Proceedings of the 37th European Peptide Symposium
Michal Lebl, Editor
European Peptide Society, 2024 http://doi.org/10.17952/37EPS.2024.YIF09

Designed cyclic peptides as inhibitors of amyloid self-assembly and cross-seeding of amyloid-beta peptide and islet amyloid polypeptide

Beatrice Dalla Volta[1], Melanie Roth[1], Yuan Tian[2], Alessia Calzi[1], Jürgen Bernhagen[2], and Aphrodite Kapurniotu[1]

[1]Division of Peptide Biochemistry, TUM School of Life Sciences, Technical University of Munich (TUM), Freising, D-85354, Germany; [2]Division of Vascular Biology, Institute for Stroke and Dementia Research (ISD), Klinikum der Universität München, Ludwig-Maximilian-University (LMU), D-81377, Munich, Germany

Introduction

Increasing evidence suggests a link between the pathogenesis of Alzheimer's disease (AD) and type 2 diabetes (T2D). In this context, the key amyloid polypeptides of AD and T2D amyloid-β peptide (Aβ (Aβ40(or 42)) and islet amyloid polypeptide (IAPP) have been reported to co-localise in brain or pancreas of AD and T2D patients [1,2]. In addition, IAPP or Aβ fibrils were found to act as cross-seeds of the amyloid self-assembly of the two polypeptides [3,4]. Therefore, molecules that inhibit amyloid self-assembly and cross-seeding of IAPP and Aβ may offer promising leads for anti-amyloid drugs in both diseases.

Our earlier studies revealed high affinity interactions between Aβ and IAPP and were consistent with cross-interactions and amyloid self-assembly being mediated by the same "hot segments" [5,6]. We used the identified IAPP "hot segments" to design peptides as inhibitors of amyloid self-assembly of IAPP, Aβ, or both polypeptides [7,8]. Our more recently developed IAPP-derived amyloid inhibitors were the 17-residue long macrocyclic peptides termed "macrocyclic inhibitory peptides" or MCIPs [8]. The lead MCIP **2e** turned out to be a nanomolar inhibitor of Aβ amyloid self-assembly and in addition it exhibited a good proteolytic stability in human plasma *in vitro* and blood-brain barrier (BBB) crossing in a cell model. However, **2e** was unable to inhibit IAPP amyloid self-assembly. The aim of the work presented here was to design novel cyclic peptides with optimized properties and functions.

Results and Discussion

Systematic MCIP shortening and optimisation yielded a new class of cyclic peptides. Peptides were synthesized by Fmoc-based solid phase peptide synthesis (SPPS) methodology. We used the amyloid specific thioflavin T (ThT) binding assay in combination with transmission electron microscopy (TEM) to study their effects on fibril formation of IAPP or Aβ42. To determine their effects on formation of cytotoxic assemblies, we used the MTT reduction assay in cultured RIN5fm for IAPP-related studies or cultured PC12 cells for Aβ42-related studies. According to the ThT binding assay and TEM, in the presence of the cyclic peptides IAPP fibril formation was fully suppressed. In addition, the cell viability assay showed that formation of cytotoxic IAPP aggregates was fully suppressed as well. Furthermore, the cyclic peptides suppressed Aβ42 amyloid self-assembly while the lead cyclic peptide suppressed IAPP/Aβ42 cross-seeding as well. Thus, the cyclic peptides of our study might be promising candidates for drugs targeting pathogenic amyloid self-assembly in both AD and T2D and studies toward their further development are in progress.

Acknowledgements

This work was supported by Deutsche Forschungsgemeinschaft (DFG) grant SFB1035 B06 to AK (part of SFB1035, project number 201302640) and by DFG under Germany's Excellence Strategy within the framework of the Munich Cluster for Systems Neurology EXC 2145 SyNergy-ID 390857198 to JB. We thank K. Hille and J. Jekel for help with peptide synthesis and purification.

References

1. Jackson, K., et al. *Ann. Neurol.* **74**, 517-26 (2013), https://doi.org/10.1002/ana.23956
2. Martinez-Valbuena, I., et al. *Ann. Neurol.* **86**, 539-551 (2019), https://doi.org/10.1002/ana.25570
3. O'Nuallain, B., et al. *J Biol Chem* **279**, 17490-17499 (2004), https://doi.org/10.1074/jbc.M311300200
4. Moreno-Gonzalez, I., et al. *Mol Psychiatry* **22**, 1327-1334 (2017), https://doi.org/10.1038/mp.2016.230
5. Yan, L.M., et al. *Angew Chem Int Ed Engl* **46**, 1246-1252 (2007), https://doi:10.1002/anie.200604056
6. Andreetto, E., et al. *Angew Chem Int Ed Engl* **49**, 3081-3085 (2010), https://doi.org/10.1002/anie.200904902
7. Andreetto, E., et al. *Angew Chem Int Ed Engl* **54**, 13095-1310 (2015), https://doi.org/10.1002/anie.201504973
8. Spanopoulou, A., et al. *Angew Chem Int Ed Engl* **57**, 14503-14508 (2018), https://doi.org/10.1002/anie.201802979