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Conformational and functional studies of gomesin analogues by CD, EPR and fluorescence spectroscopies

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Abstract

The aim of this work was to examine the bioactivity and the conformational behavior of some gomesin (*Gm*) analogues in different environments that mimic the biological membrane/water interface. Thus, manual peptide synthesis was performed by the solid-phase method, antimicrobial activity was evaluated by a liquid growth inhibition assay, and conformational studies were performed making use of several spectroscopic techniques: CD, fluorescence and EPR. [TOAC^{1}]-*Gm*; [TOAC^{1}, Ser^{2,6,11,15}]-*Gm*; [Trp^{7}]-*Gm*; [Ser^{2,6,11,15}, Trp^{7}]-*Gm*; [Trp^{9}]-*Gm*; and [Ser^{2,6,11,15}, Trp^{9}]-*Gm* were synthesized and tested. The results indicated that incorporation of TOAC or Trp caused no significant reduction of antimicrobial activity; the cyclic analogues presented a β-hairpin conformation similar to that of *Gm*. All analogues interacted with negatively charged SDS both above and below the detergent's critical micellar concentration (cmc). In contrast, while *Gm* and [TOAC^{1}]-*Gm* required higher LPC concentrations to bind to micelles of this zwitterionic detergent, the cyclic Trp derivatives and the linear derivatives did not seem to interact with this membrane-mimetic system. These data corroborate previous results that suggest that electrostatic interactions with the lipid bilayer of microorganisms play an important role in the mechanism of action of gomesin. Moreover, the results show that hydrophobic interactions also contribute to membrane binding of this antimicrobial peptide.

Keywords: Gomesin; Antimicrobial peptide; Solid-phase peptide synthesis; Electron paramagnetic resonance; Circular dichroism; Fluorescence

1. Introduction

There is an increasing interest on the pharmacological application of antimicrobial peptides (AMPs) to treat infections due to the growing problem of pathogenic organisms resistant to conventional antibiotics. AMPs are widely distributed in nature and represent an ancient host defense mechanism present in organisms across the evolutionary spectrum. They are key elements of the innate immune defense against bacteria, fungi and other pathogenic agents, generally displaying minimal toxicity towards mammalian cells [1,2].

Since the original report on cecropin [3] – the first inducible antibacterial peptide isolated from an insect – a large number of AMPs have been found, especially in arthropods, over the last two decades (http://www.bbcm.univ.trieste.it/~tossi/ pag1.htm). Most of these peptides share some common features, such as low molecular weight (less than 5 kDa), positive net charge (at physiological pH) and amphipathic character. Among them, the cysteine-rich family has been largely investigated in the past years [4], since the maintenance of at least one intramolecular disulfide bridge seems to be fundamental to maintain antimicrobial activity [5,6].

Abbreviations: AMP, antimicrobial peptide; CD, circular dichroism; cmc, critical micelle concentration; EPR, electron paramagnetic resonance; LC/ESI-MS, liquid chromatography electrospray ionization mass spectrometry; LPC, lysophosphatidylcholine; MBHAR, 4-methylbenzhydrylamine-resin; NMR, nuclear magnetic resonance; PB, poor broth; PDB, potato dextrose broth; RP-HPLC, reversed phase high performance liquid chromatography; SDS, sodium dodecyl sulfate; t-Boc, tert-butyloxycarbonyl; TEAP, triethylammonium phosphate; TFA, trifluoroacetic acid; TFE, 2,2,2-trifluoroethanol; TOAC, 2,2,6,6-tetramethylpiperidine-1-oxyl-4-amino-4-carboxylic acid

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A large number of AMPs are thought to act at the membrane level, inducing membrane thinning, pore formation, and bilayer disruption, depending on the molecular properties of both peptide and membrane lipid. Some of these transmembrane structures are termed wormholes or toroidal pores, membrane channel or barrel-stave [2,7]. The initial interaction of cationic AMP, such as the tachyplesins [8], with the membrane of microorganisms involves the binding of the peptides parallel to the membrane surface at the interface between the phospholipid head groups and fatty acid chains of the outer monolayer. It seems that some AMPs interact electrostatically with the phospholipid head groups leading the disruption of the bacterial inner membrane, whereas some others enter the cells inhibiting the synthesis of specific macromolecules [9]. Thus, diverse models proposed how the peptides reorient perpendicular to the plane of the membrane and the consequences thereof, even though the exact killing mechanism is not clearly understood.

