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ORIGINAL ARTICLE

Hylaranins: prototypes of a new class of amphibian antimicrobial peptide from the skin secretion of the oriental broad-folded frog, *Hylarana latouchii*

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Abstract Amphibian skin secretions contain a broad spectrum of biologically active compounds, particularly antimicrobial peptides, which are considered to constitute a first line of defence against bacterial infection. Here we describe the identification of two prototype peptides representing a novel structural class of antimicrobial peptide from the skin secretion of the oriental broad-folded frog, *Hylarana latouchii*. Named hylaranin-L1 (GVLSAFKNALPGIMKI-IVamide) and hylaranin-L2 (GVLSVIKNALPGIMRFIAamide), both peptides consist of 18 amino acid residues, are C-terminally amidated and are of unique primary structures. Their primary structures were initially deduced by MS/MS fragmentation sequencing from reverse-phase HPLC fractions of skin secretion that demonstrated antimicrobial activity. Subsequently, their precursor-encoding cDNAs were cloned from a skin secretion-derived cDNA library and their primary structures were confirmed unequivocally. Synthetic replicates of both peptides exhibited broad-spectrum antimicrobial activity with mean inhibitory concentrations (MICs) of 34 μM against Gram-negative *Escherichia coli*, 4.3 μM against Gram-positive *Staphylococcus aureus* and 4–9 μM against the yeast, *Candida albicans*. Both peptides exhibited little haemolytic activity (<6 %) at the MICs for *S. aureus* and *C. albicans*. Amphibian skin secretions thus continue to provide novel antimicrobial peptide structures that may prove to be lead compounds in the design of new classes of anti-infection therapeutics.

Keywords Amphibian · Antimicrobial · Peptide · Molecular cloning · Mass spectrometry

Introduction

In recent years, the emergence of multiple drug-resistant pathogenic microorganisms, such as MRSA, has stimulated the search for new and natural antibiotics which are curative (Davies and Davies 2010; Saleem et al. 2010). Since the discovery of the magainins, peptides with broad-spectrum antimicrobial activity, in the skin of the African clawed frog, *Xenopus laevis*, by Zasloff (1987), the unique antibiotic properties of these amphibian skin peptides have prompted much research (Zasloff 1987; Rinaldi 2002). Consequently, this theme has become a hot topic in the global research community.

Extensive studies have been conducted on amphibian skin antimicrobial peptides and several hundreds of such molecules with diverse structures have been isolated thus far, making amphibian skin the most productive source of these peptides in Nature (Conlon et al. 2004; Rinaldi 2002). While the peptides possess potent and broad-spectrum antimicrobial activities against numerous species of pathogenic microorganisms, including both Gram-positive and Gram-negative bacteria, fungi and yeasts such as *Candida albicans*, protozoa and viruses, they also have a relatively low cytolytic activity against normal mammalian cells (Haney et al. 2009; Simmaco et al. 1998). Furthermore, compared with conventional

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antibiotics, it is considerably more unlikely that pathogenic target organisms could develop resistance to these peptides due to their unique and very rapid membrane-disrupting antimicrobial mechanisms (Conlon et al. 2004; Haney et al. 2009; Rinaldi 2002; Simmaco et al. 1998).

Frogs of the family Ranidae have been found to produce defensive skin secretions that are complex molecular cocktails and have proven to be a particularly rich source of many different structural groups of antimicrobial peptides (Conlon et al. 2004; Thomas et al. 2012). Several hundred different antimicrobial peptides, representing at least nine different structural families, have so far been isolated from more than 20 ranid frog species and some of these peptides have been evaluated as potential lead compounds in the design of novel anti-infective therapeutics for the control of emerging microbial pathogens (Conlon et al. 2004; Thomas et al. 2012).

In this study, we have studied the skin peptidome of the oriental broad-folded frog, *Hylarana latouchii*, using both transcriptomic and peptidomic strategies and have discovered two 18-mer, C-terminally amidated peptides, named hylaranin-L1 and hylaranin-L2, that represent the prototypes of a novel class of amphibian skin antimicrobial peptide. The peptides were initially and tentatively sequenced from reverse-phase HPLC fractions of skin secretion by MS/MS fragmentation and primary structures were then unequivocally established following molecular cloning of their biosynthetic precursor-encoding cDNAs from a skin secretion-derived cDNA library. Solid-phase chemical synthesis provided sufficient quantities of both peptides to examine and establish their broad-spectrum antimicrobial activities.

Materials and methods

Specimen biodata and secretion harvesting

Specimens of broad-folded frog, *H. latouchii* ($n = 7$, snout-to-vent length 4–7 cm) were captured during expeditions in Fujian Province of the People's Republic of China. All frogs were adults of undetermined sex and secretion harvesting was performed in the field after which the frogs were released. Skin secretion was obtained by gentle transdermal electrical stimulation of the dorsal skin, as previously described (Tyler et al. 1992). Stimulated secretion was maintained at 4 °C prior to being snap-frozen in liquid nitrogen, lyophilised and stored at −20 °C prior to analyses.

