Mid-infrared absorption spectroscopy of protein aggregates using germanium on silicon waveguides

V. Mittal1, G. Devitt2, P.N. Bartlett3, H. M. H. Chong4, G. Z. Mashanovich1, S. Mahajan2 and J. S. Wilkinson

1 Optoelectronics Research Centre, University of Southampton, Southampton, SO17 1BJ United Kingdom
2 Institute for Life Sciences, University of Southampton, Southampton, SO17 1BJ United Kingdom
3 School of Chemistry, University of Southampton, Southampton, SO17 1BJ United Kingdom
4 School of Electronics and Computer Science, University of Southampton, Southampton, SO17 1BJ United Kingdom

e-mail: v.mittal@soton.ac.uk

ABSTRACT

Proteins in human samples can be used to detect the onset of a group of neurodegenerative diseases such as Alzheimer’s and Parkinson’s by studying their conformational (shape and structure) changes that can cause cognitive impairment. Proteins form aggregates from normal state (monomers) to disease state (amyloid deposition and fibril formation in central nervous system) that is associated with disease progression. These changes can be diagnosed and monitored using mid-infrared (MIR) absorption spectroscopy by studying line shapes and relative absorbance of amide bands. We have demonstrated MIR spectroscopy of proteins in three stages of aggregation: monomers, oligomers and fibrils of Bovine Serum Albumin (BSA) protein on a germanium on silicon (GOS) waveguide in the MIR wavelength region of 5.2 – 10 µm (1900 – 1000 cm⁻¹). The protein samples were also characterised by atomic force microscopy to confirm their structure.

Keywords: mid-infrared, spectroscopy, protein, waveguide, integrated optics, biosensing

1. INTRODUCTION

The MIR region corresponding to the spectral range from 2-25 µm of the electromagnetic spectrum coincides with the molecular vibrational energies. When MIR light is passed through a sample, intermolecular bonds are excited to the higher vibrational states by absorbing the same energy as the difference between its ground and excited states [1]. This enables sensing of unknown analytes using their fingerprint absorption spectra in this region to detect specific bonds. Fourier transform infrared (FTIR) spectroscopy is routinely used for the analysis of bio-chemical species to determine analytical information. However, due to the strong water absorption in the MIR, cuvette length longer than 10 -20 µm cannot normally be used and narrow cuvettes tend to clog with real samples. Attenuated total reflection (ATR) spectroscopy combined with FTIR has been used to overcome this problem. However, the number of discrete reflections in a conventional ATR element is severely limited while using an optical waveguide, which is essentially a thinner ATR element, greatly increases the effective number of reflections per unit length to the extent that in a monomode waveguide a continuous evanescent wave is achieved along the waveguide surface, significantly improving the sensitivity of the device for a given length and have sample volume. MIR evanescent field absorption spectroscopy offers high selectivity over a wide range of compounds and requires less sample volume than the other conventional techniques. Current microfabrication technologies render optical chip mass-producible and hence low-cost as well as allowing integration of various optoelectronics and microfluidics components on the same chip.

Proteins are key functional biomolecules in life and their aggregation is a pathological hallmark for determining neurodegenerative diseases. Conformational changes in aggregation results in the deposition of β-sheet rich toxic amyloids or fibril formation which are both associated with disease progression [2]. The MIR absorption in proteins predominantly occurs due to vibrations of the polypeptide backbones also referred to as amide bands. The amide I, II and III bands lie at wavelengths between 5-10 µm and are used as marker of secondary protein structure. The amide peaks can be analysed by spectral deconvolution to study the structure of proteins and their aggregates.

2. EXPERIMENTAL WORK

2.1 Waveguide fabrication and characterisation

A 3 µm thick GOS 6 inch wafer from IQE Silicon Compounds Ltd was used to fabricate the waveguides. Multimode waveguides of 20 µm wide were patterned lithographically and etched using fluorine chemistry in an inductively coupled plasma (ICP). The end facets were prepared using ductile dicing. Waveguide measurements were carried out using the apparatus shown in Fig.1 a). A quantum cascade laser (QCL) (Block Engineering Inc.) tunable from 1900-800 cm⁻¹ (wavelength of 5.3 – 12.9 µm) was used as the light source and two zinc selenide
(ZnSe) objective lenses were used for input and output coupling. Prior to taking transmission spectra the QCL was set to 12.9 µm and the waveguide output was imaged on an infrared camera (Xenics-Gobi 640) following alignment, and the output intensity profile in TM polarisation is shown in Fig. 1 b). Simulation of modal intensity distributions (COMSOL) in TM polarisation show a FWHM of 10.1 µm and 2.3 µm, along the x and y axis respectively. A thermoelectrically-cooled mercury cadmium telluride (MCT) detector (VIGO System) was used to record the signal from the collection objective lens. The signal from the MCT detector was recorded on a computer which also tuned the QCL, and a software package (LaserTune), was used to process the spectra. As a simple paper-based fluidic structure, a strip of filter paper is used to introduce aqueous analyte onto the surface of the waveguide and to define the evanescent absorption pathlength. A paraffin cover was used on top of the filter paper to avoid sample evaporation. It has been established that the presence of filter paper on the surface of waveguide does not itself significantly alter the optical transmission [3].

