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ORIGINAL ARTICLE

Dimerization of aurein 1.2: effects in structure, antimicrobial activity and aggregation of *Cândida albicans* cells

E. N. Lorenzón · P. R. S. Sanches · L. G. Nogueira · T. M. Bauab · E. M. Cilli

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Abstract Antimicrobial peptides (AMPs) are a promising solution to face the antibiotic-resistant problem because they display little or no resistance effects. Dimeric analogues of select AMPs have shown pharmacotechnical advantages, making these molecules promising candidates for the development of novel antibiotic agents. Here, we evaluate the effects of dimerization on the structure and biological activity of the AMP aurein 1.2 (AU). AU and the C- and N-terminal dimers, $(AU)_2K$ and $E(AU)_2$, respectively, were synthesized by solid-phase peptide synthesis. Circular dichroism spectra indicated that $E(AU)_2$ has a "coiled coil" structure in water while $(AU)_2K$ has an α-helix structure. In contrast, AU displayed typical spectra for disordered structures. In LPC micelles, all peptides acquired a high amount of α-helix structure. Hemolytic and vesicle permeabilization assays showed that AU has a concentration dependence activity, while this effect was less pronounced for dimeric versions, suggesting that dimerization may change the mechanism of action of AU. Notably, the antimicrobial activity against bacteria and yeast decreased with dimerization. However, dimeric peptides promoted the aggregation of *C. albicans*. The ability to aggregate yeast cells makes dimeric versions of AU attractive candidates to inhibit the adhesion of *C. albicans* to biological targets and medical devices, preventing disease caused by this fungus.

Keywords Antimicrobial peptides · Aurein 1.2 · Dimerization · Biological activity · Secondary structure

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E. N. Lorenzón · P. R. S. Sanches · E. M. Cilli (✉)
Institute of Chemistry, University Estadual Paulista Araraquara (UNESP), Araraquara, SP, Brazil
e-mail: cilli@iq.unesp.br

L. G. Nogueira · T. M. Bauab
Faculty of Pharmaceutical Sciences, University Estadual Paulista (UNESP), Araraquara, SP, Brazil

Introduction

Because of the drug resistance problem with traditional antibiotics, the development of novel antimicrobial agents becomes a very important challenge (Rossolini et al. 2007). A potential solution may lie in antimicrobial peptides (AMPs), because they have a broad-spectrum activity, act rapidly and rarely develop drug resistance (Dennison et al. 2007; McCubbin et al. 2011; Kamysz et al. 2006; Pini et al. 2012). AMPs are found in animals, plants, insects, and microorganisms, forming part of the innate defense systems (Peters et al. 2010; Ribeiro et al. 2011; Cespedes et al. 2012; Libério et al. 2011; He et al. 2013).

Most of the AMPs reported disrupt the membrane of cells via three general mechanisms. In the first, the peptides remain tightly bound to the membrane interface until reach a threshold concentration when promote bilayer damage via detergent or carpet-like mechanism. In this mechanism, peptides do not necessarily insert into the hydrophobic membrane core. On the other hand, peptides could form transmembrane pores. There are two pore-forming mechanisms. In the "barrel-stave" model, the peptides adopt a helical conformation and aggregate into a barrel-like structure that spans the membrane with the peptides lying perpendicular to the plane of the membrane. In an alternative model, named "toroidal pore", the peptides are tightly bound to the polar lipid groups of the membrane, promoting the bending of the bilayer (Ambroggio et al.

2005; Fernandez et al. 2009; Castro et al. 2006). In addition to the membrane disrupting mechanisms described above, several studies indicate that cell membrane permeabilization alone may not be enough to explain peptide antimicrobial activity. The observations of peptide-induced lipid clustering of anionic components from zwitterionic lipids led to the introduction of the "lipid clustering mechanism" (Epand et al. 2010; Teixeira et al. 2012).

Parameters, such as charge, amphipathicity, hydrophobicity and helicity have been extensively studied and correlates directly with the biological activity of AMPs (Matsuzaki 2009; Zhu et al. 2007; Huang et al. 2010). In addition, the knowledge that the aggregation of molecules of AMPs, either before or after binding to the membrane surface, is a prerequisite to pore formation, led researchers to the design of dimeric peptides. (Sengupta et al. 2008; Matsuzaki 2009; Pini et al. 2005; Hornef et al. 2004; Lee et al. 2008; Welling et al. 2007; Zhu and Shin 2009a). In this way, several bioactive sequences were dimerized obtaining pharmacotechnics advantages, like enhanced antimicrobial activity, solubility and proteases resistant (Hara et al. 2001; Dempsey et al. 2003; Pini et al. 2005; Dewan et al. 2009; Liu et al. 2010). However, the effect of this modification is unclear since dimeric versions of some AMPs lost antimicrobial activity or gained toxicity (Yang et al. 2009; Zhu and Shin 2009b; Lorenzón et al. 2012).

Here, we used the AMP Aurein 1.2 (AU) as the framework to study the effects of dimerization on its structure and biological activity. In this way, AU and the C- and N-terminal dimeric version $(AU)_2K$ and $E(AU)_2$, respectively, were synthesized. This is the first study on the effects of dimerization on a peptide proposed acting by carpet-like mechanism.

