Proceedings of the 37th European Peptide Symposium
Michal Lebl, Editor
European Peptide Society, 2024 *http://doi.org/10.17952/37EPS.2024.P2111*

Molecular determinants of the anti-amyloid function of the cross-interaction of the human cathelicidin LL-37 with islet amyloid polypeptide

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Introduction

Islet amyloid polypeptide (IAPP) is a 37-amino acid long neuroendocrine hormone which is secreted from the pancreatic β-cells [1]. IAPP is a highly amyloidogenic polypeptide. Its self-assembly into cytotoxic oligomers and amyloid fibrils is linked to inflammation, β-cell degeneration, and the pathogenesis of type 2 diabetes (T2D). Cathelicidins are a large group of multifunctional host-defense peptides (HDP) which are expressed by various cells including pancreatic β-cells and play important roles in inflammatory processes. The only known human cathelicidin is the 37-residue polypeptide LL-37. LL-37 has been long known for its broad-spectrum antimicrobial activity and its potent immunomodulatory effects [2]. Intriguingly, recent studies of our group showed that LL-37 is able to interact with IAPP with nanomolar affinity and to effectively suppress its amyloid self-assembly and related pancreatic β-cell-damaging effects *in vitro*. Our results suggested that the LL-37/IAPP interaction may play a protective role in T2D [3].

We asked which are the molecular and structural requirements of the anti-amyloid function of the LL-37/IAPP interaction. To address this, we first studied the effects of systematic N-terminal shortening of LL-37 on its structure, interactions with IAPP, and related anti-amyloid function.

Results and Discussion

Various different N-terminal truncated LL-37 analogues were synthesized by Fmoc-based solid phase peptide synthesis (SPPS) methodology. Their effects on fibril formation of IAPP were studied by using the amyloid specific thioflavin T (ThT) binding assay in combination with transmission electron microscopy (TEM). Their effects on formation of cytotoxic IAPP assemblies were determined using the MTT reduction assay in cultured rat insulinoma cells (RIN5fm). Of note, full length LL-37 was included in the studies for comparison.

We found that the deletion of the N-terminal dipeptide segment from LL-37 does not affect its ability to inhibit IAPP amyloid self-assembly and cytotoxicity. This suggests that residues Leu1 and Leu2 are not necessary for its anti-amyloid function. However, further N-terminal truncation up to LL-37(7-37) resulted in a loss of anti-amyloid activity. Thus, residues within LL-37(3-6) seem to play an important role in the anti-amyloid activity of LL-37. Interestingly, fluorescence spectroscopic titrations of fluorescently labeled IAPP with the truncated LL-37 analogues revealed that N-terminal truncation of LL-37 up to residue 14 does not impair binding affinity to IAPP.

Thus, the binding affinities of the LL-37 variants to IAPP did not correlate with their effects on IAPP amyloid formation suggesting that additional factors might be involved. Finally, far-UV CD spectroscopic studies were consistent with nearly identical secondary structure contents for LL-37 and LL-37(3-37) while further N-terminal truncation affected the secondary structure. The results of our studies should assist in deciphering the molecular basis of the anti-amyloid function of the LL-37/IAPP interaction and the design of novel molecules targeting pathogenic IAPP amyloid self-assembly in T2D.

Acknowledgments

This work was supported by Deutsche Forschungsgemeinschaft (DFG) grant SFB1035 B06 (part of SFB1035, project number 201302640).

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