Reverse-phase HPLC fractionation of lyophilised *H. latouchii* skin secretion

Five mg of lyophilised skin secretion was dissolved in 0.5 ml of 0.05/99.95 (v/v) trifluoroacetic acid (TFA)/water and clarified of microparticulates by centrifugation. The supernatant was decanted and subjected to reverse-phase HPLC fractionation using a Thermoquest binary gradient HPLC system, fitted with an analytical column (Phenomenex C-5; 0.46 cm × 25 cm), that was eluted with a linear gradient formed from 0.05/99.95 (v/v) TFA/water to 0.05/19.95/80.00 (v/v/v) TFA/water/acetonitrile over 240 min, at a flow rate of 1 ml/min. Fractions (1 ml) were collected at 1-min intervals and the effluent absorbance was monitored continuously at λ 214 nm. Samples (100 µl) were removed from each fraction, lyophilised and stored at −20 °C prior to subsequent screening for antimicrobial activity.

Identification and structural characterisation of hylaranins

Lyophilised samples from each chromatographic fraction were rapidly screened for antimicrobial activity using a zonal growth inhibition assay on confluent lawns of (*Escherichia coli*—NCTC 10418) and *Staphylococcus aureus* (NCTC 10788) grown in Petri dishes. Fractions containing antimicrobial activity were then analysed to determine the molecular masses of components using matrix-assisted laser desorption/ionisation, time-of-flight mass spectrometry (MALDI-TOF MS), on a linear time-of-flight Voyager DE mass spectrometer (Perseptive Biosystems, MA, USA), used in positive detection mode and employing alpha-cyano-4-hydroxycinnamic acid as the matrix. Internal mass calibration of the instrument with known standards established the accuracy of mass determination as ±0.1 %. Doubly charged ions $(M+2H)^{2+}$ of peptides in HPLC fractions identified as having antimicrobial activity were each subjected to MS/MS fragmentation using a Thermo Fisher Scientific LCQ Fleet electrospray ion-trap mass spectrometer.

Molecular cloning of hylaranin precursor-encoding cDNAs from an *H. latouchii* skin secretion-derived cDNA library

Five mg of lyophilised skin secretion was dissolved in 1 ml of cell lysis/mRNA protection buffer supplied by Dynal Biotec, UK. Polyadenylated mRNA was isolated using magnetic oligo-dT beads as described by the manufacturer (Dynal Biotech, UK). The isolated mRNA was subjected to 5′ and 3′-rapid amplification of cDNA ends (RACE) procedures to obtain full-length hylaranin precursor-encoding cDNAs using a SMART-RACE kit (Clontech UK) essentially as described by the manufacturer. Briefly, the 3′-RACE reactions employed an NUP primer (supplied with the kit) and a degenerate sense primer pool (S; 5′-AAR-AAYGCIHTICCIGGIHTIATG-3′) (R = A/G; Y = C/T; I = deoxyinosine; H = A/T/C) that was complementary to

the common internal amino acid sequence, –K–N–A–L/I–P–G–L/I–M–, of the hylaranins. 3′-RACE reactions were gel-purified and cloned using a pGEM-T vector system (Promega Corporation) and sequenced using an ABI 3100 automated sequencer. The sequence data obtained from these 3′-RACE products were used to design a series of hylaranin precursor cDNA-specific antisense primers. One 5′-RACE specific antisense primer was used in this reaction, AS (5′-CAAGTTTTAACATTTAGAAATGTTACTT-3′), in conjunction with the NUP RACE primer. Generated PCR products were gel-purified, cloned and then sequenced.

Peptide synthesis and secondary structure prediction analysis

Once unequivocal primary structures had been obtained, both hylaranins were synthesised by solid-phase Fmoc chemistry using a PS3 automated solid-phase synthesiser (Protein Technologies, Inc., AZ, USA). Following cleavage from the resin and deprotection, each synthetic peptide was purified by reverse-phase HPLC and analysed by electrospray mass spectrometry to establish both degree of purity and authenticity of structure. Secondary structure prediction analysis was performed using the established authentic primary structures of hylaranin-L1 and hylaranin-L2. Two programmes were employed to this end: the secondary structure and domain annotation package available within the SWISS-MODEL workspace (Arnold et al. 2006) and the helical wheel plot available on-line from the RZ lab (http://www.rzlab.ucr.edu/scripts/wheel).