Materials and methods

Chemicals and microorganisms

Only analytical grade reagents from commercial suppliers were used. All solutions were prepared with Milli-Q water (Millipore Reagent Water System, USA). Solvents for chromatographic procedures were of HPLC grade from several sources. 9-fluorenylmethyloxycarbonyl (Fmoc) amino acids and resins were purchased from Synbiosci (USA) and Novabiochem (USA). Solvents and reagents for peptide synthesis were from Sigma Aldrich Co. (USA) and Fluka (Switzerland). DCM (dichloromethane) and DMF (dimethylformamide) were purchased from Hexis Cientifica (Brazil). All lipids and surfactants were purchased from Avanti Polar Lipids (USA). We used the bacterial strains *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) and the yeast *Candida albicans* (ATCC 18804).

Peptide synthesis

All peptide syntheses were manually performed by solid-phase peptide synthesis (SPPS) using the standard Fmoc (9-fluorenylmethyloxycarbonyl) protocols on a Rink-MBHA resin. For the C-terminal dimer, Fmoc-Lys(Fmoc)-OH was attached to the resin, and after α- and ε-Fmoc group deprotection with 20 % piperidine/dimethylformamide (DMF), the two chains of AU were simultaneously elongated (Fig. 1). On the other hand, after the synthesis of AU, Boc-Glu-OH, without side chain protector, was used to link two chains of this peptide, obtaining the N-terminal dimer (Fig. 1). In all cases, the amino acids were coupled at twofold excess over the amino component in the resin, using diisopropylcarbodiimide (DIC)/N-hydroxybenzotriazole (HOBt) in 50 % (v/v) DCM (methylene chloride)/DMF. After 2 h, the qualitative ninhydrin test was performed to estimate the completeness of the coupling reaction; the recoupling procedure was done when the ninhydrin test was positive. Cleavage of the Rink MBHA resin and removal of the side chain protecting groups for all the peptides were simultaneously performed with 95 % TFA, 2.5 % water and 2.5 % TIS for 2 h. After this procedure, the crude peptides were precipitated with anhydrous ethyl ether, separated from soluble nonpeptide material by centrifugation, extracted into 0.045 % (v/v) TFA/H_2O (solvent A), and lyophilized. The crude peptides were dissolved in solvent A and purified by semi-preparative HPLC on a Beckman System Gold using a reverse-phase C18 column with a linear gradient 40–80 % of solvent B (0.036 % (v/v) TFA/acetonitrile) for 120 min. The flow rate was 5 mL/min. UV detection was carried out at 220 nm. The peptide homogeneity was checked by analytical HPLC on a Shimadzu system, using solvents A and B with a linear gradient of 5–95 % (v/v) of solvent B for 30 min, at a flow rate of 1.0 mL/min and UV detection at 220 nm. The identity of the peptide was confirmed by mass spectrometry in positive ion mode ESI on a Bruker model apparatus (Germany).

Antimicrobial assay

The minimum inhibitory concentration (MIC) was performed following the recommendations of the Clinical and Laboratory Standards Institute (CLSI 2006; CLSI 2008). The antibacterial and antifungal activity tests were performed using the broth microdilution method. In brief, bacterial cells in Mueller–Hinton media (aliquots of 80 μL containing 1.5×10^7 CFUs) were incubated with a serial dilution (128–1 μmol/L) of the synthetic peptides dissolved in Milli-Q water. After incubation for 24 h at 37 °C, the microtiter plates were analyzed visually by the addition of rezasurine. For the antifungal assay, the medium used was

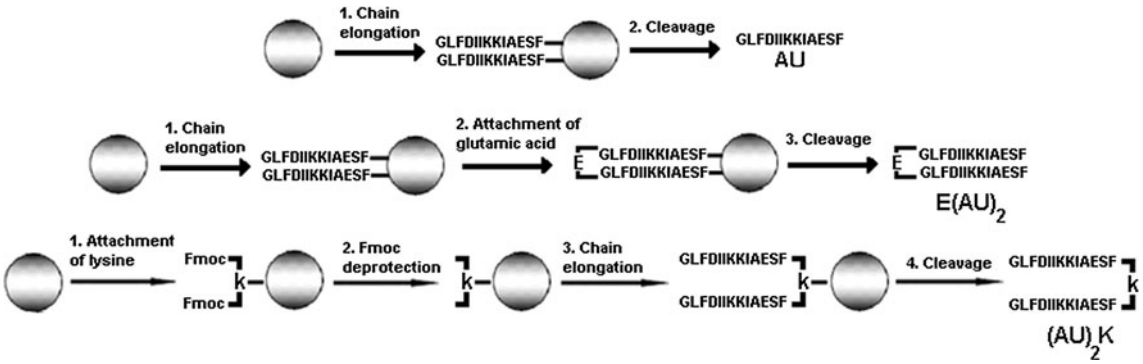

Fig. 1 Strategy of peptides synthesis

RPMI 1640 with L-glutamine buffered to pH 7.2 with 0.165 M morpholinepropanesulfonic acid (MOPS), supplemented with 2 % glucose. The cell suspension (final concentration of 1×10^3 to 2.5×10^3 CFUs/mL) was inoculated on a microdilution plate previously prepared with the synthetic peptides diluted in a concentration range from 128 to 1 μmol/L. The plates were incubated at 37 °C for 48 h. Each assay was performed in triplicates. For control, bacterial suspensions were incubated either in Milli-Q water. In addition, control drugs used were amphotericin B. and fluconazole. The MIC was defined as the lowest concentration of the peptide or other antimicrobial agent at which no growth was detectable.

