ABIN-1 senses linear ubiquitin chains: structural and biophysical insights

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The Nuclear Factor-κB (NF-κB) transcription factors are key regulators of numerous basic cellular processes, among them innate immunity, inflammation and malignant transformations. However, unrestrained NF-κB activation is associated with several inflammatory diseases. Therefore, formation and activation of these transcription factors must be tightly regulated. ABIN-1 (A20 binding and inhibitor of NF-κB) has been characterized as one of the negative regulators of NF-κB signaling. Although, this protein is known to function by facilitating A20 (ubiquitin editing protein)-mediated de-ubiquitination of NF-κB pathway regulators, more recent studies attribute ABIN-1 negative regulatory activity to its ubiquitin binding feature. ABIN-1 binds ubiquitin through the AHD-2 (ABIN homology domain-2) also called UBAN (ubiquitin binding in ABIN proteins and NEMO) motif. Previously, we showed that NEMO (NF-κB essential modulator) preferentially binds linear ubiquitin chains via its UBAN motif and this specific binding is essential for regulation of the NF-κB signaling pathway. Here, we provide structural and biophysical evidences that ABIN-1 UBAN motif, also, has higher affinity for linear over other types of ubiquitin chains. The x-ray crystal structures of ABIN-1 in the apo form and in complex with one and two linear diubiquitins are solved in this study. ABIN-1 in the apo form adopts a coiled-coil, homo-dimer structure which provides two symmetrical binding sites for linear diubiquitins. Interestingly, depending on the relative concentration of the two proteins different binding stoichimetries are observed in the crystals. The concentration-dependency of the complex formation by ABIN-1 and linear diubiquitin chains is further examined by ITC (Isothermal Titration Calorimetry) experiments. ABIN-1 UBAN domain recognizes the canonical Ile44 surface and the C-terminal tail of the distal and the newly characterized surface, adjacent to the hydrophobic patch, on proximal ubiquitins. Mutations on the ubiquitin binding surface in ABIN-1 UBAN abolished its inhibitory effects on NF-κB activation. These data explain the specificity of ABIN-1 protein for linear ubiquitin chains and in regulation of NF-κB activation.