Antimicrobial assays

The test organisms, *E. coli* (NCTC 10418)—Gram-negative, *S. aureus* (NCTC 10788)—Gram-positive and *C. albicans* (NCPF 1467)—a yeast, were grown in Mueller–Hinton Broth (MHB) for 18 h in an orbital incubator at 37 °C. Minimal inhibitory concentration (MIC) assays with antimicrobial peptides were carried out essentially as described previously (Wang et al. 2013). The lowest concentration of peptide in which no growth of each microorganism was observed was recorded as the MIC. Briefly, each organism was grown to reach log growth phase (~2 h) that reflected an organism density of ~1×10^8

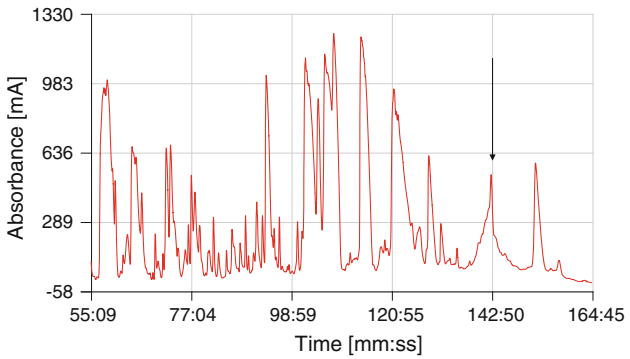

Fig. 1 Region of reverse-phase HPLC chromatogram of *Hylarana latouchii* skin secretion indicating elution position/retention time of peptide peak (*arrow*) which exhibited antimicrobial activity and that contained the novel peptides, hylaranin-L1 and hylaranin-L2

Table 1 Predicted singly and doubly charged *b*-ions and *y*-ions arising from MS/MS fragmentation of (A) hylaranin-L1 and (B) hylaranin-L2

#1	b(1+)	b(2+)	Seq.	y(1+)	y(2+)	#2
(A)						
1	58.028	29.518	G			18
2	157.097	79.052	V	1813.113	907.060	17
3	*270.181*	135.594	L	1714.044	*857.526*	16
4	*357.213*	179.110	S	1600.960	*800.984*	15
5	*428.250*	214.628	A	1513.928	757.468	14
6	*575.318*	288.163	F	*1442.891*	*721.949*	13
7	*703.413*	352.210	K	*1295.823*	*648.415*	12
8	*817.456*	409.232	N	*1167.728*	*584.367*	11
9	*888.493*	*444.750*	A	*1053.685*	*527.346*	10
10	*1001.577*	*501.292*	L	*982.648*	*491.827*	9
11	*1098.630*	549.818	P	*869.564*	435.285	8
12	*1155.652*	578.329	G	*772.511*	386.759	7
13	*1268.736*	634.871	I	*715.489*	*358.248*	6
14	*1399.776*	700.392	M	*602.405*	*301.706*	5
15	1527.871	*764.439*	K	471.365	236.186	4
16	1640.955	*820.981*	I	343.270	172.138	3
17	1754.039	*877.523*	I	230.186	115.596	2
18			V-amidated	117.102	59.054	1
(B)						
1	58.029	29.518	G			18
2	157.097	79.052	V	1841.119	921.063	17
3	270.181	135.594	L	1742.051	871.529	16
4	357.213	179.110	S	1628.967	*814.987*	15
5	*456.282*	228.644	V	1541.935	*771.471*	14
6	569.366	285.187	I	1442.866	721.936	13
7	*697.461*	*349.234*	K	1329.782	665.394	12
8	811.504	406.255	N	1201.688	601.347	11
9	882.541	*441.774*	A	1087.645	544.325	10
10	*995.625*	*498.316*	L	1016.607	508.807	9
11	1092.678	*546.842*	P	903.523	*452.265*	8
12	*1149.699*	*575.353*	G	806.471	403.738	7
13	1262.783	*631.895*	I	749.449	375.228	6
14	1393.824	*697.415*	M	636.365	318.686	5
15	1549.925	*775.466*	R	*505.325*	253.165	4
16	1696.993	*849.000*	F	*349.223*	175.115	3
17	1810.077	*905.542*	I	202.155	101.581	2
18			A-amidated	89.071	45.039	1

Observed ions are given in italic typeface

colony-forming units/ml. Peptides solutions were made by double-dilutions to effect final on plate concentrations in the range of 512–1 mg/l. Each organism was incubated with peptides for 12 h at 37 °C. Growth was assessed by monitoring absorbance at 550 nm using an ELx808 microplate reader (BioTek, USA) and data were analysed by BioTek Gen5 software. Controls included performing parallel MIC assays in the absence of peptide with vehicle alone and with the established antimicrobial peptide, melittin and with ampicillin, for comparison. Each assay datapoint was the mean value obtained from seven replicates. After this, 10 µl of the medium from each well was inoculated onto a Mueller–Hinton agar (MHA) plate and incubated for 24 h for measurement of minimum bactericidal concentrations (MBCs) which was defined as the concentration of the established antimicrobial peptide, melittin and with ampicillin from which no colonies could be grown.

