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Interaction of cyclic and linear Labaditin peptides with anionic and zwitterionic micelles

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ABSTRACT

Conformational changes of the cyclic (Lo) peptide Labaditin (VWTVWGTIAG) and its linear analogue (L_1) promoted by presence of anionic sodium dodecyl sulfate (SDS) and zwitterionic L-α-Lysophosphatidylcholine (LPC) micelles were investigated. Results from λ_{max} blue-shift of tryptophan fluorescence emission combined with Stern–Volmer constants values and molecular dynamics (MD) simulations indicated that L_1 interacts with SDS micelles to a higher extent than does Lo. Further, the MD simulation demonstrated that both Lo and L_1 interact similarly with LPC micelles, being preferentially located at the micelle/water interface. The peptide–micelle interaction elicits conformational changes in the peptides. Lo undergoes limited modifications and presents unordered structure in both LPC and SDS micelles. On the other hand, L_1 displays a random-coil structure in aqueous medium, pH 7.0, and it acquires a β-structure upon interaction with SDS and LPC, albeit with structural differences in each medium.

1. Introduction

It is well established that cyclic peptides (CPs), which are more resistant to proteolytic degradation than their linear isomers [1–4], form internal hydrogen bonds that facilitate their membrane insertion [4,5]. In addition, the more restricted conformational flexibility of CPs leads to an enhancement of their affinity and specificity to receptors [2,4–7].

CPs belonging to the *Caryophyllaceae* group consist of 7–10 aminoacids with a high proportion of hydrophobic residues [8–12] being recently named as Orbitide [13]. Biobollein (9 residues) and Labaditin (10 residues), isolated from *Jatropha Multifida*, were the first CPs of this group of peptides described in the literature [8,14,15]. One recent review about *Jatropha species* presented 19 cyclic peptides isolated from this family [16]. In particular, it has been shown that Labaditin (VWTVWGTIAG), a highly hydrophobic CP from a popular plant known as Jarak gurita (Indonesia) and also Mana (Philippines) [15], has antibacterial and acetylcholinesterase activities [14]. Moreover, Labaditin inhibits the classical pathway of human complement activation *in vitro* [14,16]. It binds to aggregated and antigen-bound IgG, mostly blocking the antibody Clq acceptor site, which is restricted to IgG subclass IgGl [15,17].

Although the biological activity of CPs is well reported, the mechanism of action of such peptides on the molecular level is poorly comprehended. In this context, it is of importance to explore the role of hydrophobic and polar environment to the peptide affinity by mimicking the biological membrane. The hydrophobic medium certainly plays an important role in peptide partitioning [18–20], since many hydrophobic amino acid residues, especially aromatic ones, are favorably distributed in the aqueous/lipid membrane interface [21,22]. This might explain structural changes imparted by bioactive molecules in contact with the membrane as well as their regulation mechanisms [23].

In this work, we investigate the behavior of the cyclic peptide Labaditin (Lo) in comparison with its linear analog (L_1) when in contact with micelles as model membranes. Lysophosphatylcholine (LPC) and sodium dodecyl sulfate (SDS) micelles were chosen because LPC is structurally similar to PC-based lipids commonly found in mammalian cells [24], whereas the presence of negative net charge in SDS micelles may help us to understand how the surface charge in the bacterial membrane influences the peptide binding. Fluorescence spectroscopy and circular dichroism (CD) techniques were employed to evaluate how the micellar environment governs the interaction and conformational changes of Lo

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and L_1 whereas molecular dynamics (MD) simulation allows for better describing the peptides location in the micelles.

2. Materials and methods

2.1. Material

All the solutions were prepared using Millipore Direct-Q ultra pure apyrogenic water. All reagents were of the highest commercially available purity grade; L-α-lysophosphatidylcholine (LPC), sodium dodecyl sulfate (SDS) and acrylamide were purchased from Sigma–Aldrich.

2.2. Peptide synthesis, cyclization and purification

Linear peptide L_1 (VWTVWGTIAG) was synthesized by Solid-Phase Peptide Synthesis procedure (SPPS) as previously described in Barbosa et al. [14]. After cleavage, Lo was obtained by L_1 cyclization. Peptides Lo and L_1 were purified by semi-preparative reversed phase HPLC C_{18} column and identified by electrospray mass spectrometry. All the details of the synthesis and purification processes are available in Barbosa et al. [14].

2.3. Circular dichroism (CD) spectroscopy

The CD spectra were recorded at 25 °C on a Jasco 810 spectropolarimeter. Lo or L_1, 100 μM, pH 7.0 were placed in a quartz cuvette with an optical path length of 0.1 cm and purged with nitrogen gas. Different concentrations of SDS (0–50 mM) or LPC (0–10 μM) were added to Lo or L_1, and each mixture was incubated for 30 min. The spectra were recorded from 250 to 190 nm, with 1 nm spectral bandwidth, scan speed of 100 nm min^{-1}, and 2 s time response, to minimize noise. The CD spectra correspond to the accumulation of ten runs after subtraction of the buffer spectrum.

