Available online at www.sciencedirect.com
ScienceDirect
Chemistry and Physics of Lipids 145 (2007) 45–62
CPL
CHEMISTRY AND PHYSICS OF LIPIDS
www.elsevier.com/locate/chemphyslip

Calorimetric study on the induction of interdigitated phase in hydrated DPPC bilayers by bioactive labdanes and correlation to their liposome stability
The role of chemical structure

Christina Matsingou, Costas Demetzos *

Department of Pharmaceutical Technology, School of Pharmacy, University of Athens, Panepistimiopolis, Zografou 15771, Athens, Greece

Received 12 May 2006; received in revised form 15 September 2006; accepted 20 October 2006
Available online 13 November 2006

Abstract

Labd-7,13-dien-15-ol (1), labd-13-ene-8α,15-diol (2), and labd-14-ene-8,13-diol (sclareol) have been found to exhibit cytotoxic and cytostatic effects. Their partitioning into phospholipid bilayers may induce membrane structure modifications, crucial in the development of liposomes. DSC was used to elucidate the profile of modifications induced in DPPC bilayers by incorporating increasing concentrations of the labdanes. Labdanes **1**, **2** and sclareol were incorporated into SUV liposomes composed of DPPC their physicochemical stability was monitored (4 °C) and was compared to liposomes incorporating cholesterol. All labdanes strongly affect the bilayer organization in a concentration dependent manner in terms of a decrease of the cooperativity, the fluidization and partially destabilization of the gel phase, the induction of a lateral phase separation and the possible existence of interdigitated domains in the bilayer. The physicochemical stability of liposomes was strongly influenced by the chemical features of the labdanes. The liposomal preparations were found to retain their stability at low labdane concentration (10 mol%), while at higher concentrations up to 30 mol% a profound decrease in intact liposomes occurred, and a possible existence of interdigitated sheets was concluded.

Keywords: Labdanes; Differential scanning calorimetry; DPPC; Interdigitation; SUV liposomes

1. Introduction

Natural products especially those derived from plant sources play a significant role as new therapeutic candidates in the drug discovery process (Newman et al., 2003). Among those, labdane-type diterpenes constitute a significant class of natural products (Demetzos and Dimas, 2001 see also references therein). Labdanes **1**, **2** and sclareol belonging to labdane-type diterpenes are similar in structure (Fig. 1). All three compounds have been investigated for their potential cytotoxic and cytostatic effects against human cancer cell lines and have been proven to be active (Demetzos and Dimas, 2001; Dimas et al., 2001; Matsingou et al., 2005, 2006; Hatziantoniou et al., 2006). The amphipathic phospholipid molecules that nature has evolved as major

Abbreviations: Labdane **1**, labd-7,13-dien-15-ol; Labdane **2**, labd-13-ene-8α,15-diol; Sclareol, labd-14-ene-8,13-diol; DPPC, dipalmitoylphosphatidylcholine; DSC, differential scanning calorimetry; SUV, small unilamelar vesicles

* Corresponding author. Tel.: +30 210 7274596; fax: +30 210 7274027.
E-mail address: demetzos@pharm.uoa.gr (C. Demetzos).

0009-3084/$ – see front matter © 2006 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.chemphyslip.2006.10.004

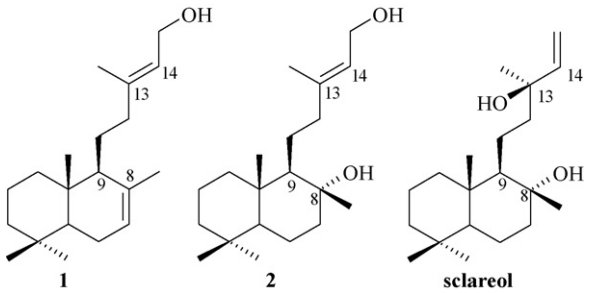

Fig. 1. Structures of labd-7,13-dien-15-ol (**1**), labd-13-ene-8α,15-diol (**2**) and labd-14-ene-8,13-diol (sclareol).