Haemolysis assay

This was carried out using horse blood essentially as described previously (Wang et al. 2013). Briefly, samples of a 4 % suspension of horse erythrocytes were incubated with the same concentrations of peptides employed for the MIC assays, but for 120 min. Controls employed included vehicle alone (0 % haemolysis) and vehicle containing Triton X-100 (100 % haemolysis). Supernatants from all incubations were assayed at a wavelength of 550 nm using the ELx808 microplate reader and data were analysed using the BioTek Gen5 software. Each sample was assayed in quadruplicate.

Results

Identification and structural analysis of hylaranins

Following reverse-phase HPLC fractionation of lyophilised *H. latouchii* skin secretion and zonal inhibition antimicrobial assay of fraction samples, a high degree of activity was detected in fraction #142 (Fig. 1). Initial analysis of this fraction by MALDI-TOF MS revealed the presence of two major singly charged peptides with *m/z* values of 1870.2 and 1898.1, respectively. Subsequently, electrospray LC/MS/MS fragmentation analysis of their doubly charged ions (*m/z* values 935.6 and 949.6, respectively) revealed the presence of two structurally related but

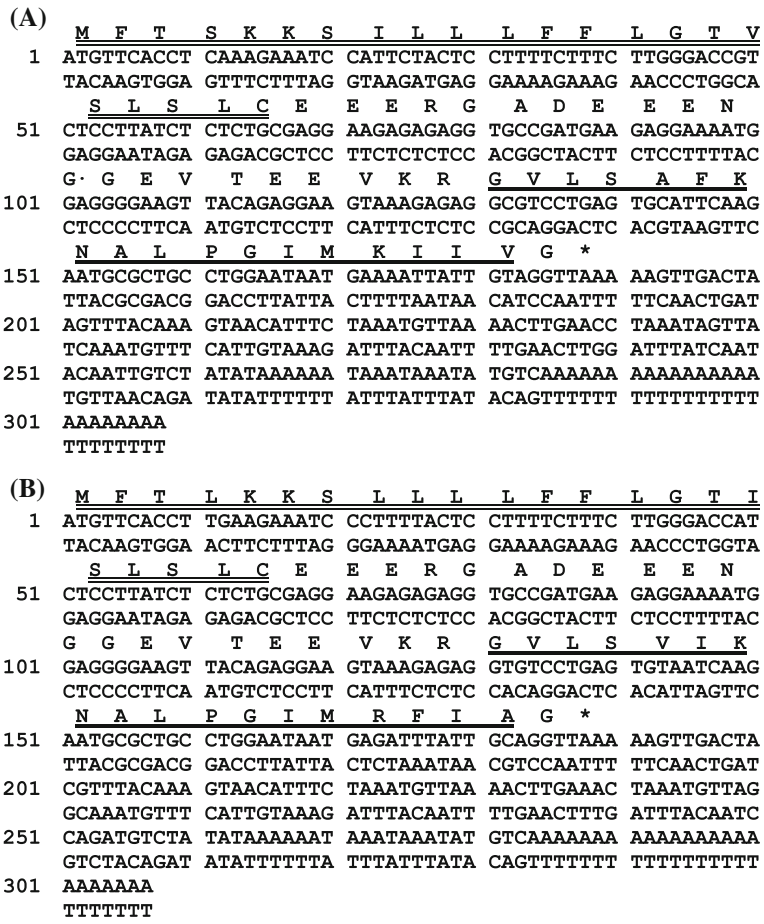

Fig. 2 Nucleotide and translated amino acid sequences of cloned skin secretion-derived cDNAs encoding the biosynthetic precursors of (**a**) hylaranin-L1 and (**b**) hylaranin-L2. Within the open-reading frame, putative signal peptide sequences are *double-underlined*, mature hylaranin sequences are *single-underlined* and stop codons are indicated with *asterisks*

distinct peptides (Table 1). As the more readily fragmented doubly charged ions from each peptide were chosen for MS/MS fragmentation, the resultant spectra contained both singly and doubly charged fragment ions. The observed and predicted fragment ions from each putative peptide are shown in Table 1. Bioinformatic analysis of the primary structures of the two novel peptides using the BLASTp programme available on-line through the National Center for Biotechnological Information (NCBI) portal indicated that they exhibited no structural similarity with any archived antimicrobial peptide from any origin. Due to their unique nature and the fact that they constituted proptotypes of a novel family of amphibian skin antimicrobial peptides, they were named hylaranin-L1 and hylaranin-L2 [hylaranin reflecting their taxonomic (generic) origin and L—*latouchii*, their species of origin]. The

