

We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900

Open access books available

185,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



Apolipoprotein A-I Associated Amyloidoses: The Intriguing Case of a Natively Unfolded Protein Fragment

Daria Maria Monti^{1,2}, Renata Piccoli^{1,2} and Angela Arciello^{1,2}

¹*Department of Structural and Functional Biology, University of Naples Federico II,
School of Biotechnological Sciences, Naples*

²*Istituto Nazionale di Biostrutture e Biosistemi (INBB),
Italy*

1. Introduction

Nowadays, a main challenge for scientists is that of drawing a comprehensive picture, in which common traits shared by the amyloidogenic proteins identified so far, structurally and functionally different from one another, are depicted. From a structural point of view, a clear relationship between protein sequences and aggregation does not exist, although proteins able to aggregate in general are characterized by a low sequence complexity (Wootton & Federhen, 1996) and/or high net charge coupled with low mean hydrophobicity (Gast et al., 1995), as well as by the abundance of residues favouring the β -sheet secondary structure (Steward et al., 2002). From a functional point of view, amyloidogenic proteins are associated to a variety of cellular functions and activities, and in some cases their biological function is unknown. As for the localization of the disease, amyloidoses may be localized in the nervous system, as those implicated in neurodegenerative diseases, or may be systemic when target tissues for amyloid deposition are in peripheral organs.

Two main classes of fibrillogenic proteins have been identified: those with a compact folding in their native state and those that are partially unfolded. Studies on pre-amyloid order-disorder transitions are central to understand both the assembly mechanisms and the disease molecular bases. In the case of amyloidogenic proteins that are natively folded, destabilizing mutations and/or changes in solution conditions are key factors responsible for the induction of fibrillogenesis, as in the case of β_2 -microglobulin ($A\beta_2M$) or the prion protein APrP (Chiti & Dobson, 2006). On the other hand, several amyloidogenic proteins or polypeptides are intrinsically disordered. Such proteins include the β -Amyloid peptide ($A\beta$), islet amyloid polypeptide (AIAPP) and α -synuclein (Abedini & Raleigh, 2009). These “natively unfolded” (Weinreb et al., 1996) proteins emerged as proteins lacking of almost any secondary structure and were shown to be extremely flexible and disordered under physiological conditions (Uversky, 2002). The main feature of these proteins is the intrinsic structural plasticity, as a disorder to order transition may occur upon functioning (Abedini & Raleigh, 2009).

In some cases, natively folded proteins generate unfolded fragments associated to the amyloid pathology. This may occur when a specific mutation diverts the fate of a globular

protein converting it to the precursor of fragments responsible for fibril formation. Is this the case of Apolipoprotein A-I (ApoA-I) and its amyloidogenic N-terminal fragment.

1.1 Apolipoprotein A-I

ApoA-I is synthesized by the liver and the intestine as a *pre-pro*-protein. After the cleavage of the *pre*- and *pro*- peptides, the mature protein (28 kDa) is secreted in the plasma, where it is either associated to lipids, or in a lipid-free state (5-10%) (Brouillette et al., 2001). ApoA-I plays a critical role in lipid metabolism (Fielding & Fielding, 1995), both in delivering cholesterol to steroidogenic tissues and in transporting it from the periphery to the liver for catabolism, in the so called reverse cholesterol transport. Therefore, ApoA-I plays an anti-atherogenic role *in vivo*, with a protecting effect against cardiovascular diseases (Pastore et al., 2004).

During HDL biogenesis, the primary acceptor of cholesterol and phospholipids from macrophages is lipid-free or lipid-poor ApoA-I, containing up to four phospholipid molecules (Duong et al., 2008). In this state, ApoA-I is the preferred substrate of the plasma membrane transporter ATP-binding cassette A1 (ABCA1) (Adorni et al., 2007; Mulya et al., 2007; Sacks et al., 2009). The conversion of unesterified cholesterol into cholesteryl ester by the enzyme lecithin:cholesterol acyltransferase (LCAT) is responsible for the conversion of the nascent, discoidal HDL into mature spherical HDL, with ApoA-I representing roughly 70% of HDL protein mass. Circulating HDL are remodelled by the action of proteins and enzymes, such as cholesteryl ester transfer protein (Rye et al., 1997), LCAT (Liang et al., 1995; Liang et al., 1996), phospholipid transfer protein (Lusa et al., 1996; Ryan et al., 1992) and hepatic lipase (Clay et al., 1992). Plasma HDL remodelling can result in the destabilization of HDL and the release of lipid-free/lipid-poor ApoA-I. Furthermore, the selective uptake of lipids from HDL through scavenger receptor B type 1 (SRB1) can yield lipid-poor ApoA-I (Acton et al., 1996). It has been demonstrated that the production of lipid-free/lipid-poor ApoA-I from mature HDL and relipidation by ABCA1 is a dynamic process in the arterial wall, which is critical in protecting macrophages from cholesteryl ester accumulation (Cavigiolio et al., 2010). Nevertheless, the molecular mechanism of the atheroprotective action of ApoA-I, as well as HDL biogenesis, is not fully understood.

HDL catabolism requires disassembly of protein and lipid components. While HDL lipid clearance is well described (Obici et al., 2006), less is known about the catabolism of the HDL protein moiety. Numerous tissue uptake studies support the view that kidneys are the principal site of ApoA-I degradation (Glass et al., 1983).

The conformational transition from the lipid-free to the lipid-bound state of ApoA-I (Cavigiolio et al., 2010) is made possible by the conformational plasticity of the protein (Obici et al., 2006). In fact, different are the structures proposed for ApoA-I as a lipid-bound or a lipid-free protein (Borhani et al., 1997; Silva et al., 2005; Ajees et al., 2006). In the absence of lipids, ApoA-I can assume a compact four-helix bundle (Silva et al., 2005), while, upon lipidation, the amphipathic α -helices substitute protein-protein contacts for protein-lipid interactions. This induces the opening of the helical bundle into an extended belt-like α -helix, which wraps around the perimeter of the nascent HDL particle (Borhani et al., 1997). Therefore, conformational plasticity of full-length ApoA-I is a functionally relevant feature, strictly related to the complex mechanism of its biological action.

1.2 The genetic basis of ApoA-I-associated amyloidosis

A variety of mutations in ApoA-I gene have been associated with familial hypercholesterolemia (Sorci-Thomas & Thomas, 2002), a prototypic “loss of function” genetic disease, whereas other mutations have been associated with familial systemic amyloidosis, a “gain of function” genetic disease (Obici et al., 2006), in that new pathological properties are associated to the protein.

Sixteen variants of ApoA-I are responsible for systemic amyloidoses, which are characterized by amyloid deposition in peripheral organs, such as heart, liver or kidneys (Obici et al., 1999; Obici et al., 2006; Eriksson et al., 2009). Although patients are invariably heterozygous for the mutated gene, only the variant isoform was detected in amyloid deposits.

In Figure 1, the map of the sixteen ApoA-I mutations associated to amyloidosis is represented.

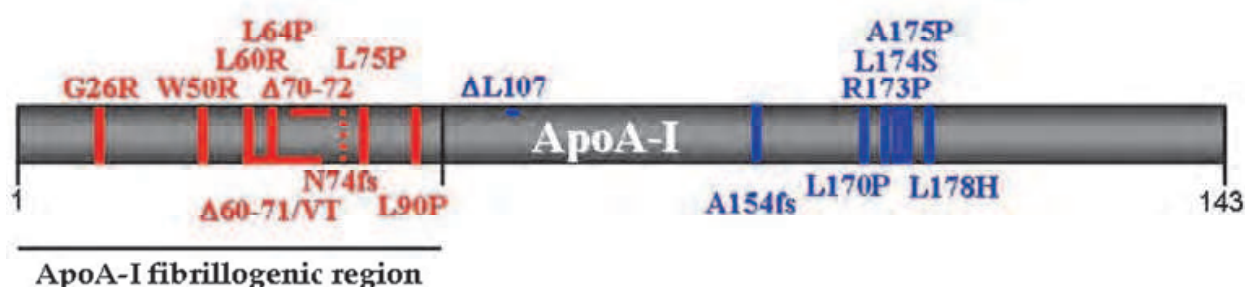


Fig. 1. ApoA-I mutations associated to systemic amyloidoses. In red, the mutations located within the N-terminal fibrillogenic region (“inside mutations”). In blue, the “outside mutations”. Δ, deletion mutation; fs, frameshift mutation.

For all these variants, amyloid fibrils isolated *ex vivo* were found to be mainly constituted by N-terminal fragments of AApoA-I, 90-100 residue long. Thus, ApoA-I variants represent the precursors of N-terminal fragments of the protein responsible for fibril formation.

The amyloidogenic mutations described so far in patients can be divided in two groups (see Figure 1): those located within the N-terminal portion of the protein that is eventually found in fibrils (“inside mutations”), and those located externally to this region (“outside mutations”) (Obici et al., 2006). Wherever an amyloidogenic mutation is located, internally or externally to the N-terminal region, a protein fragment corresponding to the N-terminal end of the protein is released, leading to the formation of fibrillar deposits. However, nothing is known about the mechanism leading *in vivo* to the release of the fibrillogenic polypeptide from a full-length amyloidogenic variant of ApoA-I, nor in which context it occurs.

The fragment corresponding to sequence 1-93 was found to be the most abundant species among all the specimens investigated so far (Obici et al., 1999). Therefore, our studies focused on this N-terminal AApoA-I peptide, denoted here as [1-93]ApoA-I, to shed light on structural and functional features relevant to the understanding of the molecular basis of the pathology. The amino acid sequence of polypeptide [1-93]ApoA-I is shown in Figure 2A.

To predict the aggregation propensity of the fibrillogenic polypeptide [1-93]ApoA-I, we performed *in silico* analyses using various algorithms. First, by the Prot Param algorithm (Guruprasad et al., 1990), the instability index of [1-93]ApoA-I was calculated and found to be 46.7, a value that classifies the polypeptide as “unstable”. The propensity of [1-93]ApoA-I to generate fibrils was analysed using the TANGO algorithm (Fernandez-Escamilla et al., 2004) (Figure 2B). The aggregation score, describing the overall protein propensity to aggregate (25°C, 0.01 M ionic strength), was found to be 372 and 428, at pH 7.0 and 4.0, respectively. This is indicative of high propensity of [1-93]ApoA-I to generate β -cross aggregated structures at both pH values, with a higher propensity at pH 4.0, as expected.

