(RFLR)3 RETRO-INVERSE PEPTIDE CONTAINING D-AMINO ACID AS ANTIMICROBIAL AGENT

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INTRODUCTION

The emerging bacterial resistance to conventional antibiotics has led to the search for new therapeutic alternatives. Antimicrobial cationic peptides are promising candidates, since they act on bacterial membranes causing their rapid destruction, with low tendency to generate resistance. However, these compounds present low stability against proteases. The aim of this study was to design a retro inverse analog containing D amino acids of oligomer (RLFR)3 (TA4R), in order to evaluate the effect of substitution by D amino acids and inverted sequence on their biological properties and enzymatic stability.

METHODOLOGY

Peptide synthesis. RI-dTA4R was synthesized by solid-phase synthesis using Fmoc chemistry.
Antimicrobial Activity. The MIC was determined against bacterial gram (+) and (-) strains
Hemolytic activity. The ability to lyse human erythrocytes was evaluated by spectrophotometric measurement of hemoglobin released at 405 nm.
Enzymatic Stability. The enzymatic stability against trypsin and chymotrypsin was determined by measuring residual antimicrobial activity by agar diffusion method and against serum enzymes was determined by HPLC after incubation at different times.
Circular Dichroism (CD) analysis. The secondary structure of the peptide was studied in water, TFE/H2O (50:50 %v/v) and in the presence of DPPC and DPPG vesicles.

RESULTS

Table 1. Phisicochemical properties of TA4R and RI-dTA4R

Peptide	Net Charge (pH=7)	MM* (g/mol)	RT° (min)
TA4R	+7	1736.01	17.7
RI-dTA4R	+7	1735.50	17.8

Figure 1. Antimicrobial activity of TA4R and RI-dTA4R

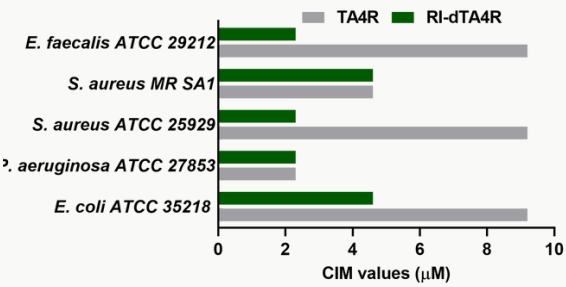
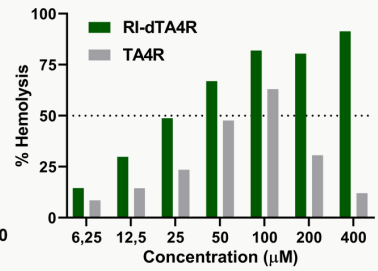


Figure 2. Hemolityc activity of TA4R and RI-dTA4R



RI-dTA4R showed antimicrobial activity against all bacteria strains tested (MIC=4,6 to 2,3 μM), increasing the inhibitory activity of TA4R (Figure 1). Substitution with D-amino acid allowed obtaining a peptide with improved enzymatic stability against trypsin, chymotrypsin and serum proteases (Figure 4). Nevertheless, this analog was more hemolytic than TA4R, presenting around 50% of hemolysis at 25 μM, but less than 20% of hemolysis at MIC concentration (Figure 2). CD analysis showed that the peptide in TFE 50% and DPPG vesicles is strongly structured, adopting a levorotatory helical structure, while in H2O and DPPC vesicles it is not structured.

Figure 3. CD spectra of RI-dTA4R

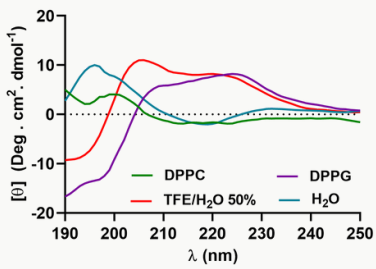
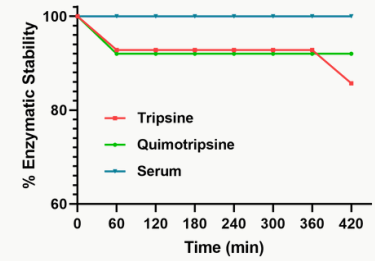


Figure 4. Stability of RI-dTA4R against proteases



CONCLUSIONS

Based on these results, retro-inverse peptides containing all D-amino acid may be considered as potential therapeutic compounds for treatment of infections produced by gram (+) and (-) bacteria with improved enzymatic stability.