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Biochimica et Biophysica Acta 1788 (2009) 1600–1609

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem

Review

Binding of amphipathic α-helical antimicrobial peptides to lipid membranes: Lessons from temporins B and L

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A R T I C L E I N F O

Article history:
Received 17 December 2008
Received in revised form 8 April 2009
Accepted 17 April 2009
Available online 24 April 2009

Keywords:
Temporin
Antimicrobial peptide
Lipid–protein interaction
Amyloid
Mode of action

A B S T R A C T

Temporins constitute a family of amphipathic α-helical antimicrobial peptides (AMP) and contain some of the shortest cytotoxic peptides, comprised of only 10–14 residues. General characteristics of temporins parallel those of other AMP, both in terms of structural features and biophysical properties relating to their interactions with membrane lipids, with selective lipid-binding properties believed to underlie the discrimination between target *vs* host cells. Lipid-binding properties also contribute to the cytotoxicity AMP, causing permeabilization of their target cell membranes. The latter functional property of AMP involves highly interdependent acidic phospholipid-induced conformational changes, aggregation, and formation of toxic oligomers in the membrane. These oligomers are subsequently converted to amyloid-type fibers, as demonstrated for *e.g.* temporins B and L in our laboratory, and more recently for dermaseptins by Auvynet et al. Amyloid state represents the generic minimum in the folding/aggregation free energy landscape, and for AMP its formation most likely serves to detoxify the peptides, in keeping with the current consensus on mature amyloid being inert and non-toxic. The above scenario is supported by sequence analyses of temporins as well as other amphipathic α-helical AMP belonging to diverse families. Accordingly, sequence comparison identifies 'conformational switches', domains with equal probabilities for adopting random coil, α-helical and β-sheet structures. These regions were further predicted also to aggregate and assemble into amyloid β-sheets. Taken together, the lipid-binding properties and structural characterization lend support to the notion that the mechanism of membrane permeabilization by temporins B and L and perhaps of most AMP could be very similar, if not identical, to that of the paradigm amyloid forming cytotoxic peptides, responsible for degenerative cell loss in *e.g.* prion, Alzheimer's and Parkinson's disease, and type 2 diabetes.
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Abbreviations: AMP, α-helical antimicrobial peptide; Arg, arginine; Aβ, Alzheimer β-peptide; CD, circular dichroism; cDNA, complementary deoxyribonucleic acid; CSSP, continuum secondary structure prediction; DPH, diphenylhexatriene; GUV, giant unilamellar vesicle; His, histidine; IAPP, islet amyloid polypeptide (also known as amylin); Lys, lysine; MD, molecular dynamics; NMR, nuclear magnetic resonance; PASTA, prediction of amyloid structure aggregation; PazePC, 1-palmitoyl-2-azelaoyl-*sn*-glycero-3-phosphocholine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PIP, phosphatidylinositol phosphate; POPG, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-*rac*-1-glycerol; PoxnoPC, 1-palmitoyl-2-(9′-oxo-nonanoyl)-*sn*-glycero-3-phosphocholine; PrP, prion protein; ROS, reactive oxygen species; SLB,, supported lipid bilayer; *sn*, stereochemical notation; SOPC, 1-stearoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; Trp, tryptophan

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0005-2736/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamem.2009.04.012

1. Introduction

Antimicrobial peptides (AMP) are ubiquitously found in higher animals, insects, arthropods, tunicates, and plants, and provide the first line of defence in eukaryotic innate immune system [1]. AMP (bacteriocins) are also secreted by some bacteria [2]. AMP generally show a wide spectrum of activity against both gram-negative and gram-positive bacteria, yeast, and fungi, and some of them are also haemolytic. Importantly, AMP are active against pathogens resistant to traditional antibiotics [3], and thus offer the possibility to develop a new class of antibiotics. In addition some AMP might be useful in treating cancer and there are already several peptides in preclinical and clinical trials [4]. Accordingly, detailed molecular level understanding of their mechanism(s) of action is needed. This task is complicated by *e.g.* the fact that the sequence diversity of AMP is vast and based on their secondary structures AMP are classified into four major classes, viz. α-helical, β-sheet, looped, and extended peptides [5].

A large variety of peptides with a broad spectrum of antibacterial and antifungal activities is synthesized in the skin of frogs and toads, protecting them against invasion by pathogenic microorganisms. Ranid frogs are a particularly rich source of AMP. Temporins were first identified in the skin of the Asian frog *Rana erythraea* and were originally described as *Vespa*-like because of their sequence similarity to chemotactic and histamine-releasing peptides isolated from the venom of wasps of the genus *Vespa*. Simmaco et al. identified in electrically stimulated skin secretions of the European common red frog *R. temporaria* a family of 10 structurally related peptides with antibacterial and antifungal properties and coined these temporins, from A to L [6]. These AMP were isolated by screening a cDNA library from the skin of *R. temporaria* using the signal peptide of the precursor of esculentin as a probe, as similarly to other AMP also temporins are synthesized as large precursors containing a single copy of the mature peptide at the C-terminus, a highly conserved region comprising a 22-residue signal peptide, and an acidic intervening propeptide sequence. While the AMP domain at the C-terminus of the precursors is hypervariable the above structural feature is highly conserved and is seen even in frogs belonging to different families [7]. Temporins likely arose through duplication from a common ancestor gene followed by local hypermutations.

New members of the temporin family have been discovered in extracts of the skin of other species of *Rana*, viz. *clamitans*, *luteiventris*, *pipiens*, and *grylio*, and temporins now include 76 peptides (Table 1). The characteristics of temporins make them interesting for in-depth investigation of their biological functions and mechanisms of action. More specifically, while some of them comprise of up to 17 amino acids, most temporins are among the shortest amphipathic α-helical AMP found, with a single 10–14 amino acid chain. Temporins are also among the most highly variable of all AMP and no single amino acid residue is invariant. All temporins isolated so far contain a prevalence of hydrophobic amino acids and are C-terminally α-amidated. Helix-stabilizing residues such as leucine, alanine and lysine are preponderant. Temporins are also unique amongst most AMPs in having a low net positive charge, with most members containing basic residues (generally Lys, alternatively His and Arg) giving a net charge ranging from 0 to +4 at physiological pH.

The mechanisms by which temporins and AMP in general execute their cytotoxicity are complex and remain incompletely understood. It has become clear that AMP contribute to innate immunity by several mechanisms, from neutralization of the lipopolysaccharide of gram-negative bacteria to the modulation of gene expression [34]. Importantly, the all-D enantiomer of temporin A has equivalent antibacterial activity when compared to the native peptide, indicating that their activities are mediated via non-chiral interactions, with an involvement of a receptor protein being unlikely [35]. Temporin L was found to be rather non-selective, killing efficiently both gram-positive and gram-negative bacteria, fungi, and cancer cells and lysing erythrocytes [36]. Studies on the interaction of temporin L with *E. coli* reveal a dose-dependent increase in the permeability of the bacterial inner membrane. At low peptide concentrations, the inner membrane becomes permeable to small molecules but does not result in the killing of the bacteria. However, at high peptide concentrations, also larger molecules leak out, which causes cell death. The above takes place without a loss of the overall structural integrity of the target cell, with the formation of ghost-like bacteria observed by electron microscopy [36].

The negative charge of bacterial membrane outer surface phospholipids, most notably phosphatidylglycerol (PG) promotes the binding of the cationic AMP [37] and it is currently widely believed that a large part of their antimicrobial effect and cytotoxicity derives from direct interactions with the lipid membrane surrounding the target cells, causing its permeabilization. The latter action has been explained in terms of different models, such as the carpet, barrel-stave, toroidal pore, and detergent-type membrane lytic mechanisms [38], all of which involve conformational changes and oligomerization upon the interaction of AMP with lipids. The lipid-binding properties of AMP further depend on a number of physicochemical properties contained in their amino acid sequence, *i.e.* net positive charge, amphipathicity and hydrophobicity, as well as peptide concentration-dependent folding and aggregation in a membrane environment. The interactions of temporins with model membranes align with the above, although upon comparing different peptides both qualitative and quantitative differences are evident.

In our own studies we focused on the non-haemolytic temporin B (LLPIVGNLLKSLL-NH_2) and the haemolytic temporin L (FVQWFSKFLGRIL-NH_2). These peptides bind to and insert into the membranes and this process is enhanced by acidic phospholipids, involving the formation of a complex with the latter, together with augmented α-helicity of the peptides. Their membrane insertion is accompanied by an increase in lipid acyl chain order. The overall interaction is cooperative and involves threshold peptide:lipid molar ratios, in keeping with the formation of aggregated structures composed of peptide–acidic phospholipid complexes. Finally, there is also a formation of more macroscopic, Congo red staining fibrillar aggregates, observed by microscopy. The above characteristics of the interactions of temporin (and AMP in general) with lipid membranes readily suggest that their sequences should contain motifs allowing for environment dependent 'conformational switching' as well as aggregation. These features are expected to be represented by relatively short stretches and be thus amenable for sequence analyses. In the following, we will discuss the above processes in more detail together with the underlying mechanisms providing the respective driving forces.

2. Lipid-binding properties of temporins B and L

Temporins lack stable secondary structure in aqueous solutions yet have the propensity to form amphipathic α-helices in a phospholipid bilayer or in a membrane-mimetic solvent, such as trifluoroethanol [39]. Accordingly, the α-helicity of temporins is augmented in the presence of phospholipids (*e.g.* [40–42]). Likewise, structural studies on temporin L in the presence of sodium dodecyl sulfate and dodecylphosphocholine micelles using CD, NMR, and MD simulation, show higher a propensity for α-helical structures in both membrane-mimetic systems [43]. The amphipathic character of AMP is enhanced upon this structural transition [38,44], with hydrophilic and hydrophobic side chains becoming accommodated on opposite faces of the helix. Exposure of a hydrophobic surface is a common motif and major driving force for the insertion of a peptide into the highly anisotropic interface of a lipid bilayer, removing hydrophobic side chains from water to contact the bilayer hydrocarbon region. Another contribution to the overall reduction in free energy comes from the shielding of peptide bonds into intramolecular H-bonds in α-helices and β-sheets [45]. Cationic hydrophilic side chains of amphipathic α-helical

peptides further interact electrostatically with negatively charged lipids, which neutralize the excess positive charge of the surface associated peptides and reduce peptide–peptide repulsion [46]. The membrane bound AMP then associate to form aggregates, oligomers, which simultaneously cause membrane permeabilization. Membranes thus provide an environment where AMP can and must adopt conformations and orientations, which promote peptide aggregation, all these processes being intimately coupled.

