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The Effect of Polysaccharide Adsorption on Surface Potential of Phospholipid Monolayers Spread at Water-Air Interface

Adam BASZKIN, Veronique ROSILIO, Francis PUISIEUX, Genevieve ALBRECHT, and Junzo SUNAMOTO†

Physico-Chimie des Surfaces et Innovation en Pharmacotechnie UA CNRS 1218, Université Paris-Sud, 5 rue J.B Clément 92296 Châtenay-Malabry, France
†Department of Polymer Chemistry, Kyoto University, Kyoto 606

Surface potential measurements were performed for the systems of egg phosphatidylcholine (PC)/cholesteryl-amylopectin and egg PC/cholesteryl-pullulan. The variations of surface potential of phospholipid monolayers on injection of polysaccharide derivatives into the aqueous subphase were monitored for various surface densities of phospholipids and polysaccharide solution concentrations. At the phospholipid surface concentrations above 10^{14} molecules/cm^2, the changes in the surface potential of monolayers were shown to be higher for amylopectin than for pullulan.

Among various factors that could affect the outcome of drug-loaded liposome therapy are low stability of the carrier and its predominant localization in the reticuloendothelial system (RES) of the liver and the spleen after intravenous injection. This preferential localization is a major obstacle to their targeting to active sites. Sunamoto et al.[1,2] have developed a successful methodology of preparation of polysaccharide derivative-coated liposomes. With this technique one obtains an attachment of a cholesterol substituted polysaccharide derivative onto a liposome membrane. The saccharide moiety behaves as a sensory device and provides targetability to specific cells or tissues. Polysaccharide coating enhances also physicochemical stability of liposomes.

In spite of numerous positive results obtained on targeting of these liposomes the action of polysaccharides at the membrane level is still poorly understood.[3] Surface potential studies of monolayers appear to be a useful approach to this question.[4-6] In such experiments, an insoluble phospholipid is spread at the air-water interface and the effect of a water soluble tensioactive substance injected beneath the monolayer on surface pressure and/or surface potential is monitored.[7,8] Egg phosphatidylcholine (Egg PC) was isolated and purified according to the method described by Singleton et al.[9] Cholesterol-substituted pullulan (CHP) and cholesterol-substituted amylopectin (CHA) were synthetized according to the previously described method.[1] The substitution degree of the cholesterol residue of the polysaccharides determined by ^{1}H-NMR method was 1.4 cholesteryl group per 100 glucose units for pullulan and 1.0 cholesteryl group per 100 glucose units for amylopectin. Their molecular weights were 52,000 and 113,000 respectively. Known

amounts of egg PC were spread from a solution in cyclohexane on an aqueous subphase of the measuring half cell of the surface potential device. The surface potential was measured with an electrometer and two identical ionizing ^{241}Am electrodes emitting α radiation, as previously described (Fig. 1).[10,11] An aqueous cholesterol-substituted polysaccharide solution was introduced through a side arm in the subphase in microliter quantities and the variations of the surface potential due to its interaction with spread monolayers were recorded until equilibrium values were reached.

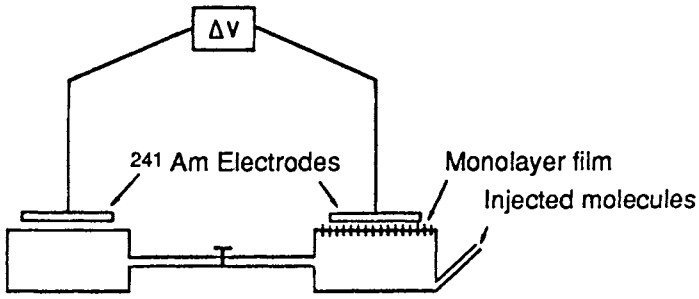

Fig. 1. Schematic representation of surface potential (ΔV) measuring device at a constant surface area (From Baszkin et al.[11]).