Moreover, at both pH values the region 13-25 shows the highest propensity to generate β -cross aggregates, as shown in Figure 2B. The high aggregation potential of region 13-25 was confirmed by other algorithms (Maurer-Stroh et al., 2010; Chou & Fasman, 1978). The data are also consistent with theoretical predictions obtained by the Zygggregator method (Tartaglia et al., 2008), indicating that residues 15-20 represent the most aggregation-prone region at pH 4.0 (Raimondi et al., 2011).

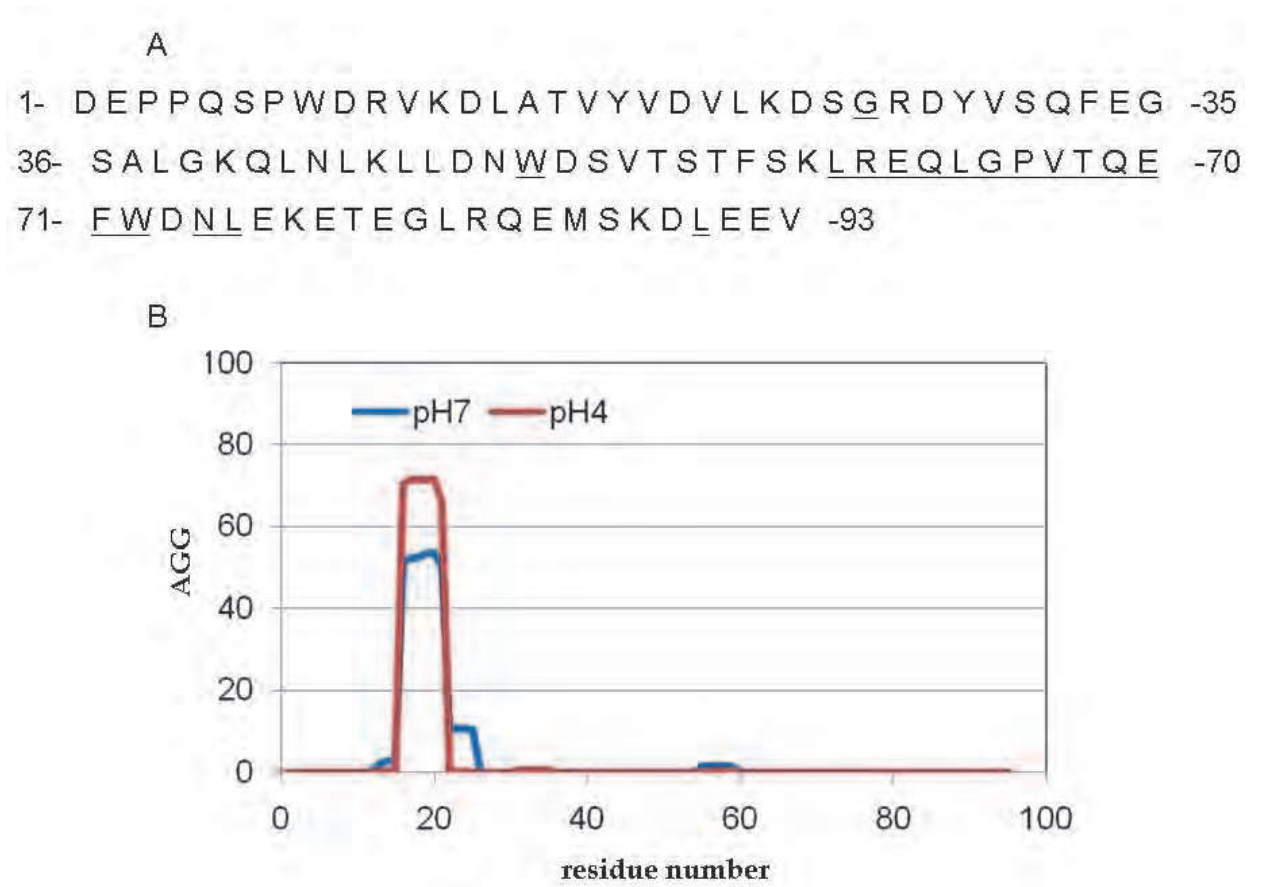


Fig. 2. AApoA-I fibrillogenic polypeptide. A, Amino acid sequence of [1-93]ApoA-I. Underlined are the sequence positions where the mutations identified in patients occur. B, Aggregation propensity (AGG) of [1-93]ApoA-I predicted by the TANGO algorithm at pH 7.0 and pH 4.0.

2. Structural and functional features of AApoA-I fibrillogenic polypeptide

2.1 A recombinant version of AApoA-I fibrillogenic polypeptide: An essential tool for structural and functional studies

The 93-residue fibrillogenic domain of AApoA-I, extracted from amyloid deposits of a patient who underwent a heart transplant for end-stage heart failure, was found to be a natively unfolded protein in water at neutral pH (Andreola et al., 2003). Acidic conditions (pH 4.0) were able to switch on a complex fibrillogenic pathway, consisting of extensive structural rearrangements of the polypeptide, that shifts from a random coil structure to an unstable helical conformation, and then aggregates into a β -sheet based polymeric structure (Andreola et al., 2003). Nevertheless, detailed structural and functional studies on the polypeptide extracted from *ex vivo* fibrils were made impossible due to the paucity of the available material.

Despite the intrinsic instability of the fibrillogenic polypeptide of AApoA-I due to its natively unfolded structure, we succeeded in the production of a recombinant version of the polypeptide, opening the way to structural and functional studies (Di Gaetano et al., 2006). The 1–93 fragment of AApoA-I was expressed in bacterial cells following an experimental strategy aimed at reducing the intracellular degradation of the polypeptide during its production. It was expressed in prokaryotic cells as a chimeric protein obtained by fusing the 93 residues polypeptide to glutathione S-transferase (GST). The [1–93]ApoA-I moiety was then released from the chimeric protein by targeted proteolysis, making use of a unique cleavage site positioned between the GST and the [1–93]ApoA-I sequences (Di Gaetano et al., 2006).

Conformational analyses of the recombinant polypeptide in solution by far-UV CD spectroscopy indicated that in physiological-like conditions the protein is largely unfolded. A pH switch from 7.0 to 4.0 induces a predominant α -helical structure, through the conversion of the protein from a random coil to a helical/molten globule state. This transition, complete within 2 seconds and fully reversible when the pH is returned to 7.0, is followed by the appearance of a significant β -sheet component. The helical conformers are thought to be key intermediates in the multistep fibrillogenic process. These observations are in good agreement with the behaviour of the natural polypeptide isolated from *ex vivo* fibrils (Obici et al., 1999).

The helical/molten globule intermediate displays a strong propensity to oligomerize, as demonstrated by atomic force microscopy (AFM) analyses. [1–93]ApoA-I, in fact, generates typical amyloid fibrils upon incubation at pH 4.0 for lengths of time comparable to those described for the natural polypeptide (Di Gaetano et al., 2006). Recently, we found that amyloid fibrils can also be obtained at neutral pH. Upon incubation of the polypeptide for 2 weeks at 37°C in buffer at pH 6.4, in the presence of 20% (v/v) trifluoroethanol (TFE), typical amyloid fibrils were obtained. In Figure 3, fibrils images obtained by AFM, following the procedure previously described (Arciello et al., 2011), are shown.

As the main features of the natural polypeptide were found to be preserved in the recombinant version of the fibrillogenic polypeptide, a valuable tool was available to generate the recombinant forms of all the variants of the polypeptide identified so far in patients affected by AApoA-I associated amyloidoses. This made possible the analysis of the structural and functional properties of the fibrillogenic polypeptides and their relationships, relevant for the comprehension of the disease.

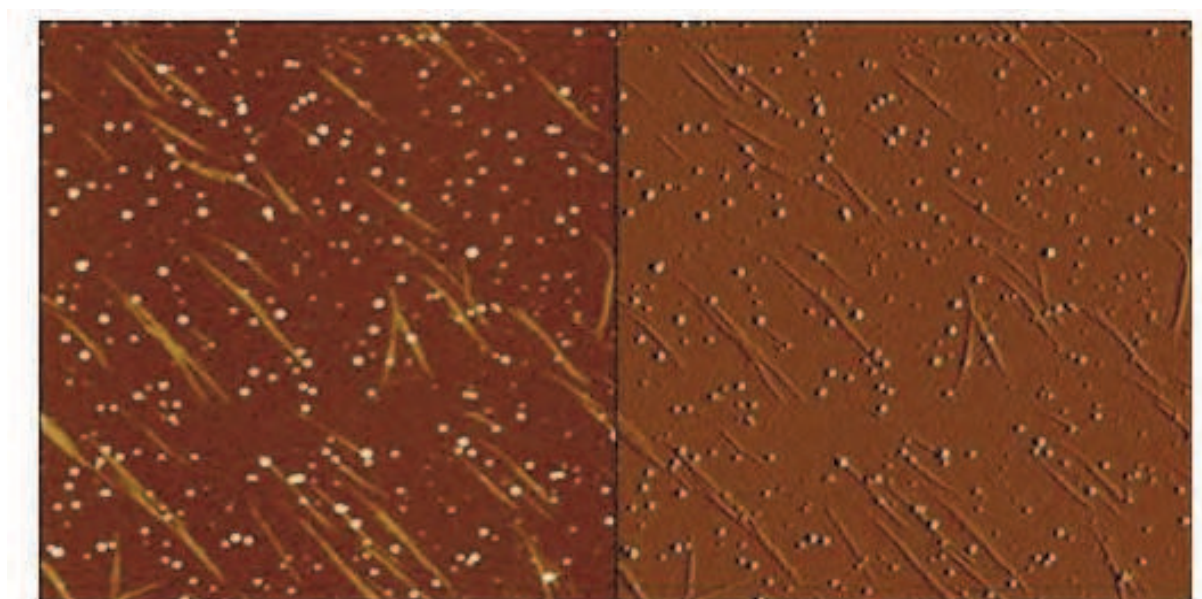


Fig. 3. AFM analysis of [1-93]ApoA-I fibrils. Tapping mode AFM image (left, height data; right, amplitude data) of [1-93]ApoA-I fibrils, obtained after 2 weeks incubation of [1-93]ApoA-I (0.3 mg/ml) at 37°C in 20% (v/v) TFE in 12 mM sodium phosphate buffer, pH 6.4. Amyloid fibrils are detected together with spheroidal aggregates. Scan size 4.0 μm , Z range 10 nm.

