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Modulation of membrane properties by silver nanoparticles probed by curcumin embedded in 1,2-Dimyristoyl-sn-glycero-3-phosphocholine liposomes

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ABSTRACT

Development of nanomaterials has drawn interest on silver nanoparticles (AgNPs), which are being incorporated in several biomedical and environmental applications, especially anti-bacterial properties of AgNPs has intense excitement for their commercial use. However, the impact of AgNPs on cell membranes, such as phospholipid membrane properties, is not clearly understood yet. By applying curcumin as a probe molecule, this work was done for the first time to investigate the effect of AgNPs on membrane properties, such as permeability and phase transition temperature using 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) liposomes as a model for phospholipid membranes. We concluded that AgNPs at low concentration decrease the partition of curcumin into DMPC liposomes by ~4-fold. In the presence of AgNPs, curcumin was found to be located close to the stern layer of DMPC liposomes by using a hydrophobic quencher, cetylpyridinium bromide (CPB). In addition, AgNPs broadened the phase transition temperature of DMPC liposomes, which ranged from 20 °C to 35 °C.

1. Introduction

Metallic nanoparticles have shown to exhibit unique physical and chemical properties, and therefore have been incorporated in several biological applications, such as, in anti-bacterial and antifungal agents, drug targeting and delivery, wound dressing, and disinfectants [1]. In addition, silver nanoparticles (AgNPs) are commonly used because of their distinctive properties like chemical stability, catalytic activity, enhanced conductivity, and optical functions [1]. Methods for synthesizing AgNPs can be categorized as chemical, physical and biological process. Green synthetic routes to synthesize AgNPs are emerging for various biomedical and other applications [2]. These routes help in minimizing the use of hazardous substances, therefore, reduce the formation of toxic products. To be considered as a green chemistry route, the used method should be evaluated based on the selection of solvent medium, eco-friendly reducing agents, and nontoxic stabilizing/capping agents. Due to their unique electrical, magnetic and size-dependent optical properties, AgNPs have been currently used in antimicrobial agents, water treatment, textile engineering, and silver-based consumer products [1]. The wide and increasing use of AgNPs in biomedical and environmental applications necessitate understanding the effect of AgNPs on human health, particularly their toxicity. How toxicity can be linked with membrane properties is not well known. At the same time, impact of AgNPs on membrane and liposome properties is not yet clearly understood.

Liposomes are synthetic vesicles prepared to resemble the structure of a cell membrane. They are made up mainly of phospholipids, which are molecules having a hydrophilic head and a hydrophobic tail [3]. Bangham and Horne were the first to show that liposomes are spontaneously formed when phospholipids are dispersed in an aqueous medium [4]. Liposomes are shown to be promising systems for drug delivery due to their size, amphipathic property and biocompatibility. Gregoriadis et al. were the first to use liposomes to deliver bioactive substances [5]. Liposomes serve as delivery systems for anticancer drugs, antifungal drugs, vaccines, and other therapeutic agents [3]. Liposomes have the ability to entrap hydrophilic molecules in their aqueous compartment and lipophilic molecules into their membrane. Liposomes can also help in reducing drug toxicity and improving its stability and bioavailability. In addition, liposomes possess tissue-targeting properties and maintain the drug intact while delivering it to the site of action [3]. New materials loaded on membrane are also being prepared to enhance membrane properties for various applications

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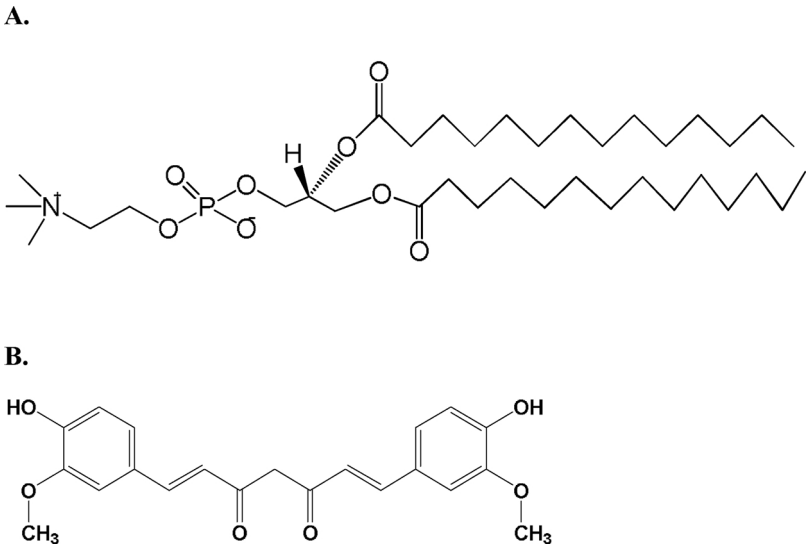

Fig. 1. (A) Chemical structure of 1,2-dimyristoyl-sn-glycero-3-phosphocholine; **(B)** Chemical structure of curcumin.

