Structure, supramolecular association and interaction of patho-active presenilin and βAPP domains by high resolution mass spectrometry

The accumulation of extracellular plaques containing a 42-amino acid neurotoxic proteolytic peptide fragment (Aβ) of the β-amyloid precursor protein (βAPP) is one of the characteristics of Alzheimer's disease. The two transmembrane protein types, βAPP and presinilin(s) (PS) have been recognised as key molecules for the patho-physiological degradation pathway, but the proteolytic pathways and interactions of βAPP, PS and other possible proteins are unclear at the molecular level. In this project the structures supramolecular associations and interactionsof βand presenilins will be investigated by means of recently developed methods of protein mass spectrometry (electrospray / ESI; matrix-assisted laser desorption / MALDI). In particular, the Fourier-transform-ICR-MS method, recently installed in our laboratory, will be used as a bajor bioanalytical tool of ultrahigh sensitivity and resolution for structural studies of βAPP and PS molecules and their interactions. βAPP polypeptides encompassing the proteolytic Aβ sequence and the transmembrane domain, and PS domains spanning transmembrane regions 6 and 7 will be prepared by chemical synthesis including sequence mutations at the critical proteolytic cleavage sites. The coupling of MS methods with the mass spectromtetric epitope mapping methods developed in our laboratory will be employed to the study of interactions of presenilins with Aβ containing sequences. In addition, a new affinity proteome MS analysis approach using the mass spectrometric epitope mapping with micro-capillary- / chip-immobilised antibodies will be applied to identify intracellular association products. Furthermore, the synthesis of relevant large Aβ–containing polypeptides should provide suitable model systems for better understanding patho-active assoiciation and interactions of PS and βAPP.