Lipid monolayers (Langmuir-films) residing on a gas/water interface provide a very easy experimental system to study peptide–lipid association under highly controlled conditions. More specifically, the initial geometry of the lipid film is restricted to 2-D, even when containing lipids such as phosphatidylethanolamine (PE, particularly species with *cis*-unsaturated chains), which because of their conical effective molecular shape are prone in bulk systems to form inverted phases, such as H_{II} [47]. The lipid lateral packing density in the monolayers is accurately controlled, which allows the extent of the insertion of AMP into the film to be investigated as a function of the initial lateral packing density. This yields (i) the dependence of peptide insertion on surface pressure, (ii) the magnitude of surface pressure increase caused by the peptide (correlating to the change in free energy), and (iii) the critical packing density abolishing the intercalation of the peptide into the monolayer [48].

Monolayer experiments revealed temporin B and L to be highly membrane active, effectively inserting into zwitterionic phosphatidylcholine (PC) monolayers. The lipid insertion was augmented by the negatively charged phosphatidylglycerol (PG), an abundant constituent of the bacterial target membranes [49]. The kinetics of the increase in surface pressure π caused by temporin B were different from those for temporin L and suggest a rapid conformational and/or orientational change following the initial intercalation of temporin B into the lipid film. These data also suggest that some PG molecules, which are electrostatically bound to the peptides, are removed from the phospholipid monolayer, becoming oriented with their long axis parallel to the monolayer plane [41,50], in a manner compatible with the orientation of lipids accommodated in the toroidal pore.

The characteristics of collisional quenching of the Trp residue of temporin L in the presence of SOPC liposomes by the water soluble acrylamide and phospholipids with brominated acyl chains demonstrate that this peptide inserts in part into the hydrocarbon region of the bilayer [41]. These data also reveal the presence of two populations of temporin L in SOPC and POPG containing membranes, with parallel and perpendicular orientation with respect to the plane of the membrane surface. Trp fluorescence of temporin L decreased upon its transfer from the buffer into a SOPC membrane and the fluorescence intensity was further decreased in the presence of cholesterol. This suggests an increase in the polarity of the environment of Trp, *e.g.* an increased exposure to the aqueous phase, confirmed by augmented acrylamide quenching. In order to span the bilayer both temporin B and L would need to dimerize, with the membrane inserted α-helices likely arranging into CN–NC dimers oriented perpendicular to the membrane plane, *i.e.* the amino-termini being juxtaposed in the bilayer center, as concluded from the fluorescence spectroscopy studies on temporin L [41].

In keeping with their membrane insertion the effects of temporin B and L on lipid dynamics in bilayers are pronounced, increment in DPH fluorescence anisotropy r revealing peptide-induced increase in acyl chain order in the presence of PG, the magnitude of this effect increasing with the content of the acidic phospholipid [48]. Lipid segregation was induced in the presence of the acidic phospholipid, with an enrichment of the latter lipid in complexes with the peptide. All these effects comply with the importance of the acidic phospholipid as well as the cationic charge of the peptides to their interaction with membranes. Interestingly, both peptides influence the quantum yields of pyrene-labelled lipids. For temporin L quenching of pyrene fluorescence was observed regardless of lipid composition while for temporin B this was abolished in the presence of POPG. Both π–π interactions between pyrene and Trp as well as contacts of the basic residues of the peptides with the fluorophore resulting in π–cation interaction could be involved [48]. Notably, collisional quenching stems from short range interactions. A significant blue shift in combination with a decrease in quantum yield suggests that Trp residue in this case fluctuates between two states, where the other state involves a contact with a charged or polar moiety, causing a diminished quantum yield, while the other state resides in a hydrophobic environment and is lacking this contact.

Experiments with giant unilamellar vesicles (GUV) demonstrated, similarly to indolicidin and magainin 2, a very rapid local aggregation of temporins B and L after their topical application onto the surface of the GUV membrane by microinjection, [48,50]. Yet, the targeted GUV were not dissolved, arguing against detergent-like mechanisms. These experiments further imply that the membrane bilayer perturbing peptide aggregate/oligomer may exist only as a transient intermediate, with the microinjected peptide causing the emergence inside the GUV of a highly refractive, dense and immobile structure at the site of the microinjection.

Supported lipid bilayers (SLB) constitute a fluid two-dimensional space allowing free translational and rotational diffusion of lipid molecules [51]. SLB are well suited to analyze membrane processes such as protein adsorption, self-assembly, and protein induced membrane reorganization [52,53]. Because of the initial well-defined bilayer geometry and confinement on a solid support, this membrane model is of particular interest when combined with surface-sensitive microscopic and spectroscopic techniques. Temporin B (wild type and two variants) and temporin L cause morphological transformations of SLB composed of phosphatidylcholine and phosphatidylglycerol. More specifically, rapid formation on SLB of flexible tubular protrusions composed of both lipid and the amphiphilic peptide is visible by fluorescence microscopy of both the fluorescent lipid analog and the labelled peptide [52]. Wild-type temporin B was found to be the most effective, giving rise to numerous long (up to several hundred micrometers) tubules. The extent and morphological features of peptide-induced perturbations were found to be very sensitive to modifications of the peptide sequence as well as to the SLB lipid composition, with lipids having negative spontaneous curvature attenuating the tubulation [52]. The driving force for tubulation is likely to be augmented lateral packing in the SLB, resulting from the insertion of the peptide into the bilayer, this pressure being relieved by escape of tubules into the bulk phase. The tubules incorporated also fluorescent peptides and were never seen to branch, which may relate to organization of temporins as linear, non-branching oligomer arrays in their surface.

The invading bacteria are exposed to reactive oxygen species (ROS) secreted by activated leukocytes at the sites of infection and inflammation [54]. As a consequence, their membrane lipids undergo a drastic modification due to oxidation, involving the formation of a myriad of phospholipids containing various polar functional groups in the ends of their acyl chains. The latter have pronounced effects on the membrane biophysical properties, including the polarity profile and overall organization. More specifically, the oxidatively modified chains no longer remain in the membrane hydrocarbon region but adopt the so-called extended conformation [55], protruding into the aqueous phase [56]. As a consequence reactive groups, such as the aldehyde present at the end of the truncated *sn*-2 chain in for example 1-palmitoyl-2-(9′-oxo-nonanoyl)-*sn*-glycero-3-phosphocholine (PoxnoPC), becomes readily available for reaction with *e.g.* amino groups of membrane associated peptides. Accordingly, the association of temporin B and L as well as the cathelicidin peptide LL-37 with both lipid monolayers and liposomes was greatly enhanced in the presence of PoxnoPC [57]. Instead, the structurally similar 1-palmitoyl-2-azelaoyl-*sn*-glycero-3-phosphocholine (PazePC) containing a carboxylic moiety was less efficient. Physiological saline reduced the binding of the above

Table 1
76 current members of the amphipathic α-helical antimicrobial peptide family of temporins and results from their analyses by algorithms predicting secondary structure, aggregation and amyloid formation.

