

Membrane Asymmetry and Surface Potential

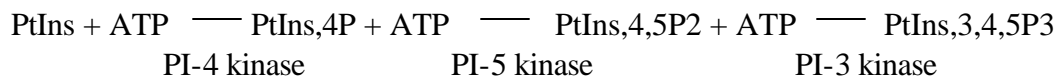
There are many consequences of membrane asymmetry. It is a critical aspect of membranes that is tied to many different cell functions.

Lipid Asymmetry and Flippases

If we consider the normal lipid composition of a plasma membrane such as the erythrocyte, the outer surface lipids are neutral except for the glycolipids and in that case the charges are separated from the membrane surface by the length of the carbohydrate molecules. At the cytoplasmic surface, we find over 90% of the phosphatidyl serine and inositol which constitute 12-20% of the total phospholipids and 24-40% of the cytoplasmic half of the bilayer. Since those lipids are negatively charged, there is a very high density of negative charges on the cytoplasmic surface (0.5-0.8 charge/nm²). There are many cationic proteins and/or cationic domains of proteins that are localized to the plasma membrane cytoplasmic surface and may neutralize a significant fraction of the lipid charges. Although binding of the lipids to proteins will decrease the effective anionic lipid concentration, increased flipping of phosphatidyl serine to the external half of the bilayer is typically found after the cytoplasmic ATP concentration drops or cells are damaged. Appearance of phosphatidyl serine on the external surface is diagnostic of cells entering apoptotic (cell death) pathways and commercial apoptosis kits utilize phosphatidyl serine binding proteins as a reliable assay for apoptosis. An ATP-dependent flippase is present that can restore the normal asymmetry of phosphatidyl serine. Thus, it appears that the asymmetry is important to the cell. Further, the asymmetry could not simply be the result of asymmetric synthesis of the lipids, which would irreversibly equalize over time without a flippase. Energy-dependent concentration of phosphatidyl serine has been observed and is a critical component in the preservation of cell viability, i.e. apoptosis occurs when lipid asymmetry is lost.

Inositol Lipids and Changes in Level of Charge

The most highly charged lipids are the most dynamic in cells. Phosphatidyl inositol mono-, di-, and tri-phosphates rapidly turn over in cells and have been linked to many signaling pathways. Hormonal signaling (calcium release), cell chemotaxis, actin assembly and secretion are all linked to the hydrolysis or synthesis of phosphorylated inositols. The basic metabolic pathways are noted below



Major roles have been proposed for PI-4,5P₂ in many cell functions. It is the most abundant phosphorylated inositol and, when hydrolyzed by phospholipase C, is the source of IP₃ (1,4,5 triphosphoinositol) and diacylglycerol. IP₃ causes calcium release from internal (ER) stores and diacylglycerol activates protein kinase C (PKC) to phosphorylate serine groups on membrane associated proteins.

Recent studies have linked the production and perhaps the dynamics of PtIns-3,4P2 and PtIns-3,4,5P3 with chemotaxis and cell asymmetry generation. The kinase, PI-3 kinase, is a major enzyme in chemotactic pathways but how kinase activity is linked to the asymmetric assembly of actin and migration is not clear.

Charge Pairs in Solution

The physical properties of charges in a water environment have important effects on the behavior of charged nucleic acids, proteins and lipids. Take the case of a charge pair such as a sodium cation and a chloride anion dissolved in water. If we go back to our consideration of diffusion, then it is clear that they will diffuse independently except that charge-charge attraction will draw them together. A balance will be struck on average between the tendency to diffuse away from each other and the attractive electrostatic force. The mathematical description of this balance is provided by the Debye-Huckel model. We can assume that the distribution of counterions around a point charge will follow the Boltzmann relation

$$n_i = n_0 e^{-U/kT} \quad (15)$$

where n_i is the number density (concentration) at the i th point which is different in energy from the 0^{th} point by U , T is the temperature, and k is the Boltzmann constant ($k = 1.38 \times 10^{-23} \text{ J}^\circ\text{K}^{-1}$). U is only electrostatic energy that is dependent upon the potential (Ψ_i) at the i th point

$$U = ze_0 \Psi_i \quad (16)$$

where z is the charge on the counterion and e_0 is a unit charge ($e_0 = 1.6 \times 10^{-19} \text{ C}$). The potential will decrease rapidly with distance from the first charge (or a protein or a lipid surface) particularly in a concentrated salt solution.

