Physicochemical and structural aspects of fibrillogenesis modulated by halogeno-chalcone molecules in the presence of lipid membranes

Fibrillogenesis, which is the process of a formation of fibril structures form native states of proteins, is responsible for neurodegenerative diseases, such as Alzheimer, Huntington, Parkinson. Most proteins, which can play many biological functions, are also able to form their fibrous form responsible for amyloid-associated diseases. Additionally, an increasing number of some types of poly amino acids (PAAs) has been described to give rise to the formation of pathological fibrils responsible for amyloid-associated diseases. It was found that genetic mutations in cells of patients with the II type of Huntington's disease can lead to aberrant poly-L-glutamine (PQ) or poly-L-leucine (PL) stretches and intranuclear fibrous aggregates.

Since the deposition of β-amyloid plaques is an early event in the development of many amyloid-associated disorders, it is important to find active inhibitors of fibrillation, which will restrain further development of these disorders or even reverse already existing pathological changes. Besides the inhibition of amyloid fibril formation, chalcone molecules have protective effects against an amyloid-induced oxidative stress and neuronal damage [1], which puts chalcone compounds at the top of the list of potent drugs against amyloid disorders. It was stated that the most effective inhibitors of fibrillogenesis have a structure of the aromatic ring(C6)-linkers-aromatic ring(C6) motive [2,3], present also in trans-chalcone molecules. Additionally, organohalogen compounds, especially organofluorine, are of exceptional interest in medical applications; in fact, approximately 20% of all currently approved drugs contain at least one fluorine atom. In structures of hundreds of proteins there are C-X…π interactions (X - halogen atoms), and carbon-bond halogen atoms (C-X) form halogen bonds with Lewis bases [4, 5]. Because C-X forms X-bonding with different amino acid groups, similarly to H-bonding, halogeno-inhibitors can modulate protein conformations and aggregations such as amyloid fibrillogenesis. In naturally appeared amyloid fibrils there is a dense network of hydrogen bonds, which determines fibril structure and properties.

The amyloid fibril assembly and the toxicity of pre- and fibrillar aggregates are strictly related to each other and are both closely membrane-associated phenomena [6-8]. Cell membrane is thought to be the direct target mediating amyloid-induced cell death. Lipid bilayer may act as an effective catalyst of fibrillogenesis, providing a generic environment, where protein molecules adopt conformation and orientation, promoting their assembly into proto-fibrillar and fibrillar structures. Additionally, amyloid cytotoxicity can arise from amyloid-induction of membrane permeabilization.

The main goal of this project will concentrate on studies of the influence of halogeno-chalcone molecules on the amyloid fibrillogenesis in the presence or absence of lipid biomembranes. In order to create a model with effective and quite universal character for the chalcone-membrane-mediated amyloid inhibition and its structural alterations, we decide to study a few examples of amyloidogenic proteins. We will use insulin and lysozyme proteins as a good model of protein fibrils. Additionally, poly-L-glutamine (PQ), poly-L-glutamic acid (PE), poly-L-lysine (PK) and poly-L-threonine (PT) will be applied as a model of a simple conversion of α-helices to cross-β structures, characteristic for amyloid fibrils. Amyloid modulators will be represented by F-, Cl-, Br- and I-derivatives of trans-chalcone (trans 1,3-diaryl-2-propen-1-one), see Fig. 1, as well as lipid bilayers of liposomes, which will be constructed with different lipids (PC, PS, PE, PG, SM and cholesterol) in order to mimic a complex structure of biomembranes.

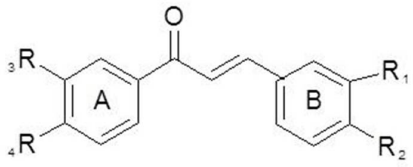

Figure 1. *Schematic representation of a structure of trans*-chalcone substituted in A nad B aromatic rings in positions of R_1, R_2, R_3, R_4 by halogen atoms X= F, Cl, Br, I

As the first step, the changes in secondary structures of natively folded, partially unfolded and aggregated in fibril manner for chosen proteins and peptides will be determined in quantitative and qualitative manner in the presence of different halogen-derivatives of chalcone. Fourier transform infrared spectroscopy (FTIR) in transmission and attenuated total reflectance (ATR) manners will be applied to analyze the amide bands, sensitive to changes of protein secondary strcutures. The role of kinetic changes in protein folding-unfolding process of amyloidogenesis will be studied by monitoring the shift of amide II bands caused by gradual H-D exchange. All the mentioned analyses will be completed by the studies of the effects of the presence of lipid membranes in protein-chalcone systems. The next step of the project schedule will be associated with VCD studies of the influence of halogeno-chalcones on the chiral superstructures of fibrils, mediated or not by lipid membranes. The latest research reports in literature stress the role of fibril supramolecular chirality in morphology and structural changes of amyloid aggregates, which determines their toxicity [9, 10]. Transmission electron microscopy with negative staining will show structural features of fibril associates accompanied by chiral alterations, which will be referred to ones of not-modulated proteins presented in the literature. The third part of the research plan will be directed toward microcalorimetric studies. This method is highly sensitive to protein folding-unfolding accompanied by changes of water hydration. Our studies will be supplemented by thermodynamic parameters, like enthalpy and partial heat capacity for heat-induced transitions going through different protein intermediates and lipid states. The next area of our studies will concentrate on fluorescence spectroscopy with intrinsic and extrinsic fluorophores. The protein folding will be estimated as well as detection of the amyloid fibril formation and its dynamic development. Lipid fluorescence probes will inform us about the changes of membrane orders, packing and hydrations, phase separation and domain formation. The principal factors analysis (PCA), multivariate curve resolution based on an alternative least squares method (MCR-ALS), and two-dimensional correlation analysis (2DCOS), and especially for

fluorescence spectra the parallel factor analysis (PARAFAC) will be used to enhance the spectral resolution and finally to elucidate maximum information.

The combination of a few techniques for one physicochemical or structural feature will give us the chance to look at one problem from different angles. The determination of the modulation of the proteins by halogeno-chalcones and membranes constructed from different lipids will shed a new light on structural requirements for effective fibrillogenesis-inhibitors. The range of physicochemical and structural changes of lipid bilayers, triggered by chalcone-modulated proteins, will be linked to the toxicity potency of these proteins.

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