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Low Molecular Mass Peptide Dendrimers that Express Antimicrobial Properties

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Abstract—A series of low-generation dendrimeric peptides was synthesized in an attempt to evaluate their antimicrobial potency. All tested dendrimeric peptides in which lysine was a starting and branching element expressed moderate activity against *Staphylococcus aureus* NCTC 4163, and *Escherichia coli* NCTC 8196.

Introduction

Dendrimers are highly ordered, hyperbranched polymers with a number of potential applications. One of the major features of dendrimers is possibility of multiplication of active elements on the dendrimer surface. In medical sciences, dendrimers are useful tools as an antibody mimics, drug transport vectors, anticancer agents, MRI contrast reagents, and so on.[1] Functionalization of dendrimers can be performed through attachment of active elements to the last generation of the growing polymer. Peptide dendrimers build from lysine residue have been used as relatively flexible scaffolds carrying multiple functions with markedly enhanced biological effectiveness of such conjugates.[2,3] There have been several attempts of designing dendrimeric compounds that are carrying antimicrobial functions. Particularly poly(propylene)imine dendrimers functionalized with quaternary ammonium salts have been found more effective than hyperbranched polymers of the same family.[4] Recently, lysine dendrimers have been used as synthetic scaffolds for attachment two to eight copies of a tetrapeptide R4 (RLYR) or an octapeptide R8 (RLYRKVYG). Both R4 and R8 contained a combination of basic and lypophilic aminoacids that have been found in protegrins and tachyplesins — natural peptides with antimicrobial activity. Dendrimeric effect was manifested by high potency in antimicrobial assays against 10 organisms in high- and low-salt conditions.[5] Bacterial membranes are charged differently than mammalian cell walls. This difference is recognized by peptides of mammalian endogenous host-defense system. All natural antibacterial peptides are multicharged, and contain several copies of basic amino acids — arginine or lysine. Therefore, we have predicted that multicharged dendrimers may have also high affinity to the bacterial membranes. Initial studies resulted in conclusion that proper topographical composition of basic and aromatic functions of small dendrimers may result in active antimicrobial properties.[6] To perform further structure–activity studies, a small library of peptide dendrimers has been synthesized and tested for antimicrobial properties.

Synthesis

All tested peptides were synthesized by the respective Boc- or Fmoc- chemistry in solid phase by previously described methods. Crude peptides were purified by gel filtration on Sephadex LH-20 (in methanol), followed by preparative HPLC. All peptides were confirmed to have correct amino acid analyses and molecular weights by ESI-MS.[7] For microbiological study, peptides in amide form were used.

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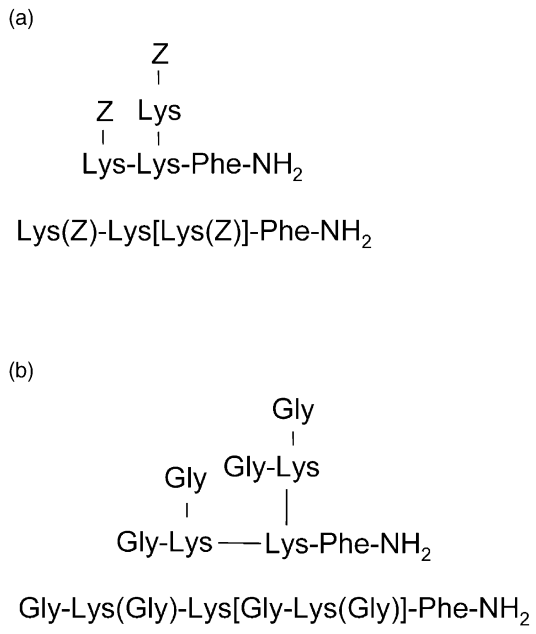

Figure 1. Schematic representations of dendrimers **4** and **11** and conventions used for abbreviated forms.

Antimicrobial activity

Antimicrobial activity was assayed against *Staphylococcus aureus* NCTC 4163, and *Escherichia coli* NCTC 8196. To determine the minimum inhibitory concentration (MIC), the microdilution broth method was used. Briefly, cells of each bacterial strain were collected in the logarithmic phase of growth and suspended in nutrient broth (Biotest AG, Germany). The concentration of colony-forming units (CFU) per milliliter was quantified by measuring absorbency at 600 nm (A_{600}).

Peptide samples were dissolved in nutrient broth (pH 7.0) and diluted serially. The sample solution (100 μL) was mixed with the diluted bacterial suspension (100 μL). Mixtures containing 105 bacterial CFU and from 1 to 0.003% of test peptides were incubated for 24 h at 37 °C. Antimicrobial activities were expressed as the minimal inhibitory concentration (MIC), the concentration at which 100% inhibition of growth was observed (Table 1). Three independent experiments were averaged and deviation was calculated.

