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Electrostatics and reduction of dimensionality produce apparent cooperativity when basic peptides bind to acidic lipids in membranes

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The binding of pentalysine to phospholipid vesicles depends in a sigmoidal manner on the mole fraction of acidic lipid in the vesicles. A simple analysis demonstrates that this apparent cooperativity is probably due to both the reduction of dimensionality that occurs when the first basic residue binds to an acidic lipid in the membrane and the Boltzmann accumulation of the peptide in the electrostatic diffuse double layer produced by the charged lipids.

Many proteins that bind to membranes can interact with acidic lipids [1,2]; examples from the calcium/phospholipid second messenger system include protein kinase C [3–5] several of its substrates [6], and phospholipase C [7,8]. These proteins contain clusters of basic residues that may act as binding sites for phospholipids. The Hill equation is often used to describe the binding of ligands to proteins and to assess the degree of cooperativity. However, the simple theoretical analysis we present here predicts that both the electrostatic potential produced by the acidic lipids [9,10] and the reduction of dimensionality [11] that occurs when the protein binds to the membrane can produce apparent cooperativity with respect to these lipids, or a Hill coefficient greater than unity. This prediction was tested by measuring the binding of the basic peptide pentalysine to membranes.

The cooperative binding of ligands, L, to proteins, P, was analyzed first by Hill [12], who showed that the fractional saturation, y, of the binding sites on hemoglobin by oxygen may be described by the semiempirical equation

$$y = (K[L])^{\alpha}/(1+(K[L])^{\alpha}) \quad (1)$$

where K and α are constants; α is now known as the Hill coefficient [13]. Standard texts [13–16], which discuss Hill coefficients and cooperativity for reactions that occur in three dimensions, make the following two points: if the n binding sites are independent and identical, by definition there is no cooperativity and $\alpha = 1$; if the binding of ligands is ideally cooperative, $P + nL \rightarrow PL_n$, α attains its maximum value of n. When the ligands are acidic lipids confined to the surface of a membrane, however, neither of these points is valid.

To illustrate the difference between reactions that occur in three and two dimensions we assume the protein, P, has only two sites. In both three and two dimensions, $P + L \rightleftarrows PL$ and $PL + L \rightleftarrows PL_2$, where L is a ligand (3D) or a lipid (2D). We assume the two sites are identical and independent. If P and L are massless, dimensionless particles and we ignore the electrostatic potential, mass action [17–21] yields Eqns. 2–4 in Table I, where k is the microscopic association constant [13].

The expressions for $[PL_2]$ differ in 3D and 2D. Our analysis follows Guggenheim [22], treating the surface as a region of finite thickness $d = 1$ nm. The concentration of acidic lipid in the surface phase is $[L]_s = (V/Ad)[L]$ where V is the cell or container volume and

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Abbreviations: d, thickness of surface phase; k, microscopic association constant defined in Eqn. 2; L, acidic lipid or ligand; $[L]_s$, concentration of acidic lipid in the surface phase; Lys_2, dilysine; Lys_3, trilysine; Lys_4, tetralysine; Lys_5, pentalysine; Mops, 4-morpholinepropanesulfonic acid; MARCKS, myristoylated alanine-rich C kinase substrate; y, fractional saturation; α, Hill coefficient.

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A is the membrane area. For a spherical cell, $V/Ad = r/3d$; for a radius $r = 3\ \mu$m, $[L]_s = 10^3[L]$. Thus when a protein binds to the first lipid it moves into the surface region, where the second site encounters a much higher concentration of lipid; the same conceptual argument has been made elsewhere for antigen–antibody interactions [17,23,24]. (Mathematically this is equivalent to the binding of the first ligand increasing the association constant k for the second site: k and $[L]_s$ appear as a product, and when $k[L]_s \gg 1$, Eqn. 4 for 2D has the same form as Eqn. 1 with a Hill coefficient $\alpha \approx n = 2$, even though the sites are by definition identical and independent. The extension to $n > 2$ is trivial.)

How high must k be for this phenomenon to be biologically important? If the membrane contains 15% acidic lipid and each lipid occupies 0.7 nm^2, $[L]_s \approx 0.35$ M. Apparent cooperativity will occur if $k[L]_s > 1$ or $k > 3\ M^{-1}$. As shown below, $k \geq 3\ M^{-1}$ for the binding of lysine and arginine residues to monovalent acidic phospholipids.

The electrostatic potential caused by the acidic lipids can also produce apparent cooperativity if the region of the protein that binds to the membrane has a cluster of positive charges and most of the negative charges on the protein are located more than a Debye length ($1/\kappa \approx 1$ nm for 0.1 M salt) from the membrane surface. The concentration of P next to the membrane will be enhanced by the Boltzmann factor, $\exp(-zF\psi/RT)$, where ψ is the electrostatic potential at the surface of the membrane, which may be adequately described by the Gouy-Chapman-Stern theory of the diffuse double layer [9], z is the effective valence of P, F the Faraday constant, T the temperature, and R the gas constant. Following through the analysis we obtain Eqn. 5. The qualitative effect of electrostatics is apparent: the magnitude of ψ increases as $[L]_s$ increases, and this can produce a Hill coefficient $\alpha > n$.

