Silvia Cassinelli
Department of Biochemistry “A. Castellani”

Pathogenesis of ApoA-I amyloidosis: new hints from cell biology and protein biochemistry

Prof. Giampaolo Merlini

Amyloidosis are conformational diseases related to protein misfolding. Fibrils are localized in the extracellular space and they produce an organ failure. A variety of amyloid diseases are associated with mutations in apolipoprotein A-I (ApoAI), for which the process of fibril formation has not yet been clarified. Amyloid fibrils of ApoAI were found to be mainly constituted by N-terminal fragments of the protein. The fragment corresponding to residues 1-93 was found to be the main building block of cardiac fibrils extracted from patients harbouring the mutation L174S in ApoA-I gene and affected by a severe hereditary systemic amyloidosis predominantly involving the heart. Mutations, about 13, are sometimes present within the N-terminal portion of the protein that is eventually found in fibrils (‘‘internal mutations’’), but can also occur in positions located outside this region of the polypeptide sequence (‘‘external mutations’’). Characterization of the polypeptides purified from natural fibrils from patients carrying the ‘‘external mutation’’ Leu174Ser has shown that the 1–93 N-terminal portion of ApoA-I is intrinsically amyloidogenic in a physiological environment. The natural 1–93 polypeptide in acidic conditions (pH 4) induce fibril formation, the polypeptide assumes a random coil structure at neutral pH, shifts into an unstable helical conformation at acidic pH, and then aggregates into a b-sheet-based polymeric structure. The target of my project is to study conformational characterization and propensity to aggregation of Apolipoprotein AI 1-93 fragment and all its mutants. First of all we produced the recombinant proteins using a system able to express the protein like a fusion protein. Our results were very good: we observed that, similarly to the polypeptide isolated ex vivo, a pH switch from 7 to 4 induces a fast and reversible conformational transition to a helical state and leads to the identification of a key intermediate in the fibrillogenesis process.
Limited proteolysis experiments suggested that the C-terminal region is involved in helix formation. The recombinant polypeptide generates fibrils at pH 4 on a time scale comparable with that of the native fragment. These findings open the way to studies on structural, thermodynamic, and kinetic aspects of ApoA-I fibrillogenesis. This happens also for all recombinant mutants that we produced.
For the future, we are trying to obtain a recombinant full-length Apolipoprotein AI.
We decided to try with different vectors and so different restriction enzymes to obtain the sticky ends to clone the vectors. At the moment I tested two different vectors (pTXB1 and pTYB11) and after digestion with restriction enzymes, transformation of different cells (NEB Turbo and BL21) we obtain great results. Now we are attending to perform the expression and than the production of the protein with a particular method; we want to use a column separation using the affinity that the protein has with chitin beads. To obtain greater results and understand the mechanism that interfere in this protein production I will go to work into the lab of Dr. Marcus Frendrich, at Max Planck Institute (Halle, Germany) that is producing yet this protein.

References

1. The N-terminal ApoA-I amyloidogenic domain is cytotoxic in the soluble unfolded state but inactive in the fibrillar form (**work in progress)**