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Stabilisation of mixed peptide/lipid complexes in selective antifungal hexapeptides

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Abstract

The design of antimicrobial peptides could have benefited from structural studies of known peptides having specific activity against target microbes, but not toward other microorganisms. We have previously reported the identification of a series of peptides (PAF-series) active against certain postharvest fungal phytopathogens, and devoid of toxicity towards *E. coli* and *S. cerevisiae* [López-García et al. Appl. Environ. Microbiol. 68 (2002) 2453]. The peptides inhibited the conidia germination and hyphal growth. Here, we present a comparative structural characterisation of selected PAF peptides obtained by single-amino-acid replacement, which differ in biological activity. The peptides were characterised in solution using fluorescence and circular dichroism (CD) spectroscopies. Membrane and membrane mimetic–peptide interactions and the lipid-bound structures were studied using fluorescence with the aid of extrinsic fluorescent probes that allowed the identification of mixed peptide/lipid complexes. A direct correlation was found between the capability of complex formation and antifungal activity. These studies provide a putative structural basis for the mechanism of action of selective antifungal peptides.

Keywords: Antimicrobial peptide; Fungicide; Postharvest; Conformational analysis; Peptide–lipid interaction

1. Introduction

Antimicrobial peptides have been isolated from different organisms, including animals and plants [1–3]. The antimicrobial peptides are of interest because their proposed mode of action has been postulated to avoid rapid development of microbial resistance. Then, peptides from natural sources, synthetic analogues or newly designed peptides may provide new leads to develop safe and effective therapies against pathogenic microbes that are resistant to more conventional antimicrobials. This prospect could be extended to the control of fungal plant pathogens, which currently is achieved by application of fungicides.

We have been interested in the identification of peptides with specific activity against fungal plant pathogens. In this field, we have identified from combinatorial libraries a series of hexapeptides synthesised with the D-stereo isomers of the natural amino acids (PAF series) [4,5] that show activity against certain fungi that cause postharvest decay in fruits—namely *Penicillium digitatum* and *Penicillium italicum*—but not against other fungi. The PAF peptides exert an inhibitory effect primary on conidia germination but also on hyphal growth [4,5]. The L-amino acid counterparts of PAF peptides were shown retain the biological activity suggesting that PAF peptides bind to and act directly on biological membranes and do not require the assistance of protein (chiral) receptor for biological activity. The newly identified peptides share, with other antimicrobial peptides, two common and functionally important requirements, net cationic charge that induces interaction with negatively charged microbial membranes and a primary sequence characterised by the presence of hydrophobic and hydrophilic residues in such a distribution that could allow peptide incorporation in biological membranes [6]. The amphipathicity is presumably related to the mode of action that, after a charge-to-charge interaction, hydrophobic side chains interact with

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the core of the lipid bilayer perturbing the biological function of the membrane. It is also known from studies on membrane proteins and model peptides that Trp residues preferentially occur at the membrane–water interface, in particular at the region near to lipid carbonyls [7–9]. The PAF peptides contain Trp residues that are essential for their biological activity [4,5] and one may hypothesize that these residues could contribute to correct positioning of the peptide molecule in the membrane and thus to antifungal activity. However, hexapeptides are believed to be too small to be induced in stable amphipathic secondary structures and only a few cases of free and co-factor assisted folded peptides have been reported [10–13, and references herein]. These findings prompted us to analyse the interaction of selected PAF peptides with synthetic lipid membranes and membrane mimetics.

2. Materials and methods

2.1. Peptide synthesis and purification

Peptides were synthesized by solid-phase methods using *N*-(9-fluorenyl) methoxycarbonyl (Fmoc) chemistry [14] and purified by preparative reverse phase high-pressure liquid chromatography (RP-HPLC), as previously described [4,5]. Matrix-assisted laser desorption ionization-time-of-flight mass spectrometry was use to confirm peptide identity. Stock solutions of each peptide were prepared at 1 mM in 5 mM 3-(*N*-morpholino)-propanesulfonic acid (MOPS-NaOH) pH 7 buffer and stored at −20 °C. For biological assays and spectroscopy studies, peptide concentrations were determined by measuring the absorbance at 280 nm (ε_{280} = 5600 M^{-1} cm^{-1} for Trp residue).

2.2. Preparation of lipid vesicles

Egg yolk L-phosphatidylcholine (1, 2-diacyl-*sn*-glycero-3-phosphocholine, PC) and brain L-phosphatidylserine (1, 2-diacyl-*sn*-glycero-3-phospho-L-serine, PS) were from Sigma. Small unilamellar vesicles (SUV, PC/PS, 9:1, mol/mol) were prepared in 5 mM MOPS-NaOH, pH 7 by sonication of phospholipid dispersions as already described [15].

