Letter to the Editor

Preferential Solvation and the Selectivity of Lipid-Protein Interactions

In a recent paper in this journal, Record and Anderson (1995) analyzed the preferential interactions of aqueous solutes with macromolecules in terms of a two-domain model that was introduced originally by Inouye and Timasheff (1972). In this model, one domain is the local region surrounding the macromolecular surface, in which preferential interactions with the macromolecule can take place. The other domain corresponds to the regions away from the macromolecular surface, in which interactions with the macromolecule are not exerted and the thermodynamic properties are those characteristic of the free solute in bulk solution. For the somewhat analogous case of the interaction of lipids with integral proteins in membranes, the two domains may be distinguished directly because of the difference in mobility of the lipid chains that is detected by electron spin resonance (ESR) of spin-labeled probe lipids (Marsh, 1985). The protein domain, also referred to as the boundary lipid layer (Jost et al., 1973), is characterized by restricted lipid chain mobility relative to that of the bulk domain, where the latter resembles fluid lipid bilayers. Previously, the selectivity of lipid-protein interactions that is observed by spin label ESR spectroscopy has been interpreted solely in terms of binding equilibria established by lipid exchange (Brotherus et al., 1981; Marsh, 1985). It is of interest, therefore, to discuss the lipid selectivity also in terms of the formalism for preferential solvation by using the two-domain approach of Record and Anderson (1995). This is especially the case because normally in spin label experiments only the *average* relative association constant is determined (cf. Marsh, 1985), and in one case for which the concentration of the competing lipid was varied systematically, no evidence was found for highly specific association sites (Powell et al., 1985).

The treatment given here restricts itself to the components intrinsic to the membrane, specifically a single solvent lipid, a single solute lipid, and the integral protein. The preferential interaction coefficient of a solute lipid, s, with the protein, p, is defined by (cf. Eisenberg, 1976):

$$\Gamma_{s,p} = \left(\frac{\partial m_s}{\partial m_p}\right)_{\mu_s,T,P}, \tag{1}$$

where m_i is the mole ratio of component i with respect to the solvent lipid t. This representation of concentration is the appropriate equivalent for membranes of molal concentrations in homogeneous solution. The preferential interaction coefficient is thus the number of moles of solute lipid that must be added to maintain its chemical potential constant when 1 mole of protein is added to the membrane containing a fixed amount of solvent lipid. Making an equivalent thermodynamic postulate to that of Record and Anderson (1995), i.e., that the activity of the solute lipid in the bulk domains is equal to that in protein-free bilayers of similar composition, Eq. 1 can be expressed as

$$\Gamma_{s,p} = \frac{m_s^{tot} - m_s^f}{m_p}, \tag{2}$$

where m_s^{tot} is the mole ratio of total solute lipid to total solvent lipid and m_s^f is the mole ratio of solute lipid with respect to solvent lipid in the bulk (or fluid) domains. Here it is understood that m_p, the mole ratio of protein with respect to total solvent lipid, is sufficiently small that the protein boundary domains are distinct and nonoverlapping. It is convenient and conventional to express some of the quantities in Eq. 2 in terms of the lipid/protein mole ratios, n_i, of component i. For the solvent lipid t, $m_p = 1/n_t$, where n_t is the mole ratio of total solvent lipid to protein, and for the solute lipid in the fluid domains: $m_s^f = n_s^f/(n_t - N_b)$, where N_b is the total number of lipids in the boundary domain of a single protein. The contribution to the latter from the solute lipids is neglected, as is appropriate to probe experiments. It is then straightforward to show that

$$\Gamma_{s,p} = n_s^b - N_b \cdot n_s^f/(n_t - N_b), \tag{3}$$

where n_s^b is the number of solute lipids in the boundary domain of a single protein. This equation is the direct analog for the membrane situation of Eq. 17 in Record and Anderson (1995), where one-to-one exchange of solute and solvent lipids at the protein boundary layer domain is assumed. Equation 3 has a simple interpretation. The preferential interaction coefficient $\Gamma_{s,p}$ is the excess population of the solute lipid in the boundary domain over that which would be obtained for an indifferent partition equilibrium with the fluid domains (viz. the second term on the right of the equation). It is also seen that $\Gamma_{s,p}$ is directly proportional to the concentration of solute lipid.

