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AUTOREFERAT ROZPRAWY DOKTORSKIEJ

"The role of conformational memory effect in propagation of structural variants of insulin amyloid fibrils"

("Rola konformacyjnego efektu pamięci w propagacji wariantów strukturalnych amyloidu insuliny")

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Under permissive i.e. slightly destabilizing conditions, proteins tend to misfold and aggregate into highly ordered linear β-aggregates – the so-called amyloid fibrils. These structures are linked to several human degenerative disorders, including Alzheimer's disease, Parkinson's disease, Creutzfeldt-Jakob disease ("prion disease"). There are many poorly understood aspects of amyloidogenesis. Unlike protein folding which leads to the native structure uniquely defined by the amino acid sequence, protein aggregation may lead to multiple structures (polymorphic forms) with different biochemical properties. Moreover, upon seeding of native protein with preformed structural variants of fibrils, conformational memory effect (termed also self-propagating polymorphism) can be observed. This effect consist in the passing structural features of mother fibrils (the seed) to daughter amyloid regardless of environmental conditions. Nowadays it is generally accepted that this type of polymorphism underlies the clinical problems of diverse neurotoxicity of Aβ fibrils (connected with Alzheimer's disease) and infectivity of prions (so-called prion strains). Another remarkable feature of amyloid fibrils is their high thermodynamic stability which is thought to cause the unusual heat resistance of prion infectivity. These features make protein amyloidogenesis not only an interesting but also clinically important research topic.

Because fibrillation patterns can be reproduced *in vitro* in nonpathogenic and more accessible proteins – such as insulin – using them as model systems has become common. Studying aggregation of this hormone is also important for pharmaceutical industry because insulin amyloid formation is a limiting factor for production, handling and long-terms storage of insulin formulations.

The central goal of this work was to study seeding-dependent fibrillation patterns of insulin. Particular attention was paid to following issues: (1) thermal stability of amyloid fibrils in terms of capacity to seed daughter fibrils, (2) relation between amyloid polymorphism and

variations in the amino acid sequence beyond the critical amyloidogenic regions, and (3) mechanisms of stable propagation of mother amyloid phenotype in daughter generation of fibrils.

The study on thermal stability of insulin fibrils highlighted several interesting aspects of the decomposition process of amyloid. Firstly, it was found that chemical degradation of covalent structures in amyloid state starts below 300°C leading to a highly cooperative release of gaseous products. Secondly, degradation of fibrils' covalent structure is preceded by disruption of the β-sheet conformation which starts well below 200°C. These changes proved to disable the correct molecular recognition taking place at fibril's end during the seeding. Moreover, the ability to catalyze aggregation through fibril surface is also diminished, although fibril's specific morphology is preserved up to 250°C. These findings are important background data to discuss the controversial issue of high temperature resistance of prions infectivity.

Relation between point mutations in amino acid sequence (beyond critical amyloidogenic region) and fibril conformation were studied using several insulin analogs. It was found that modifications in non-amyloidogenic B-chain C-terminus increases susceptibility to aggregation and affect significantly amyloid fibrils' conformation. Although fibrils from different insulins retain the common structural motif – parallel β-sheet, local β-sheet twist and inter-strand hydrogen bonds strength are diverse and specific for given insulin type. It seems that positively charged amino acid residues at C-terminus impact dynamics of this part of B-chain. This may determine which amyloidogenic pathway (leading to different polymorphic form) is taken. This observation may be important in designing of new insulin analogs.

Obtaining fibrils with distinct conformation was a prerequisite to track conformational memory effect during cross-seeding. This, in turn, was important in the context of the studies of mechanism of stable propagation of mother amyloid phenotype in daughter generation of fibrils. The experiments carried out on selected pair of insulin's fibrils ([BI] and [KR]) have shown that both types of fibrils can effectively catalyze each other's growth while transferring along fine secondary structural features with a high degree of fidelity. However mother fibrils are not able to transfer its specific morphology. The mode of protofilaments association in mature fibril depends on covalent structure of incorporated molecules. Protofilaments built of the more hydrophobic BI molecules prefer lateral association while those consisting of KR molecules with positively charged amino acids located at the B-chain C-terminus form twisted conformation. These results inspired further investigations to see if conformational memory effect can determine fibrils disaggregation pathways and stability. By utilizing the ability of DMSO to disintegrate protein aggregates, it was found that there are two different ways that insulin fibrils respond to denaturant-induced disintegration and these ways are clearly controlled by the memory effect.

It was also of interest to check if the memory effect can be observed under conditions promoting fast self-assembly of bovine insulin fibrils into chiral superstructures. It was found that the conformational memory effect may be observed but concentration of heterologous seeds needs

to be increased. It was also shown that the chirooptical properties of daughter amyloid may be controlled by the memory effect. This result may have important implications in nanotechnology.

It was also important to examine daughter fibrils phenotype after simultaneously seeding with two amyloid templates ([BI] and [KR]). It was found that daughter fibrils phenotype is determined by homologous seeds, even if its percentage in the mixture is very low (for example 5 wt. %). However, heterologous seeds remain active during aggregation process by accelerating daughter fibril's growth. This observation clearly showed that fibrillation-promoting and structure-imprinting properties of heterologous seeds can be uncoupled. Moreover, obtained results showed that both amyloid strains of [BI] and [KR] are kinetically stable and do not convert into the more thermodynamically preferred variant over prolonged period of time. This interesting results provide insights into the process of elongation of amyloid fibrils and highlighted the important role of thermodynamic control in selection of fibril phenotype. This finding may be important in the design of new drugs in amyloidosis, based on molecules that would be able to dock and block a fibril's sticky end.

Studies of conformational memory effect persistence upon multiple self-seeding passages were also performed. It was found that in the case of [KR], structural information is stably transmitted up to twelve generation of fibrils. In the case of [BI], slow conformational drift form spontaneously formed conformation of mother seed to chiral superstructures was observed. This evolution included changes at all levels of structural organization – from secondary structure – to morphology and chirooptical properties of fibrils. This interesting results highlighted that there are two different amyloidogenic pathways that leads to the same amyloid strain. First, very fast pathway is controlled by hydrodynamic forces. Second, slow pathway is controlled by self-seeding. It's seems that observed structural evolution was caused by conformational switching phenomenon.

Results obtained in this work were published in the following journals: Journal of Physical Chemistry B (2014), PLoS One (2014), Langmuir, ACS (2013), Biochemistry, ACS (2012).