So far, in patients affected by AApoA-I associated amyloidoses, nine “inside mutations” have been identified, each identifying a variant of [1-93]ApoA-I polypeptide. Six variants (G26R, W50R, L60R, L64P, L75P and L90P) present a single residue substitution. Two variants contain sequence deletions, at positions 70-72 (variant $\Delta 70-72$) or at positions 60-71 (variant $\Delta 60-71/\text{VT}$); in the latter case the deleted residues are replaced by a Val-Thr sequence. The ninth variant, recently reported (Eriksson et al., 2009), is associated to a frameshift mutation (Asn74fs). It has to be noticed that the replacement of a hydrophobic residue either with a positively charged residue (arginine), or with an α -helical interrupting residue (proline) often occurs.

2.2 Intrinsic factors influencing amyloid formation: mutations induce AApoA-I susceptibility to proteolysis

We expressed and purified recombinant forms of all the isoforms of [1-93]ApoA-I, with the exception of the truncation mutation Asn74fs. The polypeptide variants were analysed in comparison to the wild-type polypeptide to investigate the effects of each individual mutation on the aggregation propensity of the polypeptide. We investigated the induction of structural transitions by acidic conditions (pH 4.0), along with structural properties of the aggregated material, and found that all the variants adopt a highly disordered structure at neutral pH, whereas acidification of the solution induces conformational changes and the subsequent aggregation into the cross- β structure aggregates. Nevertheless, differing results were obtained when the aggregation rate of the variants was analysed: two mutations ($\Delta 70-72$ and L90P) almost abrogate the lag phase of the aggregation process; three mutations ($\Delta 60-71$, L75P and W50R) significantly accelerate the aggregation rate by two-three fold, while the remaining three variants (L64P, L60R and G26R) are not significantly different from the wild-type polypeptide.

Our results indicate that an amyloidogenic mutation may, or may not, increase the aggregation propensity of the polypeptide. Thus, the paradigm amyloidogenic mutation-increased aggregation propensity has not to be taken as a general rule. Instead, a different scenario was provided by *in silico* analyses. Sequence-based predictions of aggregation propensities and stabilities of the pathogenic variants of full-length ApoA-I revealed in almost all the variants an increase of conformational fluctuations and chain flexibility in the proximity of the region of the protein spanning approximately residues 88-110, with the consequent exposure of a putative cleavage site to a proteolytic attack that releases the fibrillogenic moiety (Raimondi et al., 2011). Therefore, all the amyloidogenic structural modifications occurring at the N-terminal region of AApoA-I have a common feature, that of dramatically affecting the stability of the whole protein, favouring the cleavage that generates the N-terminal fibrillogenic fragment. In addition, some of the mutations increase the aggregation rate of the fibrillogenic polypeptide.

2.2 Extrinsic factors that influence amyloid formation: Effects of a lipid environment

2.2.1 General aspects of membrane interaction and protein aggregation

The elucidation of the structural properties of the fibrillogenic polypeptide is a central issue in the comprehension of the pathology. To this regard, the identification of structural or environmental factors, able to activate the pathological pathway leading to amyloid fibrils, is of enormous importance to pursue strategies aimed at inhibiting this process.

Extracellular amyloid deposition *in vivo* takes place in a heterogeneous environment, in which components of the cell membrane and/or the extracellular matrix may have a central role. From a general point of view, the interaction of proteins with biological superstructures, like membranes, may dramatically affect their structural organization. The general concept that biological surfaces may influence and direct molecular assemblies is gaining increasing attention. It is known that the interaction of natively folded proteins with groups exposed on a membrane surface often modifies their conformational states (Fantini & Yahi, 2010; Shanmugam & Jayakumar, 2004; Kakio et al., 2004). On the other hand, unfolded polypeptide chains can gain ordered structures at the membrane surface or inside the bilayer (Fantini & Yahi, 2010). Conversely, proteins can alter membrane fluidity, and/or permeate the membrane bilayer and can even extract lipids from it (Hou et al., 2005).

A dramatically different behaviour may be expected for a protein in a bulk solution with respect to that in a physiological environment. It is known that the hydrophobic interior of the plasma membrane can induce structural changes in soluble intrinsically disordered proteins and peptides by favouring secondary structures often leading to aggregate nucleation (Kazlauskaitė et al., 2003; Fernandez-Escamilla et al., 2004; Shanmugam & Jayakumar, 2004; Kakio et al., 2004). The well known stability of protein α -helical structures within a membrane lipid bilayer is in line with the concept that the early formation of multimeric species is often promoted by the association of polypeptide molecules through helix-helix interaction. From this point of view, a general mechanism of membrane-catalyzed amyloid formation can be envisaged.

To this regard, factors able to induce α -helical conformers may accelerate amyloid formation. Conversely, factors able to bind to, and stabilize, helical regions by entrapping the helical intermediates in a minimum energy (kinetic trap), may have an opposite effect,

i.e. that of slowing down the fibrillogenic process. Therefore, targeting helix-helix interactions can be a valuable strategy to inhibit amyloid formation.

2.2.2 Effects of cholesterol on [1-93]ApoA-I aggregation

It is known that the *in vivo* role of ApoA-I is mediated by its interactions with lipids, that are fundamental in the maintenance of the protein native structure. It is known that the N-terminal region of ApoA-I contributes to lipid binding in the native protein (Frank & Marcel, 2000; Tanaka et al., 2006). To this regard, recently we analysed the effects of lipids on the propensity of [1-93]ApoA-I to undergo fibrillogenesis and found that a lipid environment affects [1-93]ApoA-I aggregation pathway by inducing and stabilizing helical intermediates (Monti et al., 2010). In particular, by far-UV CD spectroscopy, we observed that the presence of a lipid-mimicking detergent, namely Triton X-100, greatly affects the conformational state of [1-93]ApoA-I. We found that at pH 8.0 [1-93]ApoA-I, which is predominantly in a random coil state, adopts a helical conformation in the presence of Triton X-100. This α -helical state remains unchanged upon acidification to pH 4.0. Interestingly, we observed that in the presence of Triton X-100 the polypeptide does not aggregate over time, rather a shift towards a random coil conformation occurs. Therefore, Triton X-100 induces and stabilizes helical conformers of [1-93]ApoA-I, thus hampering aggregation. We also demonstrated that the fibrillogenic pathway of [1-93]ApoA-I can be activated even under pathophysiological conditions, as we found that [1-93]ApoA-I at pH 6.4 is predominantly in an α -helical state and aggregates over time. Again, in the presence of Triton X-100 aggregation of [1-93]ApoA-I at neutral pH was found to be strongly impaired.

Furthermore, we investigated the effects of cholesterol, a natural ApoA-I ligand, on [1-93]ApoA-I aggregation and found that cholesterol acts as an inducer of helical conformers and an inhibitor of protein aggregation in a concentration-dependent and time-dependent manner (Monti et al., 2010). When the percentage of helical species was calculated in the presence of increasing concentrations of cholesterol both at pH 8.0 and 6.4, a positive correlation was observed. In Figure 4A and B, the results of typical experiments are shown. At pH 6.4, i.e. in conditions favouring aggregation, in the absence of cholesterol a significant decrease of helical species is observed after 7 days incubation, consistent with protein aggregation (Figure 4B). In the presence of cholesterol, at concentrations close to the physiological value (1.6 mM in HDL), the aggregation process is significantly slowed down, as shown in Figure 4B.

An inverse correlation exists between the α -helical content and the aggregation rate of the polypeptide, so that the fibrillogenic process is strongly affected by a hydrophobic environment that favours the formation of α -helices.

The behaviour of [1-93]ApoA-I is in line with that of other amyloidogenic proteins, whose conformations were reported to be strongly affected by the interaction with cholesterol. It is known that cholesterol and sphingolipids are the most abundant molecules of lipid rafts. Amyloid protein precursor (A β PP) and secretases preferentially localize into ganglioside and cholesterol-rich membrane microdomains (lipid rafts) (Lee et al., 1998; Ehahalt et al., 2003; Kakio et al., 2003). Accordingly, it has been proposed that aggregation of soluble A β peptides and APrP is a raft-associated process (Ehahalt et al., 2003) and that an alteration of cholesterol homeostasis is a shared primary cause of several neurodegenerative diseases (Vedhachalam et al., 2007).

