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"Structure and dynamics of amyloidogenic peptides and proteins on the basis of serum amyloid A and human cystatin C"

The aim of my studies was to complement the current state of knowledge about the structure and interactions between amyloid proteins. In my research I focused on two proteins, which are responsible for the occurrence of the XXI century diseases: serum amyloid A (SAA) and the human cystatin C (hCC).

In the first stage of my studies I performed the theoretical modeling of spatial structure of the SAA. I used *de novo* method, based only on the amino acid sequence of the protein, in UNRES force field. I received the protein structure, which consists of seven antiparallel helices connected with the disordered N-terminal fragment. Next I compared my results with the literature data, including the homology modeling structure and two crystal SAA oligomers. I found, that my theoretical SAA structure has many similarities compared to the literature data, which mainly reflects by the antiparallel arrangement of helices and a disordered C-terminal end of the protein. The observed differences are due to the fact that the obtained structure is a dynamic structure in solution, with a high freedom of movement, while the crystal structures are static. The resulting theoretical model of a monomer of the serum amyloid A may correspond to the real structure in solution.

The next stage of my studies was to determine the structure of two proteins in solution: the hCC protein and its V57G mutant based on the analysis of multidimensional NMR spectra. I used two- (2D NMR 1 H-15N HSQC) and three-dimensional (3D HNCO, 3D HN(CO)CA, 3D HNCA, 3D CACB(CO)NH and 15N- 13C-edited NOESY-HSQC) nuclear magnetic resonance spectra for the double-labeled (hCC V57G $^{13}C,^{15}N$) and triple-labeled (hCC $^{2}D,^{13}C,^{15}N$) proteins. For the hCC protein spectra I obtained chemical shifts for the main chain atoms. On the basis of chemicals shifts I generated the three-dimensional structure of the hCC monomer. The resulting structure has all the secondary structure elements present in the known x-ray structures. The lengths of the individual elements of the secondary structures are consistent with the lengths in the known x-ray structures of the hCC protein in monomeric form. Minor differences can be observed in disordered areas – mainly in L1 and AS loops. It should be noted that the obtained structure has been generated on the basis of the chemical shifts of the main chain atoms and on the basis of homology to known protein structures, hence there is a very high similarity between the NMR structure and the x-ray structures.

For the double-labeled V57G protein, the structure was obtained on the basis of NOE signals from NOESY spectra. NOE signals for individual pairs of atoms have been converted into interproton distances. Those distances was used to generate a 3D structure of the hCC V57G variant. Next, I performed minimization of NMR structure in the AMBER program. The topology of the calculated structure is consistent with the topology of proteins belonging to the cystatin's family. Compared to them, the individual elements of the secondary structure are slightly shorter and not as regular as in the x-ray structures. The content of unstructured fragments of the structure is also greater. This is due to the fact that the obtained structure is a dynamic structure determined by using NMR technique in a solution, where the protein has freedom of movement. There is also a noticeable difference in the layout of the beta-sheet plane - they are more flat than in the known x-ray structures. Also, in the NMR structure, the position of the long N-terminal fragment and the structure of the entire AS loop were determined. These secondary structure elements are missing in the known x-ray structures of the cystatins.

These results I supplemented with studies on the structure of the SAA protein fragment which is responsible for the complex formation with hCC. I found the spatial structure of the SAA(86-104) peptide using NMR data. The results of those studies I

compared with the known structures of the SAA protein. Next I conducted research of the structures for the SAA(86-104) analogues having a Pro-Ala point mutation. I also checked whether the exclusion of proline in the amino acid sequence of the C-terminal fragment of the SAA can significantly affect the structure of those peptide, and how these changes affect to the possibility of a complex formation with the hCC protein. It turned out that it is impossible to find a correlation between structural data and the results of the affinity of the analogues to the human cystatin C.

The last stage of my studies were the molecular docking simulations studies performed for the hCC/SAA protein-peptide and protein-protein complexes using previously obtained structure of the SAA protein and the SAA(86-104) peptide. For obtained structures I have done the analysis of non-covalent interactions between the hCC and the SAA amino acid residues responsible for the formation of the complex. In the case of the hCC/SAA(86-104) I confirmed the previously obtained results: the hCC/SAA(86-104) complex structure is stabilized by the interaction between amino acid residues: Lys90 and Arg96 of the SAA and Ser98 and Tyr102 of the hCC respectively. However, molecular docking studies of the protein-protein complexes gave the result that the dominant contact, stabilizing the hCC/SAA complex, is contact between Tyr102 hCC - Lys90 SAA, supplemented by hCC Ser98 and Arg96 SAA interaction.

Undertaken studies have expanded the existing state of knowledge about the structure and interactions between amyloid proteins. The results of my work will allow to conduct further studies to design peptidomimetic which could inhibit the process of oligomerization and fibrillization of the amyloidogenic proteins: serum amyloid A and human cystatin C.