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A comparative study on the effect of Curcumin and Chlorin-p_6 on the diffusion of two organic cations across a negatively charged lipid bilayer probed by second harmonic spectroscopy

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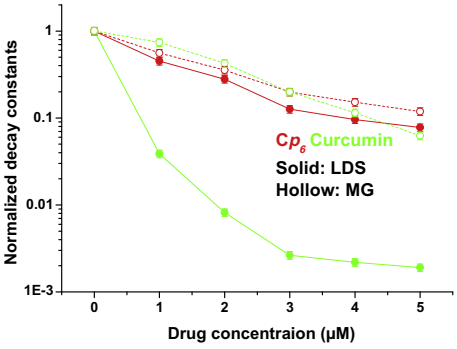
HIGHLIGHTS

- Effect of pH on drug induced membrane permeability investigated by SH spectroscopy.
- Real time diffusion kinetics of two SH probes (LDS and MG) were monitored.
- Curcumin showed substantial pH effect; Chlorin-p_6 showed modest pH effect.
- This was attributed to pH dependent interaction between the drug and the polar head group of the lipid.

GRAPHICAL ABSTRACT

Effect of pH on drug (Curcumin and Chlorin-p_6) induced membrane permeability probed by second harmonic spectroscopy: relative changes in the diffusion time constants of two organic cations (LDS & MG) with increasing drug concentration at pH 5.0 (hollow) and at pH 7.4 (solid).



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ABSTRACT

The influence of Curcumin and Chlorin-p_6 (Cp_6) on the real time diffusion kinetics of two organic cations, LDS (LDS-698) and Malachite Green (MG) across a negatively charged phospholipid bilayer is investigated by Second Harmonic (SH) spectroscopy. The diffusion time constant of LDS at neutral pH in liposomes containing either Curcumin or Cp_6 is significantly reduced, the effect being more pronounced with Curcumin. At acidic pH, the quantum of reduction in the diffusion time constant of MG by both the drugs was observed to be similar. The relative changes in the average diffusion time constants of the cations with increasing drug concentration at pH 5.0 and 7.4 shows a substantial pH effect for Curcumin induced membrane permeability, while a modest pH effect was observed for Cp_6 induced membrane permeability. Based on available evidence this can be attributed to the increased interaction between the drug and the polar head groups of the lipid at pH 7.4 where the drug resides closer to the lipid–water interface.

Introduction

The efficacy of any drug depends strongly upon its interactions with biological membranes. Therefore, studies on drug–lipid interaction are vital to understand and explain the pharmacokinetic properties of drugs. Because of the complexity of the cell

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membrane structure most of these studies are carried out on simplified artificial membrane systems, which mimic the natural bilayer lipid membrane [1]. Drug lipid interactions can alter the main biophysical properties of membranes such as membrane potential, fluidity and permeability [2–15]. These interactions are expected to depend on the chemical structure and hydrophilicity/hydrophobicity of the drug molecule. Second Harmonic (SH) spectroscopy has been used to monitor the real time diffusion of organic ions across a bilayer as this method is surface specific [16–22]. Therefore SH spectroscopy can be used to investigate drug induced changes in lipid bilayer permeability of organic ions. In our earlier studies we have observed that the binding and transport of the amphiphilic photosensitizer Chlorin-p_6 (Cp_6) across an egg lecithin bilayer depend critically on the pH of the medium [22,23]. The protonation–deprotonation process of the three carboxyl groups present in Cp_6 plays a crucial role in the binding and transport of the drug across a bilayer. Recently we have showed that the presence of the lipophilic drug Curcumin in a bilayer markedly reduces the diffusion times of a hemicyanine dye LDS-698 [21]. In this report we have compared the effect of Curcumin and Cp_6 on the diffusion of two organic cations, Malachite Green (MG) and LDS-698 (LDS). Following earlier studies, the pH of the medium was kept acidic (5.0) for MG [17] and neutral (7.4) for LDS [21] to ensure that the cationic forms of these dyes are abundant. This is necessary to facilitate electrostatic interaction between the cationic dye and the negatively charged liposome. It is pertinent to note that while Curcumin is hydrophobic at both pH 5.0 and 7.4, Cp_6 is hydrophobic at pH 5.0 and hydrophilic at pH 7.4. Therefore an additional motivation of the present work is to investigate whether pH plays a role in drug induced changes in lipid bilayer permeability. The chemical structures of the molecules used in this study are provided in Scheme 1.