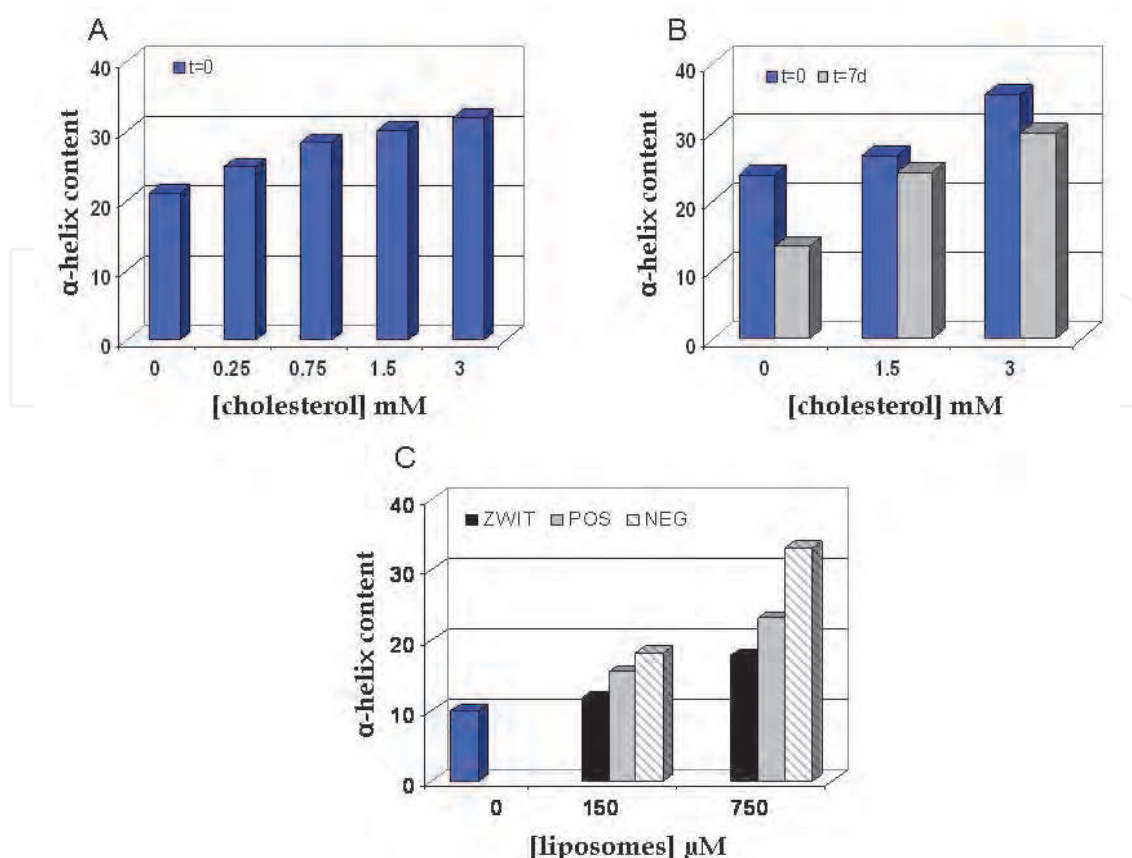


Fig. 4. Analysis of the effects of cholesterol and artificial membranes on [1-93]ApoA-I conformational state by far-UV CD spectroscopy. A, [1-93]ApoA-I (0.3 mg/ml) α -helical content in the presence of increasing concentrations of cholesterol in 3 mM glycine, 3 mM sodium acetate, and 3 mM sodium phosphate at pH 8.0. B, cholesterol concentration-dependence and time-dependence of [1-93]ApoA-I (0.3 mg/ml) α -helical content in 3 mM glycine, 3 mM sodium acetate, and 3 mM sodium phosphate at pH 6.4. C, [1-93]ApoA-I (0.3 mg/ml) α -helical content in the presence of zwitterionic (ZWIT), positively charged (POS) and negatively charged (NEG) liposomes in buffer at pH 6.4. The lipid-to-protein molar concentration ratio was 10:1 (150 μ M liposome concentration) or 50:1 (750 μ M liposome concentration). Helical content was estimated using CDPro program pack (Sreerama & Woody, 2000).

Consistent with these data are those obtained with the prion protein. In fact, PrP^c to APrP^{sc} pathological conversion seems to occur on the cell surface or during/after internalization of PrP^c, and cellular cholesterol depletion, which impairs association of PrP^c with rafts, inhibits the formation of the scrapie form in neuroblastoma cells (Taraboulos et al., 1995). However, it was also reported that the impairment of raft-association by cholesterol depletion during the early stage of PrP biosynthesis leads to protein misfolding in the ER (Sarnataro et al., 2004), suggesting a role for cholesterol as a lipochaperone. It has also been demonstrated that binding of PrP to raft-like artificial membranes induces the formation of an α -helical structure (Sanghera & Pinheiro, 2002).

Furthermore, α -synuclein, a fibrillogenic protein responsible for Parkinson's disease (PD), has been suggested to be associated with caveolae or caveolae-like domains (lipid raft domains containing caveolin), since it was found to regulate several signalling proteins

localized in these regions (Lusa et al., 1996; Ryan et al., 1992; Clay et al., 1992). Specific association of α -synuclein with lipid rafts suggests an important role for these membrane domains in the normal function of α -synuclein and raises the possibility that a perturbation of raft association could induce changes in α -synuclein conformation that contribute to PD pathogenesis (Martinez et al., 2007).

Moreover, an inverse correlation was envisaged between neurodegeneration and content in cholesterol (Lee et al., 1998; Martins et al., 2008), since reduced levels of cholesterol are present in the brains of Alzheimer disease (AD) patients (Mason et al., 1992). On the contrary, an increased membrane rigidity due to the presence of cholesterol has a protective action against aggregate cytotoxicity and membrane perturbation (Cecchi et al., 2005; Zampagni et al., 2010). In conclusion, cholesterol can modulate conformational changes of specific proteins or peptides and influence their aggregation propensity. From a general point of view, the interaction of amyloidogenic proteins with lipid rafts may play a key role in the pathogenesis of amyloid diseases.

2.2.3 Effects of liposomes on [1-93]ApoA-I aggregation

Although the mechanisms underlying amyloid diseases remain largely unknown, common features shared by some amyloidogenic proteins and peptides are emerging. One of these features is the key role of biological membranes in inducing helical structures in natively unstructured polypeptides.

Mechanistic studies with well defined model membranes have shown that natively unfolded polypeptides, upon interaction with surfaces, readily adopt helical structures that represent key intermediates in amyloid formation process (Abedini & Raleigh, 2009). In particular, anionic surfaces and anionic phospholipid-rich membranes can play key roles either in triggering protein fibrillogenesis by acting as conformational catalysts for amyloid fibrils deposition (Fantini & Yahi, 2010), or as inhibitors of fibrillogenesis (Zhu & Fink, 2003). We demonstrated that the conformation of the fibrillogenic polypeptide [1-93]ApoA-I is largely affected by membrane-mimicking structures, such as liposomes (Monti et al., 2010). Zwitterionic, negatively charged, and positively charged liposomes were prepared, made of POPC (1-Palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine), POPC/POPS (POPS, 1-Palmitoyl-2-oleoyl-sn-glycero-3-phospho-L-serine) and POPC/DOTAP (DOTAP, 1,2-Dioleoyl-3-trimethylammonium-propane), respectively. The POPC/POPS and POPC/DOTAP molar ratio was 85:15. We performed conformational analyses of [1-93]ApoA-I at pH 8.0 at 37°C in the presence of increasing concentrations of each liposome type and found a significant concentration-dependent increase of [1-93]ApoA-I helical content, particularly evident in the case of negatively charged liposomes (Figure 4C).

It has to be noticed that [1-93]ApoA-I is very rich in charged residues. Thus, interaction with charged lipids, either positive or negative, might mask intramolecular repulsion effects and favour the conformational transition towards a helical state. Furthermore, our data indicate a negative effect of liposomes on polypeptide aggregation, suggesting that membrane composition influences the fate of AApoA-I fibrillogenic polypeptide.

To this regard, the identification of molecules able to have an effect on the critical balance between folded and partially unfolded states of the fibrillogenic polypeptide would allow the development of therapies targeted to amyloidoses. To this purpose, biomimetic membranes appear of interest as effectors/modulators of the fibrillogenic process.

Our data are in line with previous observations regarding α -synuclein. Evidence was provided that α -synuclein preferentially interacts with small unilamellar vesicles (SUVs) containing acidic phospholipids, which induce and stabilize helical conformers (Wang et al., 2000). On the other hand, no interaction of α -synuclein with zwitterionic vesicles was detected (Wang et al., 2000). It has also been reported that helical conformation of α -synuclein is a prerequisite for its binding to lipids. Thus, interaction of α -synuclein with lipid vesicles might lead to preferential binding to membranes and induction of helix with a concomitant inhibition of fibrillogenesis (Zhu & Fink, 2003). It has also been demonstrated that, upon PrP binding to raft-like artificial membranes, the formation of α -helical structures is induced (Sanghera & Pinheiro, 2002), similarly to the fibrillogenic polypeptide [1-93]ApoA-I. In conclusion, our observations, in line with those collected for other fibrillogenic proteins, indicate that protein-lipid interactions induce and stabilize helical conformers, interfering with aggregation.

2.3 Membrane interaction, internalization and intracellular pathway of AApoA-I fibrillogenic polypeptide

Of primary importance in the comprehension of amyloid diseases is the elucidation of the cascade of biochemical events triggered by the exposure of cells to fibrillogenic proteins or polypeptides. Upon its release, [1-93]ApoA-I is expected to accumulate in the extracellular space. The possibility that the fibrillogenic polypeptide of AApoA-I interacts with membranes of target cells and enters the cell compartment, mimicking ApoA-I full-length protein, has to be taken into account. Recently we analysed the intracellular pathway of [1-93]ApoA-I in comparison to full-length ApoA-I using cardiac cells. Since in the case of AApoA-I associated amyloidoses the heart is a natural target for aggregate deposition *in vivo*, cardiomyoblasts were chosen as an experimental system.

We provided evidence that the polypeptide partially co-localizes with ABCA1 on rat cardiomyoblasts cell membranes (Arciello et al., 2011). Similar results were obtained for ApoA-I, in agreement with recent reports showing that the majority of cell-associated ApoA-I does not co-localize with ABCA1, although no internalization was observed in cells ABCA1^{-/-} (Zha et al., 2001). To explain this observation, a model was recently proposed (Denis et al., 2008; Vedhachalam et al., 2007) in which the interaction of a small fraction of lipid-free ApoA-I to ABCA1 is sufficient to activate ABCA1 lipid translocase activity, which in turn promotes the formation of specialized lipid domains, acting as high affinity binding sites for ApoA-I (Vedhachalam et al., 2007). Nevertheless, whether or not ABCA1 has to be considered as an ApoA-I receptor is still ambiguous, as ABCA1 could have a role in inducing modifications of membrane lipid distribution facilitating ApoA-I docking (Vedhachalam et al., 2007).

We demonstrated that the fibrillogenic polypeptide recognizes specific binding sites on cardiac cell membranes and that this binding represents a key step for [1-93]ApoA-I internalization (Arciello et al., 2011). The apparent affinity constant calculated for the binding of the polypeptide to rat cardiomyoblasts ($K_d = 5.90 \times 10^{-7} \text{ M}$) was found to be similar to those previously reported for lipid-free full-length ApoA-I binding to different cell types, such as HepG2 cells ($K_d = 0.84 \times 10^{-7} \text{ M}$) (Barbaras et al., 1994), or bovine aortic endothelial cells BAECs ($K_d = 0.8 \times 10^{-7} \text{ M}$) (Rohrer et al., 2006).

Interestingly, we demonstrated that, following binding, the polypeptide is internalized in cardiomyoblasts.

A comparative analysis of the internalization routes of the polypeptide and the full-length protein revealed that: (i) the polypeptide is internalized mostly by clathrin-mediated endocytosis and by lipid rafts, whereas a significant involvement of macropinocytosis could be excluded; (ii) ApoA-I is internalized *via* clathrin-coated pits and macropinocytosis, whereas internalization through lipid rafts was not observed (Arciello et al., 2011).

