Fluorescence Polarization Study of Membrane Processes

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The application of fluorescence polarization method provides information about the structure and dynamics of proteins, protein-receptor, receptor-ligand and protein-ion interactions. In 1926 Perrin first described the utility of fluorescence polarization to study of molecular interactions.

A common structural motif of all biological membranes is the fluid lipid bilayer. The lipid bilayer contains a large number of different lipid species and membrane proteins. One of the key roles of the lipid-bilayer components of the cell membrane is to establish a physical barrier that protects the cell to sustain osmotic gradients across the membrane. The biological lipid membrane is not a perfect chemical seal. Ions, water, and other molecular compounds may permeate passively across the membrane. Monte Carlo computer simulation on a specific microscopic model has shown various molecular compounds such as cholesterol, peptides, carotenoids and drugs incorporated into or interacting with lipid bilayers are able to change the heterogeneous microscopic lipid domains. It has been shown that lipid bilayer with incorporated low concentrations of cholesterol becomes more leaky. Certain drugs, such as anesthetics and various insecticides exert a strong disordering effect on the lipid domains. The both peripheral and integral proteins couple strongly to the local order and may take advantage of it as a mean for controlling membrane functions. Carotenoids like antioxidants transported into cell membrane can modify membrane structure and fluidity and hence movement of protein etc. within the membrane by stabilizing the system against oxidation.

Order-disorder processes and lipid-domain formation in lipid bilayers are likely to influence functions associated with membrane-bound enzymes and receptors. Lipid-lipid repulsion and protein-lipid attractions will have profound effect on the phospholipase C catalyzing the conversion of the phosphoinositides into second-messenger molecules. These messengers trigger a wide range of cellular responses.

Crystal membrane interaction and crystal-induced membranolysis have been studied using human erythrocytes. The binding of urate monohydrate crystals to membranes induces the redistribution of transmembrane proteins into clusters or aggregates leading to "pore" formation. The "pores" permit the leakage of low molecular weight soluble compounds and ion across the membrane, followed by osmotic rupture of the membrane.

The spectroscopic and photosensitizing properties of many porphyrins and their analogs have been investigated, particularly in view of their biomedical applications including the treatment of tumors by photodynamic therapy [2—3]. Phtalocyanines are class of the second generation photosensitizing agents for the treatment of tumors, preferentially localize in the outer mitochondrial membrane [4]. Fluorescence polarization assay of membrane processes is a nonradiactive, acurate, probably sensitive, simple, rapid, reproducible, enviromentally safe and minimize handling problems. Fluorescence polarization usually rely on fluorescein as an exegenous fluorophore or other fluorescent dyes as acridine orange, Hoechst 33342 or a new green protein from jelly fish. Fluorescence polarization can be adapted for the high-troughput screening of proteins and small molecule drug discovery. The lipid-receptor interactions may be required for conformational changes of the receptor and signal transmission across membrane. Delipidation of native membrane is still an area of active investigation and probably it is the requirement of specific lipids for receptor system.

The results present the experiments concerning the spectral definition of suitable membrane system for monitoring of drug toxicity.

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