Mutant ubiquitin and the proteasome in Alzheimer's disease

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Scope and outline of this thesis

Ubiquitin B\(^1\) (UBB\(^{+1}\)) was discovered to accumulate in the pathological hallmarks of Alzheimer's disease (AD) along with other frameshifted proteins, i.e. APP\(^{+1}\) (amyloid precursor protein +1) and GFAP\(^{+1}\) (glial fibrillary acidic protein +1). The mutant form of ubiquitin seemed particularly intriguing, since its aberrant C-terminal structure clearly predicted a dysfunction, compared to normal ubiquitin. Moreover, the ubiquitin proteasome system is impaired in AD brains, as well as in most other neurodegenerative diseases. In parallel to AD-related studies, UBB\(^{+1}\) was found to accumulate in many other diseases as well.

The main focus of the studies described in this thesis was the question whether UBB\(^{+1}\) plays a role in proteasome inhibition and neurodegeneration in AD. The involvement of the ubiquitin proteasome system in AD is reviewed in Chapter 1.

The specific objectives of this thesis were:

1. To investigate the influence of UBB\(^{+1}\) on cellular functioning.
2. To determine if, and by what mechanisms, UBB\(^{+1}\) inhibits proteasomal degradation.
3. To characterize other AD-related mechanisms that may have synergistic effects on proteasome inhibition by UBB\(^{+1}\).

The first objective was studied in Chapter 2 in biochemical studies demonstrating that UBB\(^{+1}\) lacks the capacity to ubiquitinate other proteins, but can be ubiquitinated itself. The ubiquitinated form of UBB\(^{+1}\) was found to be relatively stable compared to other ubiquitinated proteins. Overexpressing UBB\(^{+1}\) in human neuroblastoma cell lines revealed apoptotic-like cell death specifically caused by UBB\(^{+1}\).

In Chapter 3 we provide more insight into the mechanism by which UBB\(^{+1}\) induces this apoptotic-like cell death, addressing objective number two. With the use of a green fluorescent protein (GFP)-based reporter system for proteasome inhibition we demonstrated that ubiquitinated UBB\(^{+1}\) is a potent and specific inhibitor of proteasomal activity. This study also revealed that UBB\(^{+1}\) has
seemingly paradoxical properties, as in low concentrations, UBB\(^{+1}\) appeared to be an efficient substrate for the proteasome.

In **Chapter 4** this paradox was partly explained by demonstrating a threshold effect of UBB\(^{+1}\) accumulation in an organotypic mouse cortex slice culture model. Quantification in human neuroblastoma cells confirmed that UBB\(^{+1}\) only inhibits the proteasome after it exceeds a critical level, causing an ongoing irreversible UBB\(^{+1}\) accumulation, thereby creating a negative feedback loop in proteasome inhibition. This conclusion led to our understanding that in AD affected cells apparently have reached the threshold of UBB\(^{+1}\) accumulation, as the protein is clearly present in the pathological hallmarks. However, since UBB\(^{+1}\) is a substrate at low concentrations, it is not likely that UBB\(^{+1}\) forms the initial trigger for proteasome inhibition in AD pathogenesis.

In **Chapter 5** Aβ peptide intermediates were studied as possible initiators of proteasome inhibition, addressing objective number three. Cytosolic Aβ was found not to inhibit the proteasome. However, indirect proteasome inhibition by Aβ through ER-associated degradation (ERAD) mechanisms redirecting ER-located Aβ to the cytoplasm in a modified form, or extracellular Aβ still remain candidates for this putative function.

**Chapter 6** discusses the results presented in this thesis and addresses future research questions and directions. In addition, preliminary results are presented on human post mortem cortex slice cultures and on AD-related mechanisms other than Aβ-processing that could influence proteasome activity.