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Lateral organization of mixed, two-phosphatidylcholine liposomes as investigated by GPS, the slope of Laurdan generalized polarization spectra

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Abstract

The effect of the excitation or emission wavelengths on Laurdan generalized polarization (GP) can be evaluated by GPS, a quantitative, simplified determination of the GP spectrum slope, the thermotropic dependence of which allows the assessment of phospholipid lamellar membrane phase, as shown in a recent publication of our laboratory [J.B. Velázquez, M.S. Fernández, Arch. Biochem. Biophys. 455 (2006) 163–174]. In the present work, we applied Laurdan GPS to phase transition studies of mixed, two-phosphatidylcholine liposomes prepared from variable proportions of dimyristoyl- and dipalmitoylphosphatidylcholine (DMPC and DPPC, respectively). We have found that the GPS function reports a clear limit between the gel/liquid-crystalline phase coexistence region and the liquid-crystalline state, not only at a certain temperature T_c for liposomes of constant composition submitted to temperature scans, but also at a defined mole fraction X_c, for two-component liposomes of variable composition at constant temperature. The T_c or the X_c values obtained from GPS vs. temperature or GPS vs. composition plots, respectively, allow the construction of a partial phase diagram for the DMPC–DPPC mixtures, showing the boundary between the two-phase coexisting region and the liquid-crystalline state. Likewise, at the onset of the transition region, i.e., the two-phase coexisting region as detected by GPS, it is possible to determine, although with less precision, a temperature T_o or a mole fraction X_o defining a boundary located below but near the limit between the gel and ripple phase, reported in the literature. These GPS results are consistent with the proposal by several authors that a fraction of L_α phospholipids coexists with gel phospholipids in the rippled phase.

Keywords: Laurdan; GPS; Generalized polarization spectrum slope; DMPC–DPPC liposomes; Phase transition; Ripple phase

Cumulative evidence over the last three decades, points to heterogeneities in the lipid matrix of biological membranes as playing important roles in many cellular processes. These processes include, among others, the membrane function of enzymes, transporters and channels as well as the location and redistribution of receptors or sites for virus entry and budding [1,2]. Lipid membrane heterogeneities are likely to be related to phase separation phenomena [3]. Still, the physical behavior of complex mixtures of membrane lipids is not completely understood. It is obvious that a better knowledge of lipid miscibility or phase separation needs more studies using model systems. Consistent with this, we are seeing a revival of the classical model membrane studies of the seventies [4,5], updated by use of current approaches and methodologies [6–11].

Much of the work in our laboratory is and has been concerned with model membranes. Our interests range from the analysis of lipid surface ionization in bilayers [12–14] to studies of the effect of liposome physical state on the interfacial activation of phospholipase A_2 [15–18]. In fact, the interfacial activation of this enzyme is extremely sensitive to the fine morphological details of the organized

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