Recently, a novel AMP – gomesin (*Gm*) – was purified and characterized from hemocytes of the Brazilian spider *Acanthoscurria gomesiana* [10]. The peptide contains eighteen residues, including four cysteines that form two disulfide bridges: Cys^{2-15} and Cys^{6-11}. The native molecule (ZCRRLCYKQRCVTYC-RGR-NH_2) has a molecular mass of 2270.4 Da, contains a pyroglutamic acid (Z) at the N-terminus and is amidated at the C-terminus.

NMR studies confirmed that the structure of gomesin consists of a well-resolved two-stranded antiparallel β-sheet connected by a noncanonical β-turn [11]. It shows sequence similarities to tachyplesin [12] and polyphemusin [13] from horseshoe crabs and its structural features resemble those of protegrins (AMPs isolated from porcine leukocytes [14]) and androctonins (isolated from the blood of a scorpion [15]). A comparison between the structures of all these AMPs brings about several common features in the distribution of hydrophobic and hydrophilic residues. The N- and C-termini, the β-turn and one face of the β-sheet are hydrophilic, whereas the hydrophobicity of the other face depends on peptide sequence. These similarities suggest that all molecules interact with membranes in an analogous manner [11].

Gomesin is effective against Gram-positive and Gram-negative bacteria, fungi, yeast, and it affects the viability of the parasite *Leishmania amazonensis* [10]. Due to its strong efficacy on a broad range of microorganisms, including human pathogens, and to structural properties that confer the molecule high stability, this peptide shows an interesting potential as a model for conformational studies, especially in different solvent environments (SDS, LPC and TFE) that mimic the biological membrane/water interface.

Among the spectroscopic techniques available for the investigation of the conformation and dynamics of peptides, spin labeling electron paramagnetic resonance (EPR) has been used to a considerable extent. This approach enjoyed notable expansion following the introduction of the paramagnetic amino acid 2,2,6,6-tetramethyl-piperidine-1-oxyl-4-amino-4-carboxylic acid (TOAC), which, for the first time, allowed the incorporation of the spin probe into the peptide via a peptide bond [16]. Owing to these unique characteristics a number applications have appeared in the last years, both from our own [17,18] and from other groups [19]. Besides being employed in structural studies of peptides, TOAC has also been used advantageously to monitor peptide chain assembly in polymer backbones [20,21]. As emphasized in earlier reports, due to the special rigidity of its covalent bond to the peptide molecule, the TOAC moiety is highly sensitive to the peptide backbone conformation and dynamics. In addition, fluorescence and circular dichroism (CD) spectroscopies are very well known techniques to study peptide conformation [22].

The aim of this work was to correlate structural and functional properties of several gomesin analogues. The conformational behavior of the peptides was investigated by means of spectroscopic techniques: EPR, fluorescence and CD. EPR spectra were obtained for the analogue containing TOAC as the N-terminal residue of gomesin ([$TOAC^1$]-*Gm*) and its corresponding linear form, [$TOAC^1$, $Ser^{2,6,11,15}$]-*Gm*. Fluorescence studies were performed with tryptophan-labeled analogues, [Trp^7]-*Gm* and [Trp^9]-*Gm* and their corresponding linear analogues, [$Ser^{2,6,11,15}$, Trp^7]-*Gm* and [$Ser^{2,6,11,15}$, Trp^9]-*Gm*. CD spectra were obtained for all analogues. The analogues were synthesized chemically and characterized, and their antimicrobial activity was evaluated by a liquid growth inhibition assay against *B. megaterium*, *E. coli* and *C. albicans.*

2. Materials and Methods

2.1. Microorganisms

Three microbial strains were used in this study: *Escherichia coli* SBS 363 was donated by P.L. Poquet (CEA, Paris, France); *Candida albicans* MDM8 and *Bacillus megaterium* were obtained from the collection of the Department of Microbiology, Institute of Biomedical Sciences, University of São Paulo (São Paulo, Brazil).