Vesicle permeabilization

Large unilamellar vesicles (LUVs) composed of 95 % DPPC (1,2-dipalmitoyl-3-phosphocholine) and 5 % DPPA (1,2-dipalmitoyl-3-phosphatidic acid) were prepared by mixing the appropriate amounts of lipid in a 4:1 chloroform:methanol mixture in a round-bottom flask. The solvent was rapidly evaporated using nitrogen gas. The lipid biofilm was placed under a vacuum overnight and hydrated at 60 °C with 80 mmol/L of CF in Tris (0.01 mol/L, pH 7.4) and NaCl (0.15 mol/L) to give a final lipid concentration of 15 mmol L^{-1}. This suspension was extruded 40 times through two stacked nucleopore polycarbonate filters (100 nm pore size) using an extruder system from Avanti Polar Lipids (USA) at approximately 40 °C. Vesicles were separated from non-encapsulated CF by gel filtration on a Sephadex G-50 column using Tris (0.01 mol/L, pH 7.4) and NaCl (0.15 mol/L) for elution.

The release of carboxyfluorescein (CF) from vesicles was measured by the fluorescence intensity at a wavelength of 520 nm (492 nm excitation wavelength) after the addition of 1, 4 and 16 μmol/L of peptide. Data were acquired using a fluorescence spectrophotometer (Cary Eclipse, VARIAN). The experiments were performed at 25 °C in triplicate.

Hemolysis assay

Hemolysis assays were performed using the experimental procedure described by Castro et al. (Castro et al. 2009). In brief, before use, freshly human red blood cells (RBCs) was washed three times with 0.01 mol/L Tris–HCl pH 7.4 containing 0.15 mol/L NaCl (Tris–saline). A suspension of 1 % (v/v) erythrocytes was made with packed red blood cells re-suspended in Tris–saline. Synthetic peptides were dissolved in Tris–saline at an initial concentration of 128 mmol/L, and were serially diluted in the same buffer to determine its HC_{50} (concentration that causes 50 % hemolysis). As a positive control (100 % lysis), a 1 % (v/v) Triton X-100 solution was used. After incubation for 1 h at 37 °C, the samples were centrifuged at 1,000*g* for 5 min. Aliquots of 100 μL of the supernatant were transferred to 96-well microplates, and the absorbance was determined at 405 nm. The assay was performed in triplicate.

CD spectra

Circular dichroism spectra were obtained between 190 and 250 nm with a JASCO J-715 CD spectrophotometer (Japan) on nitrogen flush in 1 mm path length quartz cuvettes at room temperature. The peptide concentration was 60 μmol/L. To investigate the conformational changes by membrane environments, a solution containing 10 mmol/L of *lysophosphatidylcholine* (LPC) was used. CD spectra were typically recorded as an average of six scans that were obtained in millidegrees and converted to molar ellipticity [θ] (in deg cm^2 $dmol^{-1}$).

Statistics

MIC values are reported as the mode of a set of three independent assays. Hemolysis data are reported as the mean of a set of three independent assays.

Table 1 Results of peptide synthesis

Peptide	Yield (%)	Retention time (min)	Mass (Da)	
			Observed[a]	Calculated
AU	27	20.1	1,479.9	1,479.8
$(AU)_2K$	6	24.9	3,070.7	3,070.2
$E(AU)_2$	2	24.0	3,071.5	3,070.7

[a] Obtained from mass spectra of the purified peptides

Table 2 Antimicrobial activities of the synthetic peptides

Peptídeo	MIC (μmol/L)		
	E. coli	*S. aureus*	*C. albicans*
AU	16	8	32
$(AU)_2K$	128	>128	>128
$E(AU)_2$	128	>128	>128

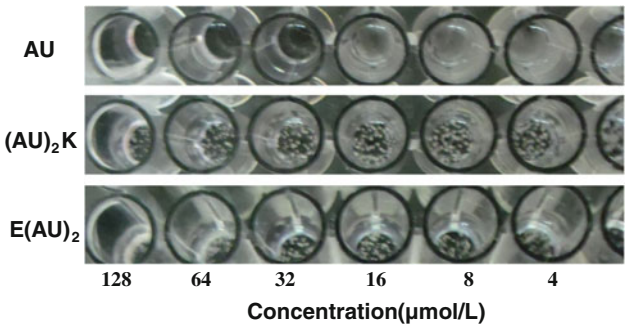

Fig. 2 Aggregation of *C. albicans* cell induced by dimeric analogues

Results

Peptide design and synthesis

The monomeric and two dimerics versions of the AMP AU 1.2 were synthesized using the SPPS (Fig. 1). The peptides were obtained with a high level of purity (above 98 %) and the identities of these molecules were confirmed by electrospray mass spectrometry (Table 1).

