another way to change $(\bar{\phi} - \phi_0)$ is via the lipids. Examples of this may be provided by drugs, such as primaricin, nystatin, and amphotericin B, which bind to the lipids and also cause protein aggregation (Kitajima et al., 1976). Phospholipases (Speth et al., 1972; Verkleij et al., 1973; Olive et al., 1978) and lipid phase transitions (Kleeman and McConnell, 1976; Cherry et al., 1978) can also make the proteins aggregate. Cross-linking agents,

such as antibodies and lecithins (Steck, 1974; Edelman, 1976; Nicholson, 1976; DeLisi and Perelson, 1976; Schreiner and Unanue, 1976) can also precipitate proteins by producing additional attractions between them. The dependence of protein aggregation on the membrane state is further evidence that it is not just an artifact of freezing.

SUMMARY AND CONCLUSIONS

We have shown that it is possible to simulate the pair distribution function of some partially aggregated membranes using a simple model for protein-lipid interactions, which generates an analytic expression for the interprotein potential energy function. We have made no attempt to specify which bilayer parameter may mediate such an interaction, although freeze-fracture studies of bacteriorhodopsin and rhodopsin in lecithins suggest a correlation between protein thickness and aggregation (Chen and Hubbell, 1973; Lewis and Engelman, 1983). Further studies on well-defined model protein-lipid systems should clarify the mechanisms involved in such an interaction and shed further light on the question of membrane homogeneity in such systems.

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