Abstract

Intermediate filament proteins create a dynamic cytoskeletal filamentous network, which due to its elastic properties, significantly contributes to the resistance of cells and tissues to mechanical stress. An important protein from the family of intermediate filaments, vimentin, is expressed mainly in cells of mesenchymal origin. Vimentin has been associated with a large number of pathophysiological conditions, and current studies consider vimentin as clinically promising target for the diagnosis, prognosis and treatment of a wide range of diseases from cancer to infectious and inflammatory diseases. Although in terms of structural characterization, vimentin belongs to one of the most studied proteins from the family of intermediate filaments, our knowledge is currently limited to the structure of the vimentin tetramer. Vimentin is capable of self-assembly into filaments formed by homo-oligomeric ULF subunits and the assembly process involves several steps of the organization of subunits. Structural characterization of the oligomeric subunits involved in the assembly of vimentin filaments is a prerequisite for elucidating the architecture of mature filaments, which can significantly contribute to understanding and connecting the mechanisms of many diseases associated with changes in vimentin expression.

This thesis is focused on the structural analysis of vimentin ULF subunits arising from the association of vimentin tetramers. Using the MIX CXMS technique based on a chemical cross-linking of isotopically labeled and unlabeled protein mixture, which enables to identify and distinguish intermolecular and intramolecular interactions, unique peptide cross-links were identified providing information on the origin of the cross-linked peptides within the ULF. Subsequent quantification of the representation of intertetrameric and intratetrameric cross-links revealed 27 unique peptide cross-links originating from the tetramer-tetramer interface, providing information on the distance constraints of tetramers in the ULF for the structural characterization of vimentin ULF subunit.

Key words: intermediate filaments, chemical cross-linking, isotopically labeled proteins, mass spectrometry

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