components of biomembranes have a crucial share in the physiological functions of living cells. Therefore, the physicochemical state of the phospholipid bilayer is of great importance since it is directly related with the physiological features of cell membranes and any drug effect resulting in alterations of the bilayer structure may affect specific membrane functions. On the other hand phospholipids are the major components of liposomes that have recently attracted a great deal of interest because of their use as artificial biocompatible vehicles in drug delivery and targeting, improving drug solubilization in body fluids and interaction with barrier membranes (Sharma and Sharma, 1997; Lia and Ho, 2001). Long-term physicochemical stability of liposomes is a requirement and may be affected by the chemical structure and properties of the incorporated molecule that is capable of modifying the physicochemical characteristics of the liposomal bilayer. Small molecules have been shown to induce alterations of phospholipid packing in model bilayer membranes inducing an interdigitated state of the opposing monolayers (McIntosh et al., 1983; Slater and Huang, 1988; Rowe and Cultera, 1990; Wang et al., 1993; Löbbecke and Cevc, 1995; Suurkuusk and Singh, 1998; Hao et al., 1998; Hata et al., 2000). Albeit bilayer interdigitation may encompass many effects on the structure of biological membranes namely the mismatching between membrane proteins and hydrophobic regions of the lipid matrix, information about the induction of interdigitation in biological membranes is virtually nonexistent. However, the interdigitated state of lipid bilayers is of great importance since it influences the stability of liposomal preparations causing hydrophobic defects in the surface area accelerating thus membrane adhesion and fusion or aggregation to larger structures (Komatsu et al., 1993; Boni et al., 1993; Komatsu and Okada, 1995; Ahl and Perkins, 2003). According to many described methods, DPPC can be considered as the most frequently used synthetic phospholipid in liposome preparation due to its biocompatibility and its low toxicity (Weiner, 1989). DSC over the years has been considered as a sensitive and non-perturbing tool in the exploration of the thermodynamic lipid phase transitions. As a thermodynamic technique, DSC has extensively been used in studies of the molecular interactions of several additives of biological relevance with model lipid bilayers (Ambrosini et al., 1998; Videira et al., 1999; Hata et al., 2000; Hauet et al., 2003; Momo et al., 2005; Butler et al., 2005). Major attention has been given in the drug incorporating processes into liposomal delivery systems. Investigations on the thermodynamic effects of drugs on structural and physicochemical characteristics of lipid bilayers as much as the nature of modifications evoked, revealed valuable in the justification of the liposomal composition to be used. In an effort to elucidate the profile of the modification in the physicochemical state of DPPC bilayers by incorporating increasing concentrations of structural related cytotoxic labdanes, DSC was used in the present work and physical properties of the new systems were discussed. The labdanes were incorporated into SUV liposomes composed of DPPC and their physicochemical stability was monitored during their storage under defined conditions. In the light of DSC findings, the behaviour of the liposomal preparations under specific conditions is discussed.

2. Experimental procedures

2.1. Materials

Labdane **1** and **2** were isolated from the resin 'Ladano' of the plant *Cistus creticus* subsp. *creticus* that grows in the island of Crete (Greece) (collector Dr. C. Demetzos) and sclareol was purchased from Sigma–Aldrich. Column chromatography for isolating and purifying labdane **1** and **2** was performed on silica gel (Merck, 230–400 mesh). Analytical thin layer chromatography (TLC) was carried out on Merck silica gel F_{254} plates. DPPC was obtained from Avanti Polar Lipids Inc. (Albaster, AL, USA). Cholesterol and Sephadex G-75 were obtained from Sigma–Aldrich. Solvents of analytical grade were obtained from Labscan Ltd. HPLC grade water was obtained by PRO^{TM}PS Labconco System.

2.2. Methods

2.2.1. Isolation of labdanes *1* and *2*

4.1 g of the CH_2Cl_2 extract of the resin "ladano" were fractionated by column chromatography according to the literature (Matsingou et al., 2005, 2006). Labdane **1** was isolated as a yellow oil [$\alpha_D = 5.57^\circ$ (*c* 0.67 mg/ml