UNIVERSITÀ DEGLI STUDI DI SALERNO

DIFARMA

UNIVERSITÀ DEGLI STUDI DI SALERNO
Dipartimento di Farmacia

PhD Program
in **Drug Discovery and Development**
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PhD Thesis in

Structural study of biomarkers for neurodegenerative diseases

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Abstract

The research activity of my PhD focused on the following topics:

• NMR conformational analysis of Aβ(1-42) in 50/50 HFIP/water v/v.

• Design and study the interaction of peptides from the binding site of antibodies targeting Aβ(1-42) epitopes.

• Study of interaction of heparin with α-synuclein1-61 region.

Based on the amyloid-β (Aβ) cascade hypothesis, abnormal accumulation of the amyloid-β (Aβ) peptides into toxic extracellular plaques is the leading cause of neurodegeneration and dementia in Alzheimer's disease (AD) patients. The main components of the amyloid plaques are Aβ(1-40) and Aβ(1-42), soluble helical peptides that in overcoming toxic environmental conditions undergo a conformational transition to form β-sheet aggregates. HFIP/water mixtures have been used as an excellent system to monitor the conformational changes induced in the stable Aβ(1-42) solution structure by increasing water amounts. Aβ(1-42) studied by NMR in 80/20 HFIP/water v/v mixture revealed regular helical-kink-helical structure, while in the mixture containing higher water content - 30/70 HFIP/water v/v mixture - it lost this structure, to assume a succession of β-bends conformations while conserving regular α-helix on ^{11}E-L^{17}.

The first part of my PhD project focused on the NMR study of Aβ(1-42) in 50/50 HFIP/water, a solvent mixture characterized by intermediate polarity compared to those previously used. Our data show that in these conditions, Aβ(1-42) is characterized by regular α-helix on ^{18}V-V^{24} residues, β-turns on ^{4}F-H^{14} and ^{32}I-G^{38} segments, short random coils, and bend structures on the remaining portions of the sequence. The C-terminus shows high flexibility and dynamic properties revealing a primary role in the transition to the β-strand conformation. Molecular dynamics simulations in water solution confirm that these flexible regions evolve to regular β-strand structures giving rise to a complex architecture of fibril β-sheet aggregates. Based on the amyloid cascade hypothesis, hundreds of molecules have been tested, as possible AD therapeutics that can disaggregate Aβ fibrils or prevent their formation. More recently, the hypothesis has spread within the scientific community that immunotherapy targeting Aβ peptide has great potential for treating or preventing AD. Accordingly, several antibodies have been developed targeting different epitopes of Aβ(1-42). In this context, part of my research activity focused on the study of new peptides as possible ligands of Aβ(1-42) designed on the model of the binding sites of different monoclonal antibodies. In particular, we selected solanezumab and crenezumab that bind ^{16}K-S^{26} and ^{13}H-D^{23} Aβ(1-42) segments and aducanumab that targets the ^{3}E-E^{11} N-terminal fragment. The designed peptides were studied for their interaction with

Aβ(1-42) using CD and NMR spectroscopies; then, we studied their effect on the Aβ(1-42) aggregation using atomic force microscopy. Our data show that WAibH and SYSTPGK are endowed with an interesting ability to bind Aβ(1-42). Although characterized by a modest dissociation constant (KD), they inhibit the formation of Aβ(1-42) mature fibrils, as shown by a quantitative decrease of fibrillation degree in AFM microscopy.

The research activity of my Ph.D. project carried out at the University of Copenhagen under the supervision of Prof. Birthe B. Kragelund was focused on the NMR study of the interaction of α-synuclein with glycosaminoglycans, in particular, heparin. The intrinsically disordered protein α-synuclein (aSN) is in its fibrillated state, the main component of Lewy bodies that are hallmarks of Parkinson's disease. Additional components include glycosaminoglycans, e.g. heparan sulfate proteoglycans. Heparan sulfate has, in an age-dependent matter, shown increased levels of sulfation. Heparin, a highly sulphated glycosaminoglycan, is a relevant mimic for heparan sulfate and has been shown to influence fibrillation of aSN. In this context, using NMR experiments, I gave my contribution to demonstrate that a region corresponding to residues 1-61 of aSN lacks intrinsic fibrillation propensity, while fibrillation can be induced by heparin in a concentration-dependent manner. Structural shifts from disorder, via type I β-turns, to β-sheet, was observed in aSN1-61. Therefore heparin can induce fibrillation of aSN1-61 as well as of the full aSN through weak binding to the N-terminal and the KTKE-motifs; it modulates the structure of aSN by inducing type I β-turn structures, which may be critical for triggering aSN fibrillation.