Journal of Colloid and Interface Science 356 (2011) 375–386

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

www.elsevier.com/locate/jcis

Feature Article

Interactions of biomacromolecules with reverse hexagonal liquid crystals: Drug delivery and crystallization applications

Dima Libster, Abraham Aserin, Nissim Garti *

Casali Institute of Applied Chemistry, The Institute of Chemistry, The Hebrew University of Jerusalem, Edmond J. Safra Campus, Givat Ram, Jerusalem 91904, Israel

ARTICLE INFO

Article history:
Received 17 November 2010
Accepted 14 January 2011
Available online 21 January 2011

Keywords:
Monoolein
Proteins
Skin penetration
Peptide
Cyclosporin A
Desmopressin
Phosphatidylcholine

ABSTRACT

Recently, self-assembled lyotropic liquid crystals (LLCs) of lipids and water have attracted the attention of both scientific and applied research communities, due to their remarkable structural complexity and practical potential in diverse applications.

The phase behavior of mixtures of glycerol monooleate (monoolein, GMO) was particularly well studied due to the potential utilization of these systems in drug delivery systems, food products, and encapsulation and crystallization of proteins. Among the studied lyotropic mesophases, reverse hexagonal LLC (H_{II}) of monoolein/water were not widely subjected to practical applications since these were stable only at elevated temperatures. Lately, we obtained stable H_{II} mesophases at room temperature by incorporating triacylglycerol (TAG) molecules into the GMO/water mixtures and explored the physical properties of these structures.

The present feature article summarizes recent systematic efforts in our laboratory to utilize the H_{II} mesophases for solubilization, and potential release and crystallization of biomacromolecules. Such a concept was demonstrated in the case of two therapeutic peptides—cyclosporin A (CSA) and desmopressin, as well as RALA peptide, which is a model skin penetration enhancer, and eventually a larger macromolecule—lysozyme (LSZ). In the course of the study we tried to elucidate relationships between the different levels of organization of LLCs (from the microstructural level, through mesoscale, to macroscopic level) and find feasible correlations between them. Since the structural properties of the mesophase systems are a key factor in drug release applications, we investigated the effects of these guest molecules on their conformations and the way these molecules partition within the domains of the mesophases. The examined H_{II} mesophases exhibited great potential as transdermal delivery vehicles for bioactive peptides, enabling tuning the release properties according to their chemical composition and physical properties. Furthermore, we showed a promising opportunity for crystallization of CSA and LSZ in single crystal form as model biomacromolecules for crystallographic structure determination.

The main outcomes of our research demonstrated that control of the physical properties of hexagonal LLC on different length scales is key for rational design of these systems as delivery vehicles and crystallization medium for biomacromolecules.

1. Introduction

Liquid crystals (LCs) are self-assembled organized mesophases with properties intermediate to those of crystalline solids and isotropic liquids [1]. In LC phases, long-range periodicity exists, although the molecules exhibit a dynamical disorder at atomic distances, as is the case in liquids. Accordingly, these materials can also be considered ordered fluids [2]. *Lyotropic LCs* (*LLCs*) are materials that are composed from at least two molecules: an amphiphilic molecule and its solvent. A hydrophilic solvent, such as water, hydrates the polar moieties of the amphiphiles via hydrogen bonding, while the flexible aliphatic tails of the amphiphiles aggregate into fused hydrophobic regions, based on van der Waals interactions. In addition to morphologic dependence on the chemical composition, LLCs are also sensitive to external parameters, such as temperature and pressure [1–3]. As a function of the molecular shape of the surfactants, packing parameters, and interfacial curvature energy considerations, LLCs can be formed with aqueous domains ranging from planar bilayer lamellae to extended, cylindrical channels, to 3-D interconnected channels and manifolds [4]. These mesophases are defined as lamellar (L_α), hexagonal (H), bicontinuous cubic [Q (or V)], and discontinuous cubic (I) phases, based on their symmetry [5]. In addition, most lyotropic mesophases exist as symmetric pairs, a "normal" (type I) oil-in-water system, consisting of lipid aggregates in a continuous water matrix, and a topologically "inverted" (type II) water-in-oil version, in which headgroups hydrated by water are arranged within a contin-

* Corresponding author. Fax: +972 2 652 0262.
E-mail address: garti@vms.huji.ac.il (N. Garti).

0021-9797/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.jcis.2011.01.047

uous non-polar matrix, which is composed of the fluid hydrocarbon chains [5]. In addition to its biological significance, inverse lipid phases could be useful as host systems for the crystallization of membrane proteins [6], for drug delivery [7], food applications [8,9] and for inorganic synthesis [10].

Reverse hexagonal mesophases (H_{II}) are characterized by densely packed, straight water-filled cylinders, exhibiting 2-D ordering. Each cylinder is surrounded by a layer of surfactant molecules that are perpendicular to the cylinder interface such that their hydrophobic moieties point outward from the water rods (Fig. 1a). There is a growing indication that inverse hexagonal mesophases play structural and dynamic roles in biological systems [5,11]. These systems are assumed to be active as transient intermediates in biological phenomena that require topological rearrangements of lipid bilayers such as membrane fusion/fission and the trans-bilayer transport of lipids and polar solutes [5,11,12].

2. H_{II} mesophase composed of GMO/triglyceride/water

It was shown that in the monoolein-based system, the cubic phase is transformed into an H_{II} mesophase upon heating at ca. 85 °C [13]. The effective Critical Packing Parameter (CPP) theory can supply a reasonable explanation to the temperature induced structural shifts from lamellar through cubic to reverse hexagonal phases, requiring greater curvature than in the lamellar phase. Increasing the thermal motion of both the hydrocarbon chains and the water molecules would increase the CPP values *via* expanding the volume of the lipophilic moiety, but decreasing the chain length and the headgroup area. This leads to an increase in curvature and therefore induces the formation of cubic and hexagonal mesophases. In addition, the hexagonal mesophase is characterized by greater packing cost than the cubic phase, but the opposite is true for curvature elastic energy. Therefore, elevated temperatures induced the tendency for interfacial curvature, which increased the curvature elastic costs of the bicontinuous cubic phase, stabilizing hexagonal symmetry [1,14].

