Chemistry and Physics of Lipids 181 (2014) 83–89

Contents lists available at ScienceDirect

Chemistry and Physics of Lipids

journal homepage: www.elsevier.com/locate/chemphyslip

An electron spin resonance study of non-ionic surfactant vesicles (niosomes)

Seongyeol Yeom, Bong Seop Shin, Sanghwa Han*

Department of Biochemistry, Kangwon National University, Chuncon 200-701, Republic of Korea

ARTICLE INFO

Article history:
Received 27 December 2013
Received in revised form 25 March 2014
Accepted 26 March 2014
Available online 8 April 2014

Keywords:
Electron spin resonance
Niosomes
Sorbitan fatty esters (Span)
Polysorbates (Tween)
Polyethoxy fatty ethers (Brij)

ABSTRACT

Certain non-ionic surfactants form lamellar vesicles called niosomes. Being elastic and deformable, niosomes have been used as an efficient vehicle for transdermal drug delivery. However, dynamic properties of niosomes have not been studied extensively. In this study we used electron spin resonance (ESR) technique to measure the membrane fluidity of niosomes. In parallel with phospholipid liposomes, the ESR spectra of 5- and 16-doxyl stearate in niosomes of sorbitan monostearate (Span 60) and sorbitan monooleate (Span 80) showed that motion of the spin label was more restricted at the region near the headgroup than near the bilayer center. Cholesterol increased fluidity of Span 60 niosomes whereas it decreased fluidity of Span 80 niosomes. Dicetyl phosphate added at 10 mol% concentration as a stabilizer had a minimal effect on the membrane fluidity throughout the bilayer. We also used ESR technique to monitor the hydration-induced transformation of Span 60 proniosome gel to niosome and showed that the niosome prepared by hydration of proniosome gel was identical to the niosome obtained from a thin film hydration method. Finally the ESR spectra of Span niosomes were compared with those of polysorbate (Tween) niosomes and polyethoxy fatty ether (Brij) niosomes. Tween niosomes had a bulky headgroup and were much less rigid than Span niosomes. This effect of headgroup size on fluidity was also manifest in Brij niosomes where fluidity increased with the number of ethoxy units in the polyethoxy headgroup.

1. Introduction

Niosomes are lamellar vesicles prepared from non-ionic surfactants such as sorbitan fatty acid esters (Span), polysorbates (Tween), and polyethoxy fatty ethers (Brij). Compared with phospholipid-based vesicles, niosomes have a low cost of production, a higher chemical stability, and a larger membrane flexibility. These characteristics make niosomes an ideal vehicle for transdermal delivery of various drugs (Hamishehkar et al., 2013). Despite the above merits, niosomes still have some disadvantages shared with other liposomes. Dispersed in an aqueous medium niosomes on a long storage suffer from hydrolysis and degradation. They often undergo sedimentation, aggregation or fusion. Proniosome gel formulation, a gelified ethanolic non-ionic surfactant that transforms into niosome upon hydration, can be an alternative to overcome these problems (Sumit et al., 2012).

Dynamic properties of a lipid bilayer membrane can be studied by various techniques (Korstanje et al., 1989; Lentz, 1993; Kato et al., 2008; Abdelkader et al., 2010; Ghatak et al., 2011) among which electron spin resonance (ESR) spectroscopy (Korstanje et al., 1989) is particularly useful for probing the motional freedom within a membrane bilayer. In an ESR experiment, stearic acid labeled with a stable doxyl radical at a specific position along the acyl chain is incorporated in the membrane (Kyrychenko and Ladokhin, 2013) and its ESR spectrum is measured to estimate local fluidity along the normal to the membrane surface (Subczynski and Wisniewska, 1996). In general 5-doxyl stearate (5-DS) in a bilayer produces an anisotropic spectrum in the slow motional regime whereas 16-doxyl stearate (16-DS) yields a more isotropic spectrum in the fast motional regime. Local fluidity near the doxyl group is reflected in the order parameter that can be obtained from an experimental ESR spectrum.

ESR has been applied extensively to study the physical properties of phospholipid membranes (Knowles and Marsh, 1991). Recently Dragicevic-Curic et al. (2011) measured ESR spectra of 5-DS and 16-DS embedded in invasomes, another formulation for transdermal drug delivery, to assess the influence of membrane-softening agents on the membrane fluidity. To our knowledge the only ESR work on niosomes was reported by Fasoli et al. (1989) but they used niosomes prepared from unusual surfactants (steroidal

* Corresponding author. Tel.: +82 33 250 8516; fax: +82 33 259 5664.
E-mail address: hansh@kangwon.ac.kr (S. Han).

