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Research paper

Effect of three pluronic polymers on the transport of an organic cation across a POPG bilayer studied by Second Harmonic spectroscopy

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ABSTRACT

Pluronic polymer induced transport of an organic cation across a negatively charged POPG membrane bilayer were studied using interfacial selective Second Harmonic (SH) spectroscopic technique. The length of either hydrophilic (poly-ethylene oxide) or hydrophobic (poly-propylene oxide) unit in the polymer was varied to investigate their effect on membrane transport. Membrane transport was observed to depend critically on the length of the hydrophobic segment present in the polymer. Membrane transport studies using polymers which were either 'incorporated' or 'incubated' with the lipid bilayer suggested that bilayer packing plays a critical role in the insertion of polymers having a long hydrophilic chain.

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1. Introduction

Several types of pluronic polymers are currently being investigated for a wide variety of bio-medical applications such as tumor therapy, chemo-sensitizing agents, drug delivery, DNA transfection, healing of injured cell membranes etc. [1]. These are amphiphilic tri-coblock polymers whose middle section consists of a hydrophobic poly-propylene oxide (PPO) unit and the two ends are hydrophilic poly-ethylene oxide (PEO) units having the general chemical structure: EO_n-PO_m-EO_n. A key prerequisite for such biomedical effects are specific interactions with biological membranes and therefore the interactions between Pluronic polymers and phospholipid membranes have been attracting increasing research interest in recent years [2–4]. Investigations employing various biophysical and microscopic techniques as well as theoretical studies have been performed to characterize the interactions between these polymers and bio-membranes [5–34]. It has been demonstrated by several studies using fluorescence spectroscopic techniques that Pluronic polymers affect membrane organization, especially membrane permeability [2,5–9,12–14,23,24,29]. A common conclusion of all the studies involving the interaction of Pluronic polymers with artificial and natural membranes is that the individual PPO and PEO units of the polymer plays a key role in deciding membrane permeability. The insertion and location of the hydrophobic part (PPO) of the polymer inside a membrane is another topic which has been studied exclusively by experimental [10,11,15,18–22] as well as theoretical [27,28,30–34] techniques.

We report in this work a comparative study on the effect of three different pluronic polymers on the bilayer permeability of an organic cation across negatively charged POPG liposomes using the interfacial selective Second Harmonic (SH) spectroscopic technique. This (SH) spectroscopic technique has the ability to monitor the transport of certain molecules across a model membrane in real time provided the molecule of interest possesses a reasonable hyperpolarizability value at the excitation wavelength [35]. The principle of this technique lies on the fact that the SH field generated from the molecules adsorbed only on the outer surface of a membrane can add coherently to generate a measurable SH signal when the diameter of the membrane are of the order of the excitation wavelength. As the molecules transport from the outer surface to the inner surface of the membrane (membrane thickness ~ 5 nm) the SH field generated from oppositely oriented molecules will cancel out because they are separated by a distance which is much less than the coherence length of this process. Therefore by monitoring the time dependent SH signal from the molecules, which is proportional to the population difference of the molecules adsorbed between the outer and inner surface of the membrane, its transport across the membrane can be monitored in real time. The capability of this technique has first been demonstrated using an organic cation Malachite Green [36] and later with other organic ions [37,38]. In this work we have studied how the transport

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properties of an organic cation (LDS-698: ([2-[4-[4-(dimethylamino)phenyl]-1,3-butadienyl]-1-ethylpyridinium monoperchlorate)) across a L-α Phosphatidyl DL-Glycerol (POPG) bilayer are affected in the presence of three different Pluronic polymers, F-127 (EO_{100} PO_{65} EO_{100}), L-61 (EO_2 PO_{30} EO_2) and F-68 (EO_{76} PO_{30} EO_{76}). The objective of this study was to investigate the role of the PPO and PEO chain lengths on bilayer permeability and how the insertion of the hydrophobic PPO unit of the polymer depends on bilayer mobility.

2. Materials & methods

LDS-698 (from Exciton), POPG (from Avanti) and the three Pluronic polymers: F-127, L-61 and F-68 (from Sigma) were used as received. Spherical liposomes from POPG lipid (details of preparation are provided in SI) were prepared in 10 mM phosphate buffer solution having a pH of 7.4. Their average size and zeta potential was 190 ± 10 nm and −80 ± 10 mV which was measured by a Brookhaven90Plus size and zeta potential analyzer. The liposome polymer complexes were prepared either by adding polymer to the liposome solution (polymer incubated liposomes) or by incorporating the polymer in the liposome. Polymer liposome complexes prepared by the first and second method are referred as 'incubated' and 'incorporated' throughout this work. The latter complex is prepared by adding a buffer solution containing the respective polymers to the dry lipid thin film and then preparing the liposomes. Addition of Pluronic polymers during the preparation of liposomes ensures that the hydrophobic PPO block of the polymer will span the bilayer. A one hour incubation time was given when these polymers were added to pre-formed liposome solution so as to ensure sufficient interaction time between the polymer and liposome [22,24].

