Structure-Activity Relationship Studies Of Single-Point D-Substituted Gomesin Analogues

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Gomesin (*Gm*) is a potent antimicrobial octadecapeptide (pGlu-CRRLCYKQRCVT YCRGR-NH_2) isolated from hemocytes of the Brazilian spider *Acanthoscurria gomesiana*. It contains two intramolecular disulfide bridges ($Cys^{2,15}$ and $Cys^{6,11}$) and shows a wide spectrum of action at micromolar concentration. NMR studies showed that *Gm* is folded in a two-stranded antiparallel beta-sheet connected by a noncanonical beta-turn. Here we describe the synthesis of gomesin analogues where the L-amino acid residues were individually replaced by its corresponding D-isomer. Thus, all peptides were synthesized by SPPS using the t-Boc strategy. They were purified by RP-HPLC and characterized by AAA and LC/MS. Antimicrobial activities were evaluated in a liquid growth inhibition assay against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans*. CD studies were performed in different environments such as TFE, SDS and LPC and revealed that the analogues exhibited the same conformational behavior than the observed with gomesin. Bioassays results showed that the replacement of the amino acid residues $pGlu^1$, Arg^4, Leu^5, Tyr^7, Gln^9, Val^{12} and Thr^{13} by its corresponding D-isomer caused a significative reduction in their antimicrobial activities. Interestingly, [D-Arg^3]-*Gm* showed to be 2-fold more potent than *Gm,* while [D-Lys^8]-*Gm* and D-Tyr^{14}]-*Gm* were equipotent to it. These results in general are in agreement with our previous studies employing Ala-scan gomesin analogues. All together our results suggest that the hydrophobic residues and the turn region are important for the peptide interaction with the microorganism membranes. No obvious correlation could be found between the biological activity and the secondary structural features inferred from the CD studies. Hence, these results are being investigated. In addition, hemolytic activities determined at 100 µM of peptides concentration showed that there is a direct correlation between antimicrobial and hemolytic activities of all peptide tested.

Palavras Chave: Gomesin, antimicrobial activity, D-scan, hemolytic activity, peptide synthesis
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