2.4. Tryptophan fluorescence spectroscopy assays

Fluorescence was measured on a Spectronic SLM 8100 spectrofluorometer. Tryptophan groups were excited at 280 nm whereas the emission spectra were recorded from 300 to 500 nm, at 25 °C, pH 7.0 and quartz cells with optical path of 0.1 cm. The slit width at the excitation and the emission of the spectroflorimeter were 1 nm. The peptide emission spectra were subtracted from the peptide-free solution spectrum.

The fluorescence intensity and maximum wavelengths (λ_{max}) were obtained using an aqueous solution containing 10 μM of each peptide (Lo and L_1) in the absence and presence of different SDS concentrations (0–50 mM) and LPC (0–50 μM) varying a peptide/detergent mol ratio up to 2×10^{-4} and 2×10^{-1}, respectively.

Peptide interaction with micelles was also characterized by tryptophan fluorescence quenching by acrylamide, in the presence and absence of micelles, according to the Stern–Volmer equation:

$$Fo/F = 1 + K_{sv} \times [Q],$$

where Fo and F are the fluorescence intensities in the absence and presence of the quencher, respectively; $[Q]$ is the concentration of the quencher; and K_{sv} is the Stern–Volmer quenching constant. Fluorescence quenching measurements of tryptophan with the quencher were accomplished by adding aliquots (0.2 μL) of acrylamide stock solution (6 M) to a cuvette containing a fixed solution of peptide (10 μM) and another one with SDS and LPC solutions containing a peptide/detergent mol ratio of 2×10^{-3} and 5×10^{-3}, respectively.

2.5. Molecular dynamics simulation

The molecular dynamics (MD) studies were performed following the scheme represented in Fig. 1. To obtain the peptide–micelle complexes, the target systems were simulated by MD using coarse-grained (CG) representations of the molecules and the Martini force field [25,26]. Then, the atomistic structures of the peptide–micelle complexes were reconstructed from their corresponding CG representations using the algorithm developed by Rzepiela et al. [27]. Finally, the resulting systems were subjected to MD simulations with the GROMOS96 53a6 force field [28], for data analysis. All these simulations were carried out with the GROMACS 4.0.5 simulation package [29].

We conducted MD studies of linear and cyclic peptide with SDS and LPC micelles. For this purpose, CG systems containing the micelles in cubic boxes were prepared and equilibrated for 500 ns. A system was constructed with 60 SDS molecules, 60 Na^+ ions and 18,244 water molecules; another system was constructed with 100 LPC molecules and 24,740 water molecules. The force field parameters for the CG model of SDS were the same as those described by Jalili and Akhavan [30], while the parameters for LPC were derived from the DPPC force field [25]. Four other systems were then constructed from these systems, each containing a peptide, cyclic or linear, close to a micelle, SDS or LPC, and their water molecules and ions. Water molecules and ions superimposed with peptide atoms were removed, and the ions were added in new positions. For systems containing the linear peptide, one Na^+ and one Cl^- ion were inserted into electrostatically favorable positions, to neutralize the peptide charges. Simulations of these systems started with energy minimization using a steepest-descent algorithm, to eliminate bad contacts and undesirable forces. The MD simulations were carried out for 5 ns, with an integration time step of 10 fs; position restrictions were applied to the atomic coordinates of the peptides. Finally, all the restrictions were removed, and the simulations were carried out for 1 μs with time step of 30 fs, at 300 K. The parameters used in these simulations are the same described by Monticelli et al. [26] and are available at http://md.chem.rug.nl/marrink/coarsegrain.html.

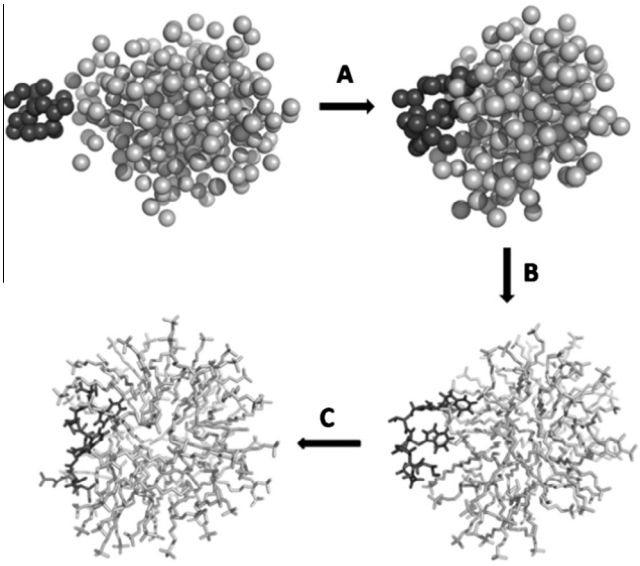

Fig. 1. Simulation scheme, considering the system containing the linear peptide with SDS micelle. The water molecules and ions have been removed for better visualization. (A) Formation of the peptide–micelle complex by CG simulation; (B) reconstruction of the atomic details of the peptide–micelle complex; (C) simulation of the complex with atomic details.