**ABSTRACT: Structural Characterization of the N-terminus of UBXD1 and its Regulation of the ATPase activity of p97**

p97 is an ubiquitary AAA ATPase with many diverse cellular functions which are mediated by a big network of different cofactors. One of these cofactors is UBXD1 which has a special role among p97 cofactors. On the one hand the interaction between UBXD1 and p97 is interrupted by IBMPFD-associated mutations of p97 while the binding of other cofactors is still intact. On the other hand UBXD1 has two p97 binding motifs (PUB domain, VIM motif) which can theoretically bind simultaneously the N domain and the C-terminus of p97. UBXD1 is involved in protein degradation in lysosomes via p97. The disruption of the interaction between p97 and UBXD1 leads to the accumulation of misfolded proteins which are responsible for the pathogenesis of neurodegenerative diseases like IBMPFD and ALS. Although UBXD1 is quite important in the development of p97-associated diseases the interaction between UBXD1 and p97 is poorly understood. As the interaction between the PUB domain of related cofactors and the C-terminus of p97 has already been studied the experiments of this work are concentrated to the N-terminus of UBXD1 including the VIM motif.

In a first step the N-terminus of UBXD1 was structurally characterized. Subsequently a model was developed on the basis of NMR- and CD-spectroscopic data as well as on data from mutational studies. This model was refined using secondary structure prediction algorithms (sequence-based, NMR resonances- and CD-based). The structural data imply a protein with α-helical regions but without any rigid tertiary structure. These features are typical for IDPs (intrinsically disordered proteins) or IDRs (intrinsically disordered regions).

Furthermore the interaction between the N-terminus of UBXD1 and the individual domains of p97 was investigated by NMR spectroscopy on an atomic level. These studies showed two interacting regions of the N-terminus of UBXD1 and the N domain. On the one hand the interaction between the VIM motif of UBXD1 and the hydrophobic cleft of the N domain was confirmed. On the other hand a new binding region was defined including the first 30 amino acids of the N-terminus of UBXD1 and the linker between the N domain and the D1 domain of p97. In addition the N-terminus of UBXD1 interacts with the D1D2 domain of p97. All these interactions were confirmed by fluorescence anisotropy studies and size exclusion chromatography.

p97 is a hexameric ATPase with flexible domains. Therefore the influence of the binding of the N-terminus of UBXD1 on the conformation of p97 was investigated biochemically (anisotropy, limited proteolysis, size exclusion chromatography and melting curves). It could be shown, that UBXD1 destabilizes the hexameric p97 and in addition fixes the p97 monomers in a more compact structure (down position of the N domain of p97).

The binding of the individual domains of p97 as well as the influence of the N-terminus of UBXD1 on the structure of the hexameric p97 suggest a inhibitory effect of the N-terminus of UBXD1 on the ATPase activity of p97 which was also confirmed experimentally. In addition the N-terminus of UBXD1 binds ATP while the physiological function of this binding has not yet been elucidated.