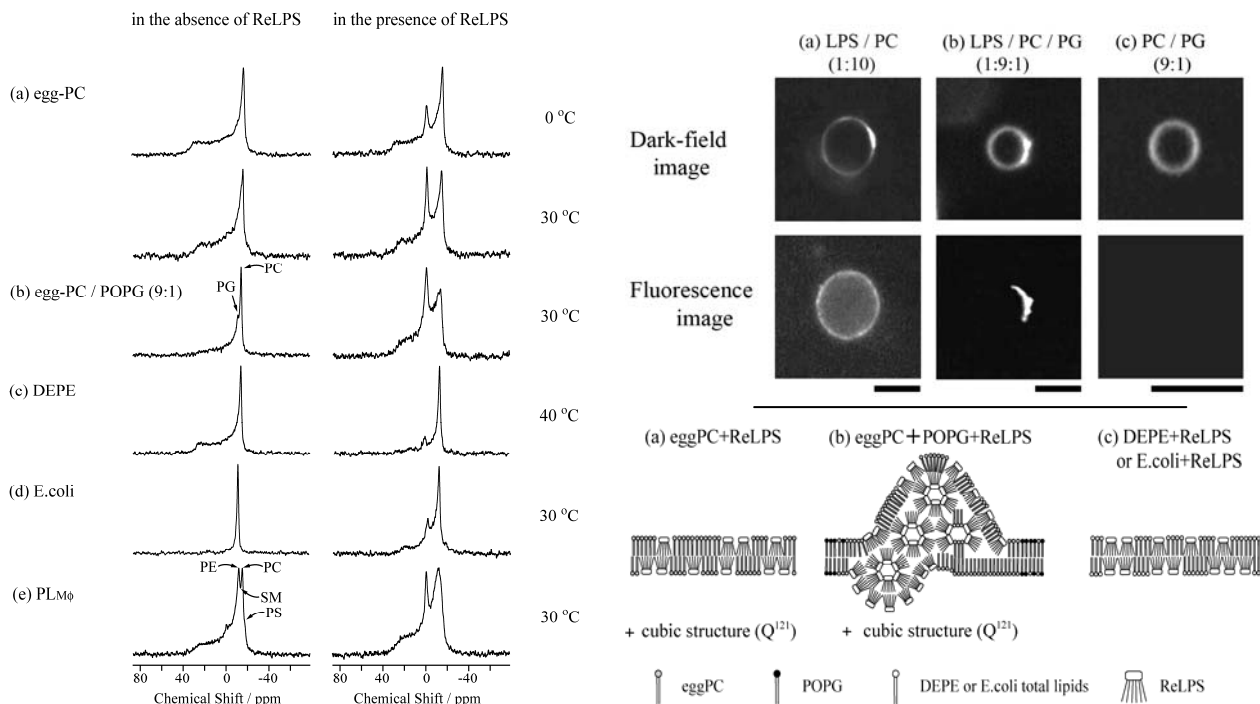


Interaction of lipopolysaccharide and phospholipid in mixed membranes: solid-state ^{31}P -NMR spectroscopic and microscopic investigations

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Biophys. J. 95, 1226-1238 (2008)

To understand the basic mode of interaction between bacterial lipopolysaccharide (LPS), which is a key molecule of innate immunity, and phospholipid cell membranes, distribution patterns were studied by various physical methods of deep rough mutant LPS (ReLPS) of *Escherichia coli* incorporated in phospholipid bilayers as simple models of cell membranes. Solid-state ^{31}P -NMR spectroscopic analysis suggested that a substantial part of ReLPS is incorporated into lipid bilayers. The aggregation structure in the presence of ReLPS was critically affected by the lipid composition. In egg L- α -phosphatidylcholine (egg-PC)-rich membranes, ReLPS undergoes micellization. In phosphatidylethanolamine (PE)-rich membranes, however, micellization was not observed. We studied the location of ReLPS in membranes and influence of ReLPS on the physicochemical properties of the membrane by microscopic techniques using giant unilamellar vesicles (GUVs) and supported planar lipid bilayers (SPBs). In both model membranes, ReLPS was uniformly incorporated in the egg-PC lipid bilayers. In the egg-PC / 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylglycerol (POPG) lipid bilayers, however, ReLPS is only partially incorporated and becomes a part of the membrane in a form of aggregates on the bilayer surface. The lipid lateral diffusion coefficient of phospholipids in ReLPS / egg-PC / POPG membrane decreased with the ReLPS composition: the reduction of the coefficient was more significant with increasing POPG concentrations. The present work demonstrated that the phospholipid composition has critical influence on the distribution of added ReLPS in the respective lipid membranes and also on the morphology and physicochemical property of the resulting membranes. A putative major factor causing these phenomena is reasoned to be the miscibility between ReLPS and individual phospholipid constituents.



(left) Effect of ReLPS on the ^{31}P NMR spectra of phospholipids in lipid bilayers. The spectra were taken at 30 °C for (a, b, c, e) and 40 °C for (d) at molar ratio of ReLPS / lipids = 1:10. (right top) Dark-field and fluorescence images of GUVs composed of (a) ReLPS / egg-PC (1:10), (b) ReLPS / egg-PC / POPG (1:9:1), and (c) PC / PG (9:1) at room temperature. Bar = 10 μm . (right bottom) Schematic model of ReLPS intercalations into the various phospholipid membranes.