Antimicrobial activities of chimera peptides composed of human neutrophil peptide 1 (HNP-1) truncated analogues and bovine lactoferrampin

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Introduction

Many pathogenic bacteria and fungi strains have become resistant to the most of commercially available antibiotics as the result of their common and excessive use in medical practice. One of the most promising strategy for searching for new compounds with antimicrobial activity is bioconjugation, covalent or non-covalent connection, of at least two chemical molecules of compounds with different activity that in the end display a different mechanism of action than the parent compounds.

The main aim of this work was to obtain a new class of compounds with antimicrobial activity. These compounds are peptidic hybrids composed of two peptides with antimicrobial activity. First peptide is a modified fragment of human neutrophil peptide 1 (HNP-1), a member of α -defensins [1]. This peptide derived from the C-terminal fragment of HNP-1, comprised of its modified (Cys¹⁹ → Cys(Acm), Gly²³ → 2Abz, Cys²⁹ → Ser) middle 15 – 29 fragment. It was reported that this peptide displayed antimicrobial activity against a broad spectrum of pathogens, the closest to that of full length HNP-1. Second peptide is bovine lactoferrampin (LFampB) which is the fragment 268 - 284 of bovine lactoferrin [2]. This peptide also exhibits broad antimicrobial action against several gram-positive and gram-negative bacteria as well as candidacidal activity [3,4]. We decided to design and synthesize series of peptidic hybrids composed of 2Abz²³S²⁹ analogues (named here as HNP) and bovine LFampB. Primary structures of synthesized chimeras and their constituent peptides are shown in Fig. 1. Two different covalent linkers were used: isopeptide bond, considered as non-cleavable in biological systems and redox-sensitive disulfide bridge. The first chimera (CH8) is composed of LFampB, which is coupled through the isopeptide bond formed by its C-terminal carboxyl group to the reference peptide HNP by the ϵ -amino group of the C-terminal Lys. In the second chimera (CH9), Cys(Acm) residue of HNP was replaced by L- α -aminobutyric acid (Abu), which is often used to substitute sulfur containing amino acids (Met or Cys). In the third chimera (CH10), constituent peptides were coupled by the disulfide bridge formed by the thiol groups of Cys⁵ of HNP and the Cys residue attached at the C-terminus of LFampB, respectively. In order to determine the cellular uptake of CH10, its fluorescently-labelled analogue (CH10-CF) and constituent peptides (HNP[Cys⁵]-CF and CF-LFampB-C) were also synthesized.

Figure 1: Primary structures of chimera peptides (A) and their constituent peptides (B).

Results and Discussion

All peptides were screened against the wide range of microorganisms, including Gram-negative, Gram-positive bacteria and fungi from *Candida* species. Broth microdilution method for MIC determination was used as a standard procedure. As a result, we obtained three peptidic hybrids that effectively inhibited the growth of selected bacterial strains, but none of the chimeric peptides was able to inhibit fungi growth. Under experimental conditions, they demonstrated significantly higher antimicrobial activity than constituent peptides. Chimeras containing the isopeptide bond as a linker (CH8 and CH9) were active against both Gram-positive and Gram-negative bacteria, whereas the one with a disulfide bridge (CH10) was specifically active against Gram-positive bacteria. CH8 and CH9 exhibited the most potent activity against all experimental strains, being the most effective against *S. epidermidis* and *P. aeruginosa*. Activity of CH10 against Gram positive bacteria was similar to that of CH8 and CH9 but this compound was practically inactive against Gram-negative strains. These results clearly show that the type of a linker used to connect AMPs determines the antibacterial activity of chimera peptides. Presence of the isopeptide bond in chimeras CH8 and CH9 resulted in their remarkable antimicrobial activity against both Gram-positive and Gram-negative bacteria, whereas connection of the two peptide chains by a disulfide bridge afforded CH10, specifically active against Gram-positive bacteria. The fact that the equimolar mixtures of constituent peptides were practically inactive, clearly indicates that antibacterial activity of chimera peptides results from the presence of a covalent linkage between the constituent peptides. The observed specificity correlated well with a significantly higher content of the helical structure determined in an environment that mimics a biological membrane in case of the chimera CH10 in compare to remaining two chimeras. The antimicrobial activity of the equimolar mixtures of studied peptides was lower than that of the individual peptides and chimeras. This observation indicates a lack of the synergistic effect of constituent peptides and importance of the covalent linkage between them for the enhancement of antibacterial activity.

In this study we also reported that antimicrobial activity depends strongly on cell penetration. Using carboxyfluorescein labeled chimera (CH10-CF) and its constituents we checked whether compounds undergo cellular uptake. Microscopic observations showed that CH10-CF as well as its constituent LFampB-C penetrate well into *S. epidermidis* cells (Fig. 2). In contrast, HNP[Cys⁵] with the lowest activities neither penetrates the cell nor interact with cell membrane. In reference to antimicrobial drugs widely used in clinic, cytotoxicity of tested chimera peptides against human cells is low and rather limited to cancer cells which possess low negative charge (in contrast to no-cancer which are neutral or slightly positive [5]). Indeed, selective toxicity of chimera peptides against pathogenic bacteria is also associated with negative charge of their cell surface. We also showed that generation of ROS in human cells is limited what can be perceived as an advantage from the point of view of eventual toxicity to the host. Nevertheless, small amount of ROS generated may be advantageous in combating pathogenic bacteria. Type of the linker has a significant impact on the proteolytic stability of studied compounds which is an important issue to consider especially in case of peptides. Taking into consideration both antimicrobial activity and selective cytotoxicity not affecting normal cells and also other presented results, we claim that such chimera peptides are very promising lead structures for the development of antimicrobial drugs.

Figure 2: Fluorescence microscope images of CH10-CF and its constituents (CF-LFampB-C and HNP[Cys5]-CF) uptake by S. epidermidis. Cells were grown to the mid-log phase and exposed to the action of the compounds at concentration 200 μ M for 30 min.

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References

- [1] Lundy F.T., Nelson J., Lockhart D., Greer B., Harriott P., Marley J. Mol Immunol 45, 190-193 (2008), DOI: 10.1016/j.molimm.2007.04.018.
- [2]. 90, 245-251 (2012), DOI: 10.1139/o2012-018 .
- [3] van der Kraan M.I.A., Groenink J., Nazmi K., Veerman E.C.I., Bolscher J.G.M., NieuwAmerongen A.V. Peptides, 25, 77-183 (2004), DOI: .
- [4] Haney E.J., Nazmi K., Lau F., Bolscher J.G.M., Vogel H.J. Biochimie 91, 141-154 (2009), DOI:
- [5] Chen B., Le W., Wang Y., Li Z., Wang D., Ren L., Lin L., Cui S., Hu J. J., Hu Y., *et al.* Theranostics 6, 1887-1898 (2016), DOI: 10.7150/thno.16358.