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Probing molecular interactions of poly(styrene-*co*-maleic acid) with lipid matrix models to interpret the therapeutic potential of the co-polymer

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ABSTRACT

To understand and maximize the therapeutic potential of poly(styrene-*co*-maleic acid) (SMA), a synthetic, pharmacologically-active co-polymer, its effect on conformation, phase behavior and stability of lipid matrix models of cell membranes were investigated. The modes of interaction between SMA and lipid molecules were also studied. While, attenuated total reflection-Fourier-transform infrared (ATR-FTIR) and static ^{31}P nuclear magnetic resonance (NMR) experiments detected SMA-induced conformational changes in the headgroup region, differential scanning calorimetry (DSC) studies revealed thermotropic phase behavior changes of the membranes. ^{1}H NMR results indicated weak immobilization of SMA within the bilayers. Molecular interpretation of the results indicated the role of hydrogen-bond formation and hydrophobic forces between SMA and zwitterionic phospholipid bilayers. The extent of membrane fluidization and generation of isotropic phases were affected by the surface charge of the liposomes, and hence suggested the role of electrostatic interactions between SMA and charged lipid headgroups. SMA was thus found to directly affect the structural integrity of model membranes.

1. Introduction

Synthetic polymers are currently generating increased interest as therapeutic agents owing to their enhanced pharmacokinetic profiles, improved efficacy and better physicochemical stability relative to small molecule drugs. The high-molecular weight nature coupled with the opportunity for polyvalent binding interactions has made polymers interesting candidates for novel chemical entities for therapeutic applications in which low-molecular weight drugs have either failed or exhibited inadequate benefits [1]. Though these polymers are presently being extensively used in the formulation of small molecule drugs, primarily as carriers or sustained release devices, they can also be used as therapeutics on their own [2].

Poly(styrene-*co*-maleic acid) (SMA) is a synthetic co-polymer with attractive chemical [3] and biological properties. The potential of SMA as a polymeric drug has already been exploited with the clinical success of SMANCS, a conjugate of immunostimulatory styrene maleic acid co-polymer with the potent yet toxic anti-tumor polypeptide neocarzinostatin (NCS), in liver and lung cancer [4–6]. Recent revelation of the potential of SMA and its derivatives in effectively inhibiting human immunodeficiency virus type 1 (HIV-1) [7,8], by preventing virus adsorption on the surface of target cell membranes, has generated considerable interest.

SMA co-polymers have also been reported to be strong inhibitors of spermatozoa motility [9]. The spermicidal activity of the co-polymer has been attributed to the presence of carboxylic groups, which induces a low-pH environment responsible for killing spermatozoa.

Our laboratory has been actively involved in researching the therapeutic applicability of styrene maleic acid/anhydride co-polymers in human sexually transmitted disease prevention and fertility control, with a special attention toward genital HIV-1 infections [10]. A new male non-hormonal contraceptive RISUG (an acronym for Reversible Inhibition of Sperm Under Guidance), with styrene maleic acid/anhydride co-polymers as active pharmaceutical ingredients, has emerged from our laboratory and is currently undergoing extended Phase III clinical trials throughout India.

The activity of the pharmacologically-active styrene maleic acid co-polymer for the treatment of a wide spectrum of human diseases is noteworthy. However, to utilize the therapeutic potential of this co-polymer to the fullest, a detailed understanding of the mechanism by which SMA molecules interact with cell membranes at the molecular level is of critical importance, especially when one considers the efficacy and safety of the polymer therapeutic, since interaction with exogenous amphiphilic SMA can directly affect the structural integrity of the cell [11]. It has already been reported that the hydrophobic regions of SMA play an important role in the penetration of the lipid bilayer of cell membranes, and the anionic portion of the co-polymer facilitates the internalization and binding of the drug-polymer conjugate with the hydrophobic milieu of the cell [12]. SMA is also known to induce surface charge imbalance on human spermatozoa membrane, which leads to the release of acrosomal enzymes hyaluronidase and acrosin. This

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ultimately leads to spermatozoa damage [13,14]. It therefore becomes imperative to elucidate the influence of SMA on lipid matrix models of cell membranes in order to improve the therapeutic potential of the co-polymer by enhancing biological activity and diminishing side effects.

This study aims to characterize the conformational changes in the SMA-doped liposomes and dry lipid/co-polymer films, detect the changes induced by the co-polymer on the phase behavior and stability of model membranes and discern the nature of intermolecular interactions at work between SMA and lipid molecules.

We have investigated the interactions of SMA with different sets of dry lipid films and multilamellar vesicles, composed of zwitterionic phospholipid distearoylphosphatidylcholine with cholesterol and charged lipids added to it, to mimic various types of cell membranes differing in composition and surface charge. ATR-FTIR studies were performed to detect the conformational changes induced by the co-polymer in the lipid acyl chain region as well as in the headgroup and interfacial regions of the lipid molecules. DSC, a thermal analytical technique, was used to study the thermotropic phase behavior of liposomes from which the molecular interactions between the co-polymer and phospholipids were quantitatively probed. ^{31}P NMR experiments were carried out to characterize the effect of the co-polymer on the structure and dynamics of multilamellar vesicles and to get a more direct indication of the effects induced by SMA in the headgroup region of the vesicles. Proton NMR spectroscopy was used to probe the existence and nature of interactions between the amphiphilic co-polymer and phospholipid molecules.