2.3. Circular dichroism (CD) measurements

CD spectra were recorded with a Jasco-820 spectropolarimeter. Unless otherwise stated, the buffer for CD assays was 5 mM MOPS-NaOH, pH 7.0 in quartz cells of 0.1-cm path length (in the far-UV, i.e., 190–250 nm) and of 1-cm path length (in the near-UV, i.e., 250–350 nm). CD spectra were the average of five to seven scans, made at 0.2-nm intervals, and always the same buffer and/or lipid solutions without peptides, used as baseline, were subtracted.

2.4. Fluorescence measurements

Fluorescence measurements were carried out at 20 ° C on a Perkin-Elmer LS-50B spectrofluorimeter using slit widths within 1 and 5 nm for excitation and emission depending on each particular experiment. Fluorescence emission spectra of the peptides and 1, 6-diphenyl-1, 3, 5-hexatrien (DPH) probe were obtained by excitation at 280 and 380 nm, respectively, using a 1-cm quartz cell. Stock solutions of DPH were prepared at 2.5 mM in tetrahydrofuran (THF) and maintained in dark. For the determination of the cmc of sodium dodecyl sulfate (SDS), microliter quantities of a concentrated SDS solution were added to 1.0 ml of MOPS-NaOH 5 mM pH 7.0 buffer containing 5 μM of DPH probe and the relative fluorescence was recorded after thorough mixing. In the binding experiments, fluorescence emission spectra of the peptides in 5 mM MOPS-NaOH pH 7.0 were monitored from 300 to 500 nm. Titrations were performed by addition of small aliquots of the liposome (or SDS) solution to the peptide at a desired concentration in 1 ml, and the data shown are representative of several independent experiments. As a control of the fluorescence of Trp residues, we used *N*-acetyl-L-tryptophan-amide (Ac-Trp-NH_2, NATA). Always the possible weak fluorescence contribution due to the buffer and/or amphiphilic solutions without peptides was used as baseline in all the experiments and subtracted.

3. Results

The active peptides PAF19 and PAF26 showed selective antifungal activity when tested against different microorganisms [4,5] (see as example previously reported data in Table 1). Remarkably, they possess antimicrobial specificity that allows discrimination of species of the genus *Penicillium*, as *P. digitatum* and *P. expansum*, two close relatives that infect fruit vegetables [16]. These two peptides have a tryptophan residue at position 4 that is critical for antifungal toxicity, as demonstrated by the marked decrease in activity against *P.*

Table 1
Biological activity of the PAF peptides

Name	Sequence[a]	Antimicrobial activity (IC_{50}, μM)[b]		
		P. digitatum PHI-26	*P. expansum* PHI-65	*E. coli* DH5α
PAF19	Ac-rktwfw-NH_2	36 ± 6[c]	NI[c,d]	NI[c]
PAF20	Ac-rktpfw-NH_2	NI[c]	NI[c]	NI[c]
PAF26	Ac-rkkwfw-NH_2	9 ± 2[c]	NI[c]	>100[c]
PAF37	Ac-rkkpfw-NH_2	51 ± 19[e]	NI[e]	NI[e]

[a] Lower case letter represents D-amino acids.
[b] IC_{50} = concentration necessary to inhibit 50% of growth as determined by a serial dilution assay.
[c] Previously reported data [4,5,16].
[d] NI, not inhibitory (i.e., no significant effect observed on growth) at 100 μM peptide.
[e] This work.

digitatum of both the Trp to Pro [5] and of the Trp to Ala (López-García et al. to be published elsewhere) substitution analogues of PAF19 and the peptide synthesized for the present study PAF37 (Table 1). With the aim of providing information to explain these noteworthy properties, in the present study we have analysed the affinity to bind to synthetic model membranes and the secondary structure properties of PAF peptides, through fluorescence and CD spectroscopies.

3.1. Interaction of peptides with model membranes using fluorescence spectroscopy

In order to evaluate the binding affinity of PAF peptides to phospholipid vesicles, we used fluorescence spectroscopy. Emission spectra of PAF19, PAF20, PAF26 and PAF37 were recorded in the absence or in the presence of vesicles containing negatively charged lipids (SUV, PC/PS, 9:1, mol/mol). The wavelength at the emission maximum of the peptides in buffer suggests totally exposed Trp residues [15,17] for all four peptides. However, the emission maximum is always blue-shifted upon peptide binding to SUV, indicating that all peptides were inserted into the lipid phase of the membrane (Fig. 1A). A more detailed analysis suggests that the microenvironment where the Trp residue of each individual peptide is placed could be different from one to another peptide. Interestingly, the tryptophans of PAF19 and PAF26 remain in a more polar microenvironment (but not totally water-exposed [18]) than those of PAF20 and PAF37 peptides. In addition, when the relative intensity of fluorescence emission was analysed (Fig. 1B), we observed that the biological active peptides (PAF19 and PAF26) exhibited a sudden increase in their fluorescence signature together with a monophasic behaviour when the phospholipid/peptide ratio (Ri=[phospholipid]/[peptide]) was increased. In contrast, PAF20 and PAF37 that have a diminished biological activity when compared with PAF19 and PAF26 showed only a very small and linear increase of the fluorescence intensity as Ri increased (Fig. 1B). The amphipathic nature of the two Trp residues at the C-terminus in PAF26 and PAF19, especially Trp-4, would anchor the antimicrobial peptides in a rather polar microenvironment at the membrane–water interface. These results are in line with those obtained for different six-amino-acid-long analogues of the antimicrobial peptide lactoferricin B [19,20] and for antimicrobial peptides belonging to the pediocin-like family of bacteriocins [21].