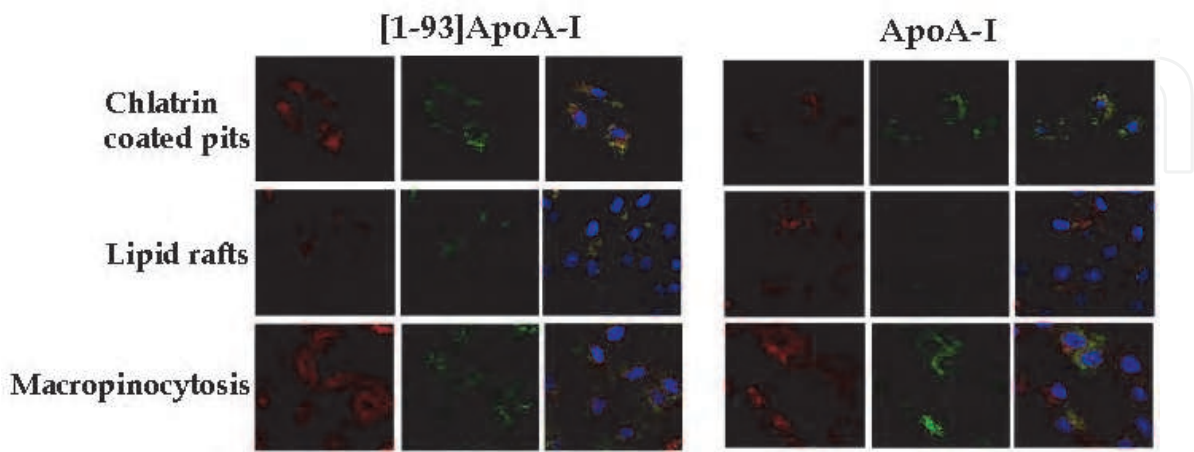


Fig. 5. Analysis of the internalization routes of the fibrillogenic polypeptide [1-93]ApoA-I and full-length ApoA-I by confocal microscopy. To analyse clathrin-mediated endocytosis, cardiomyoblasts (H9c2 cells) were transiently transfected with an expression vector for Rab5 conjugated to the red fluorescent protein. After 24 h, cells were incubated 6 h at 37°C either with FITC-labelled [1-93]ApoA-I (3 µM), or ApoA-I (1 µM). For lipid rafts-mediated internalization, cells were incubated 4 h at 37°C with rhodamine- labelled proteins (3 µM [1-93]ApoA-I, 1 µM ApoA-I) in the presence of FITC-insulin (0.1 mg/ml). To analyse macropinocytosis, cells were incubated 4 h at 37°C with rhodamine-labelled proteins as above, in the presence of FITC-dextran (5 mg/ml). Nuclei were stained with Hoechst (blue).

Thus, evidence was provided that the pathogenic polypeptide translocates from the extracellular space, where fibrils form and grow, to the intracellular space. ApoA-I and its fibrillogenic polypeptide share the endocytic route to gain access to the intracellular compartment, whereas lipid rafts and macropinocytosis represent specific routes for the fibrillogenic polypeptide and the full-length protein, respectively. Considering that lipid rafts are rich in cholesterol, which is able to induce and stabilize [1-93]ApoA-I helical states, the polypeptide internalization mediated by lipid rafts may have a key role in the pathogenesis of the disease. On the other hand, our data extend the knowledge on ApoA-I mechanism of action as, while it is known that ApoA-I interacts with plasma membrane lipid rafts to control cholesterol export (Gaus et al., 2004), we provided evidence for the first time that these domains are not involved in ApoA-I internalization.

Understanding the fate of internalized [1-93]ApoA-I is fundamental to deeply inspect the molecular bases of the pathology. It has been reported that ApoA-I, once internalized in endosomes, is resecreted to the medium as HDL. Endosomes represent an intracellular reservoir of cholesterol where ApoA-I binds to lipids to be secreted through a retroendocytic pathway involving Rab4 containing endosomes (Mukherjee et al., 1998). We found that ApoA-I and its fibrillogenic polypeptide follow different pathways, as ApoA-I was found to co-localize with Rab4, consistently with the recycling pathway (Mukherjee et al., 1998), whereas no evidence of co-localization with Rab4 was obtained for the polypeptide.

We thus analysed the intracellular fate of the pathogenic polypeptide by fluorescence microscopy. After a prolonged exposure (24 h) of cardiomyoblasts to the fibrillogenic polypeptide, the disappearance of the intracellular fluorescent signal, indicative of a massive degradation of [1-93]ApoA-I, was observed. To define the degradation route, we analysed the involvement of proteasome and lysosomes activities. In the presence of specific inhibitors of protein degradation mediated by either proteasome or lysosomes, a significant persistence of [1-93]ApoA-I associated fluorescent signal was observed. This suggests that the fibrillogenic fragment is targeted to both these stations for degradation (Arciello et al., 2011). Different results were obtained instead in the case of full-length ApoA-I, as the protein does not appear to be significantly degraded once internalized. However, we observed a strong co-localization of ApoA-I with lysosomes, in agreement with recent reports (Cavelier et al., 2006; Denis et al., 2008). Although the question concerning the physiologic role of ApoA-I in lysosomes remains controversial, it might be considered that, even if lysosomes are best known for their role in degradation, they may also fuse with the plasma membrane to release their content in the extracellular medium (Luzio et al., 2007). Some authors demonstrated that ApoA-I reaches lysosomes to be degraded (Pastore et al., 2004; Rye & Barter, 2004), while other studies support the idea that ABCA1-bound ApoA-I traffics through late endosomal vesicles and/or lysosomes. Being these stations an intracellular reservoir of cholesterol, nascent lipoprotein particles may be formed at this level and then secreted in the extracellular space (Oram, 2008; Chen et al., 2001; Chen et al., 2005).

As it is conceivable that the accumulation of the fibrillogenic polypeptide in the extracellular space leads to fibrils deposition, the question was raised: are [1-93]ApoA-I fibrils able to enter cardiac cells? We thus produced fluorescent fibrils *in vitro* by incubating the fluorescein-labelled polypeptide under suitable conditions to induce fibrillogenesis (see Fig. 3). When fibrils were tested on cardiomyoblasts, no evidence of internalization was found.

We also tested the effects of the polypeptide, either in its unaggregated form or in the fibrillar state, on cell viability. In both cases, no effects on cardiomyoblasts viability were detected. These findings are in line with the evidence that: (i) fibrils are not able to enter cardiac cells; (ii) the unaggregated polypeptide, which enters the cells, undergoes rapid intracellular degradation. The latter observation is also in line with our previous experiments of limited proteolysis (Di Gaetano et al., 2006), indicating that the largely unfolded structure of the fibrillogenic polypeptide is responsible for its susceptibility to proteolytic cleavages. This, in turn, is in line with the fact that we succeeded in the production of a recombinant form of the polypeptide in a prokaryotic expression system only upon fusion of [1-93]ApoA-I to a stable bacterial protein to avoid intracellular degradation of the polypeptide (Di Gaetano et al., 2006).

Interestingly, the elucidation of key steps of the intracellular pathway and fate of AApoA-I fibrillogenic polypeptide reveals features common to other amyloidogenic proteins.

In the case of transmissible spongiform encephalopathy, the misfolded form of APrP accumulates in the brain. It is known that, after being exported to the plasma membrane, PrP^c is internalized and recycled back to the surface. However, the compartment where the transition from PrP^c to APrP^{sc} occurs has not yet been clearly identified. It has been suggested that raft-enriched lipids represent the site of scrapie formation (Sarnataro et al., 2004). On the other hand, it is known that APrP^{sc} undergoes proteasomal degradation (Sarnataro et al., 2004), and accumulates in lysosomes, a compartment that might be involved in the transconformational event.

In the case of Parkinson's disease, α -synuclein accumulates inside the cells as fibrillar aggregates named Lewy bodies. However, although α -synuclein is a cytoplasmic protein, a small amount of the protein is secreted by cells and is present in human body fluids. It has been demonstrated that both non-fibrillar oligomeric aggregates and fibrils are able to enter the cells through the endosomal pathway and to be degraded by lysosomes. In contrast to the uptake of protein aggregates, α -synuclein monomers are able to freely diffuse across the plasma membrane and to be resecreted before being degraded by the cellular proteolytic systems. This mechanism might protect neurons from exposure to potentially toxic α -synuclein (Lee et al., 2008). However, it has to be noticed that newly synthesized α -synuclein monomers and dimers, but not protofibrils, can be degraded by the proteasome (Zhang et al., 2008). Once α -synuclein protofibrils are formed, they are able to impair proteasome activity and this phenomenon may eventually result in α -synuclein accumulation in cells (Bennett et al., 1999; Tofaris et al., 2001; McNaught et al., 2002).

Along the internalization and intracellular pathway, common aspects are shared by [1-93]ApoA-I and A β . The most abundant forms of A β are 40 and 42 residue long (A β 40/42), whose oligomeric species were found to rapidly bind and internalize in neuronal cells and accumulate in lysosomes. In contrast, aggregated polypeptides were found to associate with cells only weakly (Bateman & Chakrabartty, 2011). Furthermore, soluble A β oligomers, but not monomers, inhibit proteasomal activity *in vitro* (Tseng et al., 2008). Thus, an inverse correlation exists between A β 40/42 aggregation rate and ability to bind to cells and to be internalized (Bateman & Chakrabartty, 2011).

It has to be noticed that cellular mechanisms deputed to protein degradation, i.e. lysosomes, proteasome and autophagy, may be important targets for therapeutic approaches against amyloidoses. It is known that the pathological accumulation of abnormal proteins, improperly folded and able to impair cellular functions, is determined by different causes, such as mutations, protein overproduction or impairment of the protein degradation machineries (Casarejos et al., 2011). Since in several amyloid diseases the impairment of proteasomal activity has been pointed out, a promising therapeutic approach would be that of enhancing the activity of cellular mechanisms for protein clearance.

2.4 A model for [1-93]ApoA-I

Based on the experimental data collected so far, a model representing the possible fates of [1-93]ApoA-I polypeptide in an *in vivo* context is proposed (Figure 6).

From a general point of view, the continuous accumulation of the natively unfolded polypeptide in the cardiac tissue is expected to favour protein aggregation and fibrillogenesis leading to a progressive, massive occupancy of the extracellular space by amyloid deposits, as observed in pathological hearts, from which the natural fibrillogenic polypeptide can be isolated (Obici et al., 1999). During the fibrillogenic process, a dynamic equilibrium between monomeric species and aggregated states has been proposed (Carulla et al., 2005). In the case of [1-93]ApoA-I, internalization in target cells may represent an alternative fate to aggregation, thus subtracting the unaggregated form of [1-93]ApoA-I from the equilibrium. This would direct the polypeptide towards a non pathological route, as, once inside the cells, the fibrillogenic polypeptide is promptly cleared off. Hence, the hypothesis can be raised that internalization and subsequent degradation of the unaggregated polypeptide represent a protective mechanism against fibrillogenesis, able to balance [1-93]ApoA-I progressive aggregation and to slow down the fibrillogenic process.

This phenomenon may be relevant in the slow progression and late onset of AApoA-I-associated amyloidoses.

