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Review

FTIR spectroscopic imaging of protein aggregation in living cells ☆

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ABSTRACT

Protein misfolding and aggregation are the hallmark of a number of diseases including Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and the prion diseases. In all cases, a naturally-occurring protein misfolds and forms aggregates that are thought to disrupt cell function through a wide range of mechanisms that are yet to be fully unraveled. Fourier transform infrared (FTIR) spectroscopy is a technique that is sensitive to the secondary structure of proteins and has been widely used to investigate the process of misfolding and aggregate formation. This review focuses on how FTIR spectroscopy and spectroscopic microscopy are being used to evaluate the structural changes in disease-related proteins both in vitro and directly within cells and tissues. Finally, ongoing technological advances will be presented that are enabling time-resolved FTIR imaging of protein aggregation directly within living cells, which can provide insight into the structural intermediates, time scale, and mechanisms of cell toxicity associated with aggregate formation. This article is part of a Special Issue entitled: FTIR in membrane proteins and peptide studies.

Contents

1. Introduction

Many diseases involve the misfolding and aggregation of naturally occurring proteins in the body. These aggregates disrupt cellular function through a number of different mechanisms, often resulting in cell death [1]. This process is most prevalent in neurodegenerative diseases [2,3]. For example, Alzheimer's disease is characterized by the misfolding of the amyloid beta (Abeta) protein, which leads to amyloid plaques, whereas structural changes associated with hyperphosphorylation of the tau protein leads to the formation of neurofibrillary tangles. In Parkinson's and Huntington's disease, aggregates of α-synuclein and huntingtin are observed, respectively. A familial form of amyotrophic lateral sclerosis (ALS) is characterized by tiny aggregates of copper-zinc superoxide dismutase (SOD1). Prion diseases such as mad cow disease (bovine spongiform encephalopathy), Creutzfeldt–Jakob disease, scrapie, and chronic wasting disease are associated with the misfolding of the prion protein, which becomes infectious in its aberrant form. Not all protein-folding diseases occur in the central nervous system; other examples include cystic fibrosis, type II diabetes, and multiple myeloma

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[4]. In all cases, protein misfolding is associated with a change in secondary structure.

Fourier transform infrared spectroscopy (FTIR) has been shown to be sensitive to the secondary structure of proteins, making it a valuable technique for studying protein aggregation. A protein's FTIR spectrum has two prominent features, the Amide I (~1650 cm^{-1}) and Amide II (~1540 cm^{-1}) bands, where the former arise primarily from the C═O stretching vibration and the latter is attributed to the N – H bending and C – N stretching vibrations of the peptide backbone [5]. The frequency of the Amide I band is particularly sensitive to secondary structure based on different hydrogen-bonding environments for α-helix, β-sheet, turn, and unordered conformations. For example, α-helices and β-sheets have Amide I vibrational frequencies at approximately 1655 and 1630 cm^{-1}, respectively [6,7]. The vibrational frequency of an aggregated protein falls around 1620–1625 cm^{-1} due to the distinct hydrophobic environment [8]. For most proteins, a mixture of secondary structures exists; thus, the Amide I band represents a combination of these components (Fig. 1). As a first approximation, the Amide I band can be curve-fit to predict a protein's secondary structure, or database approaches based on crystallographic information have also been used [9].

Beyond the examination of protein secondary structure in vitro, FTIR spectroscopic microscopy can also be used to directly study protein misfolding and aggregation within cells and tissues. Protein aggregates are typically very small, where initial oligomers form at the nanoscale and the larger aggregates are only 20–30 μm in size. In addition, the spectral differences associated with changes in protein conformation are subtle, requiring spectra with a high signal-to-noise (S/N) ratio. These challenges have been addressed recently by taking advantage of the high brightness of a synchrotron infrared source, where protein misfolding and aggregation have been examined directly within diseased tissue in Alzheimer's disease [8,10–12], Parkinson's disease [13], Huntington's disease [14], amyotrophic lateral sclerosis [15], and scrapie [16–18].

In this review, we will describe how FTIR spectroscopy and microscopy have been used to examine protein misfolding and aggregation both in vitro and in situ. The challenges associated with data collection and spectral interpretation will be discussed along with recent technological developments and opportunities for future studies.

2. FTIR spectroscopy of protein structure in vitro

Since the Amide I band is sensitive to protein secondary structure, FTIR spectroscopy is frequently used to study the process of protein misfolding and aggregation in vitro. Due to unique hydrogen bonding environments for the different secondary structure elements, shifts are observed in the frequency of the Amide I band [6,19]. And by studying protein structure in vitro, the effects of temperature, pH, solvent, and protein conformation can be systematically examined.