SDS is an anionic surfactant that has been used as membrane mimetic due to its intrinsic properties [19,22,23]. SDS exists as monomer in solution or in a micellar state at concentrations above the critical micellar concentration (cmc). Then, SDS is a membrane mimetic additive that it is useful to analyse the formation of peptide/detergent mixed complexes. The cmc value for SDS strongly depends on the ionic strength [24–27]. We have determined (see Material and methods) a value of 10 ± 1 mM for

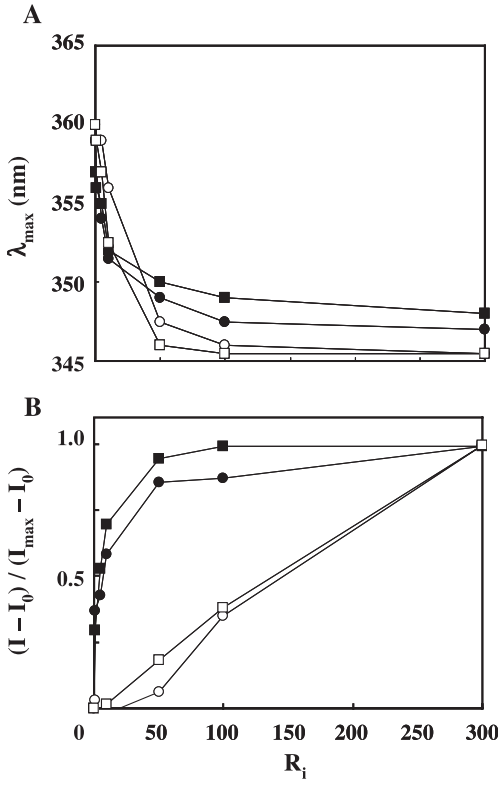

Fig. 1. Fluorescence spectroscopy analysis of the binding of PAF peptides to PC/PS SUVs. (A) Fluorescence emission maximum wavelength of the Trp residues and (B) relative variation of the fluorescence intensity expressed as the ratio $(I - I_0)/(I_{max} - I_0)$ where I_0 is the intensity at 340 nm in the absence of SUVs, I is the fluorescence intensity upon binding to SUVs and I_{max} is the maximum fluorescence intensity obtained from double reciprocal plots. The peptide concentration was 3 μM and Ri is the molar phospholipid/peptide ratio. (●) PAF19, (○) PAF20, (■) PAF26 and (□) PAF37.

the cmc of SDS in our experimental conditions. A preliminary titration by fluorescence spectroscopy of PAF peptides with SDS pointed to a peptide–SDS interaction at SDS concentrations below the cmc (not shown). In fact, the emission maximum of the Trp residues averaged at 346 nm (in plain buffered solution the emission maximum was 360 nm) in the presence of 1 mM SDS. We have further explored the formation of mixed peptide/SDS complexes with the aid of the fluorescent probe DPH. Incorporation of DPH into the core of membranes and/or micelles is accompanied by strong enhancement of its fluorescence [28]. The fluorescence at 430 nm (λ_{exc} = 380 nm) of a solution containing 5 μM DPH in the presence of 0.4 mM SDS is negligible (Fig. 2A). However, increased concentrations of PAF peptides induced a marked increase in the fluorescence emission of DPH (Fig. 2A) suggesting the incorporation of the probe into the hydrophobic core of peptide/SDS complexes. Interestingly, the biologically active peptides PAF19 and PAF26 induced a fluorescence increment larger than that induced by the less active PAF37 and the inactive PAF20, showing a good correlation with biological activity. When PAF peptides in the presence of 5 μM DPH are

