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Characterization of Dimyristoylphosphatidylcholine Liposome Aggregates Induced by Dextran Sulfate and La^{3+} by Fluorescence Spectroscopy

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The addition of dextran sulfate (DS) to DMPC vesicles in the presence of di- and trivalent cations leads to a strong aggregation, resulting in a stack-like arrangement of the opposing membrane surfaces as shown by freeze–fracture electron microscopy. The strong aggregation is connected with a lipid mixing process, especially in the presence of La^{3+} (measured by the NBD/Rh assay). The extent of lipid mixing depends on the molecular weight of DS and size of the DMPC vesicles. Additionally, a decrease in the surface dielectric constant of DMPC vesicles [measured by the emission shift of the fluorescent probe, dansylphosphatidyl-ethanolamine (DPE)] was observed. A direct dependence on the molecular weight (MW) of DS exists: the higher their MW, the higher the blue emission shift of the DPE probe. The results are discussed in terms of the theory proposed by Ohki and Arnold, which connects the decrease of the surface dielectric constant with the interaction parameters of phospholipid membranes.

KEY WORDS: Dextran sulfate; liposomes; dymyristoylphosphatidylcholine; La^{3+}; phospholipid mixing.

INTRODUCTION

The influence of anionic polymers on the physicochemical properties of phospholipid membranes has been the subject of several studies in the last years [1–6]. It was shown that glycosaminoglycans (GAG)[4] and the homologous molecule dextran sulfate can bind to the surface of cells [7], phospholipid vesicles [8], lipoproteins [9], and viruses [10]. The changed physiocochemical properties of the aggregates have a strong influence on their function. For instance, the interaction of GAG with LDL leads to a change in the thermotropic phase transition of the cholesteryl ester core of LDL [11]. The phase transition temperature of LDL was shifted to higher temperatures in the presence of GAG and Ca^{2+}.

Fusion processes have been of great interest in the last 20 years. Nevertheless, knowledge about the molecular mechanisms of the interaction is limited. Whereas for ion-induced fusion processes, more data are available, only a few publications dealing with polyanion induced membrane interaction processes exist. For the description of ion-induced fusion processes of PS vesicles, Ohki and Arnold [12] introduced the concept of the change of surface dielectric constant. Whereas also for PEG-induced fusion processes of PS vesicles, this rela-

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[4] Abbreviations used: PC, phosphatidylcholine; DPE, dansylphosphatidyl-ethanolamine; NBD-PE, 1-4-nitrobenzo-2-oxa-1,3-diazole-PE; Rh-PE, lissamine rhodamine B sulfonyl-PE; DS, dextran sulfate; SUV, small unilamellar vesicles; GAG, glycosaminoglycan; DMPC, dimyristoylphosphatidylcholine; PL, phospholipid; MLV, multilamellar vesicles; LUV, large unilamellar vesicles; MW, molecular weight.

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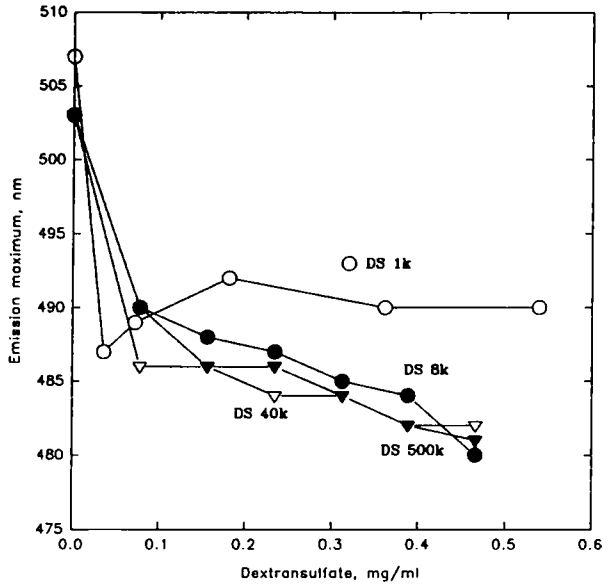

Fig. 1. Shift of the emission wavelength maximum of DPE in DMPC MLV as measured by fluorescence in dependence of 1K, 8K, 40K, and 500K DS in the presence of 4.5 m*M* La^{3+}. 0.08 m*M* DMPC, 10 mol% DPE, 10 m*M* Hepes, 100 m*M* NaCl, pH 7.4, 35°C.

tion is valid, for DS-induced PL mixing of stearylamine-containing PC vesicles [6], no changes in the surface dielectric constant were observed. On the other hand, our present results indicate a decrease in the surface dielectric constant of DMPC vesicles in the presence of di-/trivalent cations and DS.

MATERIALS AND METHODS

Dimyristoylphosphatidylcholine (DMPC) was purchased from Sigma. The purity was checked by thin-layer chromatography. Fluorophore-labeled phospholipids, dansylphosphatidyl-ethanolamine (DPE), 1-4-nitrobenzo-2-oxa-1,3-diazole-PE (NBD-PE), and lissamine rhodamine B sulfonyl-PE (Rh-PE) were obtained from Molecular Probes (USA).

Dextran sulfate (MW 1000) was from Pfeifer & Langen (Germany), dextran sulfate (MW 8000 and 40,000) was obtained from ICN Biochemicals (USA), and dextran sulfate (MW 500,000) was obtained from Pharmacia (Sweden).