Biological activity

The peptides were evaluated in term of antimicrobial, antifungal and hemolytic activity. Dimerization of AU decreases the ability to inhibit the growth of bacteria and fungi (Table 2). The MICs values of N- and C-dimers were bigger than monomer. However, dimeric peptides promoted the aggregation of *C. albicans* cells (Fig. 2). To evaluate the toxicity of the peptides, we used the hemolysis test. The results summarized in Fig. 3 show that $E(AU)_2$ has no hemolytic activity, while $(AU)_2K$ caused 10–30 % hemolysis in the range of concentration used. On the other hand, the monomer showed the bigger HC_{50} values.

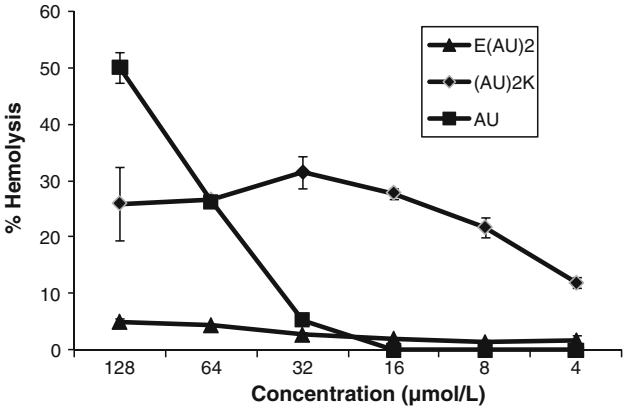

Fig. 3 Hemolytic activities of the synthetics peptides

Vesicle permeabilization

CF release from LUVs composed of DPPA/DPPC (5/95 %) was evaluated in three different peptide concentrations. The results showed that the addition of peptides promote the release of the dye, as indicated by an increase in fluorescence (Fig. 4). In addition, AU has a concentration dependence permeabilization activity, whereas this effect was less pronounced for dimeric versions.

CD spectroscopy

CD spectroscopy studies in water showed that $(AU)_2K$ has a typical α-helix spectrum with double maxima at 208 and 222 nm. The ellipticity radio (222/208 nm > 1) for $E(AU)_2$ determined a "coiled coil" structure where two helices wrap around each other (Bromley and Channon 2011). In contrast, AU displayed typical spectra for disordered structures. For all the peptides, an α-helical structure was induced by the non-polar environment of LPC micelles (Fig. 5).

Discussion

Several studies have shown that dimerization of AMPs improves antimicrobial activity, solubility and proteases resistant (Falciani et al. 2007; Lee et al. 2008; Taylor et al. 2007; Welling et al. 2007; Dempsey et al. 2003; Hara et al. 2001). Here, we study the effects of dimerization in structure and biological activity of the AMP AU 1.2. This 13-residue peptide is the most studied member of the aurein′s family (Giacometti et al. 2007; Karbalaeemohammad and Naderi-Manesh 2011; Dennison et al. 2007; Lee et al.