For an insoluble monolayer spread at the liquid-gas interface the surface potential is expressed as

$$\Delta V = \Psi o + Vp \qquad (1)$$

where Ψo is the electrostatic potential associated to the charged lipid monolayer and Vp is the potential in the plane of charged groups due to the formed ion pairs and the diffused ionic double layer. On injection of polysaccharide molecules to the aqueous subphase an increase in the surface potential can be described by

$$\Delta(\Delta V) = \Delta\Psi o + 12\pi\Delta\mu_2 / A \qquad (2)$$

where $\Delta\Psi o$ is the change in the electrostatic potential expressed in mV, $\Delta\mu_2$ the change of the vertical component of the total dipole moment expressed in mDebye and A the area occupied per molecule in $Å^2$/molecule. $\Delta\Psi o$ and ΔVp depend both on the surface density of interfacial molecules and on their mean orientation relative to the interface plane. The total surface dipole moment involves at least three contributions: the dipole moment due to the oriented water molecules, the dipole moment of the polar head, and the dipole moment of adsorbing molecules.

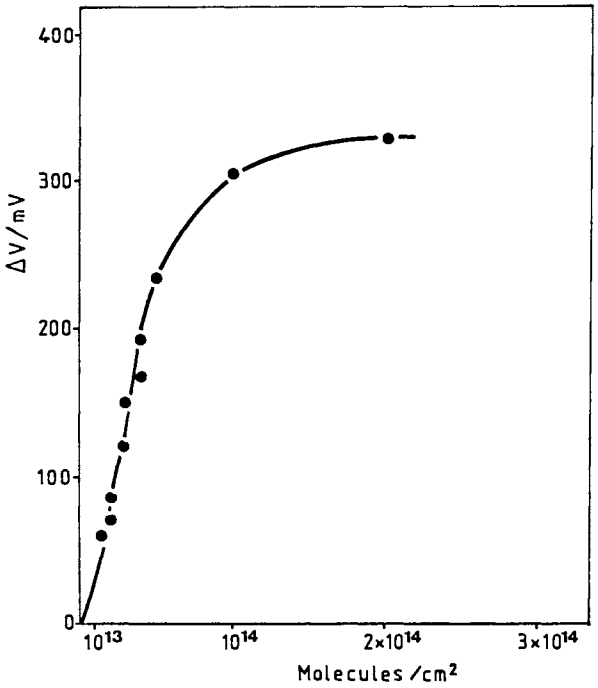

Fig. 2. Equilibrium surface potential versus surface density of spread egg phosphatidylcholine molecules.

Figure 2 shows the ΔV variation as a function of lipid surface density. It may be noted that the ΔV increases very rapidly with an increase in the lipid

surface density and that the plateau value corresponding to about 325 mV is achieved for the molecular area of 58 Å^2.

These values are in a good agreement with the literature data obtained both through the surface pressure measurements[12-14] and surface potential experiments.[8,10,15]

Injection of CHA or CHP solutions to the subphase (Figs. 3 and 4) brings about changes in the surface potential of spread phospholipid monolayers. Whilst positive $\Delta(\Delta V)$ values may be attributed to the change in the electrostatic potential of the system and are due to the penetration/adsorption of polysaccharide molecules into lipid monolayers, negative $\Delta(\Delta V)$ would reflect variations of the dipolar Vp term of the surface potential.[8, 11]

