FLUORESCENCE ANISOTROPY MEASUREMENT OF MEMBRANE FLUIDITY

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Abstract: Although fluorescence anisotropy measurements are commonly used in the biophysical research since 1980's, nowadays they still find application in investigation of living cells. Anisotropy measurements provide information on the size and shape of proteins or the rigidity of various molecular environments. The concept of fluidity, applied to phospholipidic bilayer membranes refers to orientational and dynamic properties of the lipid hydrocarbon chains in the bilayer matrix, respectively the limiting rotational amplitude of motion, quantitatively expressed by the lipid order parameter, and the rate of rotational motion related to local microviscosity. The fluid properties of biological membranes are essential for numerous cell functions including cell growth, solute transport, signal transduction, and membrane-associated enzymatic activities.

Anisotropy measurements are based on the principle of photoselective excitation of fluorophores by polarized light. Subsequently the vertically and horizontally polarized emissions are recorded. A special attention is focused on 1,6-diphenyl 1,3,5-hexatriene (DPH), the most frequently employed fluorescence polarization probe. DPH diffuses into hydrophobic regions of the lipid bilayer. On the other hand, DPH cationic derivative trimethylammoniumdiphenylhexatriene (TMA-DPH) is a hydrophobic fluorescent probe with a high quantum yield, which monitor fluidity near the surface of the cell membrane. The polar region of this probe is anchored at the lipid-water interface, while the hydrocarbon moiety enters the lipid part of the membrane and will thus provide information on this more superficial region including the surface and glycerol side chain region of the plasma membrane. Both of these fluorescence probes were sussesfully used in our laboratory for monitoring of the membrane fluidity on the red blood cells ghosts, mitochondrial membrane, as well as living yeasts cells.

Key words: membrane fluidity, order parameter, fluorescence, anisotropy

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