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Review

ATR-FTIR: A "rejuvenated" tool to investigate amyloid proteins ☆

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ABSTRACT

Amyloid refers to insoluble protein aggregates that are responsible for amyloid diseases but are also implicated in important physiological functions (functional amyloids). The widespread presence of protein aggregates but also, in most of the cases, their deleterious effects explain worldwide efforts made to understand their formation, structure and biological functions. We emphasized the role of FTIR and especially ATR-FTIR techniques in amyloid protein and/or peptide studies. The multiple advantages provided by ATR-FTIR allow an almost continuous structural view of protein/peptide conversion during the aggregation process. Moreover, it is now well-established that infrared can differentiate oligomers from fibrils simply on their spectral features. ATR-FTIR is certainly the fastest and easiest method to obtain this information. ATR-FTIR occupies a key position in the analysis and comprehension of the complex aggregation mechanism(s) at the oligomer and/or fibril level. These mechanism(s) seem to present strong similarities between different amyloid proteins and might therefore be extremely important to understand for both disease-associated and functional amyloid proteins. This article is part of a Special Issue entitled: FTIR in membrane proteins and peptide studies.

Contents

Abbreviations: AD, Alzheimer's disease; ADDLs, amyloid-beta derived diffusible ligands; ANS, 8-anilino-1-naphthalenesulfonic acid; ATR, Attenuated total reflection; Aβ, Amyloid beta; CD, circular dichroism; EM, electron microscopy; EPR, electron paramagnetic resonance; FTIR, Fourier-transform infrared spectroscopy; HETs, prion of the filamentous fungus P. anserine; hIAPP, human Islet Amyloid Polypeptide; IAPP, Islet Amyloid Polypeptide; IRE, internal reflection element; PrP, prion protein; PrP^{C}, cellular prion protein; PrP^{Sc}, pathological (scrapie) isoform of the prion protein; SDS-PAGE, Sodium dodecyl sulfate polyacrylamide gel electrophoresis; SH3 domain, Src homology 3 domain; ssNMR, solid-state Nuclear magnetic resonance; ThT, Thioflavine T; TTR, Transthyretin; WB, Western Blot

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1. Introduction

Amyloid refers to insoluble protein aggregates that are responsible for amyloid diseases (e.g. Alzheimer's, Parkinson's, Huntington's, prion's diseases, type II diabetes, and also some cancers) affecting large populations worldwide [1,2]. But they are also implicated in the binding and storage of peptide hormones in the brain, formation of bacterial biofilms, melanin formation and initiation of antiviral innate immune response (this latter category is nowadays referred to as "functional amyloids" and the number of proteins involved in these is

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expected to increase rapidly in the near future) [3–7]. The widespread presence of protein aggregates but also, in most the cases, their deleterious effects explain worldwide efforts made to understand their formation, structure and biological functions [8].

Amyloid fibrils present an unbranched filamentous morphology [9] and are characterized by β-strand repeats running perpendicular to the fiber axis. This structure named cross-β is a unique motif among protein folds [2,10]. Whether any protein is able to form amyloids is still a matter of debate and reassesses our current knowledge underlying the principles of protein folding, misfolding and aggregation. Two apparently contradictory theories are currently proposed. The first one postulates that fibril formation is a general property occurring for any polypeptide chain in some circumstances (sometimes far from any physiologically relevant conditions) [11], while the second restricts their formation to specific proteins collectively classified as the 'amylome' [12].

Extensive efforts have been carried out during the past few years to understand the pathways leading to this structural conversion [13]. Fibril formation is presently described by a two-step process. The *nucleated conformational changes* conversion of the native polypeptide chain promotes oligomer species (Fig. 1). This first step is often subdivided into a lag phase and a nucleation phase. The *elongation* process leads to mature fibrils by a self-template growth mechanism [14–16]. Fibrils can also undergo fragmentation which enhances the kinetics of the aggregation process by increasing their number and suppressing the limiting step of misfolded conformer's production [17–19]. Those two misfolding processes are not exclusive and may occur concomitantly.

The common structural motif shared by fibrils for all amyloidogenic proteins have suggested a common mechanism of aggregation and toxicity [20–22]. Why and how protein aggregates is largely not understood. Moreover, during the aggregation several species are formed and do not contribute in the same way to cell toxicity. It is not clear whether specific species (fibrils [23], oligomers [24,25]) or membrane-associated conformational changes [26,27] lead to cell death. This raises the question of a relationship between structure and cytotoxicity [28–34].

