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Analysis of membrane binding equilibria of peripheral proteins: allowance for excluded area of bound protein

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Abstract

When peripheral proteins bind to phospholipid membranes lacking discrete binding sites, steric repulsion between bound protein molecules may result in a reduction of the surface area available to additional bound protein by an amount significantly greater than the actual area occupied by bound protein. An approximate treatment of this effect demonstrates that neglect of area exclusion by bound protein may lead to significant errors in the evaluation of equilibrium association constants and the fractional coverage of membrane surface area.

Keywords

physical adsorption; steric repulsion

The binding of peripheral proteins to membranes has been the subject of extensive study (for a recent review, see [1]). It is often the case that such proteins do not bind to discrete, specific sites on the membrane, but are electrostatically attracted to the head groups of the phospholipids that make up the membrane, so that the binding process is equivalent to physical adsorption [1]. The difference between binding to discrete sites and physical adsorption appears to have been neglected or glossed over by workers in this field (see for example, [2]). The purpose of the present note is to clarify this distinction and to demonstrate that neglect of the difference can lead to significant quantitative errors in the interpretation of binding data.

We consider the following experiment. A fixed amount of phospholipid membrane, or an equivalent phospholipid surface such as that produced by coating hydrophobic beads with phospholipid [3,4], is equilibrated with varying amounts of peripheral protein. The amount of peripheral protein bound to the surface of the membrane or phospholipid-coated bead may be measured by a variety of techniques [2]. The quantitative analysis of the dependence of the equilibrium concentration of bound protein upon the total protein concentration proceeds as follows.

Let the total area of membrane per unit volume be given by

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$$A_{mem} = a_L c_{L,tot} \quad (1)$$

where a_L denotes the average cross-sectional area of a phospholipid molecule (lipid or L) in the plane of the membrane and $c_{L,tot}$ denotes the molar concentration of L. The fraction of surface area occupied by a concentration $c_{P,bound}$ of membrane-bound protein (P) is then given by

$$\phi = \frac{c_{P,bound} a_P}{c_{L,tot} a_L} = \frac{c_{P,bound} n_L}{c_{L,tot}} \quad (2)$$

where a_P denotes the area of membrane “covered” by each molecule of bound protein, and n_L the number of lipid molecules “covered” by each protein molecule. It may be shown via a general thermodynamic argument [5] that the equilibrium between free and membrane-bound protein is given by

$$K c_{P,free} = \phi \gamma_{P,bound}(\phi) \quad (3)$$

where K denotes the intrinsic equilibrium association constant for binding of protein to a bare membrane and $\gamma_{P,bound}$ denotes the thermodynamic activity coefficient of bound protein, a measure of the interaction between bound protein molecules. It may be shown that when the protein binds to discrete equivalent and independent sites, the activity coefficient of bound protein is simply $1/(1 - \phi)$ [5], and that as a result, the membrane binding isotherm may be described by the familiar Langmuir isotherm

$$K c_{P,free} = \frac{\phi}{1 - \phi} \quad (4)$$

which may be combined with equation (2) to yield

$$c_{P,bound} = \frac{c_{L,tot}}{n_L} \frac{K c_{P,free}}{1 + K c_{P,free}} \quad (5)$$

Equation (5) has been used to model data on the reversible binding of peripheral proteins to membranes. However, to the extent that protein does not bind to discrete and independent binding sites, equation (5) is not a correct description of the binding equilibrium, since it does not allow for the fact that bound protein excludes surface area, and hence potential locations for binding, to other proteins, leading to values of $\gamma_{P,bound}$ that substantially exceed $1/(1 - \phi)$ with increasing ϕ .

The dependence of $\gamma_{P,bound}$ on ϕ depends upon many factors, including the size and shape of the cross-section of the bound protein in the plane of the membrane, as well as the presence and magnitude of any interactions between bound protein over and above simple steric repulsion [5]. These details are generally unknown to the experimenter in advance, but in some instances may be deduced by fitting sufficiently comprehensive data with sufficiently sophisticated models [6]. The inadequacy of the naïve Langmuir analysis of membrane binding may be demonstrated by application of the simplest model allowing for area exclusion by bound protein to the analysis of published experimental data.