Name	Sequence	Region identified by							Ref
		CSSP	SecStr	AGGRESCAN		TANGO	PASTA		
		a	b	c	d	e	F	g	
Temporin A	FLPLIGRVLSGIL-NH_2	4–11	7–12	4–13	52.4	1–13	8–13	−3.64 p	[6]
Temporin B	LLPIVGNLLKSLL-NH_2	7–13	5–13	4–13	44.1	–	4–13	−4.12 p	[6]
Temporin C	LLPILGNLLNGLL-NH_2	7–12	5–13	–	37.2	4–13	4–10	−2.99 p	[6]
Temporin D	LLPIVGNLLNSLL-NH_2	6–12	7–10	–	40.7	5–13	4–13	−5.48 p	[6]
Temporin E	VLPIIGNLLNSLL-NH_2	6–12	4–10	4–13	43.5	1–13	4–13	−5.43 p	[6]
Temporin F	FLPLIGKVLSGIL-NH_2	4–11	7–11	4–13	54.8	1–13	8–13	−3.64 p	[6]
Temporin G	FFPVIGRILNGIL-NH_2	4–10	6–10	4–13	49.1	4–13	4–13	−4.94 p	[6]
Temporin H	LSPNLLKSLL-NH_2	3–5	7–10	4–10	8.9	–	4–10	−1.71 ap	[6]
Temporin K	LLPNLLKSLL-NH_2	2–5	6–10	4–10	22.3	–	4–10	−1.71 ap	[6]
Temporin L	FVQWFSKFLGRIL-NH_2	10–12	5–10	1–13	41	1–13	1–9	−4.60 p	[6]
Temporin 1ARa	FLPIVGRLISGLL-NH_2	4–11	5–9	4–13	53.8	5–13	4–9	−3.64 p	[8]
Temporin 1AUa	FLPIIGQLLSGLL-NH_2	7–12	7–10	4–13	52.2	1–13	1–5	−3.02 p	[9]
Temporin 1AUa	PLPIIGQLLSGLL-NH_2	7–12	–	4–13	42.6	5–13	4–9	−2.76 p	[10]
Temporin 1BYa	FLPIIAKVLSGLL-NH_2	9–11	4–8	4–13	60	1–13	4–9	−4.91 p	[11]
Temporin 1Ca	FLPFLAKILTGVL-NH_2	9–11	4–9	4–13	60.3	4–13	4–13	−3.85 p	[12]
Temporin 1Cb	FLPLFASLIGKLL-NH_2	10–12	4–9	4–13	56.8	1–13	4–9	−3.86 p	[12]
Temporin 1Cc	FLPFLASLLTKVL-NH_2	–	4–10	4–13	58.7	5–13	4–13	−3.93 ap	[12]
Temporin 1Cd	FLPFLASLLSKVL-NH_2	–	4–10	4–13	57.5	5–13	4–13	−3.94 ap	[12]
Temporin 1Ce	FLPFLATLLSKVL-NH_2	–	4–10	4–13	58.5	–	4–13	−3.93 ap	[12]
Temporin 1CSa	FLPIVGKLLSGLL-NH_2	3–12	4–10	4–13	52.7	–	1–5	−3.07 p	[13]
Temporin 1CSb	FLPIIGKLLSGLL-NH_2	4–11	5–10	4–13	54.5	1–13	1–5	−3.02 p	[13]
Temporin 1CSc	FLPLVTGLLSGLL-NH_2	3–11	–	4–13	54.6	–	4–9	−2.43 p	[13]
Temporin 1CSd	NFLGTLVNLAKKIL-NH_2	–	4–9	3–9	24.1	5–14	1–9	−4.01 p	[13]
Temporin 1DRa	HFLGTLVNLAKKIL-NH_2	–	4–9	1–9	25.2	5–14	1–9	−4.40 p	[10]
Temporin 1DRb	NFLGTLVNLAKKIL-NH_2	–	4–9	3–9	24.1	5–14	1–9	−4.01 p	[10]
Temporin 1DRc	FLPIIASVLSSLL-NH_2	–	5–9	4–13	66.7	1–13	4–13	−6.73 p	[10]
Temporin 1DYa	FIGPIISALASLFG-NH_2	–	5–8	5–14	51.8	5–13	5–13	−4.89 p	[14]
Temporin 1Ec	FLPVIAGLLSKLF-NH_2	7–12	4–10	4–13	58	–	4–13	−3.85 p	[15]
Temporin 1Ga	SILPTIVSFLSKVF-NH_2	–	9–11	5–14	59.4	2–14	6–14	−7.53 p	[16]
Temporin 1Gb	SILPTIVSFLSKFL-NH_2	–	9–11	5–14	58.7	2–14	6–14	−6.27 p	[16]
Temporin 1Gc	SILPTIVSFLTKFL-NH_2	–	7–11	5–14	59.9	2–14	6–14	−6.27 p	[16]
Temporin 1Gd	FILPLIASFLSKFL-NH_2	–	4–11	5–14	66.8	2–14	1–14	−5.40 p	[16]
Temporin 1HKa	SIFPAIVSFLSKFL-NH_2	–	5–11	5–14	62.3	2–14	6–14	−6.27 p	[17]
Temporin 1Ja	ILPLVGNLLNDLL-NH_2	6–13	4–9	–	26.8	4–14	4–10	−3.05 p	[18]
Temporin 1La	VLPLISMALGKLL-NH_2	8–13	2–5	4–13	49.5	1–7	4–9	−2.66 p	[19]
Temporin 1Lb	NFLGTLINLAKKIM-NH_2	–	4–9	3–9	23.7	3–14	1–9	−3.95 p	[19]
Temporin 1Lc	FLPILINLIHKGLL-NH_2	8–12	4–8	4–14	52	2–14	4–10	−8.16 p	[19]
Temporin 1M	FLPIVGKLLSGLL-NH_2	4–11	4–10	4–13	52.7	–	1–5	−3.07 p	[20]
Temporin 1Oa	FLPLLASLFSRLL-NH_2	–	4–10	4–13	53.2	5–13	4–9	−2.66 p	[16]
Temporin 1Ob	FLPLIGKILGTIL-NH_2	4–12	8–12	4–13	57.2	1–13	8–13	−3.97 p	[21]
Temporin 1Oc	FLPLLASLFSRLF-NH_2	–	2–10	4–13	54.9	5–13	4–13	−2.88 p	[16]
Temporin 1Od	FLPLLASLFSGLF-NH_2	–	2–10	4–13	60.4	5–13	4–13	−2.80 p	[21]
Temporin 1Oe	ILPLLGNLLNGLL-NH_2	7–12	4–10	–	35.2	4–13	7–10	−1.84 p	[16]
Temporin 1Of	SLLLKGLASIAKLF-NH_2	2–7	–	1–6, 8–14	39.1	6–14	10–14	−2.18 p	[21]
Temporin 1Og	FLSSLLSKVVSLFT-NH_2	9–12	5–11	1–14	53.5	8–14	1–13	−7.25 p	[16]
Temporin 1OLa	FLPFLKSILGKIL-NH_2	8–11	5–11	4–13	53.2	4–14	4–9	−3.27 p	[17]
Temporin 1OLb	FLPFFASLLGKLL-NH_2	10–12	3–10	4–13	56.8	1–13	4–9	−3.16 p	[17]
Temporin 1P	FLPIVGKLLSGLL-NH_2	4–11	4–9	4–13	52.7	–	1–5	−3.07 p	[19]
Temporin 1PL	FLPLVGKILSGLI-NH_2	4–11	4–10	4–13	54.1	4–13	8–13	−3.58 p	[22]
Temporin 1PLa	FLPLVGKILSGLI-NH_2	4–11	4–10	4–13	54.1	4–13	8–13	−3.58 p	[23]
Temporin 1PRa	ILPILGNLLNGLL-NH_2	3–12	8–10	–	39.3	4–13	4–10	−2.99 p	[9]
Temporin 1PRb	ILPILGNLLNSLL-NH_2	6–12	4–10	–	41.1	4–13	4–13	−4.23 p	[9]
Temporin 1Sa	FLSGIVGMLGKLF-NH_2	7–11	8–10	1–13	45.3	1–6	1–6	−4.15 p	[24]
Temporin 1Sb	FLPIVTNLLSGLL-NH_2	7–12	–	4–13	52.8	5–13	4–9	−5.38 p	[24]
Temporin 1Sc	FLSHIAGFLSNLF-NH_2	7–11	1–6	1–13	34	1–13	1–13	−5.97 p	[24]
Temporin 1SKa	FLPVILPVIGKLLNGIL-NH_2	4–7, 12–15	11–14	8–17	61.3	12–17	4–17	−5.83 p	[25]
Temporin 1SKb	FLPVILPVIGKLLSGIL-NH_2	4–7, 12–15	11–14	8–17	68.4	12–17	4–9	−5.80 p	[25]
Temporin 1SPa	FLSAITSILGKFF-NH_2	8–11	5–10	1–13	47.6	1–13	1–9	−5.20 p	[26]
Temporin 1SPb	FLSAITSLLGKLL-NH_2	10–12	2–9	1–13	40.2	1–13	1–9	−4.00 p	[26]
Temporin 1SPc	FLSAITSILGKLF-NH_2	9–11	4–9	1–13	45.3	1–13	1–9	−5.20 p	[26]
Temporin 1TGa	FLPILGKLLSGIL-NH_2	3–11	11–14	4–13	53.8	–	8–13	−2.38 p	[27]
Temporin 1TGb	AVDLAKIANKVLSSLF-NH_2	11–15	7–13	9–16	19.1	–	7–16	−4.51 p	[27]
Temporin 1TGc	FLPVILPVIGKLLSGIL-NH_2	12–15	11–14	8–17	68.4	12–17	4–9	−5.80 p	[27]
Temporin 1TSa	FLGALAKIISGIF-NH_2	–	8–10	6–13	47.5	–	5–13	−4.22 p	[28]
Temporin 1TSb	FLPLLGNLLNGLL-NH_2	6–11	3–10	–	34.9	–	7–10	−1.84 p	[28]
Temporin 1TSc	FLPLLGNLLRGLL-NH_2	6–12	3–10	–	35.4	4–13	4–9	−1.64 p	[28]
Temporin 1TSd	FLPLLASLIGGML-NH_2	8–10	3–13	4–13	54.0	5–13	4–9	−3.35 p	[28]
Temporin 1Va	FLSSIGKILGNLL-NH_2	2–11	8–11	6–11	30.6	1–13	1–13	−3.44 p	[29]
Temporin 1Vb	FLSIIAKVLGSLF-NH_2	8–11	1–7	1–13	61.7	1–13	1–13	−6.80 p	[29]
Temporin 1Vc	FLPLVTMLLGKLF-NH_2	10–12	4–9	4–13	62.2	–	4–9	−4.11 p	[29]
Temporin 1VE	FLPLVGKILSGLI-NH_2	4–11	4–10	4–13	54.1	4–13	8–13	−3.58 p	[30]

(continued on next page)

Table 1 (*continued*)

Name	Sequence	Region identified by							Ref
		CSSP	SecStr	AGGRESCAN		TANGO	PASTA		
		a	b	c	d	e	F	g	
Temporin Ala	FLPIVGKLLSGLSGLL-NH_2	4–7, 10–13	–	4–11	46.3	–	1–5	−3.07 p	[31]
Temporin GH	FLPLLFGAISHLL-NH_2	3–12	4–10	4–13	56.4	2–13	4–13	−4.35 p	[32]
Temporin GUa	FLQHIIGALSHIF-NH_2	7–11	1–7	3–8	32.1	–	1–13	−7.43 p	[33]
Temporin GUb	FLPLLFGAISHIL-NH_2	3–12	3–10	4–13	59.1	2–13	4–13	−5.55 p	[33]
Temporin GUc	FFPLIFGALSSILPKIL-NH_2	3–7, 11–15	–	4–13	62.6	1–17	4–13	−4.70 p	[33]

a. Continuum secondary structure predictor identified region.
b. Region of ambivalent secondary structures.
c. Aggregation-prone segments (hot spots) predicted by AGGRESCAN.
d. Aggregation score by AGGRESCAN.
e. Amyloidogenic regions predicted by TANGO.
f. Amyloidogenic regions predicted by PASTA.
g. Free energy obtained from PASTA and relative orientation of neighbouring β-strand in the fibril core, p = parallel, ap = antiparallel.
h. –, no match detected by the algorithms.
See text for details.

peptides to membranes containing PG, whereas interactions with PoxnoPC were found to be insensitive to the ionic strength. Intercalation of temporin L into PoxnoPC containing membranes was blocked by methoxyamine, suggesting Schiff base formation between peptide amino groups and the lipid aldehyde function. PoxnoPC and similar aldehyde bearing oxidatively modified phospholipids could thus represent potential molecular targets for AMP [57]. This suggests that ROS secretion by leucocytes and subsequent oxidation of the phospholipids in bacteria could make the latter vulnerable by generating molecular targets for AMP, possibly further relating to their other membrane perturbing properties and capability to modulate both innate and adaptive immunity via different mechanisms, including induction of cytokine production, stimulation of leukocyte chemotaxis, and promotion of monocyte-derived dendritic cell maturation [58].