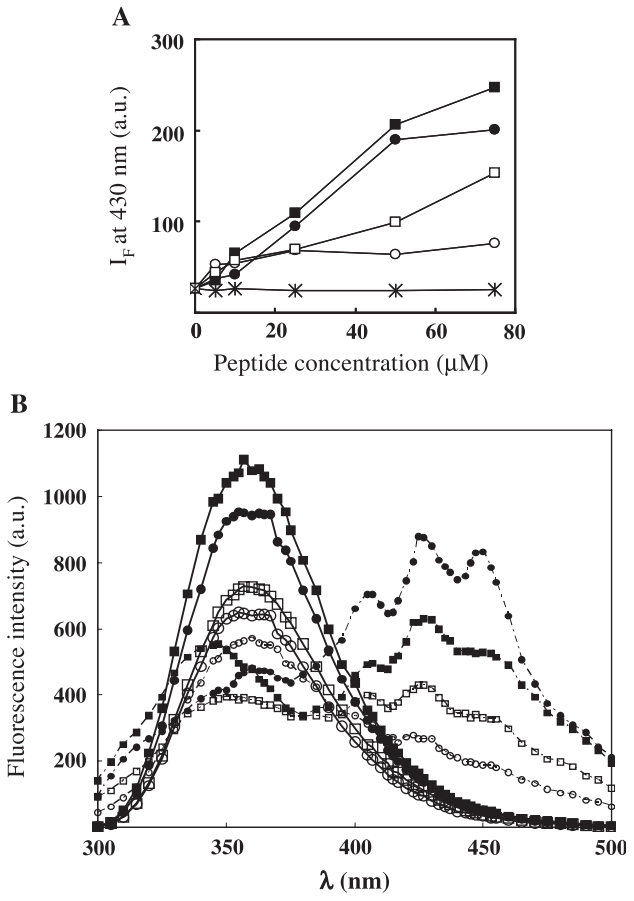

Fig. 2. Fluorescence spectroscopy analysis of the formation of peptide/SDS mixed micelles. (A) Fluorescence emission intensity of the probe DPH at 430 nm upon excitation at 380 nm in the absence (*) and in the presence of 0.4 mM SDS at different peptide concentrations. (B) Fluorescence emission spectra obtained by excitation at 280 nm of a mixture of DPH (5 μM) and PAF peptides (20 μM) in the absence (full line and large symbols) and in the presence (dotted line and small symbols) of SDS (0.4 mM). Symbols as in Fig. 1.

excited at 280 nm, only the fluorescence emission of Trp residues is recovered with emission maxima values close to 360 nm (Fig. 2B, solid lines). However, when the peptides and the probe are excited at 280 nm in the presence of 0.4 mM SDS, two emission maxima are observed (Fig. 2B, dashed lines) corresponding to the Trp fluorescence at short wavelengths (emission maxima around 346 nm) and the probe fluorescence at longer wavelengths (emission maxima centered at 430 nm). In these conditions, the fluorescence intensity of the Trp emission is decreased when compared to the former conditions. This quenching can be due to energy transfer, then the Trp residues of the peptides should be close to the DPH probe and they contribute to its fluorescence because of energy transfer.

The process of energy transfer between the Trp residues of the active peptides (PAF26 as an example) and the DPH probe was analysed in the presence of different concentrations of SDS (Fig. 3). Upon excitation at 280 nm, the fluorescence emission at 350 nm decreased (Fig. 3A, black squares) concomitant with an increase at 430 nm (Fig. 3B) at SDS concentrations below 1 mM. When the concentration of SDS was increased, the emission at 350 nm (Fig. 3A) was stabilised while the emission at 430 nm (Fig. 3B) decreased, suggesting a diminished transfer of energy from Trp residues to the DPH when the molecular organization of the system changes from peptide/SDS complexes to premicellar aggregates or well-organized SDS micelles with peptide incorporated on it. In contrast, the fluorescent behaviour of the system in the presence of the less active