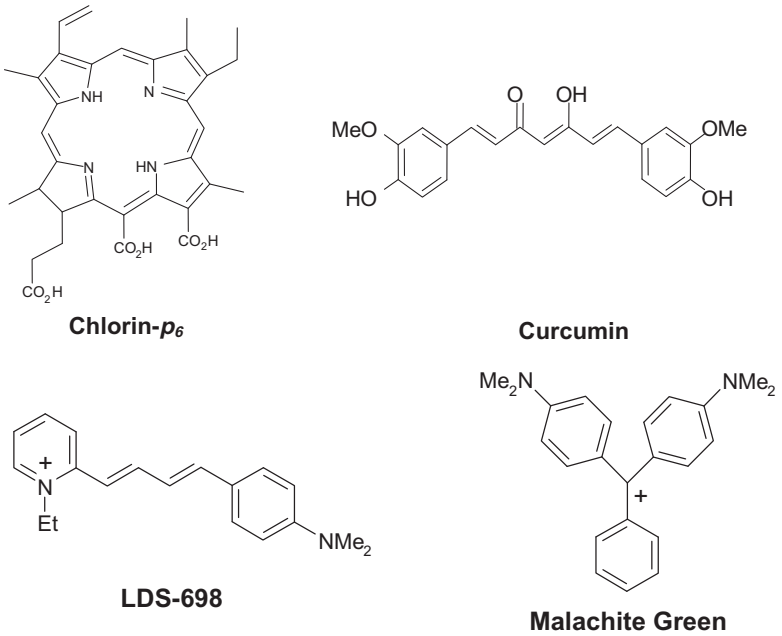
Materials and methods

LDS (from Exciton) was a gift from Prof. N. Sarkar and was used as received. Cp_6, Curcumin and MG were purified before use (see SI). L-α Phosphatidyl DL-Glycerol (POPG) from Sigma were used as received. Unilamellar liposomes (for preparation details, see SI) were suspended in 20 mM phosphate buffer solution having a pH of either 5.0 or 7.4. The size and zeta potential of the liposomes were measured by dynamic light scattering technique at every experimental conditions used in this study (pH and with/without drugs) and these values are provided in supporting information. No significant changes in these parameters (size and zeta potential) were observed, thus ruling out any changes in the size/structure of the liposomes due to the change in pH and/or presence of 4 μM Curcumin and Cp_6.

SH measurements were performed using the 800 nm quasi-CW output of a Ti-Sapphire (Coherent Mira) laser pumped by a green (532 nm, Coherent Verdi 5 W) laser. The average laser power used in the experiments was 600 mW and the pulse width at this wavelength was ~150 fs. The polarization of the laser was fixed in vertical plane by using a quarter wave-plate. The laser beam was focused into the sample by a convex lens having a focal length of 10 cm. The generated SH light was detected using an Edinburgh Instruments LifeSpec single photon counting system. A band pass filter was placed before the monochromator to reject the fundamental. The wavelength resolution of the monochromator was 2 nm. The SH light (at 400 nm) was detected at right angles with respect to the fundamental (800 nm) by a PMT using single photon counting technique. The SH signal were collected every second. The sample in the cuvette was constantly stirred during the measurement using a magnetic stirrer. Sample temperature was controlled by a Neslab circulating water chiller. SH experiments were done as follows: first the signal from 2 mL of buffer containing LDS (pH 7.4) or MG (pH 5.0) or the drugs (at pH 5.0 & 7.4) was recorded followed by addition of 20 μL of liposome solution at 50 s time point. The final concentration of lipid was 20 μM. To investigate the effect of drug–liposome interaction on membrane permeability of MG and LDS micro-liter aliquots from a concentrated stock solution of the drugs were added to the liposome solution and incubated for at least 30 min for attaining equilibrium between the drug and the liposome. The liposomal drug solution (20 μL) was then added to 2 mL buffer solution (final lipid concentration: 20 μM) containing either LDS or MG dye.

The observed SH signal was then fitted exponentially as:

$$I_{SH}(t) = \sum a_i \exp(-t/\tau_i) + A_0$$



Scheme 1. Chemical structures of organic cations Malachite Green (MG) and LDS (LDS-698) and the drugs Chlorin-p_6 (Cp_6) and Curcumin.