Details of the SH experimental setup were similar to our previously published reports and therefore provided in the SI section. For temperature variation experiments, the sample temperature was controlled by a Neslab circulating water chiller having a temperature accuracy of ±1 °C. The electric field of the SH signal ($E_{2\omega}$) were obtained from the observed SH signal ($I_{2\omega}$) as [35]:

$$E_{2\omega}(t) = \sqrt{I_{(2\omega)dye+liposome}(t) - I_{(2\omega)background}} \quad (1)$$

where $I_{(2\omega)dye+liposome}$ (t) is the SH signal detected at 2ω at time t and $I_{(2\omega)background}$ represents the contributions from the SH signal generated by the buffer solution alone. The $E_{2\omega}$ field of LDS cation, before addition of the liposomes originates due to hyper-Rayleigh scattering which is confirmed in a previous study [38].

3. Results

The various physico-chemical properties of the three Pluronic polymers used in this study are listed in Table 1. While L-61 and F-68 have similar number of propylene oxide (PO) units but differ significantly in the number of ethylene oxide (EO) units, F-68 and F-127 have more-or-less similar number of EO units but differ significantly in the number of PO units. We have studied the average size, poly-dispersity and zeta potential of POPG liposomes incubated with the three pluronics at various lipid:pluronic molar ratio and also when they are incorporated within the POPG bilayer. The results are presented in Fig. S1 in SI which shows that no significant changes of these three parameters are occurring due to incubation/incorporation with the polymers. This is consistent with previous reports [6,15,18,20,12,23,28] where it was observed that liposome integrity is not compromised at polymer concentrations lying well below their CMC values.

Table 1
The various physico-chemical properties of the three Pluronic polymers used in this study.

Pluronic polymer	Chemical formula	[a]HLB	[a]CMC	[b]Log $K_{p\ water/hexane}$
L-61	$EO_2PO_{30}EO_2$	3	1.1×10^{-4}	−0.24 ± 0.037
F-68	$EO_{76}PO_{30}EO_{76}$	29	4.8×10^{-4}	−3.5 ± 0.53
F-127	$EO_{100}PO_{65}EO_{100}$	22	2.8×10^{-6}	N.A.

[a] Ref. [6]
[b] Ref. [14]

Fig. 1 describes the changes in the SH electric field of LDS cation ($E_{2\omega}$) at 25 °C before and after addition of POPG liposomes (added at 50 s) for:

(1) Only POPG liposomes (black curve).
(2) POPG liposomes which were earlier incubated with 100 nano-molar F-127 pluronic polymer (red curve).
(3) POPG liposomes which were earlier incubated with 3000 nano-molar L-61 pluronic polymer (green curve).
(4) POPG liposomes which were earlier incubated with 10000 nano-molar F-68 pluronic polymer (blue curve).

Immediately after addition of liposomes, the SH signal increases instantaneously (<1 s) which is attributed to the electrostatic adsorption of the LDS cation on the outer surface of the POPG liposomes as demonstrated earlier [38]. After addition of POPG liposomes $E_{2\omega}$ of LDS decreases gradually due to transport of the LDS cation from the outer leaflet to the inner leaflet of the liposomes. It is obvious that presence of Pluronic polymers enhances the transport of the LDS cation across the membrane. The transport time constants (T_{av}) were obtained by exponential fitting of the curves from their maximum i.e. just after addition of the liposomes and their values are indicated in the legend in Fig. 1. The T_{av} values of LDS across the POPG membrane becomes ~ 9 times faster in the presence of three polymers. However as indicated in Fig. 1, the concentrations of the individual polymers to induce such a change vary widely. This suggests that permeability of the POPG membrane against the LDS cation is critically dependent on the chemical architecture of the pluronic polymers. Fig. 2 describes how the number of polymer molecules per liposome affects the transport rate constant (k_{av} i.e. $1/T_{av}$) of the LDS cation. In order to capture

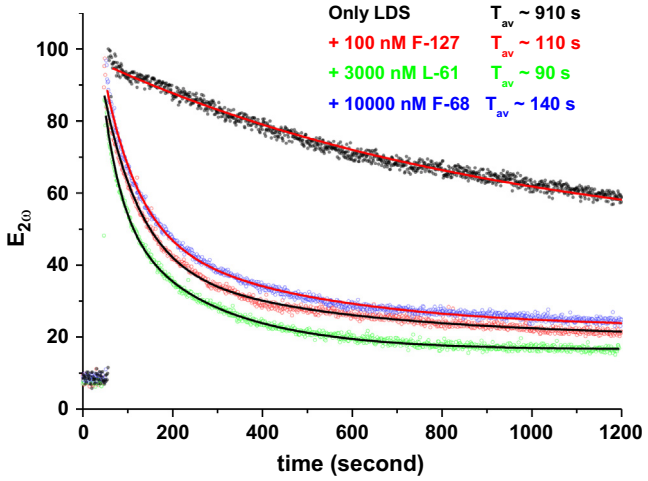

Fig. 1. Changes in the SH electric field of LDS cation with time after addition of POPG liposomes (added at 50 s) incubated with or without three different Pluronic polymers at 25 °C. The legends are colour matched with the data for clarity. The solid lines passing through the data points represent the exponential fits of the data. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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