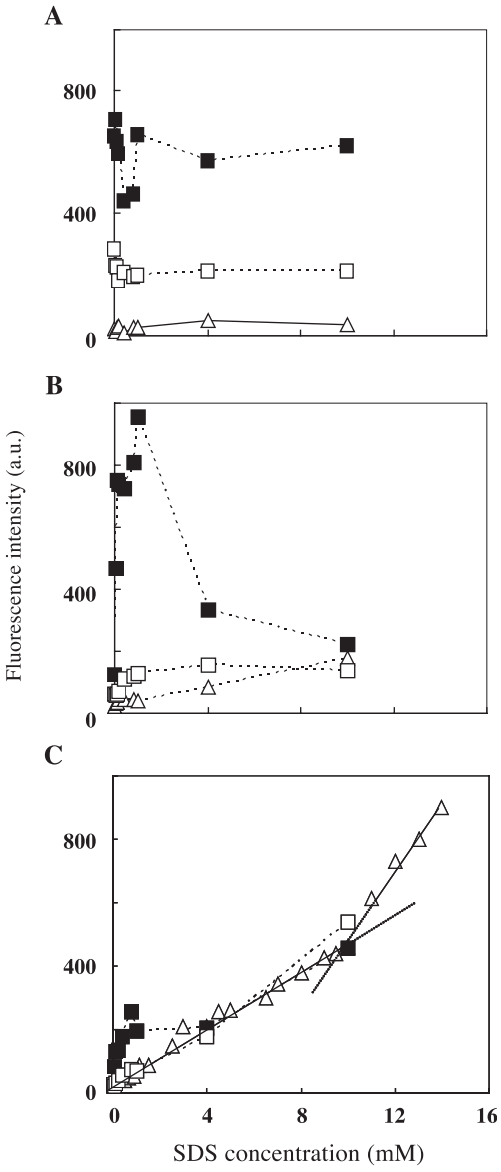

Fig. 3. Fluorescence spectroscopy analysis of the SDS concentration dependence in the formation of peptide/SDS mixed micelles. Fluorescence emission intensity upon excitation at 280 nm of a mixture of DPH (5 μM) and PAF peptides (3 μM) at different SDS concentrations following the emission (A) at 350 nm and (B) at 430 nm. (C) Fluorescence emission intensity of a mixture of DPH (5 μM) and PAF peptides (50 μM) upon excitation at 380 nm. (■) PAF26; (□) PAF37. (△) Control sample of DPH alone that was used for the determination of the cmc of SDS in the present experimental conditions (see text for details).

peptide PAF37 (white squares in Fig. 3, as an example) was quite similar to that of the DPH probe in the absence of peptide (Fig. 3A and B, white triangles). Furthermore, the formation of SDS–peptide complexes was also followed by the changes in fluorescence characteristics of DPH at 430 nm upon excitation at 380 nm (Fig. 3C). A noticeable increase in the fluorescence intensity was only observed for PAF26 at detergent concentrations below 1 mM. This provides support to the formation of a specific peptide–surfactant complex at premicellar concentrations. Premicellar surfactant solutions (surfactant concentration below cmc) may be considered to consist in a distribution of surfactant aggregates, which are not fully formed micelles [26]. Moreover, the peptide was capable of decreasing the cmc of SDS (from 10 to 4 mM) due to the formation of mixed peptide–SDS micelles [25].

3.2. Structural analysis by CD spectroscopy

The conformational behaviour of the D-amino-acid-containing PAF peptides was further analysed by means of CD spectroscopy (it should be taken into account that the CD spectra of D-amino acid-based peptides are the mirror image of L-amino acid-based peptides). All four peptides analysed (PAF19, PAF20, PAF26 and PAF37) adopted a random conformation in aqueous solution at pH 7.0 (data not shown). At concentrations below the cmc of the SDS, the far-UV CD spectra of peptides PAF20, and PAF37 are characterised by the presence of two maxima at 202 and 225 nm that can be associated with a helix-like conformation. In fact, the CD spectrum of PAF37 is similar to those described for short 12- to 14-amino-acid-long designed helical peptides containing a Tyr residue [29] and with those obtained for the TPG tritrpticin analogue (13 amino acids) in SDS micelles [30]. The spectrum obtained for the peptide PAF26 showed the presence of two maxima at 207 and 222 nm (Fig. 4A) that suggest the induction in part of an α-helical conformation [29]. The stabilisation of α-helical conformations has been previously reported for other antimicrobial peptides in membrane–mimetic environments [30,31]. In contrast, the CD spectra of PAF19 showed minimum and maximum molar ellipticity values at 195 and 208 nm, respectively, at SDS concentrations from 0.3 mM up to 3.0 mM (Fig. 4B). This spectrum is similar to the CD spectrum obtained for the six-residue (RRWQWR-NH_2) active center of lactoferricin B bound to SDS micelles previously reported by Schibli et al. [19]. These authors have suggested that such spectrum can be due to the indole transitions of two Trp residues in a defined structure stabilised by the micellar media. Furthermore, the adoption of a polyproline II-like conformation [32] could be also consistent with the CD signatures of PAF19 as also suggested for the CD spectrum of TWF tritrpticin analogue in both aqueous buffer and SDS micelles [30].

The aromatic CD spectrum (near-UV CD) is useful for examining the occurrence of peptide/protein conformations