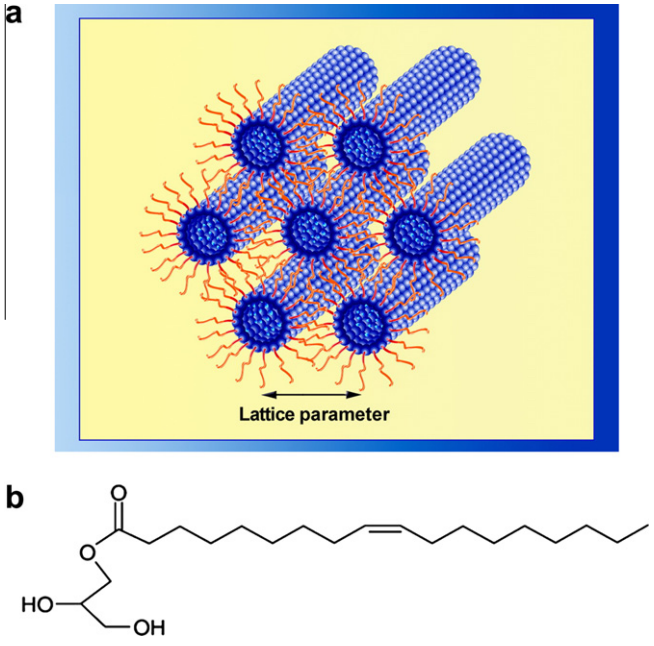

Fig. 1. (a) Schematic presentation of H_{II} mesophase, showing the packing of water-filled rods, surrounded by lipid layers. (b) Chemical structures of glycerol monooleate (GMO).

Bearing in mind the thermodynamic and structural considerations noted above, systematic research was conducted in our laboratory to decrease the cubic to hexagonal temperature transition and stabilize the GMO-based (Fig. 1b) H_{II} mesophase at room temperature [15]. As such, these mesophases that are stable at room temperature can potentially be used as efficient drug delivery vehicles. To achieve this goal, experiments relative to incorporation of triglycerides (TAGs) with various chain lengths to the binary GMO/water system were conducted. Amar-Yuli and Garti [15] surmised that immobilization of a TAG between GMO tails would lead to a change in the geometry of monoolein molecules from cylindrical to wedge-shaped and thereby an increase in the CPP value of the system. This should, therefore, encourage transition from lamellar or cubic phases to hexagonal structures. In addition, the immobilization of TAG in the system was expected to reduce the packing frustration, stabilizing the hexagonal LLC at room temperature. These experimental results showed that a critical and optimal chain length of the triglyceride is required to induce the formation of H_{II} at room temperature. Among all the examined TAGs, tricaprylin (C_8) attained the largest region of hexagonal structure in the phase diagram (Fig. 2), indicating its flexible accommodation between the tails of the GMO. It was suggested that the high mobility of tricaprylin in the surfactant aggregate provided direct transformation of the lamellar mesophase to the hexagonal phase, avoiding the formation of a cubic structure.

The structural properties of ternary hexagonal mesophases composed of GMO, tricaprylin, and water were extensively and systematically studied in our laboratory [16–20]. Several additives, including dermal penetration enhancers, were solubilized to control the physical properties of these carriers [16–18] such as viscosity and thermal stability. For instance, the synergistic solubilization of two major hydrophilic (vitamin C, ascorbic acid, AA) and lipophilic (vitamin E, D-alpha-tocopherol, VE) antioxidants within H_{II} mesophases was reported by Bitan-Cherbakovsky et al. [21,22]. This enabled expanding conditions to obtain stable H_{II} mesophase at room temperature.

In addition, it was shown that phosphatidylcholine (PC) can be embedded into the ternary GMO/TAG/water system [23–26]. PC was incorporated into the ternary mesophases, as it is known that phospholipid-based structures possess relatively high thermal stability, and enhance transdermal drug permeation [27–29] and transmembrane transport across the digestive tract [30,31].

Incorporation of PC to the ternary system (Fig. 3A and B) caused competition for water binding between the hydroxyl groups of

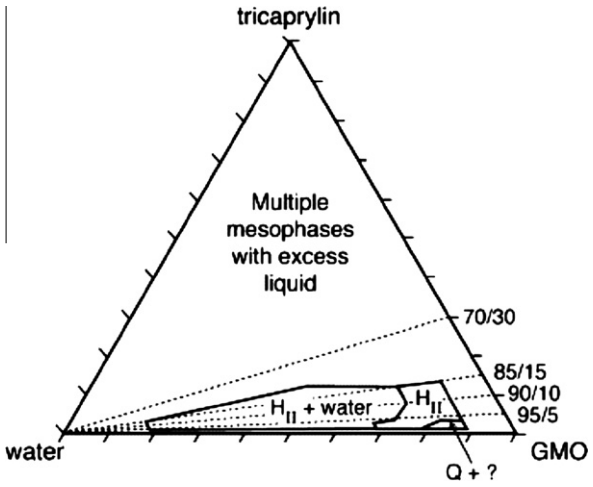

Fig. 2. The ternary phase diagram of GMO/tricaprylin/water at 25 °C. The dilution lines represent the surfactant/oil weight ratio (from Ref. [15] with permission).