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SHORT COMMUNICATION

Interaction between the antimicrobial peptide Aurein 1.2 dimer and mannans

Esteban N. Lorenzón · Julia P. Piccoli · Eduardo M. Cilli

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Abstract We have previously described the structure and the ability of a dimeric analog of the antimicrobial peptide Aurein 1.2 to aggregate *Candida albicans*. In this study, circular dichroism and fluorescence spectroscopy data showed that this aggregation is related to the interaction between the peptide and mannans, the main component of yeast cell wall. In this context, we propose a model in which dimers interact with the polysaccharide leading to cells aggregation.

Keywords Aurein 1.2 · Dimerization · *Candida albicans* · Aggregation · Mannans

Introduction

Antimicrobial peptides (AMPs) can be considered as promising candidates for the development of novel therapeutic agents acting against pathogens. Cationic AMPs (cAMPs) have broad-spectrum activity, act rapidly, rarely develop drug resistance and also have anti-biofilm activity (Castro et al. 2006; Ahmad et al. 2012; Cespedes et al. 2012; da Silva et al. 2012, 2013; Li et al. 2012; Delattin et al. 2014; Di Luca et al. 2014; Gopal et al. 2014). Amphibian skin is one of the most generous sources of these peptides (Giacometti et al. 2007; Mangoni et al. 2008; Castro et al. 2009). For example, the cAMPs aureins were originally isolated from the skin secretions of the Australian bell frogs *Litoria aurea* and *Litoria raniformis* (Dennison et al. 2007). In this family, Aurein 1.2 (AU) is the most studied member.

Although the exact mode of action of cAMPs has not been established, it is accepted that the cytoplasmic membrane is their main target (Vicente et al. 2013). Most of the cAMPs reported disrupt the membrane of cells via three general mechanisms: (1) barrel-stave, (2) toroidal-pore, and (3) carpet. In the "barrel-stave" model, the peptides 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", lipids are intercalated with peptides in the transmembrane channel. Finally, in the "carpet" mechanism, the peptides remain tightly bound to the membrane interface until reaching a threshold concentration that promotes bilayer damage via detergent mechanism (Ambroggio et al. 2005). It is well known that parameters such as charge, helicity, hydrophobicity, and amphipathicity are important for the activity of cAMPs (Huang et al. 2010; Crusca et al. 2011; Cespedes et al. 2012; Huang et al. 2014). In addition, recent studies have shown that dimerization of cAMPs is a new parameter to be considered in the design of new molecules. However, the effect of this modification remains unclear, since dimerization of cAMPs could increase or decrease antimicrobial activity (Dempsey et al. 2003; Zhu and Shin 2009; Lorenzón et al. 2012).

Regardless of the mechanism of action, these molecules have to cross the cell wall of the microorganisms to reach the plasmatic membrane, its main target. In addition, recent studies showed that cAMPs can bind and dissociate lipopolysaccharides (LPS) aggregates with greater efficacy (Srivastava and Ghosh 2013; Kushibiki et al. 2014; Mohanram and Bhattacharjya 2014). In this way, the interaction between AMPs and cell wall components needs to be considered for the design of novel and more active antimicrobial agents.

E. N. Lorenzón · J. P. Piccoli · E. M. Cilli (✉)
Institute of Chemistry, UNESP - Univ Estadual Paulista, Rua Prof. Francisco Degni, 55, Bairro, Quitandinha, CEP 14800-060 Araraquara, São Paulo, Brazil
e-mail: cilli@iq.unesp.br