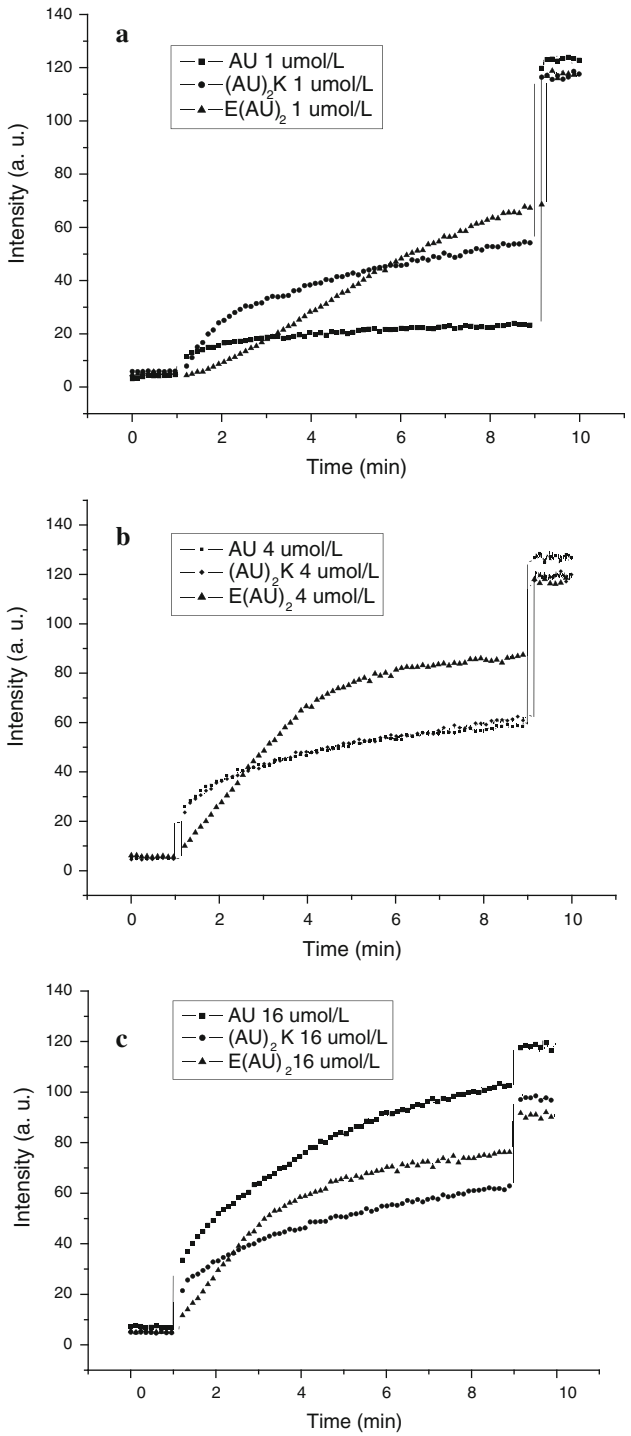

Fig. 4 Carboxyflourescein release. Peptide concentration: 1 (**a**), 4 (**b**) and 16 μmol/L (**c**)

2010; Li et al. 2006). Previous studies have proposed that this peptide acts by a carpet-like mechanism (McCubbin et al. 2011; Cheng et al. 2009; Ambroggio et al. 2005; Chen and Mark 2011). It is supported by the fact that it is too short to span the membrane. We decided to use this AMP as the scaffold because there is no information about the effects of dimerization of AMPs described as having the carpet-like mechanism. In addition, many studies have shown that the modifications of C- and N-terminus of AMPs are important to biological activity (Crusca et al. 2011; Sforça et al. 2004). Sforça et al. (2004) showed that the amidation of C-terminus promotes a structural perturbation of the amphipathic α-helix and affects its biological activity. In addition, Crusca et al. (2011) demonstrated that the charge of N-terminal is important to the structure and selectivity of the antimicrobial peptide Hyl-A1. Taking it into consideration, we designed the C- and N-terminal dimeric versions of AU to evaluate the structure and the biological activity of these analogues. As shown in Table 2, dimerization decreases the ability of the peptide to inhibit the growth of bacteria and fungi. These results are in agreement with Yang et al. (2009) and Mäntylä et al. (2005) whose have shown negative effect with dimeric versions of PST13-RK and Temporin A, respectively. However, others studies with dimeric forms of AMPs have shown pharmacotechnical advantages, such as enhanced antimicrobial potency, solubility, and resistance to proteases (Hara et al. 2001; Dempsey et al. 2003; Pini et al. 2005; Dewan et al. 2009; Liu et al. 2010). These controversial results show that the effects of dimerization of AMPs needs to be better studied.

In antifungal studies, the dimeric version of AU promoted the aggregation of *C. albicans* cells (Fig. 2). This may be due to the interaction of the peptides with yeast cell wall carbohydrates. Tsai et al. (2011) showed that the peptide LL-37 interact with mannans, the main component of the *C. albicans* cell wall, promoting the aggregation of these cells. It has been reported also that AMPs interact with lipopolysaccharides and lipoteichoic acid, the distinct components of the gram-negative and gram-positive bacteria, respectively (Bucki and Janmey 2006; Giacometti et al. 2006). Previous studies have shown that dimeric peptides may be inhibited from passing through the cell walls of prokaryotic cells, (Jiang et al. 2011; Lorenzón et al. 2012). These interactions with cell wall components could explain the lower capacity of dimeric AMPs to reach the membrane of bacteria and fungi.

Hemolytic activity is always a potential barrier preventing the use of AMPs as systemic therapeutics. To evaluate the toxicity of the peptides, we used the hemolysis test, the most commonly procedure to characterize the eukaryotic membrane damage caused by AMPs. The results summarized in Fig. 3 shows that dimeric peptides have little to no hemolytic activity and seem to be not concentration dependent. In contrast, AU has a concentration dependence activity above 32 μmol/L.

CF release from LUVs also showed that AU has a concentration dependence permeabilization activity, whereas this effect was less pronounced for dimeric versions (Fig. 4).

