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Institutionen för Medicinsk Biokemi och Biofysik

Molecular Mechanisms of Amyloid Self-Regulation

AKADEMISK AVHANDLING
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ABSTRACT

Amyloid is associated with both pathological protein deposits and the formation of functional protein structures. Therefore, several strategies have evolved to control the formation or inhibition of amyloid *in vivo*. In this thesis, three separate systems were investigated in which amyloidogenic protein segments are coupled to regulatory elements that prevent or promote fibrillation. We describe the molecular mechanism for how (a) a propeptide segment prevents the uncontrolled aggregation of the mature peptide, (b) a chaperone domain inhibits amyloid formation, and (c) a pH-dependent relay controls protein assembly. For this purpose, mass spectrometry (MS)-based approaches to structural biology were applied and extended, involving gas phase interaction studies and hydrogen/deuterium exchange MS.

(a) Proinsulin C-peptide is beneficial for the preservation of insulin activity. We show that C-peptide interferes with insulin amyloid fibril formation at low pH and how conserved glutamate residues in C-peptide mediate reversible co-precipitation with insulin. A mechanism is proposed for how the balance between zinc and C-peptide mediates sorting of insulin into slow acting and rapid acting forms inside the secretory granules of the pancreatic β-cells, which potentially links C-peptide to diabetes type 1 and 2.
(b) Lung surfactant protein C (SP-C) is a highly amyloidogenic transmembrane polypeptide that controls surface tension in the alveolar phospholipid bilayer. Its proprotein includes a conserved chaperone domain termed BRICHOS, which is also associated with neurodegenerative disorders. It is shown here how BRICHOS and its N-terminal linker recognize hydrophobic residues and trap the SP-C segment in a β-hairpin conformation to prevent amyloid formation.
(c) Spider silk is synthesized as a highly soluble protein that assembles into silk in a pH-dependent fashion. It is shown that the spider silk protein N-terminal (NT) domain dimerizes at the same pH interval that triggers silk assembly, and we define the associated structural changes. Furthermore, the use of the NT domain as a solubility tag for the expression of aggregation-prone proteins is demonstrated.

In summary, we have determined the molecular basis for three distinct mechanisms by which fibril formation is controlled through autoregulatory elements and provide insights into nature’s strategies to control amyloid formation and prevention. Based on these findings, we can now make conclusions about nature’s handling of amyloidogenic proteins and their function in general.

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