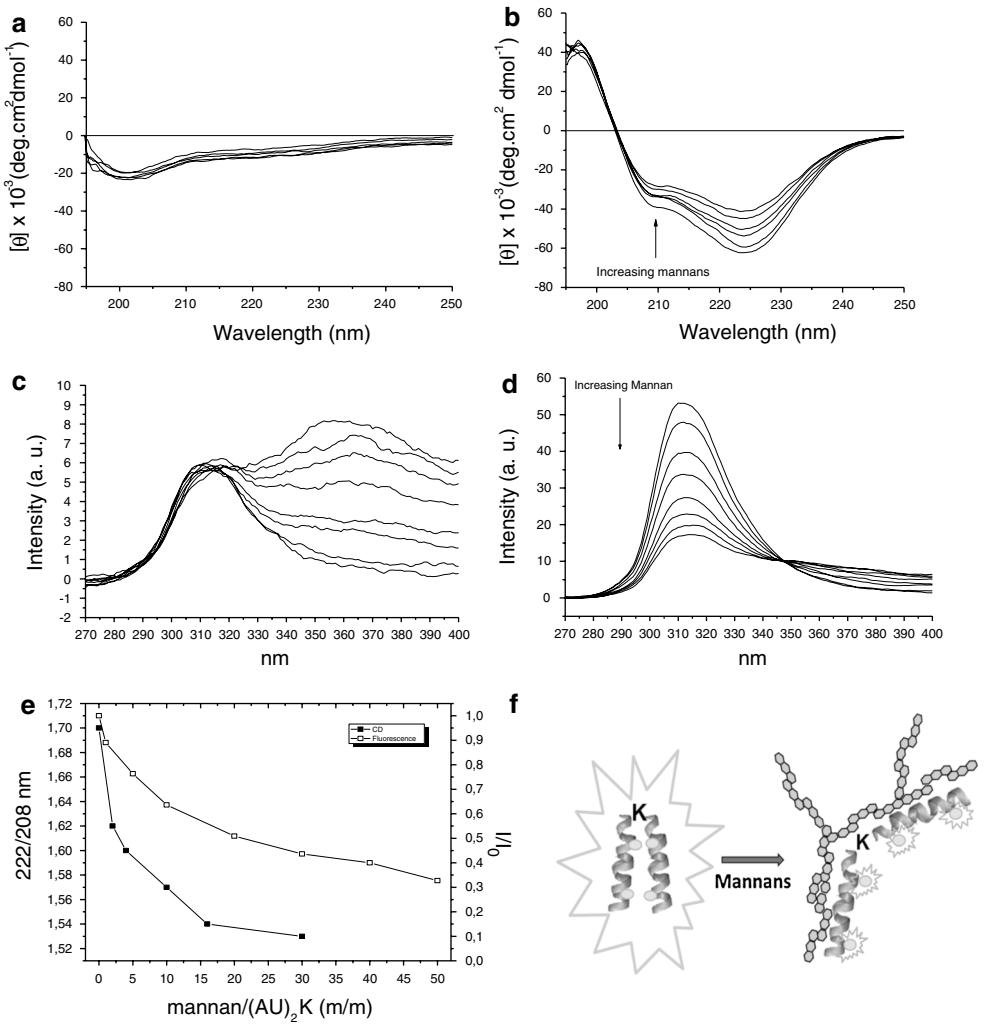

Fig. 1 CD spectra of the 40 μmol/L peptides AU (**a**) and $(AU)_2K$ (**b**) as a function of mannans mass. The mannans range was from 0- to 30-fold of the peptide mass. Fluorescence spectra of the 90 μmol/L peptides AU (**c**) and $(AU)_2K$ (**d**) as function of mannans mass. The mannans range was from 0- to 50-fold of the peptide mass. CD and fluorescence spectra of AU do not change. Contrarily, the helical content (222/208 nm) and relative fluorescence intensity (I maximum intensity at each mannan/peptide ratio, I_0 maximum intensity in the absence of mannan) of $(AU)_2K$ are reduced with the addition of mannans (**e**). Scheme of the proposed model for the interaction between $(AU)_2K$ and mannans. $(AU)_2K$ interacts with the polysaccharide chains decreasing the coiled-coil conformation and exposing the fluorophore to the aqueous solution, decreasing the fluorescence intensity. *Oval circle* represents phenylalanine residues (**f**)

Candida albicans is the most prevalent fungal pathogen in humans, causing infections ranging from superficial mucosal infections to hematogenous candidiasis. *C. albicans* adhesion to host epithelial cells is the first step in infection. This adhesion is mediated by cell wall components that interact with the host cells. The cell wall of *C. albicans* contains different carbohydrates that come into contact with epithelial cells and facilitate cell–cell interconnections (Klotz et al. 2004; Salgado et al. 2011; Tsai et al. 2011). Between these carbohydrates, mannans are the main and the major antigenic component of the cell wall of yeast (Nelson et al. 1991; Tsai et al. 2011; Hardison and Brown 2012).