3. Discriminating between the target and the host cell: role of lipid composition

The mechanism of discrimination between the target cells and AMP-secreting eukaryote hosts is not clear. While PG is abundant in bacteria, the outer leaflet of eukaryotic plasma membranes normally lacks acidic phospholipids and it is this difference which has been suggested to enable the survival of the host [59]. The presence of sphingomyelin and cholesterol in the plasma membrane of eukaryotes is another major difference to the lipid composition of the bacterial membrane. Interestingly, it is this combination of cholesterol and sphingomyelin which seems to provide the most efficient barrier against the insertion of AMP [60]. Sterols increase the conformational order of the lipid acyl chains and reduce membrane permeability [61]. Further, sterols regulate the membrane lateral organization and the membrane hydrophobic thickness that is responsible in part for the regulation of lipid–protein interactions [62]. Cholesterol in particular has cohesive interactions with saturated lipids [63] and has been shown to attenuate the membrane association of some AMP. This effect has been suggested to protect the host cell from membrane permeabilization by these peptides. Membrane association of temporin L (as well as LL-37) in the presence of $X_{Sterol} = 0.5$ was dependent on the type and content of sterol in the model membranes, with cholesterol being the most effective, followed by lanosterol and ergosterol [64]. To this end, the chemical evolution of sterol structure could have been driven by improving the resistance of host cell membranes to AMP, thus promoting their survival without compromising the efficiency of AMP for target cell killing. Combining i) high toxicity of AMP to targets and ii) highly improved insensitivity of the host to these peptides imparted by the outer leaflet lipids (cholesterol and sphingomyelin) must have had a highly decisive impact to the cell and species survival.

4. Formation of amyloid-like fibers by temporin B and L

Amyloid forming peptides/proteins constitute a particularly important class of cytotoxic biomolecules, which similarly to AMP are believed to exert their action by permeabilization of cellular membranes [65]. The cytotoxic action of amyloid forming proteins seems to be generic and due to transient protofibrils in the folding/aggregation free energy landscape preceding the formation of inert, non-toxic mature amyloid [66,67]. The role of membranes is emphasized by the lack of effect by amino acid chirality on the toxicity of small amyloidogenic peptides [68]. Protofibrillar intermediates have been observed during the fibrillogenesis of all amyloid forming proteins and were further shown to have channel- or pore-like properties *in vitro* [66]. These oligomers then convert into amyloid, the latter corresponding to the minimum in the folding/aggregation free energy landscape of the peptide [69]. X-ray fiber diffraction studies have demonstrated that the amyloid fibrils consist of a cross-β structure aligning perpendicular to the fibril long axis [70]. The formation of fibers by amyloid forming peptides and their cytotoxic action are interconnected, membrane associated processes [71], with lipid–protein interactions greatly enhancing the rates of peptide aggregation and fibrillogenesis observed in the presence of membranes, especially when containing anionic phospholipids, as demonstrated for Aβ [72], prion [73], α-synuclein [74], and IAPP [75], for instance. Formation of amyloid-type fibrils was also induced by negatively charged phospholipids for several other cytotoxic and apoptotic proteins [71,76]. Uptake of membrane phospholipids in fibers formed by amyloid forming proteins IAPP, transthyretin, lysozyme, cytochrome *c*, endostatin, and insulin [71,76–78] could additionally contribute to their cytotoxic action [78].

Membranes provide a unique environment that can facilitate spatial protein enrichment and destabilization of their native structure, induce orientational anisotropy and conformational changes, alleviate electrostatic repulsion between charged monomers, and eventually drive an ordered polymerization of the cytotoxic proteins/peptides [71,75,76,79]. On the other hand, changes in the secondary structure of AMP enable them to interact with and destabilize lipid bilayers of their target cells, exposing hydrophobic regions [80] needed for membrane incorporation and subsequent peptide aggregation. Magainin-2, for example has no well-defined secondary structure in an aqueous solution at neutral pH, while α-helical and β-sheet structures are observed in different populations in acidic phospholipid bilayers [81]. Interestingly, there is recent evidence also for several AMP [60,76,82] forming in the presence of acidic phospholipids amyloid-type, Congo red and Thioflavin T staining fibrils, as demonstrated for temporin B and L, LL-37, plantaricin A, magainin-2, sakacin P and melittin in our laboratory [76,82], and more recently also for dermaseptins [83]. We have further

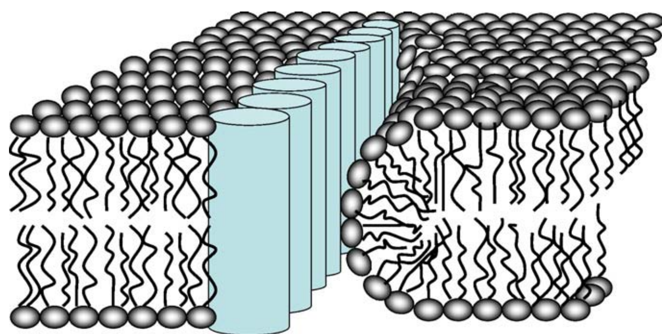

Fig. 1. 'Leaky slit' membrane defect caused by an amphipathic ribbon formed by an oligomeric peptide. The hydrophobic facet of the ribbon seals with the hydrocarbon chain of the bilayer, while the hydrophilic facet forces the lipids to have a high positive curvature. See text for more details (from [82]).

suggested that the membrane permeabilization by AMP would be the same as for amyloid forming peptides and proteins in general [76,82]. On the basis of the above we have proposed the "leaky slit" model (Fig. 1) for the membrane damaging action of AMP amyloid protofibrils [82]. More specifically, this model would represent the minimal requirement for cytotoxicity and involves the formation of an oligomer, a membrane spanning amphipathic ribbon with one facet constituted by hydrophobic and the other by hydrophilic side chains, the ribbon long axis lying parallel to the membrane plane. The actual conformation of the peptide in the ribbon is irrelevant and also a structure such as the α-sheet [84] could be involved. In fact, an α-helical state has been identified as an obligatory intermediate in amyloid formation [85,86]. The length of the amphipathic ribbon can vary, with higher content of AMP yielding longer oligomers and generating larger openings in the membrane. Likewise, both linear and circular structures are allowed. The toxic state should be transient only, with the mature amyloid being rather inert and non-toxic. To this end, amyloid formation process represents in many ways an ideal mechanism for host defence. Secreted by *e.g.* macrophages at the site of infection the local concentration of the peptides is high, sufficient for the formation of transient, toxic oligomers allowing leakage of the contents of their target cells. At the end, the oligomers would convert into amyloid, detoxifying the peptides. At this point it is necessary to emphasize that mechanisms such as described above need to be confirmed and a more detailed molecular level understanding of the process of amyloid-like fiber formation by AMP and the roles played by specific lipids at different stages of aggregation/folding is needed.

5. Prediction of peptide structural characteristics

The information about the secondary structure and oligomerization of α-helical AMP as well as other lipid-induced amyloid forming peptides, critical for their activity, is coded in their sequences. We subjected the current temporin family (76 peptides, Table 1) as well as a more diverse group of α-helical AMP, in total 188 peptides (Table S1 in Supplementary material) to analyses by algorithms developed to identify regions with conformational ambiguity, as well as propensity to aggregation and amyloid formation, viz. CSSP [87], SecStr [88], AGGRESCAN [89], TANGO [90] and PASTA [91] (see Supplementary material for more details).

CSSP (continuum secondary structure prediction) identifies and gives the entropy for sequences, which can undergo a conformational change from a random coil to an α-helix [87]. Interestingly, for 58 out of the 76 α-helical temporins CSSP identifies *ambivalent* regions, which have more than 0.5 entropy of predicted class probabilities, the disorder arising due to the lack of preference for neither the random coil nor the α-helix (Table 1). Pursuing this structural ambivalence further these sequences were analyzed also by SecStr to identify amino acid stretches that have propensities for both α-helix and β-strand, *i.e.* regions which are potentially capable of undergoing a conformational change, representing possible 'conformational switches' [92]. Importantly, SecStr predicts 70 out of the 76 temporins to contain class switching elements with intrinsic equal probability for both α-helix and β-sheet (Table 1). It is further evident that the regions, which have preference either for random coil or α-helix as observed by CSSP are predicted by SecStr to prefer β-sheet or α-helix (Table 1).

A structural transformation of fibril forming protein is a prerequisite to form amyloid aggregates. Amyloidogenic proteins/peptides contain short sequence motifs called 'hot spots' that, once exposed, are highly prone to aggregation [93], responsible for their amyloidogenic behaviour. Hydrophobicity, propensity to form α-helical or β-sheet secondary structure, and net charge of the polypeptide chain modulate aggregation of the partially or totally unfolded state of a protein [94]. Motifs consisting of more than 90% of hydrophobic residues are a major contributor in β-sheet structures found in amyloid [95]. Aggregating and amyloidogenic regions (regions of high packing density) and short specific amino acid stretches ('hot spots') can act as facilitators [96]. Out of the 76 temporin sequences 68, 61 and 36 were identified with regions potentially forming aggregates and amyloid, respectively, by the AGGRESCAN [89], TANGO [90] and PASTA [91] algorithms, the latter two predicting sequences with a tendency to aggregate as β-sheets (Table 1). Some sequences, such as temporin C, D, 1Ja, 1Oe, 1PRa, 1TSb, and 1TSc display high positive aggregation score in AGGRESCAN without having a 'hot spot', which also suggests an overall high aggregation propensity [90].

Importantly, the conformational switches predicted by CSSP [87], and SecStr [88] in temporins coincide very well (Table 1) with the high packing density aggregating and amyloidogenic regions predicted by AGGRESCAN [89], TANGO [90] and PASTA [91]. Our analyses thus imply that the short amino acid stretches in temporins, accessible for intermolecular interactions, show propensities for random coil, α-helix and β-sheet formation, as well as for self-assembly, aggregation and oligomerization into amyloid fibrils.

Interestingly, very similar results were obtained for a more extensive collection of amphipathic α-helical peptides by the above algorithms. Of these 112 α-helical AMP 103 were identified by CSSP to have the propensity to switch conformation from a random coil to an α-helix while 82 were further selected by SecStr to contain motifs of varying length and with a preference for both α-helix and β-sheet (Table S1, Supplementary material). Notably, out of the 112 α-helical AMP 107 were identified by AGGRESCAN to have aggregation prone segments in their sequences, while PASTA and TANGO further recognized 82, and 95 peptides, respectively, to contain amyloidogenic motifs.

