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Effect of PEG-lipid conjugates on the phase behavior of phosphatidylethanolamine dispersions

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Abstract

The phase behavior of binary mixtures of hydrated dielaidoylphosphatidylethanolamine (DEPE) with two different PEG-lipid conjugates at a molar fraction below 0.2 has been studied by using time-resolved X-ray diffraction, and partial phase diagrams have been constructed. The studied conjugates comprise two saturated hydrocarbon acyl chains 14 carbon atoms long and PEG550 or PEG5000 chains covalently attached to a phosphoethanolamine polar head group, DMPE(PEG550) and DMPE(PEG5000), respectively. When added in small amounts (10–20 mol%) to DEPE aqueous dispersions, both PEG-lipids favor the lamellar liquid crystalline (L_α) phase at the expense of the lamellar gel (L_β) and the inverted hexagonal (H_{II}) phases. One of the conjugates, DMPE(PEG550), shifts the L_α–H_{II} transition of DEPE to higher temperatures by 2.5°C per mol% PEG-lipid, and induces the spontaneous formation of a cubic phase of space group Im3m in the DEPE dispersions. The cubic phase intrudes between the lamellar liquid crystalline and the inverted hexagonal phases in the DEPE/DMPE(PEG550) phase diagram. Low amounts of the DMPE(PEG5000) conjugate only shift the L_α–H_{II} transition of DEPE to higher temperatures, at 5.2°C per mol% PEG-lipid, but does not promote the formation of additional phases. The respective slopes for the L_β–L_α transition temperature depression are 10–15 times smaller. At >15 mol% DMPE(PEG550) and at >5 mol% DMPE(PEG5000), the non-lamellar phases are eliminated from the phase diagrams. Structural data on the organization of the pure hydrated PEG-lipid conjugates are also provided, suggesting that these lipids form micelles and lamellae.

Keywords: Cubic phase; Im3m; Liposome; Mesophase; PEG-lipid; Phase diagram; TRXRD

1. Introduction

Synthetic lipids conjugated with PEG polymer have attracted attention recently due to their application in drug delivery systems, for the prolongation of blood circulation time [1–4]. Knowledge concerning their interactions with membrane lipids is essential for optimizing their use in the pharmaceutical liposome preparations. Such information is of basic scientific interest as well, since the physico-chemical properties of the recently developed PEG-lipids and their interactions with membrane lipids are still poorly studied.

Here, we report the effect of small amounts of two different PEG-lipid conjugates, DMPE(PEG550) and DMPE(PEG5000), on the phase behavior of hydrated DEPE dispersions. Partial phase diagrams of the two hydrated mixtures, DEPE/DMPE(PEG550) and DEPE/DMPE

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(PEG5000), in the range of up to 20 mol% DMPE(PEG550), and up to 10 mol% DMPE (PEG5000), have been constructed, based on data from time-resolved X-ray diffraction. Recently, the induction of cubic phase formation in DEPE by 5–10 mol% of DMPE(PEG550) was reported [5].

This work has been reported at the 9th ICSCS, Sofia 1997 [6].

2. Materials and methods

1,2 - Dielaidoyl - *sn* - glycero - 3 - phosphoethanolamine (DEPE), 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[poly(ethylene glycol) 550] (DMPE(PEG550)), and 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[poly(ethylene glycol) 5000] [DMPE(PEG5000)] (Avanti Polar Lipids, Birmingham, AL) were used without further purification. The DEPE/PE(PEG) samples were prepared by mixing appropriate amounts of lipids as chloroform solutions, the chloroform was removed by rotary evaporation under nitrogen, and the lipid mixtures were dried under vacuum for at least 48 h. Double-distilled deionized water was added, and the dispersions were hydrated overnight at 20°C. Samples were homogenised by at least 10 successive cycles of freezing to −20°C, followed by thawing at room temperature and vortexing during the thawing step. The lipid concentration was 10 wt%. The samples were filled into glass capillaries (d=1.0 mm) (Hilgenberg, Malsfeld, Germany), flame-sealed, and stored at room temperature for 1–2 days before measurements.

