Summary

Human serum amyloid A protein (hSAA) is a small, 104-amino acid residues protein, which under physiological conditions exists mainly in oligomeric form. hSAA plays an important role in inflammatory response, and also in the transport and metabolism of cholesterol. However, it is still unknown whether this is the primary role of this protein. The concentration of SAA in the plasma/serum of a healthy person is approximately 3 mg / l, but it changes drastically in response to injury, inflammation and infection, increasing up to 1,000 times. The accumulation of SAA particles favors their assembly and formation of amyloid aggregates, which deposits extracellularly in many organs, causing their dysfunction and eventually even death.

Since the native form of amyloidogenic hSAA1.1 protein is quite stable and it is difficult to conduct aggregation research on it, a less stable MetSAA1.1 was chosen, whose oligomerization /aggregation process was much faster. This isoform has an additional methionine residue at the N-terminus. The studies presented in the dissertation verify and supplement the information available in the literature on the possible mechanism of aggregation of this isoform. Native electrophoresis showed that several oligomeric forms were involved in the protein aggregation process. In addition, the fluorescence of internal and external fluorescent dyes allowed to detect significant structural transformations in the protein during the aggregation process, that, however, did not lead to fibril-like species.

The main goal of the dissertation was to obtain an effective inhibitor of SAA protein fibrillization/ aggregation. 24 small-molecule compounds were designed as potential inhibitors and tested in the fibrillization process of the highly amyloidogenic fragment 1-27 of hSAA (SAA1-27). The inhibitors were designed based on the *N*-terminal sequence of the SAA protein (RSFFS) and the sequence of the β-amyloid peptide (KLVFF). It was assumed that these peptides would interact with the amyloidogenic core located at the N-terminus of SAA and thus block this fragment, preventing the protein aggregation. The research proved that most of the designed small-molecule compounds were able to inhibit the SAA1-27 fibrillization process. From the pool of inhibitors for further research only one compound, saa3Dip, was chosen, in which a non-protein amino acid, β, β-diphenylalanine, substituted the Phe3 residue.

The results of experiments with the MetSAA1.1 isoform show that saa3Dip inhibits also aggregation of the whole protein. As indicated results of the tests conducted using various physicochemical techniques, the inhibitor was able to stabilize some elements of the native secondary structure and influenced the native tertiary structure of the protein. Moreover, saa3Dip also significantly influenced the size of the formed oligomers / aggregates and their morphology. In addition, the results of the soluble fraction quantitative studies showed that the protein is partially hydrolyzed under the incubation conditions. Protein hydrolysis appears to occur more rapidly in the presence of the aggregation inhibitor, saa3Dip. Therefore, it is very likely that the interaction of MetSAA1.1 with saa3Dip promotes the exposure of protein sequence fragments sensitive to the hydrolysis reaction.

An effort to obtain the MetSAA1.1 protein using native chemical ligation was also performed. This method is based on the chemoselective linkage of two peptides with an amide bond. The reaction requires one peptide possessing an active thioester at the *C*-terminus and the other with a free cysteine group at the N-terminus. Since there is no cysteine in the SAA amino acid sequence, an additional, cysteine desulfurization step was needed to re-create alanine present in the native sequence. Two polypeptide fragments: -1-44 and 45-104 of the MetSAA1.1 was linked through native chemical ligation. Several analytical methods, such as CD, FTIR-ATR and native electrophoresis proved that the protein obtained by this method was capable of creating a native structure and native oligomeric forms. The developed method will not only facilitate the production of the SAA protein, but more importantly, it will allow easy introduction of modifications in the native sequence, which is important in the context of studying the mechanism of SAA oligomerization/ aggregation.