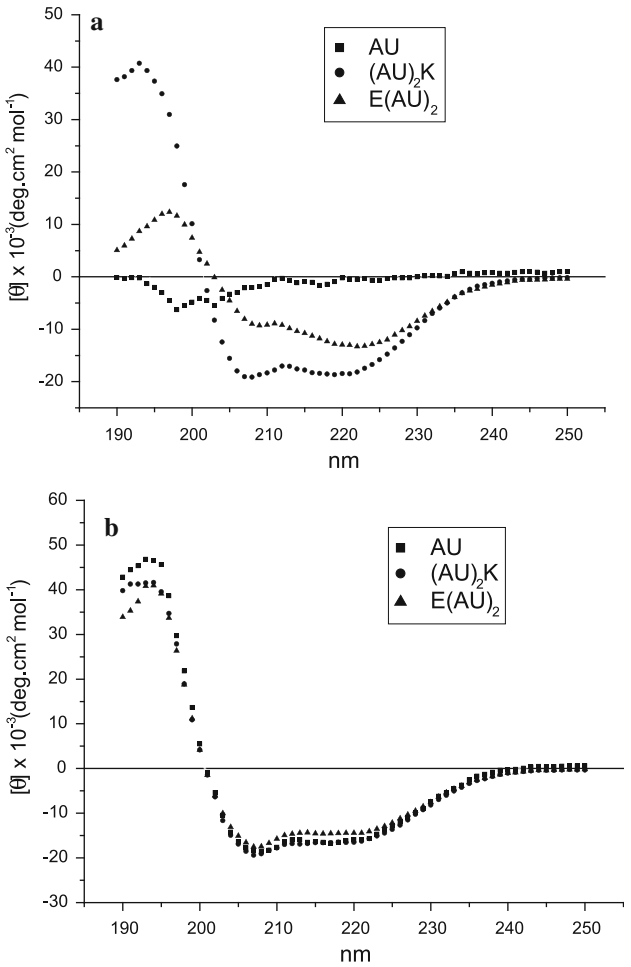

Fig. 5 CD spectra of the AU and analogues in aqueous solution (**a**) and LPC (**b**)

These results are explained by the aggregation among peptide chain of the dimeric version. This hypothesis was confirmed by CD studies (Fig. 5). Vesicles used in CF release have no cell wall components, including carbohydrates, to interact with the peptides. This data showed that the difference in biological activity is not only due the peptide-cell wall interactions.

To evaluate the correlation among the above results and the structure of the peptides, CD spectroscopy was used. When compared with AU, dimeric versions have more defined secondary structures in aqueous solution. C-terminal dimerization has induced α-helix structure while N-terminal dimerization promotes an interaction between the two peptide chains, leading to a "coil-coiled" structure. It is well known that the first step in any mechanism of action is the electrostatic attraction of the cationic peptide to the anionic membrane. In general, peptides are in a random coil conformation in solution but adopt an α-helical conformation when associated with the lipid membrane, the second step in the peptide-membrane binding process (Seelig 2004). Our results indicated that the structured-state in solution imposed by dimerization is more definite and it may modify the capacity of the peptide to bind the membrane.

Besides that, in the presence of LPC micelles, all peptides acquired the same content of α-helical conformation, as indicated by one positive band at 190 nm and negative bands in 208 and 222 nm. These results indicated that the difference in biological activity among monomeric and dimeric version of AU occurs mainly before (with cell wall component) or in the first step of the interaction with the phospholipid bilayer.

Conclusion

The analysis of the whole data indicates that dimerization of AU decrease the antimicrobial activity. In this study, two factors could explain these data. The first is the interaction of AMP with cell wall components, as confirmed by the aggregation of *C. albicans* cells. The other is due to the structure-state in solution that promotes different initial interaction with the membrane and could change the mechanism of action. In addition, the ability of aggregating yeast cells, make the dimeric versions of AU future drug candidates to prevent *C. albicans* adhesion to biological targets and medical devices, such as prostheses and catheters, preventing disease caused by this fungus.

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References

Ambroggio EE, Separovic F, Bowie JH, Fidelio GD, Bagatolli LA (2005) Direct visualization of membrane leakage induced by the antibiotic peptides: maculatin, citropin, and aurein. Biophys J 89(3):1874–1881

Bromley EH, Channon KJ (2011) Alpha-helical peptide assemblies giving new function to designed structures. Prog Mol Biol Transl Sci 103:231–275

Bucki R, Janmey PA (2006) Interaction of the gelsolin-derived antibacterial PBP 10 peptide with lipid bilayers and cell membranes. Antimicrob Agents Chemother 50(9):2932–2940

Castro MS, Cilli EM, Fontes W (2006) Combinatorial synthesis and directed evolution applied to the production of alpha-helix forming antimicrobial peptides analogues. Curr Protein Pept Sci 7(6):473–478

Castro MS, Ferreira TC, Cilli EM, Crusca Jr. E, Mendes-Giannini MJS, Sebben A, Ricart CA, Sousa MV, Fontes W (2009) Hylin a1, the first cytolytic peptide isolated from the arboreal South American frog Hypsiboas albopunctatus ("spotted treefrog"). Peptides 30(2):291–296

Cespedes GF, Lorenzón EN, Vicente EF, Mendes-Giannini MJ, Fontes W, Castro MS, Cilli EM (2012) Mechanism of action and relationship between structure and biological activity of Ctx-Ha: a new ceratotoxin-like peptide from Hypsiboas albopunctatus. Protein Pept Lett 19(6):596–603

Chen R, Mark AE (2011) The effect of membrane curvature on the conformation of antimicrobial peptides: implications for binding and the mechanism of action. Eur Biophys J 40(4):545–553

Cheng JT, Hale JD, Elliot M, Hancock RE, Straus SK (2009) Effect of membrane composition on antimicrobial peptides aurein 2.2 and 2.3 from Australian southern bell frogs. Biophys J 96(2): 552–565

CLSI. Manual Clinical and Laboratory Standards Institute. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standards-6[a] ed. Document M7-A6 performance standards for antimicrobial susceptibility testing. Clinical and Laboratory Standards Institute, Wayne, PA., 2006

CLSI. Manual Clinical and Laboratory Standards Institute. Reference methods for broth dilution antifungal susceptibility tests for yeasts; approved standards, CLSI document M27-A3. Clinical and Laboratory Standards Institute,Wayne, PA., 2008