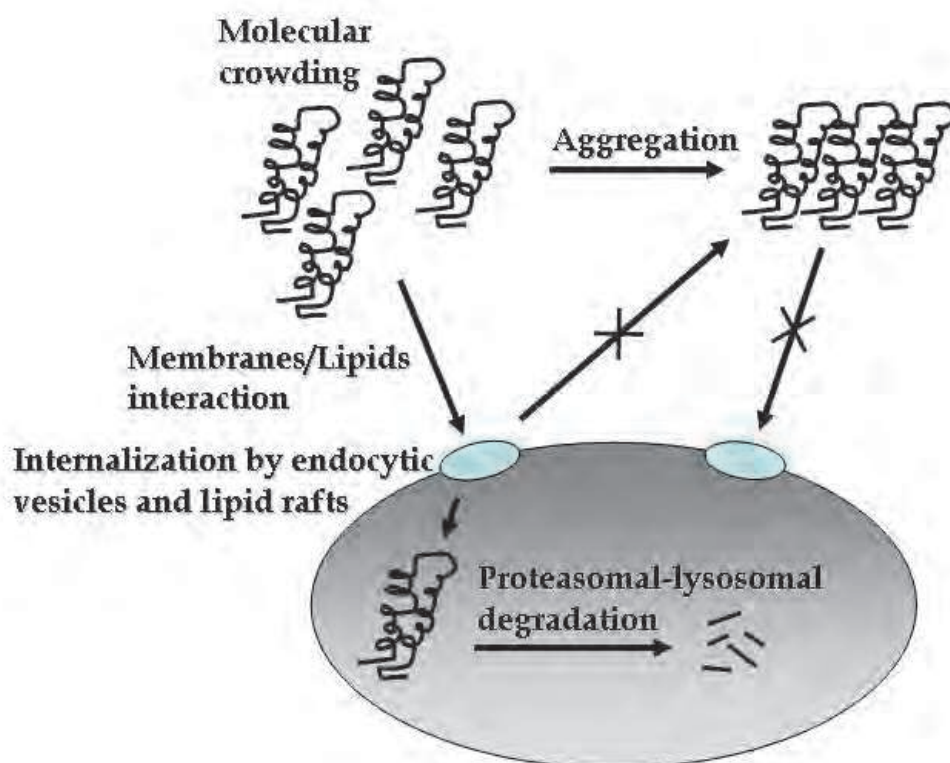


Fig. 6. A schematic representation of the possible fates of AApoA-I fibrillogenic polypeptide. Upon accumulation in the extracellular space of target tissues, the soluble polypeptide is prone to aggregate in fibrillar structures. Viceversa, the interaction with membrane components, presumably lipids, induces conformational changes generating stable helical species with low propensity to aggregation. Upon membrane binding, the polypeptide can gain the intracellular compartment via endocytic vesicles and lipid rafts. Inside the cells, the polypeptide is promptly degraded by the lysosomal and proteasomal machineries. On the contrary, once aggregated in amyloid fibrils, the polypeptide is unable to be internalized.

3. Conclusions

The elucidation of the cascade of biochemical events triggered by the exposure of cells to a fibrillogenic protein is of primary importance in the comprehension of the molecular bases of amyloid diseases. Presently, the molecular mechanism of fibril formation in patients bearing AApoA-I amyloidogenic mutations is largely unknown, as unknown are the molecular bases of the etiology of this pathology.

Our work is aimed at elucidating the molecular bases of the process responsible for fibril deposition in amyloid diseases associated to AApoA-I. ApoA-I plays a key role in lipids metabolism acting as an antiatherogenic factor in humans. Nevertheless, specific mutations convert it into the precursor of a pathogenic polypeptide named [1-93]ApoA-I, whose largely unfolded structure correlates with a high propensity to aggregate in fibrillar deposits. For both the full-length protein and its fibrillogenic fragment, conformational

plasticity is an essential feature that makes possible protein adaptation to different environmental conditions.

We shed light on key steps of the possible routes accessible to the pathogenic polypeptide in a physiological-like environment, such as that of cultured cardiomyoblasts, which mimic one of the most frequent target tissues of the pathology, i.e. the heart. We observed that the polypeptide is able to bind lipids undergoing extensive conformational changes. We thus hypothesized that in a cellular environment the interaction of the polypeptide with lipids may play a critical role in slowing down the fibrillogenic process by entrapping the polypeptide in stable helical structures unable to aggregate. Through endocytic vesicles and lipid rafts the polypeptide may be conveyed from the extracellular to the intracellular compartment of target cells. Here, the polypeptide gains access to the protein degradation machinery.

We thus envisaged two distinct mechanisms acting synergistically to prevent the progressive aggregation in fibrillar structures. The interaction of extracellular polypeptide molecules with the cell surface may “freeze” non amyloidogenic conformational states of the polypeptide. On the other side, the activation of the internalization-degradation pathway subtracts polypeptide molecules from the extracellular space, interfering with amyloid deposition.

In this chapter, we underlined some structural and functional features shared by AApoA-I fibrillogenic polypeptide and other natively unfolded amyloidogenic proteins. We believe that pointing out such correlations might greatly contribute to draw a still more comprehensive picture of amyloid diseases.

The comprehension of the molecular mechanisms of the pathology will certainly benefit from future studies aimed at identifying the intracellular partners of the fibrillogenic polypeptide, as well as membrane and extracellular components that may alter the balance between aggregated and unaggregated states of the polypeptide.

4. Acknowledgements

The Authors wish to thank Dr A. Relini, University of Genoa, for AFM analyses and Dr F. Guglielmi for contribution in spectroscopic analyses.

5. References

- Abedini, A. & Raleigh, D.P. (2009). A critical assessment of the role of helical intermediates in amyloid formation by natively unfolded proteins and polypeptides. *Prot. Eng. Des. Sel.*, 22: 453-459.
- Acton, S.; Rigotti, A.; Landschulz, K.T.; Xu, S.; Hobbs, H.H.; Krieger, M. (1996). Identification of scavenger receptor SR-BI as a high density lipoprotein receptor. *Science.*, 271(5248): 518-520.
- Adorni, M.P.; Zimetti, F.; Billheimer, J.T.; Wang, N.; Rader, D.J.; Phillips, M.C.; Rothblat, G.H. (2007). The roles of different pathways in the release of cholesterol from macrophages. *J Lipid Res.*, 48(11): 2453-2462.
- Ajees, A.A.; Anantharamaiah, G.M.; Mishra, V.K.; Hussain, M.M.; Murthy, H.M. (2006) Crystal structure of human apolipoprotein A-I: insights into its protective effect against cardiovascular diseases. *Proc. Natl. Acad. Sci. USA*, 103: 2126-2131. This paper is currently under dispute as reported in a PNAS editorial expression of

- concern (<http://www.pnas.org/content/107/14/6551.3.full?sid=daacdccb-0537-41fb-87c3-8%2067aba380ca7>)
- Andreola, A.; Bellotti, V.; Giorgetti, S.; Mangione, P.; Obici, L.; Stoppini, M.; Torres, J.; Monzani, E.; Merlini, G.; Sunde, M. Conformational switching and fibrillogenesis in the amyloidogenic fragment of apolipoprotein A-I. (2003). *J Biol Chem.*, 278: 2444-2451.
- Arciello, A.; De Marco, N.; Del Giudice, R.; Guglielmi, F.; Pucci, P.; Relini, A.; Monti, D.M.; Piccoli, R. (2011). Insights into the fate of the N-terminal amyloidogenic polypeptide of ApoA-I in cultured target cells. *J. Cell. Mol. Med.*, DOI: 10.1111/j.1582-4934.2011.01271.x
- Barbaras, R.; Collet, X.; Chap, H.; Perret, B. (1994). Specific binding of free apolipoprotein A-I to a high-affinity binding site on HepG2 cells: characterization of two high-density lipoprotein sites. *Biochemistry*, 33: 2335-2340.
- Bateman, D.A. & Chakrabartty, A. (2011). Cell surface binding and internalization of $\alpha\beta$ modulated by degree of aggregation. *Int J Alzheimers Dis.*, 2011: 962352.
- Bennett, M.C.; Bishop, J.F.; Leng, Y.; Chock, P.B.; Chase, T.N.; Mouradian, M.M. (1999). Degradation of α -Synuclein by Proteasome *J. Biol. Chem.* 274: 33855-33858.
- Borhani, D.W.; Rogers, D.P.; Engler, J.A.; Brouillette, C.G. (1997). Crystal structure of truncated human apolipoprotein A-I suggests a lipid-bound conformation. *Proc Natl Acad Sci U S A.*, 94(23): 12291-12296.
- Brouillette, C.G.; Anantharamaiah, G.M.; Engler, J.A.; Borhani, D.W. (2001). Structural models of human apolipoprotein A-I: a critical analysis and review. *Biochim Biophys Acta*, 1531:4-46.
- Carulla, N.; Caddy, G.L.; Hall, D.R.; Zurdo, J.; Gairí, M.; Feliz, M.; Giralt, E.; Robinson, C.V.; Dobson, C.M. (2005). Molecular recycling within amyloid fibrils. *Nature*, 436(7050): 554-558.
- Casarejos, M.J.; Solano, R.M.; Gómez, A.; Perucho, J.; de Yébenes, J.G., Mena, M.A. (2011). The accumulation of neurotoxic proteins, induced by proteasome inhibition, is reverted by trehalose, an enhancer of autophagy, in human neuroblastoma cells. *Neurochem Int.*, 58(4): 512-520.
- Cavelier, C.; Lorenzi, I.; Rohrer, L.; von Eckardstein, A. (2006). Lipid efflux by the ATP-binding cassette transporters ABCA1 and ABCG1. *Biochim Biophys Acta*, 1761: 655-666.
- Cavigiolio, G.; Geier, E.G.; Shao, B.; Heinecke, J.W.; Oda, M.N. (2010). Exchange of apolipoprotein A-I between lipid-associated and lipid-free states: a potential target for oxidative generation of dysfunctional high density lipoproteins. *J Biol Chem.*, 285(24): 18847-18857.
- Cecchi, C.; Baglioni, S.; Fiorillo, C.; Pensalfini, A.; Liguri, G.; Nosi, D.; Rigacci, S.; Bucciantini, M.; Stefani, M. (2005). Insights into the molecular basis of the differing susceptibility of varying cell types to the toxicity of amyloid aggregates. *J. Cell Sci.*, 118 (Pt 15): 3459-3470.
- Chen, W.; Sun, Y.; Welch, C.; Gorelik, A.; Leventhal, A.R.; Tabas, I.; Tall, A.R. (2001). Preferential ATP-binding cassette transporter A1-mediated cholesterol efflux from late endosomes/lysosomes. *J Biol Chem.*, 276: 43564-43569.