Importantly, the algorithms used were built on the basis of amyloid structures found *in vivo* and they do not take into account the complex environment prevailing in membranes, which induce the conformational changes, aggregation, and amyloid formation of α-helical AMP. The fact that the above algorithms performed so well, in spite of the above limitation, is likely to indicate that these features, *i.e.* conformational switching, aggregation, and amyloid formation are indeed inherent to the sequences of α-helical AMP, and dominate the behaviour of these peptides even in a membrane environment. Yet, in this context it is also worth noticing, that recent studies on amyloid formation have revealed this process to be enhanced by a membrane environment, in particular when containing acidic phospholipids [79]. Further, there is a current consensus that the toxicity of amyloid formation derives from the interactions of the toxic oligomer 'protofibril' with lipids, resulting in membrane permeabilization. Accordingly, although the algorithms were not knowingly developed with the lipid environment in mind, at the end, they have been developed for sequences involved in a process, which in fact does take place in membranes.

To conclude, out of the 76 α-helical temporins 58, 70, 68, 61 and 36 peptides were found by CSSP, SecStr, AGGRESCAN, TANGO, and PASTA, respectively, to contain regions complying with the view that these peptides possess considerable conformational flexibility and the potential to form amyloid structure. Moreover, the regions identified by the above algorithms reveal a significant overlap (Table 1). Importantly, this feature seems to be inherent also to other AMP (Table S1 in Supplementary material). The recognition of ambivalent sequences which can be easily converted between α-helical and β-sheet structures and amyloid fibrils in a proper environment can be expected to reveal important further clues about the molecular level mechanisms of amphipathic α-helical AMP in general. The above sequence analyses further support the notion that the cytotoxic actions of α-helical AMP and amyloid forming peptides/proteins are closely related, these two classes of peptides possessing similar specific sequence motifs encoding the amyloid core structure.

6. Concluding remarks

A given amino acid sequence can adopt different conformations depending on its solvent environment [97], its environment inside a protein [98] or upon the interaction between an enzyme and its substrate [99]. A large fraction (approxim. 30%) of the human genome is estimated to code for integral, membrane spanning proteins. The number of peripheral membrane associating proteins is likely to be significantly higher. Lipids play an important role in modulating the conformations of soluble proteins/peptides, as demonstrated for AMP [100,101], with different membrane environments (*e.g.* lipid lateral packing, lateral pressure profile, density of negative charge, presence of cholesterol and sphingomyelin, PIPs, oxidized phospholipids) influencing the outcome. Such environment determined folding/aggregation free energy landscapes are the hallmark of natively unfolded peptides/proteins [102].

For AMP the toxic function is intimately coupled to folding/aggregation. This can be rationalized in the following highly interconnected sequence of events (Fig. 2):

i) Electrostatically enhanced initial association of a random coil peptide to the membrane surface.
ii) The ensuing second step involves several simultaneous processes: intercalation into the bilayer, with the peptide long axis parallel to the membrane layer plane, a conformational change from a random coil to an amphipathic α-helix [45,103], and ion-pairing of acidic phospholipids with positive residues of the peptide.
iii) Reorientation and membrane insertion of the peptide, its long axis becoming perpendicular to the monolayer surface [41,48]. In this step some peptide associated acidic phospholipids can removed from their initial location, so as to have their acyl chains oriented more or less parallel to the plane of the bilayer [41,50], as required for instance by the toroidal pore model.
iv) Aggregation of the α-helical peptides into oligomers. It is possible that this intermediate represents the toxic 'protofibril'. Further, iii) and iv) could be coupled. For the sake of clarity possible formation of *e.g.* tail-to-tail dimers is not depicted.
v) The above process may further involve the formation of an α-sheet [84].
vi) Conversion of the α-helical fibril into a β-sheet, with subsequent further aggregation of the fibrils into inert, non-toxic amyloid. Both v) and vi) are driven by the free energy gain from intermolecular hydrogen bonding, analogously to the folding driven by intramolecular H-bonds [45].

Steps ii) and iii) can be attenuated by augmented lipid lateral packing such as prevailing in the outer leaflet of eukaryote plasma membrane containing both cholesterol and sphingomyelin in the liquid ordered phase [64], thus shifting the equilibrium towards partitioning of the peptide into the aqueous phase. On the other hand, membrane partitioning is promoted by the presence of acidic phospholipids, such as PG in the bacterial membrane. Also a change in the orientation of the peptide long axis from parallel to perpendicular to the membrane plane is greatly promoted by negatively charged lipids. The latter further promote peptide aggregation by abolishing electrostatic repulsion between the cationic peptides, in keeping with the general gatekeeping role of Arg and Lys, capping aggregating hydrophobic sequences [104]. Peptide aggregation by acidic phospholipids further enhances a conformational transition from an α-helix to a β-sheet, forming Congo red and Thioflavin T staining fibers, characteristically to amyloid.

Paradigms of amyloidogenic peptides are Aβ, α-synuclein, and amylin (also known as IAPP, islet associated polypeptide), involved in Alzheimer's and Parkinson's diseases, and type 2 diabetes, respectively. Similarly to temporin B and L, discussed above, also for these peptides enhanced fibril formation in the presence of acidic lipids has been demonstrated [79]. Because of their parallel behaviour to AMP, we have postulated, that all these peptides execute their cytotoxic function by very similar molecular level mechanisms, with membrane–lipid interactions providing the driving force. For membrane associated amyloid diseases the toxic form is currently thought to be an amphipathic fibrillar intermediate, 'protofibril', which permeabilizes membranes, triggering cell death. Similarly to the Aβ peptide [79], temporins B and L cause concentration-dependent membrane permeabilization, larger molecules leaking out at higher concentrations of peptide [79]. The kinetics (cell death *vs* dose *vs* time dependence, Fig. 3) shows a fast decay in live cell number, yet with an abrupt levelling off of the cytotoxicity, observed at all concentrations of temporin L added [105]. These data comply with the toxic oligomer converting into an inactive form in a transition like manner, as *e.g.* lateral diffusion of the peptide in the target cell membrane would otherwise result in cell death to continue and slow down asymptotically. We have previously observed the macroscopic aggregation of

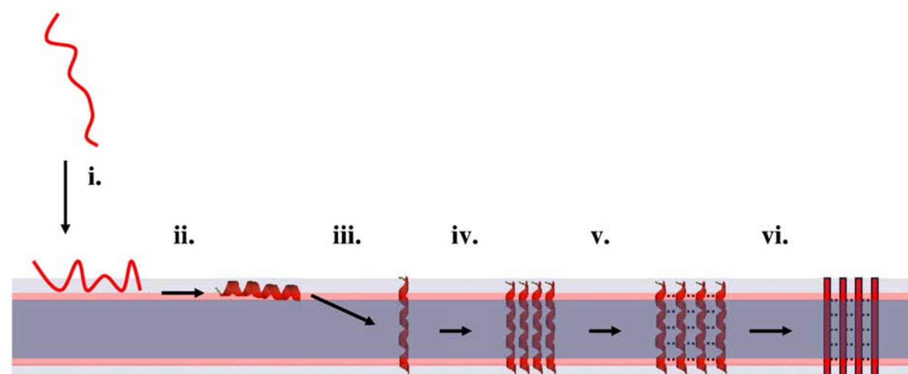

Fig. 2. Schematic illustration of the stages in the membrane association/folding/aggregation pathway (adapted and modified from [103]). See text for details.

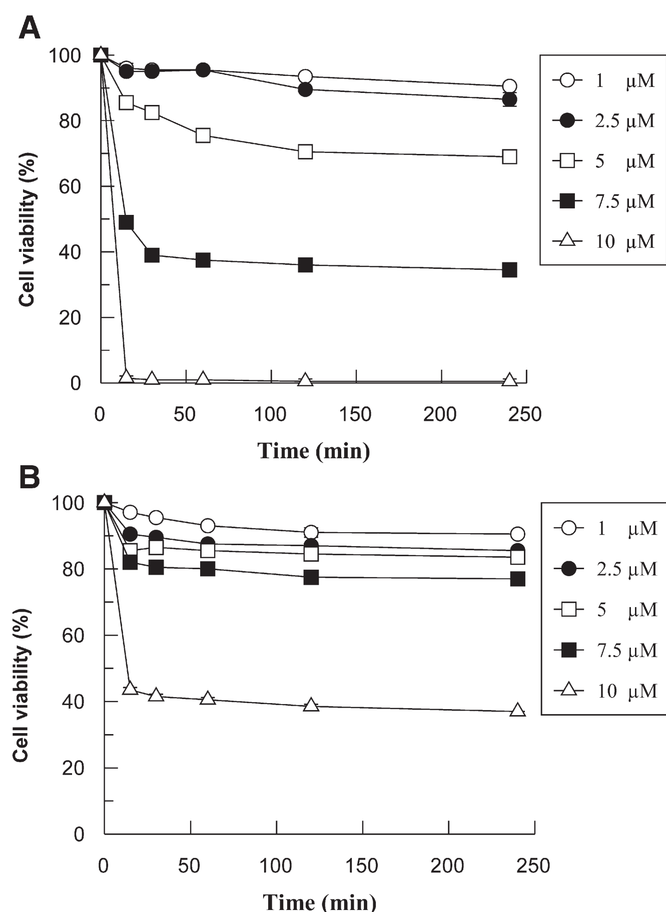

Fig. 3. Cytotoxicity of temporin L to Hut-78 (panel A) and K-562 (panel B) tumour cells. Reprinted with permission from [105].

the peptide in giant unilamellar vesicles to be very rapid, ending up as a seemingly inert, highly refractive aggregate [48]. In this process the integrity of the GUV is retained and after the formation of the aggregate there are no further macroscopic changes in the assembly. Based on these observations we suggested that the membrane perturbation by these peptides is transient and due to the process itself, *i.e.* the binding and subsequent aggregation (oligomerization) of the peptide in the bilayer [41,50], leading at the end to the formation of an inert, non-toxic peptide–lipid aggregate.