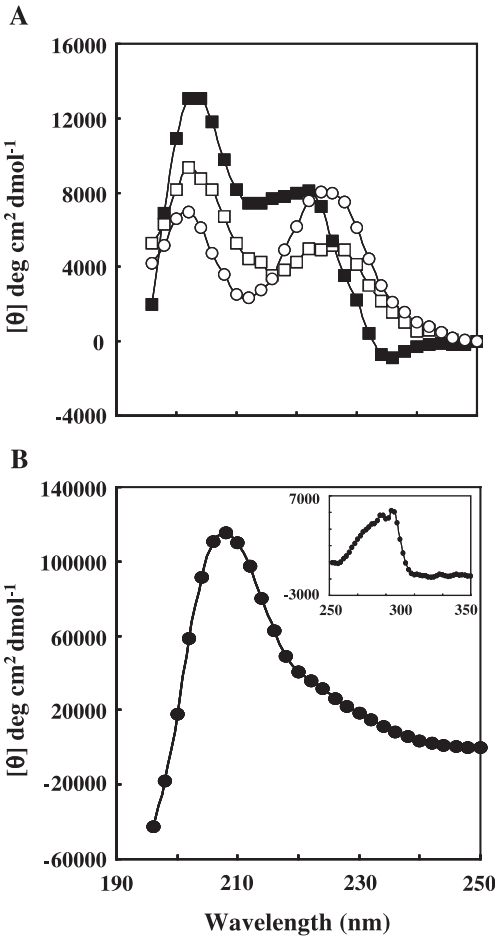

Fig. 4. Conformational behaviour of PAF peptides in the presence of SDS below cmc. (A) Far-UV CD spectra of (○) PAF20, (■) PAF26 and (□) PAF37 at 50 μM concentration in the presence of 1 mM SDS. (B) Far-UV and near-UV (insert) CD spectra of (●) PAF19 at the same experimental conditions. Notice the different scale on the *y* axes.

that have aromatic residues in a well-ordered structure. Native proteins typically show CD spectra in the near-UV that arise from the asymmetric environments of the aromatic amino acids. On the contrary, a weak and monotonous spectrum suggests a disordered or fluctuating tertiary structure. The CD signal observed in the near-UV spectrum of PAF19 (inset in Fig. 4B) suggested the presence of unique side-chain interactions in a well-defined conformation. In contrast, peptides PAF20, PAF26 and PAF37 do not have CD signal in the near-UV.

4. Discussion

We have analysed the structural behaviour of a series of antifungal all-D-amino acid hexapeptides (PAF peptides) identified from the screening of combinatorial libraries. At the screening process we included two parameters for library deconvolution, antifungal activity against *P. digitatum* and low antimicrobial activity against non-target

microbes (i.e., *E. coli*), in order to define the selective peptides PAF19 and PAF26. In fact, these PAF peptides were as potent in vitro as the natural antifungal peptide melittin but, very remarkably, they did not share with melittin its high nonspecific toxicity to *E. coli* and *S. cerevisiae* [4]. A main objective of our current work is the identification of structural properties associated to PAF selective biological activity.

Fluorescence and CD spectroscopies showed that all the PAF peptides are essentially unstructured in buffer, with a water exposure of aromatic amino acids. The binding to model membranes (PC/PS SUVs), when followed by the analysis of the maximum emission wavelength of the Trp fluorescence, looked similar for all peptides (Fig. 1A). However, the fluorescence intensity changes associated to the binding to the membranes were different when the more active PAF19 and PAF26 are compared with PAF20 and PAF37 (Fig. 1B). These results suggest that all four PAF peptides are able to bind to SUV, although the relative orientation in the membrane plane and/or the peptide-induced membrane disruption could be different for active and inactive peptides.

It is generally accepted that when antimicrobial peptides seek to interact with phospholipid membranes, there is an initial electrostatic interaction involving positively charged amino acids of the peptides [6,33]. Afterwards, the hydrophobic amino acids, which are present in most of this class of peptides, have a high tendency to be allocated in the hydrophobic core of the membrane. There are two mechanism hypotheses that aim to define how the peptide/membrane system became energetically stabilised, the 'barrel stave' and the 'carpet' mechanism [3,34]. In the 'barrel stave' model, the peptides insert into the lipid core of the membrane following recruitment of additional monomers and the formation of pores. This model could serve well for membrane spanning peptides; however, it is believed to hardly explain the mode of action of short antimicrobial peptides where the formation of pores is not probable due to the length of the peptide sequence [35]. The 'carpet' mechanism postulates that membrane destabilisation is a direct consequence of the formation of mixed peptide/lipid micelles when a threshold peptide concentration is reached. Thus, peptides that follow the 'carpet' mechanism for membrane interaction should be able to form mixed peptide/lipid complexes. We have explored the formation of such complexes using SDS as a membrane mimetic system with the aid of the fluorescent probe DPH (Figs. 2 and 3). From our results it could be suggested that the monomeric peptide in solution binds to SDS monomers and the formation of a mixed peptide/SDS complex can be induced. A minimum stoichiometry of eight SDS per peptide molecule can be deduced (3:1 SDS/lysine (arginine) ratio for PAF26 and 4:1 SDS lysine (arginine) ratio for PAF19) in SDS at 0.4 mM concentration. These complexes should expose to the solvent the sulfate groups of SDS and the polar (lysine, arginine) amino acids of peptides. The SDS tails and nonpolar amino acid residues will build the hydrophobic core of the complex that can host hydrophobic fluorescent probes as DPH. The molecular organisation seems to be well defined because it allows the process of energy transfer to occur between the indole ring of Trp residues and the probe (Fig. 2B). Furthermore, the PAF peptides and, in particular, PAF19 adopts a well-defined conformation that should induce a highly restricted environment for the Trp residues as deduced from both the far- and near-UV CD spectra (Fig. 4) in the presence of SDS concentrations that induce the formation of the peptide/SDS complex. Thus, it is conceivable that the two Trp residues in PAF26 and PAF19 could be located in each other's vicinity, which could give rise to an additional important interaction for stabilising the active structure [21].

