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Effects of gemini amphiphilic pseudopeptides on model lipid membranes: A Langmuir monolayer study

Jenifer Rubio-Magnieto[a,b], Santiago V. Luis[b,**], Monika Orlof[c], Beata Korchowiec[c], Guillaume Sautrey[a], Ewa Rogalska[a,*]

[a] Structure et Réactivité des Systèmes Moléculaires Complexes, BP 239, CNRS/Université de Lorraine, 54506 Vandoeuvre-lès-Nancy cedex, France
[b] Departamento de Química Inorgánica y Orgánica, Universidad Jaume I, Av. Sos Baynat, s/n 12071 Castellon, Spain
[c] Department of Physical Chemistry and Electrochemistry, Faculty of Chemistry, Jagiellonian University, ul. R. Ingardena 3, 30-060 Krakow, Poland

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ABSTRACT

Monolayers formed with 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine, 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine, 1,2-dimyristoyl-*sn*-glycero-3-[phospho-*rac*-(1-glycerol)] and 1,2-dipalmitoyl-*sn*-glycero-3-[phospho-*rac*-(1-glycerol)] at the air/water interface were used as model membranes for studying a potential biological activity of four newly synthesized gemini amphiphilic pseudopeptides (GAPs); some of the GAPs studied showed interesting self-assembly properties. The capacity of GAPs to self-assemble in different environments let us think that these molecules may find biomedical applications in, e.g., drug delivery or transfection. The surface pressure–area and surface potential–area compression isotherms, as well as Brewster angle microscopy and polarization-modulation infrared reflection–absorption spectroscopy were used to study monolayers formed with pure GAPs, pure lipids and lipid/GAPs mixtures.

The results obtained show that all four GAPs studied can be incorporated in lipid monolayers. The monolayers containing GAPs are expanded and more liquid-like compared to pure lipids. The overall results indicate that the important changes of the properties induced in the model membranes by GAPs are related to their intrinsic conformational flexibility. This feature of GAPs can be easily adjusted by engineering the structure of the spacer present in the polar head, with the aim to modify lipid membranes in a controlled way.

1. Introduction

Gemini amphiphiles [1] consist of two polar and two apolar moieties linked together with a spacer; in this work gemini amphiphilic pseudopeptides (GAPs) were used [2–5]. Majority of geminis have symmetrical structures with two identical polar groups and two identical apolar chains [6]. The combination of polar amino acids/peptides (hydrophilic moiety) [7] and apolar long-chain molecules (hydrophobic moiety) yielded amphiphiles with high surface activity [8]. This property makes gemini amphiphiles more interesting from the point of view of possible applications compared to the conventional, one tail–one head surfactants [9]. In recent years, these compounds attracted considerable attention in soil remediation, enhanced oil recovery, drug entrapment and release, as well as in preparation of high-porosity materials [10,11]. More recently, gemini amphiphiles were studied as potential synthetic vectors for delivering genes into cells to induce protein expression [12–17].

The present work involves new GAPs synthesized recently [18–20]. Because these molecules are amphiphilic [21–23], it was interesting to study their behavior in membrane-like lipid environment. Indeed, depending on the interaction with biological barriers, GAPs could be possibly used in, e.g., drug delivery or transfection [14,24]. However, before any potential application of GAPs related to membranes could be envisaged, a molecular-level understanding of interaction with phospholipids is necessary. As demonstrated in the literature, changes in the lipid composition of cells and tissue may alter biophysical interactions, which could be explored to develop target-specific drugs and drug delivery systems [25]. In particular, the effects linked to the charge of the lipid polar head and to the length of the hydrocarbon chains should be discerned. To this end, single-component model membranes must be used. While such models are different compared to multicomponent biological membranes, they offer a handle on the mechanism of lipid-exogenous molecule interaction. Here, to get more insight in the behavior of GAPs in a membrane environment, a recently

* Corresponding author. Tel.: +33 3 83 68 43 45; fax: +33 3 83 68 43 22.
** Corresponding author. Tel.: +34 964 72 82 39; fax: +34 964 72 82 14.
E-mail addresses: luiss@qio.uji.es (S.V. Luis), ewa.rogalska@univ-lorraine.fr, rogalska@uhp-nancy.fr (E. Rogalska).

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synthesized family of four structurally closely related molecules with C_2 symmetry [22] was studied in mixed monolayers formed with chosen phospholipids.

The Langmuir technique allows preparation of model membranes by spreading phospholipid monolayers at the air/water interface [26,27]. This technique offers a unique possibility of investigating the interaction between membranes formed and different molecules [25,28–34]. The choice of the lipids used in this study was dictated by the information, which can be obtained with some of them and was unrelated to the composition of defined cell membranes. Namely, 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine (DMPE) was used as a model zwitterionic lipid because, contrary to phosphocholines, it offers a well-known possibility of studying the morphology of the monolayer in the phase transition region with Brewster angle microscopy (BAM) [27,35,36]. Consequently, 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine (DPPE) was used, to compare the effect of the chain length. These two zwitterionic lipids were compared in turn with the negatively charged 1,2-dimyristoyl-*sn*-glycero-3-[phospho-*rac*-(1-glycerol)] (DMPG), which can be studied using BAM as well [37], and 1,2-dipalmitoyl-*sn*-glycero-3-[phospho-*rac*-(1-glycerol)] (DPPG); lipid/GAPs mixtures in different molar proportions were used as well. It is important to mention that the Langmuir technique allows adjusting the physical state of the monolayer. Consequently, the fluidity of the monolayers formed with phospholipids bearing saturated hydrocarbon chains can be compared to that of biological membranes. The monolayers were studied using surface pressure and surface potential measurements, polarization modulation-infrared reflection–absorption spectroscopy (PM-IRRAS), as well as BAM. The results obtained show that GAPs studied in this work modify properties of phosphoglyceride films. On the other hand, GAPs undergo conformational rearrangement upon the effect of the phospholipid monolayer. We propose that the conformation of GAPs could be modified via different parameters such as pH or ion complexation and thus be used as a switch for modifying lipid membranes in a controlled manner.

