Structural effects of PAMAM (G5) dendrimers in multilamellar DPPC-water vesicles: comparative SAXS and FF-TEM studies

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Dendrimers are a relatively new class of polymers. These spherical, highly branched macromolecules are ideal nanocarriers for drugs, because drug molecules can be loaded into the internal cavities between the branches or attached covalently to the surface functional groups. In addition more molecules can be attached to the same carrier for targeting and imaging. Nanoparticles used for drug delivery applications have to be nontoxic and biocompatible, but they also may have to cross barriers in human body, like cell membranes. In view of these aspects it is very important to investigate how these nanoparticles affect cell membranes.

Here, we have investigated the effect of 5th generation polyamidoamine dendrimers (PAMAM G5, Fig. 1), (with ethylenediamine core and 128 terminal amino groups) on multilamellar vesicles (MLVs) formed from dipalmitoylphosphocholine (DPPC) and water.

Figure 1: Structure of the PAMAM (G5) dendrimer (diameter approx. 4.5 nm)

DPPC/water vesicles (or liposomes) are conventional models of cell membranes because of their similar lipid bilayers. From the structural changes occurred in the system on addition of the guest molecule, one can derive the features of interactions between dendrimers and lipids. Depending on temperature, fully hydrated DPPC vesicles can assume three different – and biologically relevant – multilamellar structures denoted as the gel (Lβ'), the rippled-gel (Pβ') and the liquid crystalline (Lα) phases with 6.4, 7.05 and 6.7 nm characteristic periodicities, respectively. It has been reported that hydrophilic dendrimers can be located near the head groups, while hydrophobic ones interact with the alkyl chains of the lipids and may cause loss of integrity in the membrane [1].

A mixture of PAMAM dendrimer and DPPC was prepared at 10⁻² molar ratio, and the final lipid/water ratio was 20 wt%. According to the thermotropic behavior of the fully hydrated DPPC MLVs, the measurements were carried out at 25, 38 and 46°C, corresponding to the three above mentioned characteristic phases.

SAXS measurements were performed at the synchrotron beamline B1 of the storage-ring DORIS III in HASYLAB/DESY, Hamburg, Germany. The beam used was point-collimated (1×0.7 mm) and monochromatized with a Si (311) double crystal monochromator. Correction for instrumental effects and calibrating into absolute intensity units, as well as producing radial scattering curves from the corrected scattering images was done using the standard Matlab software package of the beamline.

Freeze-fracture TEM measurements were used for direct visualization of the evolved structures. The gold sample holders used in freeze-fracture were preincubated at 24°C, the same temperatures as the samples. Droplets of 1-2 μL of the sample were pipetted onto a gold sample holder and were frozen by plunging the holder immediately into partially solidified Freon for 20 seconds and were then stored in liquid nitrogen.
Fracturing was performed at -100°C in a Balzers freeze-fracture device (Balzers BAF 400D, Balzers AG, Vaduz, Liechtenstein). Replicas of the fractured faces etched at -100°C were made by platinum-carbon shadowing then cleaned with a water solution of surfactant and washed with distilled water. The replicas were placed on 200 mesh copper grids and examined in a MORGAGNI 268D TEM apparatus.

Here we show that the presence of G5 dendrimer caused drastic changes in the characteristic layer structure of DPPC/water system (Figure 2, left side). The SAXS patterns differ significantly from the known pattern of the pure DPPC MLVs. At 25°C the positions of Bragg reflections of the system do not follow the equidistant sequence characteristic for a periodical stacks of lamellae \( q_n = \frac{2\pi n}{d} \) where \( n \) is an integer, \( d \) is the periodicity, \( q = \frac{4\pi \sin(\theta)}{\lambda} \) with \( \lambda \) denoting the X-ray wavelength, \( 2\theta \) the scattering angle, but the normalized peak positions are 1, \( \sqrt{4} \), \( \sqrt{8} \), \( \sqrt{12} \), indicating a nonlamellar structure. It is difficult to distinguish between the hexagonal and cubic phases as the diffraction peaks are diffuse. Taking into account the morphology revealed by means of transmission electron microscopy (TEM) combined with freeze-fracture methodology we have determined the appearance of cubic phase. Closely packed coarse grains consisting of also closely packed smaller, isotropic grains as structural units appeared in TEM micrographs (Figure 2, right side). The characteristic size of these coarse structural units was found to lie between 12-14 nm, in agreement with the SAXS data.

![SAXS patterns](image)

**Figure 2:** characteristic SAXS patterns of DPPC/PAMAM/water systems (left) and the characteristic surface morphology of lamellar (top right) and cubic phases (bottom right).

At 38 °C the patterns are very similar to that measured at 25 °C, but the reflections are more diffuse. Surprisingly, in the temperature domain of the liquid crystalline phase (46 °C) the pattern provides equidistant Bragg reflections in four orders with an unusually high periodic distance (13.4 nm). Presumably, this increased period is the consequence of dendrimers embedded between lipid bilayers which results in an enlargement from 6.5 nm to 13.4 nm.

These findings demonstrated that the G5 dendrimer can enter into the membranes inducing non-bilayer type local structures, the self-assembly being guided by the chemical characters of the local membrane constituents.

**References**