Antimicrobial aza-β³-peptides: Structure-activity relationship?

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1. Introduction

Designing antimicrobial molecules based on pseudopeptides to increase the activity, selectivity and bioavailability of natural peptides is now widespread. Recently, numerous peptidomimetics have been developed for biological applications including azapeptides, β-peptides, peptoids, oligoureas. In this context, aza-β³-aminoacids were used as new blocks to compose antimicrobial peptide sequences. From a natural antimicrobial peptide, H-ALSGDAFLRF-NH₂ (AD), depending on the aza-β³ residue insertions, the modifications can result either in inactive pseudopeptides or in a drastic enhancement of the antimicrobial activity without cytotoxicity. We present here, the first NMR solution structures of peptides containing aza-β³ amino acids to study their structure-activity relationship.

2. Results and Discussion

In SDS micelles, the global fold of AD and AK displays disordered N-terminal regions and well-defined amphipatic helical C-terminal moieties (5-10) (Fig 1). The Aβ³K peptide is based on AK, substituting the lysine in position 5 by an aza-β³-lysine (Aβ³K). Its global fold is very different from the two previous peptides and do not exhibit a helical C-terminal part. A typical aza-β³ residues hydrazino turn, previously described in crystals, can be also observed in solution. Despite the two possible conformations of the aza-β³ lysine nitrogen stereocenter, only one set of signals is observable on the NMR spectra and the NOESY spectrum have numerous NOEs. The configuration of the stereocenter of the aza-β³-Lys5 was not defined in the CNS topology and parameter files, and two set of conformations can be obtained from the NMR restraints according to the configuration R or S of the Nα stereocenter. Based on the NMR spectra, one cannot discriminate if only one conformation is present in solution or if two configurations are in fast exchange.

![Fig. 1 AD natural cuttlefish peptide NMR structure.](image)
Table 1  Sequences and MIC activity of the natural peptide and of five synthetic analogues.
(MIC: Minimum Inhibitory Concentration in µM; na: not active; nd: not determined)

<table>
<thead>
<tr>
<th>Name</th>
<th>Primary sequence</th>
<th>Staphylococcus aureus</th>
<th>Escherichia coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>AD</td>
<td>H-ALSGDAFLRF-NH₂</td>
<td>na</td>
<td>584</td>
</tr>
<tr>
<td>AK</td>
<td>H-ALSGKAFLRF-NH₂</td>
<td>262</td>
<td>65</td>
</tr>
<tr>
<td>Aβ³K</td>
<td>H-ALSG-aza-β³K-FLRF-NH₂</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>K-2Nal7</td>
<td>H-ALSGKA-aza-β³-1Nal-LRF-NH₂</td>
<td>nd</td>
<td>268</td>
</tr>
<tr>
<td>K-1Nal</td>
<td>H-ALSGKA-aza-β³-1Nal-LRF-aza-β³-1Nal-NH₂</td>
<td>15</td>
<td>30</td>
</tr>
</tbody>
</table>

Its global fold is very different and do not exhibit a helical C-terminal part as the two previous peptides anymore. A typical aza-β³ residues hydrazino-turn, previously described in crystals with a characteristic geometry, can be also observed in solution (Fig 2).

Two new peptides, named K2Nal7 and K2Nal, were designed with subsequent substitution of the phenylalanine by aza-β³-naphthylalanine amino acids (aza-β³Nal). As AD and AK, only their C-terminal moieties are ordered but not fold in helix. They share a similar turn which approach the two hydrophobic aromatic residues in position 7 and 10. Nevertheless, in each case, they are too far to pack each other (~9 Å) and no NOEs can be detected between the residues 7 and 10 aromatic protons. The K2Nal7 and K2Nal dominant structures do not have a hydrazino-turn, indeed the hydrazino-turn NOEs marker previously noticed on the Aβ³K NOESY spectrum cannot be detected for these peptides.

The incorporation of aza-β³ residues broke the helical structures to induce different peptide folds. The Aβ³K structure is quite rigid and shows a hydrazino-turn surrounded by two β-turn (Fig 2). However, despite the presence of aza-β³ amino-acids the K-2Nal7 and K-2Nal appears to be quite flexible, their structures do not have a stable hydrazino-turn and only the C-terminal part is well defined.

![Fig. 2 Aβ³K peptide NMR Structure.](image)

3. Conclusion

Structural parameters, such as peptide helicity, hydrophobicity, hydrophobic moment, peptide charge and the size of the hydrophobic/hydrophilic domain, on membrane activity and selectivity could increase the antibacterial activity and improve the prokaryotic selectivity of natural and peptides analogues.

References