Electrostatic Interaction of Myristoylated Proteins with Membranes

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Abstract

Membrane binding of a number of important peripheral proteins (e.g. Src, MARCKS, HIV-1 Gag, and K-Ras) requires nonspecific electrostatic interactions between a cluster of basic residues on the protein and acidic phospholipids in the membrane. Simple theoretical models based on the Poisson-Boltzmann equation can describe the experimentally measured electrostatic interactions and illustrate how electrostatics contributes to the formation of lateral domains.

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Hydrophobic and electrostatic interactions act in concert to anchor several important myristoylated (e.g. Src, HIV-1 Gag, MARCKS) and farnesylated (e.g. K-Ras) proteins to membranes. Two recent reviews focus on how the attachment of acyl and isoprenyl groups to proteins influences membrane binding (Resh, 1996; Bhatnagar and Gordon, 1997). We stress the role electrostatics plays in binding myristoylated proteins to membranes and in forming lateral domains.

Recent structural studies of c-Src have revealed how the binding of a phosphorylated tyrosine (Tyr 527) in the C-terminal tail to the SH2 domain juxtaposes the SH3 domain with the polyproline type-II helix that links the SH2 and kinase domains (Xu et al., 1997). The intramolecular interaction between the SH3 and kinase domains holds the protein in an inactivate conformation. Membrane association of Src requires both the N-terminal myristate and cluster of basic residues. Membrane binding increases the effective concentration of Src in a thin ($d \sim 1$ nm) surface layer adjacent to the membrane. For a spherical cell with a radius equal to a few micrometers, the volume of the surface phase ($V = 4\pi R^2 d$) is about 1/1000 the volume of the cell ($V = 4\pi R^3/3$); thus anchoring Src to the membrane increases its effective concentration 1000-fold and enhances its ability to phosphorylate its membrane-bound substrates.

Myristate (14-carbon fatty acid) is attached co-

translationally to the N-terminal glycine of Src by the enzyme N-myristoyl transferase (Resh, 1996; Bhatnagar and Gordon, 1997). Myristate is required for Src membrane binding and binding is required for function: nonmyristoylated v-Src mutants are found in the cytoplasm and do not transform cells, even though the kinase activity of the protein is unaffected. Simple myristoylated peptides bind to electrically neutral phospholipid vesicles with a unitary binding energy of 8 kcal/mol or a molar partition coefficient of $10^4 M^{-1}$ (Peitzsch and McLaughlin, 1993; Buser et al., 1994). Measurements of the membrane partitioning of acylated peptides show that the binding energy increases 0.8 kcal/mol for each CH_2 group added to the acyl chain, in agreement with Tanford's observations on the hydrophobic partitioning of fatty acids into oil from water (Peitzsch and McLaughlin, 1993; Tanford, 1991). Thus (8 kcal/mol)/(0.8 kcal/mol per CH_2) = 10 CH_2 groups penetrate the hydrocarbon core of the membrane, 4 traverse the polar head group region, while the N-terminal glycine is located just outside the envelope of the polar head group region. Recent spin label (Cafiso, 1997), monolayer, and circular dichroism (Buser et al., 1994, Murray et al., 1997) measurements show that the N-terminal residues of Src do not penetrate the membrane and indicate that a myristoylated peptide corresponding to this region has an extended conformation when it binds to a membrane.

Although myristate is required for Src membrane binding, it is not sufficient (McLaughlin and Aderem, 1995). The Src protein binds to electrically neutral membranes with a molar partition coefficient of $10^3 M^{-1}$ (Sigal et al., 1994); the concentration of lipid in the plasma membrane of a 10 μm radius cell is about 10^{-3}M, so myristate alone would bind only half the protein. Src's N-terminal cluster of basic residues augments the binding due to myristate. Studies with peptides corresponding to the N-terminus of Src show that adding 33% acidic lipid to electrically neutral membranes increases the binding 1000-fold (Buser et al., 1994). The same 1000-fold enhancement is seen with the intact Src protein (Sigal et al., 1994). Mutating away the N-terminal basic residues weakens the binding *in vitro* and produces non-transforming phenotypes *in vivo* (Sigal et al., 1994). These observations provide strong evidence that the N-terminal basic residues contribute to the membrane binding of Src by interacting electrostatically with acidic lipids.

To a first approximation, the hydrophobic and electrostatic binding energies add (or the molar partition coefficients multiply). This observation follows from simple "ball and string" models that consider the acyl chain and basic cluster as small balls connected by a flexible string of length r (Buser et al., 1994; Ghomashchi et al., 1995). Binding of myristate confines the basic cluster to a hemisphere of radius r above the membrane surface and facilitates its binding. Although the simple models account for the synergism between electrostatic and hydrophobic interactions, they are descriptive rather than predictive. Using atomic models of Src's N-terminus and phospholipid bilayers, a continuum representation of the solvent, and the nonlinear Poisson-Boltzmann equation we can describe theoretically the electrostatic binding (Ben-Tal et al., 1996; Ben-Tal et al., 1997; Murray et al., 1997).