In conclusion, our data suggest that peptides from the PAF series with specific antimicrobial properties have a high tendency to form peptide/lipid mixed complexes. It is feasible that such a property could define the basis of their biological activity that is linked to a strong perturbation of fungal membranes. The data derived from the present study could enlighten our knowledge in the molecular mechanism that orchestrates selectivity in antimicrobial activity. However, it still remains to more definitively settle whether or not there exists a parallelism between the perturbation induced by this class of peptides on cell membranes of different composition and the nature and detailed structure of the peptide/lipid mixed complexes here detected. Future work will address these questions and to which extent the peptide/lipid mixed complexes are involved in the mechanism of action of selective antifungal peptides.

Acknowledgements

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References

[1] D. Andreu, L. Rivas, Animal antimicrobial peptides: an overview, Biopolymers 47 (1998) 415–433.
[2] F. Garcia-Olmedo, A. Molina, J.M. Alamillo, P. Rodriguez-Palenzuela, Plant defense peptides, Biopolymers 47 (1998) 479–491.
[3] R.E. Hancock, D.S. Chapple, Peptide antibiotics, Antimicrob. Agents Chemother. 43 (1999) 1317–1323.
[4] B. Lopez-Garcia, E. Pérez-Payá, J.F. Marcos, Identification of novel hexapeptides bioactive against phytopathogenic fungi through screening of a synthetic peptide combinatorial library, Appl. Environ. Microbiol. 68 (2002) 2453–2460.
[5] B. Lopez-Garcia, L. Gonzalez-Candelas, E. Pérez-Payá, J.F. Marcos, Identification and characterization of a hexapeptide with activity against phytopathogenic fungi that cause postharvest decay in fruits, Mol. Plant-Microb. Interact. 13 (2000) 837–846.