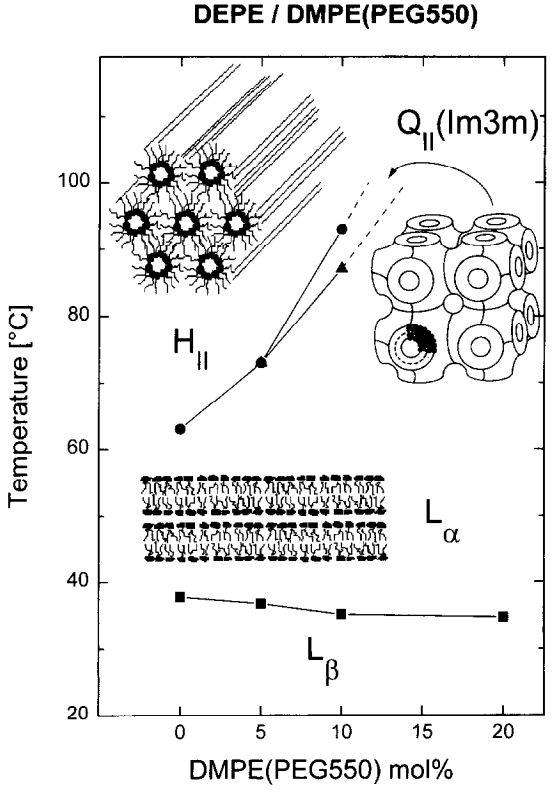

Fig. 1. Partial temperature/composition phase diagram of the hydrated mixture DEPE/DMPE(PEG550) constructed from TRXRD data.

Time-resolved X-ray diffraction experiments were carried out on beam line X13 of the EMBL outstation at DESY in Hamburg. In brief, this camera comprises a double focusing monochromator-mirror arrangement [7] with an X-ray wavelength of 0.15 nm. X-ray reflections in the small- and wide-angle regimes were recorded simultaneously using a data-acquisition system described recently [8]. Detectors were calibrated using dry rat tail tendon collagen (long spacing 65 nm) and Ag-behenate in the SAXS and p-bromobenzoic acid in the WAXS region. Data were normalised for incident intensity and analysed using the interactive data evaluating program OTOKO [9]. Diffraction patterns were recorded during heating–cooling scans at scan rates of 0.5–5°C min^{-1} as previously described [10,11]. Temperature cycling at 10°C min^{-1} was also applied.

3. Results and discussion

Aqueous dispersions of DEPE were observed to form a lamellar gel (L_β) phase at low temperatures, with a lamellar repeat period d=6.5 nm at 35°C, lamellar liquid crystalline (L_α) phase at intermediate temperatures (d=5.5 nm at 40°C and 5.2 nm at 60°C), and inverted hexagonal (H_{II}) phase at high temperatures, with a lattice constant $a=2d/\sqrt{3}$=7.5 nm at 65°C and 7.3 nm at 75°C. The L_β–L_α transition was at 37.8°C, and the L_α–H_{II}

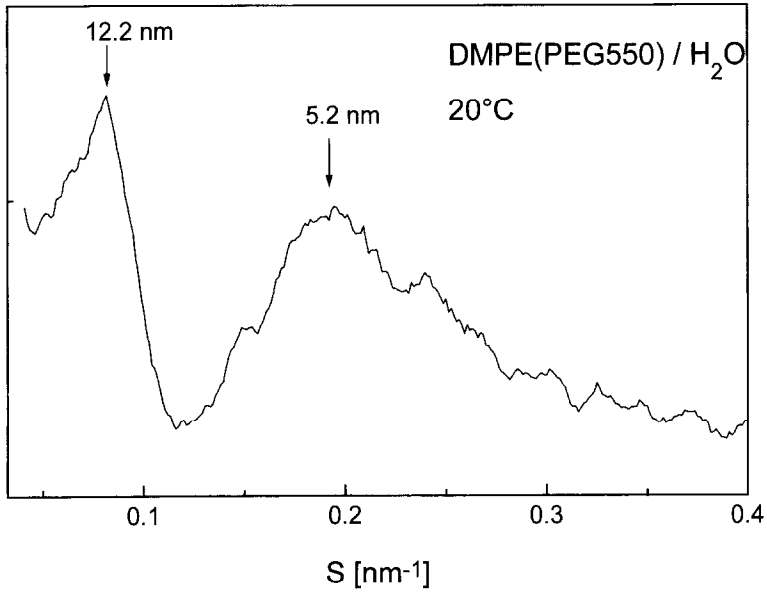

Fig. 2. Small-angle X-ray diffraction pattern of the aqueous DMPE(PEG550) dispersion recorded for 20 s at 20°C.

transition was at 63.0°C, in agreement with published data [12,13].

