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Influence of nanoparticle–membrane electrostatic interactions on membrane fluidity and bending elasticity

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ABSTRACT

The aim of this work is to investigate the effect of electrostatic interactions between the nanoparticles and the membrane lipids on altering the physical properties of the liposomal membrane such as fluidity and bending elasticity. For this purpose, we have used nanoparticles and lipids with different surface charges. Positively charged iron oxide (γ-Fe_2O_3) nanoparticles, neutral and negatively charged cobalt ferrite ($CoFe_2O_4$) nanoparticles were encapsulated in neutral lipid 1-stearoyl-2-oleoyl-sn-glycero-3-phosphocholine and negatively charged 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-L-serine lipid mixture. Membrane fluidity was assessed through the anisotropy measurements using the fluorescent probe 1,6-diphenyl-1,3,5-hexatriene. Though the interaction of both the types of nanoparticles reduced the membrane fluidity, the results were more pronounced in the negatively charged liposomes encapsulated with positively charged iron oxide nanoparticles due to strong electrostatic attractions. X-ray photoelectron spectroscopy results also confirmed the presence of significant quantity of positively charged iron oxide nanoparticles in negatively charged liposomes. Through thermally induced shape fluctuation measurements of the giant liposomes, a considerable reduction in the bending elasticity modulus was observed for cobalt ferrite nanoparticles. The experimental results were supported by the simulation studies using modified Langevin–Poisson–Boltzmann model.

1. Introduction

Liposomes with the potential to encapsulate the nanoparticles (NPs) find extensive applications in biomedical and pharmaceutical fields due to their ability to carry huge payload, improved stability, targeted delivery of the encapsulated material and to minimize the NPs toxicity (Torchilin, 2005; Uhumwangho and Okorr, 2005). For instance, liposomes encapsulated with ultrasmall superparamagnetic iron oxide NPs are gaining popularity in magnetic resonance imaging of cancer cells, diagnosis as well as treatment (Weinstein et al., 2010; Laurent and Mahmoudi, 2011). Since the NPs usage for *in vivo* applications are gradually increasing, it is crucial to investigate the various modes of NPs interaction with the bilayer lipids. Depending on the NPs concentration, their interaction with the bilayer lipids may alter the membrane properties and their functions (Albanese et al., 2012).

High density encapsulation of NPs in the liposomes is preferred in many clinical applications such as drug delivery and hyperthermia to achieve better results. Electrostatic interactions based on the surface charge of the liposomes and NPs plays an important role in determining the efficiency of NPs encapsulation in liposomes (Sipai Altaf Bhai et al., 2012). The surface charge of the liposomes can be altered by varying the lipid composition, pH and the external environment during liposome preparation. When charged lipids are used to prepare liposomes, they are more stable due to electrostatic repulsions which can prevent the aggregation and fusion of

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neighbouring vesicles (Sybachin et al., 2012). Frokjaer et al. (1982) has demonstrated increased stability of phosphatidylcholine liposomes by decreasing the ionic strength and increasing the surface charge. The surface charge of the NPs can be modified by changing the functional groups attached to the NPs surface or by changing the coating material (Jiang et al., 2009). The NPs are coated with different materials such as polyethylene glycol, citric acid and dextran to increase their stability. The nature of the coating material greatly influences the zeta potential value of NPs. High zeta potential values of the NPs are a key factor to stabilize them in suspensions and to reduce their cytotoxicity (Wu et al., 2008).

To study the effect of surface charges and electrostatic interactions in detail, we have used a neutral lipid 1-stearoyl-2-oleoyl-sn-glycero-3-phosphocholine (SOPC) and its mixture with negatively charged 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-L-serine (POPS) to prepare the liposomes. Similarly we have used NPs with varying surface charges by adding different functional groups to their surface. Neutral and negatively charged $CoFe_2O_4$ NPs and positively charged γ-Fe_2O_3 NPs were encapsulated in both the type of liposomes. We have chosen magnetic NPs such as iron oxide and cobalt ferrite NPs for our work as they have vast expanding applications such as magnetofection, cell labeling, immunoassays and cancer therapy (Akbarzadeh et al., 2012). By the application of an external magnetic field, they can be easily manipulated to reach the target region for diagnosis, drug delivery and treatment (Santhosh and Ulrih, 2013).

The interaction of NPs with the phospholipid membrane affects the fluidity which can be studied through the anisotropy measurements using the fluorescent probes such as 1,6-diphenyl-1,3,5 hexatriene (DPH) (Hianik and Passechnik, 1995). Bilayer fluidity is related to the viscosity of the lipid membrane which is an important feature of the cell membrane. Increased fluidity enhances the free movement of phospholipid molecules and protein moieties in the membrane to facilitate various biological functions like ion transport, cell signaling and cell growth (Park et al., 2005). The fluidity of the bilayer/membrane can be affected by the membrane bound or encapsulated NPs (Roiter et al., 2008). Similarly the NPs adsorbed to the membrane surface or entrapped in the bilayer have a significant effect on the elastic properties of the membrane which is essential for the cell to perform the fundamental functions like adhesion, migration and interaction (Lai et al., 2013). Hence in order to better understand the influence of NPs in membranes, it is necessary to study the various effects of NPs interactions on cell membranes and model membranes.