http://dx.doi.org/10.1016/j.chemphyslip.2014.03.004
0009-3084/

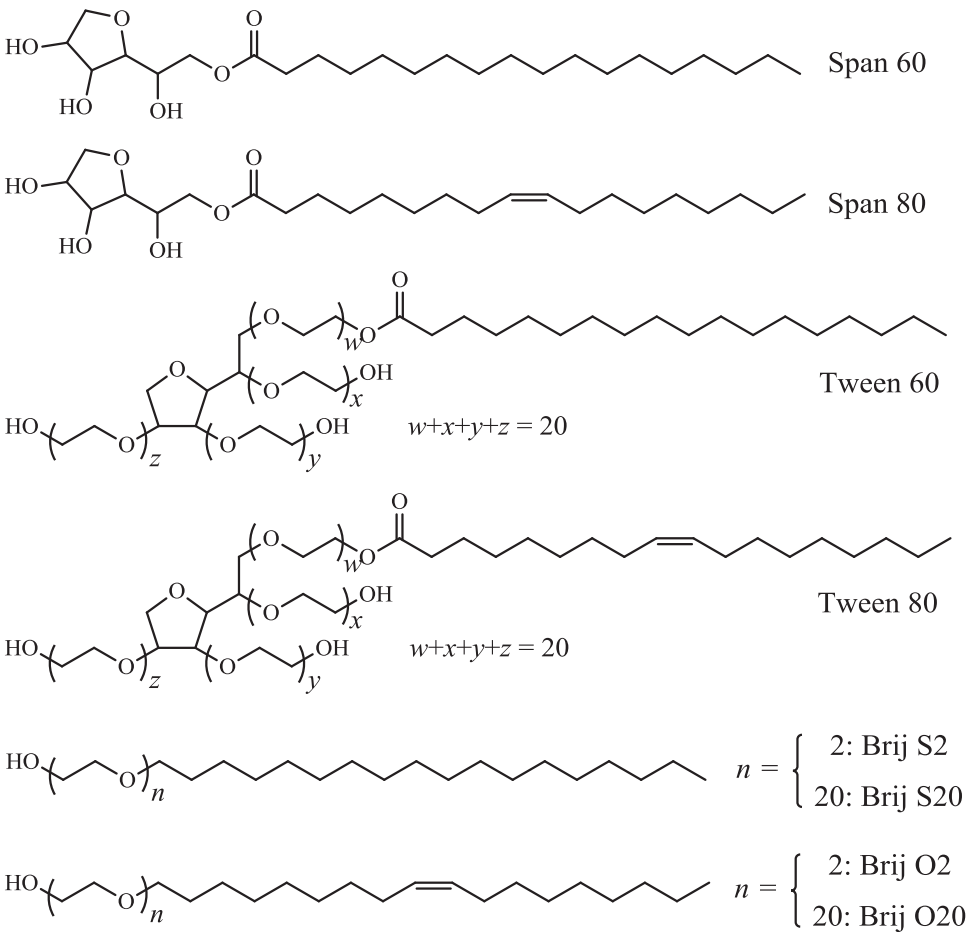

Fig. 1. Structures of surfactants used in the preparation of niosomes.

lariat ethers) rather than common surfactants such as Span, Tween, and Brij.

We have been studying the structures and properties of lipid bilayers using molecular dynamics simulation (Han, 2013a,b). In this study we measured the ESR spectra of niosomes from Span, Tween, and Brij to assess their dynamic properties. ESR was found to be a convenient tool to monitor the hydration-induced conversion of proniosome gel into niosome. We also studied the effect of cholesterol, dicetyl phosphate, and β-estradiol (an example drug) on the membrane fluidity.

2. Materials and methods

2.1. Materials

Sigma–Aldrich Chemical (St. Louis, USA) was the source of the chemicals except the following: Merck-Millipore (Darmstadt, Germany) for Span 80, Avanti Polar Lipids (Alabaster, USA) for distearoyl phosphatidylcholine and dioleoyl phosphatidylcholine, Showa Chemical (Tokyo, Japan) for Tween 80, and Santa Cruz Biotechnology (Dallas, USA) for 5- and 16-doxyl stearate.

2.2. Preparation of niosomes

Niosomes were prepared by using a thin film hydration method (Kunitake and Okata, 1976). Briefly, 10 mg total lipids (a non-ionic surfactant plus additives such as cholesterol, dicetyl phosphate, and β-estradiol) were dissolved in chloroform and the solvent was removed completely by evaporation and evacuation. The resulting lipid thin film on the wall of a flask was hydrated with 1 mL PBS (pH 7.4) to form multilamellar suspension, which was then sonicated to obtain niosomes.

2.3. Preparation and hydration of Span 60 proniosome gel

Proniosome gel was prepared according to a published method (Mokhtar et al., 2008). Span 60 (0.6 mmol) and cholesterol (0.4 mmol) were added to 500 μL ethanol and the mixture was heated up to 65 °C to obtain a clear solution. Proniosome gel was formed by adding a small amount (typically 160 μL, ~50 mol%) of water at 65 °C to the hot ethanolic solution and cooling it down. In this work, however, we varied the amount of water from 0 to 100 mol% to monitor the formation of proniosome gel and its subsequent conversion to niosome. The proniosome gel (with 50 mol% water) was white and appeared to be homogeneous but high speed centrifugation resulted in a denser gel and supernatant. Doxyl stearate spin label was added together with lipids in the samples for the hydration experiment. Concentration of the spin label was adjusted by the dilution factor of hydration so that all the samples used in the hydration experiment contained 30 μM spin label.

2.4. ESR spectroscopy

Doxyl stearate spin label (3 mM in ethanol) was added to a niosome suspension (10 mg/mL) at 30 μM final concentration. Ethanol in the final sample (1% v/v) did not affect the ESR spectra. The sample was transferred to a quartz flat cell and ESR spectrum was