Abstract

Intermediate filaments are cytoskeleton components formed by a large family of fibrous proteins specifically expressed in nearly all differentiated cells. Under physiological conditions, they spontaneously assemble into fibers in a process that involves several stages in the organization of subunits. These fibers provide elastic properties to the cells, allowing them to maintain their structural and mechanical integrity. While the structure of other cytoskeletal components is now well researched, detailed information on the structure of intermediate filaments at various stages of assembly is still not available. Thus, new insights into the structure of these proteins could be of great benefit in understanding of various pathological mechanisms associated with changes in their expression in cells.

This thesis studies interactions of dimeric subunits in the tetrameric assembly of vimentin, class III protein of intermediate filaments. By chemical cross-linking of isotopically labeled and unlabeled tetrameric vimentin mixture, followed by proteolytic cleavage and mass spectrometry analysis, interdimeric, intradimeric and intrapeptide cross-linking products were identified. Quantification yielded information on interdimeric and intradimeric distance constraints, which allow the characterization of a more detailed tetrameric vimentin structure.

Key words: intermediate filaments, cytoskeleton, chemical cross-linking, mass spectrometry