In a conventional ESR probe experiment, the relative selectivities usually are of more direct interest than are the absolute values of the spin label concentrations. These can be expressed as the excess population of the spin-labeled lipid in the boundary domain, $\Gamma_{s,p}$, normalized to the population that would be obtained in the absence of selective interactions. This quantity is termed the *relative* preferential interaction, designated by $\Gamma^r_{s,p}$. From Eq. 3 and the accompanying discussion, it is found immediately that

$$\Gamma^r_{s,p} = \left(\frac{n_s^b}{n_s^f}\right)\left(\frac{n_t}{N_b} - 1\right) - 1, \tag{4}$$

where $\Gamma^r_{s,p}$, unlike $\Gamma_{s,p}$, is independent of the spin label concentration. Equation 4 is exactly of the form routinely used for analyzing lipid spin label equilibria in lipid-protein systems, as derived from multiple binding models (Brotherus et al., 1981; Marsh, 1985). In terms of the latter, the

average association constant of the spin-labeled solute lipid relative to that of the unlabelled solvent lipid is given by $K_r^{av} = 1 + \Gamma_{s,p}^r$, essentially in agreement with the treatment of Schellman (1990) for soluble proteins. In the two formalisms, lack of preferential interaction is given consistently by $K_r^{av} = 1$ and $\Gamma_{s,p}^r = 0$, respectively. A preferential interaction is characterized by $K_r^{av} > 1$, $\Gamma_{s,p}^r > 0$ and a selective exclusion by $0 \leq K_r^{av} < 1$, $-1 \leq \Gamma_{s,p}^r < 0$. Typical values of $\Gamma_{s,p}^r$ ($=K_r^{av} - 1$) obtained from ESR measurements with various integral proteins lie in the range -0.5 to 9.4, where phosphatidylcholine is the reference lipid (Marsh, 1995).

It is now possible, from Eqs. 3 and 4, to derive the preferential interaction coefficients, $\Gamma_{s,p}$, appropriate to spin label probe measurements in membranes. The solute lipid populations in the two domains are related by $m_s^{tot} n_t = n_s^b + n_s^f$. It is then found that the preferential interaction coefficient is given by

$$\frac{\Gamma_{s,p}}{m_s^{tot}} = \frac{N_b \Gamma_{s,p}^r \cdot n_t}{n_t + N_b \Gamma_{s,p}^r}. \quad (5)$$

A wide range of ESR experiments have demonstrated that N_b and $\Gamma_{s,p}^r$ ($=K_r^{av} - 1$) are constant, independent of n_t, in the limit of non-overlapping boundary domains, i.e. for $n_t > N_b$ (cf. Marsh, 1985). Therefore, $\Gamma_{s,p}$ increases with increasing protein dilution, reaching a constant, limiting value of $\Gamma_{s,p}/m_s^{tot} = N_b \Gamma_{s,p}^r$ at high n_t. The latter is the situation obtaining frequently in homogeneous solution, where the concentrations are relatively smaller than in the two-dimensional membrane case. Representative values of $\Gamma_{s,p}/m_s^{tot}$ in the high dilution limit, derived from ESR experiments with integral membrane proteins, lie in the range -12 to 200 mol lipid/mol protein, again with phosphatidylcholine as the reference lipid (Marsh, 1985; 1995). For a typical ESR experiment, the spin label concentration is $m_s^{tot} \approx 0.01$.

In summary, at low concentrations of spin-labeled lipid, both the multiple binding and preferential solvation approaches give rise to equivalent forms for analyzing ESR experiments on lipid-protein interactions in membranes, where the two lipid domains are resolved spectroscopically. Physically, the two models are equivalent when all solvation sites have a generalized increase in specificity for a particular solute lipid with respect to the reference solvent lipid (e.g., phosphatidylcholine), rather than there being a limited number of sites with high specificity for association. In such cases, the preferential interaction model is useful if the total number of lipids that are preferentially associated with the protein is of importance, e.g., in activating the protein. It will be noted that just as for macromolecules in solution (Inouye and Timasheff, 1972), picturing the interactions of lipids with integral proteins as a solvation process implies that these are essential for the stability and function of proteins in membranes.

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Derek Marsh

Max-Planck-Institut für biophysikalische Chemie
Am Faßberg 11, D-37077 Göttingen, Germany