2.2. Chemicals

Nα-*tert*-butyloxycarbonyl (Boc) amino acids were from Bachem, Torrance, CA. Solvents, SDS, LPC and reagents were from Sigma-Aldrich Co, St. Louis, MO. DMF was distilled (over P_2O_5 and ninhydrin under reduced pressure) before use. All chemicals met ACS standards.

2.3. Peptide synthesis

Peptides were synthesized manually by solid-phase methodology on 4-methylbenzhydrylamine-resin (MBHAR) (0.8 mmol/g) using the t-Boc strategy [23]. Due to the presence of five Arg (Tos), full deprotection and cleavage from the resin were carried out using anhydrous HF treatment with anisole and dimethyl sulfide (DMS) as scavengers during 2 h at 0 °C. Fmoc-TOAC was synthesized in our laboratory, following the procedure published elsewhere [16,24,25]. Disulfide bridge formation was achieved on crude peptides following HF cleavage at pH 6.8–7.0 and 5 °C for 72 h. The resulting lyophilized peptides were purified by preparative RP-HPLC (column Vydac C_{18} (25×250 mm, 300 Å pore size, 15 μm particle size) in two steps. The first was performed by using TEAP pH 2.25 as solvent A and 60% CH_3CN in A as solvent B. The second step was carried out using 0.1% TFA/H_2O as solvent A and 60% CH_3CN/H_2O as solvent B. Pure peptides were characterized by amino acid analysis and reverse-phase liquid chromatography coupled to mass spectrometry electrospray ionization (LC/ESI-MS).

2.4. Antimicrobial activity

The microbial strains used were *Bacillus megaterium* (Gram-positive bacteria), *Escherichia coli* (Gram-negative bacteria) and *Candida albicans*

(yeast). The antibacterial and antifungal activities were monitored by a liquid growth inhibition assay as described earlier [26]. Microbial growth was assessed by changes in optical density of the culture suspension as an increase in $A_{595\,nm}$. The minimal inhibitory concentration (MIC) was determined after 16 h incubation at 30 °C. Cells (10^6 CFU/mL) were incubated in PB or 1/2PDB media, and MICs were expressed as the [*a*]–[*b*] interval of concentrations, where [*a*] is the highest concentration tested at which the microorganisms grow and [*b*] is the lowest concentration that causes 100% growth inhibition [27]. All experiments were performed in triplicate.

2.5. CD studies

CD measurements were performed on a Jasco J-810 spectropolarimeter thermostated at 20 °C and continually flushed with ultra-pure nitrogen. CD spectra were recorded using a 1 mm path length rectangular quartz cell, with four accumulations at 50 nm/min scan speed, 8 s response time, 0.5 nm spectral bandwidth and wavelength range of 260 to 190 nm. The spectra were obtained in water, water: TFE (0–90%, v/v) and in the presence of SDS (1–50 mM) and LPC (1–50 mM). Samples were prepared by diluting the peptides stock solutions (1 mM solution in water, pH 4) to obtain a final concentration of 0.1 mM. The results are expressed in terms of molar ellipticity [θ] in units of degrees cm^2 $dmol^{-1}$.

2.6. EPR studies

EPR measurements were carried out at 9.5 GHz in a Bruker ER 200D-SRC spectrometer at room temperature (22±2 °C) using flat quartz cells. The magnetic field was modulated with amplitudes less than one-fifth of the line widths, and the microwave power was 5 mW to avoid saturation effects. Rotational correlation times, τ_C and τ_B, were calculated according to Schreier et al. [28].

2.7. Fluorescence studies

Emission spectra were recorded on a Hitachi F4500 spectrofluorometer linked to a PC microprocessor with Spectro WinLab software. The excitation wavelength was 295 nm, and the excitation and emission slit widths were 5 and 10 nm, respectively. Emission spectra were acquired in the 310–400 nm range at 1 nm increments with a scan speed of 20 nm/min at 25 °C. The peptide concentrations were approximately 0.3 μM in water, while SDS and LPC concentrations varied from 1 to 50 mM.