In a previous work, our group showed that the dimeric analog of AU, called as $(AU)_2K$, was unable to inhibit the growth of *C. albicans* but led to the aggregation of it cells. However, $(AU)_2K$ promoted carboxyfluorescein release from large unilamellar vesicles, suggesting that there are components interacting with the peptide, hindering the reach of the plasmatic membrane (Lorenzon et al. 2013). We hypothesized that the reduced antifungal activity could be due to the interaction of the peptide with cell wall carbohydrates like mannans. To confirm this hypothesis, in this study, circular dichroism (CD) and fluorescence spectroscopy were employed to evaluate the interaction between the peptides AU and $(AU)_2K$ with mannans.

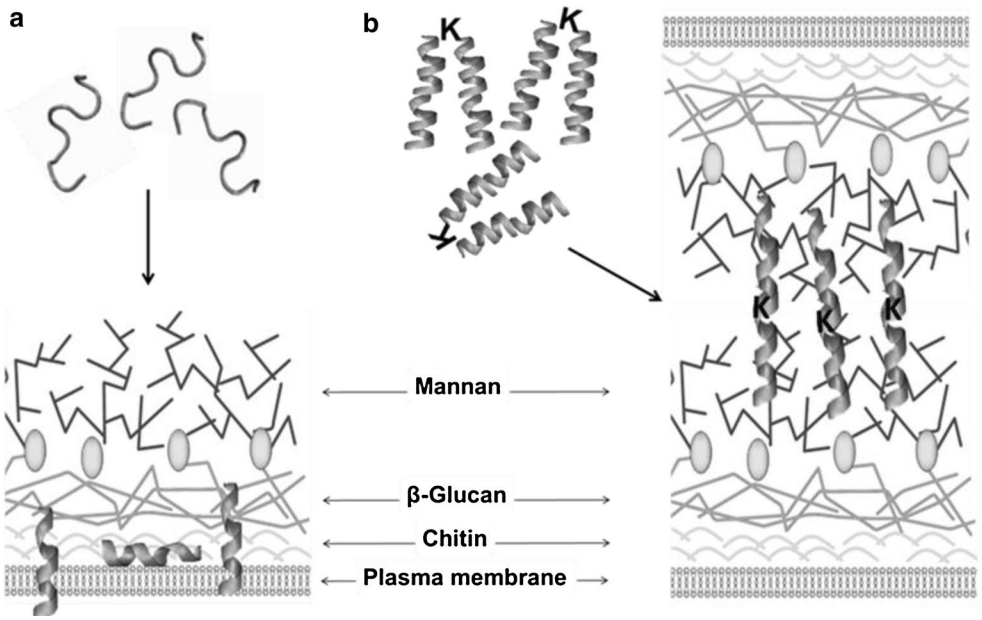

Fig. 2 Proposed model for the interaction of AU (**a**) and $(AU)_2K$ (**b**) with yeast cells. AU cross the cell wall of *C. albicans* and reach its target, while the interaction between $(AU)_2K$ and mannans hinders the peptide to reach the plasmatic membrane (Adapted from Hardison and Brown 2012)