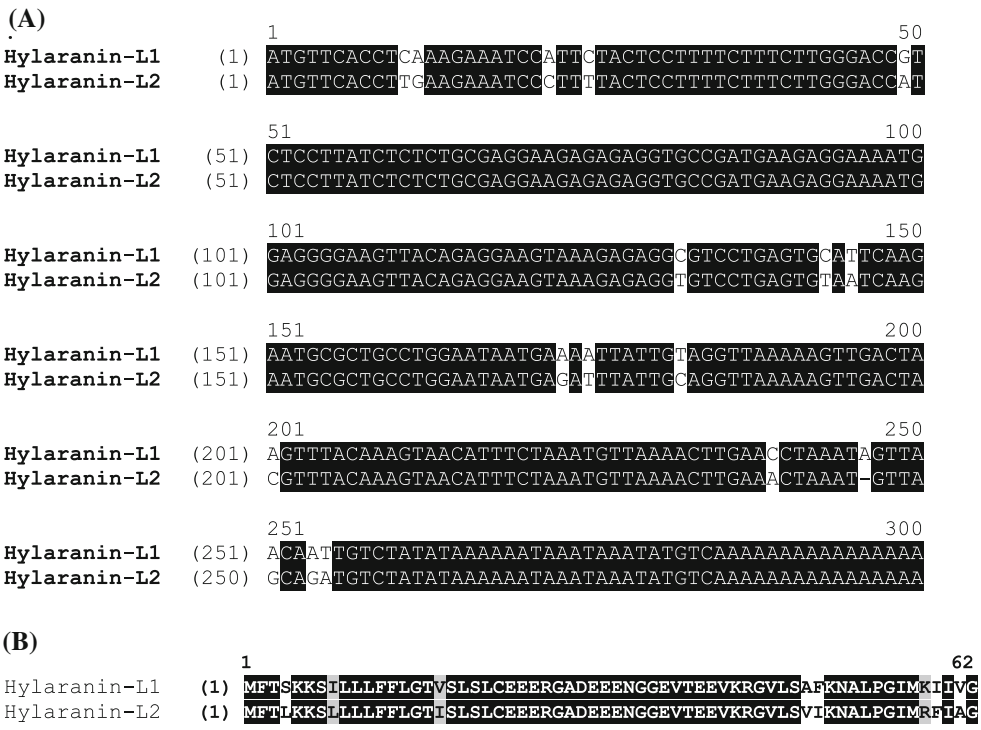

Fig. 3 Alignment of nucleotide sequences of (**a**) cloned cDNAs encoding the biosynthetic precursors of the hylaranins and (**b**), their translated open-reading frame amino acid sequences. In (**b**), identical amino acid residues are *shaded black*, conservative substitutions in are *shaded grey* and non-conservative substitutions are *unshaded*

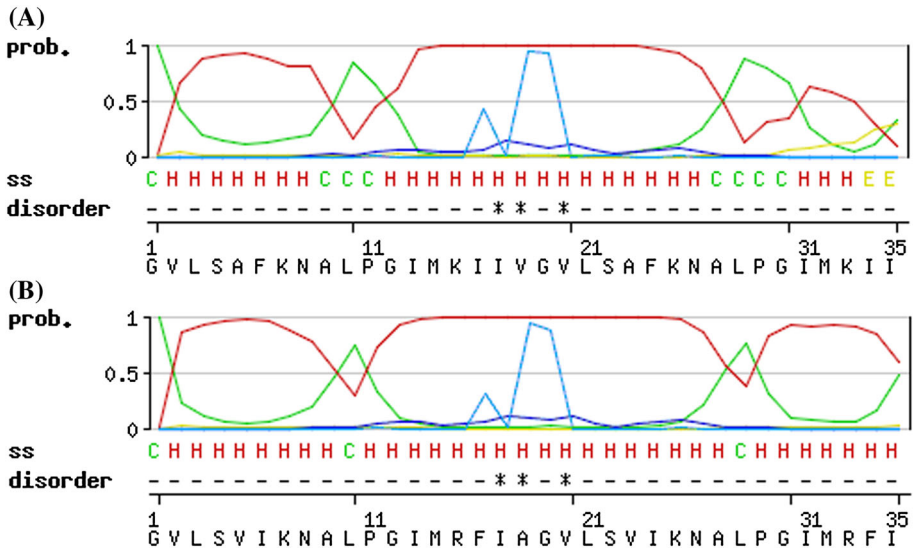

Fig. 4 Secondary structure predictions (SWISSMODEL workspace) of (**a**) hylaranin-L1 and (**b**) hylaranin-L2. Double tandem repeat sequences of each peptide were used for this programme as single sequences were too short for the analyses. (*C* coil, *H* α-helix, *E* extended sheet). Clearly the peptides are essentially helical with breaks in continuity caused by the Pro residue at position 11 in each

nucleotide sequences of the precursor-encoding cDNAs of both peptides have been deposited in the EMBL Nucleotide Sequence Database under the accession codes HF912233 (Hylaranin-L1) and HF912234 (Hylaranin-L2).