Several oligomer geometries are possible and have been observed, from circular well-defined pores to large, highly leaking openings in the membrane. This together with the sequence diversity of temporins and AMP in general all point to the conclusion that toxicity is due to generic physicochemical characteristics of the oligomer. Highlighting in this respect the similarity between *e.g.* temporins and short amyloidogenic peptides is the independence of their toxicity from amino acid chirality [35,68]. The minimal physicochemical property required for cytotoxicity is the amphipathic nature of the oligomer, one surface being hydrophilic and the other hydrophobic. For a channel-like oligomer the pore is lined by hydrophilic residues. Much less defined is the toxic structure constituted by an amphipathic ribbon causing a 'leaky slit' type membrane defect (Fig. 1). The actual length of the oligomer can vary, higher peptide contents making longer fibrils and allowing leakage of larger molecules, as observed [36,65]. Most notably, the only requirement for cytotoxicity is the amphipathic nature of the fibril, with a proper thickness to match the lipid bilayer. Accordingly, both β-sheet fibrils as well as aggregated α-helices and α-sheets [84] would be feasible. To this end, it is of interest that an obligatory α-helical intermediate has been demonstrated for amyloid formation [85,86]. The adoption of an α-helical structure upon binding to lipid surfaces is not limited to temporins and other AMP of this type but is a property shared by many disease-associated amyloid proteins. Accordingly, α-synuclein [106,107], Aβ [108], IAPP [109], prion protein (PrP) [110], and amyloid A [111] have all been shown to acquire increased α-helical structure upon association with charged amphiphile surfaces, indicating common mechanisms. Last, apart from obstructive macroscopic accumulation, the mature amyloid is considered to be inert and non-toxic. The above scenario is supported by the sequence analyses of temporins (Table 1), together with several other families of α-helical amphipathic AMP (Table S1 in Supplementary material). In brief, temporins and other amphipathic α-helical AMP contain 'conformational switches', regions with equal probability for different conformations. These domains overlap with those predicted to aggregate and form amyloid. Proper lipid bilayer environment involving anionic and oxidized lipids would thus control 'conformational switching' in AMP, causing them to expose hydrophobic residues and promoting membrane insertion, which subsequently allows self-association of the peptides by hydrogen bonding in the target cell membrane, leading to extensive aggregation, formation of toxic oligomers and finally non-toxic amyloid for degradation and removal. The above partly tentative molecular level mechanisms of membrane association and cytotoxicity highlight the great degree of similarity between AMP and the disease-associated amyloid forming peptides. However, it needs to be recognized that the physicochemistry of AMP was optimized during evolution for maximal benefit to the host defence, while the cytotoxicity of the pathology associated peptides is likely to reflect a weak point in the current machinery of organisms such as man. This means that also differences are to be expected in *e.g.* the kinetics of the pathways in the folding/aggregation free energy landscapes for peptide/membrane assemblies. Finally, an interesting and important new area concerns the synergistic effects of AMPs, both as combinations of different AMPs [112] as well as in combination with other host defence mechanisms [113,114], which both add to the potential of AMP and understanding of innate immunity to the development of new means to combat microbes.

Acknowledgements

The authors thank HBBG members for stimulating discussions. HBBG is supported by grants from EU FP6 and FP7, Sitra (A.K.M.), Finnish Academy, and Sigrid Juselius Foundation.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbamem.2009.04.012.

References

[1] A. Tossi, L. Sandri, A. Giangaspero, Amphipathic, alpha-helical antimicrobial peptides, Biopolymers 55 (2000) 4–30.
[2] V. Sosunov, V. Mischenko, B. Eruslanov, E. Svetoch, Y. Shakina, N. Stern, K. Majorov, G. Sorokoumova, A. Selishcheva, A. Apt, Antimycobacterial activity of bacteriocins and their complexes with liposomes, J. Antimicrob. Chemother. 59 (2007) 919–925.
[3] E. Tiozzo, G. Rocco, A. Tossi, D. Romeo, Wide-spectrum antibiotic activity of synthetic, amphipathic peptides, Biochem. Biophys. Res. Commun. 249 (1998) 202–206.
[4] A.R. Koczulla, R. Bals, Antimicrobial peptides: current status and therapeutic potential, Drugs 63 (2003) 389–406.
[5] R.E. Hancock, R. Lehrer, Cationic peptides: a new source of antibiotics, Trends Biotechnol. 16 (1998) 82–88.
[6] M. Simmaco, G. Mignogna, S. Canofeni, R. Miele, M.L. Mangoni, D. Barra, Temporins, antimicrobial peptides from the European red frog *Rana temporaria*, Eur. J. Biochem. 242 (1996) 788–792.
[7] D. Vanhoye, F. Bruston, P. Nicolas, M. Amiche, Antimicrobial peptides from hylid and ranin frogs originated from a 150-million-year-old ancestral precursor with a conserved signal peptide but a hypermutable antimicrobial domain, Eur. J. Biochem. 270 (2003) 2068–2081.
[8] M.F. Ali, K.R. Lips, F.C. Knoop, B. Fritzsch, C. Miller, J.M. Conlon, Antimicrobial peptides and protease inhibitors in the skin secretions of the crawfish frog, *Rana areolata*, Biochim. Biophys. Acta 1601 (2002) 55–63.

