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Oligomerization and structural characterization of native human cystatin C and its single-point mutants

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The foundation of properly operating human body are the processes of protein production and degradation and also the balance between them. One of the enzymes responsible for regulating these processes in human is a small monomeric protein Cystatin C (HCC). It belongs to the type 2 cystatin family, which function is the inhibition of papain- and legumain-like proteases [1]. This protein consists of 120 amino acids forming 5 anti-parallel beta sheets surrounding a long helical fragment [2]. This protein can form aggregates throughout the domain swapping mechanism [3].

The study of the oligomerization is extremely important because during this process the protein undergoes from soluble non-toxic monomeric form to insoluble, amyloid deposits, which are associated with neurodegenerative diseases [4]. Therefore, the intermediate forms are of great interest in order to find a way to counteract the development of these diseases. However, they are highly unstable and difficult to study. In case of HCC, it was shown that insertion of a disulfide bridge prevents transition into amyloid fibrils [5] and also the disulfide-stabilized cystatin C was used to produce stable intermediary oligomers of cystatin C [6].

Hereby we present a study conducted to obtain high weight oligomers from not stabilized HCC. In order to do that, we tested various conditions, using the protocol applied for stab-HCC as a starting point. Also, we tested proteins with single-point mutations, particularly concerning the residues 68 and 57. The first, because it is the location of naturally occurring mutation in cystatin C gene, changing L68 into Q68, leading to hereditary cystatin C amyloid angiopathy (Icelandic type, HCCAA-I) and the second, as the source of instability leading the dimer formations [7]. The formation of aggregates was detected by native gel electrophoresis and fluorescent studies using Thioflavin T assay. Both, oligomers and fibers, were then visualized by atomic force microscopy and transmission electron microscopy. Additionally, we assessed the secondary structure content in the wide range of temperatures for all studied proteins using infrared spectroscopy and circular dichroism spectroscopy.

We were able to produce aggregates form all studied proteins and examine the dependence of oligomerization process on factors like protein concentration, type of movement or type of confinement. The obtained results showed that mutants at the same conditions differ significantly in the time needed to form fibrils. They also vary in the content of secondary structure forms. For example, protein with a point mutation in the residue 68 (L68V) has the highest content of beta sheets and also undergoes the processes of oligomerization almost instantly whereas wild type cystatin C requires more than 24 h to form aggregates.

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