Available online at www.sciencedirect.com
ScienceDirect
ELSEVIER
Biochimie 88 (2006) 1883−1888
BIOCHIMIE
www.elsevier.com/locate/biochi

The structure and phase behaviour of α-tocopherol-rich domains in 1-palmitoyl-2-oleoyl-phosphatidylethanolamine

Xiaoyuan Wang[1], Peter J. Quinn*

Department of Biochemistry, King's College London, 150 Stamford Street, London SE1 9NH, UK

Received 13 March 2006; accepted 19 July 2006
Available online 11 August 2006

Abstract

The effect of α-tocopherol on the structure and thermotropic phase behaviour of 1-palmitoyl-2-oleoyl-phosphatidylethanolamine dispersed in excess water was examined by synchrotron X-ray diffraction and differential scanning calorimetry. Small- and wide-angle X-ray scattering intensity profiles were recorded from mixed dispersions containing up to 20 mol% α-tocopherol during temperature scans over the range 10−75 °C. These showed that a domain enriched in α-tocopherol phase separated from pure phospholipid in the mixture. This domain tends to have inverted hexagonal structure which coexists with phospholipid bilayers depleted of α-tocopherol. The scattering intensity and dimensions of the phase are dependent on the temperature and proportion of α-tocopherol in the mixture. Phase separations were also manifest in calorimetric scans of the mixed dispersions evidenced from the appearance of multiple peaks at temperatures corresponding to transitions observed in the X-ray scattering experiments. The effect of α-tocopherol in the range 0−20 mol% on the phase behaviour and structure of the phospholipid as observed from the X-ray scattering and calorimetric results have been used to construct a partial phase diagram of the mixture in the temperature range 10−75 °C. This shows that α-tocopherol has a marked tendency to partition from bilayers of the phospholipid to form an enriched domain in which the phospholipid assumes a hexagonal-II structure.
© 2006 Elsevier Masson SAS. All rights reserved.

Keywords: Vitamin E; α-Tocopherol; Model membranes; Phospholipids; X-ray scattering; Calorimetry

1. Introduction

α-Tocopherol is a ubiquitous lipid component of biological membranes. It has received considerable attention as an antioxidant acting to prevent free radical damage to tissues [1−5]. There is also evidence that α-tocopherol modulates the activity of certain transcription factors and a variety of enzymes [6,7] but the detailed mechanisms of action of these functions are presently unknown.

Because of its lipophilic properties α-tocopherol is distributed throughout the body in various cell membranes and lipid depots. A general approach to probe the location and function of α-tocopherol in membranes has been to examine its interaction with phospholipids model membranes. Such studies have revealed that the effect of α-tocopherol on the phase behaviour of aqueous dispersions of phospholipids depends on the acyl chain composition [8,9] as well as the headgroup of the phospholipid [10,11]. Differential scanning calorimetric studies of mixed dispersions of phosphatidylethanolamines have shown that the presence of α-tocopherol results in the appearance of multi-endothermic peaks while for phosphatidylcholines α-tocopherol only broadens the main transition peak and decreases the transition temperature [12,13]. The structure of mixed aqueous dispersions as revealed by X-ray diffraction [5,14] indicates that an α-tocopherol enriched domain is formed in the mixture that preferentially adopts H_{II} structure if the mixture contains phosphatidylethanolamine and ripple

Abbreviations: POPE, 1-palmitoyl-2-oleoyl-*sn*-phosphatidylethanolamine; SAXS, small-angle X-ray scattering ($2\theta = 0.043°-7.9°$); WAXS, wide-angle X-ray scattering ($2\theta = 8°-60°$); DSC, differential scanning calorimetry; α-T, α-tocopherol; L_α, lamellar liquid-crystalline phase; L_β, lamellar gel phase; H_{II}, inverted hexagonal phase.

* Corresponding author. Tel./fax: +44 2078484408.
E-mail address: p.quinn@kcl.ac.uk (P.J. Quinn).
[1] Present address: Department of Biochemistry, Duke University Medical Center, P.O. Box 3711, Durham, NC 27710, USA.

0300-9084/$ - see front matter © 2006 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.biochi.2006.07.017

structure if it contains phosphatidylcholine. These effects are presumably a reflection of the particular packing constraints imposed by the presence of α-tocopherol in the phospholipid bilayers.

The presence of α-tocopherol in phospholipid bilayers acts in certain respects in a similar manner to cholesterol [8]. Thus α-tocopherol forms stoichiometric complexes with saturated phosphatidylcholines in molar ratios of 10:1 phospholipid: α-tocopherol [15] that restrict the trans-bilayer mobility of the tocopherol [16,17]. The formation of the complex is associated with a condensing effect on the surface area occupied by the phospholipid at the interface with water [18]. Nevertheless, the phytanoyl chain of α-tocopherol resembles the side chain of cholesterol only when extended in the all-*trans* conformation and flexibility of the chain prevents more extensive van der Waals interactions and hence the attractive forces of the complex are weaker.