[6] A. Tossi, L. Sandri, A. Giangaspero, Amphipathic, alpha-helical antimicrobial peptides, Biopolymers 55 (2000) 4–30.
[7] J.A. Killian, G. Von Heijne, How proteins adapt to a membrane–water interface, Trends Biochem. Sci. 25 (2000) 429–434.
[8] M.R. De Planque, J.A. Kruijtzer, R.M. Liskamp, D. Marsh, D.V. Greathouse, R.E. Koeppe II, B. De Kruijff, J.A. Killian, Different membrane anchoring positions of tryptophan and lysine in synthetic transmembrane alpha-helical peptides, J. Biol. Chem. 274 (1999) 20839–20846.
[9] A.N. Ridder, S. Morein, J.G. Stam, A. Kuhn, B. De Kruijff, J.A. Killian, Analysis of the role of interfacial tryptophan residues in controlling the topology of membrane proteins, Biochemistry 39 (2000) 6521–6528.
[10] F. Eker, X. Cao, L. Nafie, R. Schweitzer-Stenner, Tripeptides adopt stable structures in water. A combined polarized visible raman, FTIR, and VCD spectroscopy study, J. Am. Chem. Soc. 124 (2002) 14330–14341.
[11] J.R. Lai, B.R. Huck, B. Weisblum, S.H. Gellman, Design of non-cysteine-containing antimicrobial beta-hairpins: structure–activity relationship studies with linear protegrin-1 analogues, Biochemistry 41 (2002) 12835–12842.
[12] Z. Oren, J. Ramesh, D. Avrahami, N. Suryaprakash, Y. Shai, R. Jelinek, Structures and mode of membrane interaction of a short alpha helical lytic peptide and its diastereomer determined by NMR, FTIR, and fluorescence spectroscopy, Eur. J. Biochem. 269 (2002) 3869–3880.
[13] V. Esteve, S. Blondelle, B. Celda, E. Pérez-Payá, Stabilization of an alpha-helical conformation in an isolated hexapeptide inhibitor of calmodulin, Biopolymers 59 (2001) 467–476.
[14] G.B. Fields, R.L. Noble, Solid phase peptide synthesis utilizing 9-fluorenylmethoxycarbonyl amino acids, Int. J. Pept. Protein Res. 35 (1990) 161–214.
[15] E. Pérez-Payá, J. Dufourcq, L. Braco, C. Abad, Structural characterisation of the natural membrane-bound state of melittin: a fluorescence study of a dansylated analogue, Biochim. Biophys. Acta 1329 (1997) 223–236.
[16] B. Lopez-Garcia, A. Veyrat, E. Pérez-Payá, L. Gonzalez-Candelas, J.F. Marcos, Comparison of the activity of antifungal hexapeptides and the fungicides thiabendazole and imazalil against postharverst fungal pathogens, Int. J. Food Microbiol. 89 (2003) 163–170.
[17] E. Pérez-Payá, I. Porcar, C.M. Gomez, J. Pedros, A. Campos, C. Abad, Binding of basic amphipathic peptides to neutral phospholipid membranes: a thermodynamic study applied to dansyl-labeled melittin and substance P analogues, Biopolymers 42 (1997) 169–181.
[18] D. Salom, E. Pérez-Payá, J. Pascal, C. Abad, Environment- and sequence-dependent modulation of the double-stranded to single-stranded conformational transition of gramicidin A in membranes. PG, Biochemistry 37 (1998) 14279–14291.
[19] D.J. Schibli, P.M. Hwang, H.J. Vogel, The structure of the antimicrobial active center of lactoferricin B bound to sodium dodecyl sulfate micelles, FEBS Lett. 446 (1999) 213–217.
[20] B.E. Haug, J.S. Svendsen, The role of tryptophan in the antibacterial activity of a 15-residue bovine lactoferricin peptide, J. Pept. Sci. 7 (2001) 190–196.
[21] G. Fimland, V.G. Eijsink, J. Nissen-Meyer, Mutational analysis of the role of tryptophan residues in an antimicrobial peptide, Biochemistry 41 (2002) 9508–9515.
[22] A.S. Ladokhin, M.E. Selsted, S.H. White, CD spectra of indolicidin antimicrobial peptides suggest turns, not polyproline helix, Biochemistry 38 (1999) 12313–12319.
[23] G.A. Dykes, S. Aimoto, J.W. Hastings, Modification of a synthetic antimicrobial peptide (ESF1) for improved inhibitory activity, Biochem. Biophys. Res. Commun. 248 (1998) 268–272.
[24] S.E. Blondelle, J.M. Ostresh, R.A. Houghten, E. Pérez-Payá, Induced conformational states of amphipathic peptides in aqueous/lipid environments. PG-351-9, Biophys. J. 68 (1995) 351–359.
[25] R. Montserret, M.J. Mcleish, A. Bockmann, C. Geourjon, F. Penin, Involvement of electrostatic interactions in the mechanism of peptide folding induced by sodium dodecyl sulfate binding, Biochemistry 39 (2000) 8362–8373.
[26] A. Mishra, G.B. Behera, M.M.G. Krishna, N. Periasamy, Time-resolved fluorescence studies of aminostyryl pyridinium dyes in organic solvents and surfactant solutions, J. Lumin. 92 (2001) 175–188.
[27] A.G. Krishna, S.T. Menon, T.J. Terry, T.P. Sakmar, Evidence that helix 8 of rhodopsin acts as a membrane-dependent conformational switch, Biochemistry 41 (2002) 8298–8309.
[28] G. Lantzsch, H. Binder, H. Heerklotz, P. Welzel, G. Klose, Aggregation behavior of the antibiotic Moenomycin A in aqueous solution, Langmuir 14 (1998) 4095–4104.
[29] R. Banerjee, G. Basu, Direct evidence for alteration of unfolding profile of a helical peptide by far-ultraviolet circular dichroism aromatic side-chain contribution, FEBS Lett. 523 (2002) 152–156.
[30] S.T. Yang, S.Y. Yub Shin, Y.C. Kim, Y. Kim, K.S. Hahm, J.I. Kim, Conformation-dependent antibiotic activity of tritrpticin, a cathelicidin-derived antimicrobial peptide, Biochem. Biophys. Res. Commun. 296 (2002) 1044–1050.
[31] C.L. Friedrich, A. Rozek, A. Patrzykat, R.E. Hancock, Structure and mechanism of action of an indolicidin peptide derivative with improved activity against gram-positive bacteria, J. Biol. Chem. 276 (2001) 24015–24022.
[32] F. Rabanal, M.D. Ludevid, M. Pons, E. Giralt, CD of proline-rich polypeptides: application to the study of the repetitive domain of maize glutelin-2, Biopolymers 33 (1993) 1019–1028.
[33] S. Castano, I. Cornut, K. Buttner, J.L. Dasseux, J. Dufourcq, The amphipathic helix concept: length effects on ideally amphipathic LiKj(i=2j) peptides to acquire optimal hemolytic activity, Biochim. Biophys. Acta 1416 (1999) 161–175.
[34] Z. Oren, Y. Shai, Mode of action of linear amphipathic alpha-helical antimicrobial peptides, Biopolymers 47 (1998) 451–463.
[35] K. Anzai, M. Hamasuna, H. Kadono, S. Lee, H. Aoyagi, Y. Kirino, Formation of ion channels in planar lipid bilayer membranes by synthetic basic peptides, Biochim. Biophys. Acta 1064 (1991) 256–266.