Materials and methods

Aurein 1.2 (AU, sequence: GLFDIIKKIAESF-NH_2) was manually synthesized by solid-phase peptide synthesis using standard 9-fluorenylmethyloxycarbonyl (Fmoc) protocols on a Rink MBHA resin. For the synthesis of the lysine-linked dimeric peptide, $(AU)_2K$, Fmoc-Lys(Fmoc)-OH was first attached to the resin, and after α- and ε-Fmoc group deprotection, the two chains were simultaneously elongated. The peptides were obtained with a high level of purity (above 98 %) and the identities of these molecules were confirmed by electrospray mass spectrometry (positive ion mode on a Bruker model apparatus; 1,479.9 and 3,070.7 g/mol for AU and $(AU)_2K$, respectively). CD spectra (JASCO J-715 CD spectrophotometer) were recorded between 195 and 250 nm at 25°Con 0.06 and 0.12 mg/mL, for AU and $(AU)_2K$, respectively (40 μmol/L), peptide solution in phosphate-buffered saline (PBS) 20 mmol/L, pH 7.2. Different quantities of mannans (Sigma M7504; O-and N-linked mannose polymers from *Saccharomyces cerevisiae* with average molecular weight ranged from 31,700 to 64,100 Da) were added to investigate the conformational changes (0-, 2-, 4-, 10-, 16- and 30-fold of the peptide mass). Fluorescence experiments were performed on 0.13 and 0.26 g/mL, for AU and $(AU)_2K$, respectively (90 μmol/L), peptide solution in PBS on a spectrofluorimeter with an excitation wavelength of 257 nm (phenylalanine). Emission spectra were recorded between 270 and 400 nm. Interaction of the peptides with mannans was monitored varying the polysaccharide concentration (ranging from 0-, 1-, 5-, 10-, 20-, 30-, 40- and 50-fold of the peptide mass).

Results and discussion

CD studies in PBS showed that AU displayed a typical spectrum for disordered structures (Fig. 1a), while $(AU)_2K$ has a typical helical spectrum with double minima at 208 and 222 nm and a maximum around 195 nm (Fig. 1b). Furthermore, CD spectra of $(AU)_2K$ were typical of α-helices in a coiled-coil conformation, where helices wrap around each other, with greater ellipticity values at 222 nm than at 208 nm (Bromley and Channon 2011). In the presence of mannans, CD spectra of AU do not change (Fig. 1a). Contrarily, the helical content of $(AU)_2K$ is reduced and the ratio 222/208 nm is decreased with the addition of mannans (Fig. 1b). Thus, the mannans induce changes in the peptide conformation, decreasing the peptide chain association and coiled-coil structure. Similar to CD, the fluorescence emission of AU does not change with the addition of mannans (differences above 330 nm are due to mannans contribution) (Fig. 1c). However, fluorescence intensity of $(AU)_2K$ decreased and the maximum emission wavelength underwent a red shift of 6 nm (from 310 to 316 nm) with the increasing of mannans mass (Fig. 1d). Therefore, both helical content and relative fluorescence intensity of $(AU)_2K$ decrease with the addition of mannans (Fig. 1e). This is a direct evidence of the interaction between $(AU)_2K$ and the polysaccharide. In the absence of mannans, the coiled-coil structure shielded the fluorophore (phenylalanine) from the solvent. With the addition of mannans, the peptide molecules interact with the polysaccharide chains, and the fluorophore is more exposed to the aqueous solution, decreasing the fluorescence intensity (Fig. 1f). Hydrogen bonds and electrostatic interactions between negatively charged polysaccharides and cAMPs are the main forces that lead to

polysaccharide–peptide complexes (Srivastava and Ghosh 2013).

According to the CD and fluorescence data and the reported in our previous work, we propose a model in which AU cross the cell wall of *C. albicans*, reach the plasmatic membrane and kill the cell (Fig. 2a). In contrast, the interaction between $(AU)_2K$ and mannans hinders the peptide to reach the plasmatic membrane (Fig. 2b). Because of this interaction, the antifungal activity is lost but leads to cells aggregation.

In conclusion, our results provide strong evidences of the interaction between $(AU)_2K$ and mannans. This finding showed that the interaction between cAMPs and polysaccharides needs to be taken into account in the design of new and more active antifungal agents.

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Conflict of interest None.

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