Crusca E, Rezende A, Marchetto R, Mendes-Giannini M, Fontes W, Castro MS, Cilli E (2011) Influence of N-Terminus Modifications on the biological activity, membrane interaction, and secondary structure of the antimicrobial peptide Hylin-a1. Biopolymers 96(1):41–48

Dempsey CE, Ueno S, Avison MB (2003) Enhanced membrane permeabilization and antibacterial activity of a disulfide-dimerized magainin analogue. Biochemistry 42(2):402–409

Dennison SR, Harris F, Phoenix DA (2007) The interactions of aurein 1.2 with cancer cell membranes. Biophys Chem 127(1–2):78–83

Dewan PC, Anantharaman A, Chauhan VS, Sahal D (2009) Antimicrobial action of prototypic amphipathic cationic decapeptides and their branched dimers. Biochemistry 48(24):5642–5657

Epand RF, Maloy L, Ramamoorthy A, Epand RM (2010) Amphipathic helical cationic antimicrobial peptides promote rapid formation of crystalline states in the presence of phosphatidylglycerol: lipid clustering in anionic membranes. Biophys J 98(11):2564–2573

Falciani C, Lozzi L, Pini A, Corti F, Fabbrini M, Bernini A, Lelli B, Niccolai N, Bracci L (2007) Molecular basis of branched peptides resistance to enzyme proteolysis. Chem Biol Drug Des 69(3):216–221

Fernandez DI, Gehman JD, Separovic F (2009) Membrane interactions of antimicrobial peptides from Australian frogs. Biochim Biophys Acta 1788(8):1630–1638

Giacometti A, Cirioni O, Ghiselli R, Mocchegiani F, Orlando F, Silvestri C, Bozzi A, Di Giulio A, Luzi C, Mangoni ML, Barra D, Saba V, Scalise G, Rinaldi AC (2006) Interaction of antimicrobial peptide temporin L with lipopolysaccharide in vitro and in experimental rat models of septic shock caused by gram-negative bacteria. Antimicrob Agents Chemother 50(7):2478–2486

Giacometti A, Cirioni O, Riva A, Kamysz W, Silvestri C, Nadolski P, Della Vittoria A, Łukasiak J, Scalise G (2007) In vitro activity of aurein 1.2 alone and in combination with antibiotics against gram-positive nosocomial cocci. Antimicrob Agents Chemother 51(4):1494–1496

Hara T, Kodama H, Kondo M, Wakamatsu K, Takeda A, Tachi T, Matsuzaki K (2001) Effects of peptide dimerization on pore formation: antiparallel disulfide-dimerized magainin 2 analogue. Biopolymers 58(4):437–446

He X, Yang S, Wei L, Liu R, Lai R, Rong M (2013) Antimicrobial peptide diversity in the skin of the torrent frog, Amolops jingdongensis. Amino Acids 44(2):481–487

Hornef MW, Putsep K, Karlsson J, Refai E, Andersson M (2004) Increased diversity of intestinal antimicrobial peptides by covalent dimer formation. Nat Immunol 5(8):836–843

Huang Y, Huang J, Chen Y (2010) Alpha-helical cationic antimicrobial peptides: relationships of structure and function. Protein Cell 1(2):143–152

Jiang ZQ, Vasil AI, Gera L, Vasil ML, Hodges RS (2011) Rational design of alpha-helical antimicrobial peptides to target gram-negative pathogens, acinetobacter baumannii and pseudomonas aeruginosa: utilization of charge, 'Specificity Determinants,' Total Hydrophobicity, Hydrophobe type and location as design parameters to improve the therapeutic ratio. Chem Biol Drug Des 77:225–240

Kamysz W, Mickiewicz B, Rodziewicz-Motowidio S, Greber K, Okroj M (2006) Temporin A and its retro-analogues: synthesis, conformational analysis and antimicrobial activities. J Pept Sci 12(8):533–537

Karbalaeemohammad S, Naderi-Manesh H (2011) Two novel anticancer peptides from Aurein1.2. Int J Pept Res Ther 17(3):159–164

Lee JY, Yang ST, Lee SK, Jung HH, Shin SY, Hahm KS, Kim JI (2008) Salt-resistant homodimeric bactenecin, a cathelicidin-derived antimicrobial peptide. Febs J 275(15):3911–3920

Lee TH, Heng C, Swann MJ, Gehman JD, Separovic F, Aguilar MI (2010) Real-time quantitative analysis of lipid disordering by aurein 1.2 during membrane adsorption, destabilisation and lysis. Biochim Biophys Acta 1798(10):1977–1986

Li X, Li Y, Peterkofsky A, Wang G (2006) NMR studies of aurein 1.2 analogs. Biochim Biophys Acta 1758(9):1203–1214

Libério MS, Joanitti GA, Azevedo RB, Cilli EM, Zanotta LC, Nascimento AC, Sousa MV, Pires Júnior OR, Fontes W, Castro MS (2011) Anti-proliferative and cytotoxic activity of pentadactylin isolated from Leptodactylus labyrinthicus on melanoma cells. Amino Acids 40(1):51–59