3. Results and discussion

The MICs of gomesin analogues against strains of bacteria and yeast were determined using liquid growth inhibition assays (Table 1). The linear analogues were always less active against *B. megaterium*, *E. coli.* and *C. albicans* than the corresponding cyclic ones, corroborating our previous data that revealed that at least one disulfide bridge is essential to maintain gomesin secondary structure and antimicrobial activity [6]. In fact, the linear analogues were 8 to 32-fold less active than the native peptide in all tests. On the other hand, the cyclic analogues were almost as active as gomesin (up to 4-fold less active) in all microorganism strains. Taken together these results indicate that the incorporation of TOAC in position 1 or the replacement of Tyr^7 and Gln^9 by Trp in the primary sequence of gomesin was well tolerated and generated active analogues.

The above observations correlate very well with CD spectra of the analogues (Fig. 1) that show that gomesin and the cyclic analogues have the tendency to adopt β-turn conformation in water, SDS (Fig. 1A and G), LPC (Fig. 1B and H), and TFE (Fig. 1C and I), as evinced by the strong negative band at 205 nm and the positive one at 232 nm, characteristic of a β-hairpin-like structure. Varying the environment did not significantly affect their conformations, as observed in Fig. 1. Such features are fundamental for the high efficacy of cyclic analogues against the microorganisms tested.

The CD spectra of the linear analogues in water showed a negative band at 198 nm, indicative of unordered structure (Fig. 1). When 1 mM SDS was added (Fig. 1D and J), the spectra presented a positive band at 192 nm and a negative one at 216 nm, characteristic of β-sheet conformation [29]. As the critical micelle concentration (cmc) of this surfactant in water is ca 8 mM [30], the CD spectra show that SDS monomers are able to induce drastic conformational changes in the linear peptides. In addition, increasing SDS concentrations did not cause additional significant conformational modifications, even above the cmc.

In the presence of LPC micelles, spectra of the linear analogues (Fig. 1E and K) showed a negative band at 198 nm, indicating an unordered structure. These results suggest that linear peptides did not interact with LPC, which was expected because LPC carries a zwitterionic head group that does not interact electrostatically with cationic peptides. Consequently, increasing LPC concentration did not affect the conformation of the peptides.

The spectra of the linear analogues in TFE (Fig. 1F and L) evidenced the important contribution of this organic solvent to conformation. As can be seen, a tendency to adopt α-helical conformation with increasing TFE concentration was observed,

Table 1
Antimicrobial activity of gomesin and its analogues

Peptide	MIC (μM)[a]		
	B. megaterium	*E. coli*	*C. albicans*
Gomesin *(Gm)*	0.32–0.64	0.64–1.28	0.64–1.28
[$TOAC^1$]-*Gm*	0.64–1.28 (2)	0.64–1.28 (1)	1.28–2.56 (2)
[$TOAC^1$, $Ser^{2,6,11,15}$]-*Gm*	2.56–5.12 (8)	20.48–40.96 (32)	5.12–10.24 (8)
[Trp^7]-*Gm*	1.28–2.56 (4)	1.28–2.56 (2)	0.64–1.28 (1)
[$Ser^{2,6,11,15}$, Trp^7]-*Gm*	2.56–5.12 (8)	10.24–20.48 (16)	5.12–10.24 (8)
[Trp^9]-*Gm*	0.64–1.28 (2)	0.64–1.28 (1)	0.64–1.28 (1)
[$Ser^{2,6,11,15}$, Trp^9]-*Gm*	5.12–10.24 (16)	5.12–10.24 (8)	5.12–10.24 (8)

[a] MICs were obtained in Poor Broth (217 mOsM; 1.0 g Peptone + 86 mM NaCl in 100 mL of H_2O) or 1/2PDB (79 mOsM; 1.2 g potato dextrose in 100 mL of H_2O) nutrients mediums. MICs were expressed as the interval of concentrations [*a*]–[*b*], where [*a*] is the highest concentration tested at which the microorganisms were growing and [*b*] is the lowest concentration that causes 100% growth inhibition. Numbers in the parentheses mean: analogue MIC/gomesin MIC.