Multilamellar DMPC liposomes (MLV) were prepared using the method of Bangham *et al.* [13]. Large unilamellar vesicles (LUV) were prepared by five freeze–thawing cycles of MLV and following extrusion (five times) through 0.1-μm Nucleopore filter membranes using an extruder (Lipex Biomembranes) at 30°C.

Small unilamellar vesicles (SUV) were prepared by sonication of MLV for 40 min in a bath type sonifier. Alternatively, a Branson 250 tip sonifier was used (10 min).

The phospholipid demixing assay (NBD/Rh) was performed according to Ref. 14. The 100% value of fusion was defined as the value obtained by solubilization of the vesicles in 0.2% (v/v) Triton X-100.

The content mixing assay was done according to Ref. 15.

The measurements of the surface dielectric constant were done following the protocols in Refs. 12 and 16.

RESULTS

Using DMPC MLV and LUV, but not with SUV, we measured a shift in the emission wavelength of DPE incorporated in the liposomes after the addition of DS in the presence of La^{3+}. In Fig. 1 the values of the emission wavelength maximum of the DPE probe are given in dependence on the DS concentration. The measurements were carried out at 35°C, because the shift of DPE in the presence of DS and di/trivalent cations is strongly temperature dependent. In the absence of DS and di-/trivalent cations, the emission peak of DPE in DMPC MLV is shifted only slightly in dependence on temperature. We chose 35°C because DSC measurements indicate that this temperature is above the high-temperature transition peak in the presence of DS and di-/trivalent cations. After the addition of DS a shift of the DPE emission wavelength maximum to a lower wavelength can be observed. DS with a molecular weight higher than 8K shift the emission more to a lower wavelength compared to DS with a molecular weight of 1K.

In Fig. 2 the changes in the extent of phospholipid mixing (NBD/Rh assay) of DMPC LUV after the addition of 1K and 8K DS in the presence of 1 m*M* La^{3+} are shown. It can be seen that at DS concentrations of about 0.1 mg/ml, substantial phospholipid mixing occurs (20–30%). DS at 40K and 500K does not induce substantial PL mixing of DMPC LUV in presence of 1 m*M* La^{3+}. It should be noted that using DMPC SUV mixing rates of about 55% for 1K and 8K DS and about 20–25% for 40K and 500K DS in the presence of 1 m*M* La^{3+} were measured.

DISCUSSION

Using freeze-fracture electron microscopy, it was demonstrated that DMPC LUV with a high homogeneity

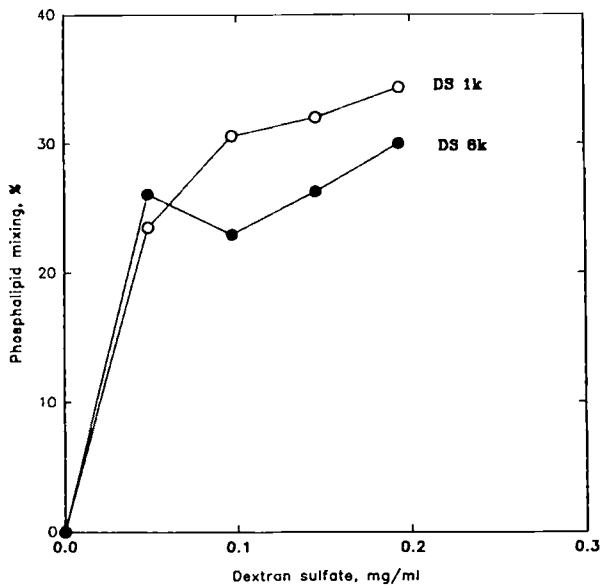

Fig. 2. Phospholipid mixing as measured by the NBD/Rh assay of DMPC LUV induced by 1 m*M* La^{3+} and DS of different molecular weights: 10 m*M* Hepes, 10 m*M* NaCl, pH 7.4, 35°C.

in diameter form large aggregates with long stacked lamellae after the addition of La^{3+} and DS.

As measured by the Stoke's shift of the fluorescent probe DPE, the DMPC/DS/ion complexes exhibit a reduced surface dielectric constant dependent on the molecular weight of DS. The decreased surface dielectric constant is an indication of a reduced water content near the membrane surface.

The induction of PL mixing of PC SUV by Ca^{2+} and 500K DS was described by Arnold *et al.* [4]. These studies were extended to other cations and DS of different MW. The induction of PL mixing between DMPC LUV is in the order $La^{3+}>Ca^{2+}>Mn^{2+}>Mg^{2+}$ at a constant DS concentration (data not shown).

The question whether there is true fusion, i.e., mixing of the aqueous contents of the vesicles, is still unsolved. These problems are currently under investigation. One problem responsible for the failure of the content mixing assay is the low efficiency to entrap fluorescent water-soluble markers such as ANTS/DPX into DMPC LUV.

The fusion of liposomes induced by cationic and anionic polymers, respectively, could have properties in common with Ca^{2+}-induced fusion. The polymers are sandwiched between the bilayers. Therefore, it seems to be clear that a major function of charged polymers in liposome fusion is the promotion of liposome aggregation by neutralization of the surface charge. Further studies are required to elucidate the additional molecular events of membrane destabilization. It is possible that lipid reorganization leads to the fusion or mixing of membranes at points where close contact of phospholipid membranes is mediated by the polymer.

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