ABSTRACT

Amyloidoses are a group of diseases directly related to abnormalities in the metabolism of misfolded proteins, often resulting from defects in degradative machinery. Proteolytic systems controlling protein homeostasis include the 20S proteasome. In the physiological state, it is an important component of the proteasome pool, and under conditions of oxidative stress, when it is additionally released by dissociation of the 26S complex, it is the main proteolytic form that has to deal with the increasing amount of damaged proteins showing aggregation tendencies. Unfortunately, under these conditions, the activity of the 20S proteasome is usually inhibited. For many years it was thought that fibrillar forms of proteins were responsible for the observed inhibition. The current approach assumes that soluble oligomers, which precede fibrillar deposits in the aggregation pathway, are the most toxic to cells. Their causal role in proteasome inhibition has been discovered in the case of Alzheimer's disease-associated Aβ peptide, Parkinson's disease-associated α-synuclein, and mutant huntingtin, whose deposits have been found in Huntington's disease patients. Blocked by soluble oligomers, the proteasomal system loses its biological activity, resulting in the accumulation of cytotoxic oligomers and their subsequent transformation into protofibrils, fibrils and then amyloid fibrils. According to research by Smith's group [1], Aβ 1-42 oligomers inhibit the activity of the 20S proteasome in an allosteric manner. These researchers also showed that short peptides encompassing the *C*-terminal motif of HbYX, characteristic of natural protein activators, can stimulate the Aβ oligomer-inhibited 20S proteasome.

Because amyloid deposits have been observed in most diabetic patients, type 2 diabetes has begun to be considered in the context of amyloidosis. The hallmark of amyloidosis is the accumulation of cytotoxic aggregates, which in the case of type 2 diabetes are deposited mainly in the pancreas, and consist of amylin molecules, also known as IAPP. IAPP is a 37-residue peptide with stronger aggregation propensity than Aβ. In addition to sharing amyloidogenic properties, the two peptides share biochemical characteristics, suggesting also a common pathogenic mechanism. For the Aβ peptide, it is already known that the 20S proteasome is involved in this mechanism.

Therefore, the main goal of this dissertation was to characterize the oligomerization process of human amylin and investigate whether the accumulation of its oligomeric forms can be prevented by activating a proteolytic system based on the 20S proteasome.

To achieve my goals, I needed significant amounts of human amylin, so in the first step I synthesized this protein and then optimized the approach to study its oligomerization. To characterize the oligomeric forms, I applied both native and denaturing conditions, stabilizing the resulting oligomers using chemical crosslinking and photocrosslinking. In the next step, I examined the strength of interaction of fractions containing soluble amylin oligomers with the 20S proteasome isolated from human blood. The determined EC50 values, indicating a strong interaction between these molecules, were the starting point for trying to determine the site of their interaction. To this end, I also synthesized amylin variants with a photocrosslinking residue or fluorophores. To determine the binding site of the oligomers to the proteasome, I used two independent techniques: mass spectrometry and electron cryomicroscopy. Referring to the results presented by Smith's group, I also set out to verify the effect of amylin oligomers on the proteolytic activity of the 20S proteasome. To this end, I conducted a series of experiments using commercially available fluorogenic substrates, confirming the inhibitory effect of the oligomers on h20S, as well as determining the kinetics of inhibition. I also performed tests with the A11 antibody. The results obtained by Smith's group [1] indicate that fractions of oligomers interacting with the A11 antibody are responsible for the inhibitory effect on 20S proteasome activity. Similar experiments conducted for amylin proved that, unlike the Aβ oligomers, interaction of the amylin oligomers with the antibody does not block their ability to inhibit h20S. Using peptidomimetics from our pool of top activators, I proved that it is possible to overcome h20S inhibition by oligomers. I carried out the above study not only on isolated 20S proteasome, but also on lysate of human HEK293-T cells. In addition, I verified the cytotoxicity of fractions containing low molecular weight amylin oligomers against the above cell line. In the next step, I demonstrated that low-molecular-weight amylin oligomers can be a substrate for the 20S proteasome, and that the activating compounds we possess are able to stimulate their proteolysis. The results of the presented studies shed new light on the mechanisms that may contribute to the development of type 2 diabetes. Considering this disease entity in the context of amyloidosis makes it appropriate to include methods of preventing protein aggregation in its therapy. The efficacy of peptidomimetic activators based on a fragment of the Blm10 protein, which I have demonstrated, may provide a good basis for the development of new therapies for the treatment

of amyloidosis associated with type 2 diabetes, and thus reduce the negative consequences of this disease.

[1] T. A. Thibaudeau et al., ‘A common mechanism of proteasome impairment by neurodegenerative disease-associated oligomers’, *Nature Communications*, 9 (2018) 1–14