Addition of 5 mol% DMPE(PEG550) results in (1) a decrease in temperature of the L_β–L_α transition by c. 1°C (Fig. 1), (2) an increase in L_α–H_{II} transition temperature by c. 10°C (Fig. 1), and (3) considerable broadening of the SAXS lamellar reflections of both L_β and L_α phases, which is attributed to strong perturbation of the interlamellar correlation. Concurrently with the H_{II} phase, additional trace reflections appear at small angles, seen better upon cooling. During the first heating–cooling course, these reflections are weak but become more pronounced with temperature cycling, and gradually start to dominate over the H_{II} phase reflections. After 10 cycles between 52.5 and 76.5°C, reflections at 15.9, 11.2, 9.2, 8.0 and 7.1 nm are observable, with the spacing ratios $\sqrt{2}$:$\sqrt{4}$:$\sqrt{6}$:$\sqrt{8}$:$\sqrt{10}$, characteristic of the cubic phases of space groups Pn3n or Im3m [14]. Unambiguous identification of the cubic phase from the X-ray pattern is not possible.

In the aqueous dispersion of DEPE/DMPE(PEG550) mixture containing 10 mol% DMPE(PEG550), the L_β–L_α transition is shifted down in temperature by an additional 1.5°C and takes place at 35.2°C upon heating at a rate of 1°C min^{-1}. Upon further heating, reflections characteristic of a cubic phase appear in the small-angle scattering region at 87°C. The spacings of these reflections are in the ratio: $\sqrt{2}$:$\sqrt{4}$:$\sqrt{6}$:$\sqrt{8}$:$\sqrt{10}$:$\sqrt{12}$:$\sqrt{14}$:$\sqrt{16}$:$\sqrt{18}$:$\sqrt{20}$:$\sqrt{22}$:$\sqrt{24}$:$\sqrt{26}$:$\sqrt{30}$:$\sqrt{32}$:$\sqrt{42}$:$\sqrt{48}$ [5], consistent with the cubic phase of space group Im3m [14]. The indexing of the diffraction pattern unambiguously supports the cubic phase identification [5]. The cubic unit cell lattice parameter at 89.8°C is a= 20.5 nm. Further heating results in the appearance of an inverted hexagonal (H_{II}) phase at c. 93°C that coexists with the cubic phase up to 100°C. Upon cooling, the reverse phase sequence $H_{II}+Q_{II}\rightarrow Q_{II}\rightarrow L_\alpha\rightarrow L_\beta$ is observed.

In a DEPE/DMPE(PEG550) sample containing 20 mol% DMPE(PEG550), an L_β–L_α transition takes place at 34.8°C. Only the L_α phase is observed up to 100°C (Fig. 1).

At the opposite end of the phase diagram, pure PEG-lipids in water have been supposed to form micelles [15]. The X-ray patterns recorded in the present study indicate a more complicated DMPE(PEG550) organization that is difficult to decipher unambiguously. The SAXS diffraction

pattern observed at 20°C comprises a relatively sharp reflection at 12.2 nm, possibly reflecting a lamellar arrangement, and a broad diffuse band centerd at 5.2 nm (Fig. 2). Similar patterns are observed in the whole temperature range of 0–100°C, with both peaks becoming broader at high temperatures. The recorded WAXS patterns are typical for disordered hydrocarbon chains in the whole studied temperature range, i.e. hydrated DMPE(PEG550) does not form a gel phase.

We also examined the effect of another PEG-lipid with a much larger head group, DMPE (PEG5000), on the phase behavior of DEPE aqueous dispersion. Addition of 0.5, 1, and 2.5 mol% DMPE(PEG5000) to DEPE shifts the L_α–H_{II} transition upwards in temperature but does not induce the formation of other mesomorphic phases (Fig. 3). In a preparation with 2.5 mol% DMPE(PEG5000), the L_α–H_{II} transition is at 76°C, and the lattice constant of the H_{II} phase is 6.9 nm at 80°C. The L_β–L_α transition takes place at 36.8°C. Higher concentrations of DMPE (PEG5000) (5 and 10 mol%) rule out the L_α–H_{II} transition from the temperature scale up to 100°C.

Pure hydrated DMPE(PEG5000) displays a single relatively sharp SAXS reflection at 12.8 nm at room temperature (Fig. 4), possibly due to the lamellar arrangement of the PEG-lipid. This pattern is retained upon cooling to low temperatures and disappears at c. −15°C upon water freezing, as evidenced by the appearance of the sharp ice reflections in the WAXS region. Below that temperature, diffraction maxima are not observed in the SAXS range. Upon heating to above 40–50°C, the sharp SAXS reflection transforms into a diffuse scattering halo centerd at