In this study, we chose the system α-tocopherol and 1-palmitoyl-2-oleoyl-phosphatidylethanolamine to define more precisely the effect of α-tocopherol on unsaturated phosphatidylethanolamine, using synchrotron X-ray diffraction and differential scanning calorimeter. 1-Palmitoyl-2-oleoyl-phosphatidylethanolamine is the major phospholipid in the inner membrane of *Escherichia coli* [19]. Mutants of *E. coli* lacking phosphatidylethanolamine cannot survive in normal media. Membrane proteins in liposomes lacking phosphatidylethanolamine also have different topologies and therefore their functions cannot be fully reconstituted [20]. The effect of α-tocopherol on the structure and thermotropic phase behaviour of 1-palmitoyl-2-oleoyl-phosphatidylethanolamine dispersed in excess water was examined by synchrotron X-ray diffraction and differential scanning calorimetric methods. Temperature scans over the range 10−75 °C showed that an α-tocopherol enriched domain was formed in the mixture of α-tocopherol and phosphatidylethanolamine. This domain tends to form inverted hexagonal phase, whose intensity and size are dependent on the temperature and concentration of α-tocopherol in the mixture.

2. Materials and methods

2.1. Materials

POPE was purchased from Avanti Polar Lipids (Alabaster), and α-tocopherol from Acros Organics (Geel, Belgium). The lipids were dissolved in chloroform and mixed in appropriate proportions to achieve the desired molar fractions. The solvent was evaporated under a stream of oxygen-free dry nitrogen and stored for 24 h under vacuum to remove any remaining traces of solvent. The lipid mixtures were hydrated with water (pH 7) at 80 °C for at least 1 h and dispersed by a rotamixer until homogeneous dispersions were obtained. The lipid dispersions were stored at 4 °C for 24 h before examination. The method of preparation and storage gave reproducible phase behaviour when samples prepared at different times were examined by X-ray diffraction.

2.2. Synchrotron X-ray diffraction

Synchrotron X-ray diffraction experiments were performed using a monochromatic (0.15405 nm) focused X-ray beam at station 8.2 of the Daresbury Synchrotron Radiation Laboratory, UK. The camera configuration allowed detection of small-angle and wide-angle X-ray scattering with a minimum of parallax error [21]. The beamline generates a flux of 4×10^{10} photons/s with a focal spot size of $0.3 \times 3\ mm^2$ ($V \times H$) when the synchrotron radiation source is operating at a nominal 200 mA. The samples were mounted in a slot (1 × 5 mm) cut in a 1 mm thick copper plate sandwiched between a pair of thin mica sheets. The sandwich was clamped to the silver block containing the temperature sensing and modulating elements of a cryomicroscope stage (Linkam Scientific Instruments Ltd., UK). X-ray scattering intensities at small-angles ($2\theta = 0.043°-7.9°$) were recorded using a multiwire quadrant detector. X-ray scattering intensities at wide-angles ($2\theta = 8°-60°$) were recorded using an INEL curved linear-wire detector (*IN*strumentation *El*ectronique, France). Data were acquired in 400 consecutive time frames of 5 s separated by a dead time between frames of 50 ms. Experimental data were analysed using the OTOKO software (EMBL, Hamburg, Germany) programme [22]. Scattering intensities at low-angles were corrected for fluctuations in beam intensity and detector response recorded from an ^{59}Fe source. Spatial calibrations were obtained using 21-orders of wet rat-tail collagen ($d = 67$ nm) [23]. The scattering intensity data recorded by the INEL detector were corrected for scattering from an empty cell and spatial calibrations were established from high-density polyethylene (0.4166, 0.3780, 0.3014 nm) [24]. The reciprocal spacing $S = 1/d = 2\sin(\theta)/\lambda$, where d, λ, θ are the repeat distance, X-ray wave length and the diffraction angle, respectively.

2.3. Differential scanning calorimetry

Differential scanning calorimeter (DSC) experiments were performed using a Perkin−Elmer DSC-2 calorimeter. Lipid dispersions were sealed in aluminium pans, and heating and cooling scans at 1.25°/min were used. The transition in cooling scan was reversible with a temperature hysteresis less than 0.5°.

3. Results

X-ray diffraction patterns of the pure hydrated POPE recorded during a heating scan are shown in Fig. 1. It can be seen that the phospholipid undergoes a gel to liquid−crystalline ($L_\beta \rightarrow L_\alpha$) phase transition at 25 °C and a liquid−crystalline to inverted hexagonal ($L_\alpha \rightarrow H_{II}$) phase transition at 71 °C. The former is characterised by a decrease in the lamellar repeat spacing from 6.3 nm (12 °C) to 5.3 nm (32 °C) in the SAXS region (Fig. 1a), which coincides with a change from sharp symmetrical diffraction peaks at 0.43 nm, typical of gel phases, to broad peaks centred at 0.46 nm in the WAXS region (Fig. 1b). The H_{II} phase is characterised by a series of reflections in the order of 1,

Download English Version:

https://daneshyari.com/en/article/1953266

Download Persian Version:

https://daneshyari.com/article/1953266

Daneshyari.com