To understand the relationship between the salt concentration and the change in potential with distance, a useful parameter is the Debye length, which corresponds to the effective distance between the two counterions. Debye length is calculated from the equations above using a number of approximations and derivations for Ψ_i .

$$\kappa^{-1} = L_D = (\epsilon_0 \epsilon kT / 2e_0^2 N_A I)^{1/2} \quad (17)$$

where ϵ_0 is the dielectric constant, ϵ_0 is the permittivity of free space ($\epsilon_0 = 8.854 \times 10^{-12} \text{ C}^2\text{N}^{-1}\text{m}^{-2}$), N_A is Avagadro's number ($N_A = 6.023 \times 10^{23} \text{ molecules/mole}$) and I is the ionic strength ($I = 1/2 \sum c_i z_i^2$, where c_i is the concentration of the i th ion and z_i is the charge).

Debye length for	0.1 M NaCl,	$L_D = 0.96 \text{ nm}$,
	0.01 M NaCl	$L_D = 3.04 \text{ nm}$
	0.01 M MgCl2	$L_D = 1.75 \text{ nm}$

Surface Potentials (Guoy Chapman Double layer)

Because density of anionic charges is very high on the cytoplasmic surface of the plasma membrane, we should consider the consequences when the overall concentration of ions is low the counterions can diffuse very far from the surface, giving rise to a surface potential. The Debye length is the distance from the surface at which the surface potential drops to $1/e$ of the original potential.

$$\Psi_x = \Psi_0 e^{-\kappa x} \quad (19)$$

Debye length increases with increasing temperature and with decreasing ion concentration.

Concentration at Charged Surfaces

We have talked about the concentration of proteins at membrane surfaces as a means of increasing protein-protein interactions. The surface potential at the cytoplasmic face of the plasma membrane can affect the concentration of ions in the region as well as the pH. If we assume that the concentration of anionic lipids is 33% of the total lipids on that surface, then the surface potential in 0.1 M NaCl will be about 50 mVolts negative. According to the Boltzmann distribution, the concentration of a cation at the surface (c_s) will be greater than the concentration in cytoplasm (c_c) because of a lower energy due to interaction with the surface charge.

$$c_s = c_c e^{+ze \cdot 0.05/kT} = c_c e^{+8 \times 10^{-21}/(1.38 \times 10^{-23} \times 300^\circ)} = c_c e^{1.9} \quad (20)$$

This applies for all singly charged ions and corresponds to roughly a 4.5-fold increase in the concentration. For a divalent cation, the concentration would be about twenty-fold greater ($e^{3.8}$). These are theoretical values and the large size of hydrated ions in solution will change the observed values dramatically.

Because at 0.1 M salt the potential drops to 1/e in about 1 nm, the surface potential is only 50 mV at the surface and at 5 nm from the surface the potential will be only 1/e⁵ of what it is at the surface. Regional changes in the distribution of anionic lipids or the binding of proteins to the surface through cationic domains would result in major inhomogeneities in the surface potential (Peitzsch et al., 1995; Murray et al., 1999).

Problems:

1. A membrane channel has a large cytoplasmic domain that covers a circular area 4 nm in diameter (pore is in the center) at the membrane surface and neutralizes the negative lipid charges beneath it. If the potential at the uncovered membrane surface is -50 millivolts, and the solution contains 0.1 M of monovalent salt. What is the pH at the mouth of the pore and edge of the protein?
2. We want to understand the importance of the cytoplasmic surface potential to the transmembrane potential. Transmembrane potentials are typically -100 millivolts (negative inside the cell). If the cytoplasmic surface has a charge of -50 millivolts, what is potential gradient across the 5 nm of the bilayer? Dielectric breakdown of biological membrane occurs at about 1 volt across the membrane. What is the fraction of the potential gradient across the membrane at dielectric breakdown that is contributed by the cytoplasmic surface potential given above?

References:

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- Peitzsch, R. M., M. Eisenberg, K. A. Sharp, and S. McLaughlin. 1995. Calculations of the electrostatic potential adjacent to model phospholipid bilayers. *Biophys J.* 68:729-738.