Liu S, Zhou L, Lakshminarayanan R, Beuerman R (2010) Multivalent antimicrobial peptides as therapeutics: design principles and structural diversities. Int J Pept Res Ther 16(3):199–213

Lorenzón EN, Cespedes GF, Vicente EF, Nogueira LG, Bauab TM, Castro MS, Cilli EM (2012) Effects of dimerization on the structure and biological activity of antimicrobial peptide Ctx-Ha. Antimicrob Agents Chemother 56(6):3004–3010

Mäntylä T, Sirola H, Kansanen E, Korjamo T, Lankinen H, Lappalainen K, Välimaa AL, Harvima I, Närvänen A (2005) Effect of temporin A modifications on its cytotoxicity and antimicrobial activity. APMIS 113:497–505

Matsuzaki K (2009) Control of cell selectivity of antimicrobial peptides. Biochimica et Biophysica Acta-Biomembranes 1788(8):1687–1692

McCubbin GA, Praporski S, Piantavigna S, Knappe D, Hoffmann R, Bowie JH, Separovic F, Martin LL (2011) QCM-D fingerprinting of membrane-active peptides. Eur Biophys J 40(4):437–446

Peters BM, Shirtliff ME, Jabra-Rizk MA (2010) Antimicrobial peptides: primeval molecules or future drugs?. Plos Pathogens 6(10):e1001067

Pini A, Giuliani A, Falciani C, Runci Y, Ricci C, Lelli B, Malossi M, Neri P, Rossolini GM, Bracci L (2005) Antimicrobial activity of novel dendrimeric peptides obtained by phage display selection and rational modification. Antimicrob Agents Chemother 49(7):2665–2672

Pini A, Lozzi L, Bernini A, Brunetti J, Falciani C, Scali S, Bindi S, Di Maggio T, Rossolini GM, Niccolai N, Bracci L (2012) Efficacy and toxicity of the antimicrobial peptide M33 produced with different counter-ions. Amino Acids 43(1):467–473

Ribeiro SM, Almeida RG, Pereira CA, Moreira JS, Pinto MF, Oliveira AC, Vasconcelos IM, Oliveira JT, Santos MO, Dias SC, Franco OL (2011) Identification of a passiflora alata curtis

dimeric peptide showing identity with 2S albumins. Peptides 32(5):868–874

Rossolini GM, Mantengoli E, Docquier JD, Musmanno RA, Coratza G (2007) Epidemiology of infections caused by multiresistant gram-negatives: ESBLs, MBLs, panresistant strains. New Microbiol 30(3):332–339

Seelig J (2004) Thermodynamics of lipid-peptide interactions. Biochim Biophys Acta 1666(1–2):40–50

Sengupta D, Leontiadou H, Mark AE, Marrink SJ (2008) Toroidal pores formed by antimicrobial peptides show significant disorder. Biochimica et Biophysica Acta (BBA) Biomembranes 1778(10):2308–2317

Sforça ML, Oyama S, Canduri F, Lorenzi CC, Pertinhez TA, Konno K, Souza BM, Palma MS, Ruggiero Neto J, Azevedo WF, Spisni A (2004) How C-terminal carboxyamidation alters the biological activity of peptides from the venom of the eumenine solitary wasp. Biochemistry 43(19):5608–5617

Taylor K, McCullough B, Clarke DJ, Langley RJ, Pechenick T, Hill A, Campopiano DJ, Barr PE, Dorin JR, Govan JRW (2007) Covalent dimer species of beta-defensin Defr1 display potent antimicrobial activity against multidrug-resistant bacterial pathogens. Antimicrob Agents Chemother 51(5):1719–1724

Teixeira V, Feio MJ, Bastos M (2012) Role of lipids in the interaction of antimicrobial peptides with membranes. Prog Lipid Res 51(2):149–177

Tsai P, Yang C, Chang H, Lan C (2011) Human antimicrobial peptide LL-37 inhibits adhesion of Candida albicans by interacting with Yeast cell-wall carbohydrates. Plos One 6(3):e17755

Welling MM, Brouwer CPJM, t Hof W, Veerman ECI, Amerongen AVN (2007) Histatin-derived monomeric and dimeric synthetic peptides show strong bactericidal activity towards multidrug-resistant Staphylococcus aureus in vivo. Antimicrob Agents Chemother 51(9):3416–3419

Yang ST, Kim JI, Shin SY (2009) Effect of dimerization of a beta-turn antimicrobial peptide, PST13-RK, on antimicrobial activity and mammalian cell toxicity. Biotechnol Lett 31(2):233–237

Zhu WL, Shin SY (2009a) Antimicrobial and cytolytic activities and plausible mode of bactericidal action of the cell penetrating peptide penetratin and Its Lys-linked two-stranded peptide. Chem Biol Drug Des 73(2):209–215

Zhu WL, Shin SY (2009b) Effects of dimerization of the cell-penetrating peptide Tat analog on antimicrobial activity and mechanism of bactericidal action. J Pept Sci 15(5):345–352

Zhu WL, Nan YH, Hahm KS, Shin SY (2007) Cell selectivity of an antimicrobial peptide melittin diastereomer with D-amino acid in the leucine zipper sequence. J Biochem Mol Biol 40(6): 1090–1094