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Structure – membrane activity relationship in a family of peptide-based gemini amphiphiles: An insight from experimental and theoretical model systems

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ABSTRACT

A study of the interaction between five gemini amphiphilic valine-based pseudopeptides (GAPs) differing by the length of the central aliphatic spacer linking two amino acid subunits, and a model bacterial membrane lipid, 1,2-dimyristoyl-*sn*-glycero-3-phospho-*rac*-(1-glycerol) (DMPG), is here presented. Pure DMPG, pure GAPs and mixed GAPs/DMPG monolayers were formed at the air-water interface using Langmuir technique. The properties of the Langmuir films were investigated using surface pressure measurements, polarization modulation-infrared reflection-absorption spectroscopy, and Brewster angle microscopy. The atomic level information concerning the orientation of molecules in the monolayer and hydration of the polar headgroups was obtained from molecular dynamics simulations. It was demonstrated that the length of the central spacer in the GAPs structure is important for the properties of the mixed films; the disorganization of the membrane increases with the length of the spacer. The latter point is important for developing possible antimicrobial agents based on GAPs.

1. Introduction

Peptide-based gemini amphiphiles represent a class of molecules derived from amino acids and consisting of two hydrophilic headgroups and two hydrophobic tails linked together by a spacer [1–3]. The peptide-based gemini amphiphiles have a high surface activity compared to the conventional, one head-one tail surfactants [4,5]. These molecules have a high potential for practical applications in drug/gene delivery [6–9] and soil remediation [10–13]. Moreover, peptide-based gemini amphiphiles are biocompatible and biodegradable. The latter is important from the point of view of environmental protection [14]. Consequently, GAPs attract considerable attention in the domain of formulation and in developing of new products.

Lipid membranes play essential roles in living cells; their properties are associated with the presence of a very complex set of biomolecules including a large variety of lipid compounds. Different properties of the membranes are related to specific lipid structures [15,16]. In this regard, the analysis of the interaction of different molecules, including pseudopeptide gemini amphiphiles with membrane lipids is biologically relevant, as it can open the way to developing new antimicrobials and drug or gene delivery systems [17,18]. Phosphatidylglycerol (PG) is one of the main components of some bacterial cell membranes (20% in the case of *E. coli* [19]); it is present as well in plant and animal membranes. PG is involved in essential cellular processes such as DNA replication and protein translocation across the cytoplasmic membrane. Its absence results in defective DNA replication and lack of lipoprotein, leading to membrane welding and eventually cell death [20,21]. Moreover, in cyanobacteria and plants, PG appears to be essential for photosynthesis and growth [15]. In animal tissues, PG is the most abundant anionic phospholipid in lung surfactant and its deficiency in the pulmonary surfactant of infants leads to the respiratory distress syndrome, a potentially fatal diseases in newborns [22,23]. Phospholipid Langmuir films are often used as model systems to better understand the properties and function of biological cell membranes. This approach was used to study the interaction between membranes and antimicrobial peptides [24,25]. 1,2-dimyristoyl-*sn*-glycero-3-phospho-*rac*-(1-glycerol) (DMPG) is

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often used in such studies as a model bacterial membrane lipid. DMPG displays a characteristic thermo-structural behaviour that can be regulated by pH and the presence of ions and other species in the medium. It shows a gel-fluid transition at 23 °C under physiological conditions and may exhibit a melting regime (intermediate phase between the gel and the fluid) in which different thermodynamic phases and micro-domains can coexist [26,27]. Thus, DMPG is often considered as a good choice for the studying lipid-peptide interactions.

The results concerning the interaction of several gemini amphiphilic pseudopeptides (GAPs) with another membrane phosphoglyceride, namely cardiolipin (CL), were published in our previous paper [28]. In the present work, five valine-based GAPs, differing in the length of the central aliphatic spacer linking the amino acid subunits, were synthesized using previously reported synthetic procedures [29–32] and their interaction with 1,2-dimyristoyl-*sn*-glycero-3-phospho-*rac*-(1-glycerol) was investigated using surface pressure measurements, polarization modulation-infrared reflection-absorption spectroscopy (PM-IRRAS), as well as Brewster angle microscopy (BAM). Pure DMPG or GAPs as well as mixed GAP/DMPG monolayers were formed at the air-water interface using the Langmuir technique [33–36] and studied using different experimental methods. Moreover, molecular dynamics were performed to get more information concerning the degree of hydration of the lipid polar heads, as well as ordering of molecules in the pure and mixed monolayers.

2. Materials and methods

2.1. Materials

Synthetic DMPG (sodium salt, 99% pure) was purchased from Avanti Polar Lipids. The gemini amphiphilic pseudopeptides (GAPs) used here were:

GAP II: (2*S*)-2-({[4-(decyloxy)phenyl]methyl}amino)-*N*-{2[(2*S*)-2-({[4-(decyloxy) phenyl]methyl}amino)-3methylbutanamido] ethyl}-3-methylbutanamide, GAP III: (2*S*)-2-({[4-(decyloxy) phenyl]methyl}amino)-*N*-{3[(2*S*)-2-({[4-(decyloxy) phenyl] methyl}amino)-3methylbutanamido]propyl}-3-methylbutanamide, GAP IV: (2*S*)-2-({[4-(decyloxy)phenyl]methyl}amino)-*N*-{4[(2*S*)-2-({[4-(decyloxy) phenyl]methyl}amino)-3methylbutanamido]butyl}-3-methylbutanamide, GAP V: (2*S*)-2-({[4-(decyloxy)phenyl]methyl}amino)-*N*-{5[(2*S*)-2-({[4-(decyloxy) phenyl]methyl}amino)-3methylbutanamido]pentyl}-3-methylbutanamide, GAP VI: (2*S*)-2-({[4-(decyloxy)phenyl]methyl} amino)-*N*-{6[(2*S*)-2-({[4-(decyloxy) phenyl]methyl}amino)-3methylbutanamido]hexyl}-3-methylbutanamide.