[9] J.M. Conlon, A. Sonnevend, C. Davidson, A. Demandt, T. Jouenne, Host-defense peptides isolated from the skin secretions of the Northern red-legged frog *Rana aurora aurora*, Dev. Comp. Immunol. 29 (2005) 83–90.
[10] J.M. Conlon, N. Al-Ghafari, L. Coquet, J. Leprince, T. Jouenne, H. Vaudry, C. Davidson, Evidence from peptidomic analysis of skin secretions that the red-legged frogs, *Rana aurora draytonii* and *Rana aurora aurora*, are distinct species, Peptides 27 (2006) 1305–1312.
[11] J.M. Conlon, A. Sonnevend, M. Patel, C. Davidson, P.F. Nielsen, T. Pal, L.A. Rollins-Smith, Isolation of peptides of the brevinin-1 family with potent candidacidal activity from the skin secretions of the frog *Rana boylii*, J. Pept. Res. 62 (2003) 207–213.
[12] T. Halverson, Y.J. Basir, F.C. Knoop, J.M. Conlon, Purification and characterization of antimicrobial peptides from the skin of the North American green frog *Rana clamitans*, Peptides 21 (2000) 469–476.
[13] J.M. Conlon, A. Al-Dhaheri, E. Al-Mutawa, R. Al-Kharrge, E. Ahmed, J. Kolodziejek, N. Nowotny, P.F. Nielsen, C. Davidson, Peptide defenses of the Cascades frog *Rana cascadae*: implications for the evolutionary history of frogs of the Amerana species group, Peptides 28 (2007) 1268–1274.
[14] J.M. Conlon, J. Kolodziejek, N. Nowotny, J. Leprince, H. Vaudry, L. Coquet, T. Jouenne, S. Iwamuro, Cytolytic peptides belonging to the brevinin-1 and brevinin-2 families isolated from the skin of the Japanese brown frog, *Rana dybowskii*, Toxicon 50 (2007) 746–756.
[15] M.F. Ali, F.C. Knoop, H. Vaudry, J.M. Conlon, Characterization of novel antimicrobial peptides from the skins of frogs of the *Rana esculenta* complex, Peptides 24 (2003) 955–961.
[16] J.B. Kim, T. Halverson, Y.J. Basir, J. Dulka, F.C. Knoop, P.W. Abel, J.M. Conlon, Purification and characterization of antimicrobial and vasorelaxant peptides from skin extracts and skin secretions of the North American pig frog *Rana grylio*, Regul. Pept. 90 (2000) 53–60.
[17] J.M. Conlon, L. Coquet, J. Leprince, T. Jouenne, H. Vaudry, J. Kolodziejek, N. Nowotny, C.R. Bevier, P.E. Moler, Peptidomic analysis of skin secretions from *Rana heckscheri* and *Rana okaloosae* provides insight into phylogenetic relationships among frogs of the Aquarana species group, Regul. Pept. 138 (2007) 87–93.
[18] T. Isaacson, A. Soto, S. Iwamuro, F.C. Knoop, J.M. Conlon, Antimicrobial peptides with atypical structural features from the skin of the Japanese brown frog *Rana japonica*, Peptides 23 (2002) 419–425.
[19] J. Goraya, Y. Wang, Z. Li, M. O'Flaherty, F.C. Knoop, J.E. Platz, J.M. Conlon, Peptides with antimicrobial activity from four different families isolated from the skins of the North American frogs *Rana luteiventris*, *Rana berlandieri* and *Rana pipiens*, Eur. J. Biochem. 267 (2000) 894–900.
[20] L.A. Rollins-Smith, D.C. Woodhams, L.K. Reinert, V.T. Vredenburg, C.J. Briggs, P.F. Nielsen, J.M. Conlon, Antimicrobial peptide defenses of the mountain yellow-legged frog (*Rana muscosa*), Dev. Comp. Immunol. 30 (2006) 831–842.
[21] A. Ohnuma, J.M. Conlon, K. Yamaguchi, H. Kawasaki, L. Coquet, J. Leprince, T. Jouenne, H. Vaudry, S. Iwamuro, Antimicrobial peptides from the skin of the Japanese mountain brown frog *Rana ornativentris*: evidence for polymorphism among preprotemporin mRNAs, Peptides 28 (2007) 524–532.
[22] M. Zhou, L. Wang, D.E. Owens, T. Chen, B. Walker, C. Shaw, Rapid identification of precursor cDNAs encoding five structural classes of antimicrobial peptides from pickerel frog (*Rana palustris*) skin secretion by single step "shotgun" cloning, Peptides 28 (2007) 1605–1610.
[23] Y.J. Basir, F.C. Knoop, J. Dulka, J.M. Conlon, Multiple antimicrobial peptides and peptides related to bradykinin and neuromedin N isolated from skin secretions of the pickerel frog, *Rana palustris*, Biochim. Biophys. Acta 1543 (2000) 95–105.
[24] F. Abbassi, B. Oury, T. Blasco, D. Sereno, G. Bolbach, P. Nicolas, K. Hani, M. Amiche, A. Ladram, Isolation, characterization and molecular cloning of new temporins from the skin of the North African ranid *Pelophylax saharica*, Peptides 29 (2008) 1526–1533.
[25] H. Suzuki, S. Iwamuro, A. Ohnuma, L. Coquet, J. Leprince, T. Jouenne, H. Vaudry, C.K. Taylor, P.W. Abel, J.M. Conlon, Expression of genes encoding antimicrobial and bradykinin-related peptides in skin of the stream brown frog *Rana sakuraii*, Peptides 28 (2007) 505–514.
[26] C.R. Bevier, A. Sonnevend, J. Kolodziejek, N. Nowotny, P.F. Nielsen, J.M. Conlon, Purification and characterization of antimicrobial peptides from the skin secretions of the mink frog (*Rana septentrionalis*), Comp. Biochem. Physiol. C. Toxicol. Pharmacol. 139 (2004) 31–38.
[27] S. Iwamuro, M. Nakamura, A. Ohnuma, J.M. Conlon, Molecular cloning and sequence analyses of preprotemporin mRNAs containing premature stop codons from extradermal tissues of *Rana tagoi*, Peptides 27 (2006) 2124–2128.
[28] J.M. Conlon, N. Al-Ghaferi, B. Abraham, A. Sonnevend, L. Coquet, J. Leprince, T. Jouenne, H. Vaudry, S. Iwamuro, Antimicrobial peptides from the skin of the Tsushima brown frog *Rana tsushimensis*, Comp. Biochem. Physiol. C. Toxicol. Pharmacol. 143 (2006) 42–49.
[29] J.M. Conlon, B. Abraham, A. Sonnevend, T. Jouenne, P. Cosette, J. Leprince, H. Vaudry, C.R. Bevier, Purification and characterization of antimicrobial peptides from the skin secretions of the carpenter frog *Rana virgatipes* (Ranidae, Aquarana), Regul. Pept. 131 (2005) 38–45.
[30] T. Chen, M. Zhou, P. Rao, B. Walker, C. Shaw, The Chinese bamboo leaf odorous frog (*Rana* (*Odorrana*) *versabilis*) and North American Rana frogs share the same families of skin antimicrobial peptides, Peptides 27 (2006) 1738–1744.
[31] Y. Lu, J. Li, H. Yu, X. Xu, J. Liang, Y. Tian, D. Ma, G. Lin, G. Huang, R. Lai, Two families of antimicrobial peptides with multiple functions from skin of rufous-spotted torrent frog, Amolops loloensis, Peptides 27 (2006) 3085–3091.
[32] J. Zhou, S. McClean, A. Thompson, Y. Zhang, C. Shaw, P. Rao, A.J. Bjourson, Purification and characterization of novel antimicrobial peptides from the skin secretion of *Hylarana guentheri*, Peptides 27 (2006) 3077–3084.
[33] J.M. Conlon, G.J. Power, Y.H. Abdel-Wahab, P.R. Flatt, H. Jiansheng, L. Coquet, J. Leprince, T. Jouenne, H. Vaudry, A potent, non-toxic insulin-releasing peptide isolated from an extract of the skin of the Asian frog, *Hylarana guntheri* (Anura: Ranidae), Regul.Pept. 151 (2008) 153–159.
[34] Y. Rosenfeld, Y. Shai, Lipopolysaccharide (Endotoxin)-host defense antibacterial peptides interactions: role in bacterial resistance and prevention of sepsis, Biochim. Biophys. Acta 1758 (2006) 1513–1522.
[35] D. Wade, J. Silberring, R. Soliymani, S. Heikkinen, I. Kilpelainen, H. Lankinen, P. Kuusela, Antibacterial activities of temporin A analogs, FEBS Lett. 479 (2000) 6–9.
[36] M.L. Mangoni, N. Papo, D. Barra, M. Simmaco, A. Bozzi, A. Di Giulio, A.C. Rinaldi, Effects of the antimicrobial peptide temporin L on cell morphology, membrane permeability and viability of *Escherichia coli*, Biochem. J. 380 (2004) 859–865.
[37] K. Matsuzaki, Why and how are peptide–lipid interactions utilized for self-defense? Magainins and tachyplesins as archetypes, Biochim. Biophys. Acta 1462 (1999) 1–10.
[38] Y. Shai, Mechanism of the binding, insertion and destabilization of phospholipid bilayer membranes by alpha-helical antimicrobial and cell non-selective membrane-lytic peptides, Biochim. Biophys. Acta 1462 (1999) 55–70.
[39] M. D'Abramo, A.C. Rinaldi, A. Bozzi, A. Amadei, G. Mignogna, A. Di Nola, M. Aschi, Conformational behavior of temporin A and temporin L in aqueous solution: a computational/experimental study, Biopolymers 81 (2006) 215–224.
[40] J. Turner, Y. Cho, N.N. Dinh, A.J. Waring, R.I. Lehrer, Activities of LL-37, a cathelin-associated antimicrobial peptide of human neutrophils, Antimicrob. Agents Chemother. 42 (1998) 2206–2214.
[41] H. Zhao, P.K.J. Kinnunen, Binding of the antimicrobial peptide temporin L to liposomes assessed by Trp fluorescence, J.Biol.Chem. 277 (2002) 25170–25177.
[42] R. Sood, Y. Domanov, P.K.J. Kinnunen, Fluorescent temporin B derivative and its binding to liposomes, J. Fluoresc. 17 (2007) 223–234.
[43] A. Carotenuto, S. Malfi, M.R. Saviello, P. Campiglia, I. Gomez-Monterrey, M.L. Mangoni, L.M. Gaddi, E. Novellino, P. Grieco, A different molecular mechanism underlying antimicrobial and hemolytic actions of temporins A and L, J. Med. Chem. 51 (2008) 2354–2362.
[44] R.M. Epand, H.J. Vogel, Diversity of antimicrobial peptides and their mechanisms of action, Biochim. Biophys. Acta 1462 (1999) 11–28.
[45] S.H. White, A.S. Ladokhin, S. Jayasinghe, K. Hristova, How membranes shape protein structure, J. Biol. Chem. 276 (2001) 32395–32398.
[46] T. Abraham, S. Marwaha, D.M. Kobewka, R.N. Lewis, E.J. Prenner, R.S. Hodges, R.N. McElhaney, The relationship between the binding to and permeabilization of phospholipid bilayer membranes by GS14dK4, a designed analog of the antimicrobial peptide gramicidin S, Biochim. Biophys. Acta 1768 (2007) 2089–2098.
[47] P.K.J. Kinnunen, On the mechanisms of the lamellar–hexagonal H_{II} phase transition and the biological significance of the H_{II} Propensity, in: D.D. Lasic, Y. Barenholz (Eds.), Nonmedical Application of Liposomes, 1, CRC Press, Florida, 1996, pp. 153–171.
[48] H. Zhao, A.C. Rinaldi, A. Di Giulio, M. Simmaco, P.K.J. Kinnunen, Interactions of the antimicrobial peptides temporins with model biomembranes. Comparison of temporins B and L, Biochemistry 41 (2002) 4425–4436.
[49] W. Dowhan, Molecular basis for membrane phospholipid diversity: why are there so many lipids? Annu. Rev. Biochem. 66 (1997) 199–232.
[50] H. Zhao, J.P. Mattila, J.M. Holopainen, P.K.J. Kinnunen, Comparison of the membrane association of two antimicrobial peptides, magainin 2 and indolicidin, Biophys. J. 81 (2001) 2979–2991.
[51] R.P. Richter, R. Berat, A.R. Brisson, Formation of solid-supported lipid bilayers: an integrated view, Langmuir 22 (2006) 3497–3505.
[52] Y.A. Domanov, P.K.J. Kinnunen, Antimicrobial peptides temporins B and L induce formation of tubular lipid protrusions from supported phospholipid bilayers, Biophys. J. 91 (2006) 4427–4439.
[53] Y.A. Domanov, P.K.J. Kinnunen, Islet amyloid polypeptide forms rigid lipid–protein amyloid fibrils on supported phospholipid bilayers, J. Mol. Biol. 376 (2008) 42–54.
[54] G.O. Fruhwirth, A. Loidl, A. Hermetter, Oxidized phospholipids: from molecular properties to disease, Biochim. Biophys. Acta 1772 (2007) 718–736.
[55] P.K.J. Kinnunen, Fusion of lipid bilayers: a model involving mechanistic connection to HII phase forming lipids, Chem. Phys. Lipids 63 (1992) 251–258.
[56] K. Sabatini, J.P. Mattila, F.M. Megli, P.K.J. Kinnunen, Characterization of two oxidatively modified phospholipids in mixed monolayers with DPPC, Biophys. J. 90 (2006) 4488–4499.
[57] J.P. Mattila, K. Sabatini, P.K.J. Kinnunen, Oxidized phospholipids as potential molecular targets for antimicrobial peptides, Biochim. Biophys. Acta 1778 (2008) 2041–2050.
[58] D.M. Bowdish, D.J. Davidson, R.E. Hancock, A re-evaluation of the role of host defence peptides in mammalian immunity, Curr. Protein Pept. Sci. 6 (2005) 35–51.
[59] M.R. Yeaman, N.Y. Yount, Mechanisms of antimicrobial peptide action and resistance, Pharmacol. Rev. 55 (2003) 27–55.
[60] R. Sood, Y. Domanov, M. Pietiainen, V.P. Kontinen, P.K.J. Kinnunen, Binding of LL-37 to model biomembranes: insight into target vs host cell recognition, Biochim. Biophys. Acta 1778 (2008) 983–996.
[61] L. Miao, M. Nielsen, J. Thewalt, J.H. Ipsen, M. Bloom, M.J. Zuckermann, O.G. Mouritsen, From lanosterol to cholesterol: structural evolution and differential effects on lipid bilayers, Biophys. J. 82 (2002) 1429–1444.
[62] J. Pencer, M.P. Nieh, T.A. Harroun, S. Krueger, C. Adams, J. Katsaras, Bilayer thickness and thermal response of dimyristoylphosphatidylcholine unilamellar vesicles containing cholesterol, ergosterol and lanosterol: a small-angle neutron scattering study, Biochim. Biophys. Acta 1720 (2005) 84–91.