2. Materials and methods

2.1. Materials

1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), dimethyldioctadecylammonium bromide (DODAB), dicetylphosphate (DCP) and cholesterol (CHOL) (Fig. 1A–D) were purchased from Sigma Chemicals Co., USA. 4-2-hydroxyethyl-1-piperazineethanesulfonic acid (HEPES) was obtained from Sisco Research Laboratories Pvt. Ltd., India. Deuterium oxide (D_2O) and deuterated methanol (CD_3OD) were bought from Cambridge Isotope Laboratories Inc., USA. HPLC grade chloroform and methanol and analytical grade sodium chloride were purchased from Merck, India. All chemicals were used as obtained without further purification. Milli-Q water obtained from Milli-Q Integral 3 system (Millipore, France) was used for all experiments.

2.2. Methods

2.2.1. Synthesis of poly(styrene-co-maleic acid)

Styrene maleic acid co-polymer (Fig. 1E) was prepared according to the method described in the United States patent number 5488075 [15]. Briefly, maleic anhydride (MAn) and styrene (St) monomers in 1:1 (w/v) ratio were taken in glass bottles to which ethyl acetate was added and dry nitrogen gas purged. Polymerization was achieved by gamma irradiation at 37 °C, using a dose rate of 0.3 Gy/s and a total dosage of 2.4 Gy. The co-polymer was precipitated with petroleum ether and soxhlet distilled using 1,2-dichloro ethane and Milli-Q water respectively. Styrene maleic anhydride co-polymer obtained after careful purification from unreacted monomers was subjected to base catalyzed hydrolysis by refluxing with sodium hydroxide solution. The co-polymer was then recovered by acid precipitation using hydrochloric acid. The obtained SMA was washed several times with acidified Milli-Q water to remove any sodium salts present and then dried and used for further experiments. The weight-average molecular weight (M_w = 850,000 Da) of the hydrolysed co-polymer was determined by gel permeation chromatography at 35 °C (Viscotek, Malvern, USA).

2.2.2. Preparation of multilamellar vesicles (MLVs)

Multilamellar vesicles were prepared according to the lipid film hydration method [16] with slight modifications. Briefly, required

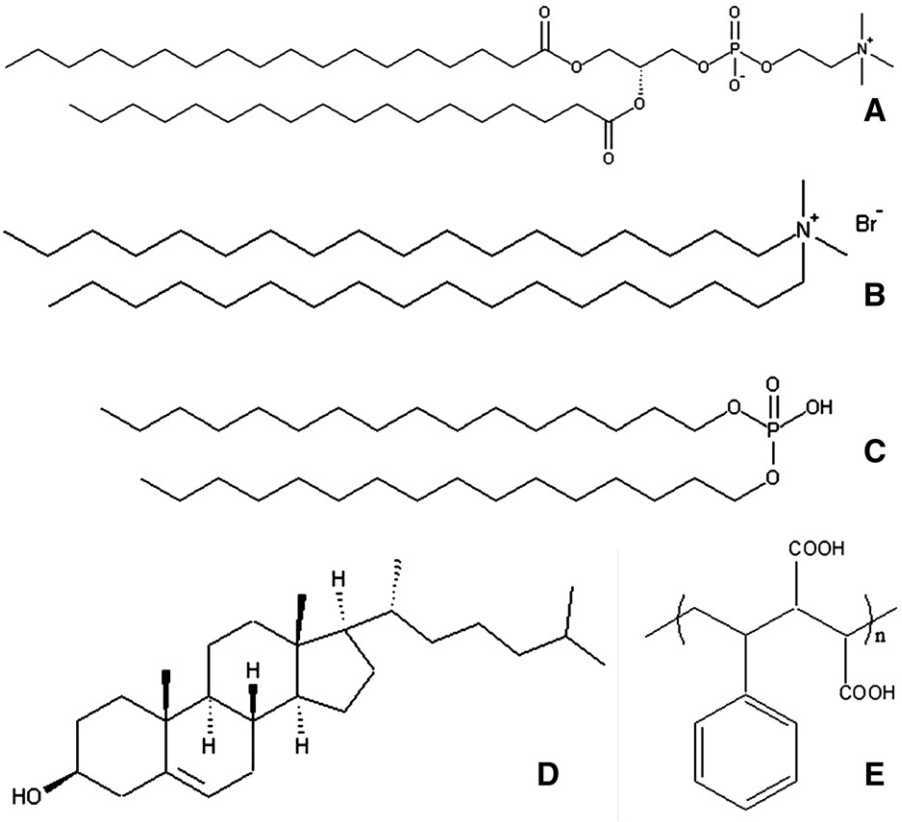

Fig. 1. Chemical structures of A, 1, 2-distearoyl-sn-glycero-3-phosphocholine (DSPC); B, dimethyldioctadecylammonium bromide (DODAB); C, dicetylphosphate (DCP); D, cholesterol (CHOL) and E, styrene maleic acid (SMA) co-polymer.