2.2 Protein sample preparation

A 900 µM BSA (Sigma Aldrich) solution of the monomeric protein was prepared by mixing it in double distilled water (pH = 3). The monomer solution was kept in an Eppendorf tube in a water bath maintained at 65ºC for 10 min to form agglomerates called Oligomers. To make fibrils, the monomer solution in an Eppendorf tube was kept for 24 hours in the water bath maintained at 65 ºC. For AFM measurement, the fresh protein samples were diluted 1/1000 and then allowed to bind to a mica substrate for two minutes, which was then washed 4 times in double distilled water and allowed to dry. For MIR absorption spectroscopy, the proteins samples stored in Eppendorf tube were introduced on the waveguide surface by using a pipette.

3. RESULTS AND DISCUSSION

AFM was performed to confirm protein aggregation before taking the MIR measurements. Fig. 2 (a), (b) and (c) shows the 1µm x 1µm AFM images of the monomers, oligomers and fibrils of BSA protein respectively. It is clearly observed from the images that the monomers aggregate together to form bigger assemblies known as oligomers as seen in Fig. 2 (b) and to form ribbon-like structures known as fibrils as observed in Fig. 2 (c).

The BSA absorption spectra aqueous buffer and the monomers, oligomers and fibrils at the surface of the waveguide were measured using a 2 mm wide, 4 cm long filter paper strip placed on the surface of the waveguide as shown in Fig. 1. The aqueous buffer (water) from the Eppendorf tube was pipetted onto the tail of the filter paper to allow it to travel to the waveguide and a transmission spectrum with buffer alone was first recorded as a reference spectrum. Then the waveguide surface was cleaned and a monomer solution was pipetted on a fresh filter paper of a similar width. The transmission spectrum for the monomer sample was then recorded. The transmitted power
spectrum from the monomer sample was divided by the transmitted power spectrum from the buffer reference spectrum, and the resultant monomer absorption spectrum was obtained. 10 sample scans were taken and averaged and the data were smoothed using 10-point adjacent averaging. Oligomer and fibril samples were measured in the same way and their absorption spectra are shown in Fig. 3(a). The amide I, II and III peaks at 1650 cm\(^{-1}\), 1540 cm\(^{-1}\) and between 1200-1350 cm\(^{-1}\) respectively, are clearly observed. Fig. 3 (b) shows the amide I and II region of the absorption spectra taken from Fig. 3 (a) to clarify that the absorption peaks shift towards higher frequencies with aggregation. For the amide I peak, the absorption frequency for monomers and oligomers are both at 1650 cm\(^{-1}\), whereas for fibrils it has shifted to 1660 cm\(^{-1}\). For the amide II peak, the absorption frequency for monomers is at 1540 cm\(^{-1}\), that of oligomers is at 1545 cm\(^{-1}\) and that of fibrils are at 1550 cm\(^{-1}\).

Shifts in peak absorption frequencies and an increase in both amide I and II has been observed from monomer to fibril formation and indicates in β-sheet secondary structure. The increase in absorbance may be due to an increased amount of sample in the focal volume because fibrils are far larger than monomers. Fibrils are also insoluble in water so they may deposit onto the surface of the waveguide as a solid film hence increasing the absorbance.

![Waveguide absorption spectra of BSA for monomers, oligomers and fibrils of BSA and (b) zoomed in spectra from (a) to show the peak shifts for the amide I and II peaks.](image)

4. CONCLUSIONS

Germanium on silicon waveguides were fabricated and evanescent field absorption spectroscopy was demonstrated using aqueous solutions of bovine serum albumin. The spectroscopy of monomers and both oligomers and fibrils clearly demonstrated their Amide I, II and III absorption peaks in the MIR region between 1900 cm\(^{-1}\) and 1000 cm\(^{-1}\). Changes in the absorption frequencies, absorption strength and lineshapes of the Amide I and II peaks is observed which can potentially be used to distinguish between the three stages of agglomerate formation and can help in early diagnosis of clinically relevant neurodegenerative diseases. In future, deconvolution of line shapes for different secondary structures (α-helix, β-sheets and random coils and turns) of amide bands will be studied in detail using multivariate analysis.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the EPSRC Electronic-Photonic Convergence Platform grant (EP/N013247/1), a Royal Society Wolfson Research Merit Award, European Research Council (IDEAS-ERC) (FP7/2007-2013, No. 291216) and ERC grant (NanoChemBiovision 638258).

REFERENCES