Cloning of hylaranin biosynthetic precursor-encoding cDNAs

Two different preprohylaranin-encoding cDNAs were consistently cloned from the skin secretion cDNA library and these encoded a single copy of hylaranin-L1 or hylaranin-L2, respectively (Fig. 2). Both open-reading frames consisted of 66 amino acid residues. Alignments of both open-reading frame DNA sequences and amino acid sequences, using the AlignX programme of the Vector NTI Bioinformatics Suite (Informax), revealed that both displayed high degrees of structural identity (Fig. 3a, b). The highest degree of amino acid differences occurred in the C-terminal hylaranin-encoding domains of the precursor proteins. Secondary structure prediction analysis of both hylaranins showed that each contained two α-helical domains interrupted by the centrally positioned prolyl (P) residue (Fig. 4). The programme required that two peptide sequences in tandem were used as each peptide was of too short a chain length for the programme to operate. The helical wheel models of both hylaranins again showed a conserved secondary structure with all but one hydrophobic residue side-chain located on one side of the molecule and the three positively charged amino groups (α-amino group, Lys-7, Lys/Arg-15) located on the opposite side (Fig. 5).

Antimicrobial and haemolytic activities

The MICs and MBCs obtained with hylaranin-L1 and hylaranin-L2, using the three test microorganisms, are summarised in Table 2. For hylaranin-L1, MICs obtained were 64 mg/l (34.2 μM) for *E. coli*, 8 mg/l (4.3 μM) for *S. aureus* and 16 mg/l (8.6 μM) for *C. albicans*. For hylaranin-2, MICs obtained were 64 mg/l (33.7 μM) for *E. coli*, 8 mg/l (4.2 μM) for *S. aureus* and 8 mg/l (4.2 μM) for *C. albicans*. Dose–response curves for hylaranin effects on test microorganism growth are shown in Fig. 6. For hylaranin-L1, MBCs obtained were 128 mg/l (68.4 μM) for *E. coli*, 32 mg/l (17.1 μM) for *S. aureus* and 32 mg/l (17.1 μM) for *C. albicans*. For hylaranin-2, MBCs obtained were 128 mg/l (67.4 μM) for *E. coli*, 32 mg/l (16.9 μM) for *S. aureus* and 16 mg/l (5.6 μM) for *C. albicans*. While the honeybee venom peptide, melittin, was found to be a more potent antimicrobial agent than the hylaranins, it was also found to be extremely haemolytic at these effective concentrations (Table 2). While ampicillin

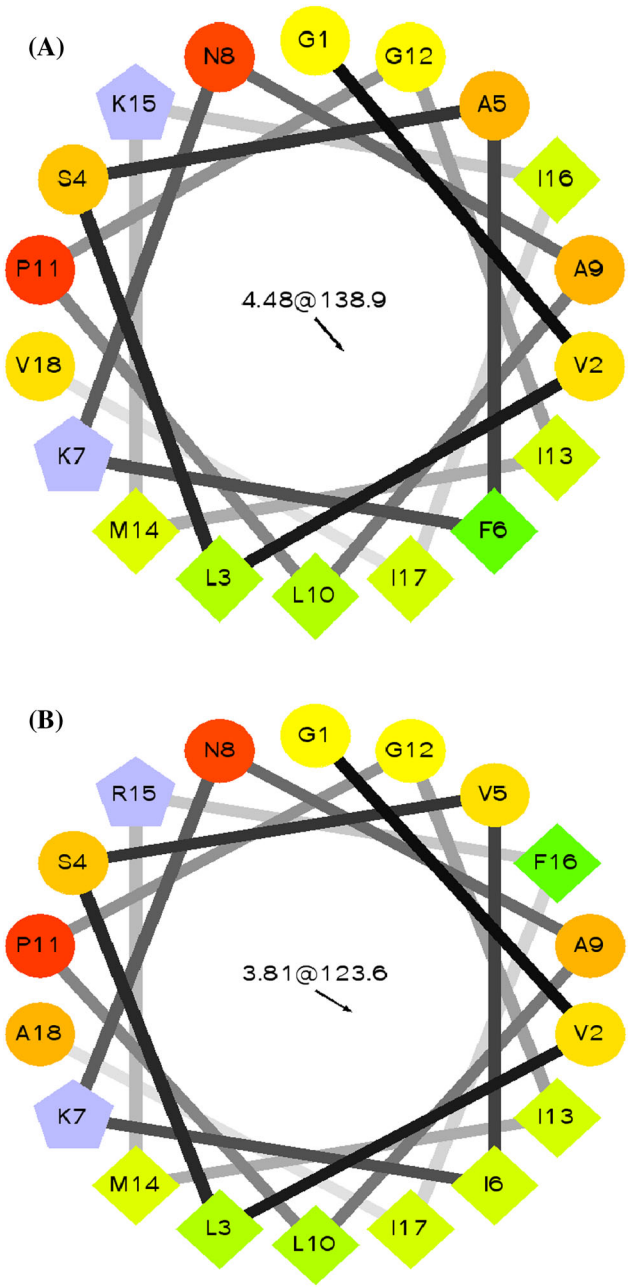

Fig. 5 Helical wheel plots of (**a**) hylaranin-L1 and (**b**) hylaranin-L2. Note the amphipathic character of each peptide with the side chains of the hydrophobic residues of hylaranin-L1 (A5, I16, A9, V2, I13, F6, I17, L10, L3 and M14) occurring on one side of the molecule and the cationic amino groups (α-amino of G1, side-chain amino groups of K7 and K15) occurring on the opposite side. A similar situation was observed for hylaranin L2. Hydrophobic moments of each peptide are indicated in the centres of respective plots

was, as expected, found to be highly potent against the Gram-positive bacterium, *S. aureus*, it was of similar potency to the hylaranins against the Gram-negative bacterium, *E. coli*, and ineffective against the yeast, *C. albicans* (Table 2).