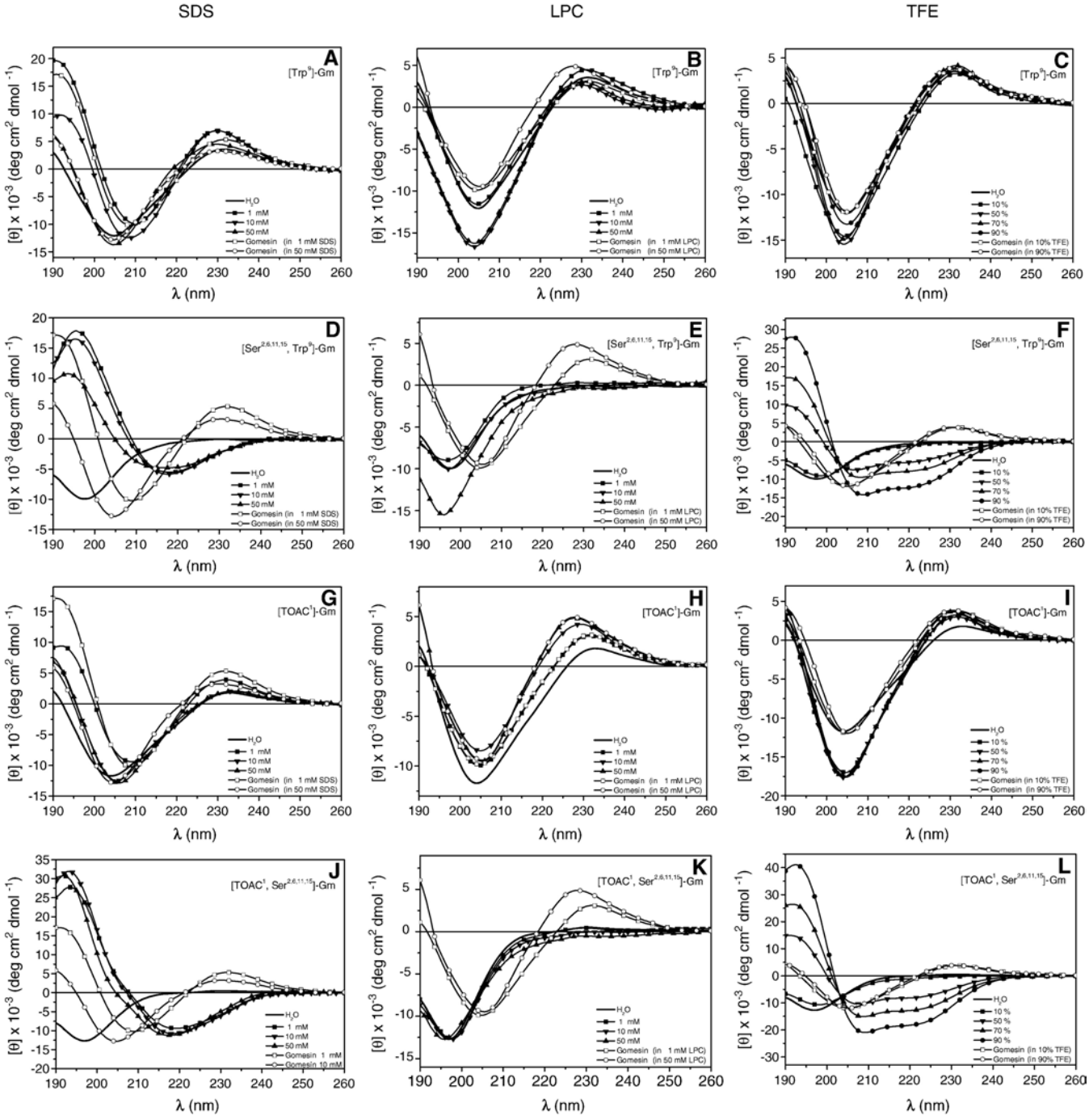

Fig. 1. CD spectra of [Trp^9]-*Gm* (A, B, C), [Ser^{2,6,11,15}, Trp^9]-*Gm* (D, E, F), [TOAC^1]-*Gm* (G, H, I) and [TOAC^1, Ser^{2,6,11,15}]-*Gm* (J, K, L) as a function of concentration of SDS (A, D, G, J), LPC (B, E, H, K), and TFE (C, F, I, L). Gomesin in the presence of 1 mM SDS, 1 mM LPC, or 10% TFE (□); and in 50 mM SDS, 50 mM LPC, or 90% TFE (○). Peptides in: H_2O, (⁻); 1 mM SDS, 1 mM LPC, or 10% TFE, (■); 10 mM SDS, 10 mM LPC, or 50% TFE, (▼); 50 mM SDS, 50 mM LPC, or 70% TFE, (▲); and 90% TFE, (●). [Peptide] = 0.1 mM.

since the CD spectra present a strong positive band at 198 nm and two negative bands at ca. 208 and 222 nm [31], mainly in 70% TFE. On the other hand, gomesin and the cyclic analogues (Fig. 1C and I) retained the β-turn-like conformation even in 90% TFE. From these observations we concluded that the lack of intramolecular disulfide bridges conferred high flexibility to these peptides, in allowing the linear analogues to acquire α-helical conformation.

