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Characteristic responses of a phospholipid molecular layer to polyols

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ABSTRACT

Polyols (sugar alcohols) are widely used in foods, pharmaceutical formulations and cosmetics, and therefore it is important to understand their effects on cell membranes and skin. To address this issue, we examined the effect of polyols (1,2-ethanediol (ethylene glycol), 1,3-butanediol, 1,2,3-propanetriol (glycerol), and 1,2,3,4-butanetetraol) on artificial membrane systems (liposomes, monolayers, or dry films) prepared from phospholipid (1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC)). 1,2-Ethanediol and 1,3-butanediol had little effect on the size of the DMPC liposomes or the surface pressure (π)–surface area (A) isotherm of DMPC monolayers at an air–water interface, whereas 1,2,3-propanetriol or 1,2,3,4-butanetetraol increased both liposome size and surface pressure. Attenuated total reflection Fourier transform infrared spectroscopy (ATR FT-IR) and differential scanning calorimetry (DSC) were used to evaluate the interaction between DMPC and polyols. These experimental results suggest that the chemical structure of polyol plays an important role in the characteristic interaction between polyol and DMPC.

1. Introduction

It is important to understand the effects of polyols or saccharides on cell membranes and skin permeability and stability, because polyols are widely used in foods, pharmaceutical formations and cosmetics to obtain various functionalities, such as protection and preservation [1–7]. On the other hand, artificial membrane systems, such as liposomes, are useful for not only simplifying biomembranes but also for understanding the intrinsic dynamics of a target molecule [8]. There have been various experimental investigations of interactions between saccharides or polyols and lipid molecules [1,2,5,7,9–11], as well as molecular dynamic simulations [12–14]. With regard to studies in living systems, Denda reported that saccharides affect the barrier recovery of hairless mouse skin in different ways, depending on their chemical structure of the saccharide [15]. Since barrier recovery occurs within 1 h, the effect of saccharide is not a genomic effect, but is likely due to direct interaction of saccharide molecules with the cell membrane. In addition, Fluhr et al., reviewed the beneficial effects of glycerol on skin [16]. On the other hand, there have been several reports on the characteristic effects of saccharides on artificial lipid membranes [17–21]. For example, Crowe et al., investigated the interaction between sugars (e.g., trehalose and glycerol) and dipalmitoyl phosphatidylcholine (DPPC) by using infrared spectroscopy and differential scanning calorimetry [1,9,20]. However, the relationship between the chemical structures of monosaccharides and their effects on biological or artificial membranes remains unclear, in part because they exist in two anomeric forms (α and β forms) in solution and contain multiple hydroxyl groups.

In this study, we examined the effects of polyols (1,2-ethanediol (ethylene glycol), 1,3-butanediol, 1,2,3-propanetriol (glycerol), or 1,2,3,4-butanetetraol: see Scheme 1) on the size of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) liposomes and on the surface pressure (π)–surface area (A) isotherm of DMPC monolayers. The nature of the interaction between the phospholipid and polyols was also examined by attenuated total reflection, Fourier transform infrared spectroscopy (ATR FT-IR) and measurement of the phase transition temperature of DMPC by differential scanning calorimetry (DSC). Our experimental results suggest that a phospholipid

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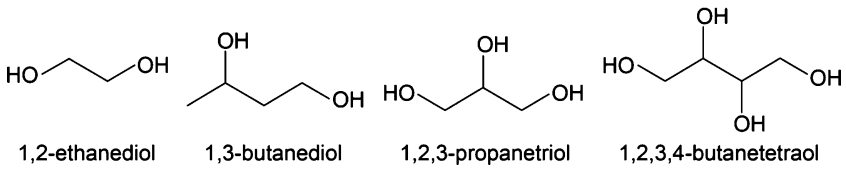

Scheme 1. Chemical structures of polyols.

molecular layer can distinguish the chemical structure of a polyol even in the absence of receptors.

2. Materials and methods

2.1. Materials

1,2-Dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) was purchased from Sigma–Aldrich (St. Louis, MO). 1,2-Ethanediol, 1,2,3-propanetriol, NaH_2PO_4, Na_2HPO_4, methanol, and chloroform were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). 1,3-Butanediol, 1,2,3,4-butanetetraol, dioctadecyl dimethyl ammonium chloride (DODAC), and HEPES were purchased from Wako Pure Chemicals (Osaka, Japan).

2.2. Preparation of liposomes

Liposomes were prepared as described previously [22,23]. A mixture of DMPC and DODAC (DMPC/DODAC molar ratio = 4/1) was dissolved in a chloroform/methanol solution (volume ratio: 2/1). DODAC was added in the liposome system to enhance the responsiveness of liposome to polyols and to reduce the aggregation of liposomes. These liposomes are subsequently referred as DMPC liposomes. The solvent was removed by evaporation in a rotary evaporator for 1 h. The residual lipid film, after being allowed to dry under vacuum for 30 min, was hydrated with 10 mM HEPES buffer solution (pH 7.0, volume: 0.5 mL) containing 1 M polyol under stirring with a vortex mixer for 30 s and left to stand for 1 h [24]. Then, Nile red (final concentration: 0.5 μM) was added to the liposome solution at 313 ± 1 K. A 35 μL aliquot of the liposome solution (concentration of DMPC: 20 mM) on a slide glass was covered with a coverslip, and the sample was observed with a fluorescence microscope (IMT-2, TS Olympus, Tokyo, Japan) equipped with a 100 W mercury lamp, emission filter (BP545), dichroic mirror (DM580, Olympus), and oil immersion objective lens (×100). Imaging data were recorded with a high-sensitivity silicon intensifier target camera (C4742, Hamamatsu Photonics, Hamamatsu, Japan). The size of the liposomes was analyzed with ImageJ 1.49 software.

