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Full Length Article

Incorporation of antimicrobial peptides in nanostructured lipid membrane mimetic bilayer cubosomes

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ABSTRACT

The inverse bicontinuous lipidic cubic phase offers a simple and robust membrane mimetic with the ability to encapsulate peptides, potentially increasing bioavailability, while also offering a platform from which functionalized, targeted nanoparticles can be developed. Herein we have investigated the use of a number of cubic phase nanoparticle systems with encapsulated antimicrobial peptides gramicidin A', melittin, and alamethicin. The optimal peptide loading ranges, over which cubic symmetry was retained, were determined using small angle X-ray scattering. A large variation in peptide loading capability of different cubosome formulations was confirmed using circular dichroism. Observations are supported by particle sizing using dynamic light scattering as well as by direct visualization of nanoparticle morphology using cryogenic transmission electron microscopy. The results are discussed in relation to bilayer properties such as the hydrophobic mismatch between bilayer and peptide, intrinsic surface curvature, and lateral pressure profile of each lipid system. The findings of this study should be of use in the further development of lipid-based peptide encapsulation systems, particularly in the field of drug delivery.

1. Introduction

Breakthroughs in the large-scale production of various types of proteins have allowed for a new class of therapeutic agents to emerge in the form of biopharmaceuticals such as therapeutic proteins and peptides, nucleic acid-based medicines and monoclonal antibody-based products [1,2]. This therapeutic approach is rapidly expanding and every year hundreds of protein based-pharmaceuticals are tested as possible treatments for cancer, auto-immune disorders or cardiovascular diseases. Ensuring optimal delivery and efficacy for these therapeutics represents a major research challenge and significant efforts have converged towards the design and development of more sophisticated delivery systems, able to ensure controlled release of these bioactive compounds as well as protect the encapsulated therapeutic from denaturing processes such as enzymatic or acidic hydrolysis.

Lipidic inverse bicontinuous cubic phase nanoparticles (cubosomes) offer a simple and effective way to encapsulate small molecule drugs [3], peptides and proteins [4] in a lipid based, biomimetic environment. Their unique structural architectures, relative structural stability, dual polar/apolar nature, and partially tunable structural parameters allow for the engineering of highly specific, application-based hosting environments. As a result, these materials have generated strong interest in the field of drug delivery, as potential hosting matrices for bioactives with otherwise poor solubility or bioavailabilty [5,6]. The internal symmetry of these nanoparticles is comprised of a lipid bilayer, curved in three-dimensional space into a triply periodic minimal surface. Three architectures are observed experimentally: the gyroid (Q_{II}^{G} with space group Ia3d), the diamond (Q_{II}^{D} with crystallographic space group Pn3m), and the primitive (Q_{II}^{P} with space group Im3m) illustrated in Fig. 1. Their bilayer structure offers an excellent cellular membrane mimetic and may assist in retention of the native conformation of hydrophobic and amphiphilic proteins and peptides [7,8], which spontaneously reconstitute within the bilayer and simplify the process of encapsulation.

Antimicrobial peptides (AMPs) play an integral role in the innate defence against bacterial infections [9,10]. The ubiquity of these compounds and their continued presence in Nature over millions

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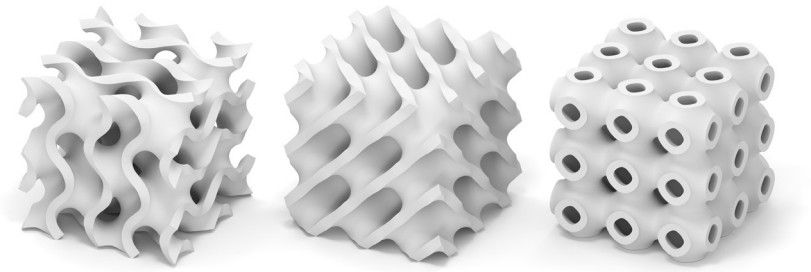

Fig. 1. 3D representations of the inverse bicontinuous cubic phases: Q_{II}^{G} with space group Ia3d (left), Q_{II}^{D} with crystallographic space group Pn3m (centre), and Q_{II}^{P} with space group Im3m (right).

of years suggests that they are less likely to induce antibiotic resistance and typically they display non-specific mechanisms of action [11]. These factors make AMPs an attractive and much needed alternative to conventional antibiotics [12]. However, AMPs have innate issues as therapeutic agents, which may include low solubility, bioavailability and stability. Their low specificity also affects their use as therapeutic agents [12]; while a given AMP may have a highly desirable activity against a range of bacteria, its haemolytic activity may preclude its use for anything but topical applications. A promising approach to address these issues is to encapsulate a peptide within a lipid-based nanoparticle, increasing its solubility and half-life, and potentially allowing for targeted delivery [13,14], which has been explored in studies of lipid nanoparticle-AMP interactions [15–19]. Moreover, due to the long polymer chains coating the outer layers of the particle, cubosomes have been shown to act as "stealth" nanoparticles, in that they remain in circulation much longer than non-structured lipid carriers (liposomes) [20].