Table 2 Mean inhibitory and mean bactericidal concentrations (MICs and MBCs) of each antimicrobial agent against the three model test microorganisms employed

	E. coli	*S. aureus*	*C. albicans*
MICs (mg/L and μM) and haemolysis (%) at corresponding MIC values			
Hylaranin-L1	64 mg/L (34.2 μM) (22.9 %)	8 mg/L (4.3 μM) (3.0 %)	16 mg/L (8.6 μM) (4.2 %)
Hylaranin-L2	64 mg/L (33.7 μM) (58.6 %)	8 mg/L (4.2 μM) (5.8 %)	8 mg/L (4.2 μM) (5.8 %)
Melittin	16 mg/L (5.6 μM) (75.2 %)	8 mg/L (2.8 μM) (73.8 %)	8 mg/L (2.8 μM) (73.8 %)
Ampicillin	8 mg/L (22.9 μM) (0 %)	0.0625 mg/L (0.18 μM) (0 %)	NE
MBCs (mg/L and μM)			
Hylaranin-L1	128 mg/L (68.4 μM)	32 mg/L (17.1 μM)	32 mg/L (17.1 μM)
Hylaranin-L2	128 mg/L (67.4 μM)	32 mg/L (16.9 μM)	16 mg/L (8.4 μM)
Melittin	32 mg/L (11.2 μM)	16 mg/L (5.6 μM)	16 mg/L (5.6 μM)
Ampicillin	16 mg/L (45.8 μM)	0.125 mg/L (0.36 μM)	NE

Percentages of haemolysis are shown at each MIC for each microorganism

NE not effective

Haemolytic activity assay

Hylaranin-L1 was found to be devoid of haemolytic activity up to 16 mg/l and hylaranin-L2 was found to be devoid of haemolytic activity up to 8 mg/l (Fig. 7).

Discussion

The C-terminally amidated octadecapeptides, hylaranin-L1 and hylaranin-L2, described here from the skin secretion of the oriental broad-folded frog, *H. latouchii*, represent the prototypes of a novel structural class of amphibian skin antimicrobial peptide. BLASTp analysis using the NCBI database (July 2013) revealed no structural similarity with any archived antimicrobial peptide from any species source.

H. latouchii is a typical ranid frog both in terms of morphology and life history which would not suggest the likelihood of the discovery of novel antimicrobial peptides in such a species. However, ranid frogs have proven to be one of the richest sources of diverse antimicrobial peptides that include the brevinins, esculentins, ranatuerins, ranalexins, palustrins, temporins and tigerinins (Conlon et al. 2004). Among the reasons suggested for the structural diversity of amphibian skin peptides, even within a single species, is that having such a cocktail of actives renders the possibility of development of pathogen resistance unlikely. Also, different peptides may act synergistically to combat infectious agents and indeed some evidence for this has been reported (Bowie et al. 2012; Conlon 2011; Park and Hahm 2005). Pharmacologically-active amphibian skin peptides, such as tachykinins, bradykinins and bombesins, mediate their effects through endogenous predator membrane bound receptors (Bevins and Zasloff 1990; Erspamer 1994) and thus exhibit and require a high degree of structural determinants to facilitate this. In contrast, antimicrobials peptides have a much lower stringency for primary structural conservation as long as their essential cationic amphipathic character is maintained. Thus the hylaranins, described here for the first time, may have no reported homologues with respect to primary structure, but nevertheless possess the requisite cationic amphipathic nature as demonstrated through secondary structure prediction modelling.

When determining the effective inhibitory and bactericidal concentrations of antimicrobial agents, particularly membranolytic peptides, it is important to assess their relative cytotoxic effects on eukaryotic host cells (Yeaman and Yount 2003; Yount et al. 2006). Erythrocytes are most often employed for this purpose as they are readily sourced and membrane damage is easily determined through measurement of haemoglobin release into their suspension medium. In this study, haemolytic activity of both peptides was found to be minimal at the MICs for all of the microorganisms examined. This was an encouraging observation for future development of the hylaranins as therapeutic agents as many previous antimicrobial peptides from a variety of structurally related families do not display such discriminatory activity between bacterial and host eukaryotic cell membranes (Yeaman and Yount 2003; Yount et al. 2006).