[63] M.R. Ali, K.H. Cheng, J. Huang, Assess the nature of cholesterol–lipid interactions through the chemical potential of cholesterol in phosphatidylcholine bilayers, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 5372–5377.
[64] R. Sood, P.K.J. Kinnunen, Cholesterol, lanosterol, and ergosterol attenuate the membrane association of LL-37(W27F) and temporin L, Biochim. Biophys. Acta 1778 (2008) 1460–1466.
[65] J.I. Kourie, A.A. Shorthouse, Properties of cytotoxic peptide-formed ion channels, Am. J. Physiol. Cell. Physiol. 278 (2000) C1063–C1087.
[66] R. Kayed, Y. Sokolov, B. Edmonds, T.M. McIntire, S.C. Milton, J.E. Hall, C.G. Glabe, Permeabilization of lipid bilayers is a common conformation-dependent activity of soluble amyloid oligomers in protein misfolding diseases, J. Biol. Chem. 279 (2004) 46363–46366.
[67] C.M. Dobson, Protein folding and misfolding, Nature 426 (2003) 884–890.
[68] M.T. Pastor, N. Kummerer, V. Schubert, A. Esteras-Chopo, C.G. Dotti, M. Lopez de la Paz, L. Serrano, Amyloid toxicity is independent of polypeptide sequence, length and chirality, J. Mol. Biol. 375 (2008) 695–707.
[69] T.R. Jahn, S.E. Radford, Folding versus aggregation: polypeptide conformations on competing pathways, Arch. Biochem. Biophys. 469 (2008) 100–117.
[70] M. Sunde, L.C. Serpell, M. Bartlam, P.E. Fraser, M.B. Pepys, C.C. Blake, Common core structure of amyloid fibrils by synchrotron X-ray diffraction, J. Mol. Biol. 273 (1997) 729–739.
[71] H. Zhao, E.K. Tuominen, P.K.J. Kinnunen, Formation of amyloid fibers triggered by phosphatidylserine-containing membranes, Biochemistry 43 (2004) 10302–10307.
[72] E. Terzi, G. Holzemann, J. Seelig, Alzheimer beta-amyloid peptide 25–35: electrostatic interactions with phospholipid membranes, Biochemistry 33 (1994) 7434–7441.
[73] M. Morillas, W. Swietnicki, P. Gambetti, W.K. Surewicz, Membrane environment alters the conformational structure of the recombinant human prion protein, J. Biol. Chem. 274 (1999) 36859–36865.
[74] H.J. Lee, C. Choi, S.J. Lee, Membrane-bound alpha-synuclein has a high aggregation propensity and the ability to seed the aggregation of the cytosolic form, J. Biol. Chem. 277 (2002) 671–678.
[75] J.D. Knight, A.D. Miranker, Phospholipid catalysis of diabetic amyloid assembly, J. Mol. Biol. 341 (2004) 1175–1187.
[76] H. Zhao, A. Jutila, T. Nurminen, S.A. Wickstrom, J. Keski-Oja, P.K.J. Kinnunen, Binding of endostatin to phosphatidylserine-containing membranes and formation of amyloid-like fibers, Biochemistry 44 (2005) 2857–2863.
[77] M.F. Engel, L. Khemtemourian, C.C. Kleijer, H.J. Meeldijk, J. Jacobs, A.J. Verkleij, B. de Kruijff, J.A. Killian, J.W. Hoppener, Membrane damage by human islet amyloid polypeptide through fibril growth at the membrane, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 6033–6038.
[78] E. Sparr, M.F. Engel, D.V. Sakharov, M. Sprong, J. Jacobs, B. de Kruijff, J.W. Hoppener, J.A. Killian, Islet amyloid polypeptide-induced membrane leakage involves uptake of lipids by forming amyloid fibers, FEBS Lett. 577 (2004) 117–120.
[79] G.P. Gorbenko, P.K.J. Kinnunen, The role of lipid–protein interactions in amyloid-type protein fibril formation, Chem. Phys. Lipids 141 (2006) 72–82.
[80] A. Giangaspero, L. Sandri, A. Tossi, Amphipathic alpha helical antimicrobial peptides, Eur. J. Biochem. 268 (2001) 5589–5600.
[81] D.J. Hirsh, J. Hammer, W.L. Maloy, J. Blazyk, J. Schaefer, Secondary structure and location of a magainin analogue in synthetic phospholipid bilayers, Biochemistry 35 (1996) 12733–12741.
[82] H. Zhao, R. Sood, A. Jutila, S. Bose, G. Fimland, J. Nissen-Meyer, P.K.J. Kinnunen, Interaction of the antimicrobial peptide pheromone Plantaricin A with model membranes: implications for a novel mechanism of action, Biochim. Biophys. Acta 1758 (2006) 1461–1474.
[83] C. Auvynet, C. El Amri, C. Lacombe, F. Bruston, J. Bourdais, P. Nicolas, Y. Rosenstein, Structural requirements for antimicrobial versus chemoattractant activities for dermaseptin S9, FEBS J. 275 (2008) 4134–4151.
[84] V. Daggett, Alpha-sheet: the toxic conformer in amyloid diseases? Acc. Chem. Res. 39 (2006) 594–602.
[85] M.D. Kirkitadze, M.M. Condron, D.B. Teplow, Identification and characterization of key kinetic intermediates in amyloid beta-protein fibrillogenesis, J. Mol. Biol. 312 (2001) 1103–1119.
[86] D.K. Klimov, D. Thirumalai, Dissecting the assembly of Abeta16-22 amyloid peptides into antiparallel beta sheets, Structure 11 (2003) 295–307.
[87] M. Boden, Z. Yuan, T.L. Bailey, Prediction of protein continuum secondary structure with probabilistic models based on NMR solved structures, BMC Bioinformatics 7 (2006) 68.
[88] S.J. Hamodrakas, A protein secondary structure prediction scheme for the IBM PC and compatibles, Comput. Appl. Biosci. 4 (1988) 473–477.
[89] O. Conchillo-Sole, N.S. de Groot, F.X. Aviles, J. Vendrell, X. Daura, S. Ventura, AGGRESCAN: a server for the prediction and evaluation of “hot spots” of aggregation in polypeptides, BMC Bioinformatics 8 (2007) 65.
[90] A.M. Fernandez-Escamilla, F. Rousseau, J. Schymkowitz, L. Serrano, Prediction of sequence-dependent and mutational effects on the aggregation of peptides and proteins, Nat. Biotechnol. 22 (2004) 1302–1306.
[91] A. Trovato, F. Seno, S.C. Tosatto, The PASTA server for protein aggregation prediction, Protein Eng. Des. Sel. 20 (2007) 521–523.
[92] M. Young, K. Kirshenbaum, K.A. Dill, S. Highsmith, Predicting conformational switches in proteins, Protein Sci. 8 (1999) 1752–1764.
[93] M. Lopez de la Paz, L. Serrano, Sequence determinants of amyloid fibril formation, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 87–92.
[94] M. Calamai, N. Taddei, M. Stefani, G. Ramponi, F. Chiti, Relative influence of hydrophobicity and net charge in the aggregation of two homologous proteins, Biochemistry 42 (2003) 15078–15083.
[95] S. Yan, G. Gawlak, K. Makabe, V. Tereshko, A. Koide, S. Koide, Hydrophobic surface burial is the major stability determinant of a flat, single-layer beta-sheet, J. Mol. Biol. 368 (2007) 230–243.
[96] S. Ventura, J. Zurdo, S. Narayanan, M. Parreno, R. Mangues, B. Reif, F. Chiti, E. Giannoni, C.M. Dobson, F.X. Aviles, L. Serrano, Short amino acid stretches can mediate amyloid formation in globular proteins: the Src homology 3 (SH3) case, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 7258–7263.
[97] D.V. Waterhous, W.C. Johnson, Importance of environment in determining secondary structure in proteins, Biochemistry 33 (1994) 2121–2128.
[98] S. Rozovsky, A.E. McDermott, The time scale of the catalytic loop motion in triosephosphate isomerase, J. Mol. Biol. 310 (2001) 259–270.
[99] S. Rozovsky, G. Jogl, L. Tong, A.E. McDermott, Solution-state NMR investigations of triosephosphate isomerase active site loop motion: ligand release in relation to active site loop dynamics, J. Mol. Biol. 310 (2001) 271–280.
[100] A. Latal, G. Degovics, R.F. Epand, R.M. Epand, K. Lohner, Structural aspects of the interaction of peptidyl–glycylleucine–carboxyamide, a highly potent antimicrobial peptide from frog skin, with lipids, Eur. J. Biochem. 248 (1997) 938–946.
[101] W. Wang, D.K. Smith, K. Moulding, H.M. Chen, The dependence of membrane permeability by the antibacterial peptide cecropin B and its analogs, CB-1 and CB-3, on liposomes of different composition, J. Biol. Chem. 273 (1998) 27438–27448.
[102] V.N. Uversky, C.J. Oldfield, A.K. Dunker, Intrinsically disordered proteins in human diseases: introducing the D2 concept, Annu. Rev. Biophys. 37 (2008) 215–246.
[103] S.H. White, G. von Heijne, How translocons select transmembrane helices, Annu. Rev. Biophys. 37 (2008) 23–42.
[104] F. Rousseau, L. Serrano, J.W. Schymkowitz, How evolutionary pressure against protein aggregation shaped chaperone specificity, J. Mol. Biol. 355 (2006) 1037–1047.
[105] A.C. Rinaldi, M.L. Mangoni, A. Rufo, C. Luzi, D. Barra, H. Zhao, P.K.J. Kinnunen, A. Bozzi, A. Di Giulio, M. Simmaco, Temporin L: antimicrobial, haemolytic and cytotoxic activities, and effects on membrane permeabilization in lipid vesicles, Biochem. J. 368 (2002) 91–100.
[106] M. Zhu, A.L. Fink, Lipid binding inhibits alpha-synuclein fibril formation, J. Biol. Chem. 278 (2003) 16873–16877.
[107] H. Shao, S. Jao, K. Ma, M.G. Zagorski, Solution structures of micelle-bound amyloid beta-(1–40) and beta-(1–42) peptides of Alzheimer's disease, J. Mol. Biol. 285 (1999) 755–773.
[108] V. Rangachari, D.K. Reed, B.D. Moore, T.L. Rosenberry, Secondary structure and interfacial aggregation of amyloid-beta(1–40) on sodium dodecyl sulfate micelles, Biochemistry 45 (2006) 8639–8648.
[109] S.A. Jayasinghe, R. Langen, Lipid membranes modulate the structure of islet amyloid polypeptide, Biochemistry 44 (2005) 12113–12119.
[110] N. Sanghera, T.J. Pinheiro, Binding of prion protein to lipid membranes and implications for prion conversion, J. Mol. Biol. 315 (2002) 1241–1256.
[111] C.M. Uhlar, A.S. Whitehead, Serum amyloid A, the major vertebrate acute-phase reactant, Eur. J. Biochem. 265 (1999) 501–523.
[112] Y. Rosenfeld, D. Barra, M. Simmaco, Y. Shai, M.L. Mangoni, A synergism between temporins toward Gram-negative bacteria overcomes resistance imposed by the lipopolysaccharide protective layer, J. Biol. Chem. 281 (2006) 28565–28574.
[113] H. Zhao, P.K.J. Kinnunen, Modulation of the activity of secretory phospholipase A2 by antimicrobial peptides, Antimicrob. Agents Chemother. 47 (2003) 965–971.
[114] C. Code, Y.A. Domanov, J.A. Killian, P.K.J. Kinnunen, Activation of phospholipase A (2) by temporin B: formation of antimicrobial peptide-enzyme amyloid-type cofibrils, Biochim. Biophys. Acta (2009).