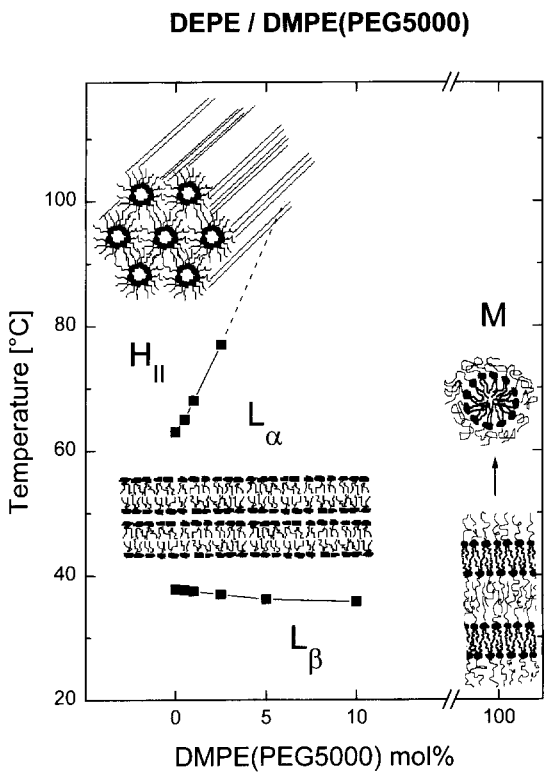

Fig. 3. Partial temperature/composition phase diagram of the hydrated mixture DEPE/DMPE(PEG5000) constructed from TRXRD data.

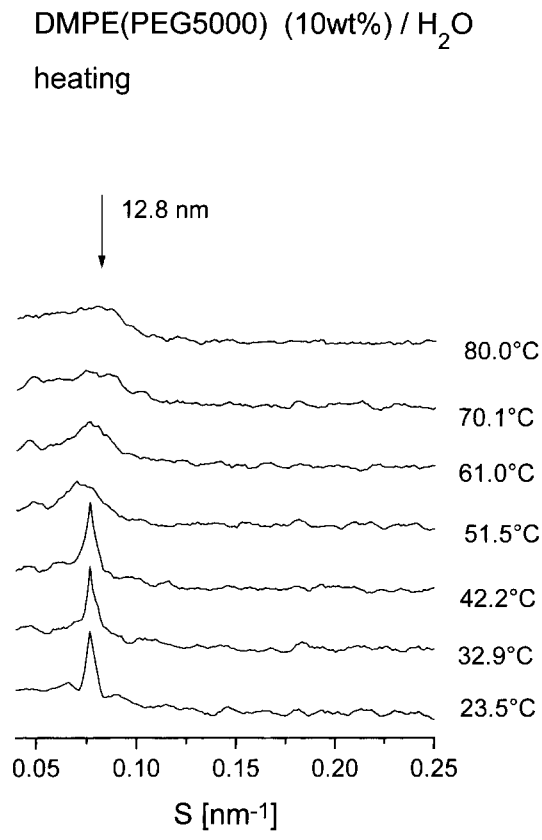

Fig. 4. Small-angle X-ray diffraction patterns of the aqueous DMPE(PEG5000) dispersion recorded upon heating in the temperature range 20–80°C.

about the same spacing (Fig. 4) that might reflect micelle formation. This transformation is not readily reversible—the diffuse pattern is preserved upon subsequent cooling to room temperature. The recorded WAXS patterns in the temperature range of −15°C to +80°C are typical for disordered hydrocarbon chains, i.e. hydrated DMPE (PEG5000) does not form a gel phase as well.

Thus, the studied PEG-lipid conjugates favor the lamellar liquid crystalline phase, providing the largest area per head group, at the expense of the lamellar gel (L_β) and the inverted hexagonal (H_{II}) phases in DEPE. They raise the temperature of the L_α–H_{II} transition by 2.5°C per mol% in the case of DMPE(PEG550) and by an impressive 5.2°C per mol% in the case of DMPE(PEG5000). The L_β–L_α transition is depressed by about 0.25°C per mol% of the PEG-lipid. These tendencies are certainly a result of the packing constraints imposed by the bulky hydrophilic moieties of the conjugates. The detected ability of a phosphatidylethanolamine/PEG-lipid mixture to adopt the bicontinuous cubic phase structure is also undoubtedly important from pharmacological aspect. The utilisation of bicontinuous cubic mesomorphs formed in lipid/water systems for controlled drug release comprises one of the essential recent advances in elaborating in vivo drug-delivery systems [16].

Acknowledgment

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Appendix

DEPE	1,2-Dielaidoyl-*sn*-glycero-3-phosphoethanolamine
PEG	Poly(ethylene glycol)
PEG550	PEG of molecular mass 550
PEG5000	PEG of molecular mass 5000
DMPE(PEG550)	1,2-Dimyristoyl-*sn*-glycero-3-phosphoethanol-amine-*N*-[poly(ethylene glycol) 550]
DMPE(PEG5000)	1,2-Dimyristoyl-*sn*-glycero-3-phospho-ethanolamine-*N*-[poly(ethylene glycol) 5000]

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