For the purposes of this demonstration we represent the cross-section of bound protein in the plane of the membrane as a circular disk. *It is emphasized that the footprint of an arbitrarily selected protein on a phospholipid surface may not be realistically modeled by a disk. This approximation is employed for didactic purposes only, and resulting parameter values obtained by fitting a model based upon this approximation should not be assumed to be physically realistic.* The activity coefficient of the bound “circular” protein may thus be estimated using the scaled particle theory for a two-dimensional fluid of hard circular particles [7]

$$\ln \gamma_{p, \text{bound}} \approx -\ln(1 - \phi) - 2 + \frac{1}{1 - \phi} + \frac{1}{(1 - \phi)^2} \quad (6)$$

An expression equivalent to equation (6) may be obtained for any footprint describable as a convex polygon [5]. We note that the value of $\gamma_{p, \text{bound}}$ obtained assuming a circular cross-section is the minimum value obtained for any convex hard particle of equal area at constant ϕ , so that the results shown below represent a lower bound to magnitude of excluded area effects expected for any real steric interaction between bound proteins.

Equation (2), Equation (3), and Equation (6) may be solved numerically to yield the dependence of $c_{p, \text{bound}}$ upon $c_{p, \text{tot}} = c_{p, \text{bound}} + c_{p, \text{free}}$ for given values of $c_{L, \text{tot}}$, K and n_L . This model was fit via nonlinear least squares to the published data of Cho et al [2] for the binding of phospholipase A₂ to phosphatidylcholine-coated beads ($c_{L, \text{tot}} = 6 \mu\text{M}$) to obtain best-fit values of $K = 4.4 (-1.0, +3.5) \times 10^6 \text{ M}^{-1}$ and $n_L = 11.2 (\pm 2)$, where the indicated uncertainties correspond to one standard error of estimate. The dependence of $c_{p, \text{bound}}$ on $c_{p, \text{tot}}$ calculated according to this model with the above-given best-fit values of K and n_L is plotted together with the data in Figure 1 (solid line).

For comparison, the Langmuir relation, equation (5), was fit to the same data to obtain best-fit values of $K = 8.5 (-2, +2.5) \times 10^6 \text{ M}$ and $n_L = 29.1 (\pm 2)$, in agreement with best-fit values reported by Cho et al [2]. The dependence of $c_{p, \text{bound}}$ on $c_{p, \text{tot}}$ calculated according to the Langmuir model with the above-given best-fit values of K and n_L is plotted together with the data in Figure 1 (dashed line).

Both models fit the data to approximately the same goodness of fit as measured by the sum of squared residuals, so the relative applicability of these two models cannot be distinguished solely on this basis. However, extrapolation of the respective best-fit curves to higher concentration, also illustrated in Figure 1, indicates that additional data obtained at significantly higher protein concentrations would enable such a distinction to be made. Additionally, if we accept the value of $n_L = 29.1$ obtained from the Langmuir analysis, then according to equation (2), the highest amount of protein bound measured in the Cho experiment (0.175 μM) would correspond to a fractional area occupancy of around 85%. Chatelier and Minton [5] demonstrated unequivocally that in the absence of attractive interactions between adsorbed proteins leading to cluster formation, it is impossible to attain such a large fractional area occupancy at equilibrium with any experimentally attainable total protein concentration. So the Langmuir model, which does not take into account either repulsive or attractive interactions between bound ligand, cannot provide a physically reasonable interpretation of the Cho data that are treated in this MS.

The present example demonstrates that failure to take into account steric repulsion between peripheral proteins bound to a membrane may lead to significant overestimates of binding affinity and the number of lipid molecules “covered” by a protein molecule. Note that this

number is not equivalent to the number of lipid molecules comprising a “binding site”, since there are no specific binding sites for protein on a featureless phospholipid membrane.

The Langmuir analysis is valid only at very low levels of area occupancy by bound protein ($\phi - 1$). This condition obtains in measurements such as those reported by Kim et al [8]. However, it follows from equation (5) that the apparent binding constant reported by these authors is not the actual binding constant K , but rather the quantity K/n_L . In order to estimate the quantities K and n_L independently, it is necessary to measure ligand binding at sufficiently high values of ϕ to enable application of the full analysis described above, and even in this case it is necessary to make assumptions regarding the cross-sectional area of adsorbing protein and tendency of adsorbed protein to self-associate, both of which affect the dependence of γ on ϕ [6]. The values of K and n_L derived from modeling the experimental data will thus be subject to more or less uncertainty depending upon the reliability of these assumptions.

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Figure 1. Concentration dependence of equilibrium binding of phospholipase A₂ to phosphatidylcholine-coated hydrophobic beads. Data from ref [2]. Solid line: best fit of equation (2), equation (3), and equation (6), calculated using parameter values given in text. Dashed line: best fit of equation (5), calculated using parameter values given in text.