2. Materials and methods

2.1. Materials and reagents

DMPE, DPPE, DMPG and DPPG (all 99% pure) were from Sigma. The GAPs used here were: **GAP I**: (2*S*)-2-({[4-(decyloxy)phenyl]methyl}amino)-*N*-{4[(2*S*)-2-({[4-(decyloxy) phenyl]methyl}amino)propanamido]butyl}propanamide.

GAP II: (2*S*)-2-({[4-(decyloxy)phenyl]methyl}amino)-*N*-{4[(2*S*)-2-({[4-(decyloxy)phenyl]methyl}amino)-3methylbutanamido]butyl}-3-methylbutanamide.

GAP III: (2*S*)-2-({[4-(decyloxy)phenyl]methyl}amino)-*N*-{4[(2*S*)-2-(([4-(decyloxy)phenyl]methyl}amino)-3phenylpropanamido]butyl}-3-phenylpropanamide.

GAP IV: (2*S*)-2-({[4-(decyloxy)phenyl]methyl}amino)-*N*-{4[(2*S*)-2-({[4-(decyloxy)phenyl]methyl}amino)-4methylpentanamido]butyl}-4-methylpentanamide.

These molecules were synthesized, purified and fully characterized as described previously [18,21,22]. All GAPs are at least 99.8% pure. MilliQ water used in the experiments had resistivity of 18.2 MΩ cm at 25 °C and a surface tension of 72.8 mN m^{-1} at 20 °C, pH 5.6. Chloroform and methanol (both ~99.9% pure), used for preparing phospholipid and GAPs solutions, were from Sigma–Aldrich.

It should be noted that at pH 5.6 an average charge in GAPs is close to 2+ due to coexistence of predominant diprotonated and minor monoprotonated species, as indicated by distribution diagrams [38].

2.2. Compression isotherms and Brewster angle microscopy

The surface pressure and electric surface potential measurements were carried out with a KSV 2000 Langmuir balance. A Teflon trough (6.5 cm × 58 cm × 1 cm) with two hydrophilic Delrin barriers (symmetric compression) was used in compression isotherm experiments. The system was equipped with an electrobalance and a platinum Wilhelmy plate (perimeter 3.94 cm) as a surface pressure sensor. Surface potential was measured using a KSV Spot 1 with a vibrating plate electrode and a steel counter electrode immersed in water (4 mm). The apparatus was closed in a Plexiglas box, and temperature was kept constant at 20 °C. Before each run, the trough and the barriers were washed using cotton soaked in chloroform and ethanol and then rinsed with MilliQ water. The platinum Wilhelmy plate was cleaned between each experiment by rinsing with purified water and ethanol and heating to a red glow. All solvents used for cleaning the trough and the barriers were of analytical grade. Aqueous subphases for monolayer experiments were prepared with MilliQ water. All impurities were removed from the subphase surface by sweeping and suction. When the surface pressure fluctuation was found to be lower than 0.2 mN m^{-1} during a compression stage, monolayers were spread from DMPE, DPPE, DMPG or DPPG solutions (concentration ~0.6 mg mL^{-1}) in chloroform/methanol mixture (4:1 v/v; 13.3% w/w of methanol) using a microsyringe (Hamilton Co., USA). In the case of GAPs, 32–36 μL of chloroform solutions with concentrations ~0.7 mg mL^{-1} were spread on the subphase. Moreover, 0.1 and 0.5 mol fraction of phospholipid/GAPs mixtures were prepared using solutions of phospholipids and GAPs (final concentration ~0.9 μmol mL^{-1}); 38–70 μL were spread on the subphase to form monolayers. The stability of the surface potential signal was checked before each experiment, after cleaning the subphase surface. After the ΔV signal had reached a constant value, it was zeroed and the film was spread on the subphase. After the equilibration time of 15 min, the films were compressed at the rate of 10 mm min^{-1} by two symmetrically moving barriers (5 mm min^{-1} per barrier; in the case of pure phospholipids it corresponds to ~2 and ~2.7 Å^2 molecule^{-1} min^{-1} at the beginning and at the end of the compression, respectively). A PC computer and KSV software were used to control the experiments. Each compression isotherm was performed at least three times. Standard deviations were ±0.5 Å^2, ±0.2 mN m^{-1} and ±0.01 V with mean molecular area, surface pressure and surface potential measurements, respectively. The compression isotherms allowed determining the compressibility modulus [26,39], C_S^{-1}, as:

$$C_S^{-1} = -A\left(\frac{\partial \Pi}{\partial A}\right)_T \quad (1)$$

The collapse parameters ΔV_{coll}, Π_{coll} and A_{coll} were determined directly from the compression isotherms at the point of the highest C_S^{-1}.

The morphology of the studied films was visualized using the same Langmuir balance combined with a Brewster angle microscope (KSV Optrel BAM 300, Helsinki, Finland). The light source was a standard HeNe laser, 10 mW, 633 nm; the spatial resolution of the device is 2 μm.

2.3. Polarization-modulation infrared reflection–absorption spectroscopy

The PM-IRRAS [40–44] spectra of pure phospholipids, pure GAPs and phospholipid/GAPs mixtures spread on pure water were acquired at 20 °C. The Teflon trough dimensions were 36.5 cm (l) × 7.5 cm (w) × 0.5 cm (d); the monolayers were prepared as described in Section 2.2. The PM-IRRAS measurements were