Biophysical Chemistry 199 (2015) 17–24

Contents lists available at ScienceDirect

Biophysical Chemistry

journal homepage: http://www.elsevier.com/locate/biophyschem

Decoding vibrational states of Concanavalin A amyloid fibrils

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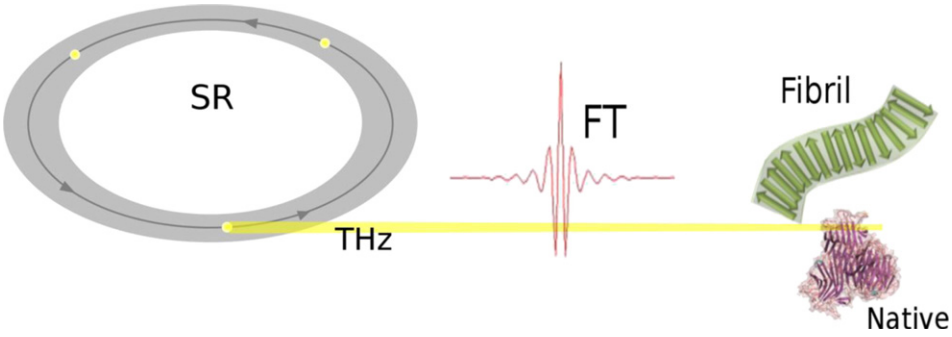
HIGHLIGHTS

- Simple coupling of low demanding techniques reveal amyloid structural details
- Raman peaks of fibrils at 1690 and 1620 cm^{-1} have been confirmed. These peaks have been rarely observed in Raman spectra.
- Fermi doublet show aromatic chains (Tyr) organization in fibrillar aggregates.
- We observe THz features of amyloid fibrils in comparison with native structure.
- THz spectroscopy highlights significant changes in hydration water around proteins building up fibril.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 3 December 2014
Received in revised form 4 February 2015
Accepted 13 February 2015
Available online 21 February 2015

Keywords:
Amyloid
Fibrils
Raman
FTIR
Terahertz spectroscopy

ABSTRACT

Amyloid and amyloid-like fibrils are a general class of protein aggregates and represent a central topic in life sciences for their involvement in several neurodegenerative disorders and their unique mechanical and supramolecular morphological properties. Both their biological role and their physical properties, including their high mechanical stability and thermodynamic inertia, are related to the structural arrangement of proteins in the aggregates at molecular level. Significant variations may exist in the supramolecular organization of the commonly termed cross-β structure that constitutes the amyloid core. In this context, a fine knowledge of the structural details in fibrils may give significant information on the assembly process and on possible ways of tuning or inhibiting it. Here we propose a simple method based on the combined use of Fourier transform infrared spectroscopy and Fourier transform Raman spectroscopy to accurately reveal structural details in the fibrillar aggregates, side-chain exposure and intermolecular interactions. Interestingly, coupled analysis of mid-infrared spectra reveals antiparallel β-sheet orientation in ConA fibrils. We also report the comparison between THz absorption spectra of Concanavalin A in its native and fibrillar state at different hydration levels, allowing obtaining corroboration of peaks assignation in this range and information on the effect of amyloid supramolecular arrangement on the network dynamics of hydration water.

1. Introduction

The processes bringing proteins to associate and form amyloid aggregates, the structural properties of the aggregation products and

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http://dx.doi.org/10.1016/j.bpc.2015.02.007
0301-4622/

their biological relevance are central topics in the current biophysical and biochemical studies [1]. In suitable physicochemical conditions, proteins can modify their native conformation and associate to form supramolecular assemblies called amyloid fibrils, which are known to be involved in the etiology of many neurodegenerative disorders like Parkinson's and Alzheimer's diseases [2,3]. Amyloid fibrils consist of elongated structures whose spine is formed by multiple β-sheets that run parallel to the fibril axis [4] and represents the most stable state for the polypeptide chain [5]. These aggregates are characterized by peculiar physical and mechanical properties (like stability, high mechanical strength, ability to form gels and films) that make them particularly appealing for their application as nanomaterials [6]. The origin of these properties is certainly structure-dependent [6–9] and the possibility of modulating and manipulating them is linked to the knowledge of the fine structural details of amyloid fibrils. Detailed structural properties rule structural flexibility, surface topology and the distribution of hydrophobic regions in the surface of amyloids thus affecting their interaction with the environment, which is fundamental for their biological function [10,11]. Advances in biophysical technologies recently gave the possibility of revealing amyloid fibrils structure in detail and their supramolecular organization by means of high-resolution techniques as X-ray crystallography, solid-state nuclear magnetic resonance (SSNMR), and cryo-electron microscopy (cryoEM) [12–14].