These compounds were synthesized as previously described [29–32]. The purity of GAPs was at least 99.8%. The chemical structures of two GAPs with the extreme length of the central spacer, namely GAP II and GAP VI are shown in Fig. 1A and B, respectively. GAPs structures are shown in the Supplementary data (Fig. A1). Spectrophotometric grade chloroform and methanol (both from Sigma-Aldrich, ~99.9% pure) were used for preparing phospholipid and GAPs solutions. Aqueous subphases for monolayer experiments were prepared with MilliQ water, which had a surface tension of 72.8 mN m^{-1} at 20 °C, pH 5.6.

2.2. Compression isotherms and Brewster angle microscopy

The surface pressure (Π–A) measurements were carried out using a KSV 2000 Langmuir balance (KSV Instruments, Helsinki). A Teflon trough with two hydrophilic Delrin barriers (symmetric compression) was used in compression isotherm experiments. The surface pressure was measured by the Wilhelmy method using

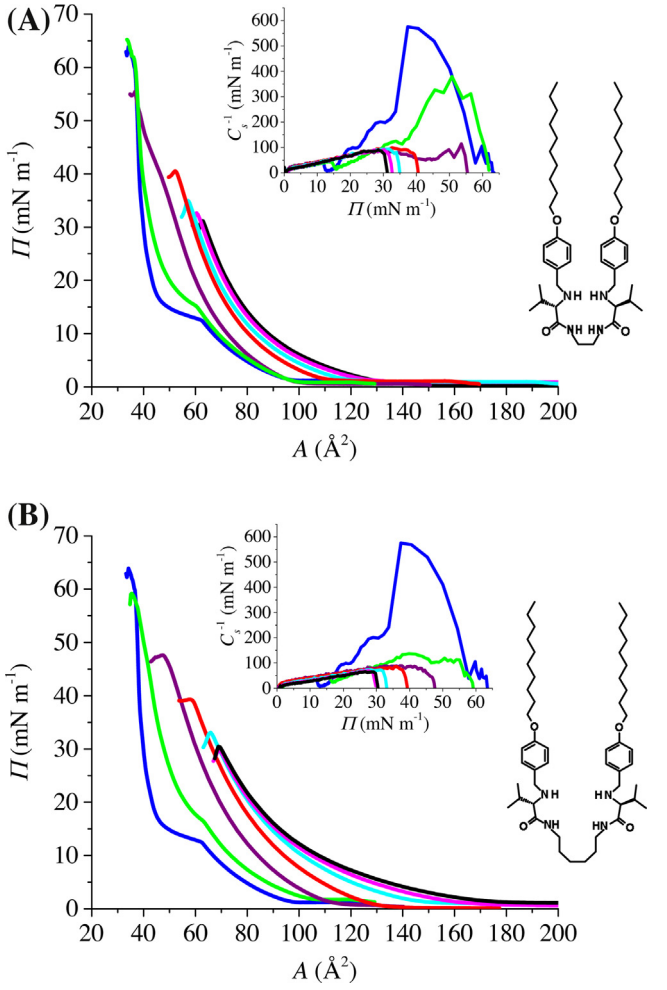

Fig. 1. Π–A isotherms of binary mixtures of GAP II/DMPG (A) and GAP VI/DMPG (B) spread on water at 20 °C. Blue: x_{GAP} = 0, green: 0.1, purple: 0.3, red: 0.5, cyan: 0.7, magenta: 0.9, and black: 1.0. Insets: C_s^{-1}–Π dependencies for mixed GAP II/DMPG (A) and GAP VI/DMPG (B) monolayers, as well as the chemical structures of GAP II (A) and GAP VI (B). The errors in C_s^{-1} are 2–3 orders of magnitude smaller than the reported values. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

platinum plate. The monolayers were spread from calibrated solutions (concentrations around 0.5 mg mL^{-1}) using a microsyringe (Hamilton Co., USA). The stock solution of DMPG was prepared in a chloroform/methanol mixture (4:1 v/v), while GAPs were dissolved in chloroform. Stock solutions of all compounds were used to prepare 0.1, 0.3, 0.5, 0.7, and 0.9 GAP/DMPG molar fraction mixtures. Each film was allowed to equilibrate and the solvent to evaporate for 15 min and then was compressed at a constant speed of 2.5 mm min^{-1} barrier^{-1}. For each monolayer composition, measurements were repeated at least three times. The isotherms were recorded at 20 ± 1 °C to avoid water evaporation at higher temperatures. The subphase temperature was maintained constant using Lauda RE 104 thermostat. It should be mentioned that calculation of thermodynamic parameters of the systems studied were done at a biologically relevant surface pressure of 28 mN m^{-1}; consequently, the impact of temperature on the interpretation of the results can be neglected.

The standard deviation obtained from compression isotherms was ±0.5 Å^2 on molecular area, and ±0.2 mN m^{-1} on surface pressure.