[6,7]. 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) is one of the vesicles forming phospholipids found in various biological systems. DMPC is a di-saturated phospholipid made up of two myristic acids, as shown in Fig. 1A. It is used in research as a model to study liposomes, lipid bilayers and biological membranes [8]. To our knowledge, little work has been done to understand the interaction of AgNPs with DMPC liposomes, which is the goal of the present study.

Curcumin, shown in Fig. 1B, fluoresces in solution and its photophysical properties depend greatly on the polarity of the environment and the pH of the medium [9,10]. This profile of curcumin has triggered to develop curcumin based fluorescence probing and sensing methods to avoid toxic chemicals like pyrene and metal nanoparticles, which are commonly used in fluorescence probing methods. Our group has successfully established curcumin as a molecular probe to investigate liposome properties [11,12]. In this study, we have applied curcumin as a molecular probe to investigate the interaction of AgNPs with DMPC liposomes and the subsequent effect of liposome properties by monitoring the fluorescence of curcumin itself. This is the first study that applies curcumin as a probe to study the interaction between AgNPs and a phospholipid membrane and therefore brings new insight on the modulation of DMPC liposome properties induced by AgNPs. The phase transition temperature of DMPC liposomes is influenced by AgNPs and it broadens with the increase in percentage of AgNPs in DMPC liposomes. The study proves that association of AgNPs with DMPC liposomes decreases the partitioning of curcumin into the membrane by modulating the permeability and fluidity of the liposomes, therefore opening the possibility of using such system for drug delivery.

2. Materials and methods

2.1. Materials

DMPC was obtained from Avanti Polar Lipids. Curcumin and cetylpyridinium bromide (CPB) were obtained from Sigma-Aldrich. Silver nitrate, used to prepare AgNPs, was also obtained from Sigma-Aldrich. The solvents chloroform and methanol used to prepare liposomes were obtained from SIAL. To increase its solubility, the stock solution of curcumin was prepared using methanol.

2.2. Preparation of curcumin conjugated AgNPs

Silver nanoparticles were synthesized by dissolving curcumin in double distilled water at 90 °C. Silver nitrate was then added while stirring at 90 °C. The solution was stirred for around an hour until its color changed from yellow to olive green. Finally, the solution was centrifuged at 20,000 rpm to sediment the prepared AgNPs. The AgNPs pellets were then dissolved in double distilled water and stored at 4 °C. The size range of the prepared AgNPs was determined using scanning electron microscopy (SEM).

2.3. Preparation of DMPC liposomes

DMPC liposomes were prepared using the lipid film hydration method [11,13]. DMPC phospholipids were dissolved in a mixture of chloroform/methanol (1:1 vol ratio). Using a rotary evaporator, the solvent mixture was evaporated at 60 °C, forming a lipid film. Adding 3 mL of double distilled water formed liposomes at a concentration of 5 mM. The mixture was then vigorously vortexed and heated at 35 °C, which was 10 °C above the phase transition temperature of DMPC, until the lipid film was hydrated and formed a homogeneous solution. The obtained liposomes were multilamellar vesicles (MLVs). Probe molecule, curcumin, used in this study is hydrophobic and MLVs are ideally suited to encapsulate hydrophobic molecule [14]

2.4. Sample preparation

Samples for each of the mentioned studies were prepared in the absence of AgNPs and in their presence at different concentrations (5, 15, and 20 pM). Since the contribution of curcumin to the absorbance of curcumin conjugated Ag NPs is negligible and within the error margin (as concluded from fluorescence signal), the concentration of AgNPs was estimated using reported extinction coefficient of AgNPs. All fluorescence measurements were done at an excitation wavelength of 425 nm and emission wavelength range of 440–650 nm. To study the effect of AgNPs on curcumin, samples were prepared at different concentrations of AgNPs whereas the concentrations of curcumin and DMPC were fixed at 9 μM and 100 μM respectively. The same experiment was repeated at different concentrations of DMPC. For partition coefficient studies, samples were prepared with different DMPC concentrations, 9 μM curcumin, and different concentrations of AgNPs. For quenching studies, samples were prepared with different concentrations of CPB, 15 μM curcumin, 100 μM DMPC and different concentrations of AgNPs. For phase transition studies, a sample of 3 μM curcumin and 100 μM DMPC was prepared. Phase transition was studied in the absence and presence of AgNPs by measuring fluorescence at different temperatures.