EPR spectroscopy was employed to study the TOAC-labeled gomesin analogues in the presence of SDS and LPC. Fig. 2 shows the EPR spectra of [TOAC^1]-*Gm* in solution and in the presence of variable concentrations of SDS and LPC. It is seen that the peptide interacts with both surfactants. The spectrum in aqueous solution (A) presents three narrow lines, typical of fast tumbling motion, as expected for a relatively low molecular weight molecule. At 1 mM SDS the spectra (B) reveal the

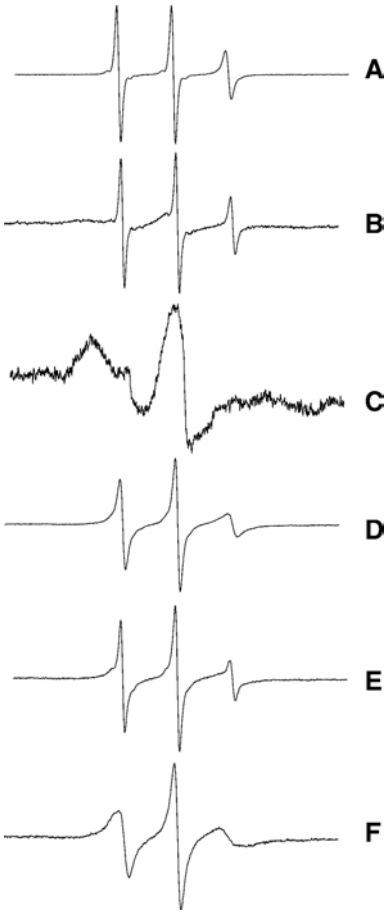

Fig. 2. EPR spectra of 0.1 mM [TOAC[1]]-*Gm*: aqueous solution, (A); 1 mM SDS, (B); 5 mM SDS, (C); 10 mM SDS, (D); 5 mM LPC, (E); and 50 mM LPC, (F). The scan width is 100 Gauss.

presence of two components, one due to the peptide in solution (narrow triplet) and another showing broad lines, typical of a strongly immobilized molecule. This SDS concentration is much lower than the surfactant cmc (8 mM). Thus, the peptide is capable of interacting with SDS monomers. This is clearly evinced at 5 mM SDS (C), where a powder spectrum, due to strongly immobilized peptide is obtained. Above the cmc, at 10 mM SDS, the spectrum (D) corresponds to the peptide totally bound to the micellar structure. This spectrum is still in the fast motion regime but the observed line broadening is indicative of the slower tumbling rate of the micelle-bound peptide.

The zwitterionic surfactant LPC was in the micellar form at all concentrations used. However, [TOAC[1]]-*Gm* only gave rise to a micelle-bound population in the sample containing 5 mM surfactant. At this concentration, a two-component spectrum was obtained (E), corresponding to free and micelle-bound peptide. With increasing LPC concentration, the latter component increased and, at 50 mM LPC, the spectrum (F) indicated that [TOAC[1]]-*Gm* was essentially totally bound. Hence, higher concentrations of zwitterionic LPC were required for total binding.

Table 2 lists the values of τ_C, τ_C/τ_B, and a_N for the spectra of [TOAC[1]]-*Gm* in aqueous solution and when totally bound to SDS and LPC micelles, as well as those calculated for [TOAC[1], Ser[2,6,11,15]]-*Gm* in aqueous solution and when totally bound to SDS (see below). It is seen that the rotational correlation time of [TOAC[1]]-*Gm* increases considerably upon binding to micelles. The larger τ_C value in the presence of LPC probably reflects the micelle slower tumbling. The τ_C/τ_B values also increase in going from water to micelle, indicating that the motion of the peptide is more anisotropic. The values of the isotropic hyperfine coupling constant are very similar in all cases, suggesting that the peptide is located at the micelle–water interface.