Recent studies on the effects of NPs on membrane stability have revealed that the incorporation of metal NPs within the membrane alters the phase behavior of the lipids by decreasing the phase transition temperature and increasing the bilayer fluidity (Bhandary et al., 2011). Since the polymorphic phase behavior of lipids influence different membrane related processes, it has become important to study the effect of NPs interaction with different lipid membranes. Enormous research has been carried out with homogeneous bilayers consisting of zwitterionic phospholipids, but very less work has been done to understand the electrostatic attraction between the negatively charged lipid bilayers and differently charged NPs and its influence on the physical and chemical properties of membranes. Therefore the aim of our work is to study these interactions in detail through simulation studies and to establish a correlation between the theoretical calculations and the experimental results.

2. Materials and methods

SOPC and POPS lipids were purchased from Avanti Polar Lipids Inc., USA. DPH and HEPES [4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid] were obtained from Sigma Aldrich Chemie GmbH, Steinheim, Germany. All the chemicals obtained have high purity (>99%) and used without any further purification. Negatively charged lipid vesicles were prepared by mixing SOPC and POPS lipids in the molar ratio of 4:1, respectively. Iron oxide and cobalt ferrite NPs were purchased from Nanotesla Institute, Ljubljana, Slovenia.

2.1. Synthesis of magnetic nanoparticles

Co-precipitation method was used to synthesize both the magnetic iron oxide and cobalt ferrite NPs. An aqueous mixture of ferric, ferrous salts and sodium hydroxide was prepared as an alkali stock solution (Wu et al., 2008; Mahnaz et al., 2013). The corresponding metal hydroxides were precipitated during the reaction between the alkaline precipitating reagent and the mixture of metal salts and subsequently oxidized in air to form γ-Fe_2O_3. The iron oxide NPs were coated with silica to ensure stability (Bumb et al., 2008) and attached with amino [NH_3^+] groups on their surface to impart positive charge. The dried iron oxide NPs were then dispersed in 20 mM HEPES buffer. The NPs were characterized using X-ray diffractometry and transmission electron microscopy (TEM) (see Fig. 2). The size of the synthesized γ-Fe_2O_3 NPs were found to be 10 ± 2 nm by TEM analysis and their zeta potential was found to be +40 mV using dynamic light scattering (DLS).

Aqueous $Fe(NO_3)_6 \cdot 9H_2O$ and $Co(NO_3)_2 \cdot 6H_2O$ solutions were mixed in stoichiometric ratios and served as precursors for the synthesis of cobalt ferrite NPs. To get the micelle solution, sodium dodecile sulfate was added and the resulting mixture was heated between 60 and 90 °C. To this mixture 10% NaOH solution was added to the mixture to set the pH between 9.5 and 11 and the synthesis temperature was maintained between 70 and 95 °C for about 4–5 h with continuous magnetic stirring. The mixture was centrifuged at a speed of 3000 rpm for 15 min. The supernatant was discarded and the remaining sample was centrifuged rapidly till a black precipitate was obtained. The precipitate was purified by washing thoroughly with water and acetone and dried in hot air oven at 100 °C for 2 h. The dried cobalt ferrite NPs were then dispersed in 20 mM HEPES buffer. To impart negative charge to their surface, the cobalt ferrite NPs were coated with citric acid. The size of the neutral and negatively charged cobalt ferrite NPs were found to be in the size range of 10–15 nm using TEM and their zeta potential values were found to be ± 34 mV and -40 mV for neutral and negatively charged cobalt ferrite NPs respectively using DLS instrument.

2.2. Preparation of nanoparticles encapsulated liposomes

Small unilamellar vesicles in the size range of 30–50 nm, encapsulated with iron oxide and cobalt ferrite NPs were prepared using thin film method. The lipids SOPC and SOPC–POPS mixture were dissolved in sufficient quantity of chloroform and then transferred into round bottomed flasks (Bangham et al., 1967). The organic solvent was evaporated completely under reduced pressure (1.7 kPa) using rotavapor to form a thin lipid film. The thin film was hydrated with a suspension of positively charged iron oxide, neutral and negatively charged cobalt ferrite NPs (1 mg/ml) suspended in 20 mM HEPES buffer at pH 7.0. The lipid suspension was then vortexed vigorously with glass beads for 10 min to prepare multilamellar vesicles (MLVs) and sonicated using a vibracell ultrasonic disintegrator VCX 750 (Sonics and Materials, Newtown, USA) with 50% amplitude and 10 s on-off cycles for 30 min to obtain small unilamellar vesicles (SUVs). The sample was then centrifuged at a speed of 14,000 rpm for 10 min to separate the debris formed after sonication (Eppendorf Centrifuge 5415C). The control SUVs were also prepared in a similar method but instead of NP suspension they were diluted with 1 mL of 20 mM HEPES buffer.