The major difficulty with measuring FTIR spectra of proteins in water arises from the strong water bending mode that overlaps the Amide I vibrational mode. Thus, transmission measurements of protein solutions are typically performed in D_2O, where the O – D bending mode is shifted to lower frequency (Fig. 2). Alternatively, spectra can be collected in water with very thin pathlengths (<10 μm), but this requires a higher protein concentration. For FTIR spectroscopy of protein solutions in D_2O, protein concentrations of 0.5–10 mM are common. Protein solutions are placed in a sandwich cell between two IR-transparent windows (typically CaF_2 or BaF_2), separated by a thin spacer (typically 25–50 μm thick). The reference (background) spectrum is collected through the buffer solution.

Secondary structure analysis of the Amide I band has been performed in a number of ways, including second derivatives, Fourier self-deconvolution, curve-fitting, and neural networks [6,7,9,19–23]. Most frequently, second-derivative analysis is used to identify the frequencies of the underlying spectral components. Then spectral deconvolution (i.e. curve-fitting) is performed on the Amide I band to define the intensities of the components (see Fig. 1). While absolute secondary structure quantification can be done, the best results are achieved when structural *changes* are studied as a function of an external perturbation, as has been demonstrated for the amyloid-beta protein [24].

2.1. Alzheimer's disease

Protein misfolding and aggregation have been studied extensively in vitro using FTIR spectroscopy. For example, Alzheimer's disease (AD) is a neurodegenerative disorder characterized by the accumulation of senile plaques and neurofibrillary tangles in gray matter areas of the brain. Plaque formation is brought about by the transformation of a small peptide known as amyloid-beta (Abeta) from a soluble form through an oligomeric intermediate to an aggregated, fibrillary structure [25].

The mechanism behind the structural changes and toxicity of Abeta during aggregate formation has been the subject of numerous in vitro studies. The seminal studies utilized circular dichroism (CD) and NMR to show the structural conversion of Abeta from a soluble α-helical protein to a fibrillar β-sheet protein [26–29]. More recently, FTIR spectroscopy has been used to study the specific alignment of β-sheet strands within Abeta fibrils. Using isotopically-labeled Abeta(16–22), Petty and coworkers showed conclusively that the β-sheets are antiparallel and in alignment across all strands. This realignment of the peptide strands is important for therapeutics because they form more stable amyloid fibrils [30].

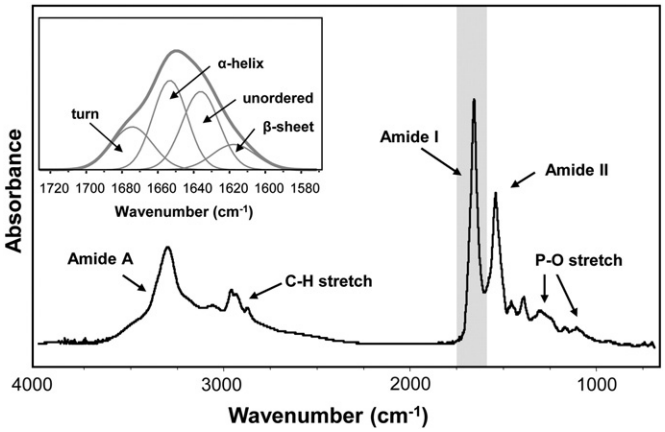

Fig. 1. FTIR spectrum of a typical protein illustrating the Amide I and Amide II bands at ~1650 cm^{-1} and ~1540 cm^{-1}, respectively. (Inset) Expanded view of the Amide I band, which can be deconvolved into its secondary structure components.

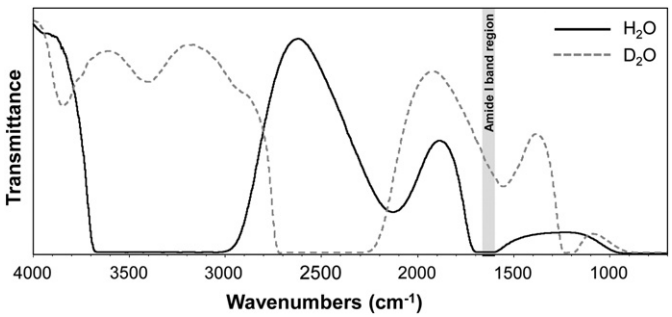

Fig. 2. FTIR transmission spectra of H_2O and D_2O. As can be seen, an O – H bending mode overlaps with the protein Amide I band at ~1650 cm^{-1}. With deuteration, the O – D bending mode falls at a lower frequency (~1225 cm^{-1}), increasing the IR transmission in the region of the Amide I band and enabling better background (i.e. solvent) subtraction.