- Chen, W.; Wang, N.; Tall, A.R. (2005). A PEST deletion mutant of ABCA1 shows impaired internalization and defective cholesterol efflux from late endosomes. *J Biol Chem.*, 280: 29277-29281.
- Chiti, F. & Dobson, C. M. (2006). Protein misfolding, functional amyloid, and human disease. *Annu. Rev. Biochem.*, 75: 333 – 366.
- Chou, P.Y. & Fasman, G.D. (1978). Prediction of the secondary structure of proteins from their amino acid sequence. *Adv Enzymol Relat Areas Mol Biol.*, 47: 45-148.
- Clay, M.A.; Newnham, H.H.; Forte, T.M.; Barter, P.I. (1992). Cholesteryl ester transfer protein and hepatic lipase activity promote shedding of apo A-I from HDL and subsequent formation of discoidal HDL. *Biochim. Biophys. Acta*, 1124(1): 52-58.
- Denis, M.; Landry, Y.D. & Zha, X. (2008). ATP-binding cassette A1-mediated lipidation of apolipoprotein A-I occurs at the plasma membrane and not in the endocytic compartments. *J Biol Chem.*, 283: 16178-16186.
- Di Gaetano, S.; Guglielmi, F.; Arciello, A.; Mangione, P.; Monti, M.; Pagnozzi, D.; Raimondi, S.; Giorgetti, S.; Orrù, S.; Canale, C.; Pucci, P.; Dobson, C.M.; Bellotti, V.; Piccoli R. (2006). Recombinant amyloidogenic domain of ApoA-I: analysis of its fibrillogenic potential. *Biochem Biophys Res Commun.*, 351(1): 223-228.
- Duong, P. T.; Weibel, G. L.; Lund-Katz, S.; Rothblat, G. H.; Phillips, M. C. (2008). Characterization and properties of pre beta-HDL particles formed by ABCA1-mediated cellular lipid efflux to apoA-I. *J. Lipid Res.*, 49(5): 1006-1014.
- Ehahalt, R.; Keller, P.; Haass, C.; Thiele, C.; Simons, K. J. (2003). Amyloidogenic processing of the Alzheimer beta-amyloid precursor protein depends on lipid rafts. *Cell Biol.*, 160: 113-123.
- Eriksson, M.; Schönland, S.; Yumlu, S.; Hegenbart, U.; von Hutten, H.; Gioeva, Z.; Lohse, P.; Büttner, J.; Schmidt, H.; Röcken, C. (2009). Hereditary apolipoprotein AI-associated amyloidosis in surgical pathology specimens: identification of three novel mutations in the APOA1 gene. *J Mol Diagn.*, 11: 257-262.
- Fantini, J. & Yahi, N. (2010). Molecular insights into amyloid regulation by membrane cholesterol and sphingolipids: common mechanisms in neurodegenerative diseases. *Expert Rev Mol Med.*, doi: 10.1017/S1462399410001602.
- Fernandez-Escamilla, A.M.; Rousseau, F., Schymkowitz, J.; Serrano, L. (2004) Prediction of sequence-dependent and mutational effects on the aggregation of peptides and proteins. *Nat Biotechnol.*, 22(10): 1302-1306.
- Fielding, C.J. & Fielding, P.E. (1995). Molecular physiology of reverse cholesterol transport. *Lipid Res.* 36(2): 211-228.
- Frank, P.G. & Marcel, Y.L. (2000). Apolipoprotein A-I: structure-function relationships. *J. Lipid Res.* 41: 853-872.
- Gast, K.; Damaschun, H.; Eckert, K.; Schulze-Forster, K.; Maurer, H.R.; Müller-Frohne, M.; Zirwer, D.; Czarnecki, J.; Damaschun, G. (1995). Prothymosin alpha: a biologically active protein with random coil conformation. *Biochemistry*, 34(40): 13211-13218.
- Gaus, K.; Kritharides, L.; Schmitz, G.; Boettcher, A.; Drobnik, W.; Langmann, T.; Quinn, C.M.; Death, A.; Dean, R.T.; Jessup, W. (2004). Apolipoprotein A-1 interaction with plasma membrane lipid rafts controls cholesterol export from macrophages. *FASEB J.*, 18: 574-576.
- Glass, C.K.; Pittman, R.C.; Keller, G.A.; Steinberg, D. (1983). Tissue sites of degradation of apoprotein A-I in the rat. *J. Biol Chem.*, 258(11): 7161-7167.

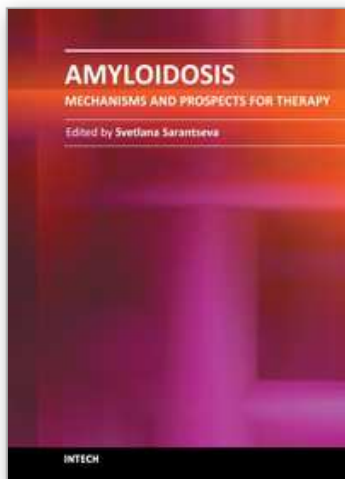
- Guruprasad, K.; Reddy, B.V. & Pandit, M.W. (1990) Correlation between stability of a protein and its dipeptide composition: a novel approach for predicting in vivo stability of a protein from its primary sequence. *Protein Eng.*, 4(2): 155-161.
- Hou, X.; Richardson, S.J.; Aguilar, M.I.; Small, D.H. (2005). Binding of amyloidogenic transthyretin to the plasma membrane alters membrane fluidity and induces neurotoxicity. *Biochemistry*, 44(34): 11618-11627.
- Kakio, A.; Nishimoto, S.-L.; Kozutsumi, Y.; Matsuzaki, K. (2003). Formation of a membrane-active form of amyloid beta-protein in raft-like model membranes. *Biochim. Biophys. Res. Commun.*, 303(2): 514-518.
- Kakio, A.; Yano, Y.; Takai, D.; Kuroda, Y.; Matsumoto, O.; Kozutsumi, Y.; Matsuzaki, K. (2004). Interaction between amyloid beta-protein aggregates and membranes. *J Pept Sci.*, 10(10): 612-621.
- Kazlauskaitė, J.; Sanghera, N.; Sylvester, I.; Vénien-Bryan, C.; Pinheiro, T.J. (2003). Structural changes of the prion protein in lipid membranes leading to aggregation and fibrillization. *Biochemistry*, 42(11): 3295-3304.
- Lee, H.J.; Suk, J.E.; Bae, E.J.; Lee, J.H.; Paik, S.R.; Lee, S.J. (2008). Assembly-dependent endocytosis and clearance of extracellular alpha-synuclein. *Int J Biochem Cell Biol.*, 40(9): 1835-1849.
- Lee, S.J.; Liyanage, U.; Bickel, P.E.; Xia, W.; Lansbury, P.T. Jr; Kosik, K.S. (1998). A detergent-insoluble membrane compartment contains A beta in vivo. *Nat Med.*, 4(6): 730-734.
- Liang, H.Q.; Rye, K.A.; Barter, P.J. (1995). Cycling of apolipoprotein A-I between lipid-associated and lipid-free pools. *Biochim Biophys Acta.*, 1257(1): 31-37.
- Liang, H.Q.; Rye, K.A.; Barter, P.J. (1996). Remodelling of reconstituted high density lipoproteins by lecithin: cholesterol acyltransferase. *J Lipid Res.*, 37(9): 1962-1970.
- Lusa, S.; Jauhiainen, M.; Metso, J.; Somerharju, P.; Ehrnholm, C. (1996). The mechanism of human plasma phospholipid transfer protein-induced enlargement of high-density lipoprotein particles: evidence for particle fusion. *Biochem. J.*, 313 (Pt 1): 275-282.
- Luzio, J.P.; Pryor, P.R. & Bright, N.A. (2007). Lysosomes: fusion and function. *Nat Rev Mol Cell Biol.*, 8: 622-632.
- Martinez, Z.; Zhu, M.; Han, S.; Fink, A.L. (2007). GM1 specifically interacts with alpha-synuclein and inhibits fibrillation. *Biochemistry*, 46: 1868-1877.
- Martins I.C.; Kuperstein I.; Wilkinson H.; Maes E.; Vanbrabant M.; Jonckheere W.; Van Gelder P.; Hartmann D.; D'Hooze R.; De Strooper B.; Schymkowitz J.; Rousseau F. (2008). Lipids revert inert Aβ amyloid fibrils to neurotoxic protofibrils that affect learning in mice. *EMBO J.*, 27(1): 224-233.
- Mason, R.P.; Shoemaker, W.J.; Shajenko, L.; Chambers, T.E.; Herbette, L.G. (1992). Evidence for changes in the Alzheimer's disease brain cortical membrane structure mediated by cholesterol. *Neurobiol. Aging*, 13: 413-419.
- Maurer-Stroh, S.; Debulpaep, M.; Kuemmerer, N.; Lopez de la Paz, M.; Martins, I.C.; Reumers, J.; Morris, K.L.; Copland, A.; Serpell, L.; Serrano, L.; Schymkowitz, J.W.; Rousseau, F. (2010). Exploring the sequence determinants of amyloid structure using position-specific scoring matrices. *Nat Methods*, 7(3): 237-242.
- McNaught, K.S.; Mytilineou, C.; Jnoubaptiste, R.; Yabut, J.; Shashidharan, P.; Jennert, P.; Olanow, C.W. (2002). Impairment of the ubiquitin-proteasome system causes dopaminergic cell death and inclusion body formation in ventral mesencephalic cultures. *J. Neurochem.*, 81: 301-306.

