DOI 10.18699/SBB-2022-175

All-D-enantiomeric peptide designed for Alzheimer´s disease treatment dynamically interacts with amyloidogenic region of membrane-bound amyloid-β peptide precursor

Okhrimenko I.[1]*, Volynsky P.[2], Zagryadskaya Y.[1], Kamynina A.[1, 2], Kuzmichev P.[1], Arseniev A.[1, 2], Efremov R.[1, 2, 3], Bocharov E.[1, 2]*

[1] *Shemyakin–Ovchinnikov Institute of Bioorganic Chemistry, RAS, Moscow, Russia*

[2] *Moscow Institute of Physics and Technology, MIPT, Dolgoprudny, Russia*

[3] *School of Applied Mathematics, Higher School of Economics, Moscow, Russia*

** okhrimenko.is@mipt.ru; edvbon@mail.ru*

Key words: Alzheimer´s disease treatment, amyloid precursor protein, transmembrane domain, protein-lipid and protein-protein interactions, NMR spectroscopy, molecular dynamics

Motivation and Aim: Alzheimer's disease (AD) is a devastating neurodegenerative disease resulting in severe dementia. Detailed information on the structure, dynamics, and various intermolecular interactions for biomolecules directly involved in the development of AD is required for the rational development of new biologically active compounds and screening of existing ones to obtain the most effective candidates for medicines [1]. Genetic evidence strongly suggests that aberrant generation and/or clearance of the neurotoxic amyloid-β peptide (Aβ), being the products of sequential cleavage of amyloid precursor protein (APP), triggers the disease. All-D-enantiomeric peptide D3 and its derivatives were recently selected with the aid of phage display to directly destroy cytotoxic Aβ aggregates [2]. Currently, one of the D3-like compounds is about to undergo a phase II clinical trial, however, high resolution molecular details of its disease preventing or pharmacological action are not completely clear.

Methods and Algorithms: To solve the problem, we used complex approach based on biochemical and biophysical methods such as protein engineering, microscopic thermophoresis, fluorescence confocal microscopy, fluorescence polarization, microfluidic diffusion calibration, circular dichroism, high resolution nuclear magnetic resonance spectroscopy (NMR) and computer simulation.

Results: We present experimental evidence showing that D3-peptide, being an intrinsically disordered peptide (IDP), can dynamically and specifically bind in IDP/IDP-like manner to the extracellular juxtamembrane (JM) region of membrane-bound Aβ precursor, CTFβ transmembrane fragment of amyloid precursor protein ($APP_{672-726}$, $Aβ_{1-55}$ in amyloid-β numeration). Namely, heteronuclear NMR spectroscopy showed that D3-peptide directly binds to the amphiphilic near-membrane JM region of $Aβ_{17-26}$, which, depending on external conditions, is capable of undergoing a conformational transition from the α-helical conformation to the β-chain, which is involved in folding of β-amyloid into fibrils. The obtained structural data in agreement with ELISA and Western-blot analyzes, which reveal that D3-peptide dynamically binds in the vicinity of α-secretase recognition site situated between the extracellular cation-binding domain and JM helix of APP. The influence of a number of pathogenic AD mutations located in different structural and functional parts of APP on the peptide binding was also tested.

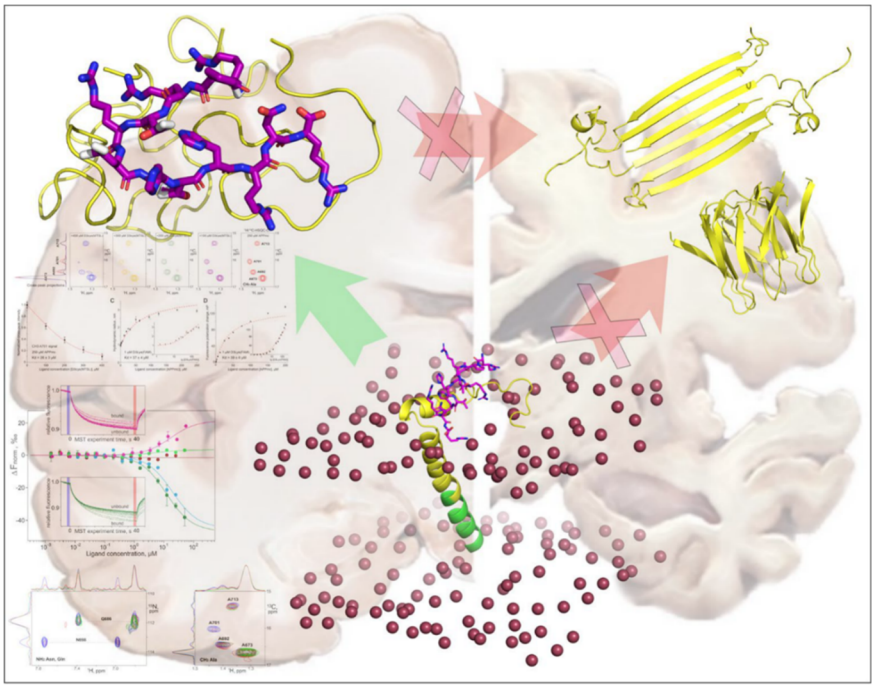

Fig. 1. Schematic representation using experimentally obtained structural data of the action of the D3-peptide (shown in purple, in the lower part of the figure) on the membrane-associated peptide $APP_{672-726}$ ($Aβ_{1-55}$), followed by the formation of D3/Aβ complexes (in the upper left part of the figure) and inhibition formation of toxic Aβ oligomers (in the upper right part of the figure)

Conclusion: The data suggest that D-enantiomeric peptide D3 recognizes the amyloidogenic region of APP also before its processing, restricting conformational diversity not compromising its α-helicity and preventing intermolecular hydrogen bond formation, which would create prerequisites for inhibition of early steps of Aβ conversion into β-conformation and its toxic oligomerization associated with early stages of AD development [3]. The achieved progress in understanding the molecular mechanism of D3-peptide action is an important step towards development of an effective AD treatment and prevention strategy.

Acknowledgements: The study is supported by Russian Science Foundation (project No. 20-64-46027).

References

1. Urban A.S. et al. Structural studies providing insights into production and conformational behavior of Amyloid-β peptide associated with Alzheimer's disease development. *Molecules*. 2021;26(10):2897.
2. Klein A.N., Gremer L., Kutzsche J., Willbold D. et al. Optimization of d-Peptides for Aβ Monomer Binding Specificity Enhances Their Potential to Eliminate Toxic Aβ Oligomers. *ACS Chem Neurosci*. 2017;8(9):1889-1900.
3. Bocharov E.V. et al. All-d-Enantiomeric peptide D3 designed for Alzheimer's disease treatment dynamically interacts with membrane-bound Amyloid-β precursors. *J Med Chem.* 2021;64(22):16464-16479.