

Abstract

α -tubulin is an essential protein for every eukaryotic cell. Together with β -tubulin, it polymerises into microtubules and participates thus in creating and maintaining cellular structures and presents a cell-wide interaction platform for a plethora of microtubule associating proteins. Primary sequences of the disordered C-termini of both α - and β -tubulin are the least conserved among tubulin isotypes and their variability is further increased by the presence of various post-translational modifications. The genetically coded, tyrosinated C-terminus of α -tubulin can be either shortened by one, two or three amino acids resulting in detyrosinated, $\Delta 2$, or $\Delta 3$ variants, respectively or it can be extended by the addition of polyglutamate or polyglycine chains.

The tubulin tyrosine ligase-like (TTL) protein family consists of 14 enzymes that participate in tubulin glutamylation, glycylation, and tyrosination. The glutamylases have two distinct activities, initiation and elongation of the polyglutamate chain. Initiases link the first glutamate residue to the γ -carboxyl group of one of the glutamates of tubulin C-termini to create a fork in the amino acid sequence. Elongases then recognise the branching glutamate and build up the polyglutamate sidechain one residue at the time. TTL11 is an elongase of α -tubulin polyglutamate sidechains. TTL11 is expressed in every tissue of the human body, and mostly found in cell cilia and flagella. Impaired function of TTL11 is implicated in various ciliopathies including retinal and vertebral defects.

The aim of this thesis was to prepare pure tyrosinated, detyrosinated, and $\Delta 3$ forms of α -tubulin and to compare these three post-translationally modified variants as substrates for TTL11-mediated polyglutamylation *in vitro*.

Key words: α -tubulin, TTL11, polyglutamylation, tubulin tyrosination, tubulin detyrosination, $\Delta 3$ tubulin