Synthesis and structural characterization of polyubiquitin conjugates using high resolution mass spectrometry

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Modification of proteins by covalent attachment of ubiquitin plays a fundamental role in the control of many biological processes including cell cycle regulation, transcription, DNA repair, and apoptosis (1, 2). Ubiquitin contains 7 lysine residues each of which in principle could be used for polyubiquitin conjugations and chain formation. Importantly, recent evidence indicates that the actual lysine residue of ubiquitin used for ubiquitin-ubiquitin conjugation determines the biochemical/biological function of the respective polyubiquitin chain. Therefore, the synthesis and structural characterization of a series of chemically defined polyubiquitin chains that differ by the lysine residue used for ubiquitin-ubiquitin attachement is a major goal of the present work. In order to synthesize such conjugates specific chemical strategies for the build-up of polyubiquitin chains are pursued that use thioether ligation of C-terminal ubiquitin-thiol residues with N-(iodacetyl)-side chain protected Lysine residues. It contains specific and original lysine residues at each distinct branching site (3). High resolution FT-ICR mass spectrometry (FT-ICR-MS) was predominantly used for the characterization of the chemically synthesized polyubiquitin chains, and affinity proteomics methods are especially suitable for identifying the conjugation structures of polyubiquitin.

[1] Nobel Prize in Chemistry, 2004 awarded to Aaron Ciechanover, Avram Hershko, and Irwin Rose for the discovery of ubiquitin-mediated protein degradation; Press release, Swedish Academy of Sciences.

[2] L.K. Linares , A.Hengstermann , A.Ciechanover , S.Müller and M.Scheffner (2003) *Proc, Natl. Acad. Sci USA.* **100**: 12009 - 12014.

[3] G. Mezö, M. Manea, A. Jakab, B. Kapuvári, S. Bösze, G. Schlosser, M. Przybylski, F. Hudecz, Synthesis and structural characterization of bioactive peptide conjugates using thioether linkage approaches. (2004) *J. Peptide Sci,* **10**, 701-713.