TABLE I

Fractional saturation equations

3D	2D	Eqn. No.
$[PL_2] = 2k[P][L]$	$[PL] = 2k[P][L]$	(2)
$[PL_2] = \frac{1}{2}k[PL][L]$	$[PL_2] = \frac{1}{2}k[PL][L]_s$	(3)
$y = \frac{k[L](1+k[L])}{1+k[L](2+k[L])}$	$y = \frac{k[L](1+k[L]_s)}{1+k[L](2+k[L]_s)}$	(4)
	with electrostatics $y = \frac{k[L]e^{-zF\psi/RT}(1+k[L]_s)}{1+k[L]e^{-zF\psi/RT}(2+k[L]_s)}$	(5)

The 2 and 1/2 in Eqns. 2 and 3 arise from statistical factors. The general expression for the fraction, f, of peptide or protein bound to a membrane is given by:

$$f = \frac{k[L]e^{-zF\psi/RT}\left(\sum_{i=0}^{n-1}\frac{n}{i+1}\binom{n-1}{i}(k[L]_s)^i\right)}{1+k[L]e^{-zF\psi/RT}\left(\sum_{i=1}^{n}\binom{n}{i}(k[L]_s)^{i-1}\right)} \quad (6)$$

where n is the number of identical independent binding sites for acidic lipids and z is the effective valence. The general expression for y is obtained by removing the $n/(i+1)$ factor from the numerator of Eqn. 6.

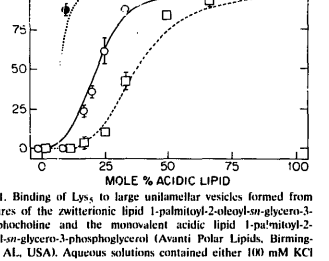

Fig. 1. Binding of Lys_5 to large unilamellar vesicles formed from mixtures of the zwitterionic lipid 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine and the monovalent acidic lipid 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphoglycerol (Avanti Polar Lipids, Birmingham, AL, USA). Aqueous solutions contained either 100 mM KCl (open symbols) or 10 mM KCl (filled circles) buffered to pH 7.0 with 1 mM Mops. Total concentrations of lipid and peptide were, respectively, 8 mM and 30 μM (circles) or 0.8 mM and 3 μM (squares). Each symbol represents average of four measurements ± S.D. when larger than symbol size. Solid curve illustrates least square fit of Eqn. 6 (Table 1) plus Gouy-Chapman-Stern theory [20] to open circles assuming $n = 4$. The free parameter, the microscopic association constant between an acidic lipid and a lysine residue, is $k = 3.5\ M^{-1}$. Independent experiments (data not shown) similar to those reported elsewhere [20,21,27] suggest the effective valence $z = 2.5$ and the intrinsic association constant of potassium ions with the acidic lipid is $1\ M^{-1}$. Dashed and dotted curves are drawn with the same parameters.

When a protein binds to acidic lipids in a membrane it is difficult to measure y, but easy to measure fraction of protein bound to the membrane, f. The analysis for this case is straightforward (Eqn. 6), and the predictions are qualitatively similar to the equations presented above. We tested the effects of dimensionality and electrostatics on apparent cooperativity by measuring the binding of pentalysine, a well characterized basic peptide [20,25,26], to phospholipid vesicles as a function of the mole fraction of an acidic lipid in large unilamellar vesicles. Large unilamellar vesicles were formed as described elsewhere and the free concentration of the peptide upon dialysis or ultrafiltration was measured by a fluorescamine assay [20,21,27].

The open circles in Fig. 1 demonstrate that the fraction of bound peptide depends on the mole fraction of acidic lipid in a sigmoidal manner, as predicted by both a Hill equation (Eqn. 1 with $\alpha = 5$, curve not shown) and the analysis above (Eqn. 6 with $k = 3.5$

M^{-1}, solid curve). Our analysis, but not the Hill equation, can account for two additional effects illustrated in Fig. 1. First, lowering the salt concentration increases the magnitude of ψ and thus increases the fraction of bound peptide (filled circles, dotted curve). Second, lowering the total concentration of lipid 10-fold (squares, dashed curve) shifts the midpoint of the curve only slightly to the right.

Ten different small peptides with a net charge of +5 exhibit binding curves with sigmoidal shapes very similar to those illustrated in Fig. 1. Peptides with five lysine or arginine residues, blocked termini, and 0, 1 or 2 alanine residues between the basic residues were studied [27], as were peptides corresponding to either the pseudosubstrate region of protein kinase C [21] or a conserved basic region of phospholipase C [8]. As predicted theoretically, Lys_4, Lys_3, and Lys_2 bind less strongly than Lys_5 [20], whereas a peptide with 13 basic residues that corresponds to the phosphorylated region of the MARCKS protein [28,29] exhibits stronger binding (McLaughlin and Hackshear, unpublished data). Thus neither chain length nor specific sequence affects the ability of the model to describe the data qualitatively.

The mass action/Gouy-Chapman model is highly oversimplified: any translational or rotational entropy losses that occur on binding to the membrane [30] are subsumed in the definition of k, and the finite size of both the lipids and the peptides is ignored. This latter assumption may account for the observation the effective valence one must use in the Boltzmann relation for charged peptides is about half the actual valence in a 0.1 M salt solution [21,27,31,32]. In spite of its simplicity, we argue that this model provides a conceptually more accurate description of the binding of small basic peptides to membranes than the Hill equation, and that the role of electrostatics and dimensionality should be considered when describing the binding of peptides and proteins to membranes.

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