- Monti, D.M.; Guglielmi, F.; Monti, M.; Cozzolino, F.; Torrassa, S.; Relini, A.; Pucci, P.; Arciello, A.; Piccoli R. (2010). Effects of a lipid environment on the fibrillogenic pathway of the N-terminal polypeptide of human apolipoprotein A-I, responsible for in vivo amyloid fibril formation. *Eur Biophys J.*, 39(9): 1289-1299.
- Mukherjee, S.; Zha, X.; Tabas, I.; Maxfield, F.R. (1998). Cholesterol distribution in living cells: fluorescence imaging using dehydroergosterol as a fluorescent cholesterol analog. *Biophys J.*, 75: 1915-1925.
- Mulya, A.; Lee, J.Y.; Gebre, A.K.; Thomas, M.J.; Colvin, P.L.; Parks, J.S. (2007). Minimal lipidation of pre-beta HDL by ABCA1 results in reduced ability to interact with ABCA1. *Arterioscler Thromb Vasc Biol.*, 27(8): 1828-1836.
- Obici, L.; Bellotti, V.; Mangione, P.; Stoppini, M.; Arbustini, E.; Verga, L.; Zorzoli, I.; Anesi, E.; Zanolli, G.; Campana, C.; Viganò, M.; Merlini, G. (1999). The new apolipoprotein A-I variant Leu¹⁷⁴→Ser causes chardiac amyloidosis, and the fibrils are constituted by the 93-residue N-terminal polypeptide. *Am J Pathol.*, 155(3): 695-702.
- Obici, L.; Franceschini, G.; Calabresi, L.; Giorgetti, S.; Stoppini, M.; Merlini, G.; Bellotti, V. (2006). Structure, function and amyloidogenic propensity of apolipoprotein A-I. *Amyloid.*, 13(4): 191-205.
- Oram, J.F. (2008). The ins and outs of ABCA. *J Lipid Res.*, 49: 1150-1151.
- Pastore, L.; Belalcazar, L.M.; Oka, K.; Cela, R.; Lee, B.; Chan, L.; Beaudet, A.L. (2004). Helper-dependent adenoviral vector-mediated long-term expression of human apolipoprotein A-I reduces atherosclerosis in apo E-deficient mice. *Gene*, 327(2): 153-60.
- Raimondi, S.; Guglielmi, F.; Giorgetti, S.; Di Gaetano, S.; Arciello, A.; Monti, D.M.; Relini, A.; Nichino, D.; Doglia, S.M.; Natalello, A.; Pucci, P.; Mangione, P.; Obici, L.; Merlini, G.; Stoppini, M.; Robustelli, P.; Tartaglia, G.G.; Vendruscolo, M.; Dobson, C.M.; Piccoli, R.; Bellotti, V. (2011). Effects of the known pathogenic mutations on the aggregation pathway of the amyloidogenic peptide of apolipoprotein A-I. *J Mol Biol.*, 407(3): 465-476.
- Rohrer, L.; Cavelier, C.; Fuchs, S.; Schlüter, M.A.; Völker, W.; von Eckardstein, A. (2006). Binding, internalization and transport of apolipoprotein A-I by vascular endothelial cells. *Biochim Biophys Acta.*, 1761: 186-194.
- Ryan, R.O.; Yokoyama, S.; Liu, H.; Czarnecka, H.; Oikawa, K.; Kay, C.M. (1992). Human apolipoprotein A-I liberated from high-density lipoprotein without denaturation. *Biochemistry*, 31(18): 4509-4514.
- Rye, K.A.; Hime, N.J.; Barter, P.J. (1997). Evidence that cholesteryl ester transfer protein-mediated reductions in reconstituted high density lipoprotein size involve particle fusion. *J Biol Chem.*, 272(7): 3953-3960.
- Rye, K.A. & Barter, P.J. (2004). Formation and metabolism of prebeta-migrating, lipid-poor apolipoprotein A-I. *Arterioscler. Thromb. Vasc. Biol.* 24(3): 421-428.
- Sacks, F.M.; Rudel, L.L.; Conner, A.; Akeefe, H.; Kostner, G.; Baki, T.; Rothblat, G.; de la Llera-Moya, M.; Asztalos, B.; Perlman, T.; Zheng, C.; Alaupovic, P.; Maltais, J.A.; Brewer, H.B. (2009). Selective delipidation of plasma HDL enhances reverse cholesterol transport in vivo. *J. Lipid Res.* 50(5): 894-907.
- Sanghera N. & Pinheiro, T.J. (2002) Binding of prion protein to lipid membranes and implications for prion conversion. *J Mol Biol.*, 315(5): 1241-1256.

- Sarnataro, D.; Campana, V.; Paladino, S.; Stornaiuolo, M.; Nitsch, L.; Zurzolo, C. (2004). PrP(C) association with lipid rafts in the early secretory pathway stabilizes its cellular conformation. *Mol Biol Cell.*, 15(9): 4031-4042.
- Shanmugam, G. & Jayakumar, R. (2004). Structural analysis of amyloid beta peptide fragment (25-35) in different microenvironments. *Biopolymers.* 76: 421-434.
- Silva, R.A.; Hilliard, G.M.; Fang, J.; Macha, S.; Davidson, W.S. (2005). A three-dimensional molecular model of lipid-free apolipoprotein A-I determined by cross-linking/mass spectrometry and sequence threading. *Biochemistry.*, 44(8): 2759-2769.
- Sorci-Thomas, M.G. & Thomas M.J. (2002). The effects of altered apolipoprotein A-I structure on plasma HDL concentration. *Trends Cardiovasc. Med.*, 12: 121-128.
- Sreerama, N. & Woody, R.W. (2000). Estimation of protein secondary structure from circular dichroism spectra: comparison of CONTIN, SELCON, and CDSSTR methods with an expanded reference set. *Anal Biochem.*, 287(2): 252-260.
- Steward, A.; Adhya S.; Clarke, J. (2002) Sequence conservation in Ig-like domains: the role of highly conserved proline residues in the fibronectin type III superfamily. *Mol. Biol.*, 318(4): 935-940.
- Tanaka, M.; Dhanasekaran, P.; Nguyen, D.; Ohta, S.; Lund-Katz, S.; Phillips, M.C.; Saito, H. (2006). Contributions of the N- and C-terminal helical segments to the lipid-free structure and lipid interaction of apolipoprotein A-I. *Biochemistry*, 45: 10351-10358.
- Taraboulos, A.; Scott, M.; Semenov, A; Avrahami, D.; Laszlo, L.; Prusiner, S.B. (1995). Cholesterol depletion and modification of COOH-terminal targeting sequence of the prion protein inhibit formation of the scrapie isoform. *J Cell Biol.*, 129(1):121-32.
- Tartaglia, G.G.; Pawar, A.P.; Campioni, S.; Dobson, C.M.; Chiti, F.; Vendruscolo, M. (2008). Prediction of aggregation-prone regions in structured proteins. *J. Mol. Biol.*, 380: 425-436.
- Tofaris, G.K.; Layfield, R. & Spillantini, M.G. (2001). alpha-synuclein metabolism and aggregation is linked to ubiquitin-independent degradation by the proteasome. *FEBS Lett.*, 509: 22-26.
- Tseng, B.P.; Green, K.N.; Chan, J.L.; Blurton-Jones, M.; Laferla, F.M. (2008). Abeta inhibits the proteasome and enhances amyloid and tau accumulation. *Neurobiol Aging.*, 29(11): 1607-1618.
- Uversky, V.N. (2002). Natively unfolded proteins: a point where biology waits for physics. *Protein Science*, 11(4): 739-756.
- Vedhachalam, C.; Duong, P.T.; Nickel, M.; Nguyen, D.; Dhanasekaran, P.; Saito, H.; Rothblat, G.H.; Lund-Katz, S.; Phillips, M.C. (2007). Mechanism of ATP-binding cassette transporter A1-mediated cellular lipid efflux to apolipoprotein A-I and formation of high density lipoprotein particles. *J. Biol. Chem.*, 282: 25123-25130.
- Wang, N.; Silver, D.L.; Costet P.; Tall, A.R. (2000). Specific binding of ApoA-I, enhanced cholesterol efflux, and altered plasma membrane morphology in cells expressing ABC1. *J Biol Chem.*, 275(42): 33053-33058.
- Weinreb, P.H.; Zhen, W. ; Poon, A.W. ; Conway, K.A. ; Lansbury, P.T. Jr. (1996). NACP, a protein implicated in Alzheimer's disease and learning, is natively unfolded. *Biochemistry*, 35(43): 13709-13715.
- Wootton, J.C. & Federhen, S. (1996). Analysis of compositionally biased regions in sequence databases. *Methods Enzymol*, 266: 554-571.

- Zampagni, M.; Cascella R.; Casamenti F.; Grossi C.; Evangelisti E.; Wright D.; Becatti M.; Liguri G.; Mannini B.; Campioni S.; Chiti F.; Cecchi C. (2010). A comparison of the biochemical modifications caused by toxic and nontoxic protein oligomers in cells. *J Cell Mol Med.*, doi: 10.1111/j.1582-4934.2010.01239.x.
- Zha, X.; Genest, J. Jr & McPherson, R. (2001). Endocytosis is enhanced in Tangier fibroblasts: possible role of ATP-binding cassette protein A1 in endosomal vesicular transport. *J Biol Chem.* 276(42): 39476-39483.
- Zhang, NY, Tang Z, Liu CW. (2008). alpha-Synuclein protofibrils inhibit 26 S proteasome-mediated protein degradation: understanding the cytotoxicity of protein protofibrils in neurodegenerative disease pathogenesis. *J Biol Chem.*, 283(29): 20288-20298.
- Zhu, M. & Fink, A.L. (2003). Lipid binding inhibits alpha-synuclein fibril formation. *J Biol Chem.*, 278(19): 16873-16877.

IntechOpen



Amyloidosis - Mechanisms and Prospects for Therapy

Edited by Dr. Svetlana Sarantseva

ISBN 978-953-307-253-1

Hard cover, 216 pages

Publisher InTech

Published online 22, September, 2011

Published in print edition September, 2011

Amyloidoses are a heterogeneous group of diverse etiology diseases. They are characterized by an endogenous production of abnormal proteins called amyloid proteins, which are not hydrosoluble, form depots in various organs and tissue of animals and humans and cause dysfunctions. Despite many decades of research, the origin of the pathogenesis and the molecular determinants involved in amyloid diseases has remained elusive. At present, there is not an effective treatment to prevent protein misfolding in these amyloid diseases. The aim of this book is to present an overview of different aspects of amyloidoses from basic mechanisms and diagnosis to latest advancements in treatment.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Daria Maria Monti, Renata Piccoli and Angela Arciello (2011). Apolipoprotein A-I Associated Amyloidoses: The Intriguing Case of a Natively Unfolded Protein Fragment, Amyloidosis - Mechanisms and Prospects for Therapy, Dr. Svetlana Sarantseva (Ed.), ISBN: 978-953-307-253-1, InTech, Available from: <http://www.intechopen.com/books/amyloidosis-mechanisms-and-prospects-for-therapy/apolipoprotein-a-i-associated-amyloidoses-the-intriguing-case-of-a-natively-unfolded-protein-fragmen>

INTech
open science | open minds

InTech Europe

University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2011 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the [Creative Commons Attribution-NonCommercial-ShareAlike-3.0 License](https://creativecommons.org/licenses/by-nc-sa/3.0/), which permits use, distribution and reproduction for non-commercial purposes, provided the original is properly cited and derivative works building on this content are distributed under the same license.

IntechOpen

IntechOpen