Fibrils have been characterized using multiple biophysical techniques such as Electron Microscopy (EM) [9,35,36], Atomic Force Microscopy (AFM) [37–40], Electron Paramagnetic Resonance (EPR) [41–44] and at higher resolution using solid state Nuclear Magnetic Resonance (ssNMR) [45–50] and X-ray diffraction [9] (for reviews see [10] and [2]). On the other hand, little information on the structure and properties of oligomers is available and no real agreement has been currently reached to describe their function in the whole aggregation process [10,45,51–53] (Fig. 1).

As previously mentioned, biophysical tools have been nicely exploited to provide important information regarding the 3D structure of fibrils [35,54–56]. The main limitation of these high resolution techniques is the important amount of protein material required, the recording time and the necessity to deal with insoluble (or at least hydrophobic or amphipathic) aggregates which above all are in constant equilibrium between different aggregated states. Fourier-transform infrared spectroscopy (FTIR) offers a unique opportunity to overcome those limitations and allows the characterization of the aggregates formed during the aggregation. Recording time is short, preventing time dependent structural changes. A few μg of protein is sufficient to evaluate the secondary structure of aggregates. Moreover, contrary to circular dichroism (CD), in FTIR β-sheet contribution has the highest absorption coefficient; therefore FTIR is particularly well-adapted to analyze β-sheet-rich proteins. Keeping all the advantages of "classical" FTIR, attenuated total reflection (ATR) (sometimes also referred to as attenuated total reflectance) FTIR provides additional extremely useful features when studying amyloid proteins. ATR-FTIR overcomes the problem of insolubility because the sample is analyzed as a thin film on the surface of an internal reflection element (IRE). Moreover, proteins (and more generally molecules) in this thin film can be oriented; therefore, linear dichroism experiments can provide useful information about the relative orientation of the corresponding dipoles which could then be related to the orientation of secondary structures. Recent ATR devices require less than 100 ng of proteins to record good quality

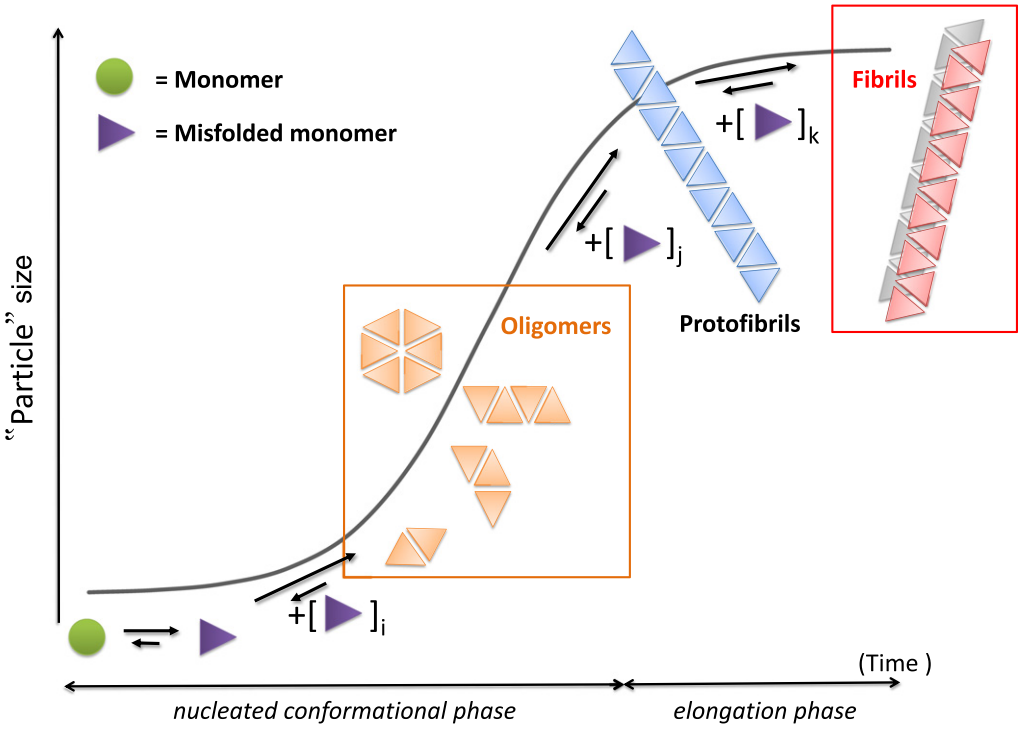

Fig. 1. Schematic representation of the aggregation process of amyloid proteins. This scheme displayed different entities or 'particles' observed along the process including oligomers at the end of the nucleation phase and before the elongation one. During the nucleation phase, native monomers are transformed into misfolded ones and then started to aggregate into bigger particles (collectively called oligomers). During the elongation phase, addition of misfolded monomers and/or oligomers at the extremities of protofibrils and association of protofibrils aimed at fibril formation.