For these calculations, each atom is assigned a radius and a partial charge, and the peptide/membrane model is mapped onto a three-dimensional lattice of points. The nonlinear Poisson-Boltzmann equation is solved numerically for the electrostatic potential adjacent to the peptide and the membrane when they are far apart and when they are close together. These potentials are used to calculate the decrease in the electrostatic free energy as the peptide approaches the membrane. The free energy curve exhibits a long-range Coulombic attraction and short-range Born repulsion that result in the free energy minimum at distance $h \sim 3$ Å. The peptide concentration at each distance h is a product of the peptide concentration in the bulk solution and the exponent of the interaction energy. (In practice, the peptide concentration at h is calculated by averaging over many orientations of the peptide with respect to the membrane in order to approximate a complete ensemble of different configurations.) Integrating the excess peptide concentration over h gives the

Gibbs surface excess, which represents the number of moles of peptide bound per unit area of membrane surface (Ben-Tal et al., 1996). The Gibbs surface excess is simply related to the molar partition coefficient that is measured experimentally.

The theoretical methodology has been used to describe the membrane binding of basic peptides (Ben-Tal et al., 1996; Murray et al., 1997) and toxins (Ben-Tal et al., 1997): the model predicts how the binding is affected by changes in the ionic strength of the solution, the net positive charge of the peptide, or the mole % acidic lipid in the membrane. For example, the model correctly predicts that the binding of charybdotoxin decreases by five orders of magnitude when the salt concentration is increased from 10 mM to 150 mM (Ben-Tal et al., 1997). These results indicate the model describes well the long-range ($h \geq 3$Å) electrostatic attraction that gives rise to the membrane binding. Nevertheless, the calculated binding energies based on electrostatics alone consistently underestimate the observed values by 1-2 kcal/mol. This implies the model ignores some attractive interactions (Ben-Tal et al., 1997), overestimates the repulsive interactions, or both. Further theoretical and experimental work is required to obtain a more accurate description of the short-range ($h < 3$ Å) interactions, particularly when hydrophobic residues penetrate the polar head group region, a phenomenon investigated experimentally by several groups (Wimley and White, 1996).

Other proteins that use either myristate or farnesyl and a cluster of basic residues to bind to membranes include HIV-1 Gag (Zhou et al., 1994), K-Ras 4B (Hancock et al., 1990; Hancock et al., 1991), and MARCKS (myristoylated alanine-rich C kinase substrate) (Swierczynski and Blackshear, 1996; Seykora et al., 1996). The N-terminal cleavage product of HIV-1 Gag, the matrix protein, contains the two membrane binding motifs (Zhou et al., 1994). Structural studies show the basic residues are clustered in a patch that forms a membrane-binding surface (Massiah et al., 1994; Hill et al., 1996). K-Ras, a small GTPase, uses farnesyl (15 carbon isoprenoid) rather than myristate to bind to membranes. The farnesyl chain, like myristate, does not provide sufficient hydrophobic energy to anchor the protein to membranes (Silvius and l'Heureux, 1994). Adding 20% acidic lipid to electrically neutral membranes enhances the binding of farnesylated peptides corresponding to the C-terminus of K-Ras 300-fold (Ghomashchi et al., 1991). Spin-label EPR experiments show the MARCKS basic effector region, MARCKS (151-175), lies at the membrane interface in an extended conformation; although most of the binding energy is due to electrostatics, its five phenylalanines penetrate the polar head group region (Qin and Cafiso, 1996). MARCKS is interesting because protein kinase C (PKC) phosphorylation of three serines within the basic effector region weakens the electrostatic interaction and causes the MARCKS protein to translocate from the plasma membrane to the cytoplasm in many cell types (Kim et al., 1994); this mechanism has been termed the "myristoyl-electrostatic" switch (McLaughlin and Aderem, 1995). Recent results show that the binding of peptides corresponding to the MARCKS effector region is a diffusion limited process and that calmodulin can produce a rapid desorption of these peptides from the membrane (Arbuzova et al., 1997).

Lateral organization at the membrane surface may provide a mechanism for facilitating or regulating the interaction of membrane-bound molecules. For example, many important signaling molecules (e.g. Src family members Lck and Fyn, G-protein alpha subunits, H-Ras, eNOS, and phosphatidylinositol 4,5-bisphosphate, PIP_2) are concentrated in plasma membrane organelles called caveolae (Simons and Ikonen, 1997). MARCKS has a punctate distribution in the plasma membrane of macrophages and is concentrated, with PKCα, in nascent phagosomes (Allen and Aderem, 1996). In phospholipid vesicles, the basic effector region of MARCKS (Glaser et al., 1996) and simple basic peptides like pentalysine (Denisov et al., 1998), which corresponds to the first five residues of the MARCKS effector region, form discrete lateral domains enriched in monovalent acidic phospholipids and PIP_2. The results with pentalysine indicate that electrostatics can play a major role in domain formation and that PIP_2 can be sequestered in domains by nonspecific electrostatic

interactions, a phenomenon with interesting physiological implications.

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