2.3. Preparation of a dry film

A dry film composed of DMPC and polyol on a slide glass was prepared as follows. A 10 μL aliquot of 1 mM DMPC dissolved in chloroform and 10 μL of 1 μM polyol dissolved in methanol was mixed as a sample solution, i.e., molar ratio of DMPC/polyol (R_m) = 1000, and 10 μL of this sample solution was dropped on a slide glass. The solvents in the sample were removed with a vacuum pump for 30 min to obtain the dry film.

2.4. Preparation of a monolayer

For preparation of a monolayer, DMPC was dissolved in chloroform, and the chloroform solution was dropped on an aqueous phase with a microsyringe (volume: several tens of μL). The amount in the sample dropped on the aqueous surface was 1.62×10^{-8} mol. As the aqueous phase, 0.1 M polyol was dissolved in a phosphate buffer solution (PBS) composed of NaH_2PO_4 and Na_2HPO_4 (pH 7.0, ionic strength: 0.2). Water was first distilled and then purified with a Millipore Milli-Q filtering system (pH of the obtained water 6.3, resistance > 18 MΩ cm).

2.5. Methods

The π–A isotherm was measured with a surface pressure meter (Kyowa Interface Science Co. Ltd., HMB, Saitama, Japan) at 293 ± 1 K. The surface area (A) was decreased from 210 to 40 cm^2 at a rate of about 16.5 $cm^2\ min^{-1}$ (e.g., 16 $Å^2\ molecule^{-1}\ min^{-1}$ for a DMPC monolayer without polyol). Compression of the monolayer was started 5 min after the addition of the chloroform solution to allow time for elimination of chloroform from the aqueous surface by evaporation. At least four examinations were performed to confirm reproducibility.

For ATR FT-IR, spectra were obtained with a FT-IR spectrophotometer (PerkinElmer Spectrum One) equipped with an ATR diamond cell (Universal ATR Sample Accessory) at room temperature. The measurements were performed for solid samples of DMPC with or without polyol, obtained by drying organic solutions (a 50 vol% chloroform and 50 vol% methanol mixture). The resolution of the wavenumber was 2 cm^{-1} and the cumulative number was 100. The amounts of DMPC and polyol added to the ATR cell were ca 3.0×10^{-7} and 1.5×10^{-7} mol, respectively, i.e., $R_m = 2$.

The phase transition temperature of DMPC with or without polyol was measured with a DSC (Thermo Plus DSC8230, Rigaku, Japan) at a scanning rate of 2 K min^{-1}. Solid samples (mass: 5–9 mg) were introduced into the aluminum vessel and measured under a N_2 atmosphere. The amounts of DMPC and polyol added to the vessel were 1.3×10^{-5} and 1.3×10^{-5} mol, respectively, i.e., $R_m = 1$. The phase transition temperature (T_p) was defined as the intersection of the baseline and the line extrapolated along the lower-temperature side of the peak signal in the heating scan.

3. Results

Fig. 1a shows typical photographs of a DMPC liposomes in the presence or absence of 1,3-butanediol or 1,2,3-propanetriol. The size of the liposomes was slightly increased in the presence of 1,2-ethanediol or 1,3-butanediol, but markedly increased in the presence of 1,2,3-propanetriol or 1,2,3,4-butanetetraol (data for 1,2-ethanediol and 1,2,3,4-butanetetraol are shown in Figs. S1 and S2 in the Supplementary Material (SM)). Liposomes were not very sensitive to polyol concentrations below 1 M. To characterize the effect of polyols on liposome size, 200 liposomes were analyzed. Histograms of the diameter (d) of DMPC liposomes in the presence and absence of 1,3-butanediol or 1,2,3-propanetriol are shown in Fig. 1b. The mean diameter was evaluated from the particle size distribution function, $f(d) = 1/(2\pi^{0.5}\sigma d) \times \exp[-(\ln d - \mu)^2/2\sigma]$, where d: liposome diameter, σ: standard deviation, and μ: mean value. The μ values of liposomes in the absence of polyol, and in the presence of 1,3-butanediol and 1,2,3-propanetriol were 0.885, 0.915, and 0.970 with $\sigma = 0.095$, so the mean values of d were 2.43, 2.51, and 2.65 μm, respectively (Fig. 1b-1–3). Liposome size distribution inevitably differed slightly from experiment to experiment due to minor inconsistencies of pipetting, and hydration process and other conditions, and therefore, the distribution of liposomes without