The EPR spectra of [TOAC[1], Ser[2,6,11,15]]-*Gm* (not shown) indicated that, while this peptide did not bind to LPC micelles, it only bound to SDS above the cmc. Similarly to [TOAC[1]]-*Gm*, the values of τ_C and τ_C/τ_B increased upon binding, and the value of a_N also pointed to interfacial location. It is worth noticing that in aqueous solution τ_C for [TOAC[1]]-*Gm* was considerably larger than that of the linear analogue, reflecting the more rigid structure of the disulfide-bridged β-hairpin.

The EPR data are in excellent agreement with CD spectroscopy. CD spectra for the peptide-SDS complex below the cmc (Fig. 1G) show that [TOAC[1]]-*Gm* acquires a somewhat different conformation under these conditions. It should be recalled that the EPR spectrum (Fig. 2C) shows that the peptide is strongly immobilized in this complex. With regard to LPC, although the features of the β-hairpin are always present (Fig. 1H), the positive band in the 215–240 nm region gradually shifts to lower wavelengths, an event that parallels the EPR-evinced gradual increase of the micelle-bound population.

As for [TOAC[1], Ser[2,6,11,15]]-*Gm*, the correlation between CD and EPR is also very good in that binding to LPC is not observed and binding to SDS above the cmc is detected by both techniques. The only exception was that, while EPR did not evince a peptide–SDS interaction below the detergent cmc, a conformational change was monitored by CD at 1 mM surfactant.

The different behavior of the disulfide-bridged peptides when compared to the linear ones can be rationalized by analyzing the amphipathicity of their structures. As previously noticed, the β-hairpin conformation of gomesin gives rise to an amphipathic structure [11] which is maintained in the cyclic analogues. In contrast, the linear derivatives are able to acquire α-helical conformation in TFE (Fig. 1F and L). Since TFE is a membrane-mimicking environment, one could expect that these

Table 2
Calculated values of τ_C, τ_C/τ_B, and a_N for the EPR spectra of [TOAC[1]]-*Gm* and [TOAC[1], Ser[2,6,11,15]]-*Gm* in aqueous solution and in the presence of micelles

	τ_C ($\times 10^{10}$ s)	τ_C/τ_B	a_N (Gauss)
[TOAC[1]]-*Gm*			
In solution	5.52	1.14	16.48
In 10 mM SDS	19.80	1.56	16.61
In 50 mM LPC	30.10	1.88	15.95
[TOAC[1], Ser[2,6,11,15]]-*Gm*			
In solution	2.68	1.15	16.45
In 50 mM LPC	16.40	1.30	15.99