For microbiological study, indolicidin, Ile-Leu-Pro-Trp-Lys-Trp-Pro-Trp-Trp-Pro-Trp-Arg-Arg-NH_2 has been used as a reference compound.[8,9] The indolicidin antibacterial property against *S. aureus* was used as a reference (Table 1).

Results and Discussion

The obtained results (Table 1; for explanation of abbreviated form of molecular formula see Fig. 1) indicate the possibility of designing antimicrobial compounds on the basis of peptidic dendrimers. The results confirmed previous conclusion that proper topographical location of two multiplicated elements, basic amino or guanidine groups and aromatic (lipophylic) components are significant factors for expressing antimicrobial properties. Surprisingly, simple dipeptide Lys-Phe-NH_2 (**2** and **3**) is the minimal element that expresses low but significant antimicrobial activity. The analogues of the respective first dendrimeric generation (**4** and **5**) are approximately 10-fold more potent that the dipeptide **2**. Interestingly, the analogue with aromatic benzyloxycarbonyl groups (*Z*) in position alpha (**4**) is less potent than the respective analogue with 2-chlorobenzyloxycarbonyl group in position epsilon (**5**).

Table 1. Antimicrobial activity of dendrimeric compounds **1**–**14**

Compd	MIC, μM[a] *S. aureus*	MIC, μM[a] *E. coli*
1 Indolicidin[a]	1.5	3.0
2 Lys-Phe-OMe	300	590
3 *Z*-Lys-Phe-NH_2	151	324
4 *Z*-Lys-Lys(*Z*-Lys)-Phe-NH_2	39	64
5 Lys(2-Cl-Z)-Lys[Lys(2-Cl-Z)]-Phe-NH_2	16	84
6 *Z*-Lys-Orn(*Z*-Lys)-Phe-NH_2	85	85
7 *Z*-Lys-Lys(*Z*-Lys)-Tyr-NH_2	1327	165
8 Lys(Z)-Lys[Lys(Z)]-Tyr-NH_2	331	331
9 *Z*-Lys-Lys(*Z*-Lys)-Gly-NH_2	94	375
10 Ala-Lys(Ala)-Lys[Ala-Lys(Ala)]-Phe-NH_2	181	181
11Arg-Lys(Arg)-Lys[Arg-Lys(Arg)]-Phe-NH_2	130	75
12 *Z*-D-Arg-Lys(*Z*-D-Arg)-Lys[*Z*-D-Arg-Lys(*Z*-D-Arg)]-Phe-NH_2	88	44
13 *Z*-D-Arg-D-Lys(*Z*-D-Arg)-D-Lys[*Z*-D-Arg-D-Lys(*Z*-D-Arg)]-Phe-NH_2	44	20
14 *Z*-Arg-D-Lys(*Z*-Arg)-D-Lys[*Z*-Arg-D-Lys(*Z*-Arg)]-Phe-NH_2	44	20

Values are means of three experiments, error has been estimated as 20–40%.
[a]Ile-Leu-Pro-Trp-Lys-Trp-Pro-Trp-Trp-Pro-Trp-Arg-Arg-NH_2.

Replacement of benzyloxycarbonyl aromatic groups with non-aromatic amino acid Ala (**10**), but with preservation of aromatic phenylalanine on C-terminus resulted with approximately ten-fold loss of activity. Location of the aromatic groups changes selectivity of the respective peptides. It appears that replacement of the core Lys by aminoacid with shorter side chain – ornityne (Orn) reduces slightly potency against *S. aureus* (**6** vs **4**). However, for every dendrimer generation the phenylalanine residue on C-terminus is optimal for expressing biological activity. Replacement of this amino acid residue with aromatic tyrosine (**7**, **8**) or non-aromatic glycine (**9**) resulted with decrease of activity.

Surprisingly, the activity is not significantly influenced by chirality of the building amino acids (compounds **12**–**14**). In most cases antibacterial potency agains *S. aureus* NCTC 4163, and *E. coli* NCTC 8196 was found to be of the same order. Only compound **7** differentiates activity against these two bacteria strains.

The obtained results may provide several working hypotheses. Likely, dendrimeric peptides of this series interact with complementary ionic and aromatic sites located on bacterial membrane, resulting with its damage. The differences in activity may suggest the existence of optimal topographical location of basic and aromatic groups that can be achieved due to molecular flexibility. Nevertheless, low differences in activity

between analogues may also suggest ability of dendrimes to adopt structure(s) proper for interactions with bacterial membrane. This example provides evidence that the presented family of dendrimers built from flexible chains of basic aminoacids can be functionalized towards bacteriostatic properties. These hypotheses will be tested in further studies, which are in progress.

Acknowledgements

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