FLUORESCENCE QUENCHING IN MODEL MEMBRANES

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Fluorescence quenching of membrane-bound fluorophors by nitroxide spin-labeled phospholipids has proved to be a useful technique for examining lipid-fluorophor contact (1–3). When the fluorophor is the tryptophanyl residue of a polypeptide, this method can yield relative binding constants of phospholipids to a membrane-bound polypeptide.

When a spin-labeled phospholipid loses contact with a tryptophanyl residue, tryptophanyl fluorescence increases. Converse behavior occurs in a bilayer lipid mixture containing spin-labeled lipid, unlabeled lipid, and a membrane protein. There, as the unlabeled lipid separates from the rest of the mixture, fluorescence decreases while the environment of the fluorophors becomes enriched in the quenching, spin-labeled phospholipid. For example, in a bilayer vesicle containing a spin-labeled phosphatidylcholine, dipalmitoylphosphatidylcholine (DPPC), and the membrane-bound polypeptide gramicidin *A'*, as the temperature is lowered below the DPPC gel-liquid crystal phase transition temperature of 41°C, the gramicidin tryptophanyl fluorescence decreases as a DPPC-rich gel phase separates, leaving the gramicidin in a spin-labeled, lipid-rich environment.

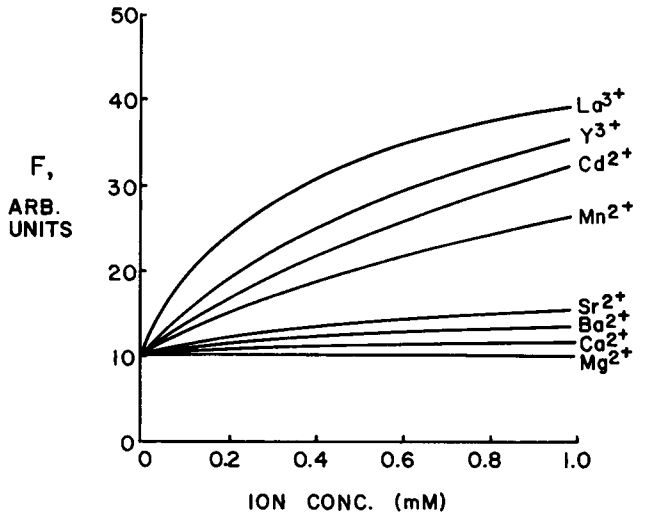

FIGURE 1 Multivalent cation effects on the fluorescence quenching of gramicidin *A'* incorporated into small unilamellar vesicles containing a mole fraction 0.2 of spin-labeled phosphatidic acid in egg phosphatidylcholine. Temperature, 21°C; overall lipid concentration, 0.5 mM, pH 7.0.

Multivalent cations bind to negatively charged phospholipids in a membrane. When this binding results in a lipid arrangement similar to that in the gel phase, the gel phase can form and separate from the rest of the membrane. For phosphatidic acid (PA), the order of cation affinities for PA is found to be (La^{3+}, Y^{3+}, Cd^{2+}, Mn^{2+}) > (Sr^{2+}, Ba^{2+}, Ca^{2+}) > Mg^{2+}. This is also the way the cations are grouped in their ability to induce the PA gel phase to form.

The separation of a cation-PA gel phase results in this region of the membrane being depleted in gramicidin *A'*. This clearing of gramicidin *A'* is monitored as a rise in fluorescence as shown in the figure. At this concentration of PA (mole fraction 0.2) the ions are clearly divided into two groups according to whether or not they induce a PA gel phase to separate.

The cation-induced clearing of membrane-bound polypeptides from a region of a membrane may be an important factor in the fusion of biological membranes.

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