Herein, we explore the ability of cubosomes to encapsulate AMPs to determine the optimal relationship between host structural symmetry, loading capacity and bioactive structure. We investigate the hierarchical relationship between the chemical structure of the host lipid, the three-dimensional structure of the cubosome, and the secondary structure of the peptide. Previous research has shown that encapsulation of an amphiphilic peptide or protein can significantly impact the nanostructure of the bulk cubic phase [21–23] and cubosomes [24], with a knock-on effect on their application as drug delivery vehicles. Using a combination of techniques, including small angle X-ray scattering (SAXS), cryogenic transmission electron microscopy (cryo-TEM) and dynamic light scattering (DLS), we have characterized the structure of the cubosome following peptide encapsulation. Circular Dichroism (CD) was used to monitor the secondary structure of encapsulated peptides and provide a qualitative indication of peptide loading efficiency.

Three AMPs, gramicidin A' (gA'), alamethicin and melittin, which vary in size, hydrophobicity and charge (Table 1) were investigated. Gramicidin A' is a 15 amino acid neutrally charged AMP, which exerts its action through the formation of a dimeric channel in the membrane of Gram-positive bacteria, allowing the free flow of monovalent cations between the intracellular and extracellular domains [25]. In a bilayer environment it forms either a double helix, or an end to end helical dimer, comprised of two β-helices, an unusual structure resulting from gramicidin's alternating D and L-residues [26]. The conformation responsible for gramicidin's antimicrobial activity remains the subject of debate [27], although recent work suggests that the helical dimer predominates in some phospholipid bilayers.

Similar to gA', alamethicin is a highly hydrophobic peptide, which exerts its action through disruption of the membrane. Neutrally charged at physiological pH, the behaviour of this α-helical, 20 amino-acid peptide in the bilayer depends on concentration. At low concentrations the peptide associates at the membrane surface,

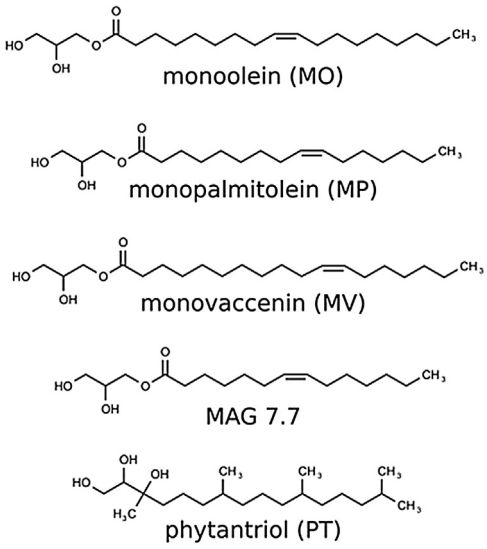

Fig. 2. The chemical structures of the five different lipids used in our cubosome formulations. Drawn using MarvinSketch 16.8.8.0.

while above a critical peptide to lipid ratio it forms pores consisting of a several monomers [28–30].

Unlike alamethicin and gA', at physiological pH melittin possesses 6 positive charges, resulting from its C-terminal glycine, 3 lysine, and 2 arginine residues. It contains a hydrophobic region in the α-helix conformation that aids in membrane association [31]. Its mechanism of action involves the formation of pores in the membrane, which similar to alamethicin occurs above a critical peptide to lipid ratio [32]. In addition to powerful antimicrobial properties, it has also demonstrated anti-cancer effects [33].

Peptides were encapsulated in cubosomes formed by monoacylglycerols (MAGs) with a range of chain architectures: monoolein (MO) C18, 9.9; monopalmitolein (MP) C16, 9.7; monovaccenin (MV) C18, 11.7 and 1-(7Z-tetradecenoyl)-rac-glycerol (MAG 7.7) C14, 7.7. In addition peptide encapsulation was investigated within cubosomes formed by the branched chain lipid phytantriol (PT). The chemical structures of these lipids are illustrated in Fig. 2. Concentrations in the range 0–10 mol% peptide were studied for gA' samples, and 0–2 mol% for alamethicin and melittin.

2. Experimental section

2.1. Materials

Monoolein (MO, ≥99%), Pluronic F127, cholesterol, gramicidin A' (sold as gramicidin D for Dubos), melittin (≥85%) and alamethicin (≥98%) were purchased from Sigma-Aldrich (St Louis, MO, USA). Monovaccenin (>99%) and monopalmi-