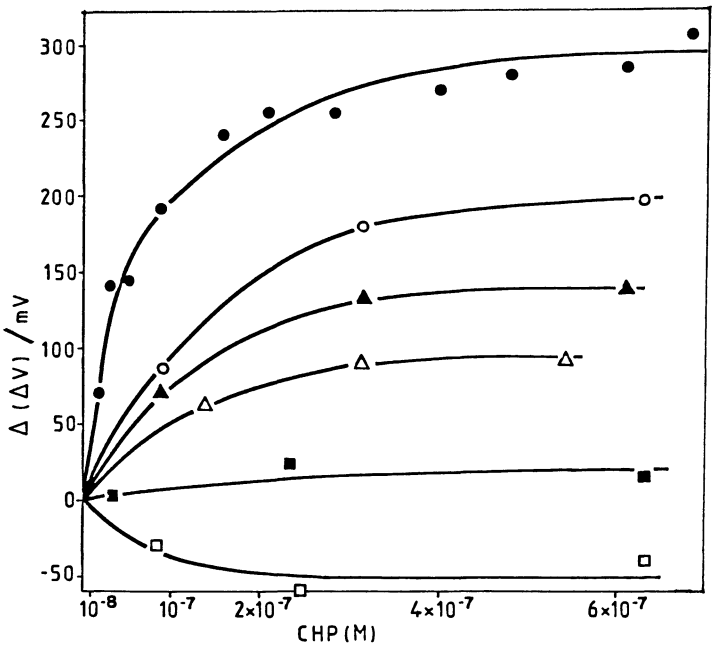

Fig.3. Evolution of the surface potential $\Delta(\Delta V)$ with increasing cholesteryl-pullulan concentration in the aqueous subphase:
●: Surface potential ΔV of CHP at the water-air interface in the absence of egg phosphatidylcholine,
○: Monolayer density (δ)=2.03 x 10^{13} molecules/cm^2; the initial surface potential (ΔVi) before polysaccharide addition was 69 mV,
■: δ=2.98 x 10^{13} molecules/cm^2; ΔVi = 153 mV,
□:δ=4.06 x 10^{13} molecules/cm^2; ΔVi = 195 mV,
▲:δ=1.015 x 10^{14}molecules/cm^2; ΔVi = 276 mV,
△:δ=2.03 x 10^{14} molecules/cm^2; ΔVi = 330 mV

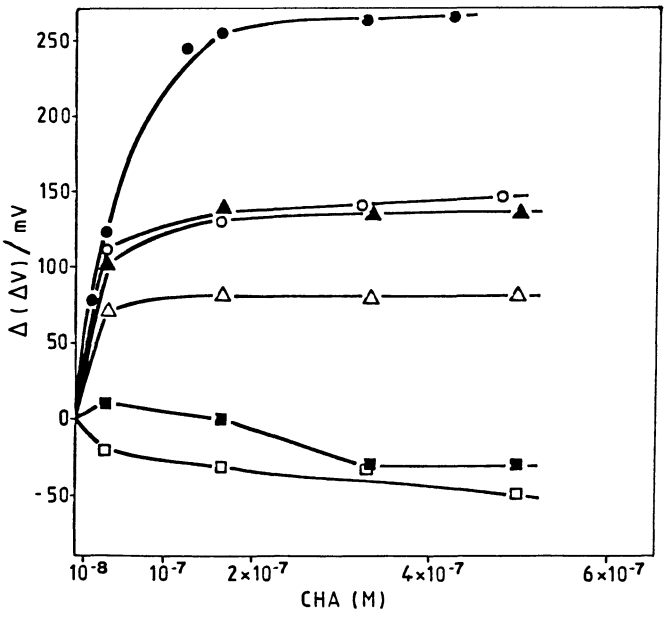

Fig.4. Evolution of the surface potential $\Delta(\Delta V)$ with increasing cholesteryl-amylopectin concentration in the aqueous subphase:
●:Surface potential ΔV of CHA at the water-air interface in the absence of egg phosphatidyl-choline,
○:Monolayer density (δ)=2.03 x 10^{13} molecules/cm^2; the initial surface potential (ΔVi) before polysaccharide addition was 85.5 mV,
■:δ = 2.98 x 10^{13} molec/cm^2; ΔVi = 120 mV,
□:δ = 4.06 x 10^{13} molec/cm^2; ΔVi = 168 mV,
▲:δ = 1.015 x 10^{14} molec/cm^2; ΔVi = 305 mV,
△:δ = 2.03 x 10^{14} molec/cm^2; ΔVi = 330 mV.

As shown in Figs. 3 and 4, for both polysaccharide derivatives, the $\Delta(\Delta V)$ values decrease with the increase in the surface density of spread lipid molecules and become negative at higher surface coverages. However, it is significant to note that at lipid surface concentration $\delta = 1.015 \times 10^{14}$ molecules/cm^2, which is within the range ($1\text{-}1.4 \times 10^{14}$ molecules/cm^2) of lipid content in liposomal membranes,[16] the $\Delta(\Delta V)$ values for CHA are negative except for low solution concentrations between 10^{-7} and 10^{-8}M. At the same lipid surface coverage and in the whole range of studied concentrations the $\Delta(\Delta V)$ values for CHP are positive. This would mean that at 10^{14} molecules/cm^2 the surface potential of egg PC is compensated by CHA to a larger extent than by CHP. The observed effect which results from the differences in molecular areas and surface properties between CHA and CHP, has most probably an incidence on a more stable configuration of the CHP/egg PC relative to the CHA/egg PC membranes.

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