Vibrational spectroscopy is known to be highly sensitive to protein structure. In particular, vibrational modes in the mid infrared region are sensitive to the secondary structural changes occurring upon amyloid fibrils formation [15–17], mainly in the amide I (amide I′ in deuterated environment) band, centered at approximately 1660 (1650) cm^{-1}. At much lower frequency, vibrational modes falling in the far infrared region (THz range) can, in principle, provide information about long-range correlations, compactness and solvent-side chains coupling in proteins and, as a consequence, represent a powerful tool to investigate protein aggregates [8,18]. However, while much information is available about mid infrared absorption profiles of amyloids, only recently the study of THz collective vibrations modes in hydrated proteins has received attention. For example, low frequency modes, falling in the THz range, have been proposed to be involved in the optimization of biological energy transport by driving biochemical reactions and to be connected to the mechanical resistance of proteins and proteins aggregates [8,18,19]. THz spectroscopy provides also information about hydration water organization around proteins. Two primary types of protein/hydration water motions have been predicted to contribute to the low frequency spectrum of proteins: local librational motions (hindered rotations) of side chains and solvent, and large-scale correlated motions of the polypeptide backbone [19–24]. However, due to the collective character of THz modes, a molecular assignment of vibrational states often requires a complex normal mode analysis that takes into account the entire protein structure and, possibly, the interactions with the environment. A. Markelz and co-workers recently provided experimental evidence of narrow features of native egg white lysozyme in the 1–3 THz region and connected their spectral position and intensity to the structural state of the protein [18]. Concerning amyloid fibrils, it has been shown that a broad absorption peak associated to the fibrillar state of insulin exists in the region around 2 THz [25]. More recently, Schirò and co-workers showed, by means of inelastic neutron scattering, that fibrillation of glucagon induces a change in the shape of the THz spectrum, which is dependent on fibril morphology and hydration state [26].

Here we show how the exploration, by a combined use of Fourier transform infrared (FT-IR) and Fourier transform Raman (FT-Raman) spectroscopy, of the broad infrared spectrum from mid infrared (MIR) to the THz range may accurately reveal structural details of amyloid-like fibrils like secondary arrangement, hydration water properties, side-chain exposure and intermolecular interactions. Our results demonstrate how the coupled use of MIR and Raman spectra allows obtaining detailed structural insights on amyloid fibrils by means of low demanding experimental and analysis techniques. The obtained information are used to support the interpretation of THz absorption spectra that reveal structure-dependent THz absorption modes of hydration water around amyloid fibrils which are not present in the native protein. This latter observation enables to speculate on the relationship between β-sheet rich aggregates and their environment.

The present study is focused on Concanavalin A (ConA) in both the native and the fibril state. ConA was chosen as a model system for its structural and aggregation properties. This protein has an "all-β" secondary structure in the native state (N-ConA) and it may form long and thin fibrils (F-ConA) *via* a highly repeatable non-nucleated aggregation mechanism [27]. Interestingly, this protein shows a large structural homology to the human serum amyloid protein, generally present in all the in vivo fibril deposits [28] and it induces apoptosis on tumoral cells with a mechanism related to its aggregation [27,29].

2. Experimental methods

2.1. Sample preparation

Concanavalin A (type IV, L7647) was purchased from Sigma Aldrich and used without further purification. Con A amyloid fibrils in powder form have been prepared as described elsewhere [26,29]. Briefly 0.5 mg/ml of freshly prepared solution was incubated at 37 °C for 150 min in potassium phosphate buffer at pH 8.9 to obtain fibrils, then aggregation was quenched putting the sample in ice and samples were dialyzed against H_2O to remove buffer salts then they have been lyophilized. Dry powders have been dissolved in D_2O (10 mg/ml protein concentration) for FT-IR measurements. The aggregation state was verified by means of Thioflavin T test and confocal fluorescence microscopy.

Samples for THz spectroscopy were prepared as previously reported [30]. Briefly, to obtain dry samples ($h = 0$) samples were vacuum dried for 24 h and mass weight variation was measured to control hydration changes. The dried powders were then held in atmosphere of H_2O and left to reach the hydration level $h = 0.2$ and $h = 0.6$, determined by measuring the mass change. Dry and hydrated F-ConA and N-Con A were prepared in parallel with scrupulously identical treatments.

2.2. FT-MIR

Lyophilized powders were dissolved in D_2O (10 mg/ml) for FT-IR analysis. Infrared measurements were carried out on protein solutions with a Bruker Vertex 70 spectrometer equipped with a DTGS (doped triglycine sulfate) detector, in a sample compartment, under continuum purging in N_2 dry atmosphere. The protein solution was loaded into a liquid cell equipped with CaF_2 windows 2 mm thick; a mylar spacer 25 μm thick was used. Measurements were performed in transmission mode, in the 400–4000 cm^{-1} frequency range. Every measurement is the sum of 256 interferograms at 4 cm^{-1} resolution. Each spectrum is the average of five independent measurements. The contribution of the background was eliminated form Amide I′ bands by subtracting buffer spectrum. The subtraction procedure was optimized in the region of H_2O bending, at about 1643 cm^{-1}. Amide I′ bands were thus normalized.

2.3. FT-Raman

FT-Raman measurements were carried out with a Bruker Vertex 70 spectrometer equipped with a Ge N_2-cooled detector, using an excitation laser with a wavelength of 1064 nm. Protein powders were loaded into a stainless steel (type 316L) sample holder. In order to avoid the damaging of the samples, the power of the excitation laser (at the source) was set around 30 mW. Measurements were performed in the 400–4000 $cm^{-}1$ frequency range. Since we were interested in the spectral position of Raman features, we choose to work with non-deuterated