Although not examined in this study, it would be worthwhile, on the basis of the antimicrobial data obtained here, to assess both peptides for possible discriminatory activity between normal eukaryotic cells and neoplastic cells as the latter often display aberrant membrane lipid compositions more reminiscent of prokaryotic cells with membrane-stabilising cholesterol depletion and increases

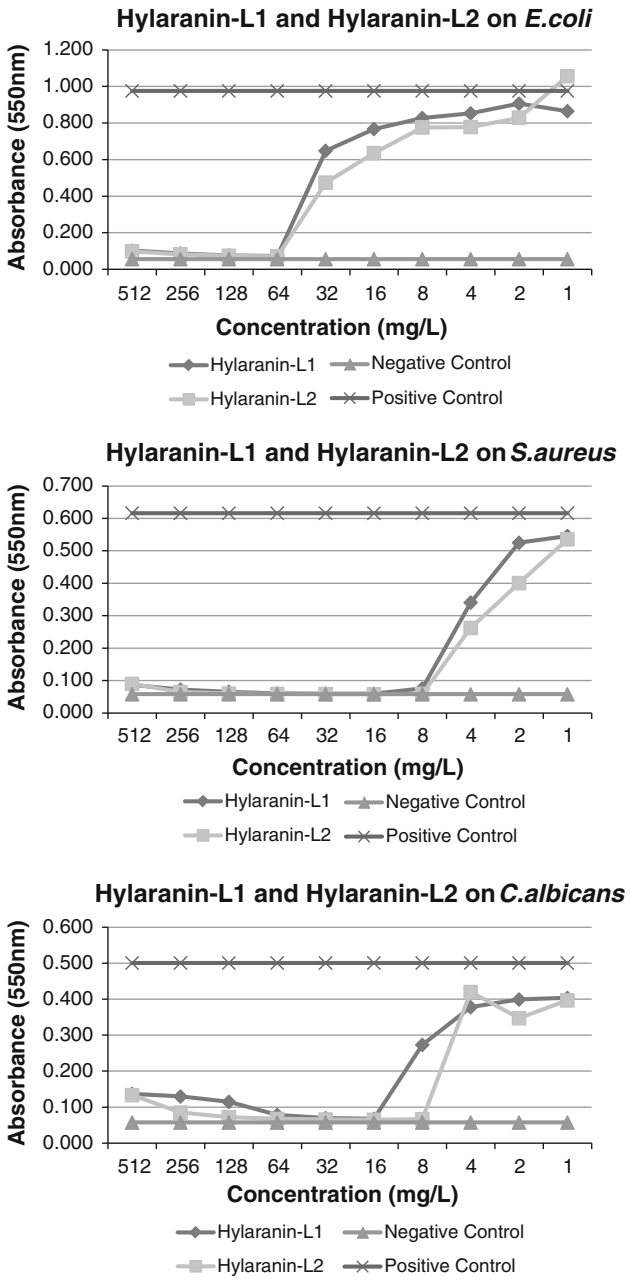

Fig. 6 Dose–response curves of synthetic hylaranin-L1 and hylaranin-L2 with the three model test microorganisms *Escherichia coli* (NCTC 10418), *Staphylococcus aureus* (NCTC 10788) and *Candida albicans* (NCPF 1467). Positive and negative control growth curves are indicated

in the net positive charges of cell surfaces (Hancock 2000; Matsuzaki 1999; Teixeira et al. 2012).

In summary, two prototypes of a novel structural class of amphibian skin antimicrobial peptide, the hylaranins, have been described in the present study. Synthetic replicates of both peptides displayed a potent and broad-spectrum antimicrobial activity with little haemolytic effects at antimicrobial minimal inhibitory concentrations (MICs).

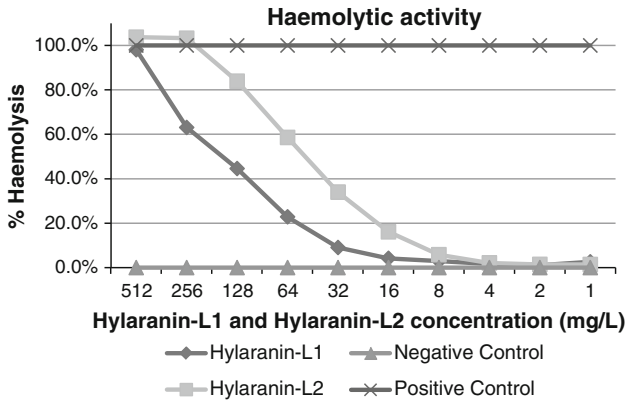

Fig. 7 Dose–response curves of the haemolytic activity of hylaranin-L1 and hylaranin-L2 using horse erythrocytes

These features warrant further in-depth investigations on this class of peptide towards their critical evaluation as putative novel anti-infection therapeutics.

Conflict of interest The authors declare that they have no conflict of interests.

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