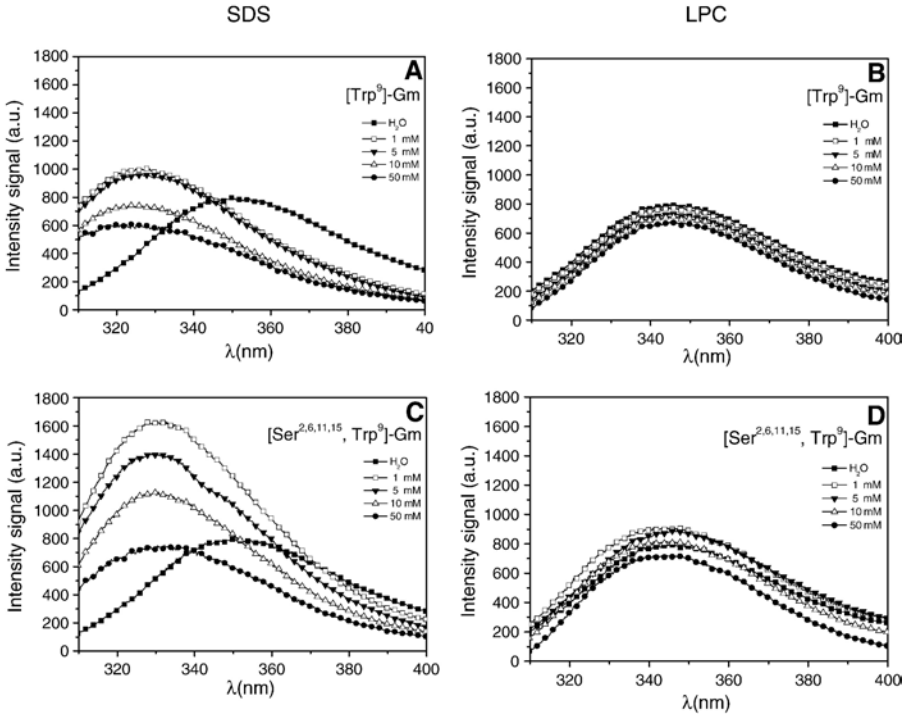

Fig. 3. Fluorescence emission spectra (λ_{exc}=295 nm) of [Trp^9]-*Gm* (A, B) and of [Ser^{2,6,11,15}, Trp^9]-*Gm* (C, D) in the presence of SDS (A, C) and LPC (B, D), at 20 °C. [Surfactant] (mM)=0 (■), 1 (□), 5 (▼), 10 (Δ), and 50 (●).

peptides would become α-helical upon binding to micelles. However, a helical wheel display of their sequences shows that the helix they give rise to does not possess amphipathic character. Instead, positively charged residues are spread over the whole helix surface. This is very likely the explanation for the fact that the linear *Gm* analogues bind to negatively charged SDS but not to zwitterionic LPC.

Fluorescence studies were performed for [Trp^9]-*Gm* and [Ser^{2,6,11,15}, Trp^9]-*Gm* in the presence of SDS and LPC (Fig. 3). Spectra of the free and bound peptides were compared. In water, the Trp residue shows a peak of maximal fluorescence (λ_{max}) at ca. 359 nm [32].

Emission spectra of [Trp^9]-*Gm* and [Ser^{2,6,11,15}, Trp^9]-*Gm* showed that λ_{max} changed in 1 mM SDS. In fact, the transfer of the peptide from the aqueous medium to the (less polar) micellar environment, caused a blue-shift [22] from around 350 nm to 325 nm (Fig. 3A and C). On the other hand, an increase in LPC concentration did not shift λ_{max}, suggesting that the Trp-containing peptide did not interact with the micellar aggregates (Fig. 3B and D).

We do not have at present an explanation for the apparently weak binding of [Trp^9]-*Gm* to LPC micelles. As suggested by both CD spectra (Fig. 1B) and the fluorescence results (Fig. 3B). This behavior is in contrast with that of the other cyclic peptides—*Gm* and [TOAC^1]-*Gm*. While both CD (Fig. 1H) and EPR (Fig. 2, spectra E and F) spectra provided evidence for the interaction between the latter peptides and LPC, only slight changes were seen in the CD spectra (Fig. 1B) and no changes were seen in fluorescence spectra (Fig. 3B) of [Trp^9]-*Gm* with increasing LPC concentration, suggesting that the interaction between [Trp^9]-*Gm* and these micelles is essentially non-existent.

Moreover, in agreement with the data for the other linear analogues, the linear Trp-substituted analogue also did not interact with LPC (Fig. 1E). In contrast, the fluorescence spectra do demonstrate that both the cyclic (Fig. 3A) and the linear (Fig. 3C) Trp-carrying analogues did interact with SDS both above and below the cmc.

In this work, three spectroscopic techniques were employed to examine the structure activity relationship for cyclic and linear analogues of the AMP gomesin. The results showed that the incorporation of TOAC or the replacement of Tyr^7 or Gln^9 by Trp caused no significant reduction of gomesin antimicrobial activity. Moreover, it was found that the more active cyclic analogues presented a β-hairpin conformation similar to that of gomesin in all environments, while the less active linear analogues presented an unordered conformation in water. All analogues strongly interacted with SDS, but not with LPC micelles, with the exception of *Gm* and [TOAC^1]-*Gm*, but in both cases the interaction with this zwitterionic detergent required higher concentration than when the negatively charged SDS was used. These findings support previous suggestions that the first step of the mechanism of action of gomesin should be an electrostatic interaction with the lipid bilayer, causing the disruption of the bacterial inner membrane. Moreover, our results show that hydrophobic interactions also contribute to membrane binding of this AMP.

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