Development of a quantitative and conformation-sensitive ATR-FTIR biosensor for Alzheimer’s disease: The effect of deuteration on the detection of the \( \text{A}\beta \) peptide

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Abstract. Alzheimer’s disease (AD) is the most common form of dementia worldwide and represents a growing socio-economical issue. To date, no reliable diagnosis can be obtained at an early-stage of the disease, though it is now recognized that the aggregation of the amyloid \( \beta \) (A\( \beta \)) peptide is responsible for the onset of the disease. Recent studies have shown that soluble amyloid oligomers present in the physiological fluids were the most neurotoxic species and correlated best with the first signs of cognitive decline, which makes them good biomarkers in the development of a diagnostic tool.

We describe here a new type of biosensor, based on attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy, that would be able to specifically detect and quantify the presence of the different forms of the A\( \beta \) peptide in solution. The principle of the detection relies on the recognition of the peptides by specific antibodies that were previously grafted on the surface of an ATR element, consisting of a functionalized germanium crystal. We show that the BIA-ATR technology is able to detect the presence of A\( \beta \) if incubated in deuterated water and that this step is crucial in the development of our conformation-sensitive biosensor for AD.

Keywords: Alzheimer’s disease, amyloid \( \beta \) peptide, biosensor, diagnosis, ATR-FTIR spectroscopy

1. Introduction

Amyloidoses comprise a series of pathologies sharing a common mechanism of aggregation of misfolded proteins after a conformational change. Alzheimer’s disease (AD) is a widespread brain-specific amyloidosis affecting more than 30 million people worldwide that is only reliably diagnosed postmortem by the observation of histopathological features of the illness during brain autopsy. Two typical hallmarks of this neurodegenerative disorder consist in fibrillar deposits or amyloid plaques in the extraneuronal spaces and neurofibrillary tangles inside the neurons [2].

These so-called senile plaques are mainly composed of the 38–43 residues amyloid \( \beta \) peptide (A\( \beta \)), which results from the proteolytic cleavage of the Amyloid Precursor Protein (APP). A\( \beta \)(1-40) and

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Aβ(1-42) are the most encountered forms of the peptide. Though less abundant, Aβ(1-42) has a higher propensity to aggregate, which has been related to two additional hydrophobic amino acids at the C-terminus [13]. Studies report that the Aβ peptide plays a major role in the onset of the disease and becomes neurotoxic upon aggregation. Aβ can adopt different conformations, ranging from monomers to soluble higher molecular weight oligomers and eventually insoluble fibrils.

The amyloid cascade hypothesis has considered for a long time the fibrillar cores as responsible for the neurodegeneration but there has been a paradigm shift now supporting that soluble oligomers are the most neurotoxic species [11]. Multiple lines of evidence have demonstrated that the amyloid oligomers are the primary pathogenic structure and correlate better with the first signs of memory loss and cognitive impairment, rather than the mature amyloid fibrils [4]. During the aggregation process leading to fibril formation, oligomeric entities of different size coexist in a dynamic equilibrium. Using attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy, our group has recently shown that these species possess distinct spectral features: fibrils are characterized by a parallel β-sheets conformation while oligomers display an anti-parallel β-sheets structure [3]. Moreover several studies have revealed a particularly high neurotoxic effect for some of these oligomers, such as dimers, trimers or the dodecamer of Aβ(42) [6,16].

Any further structural information about Aβ oligomers could help to unravel the causative role of AD and overcome the limitations with the current diagnostic methods. There is indeed a widespread consensus among the medical and Alzheimer disease research communities that the development of a sensitive and specific diagnostic standard taking into account the presence of the oligomeric forms of Aβ remains a challenging issue.

Besides the classical ELISA tests, several biosensors have been imagined to detect the presence of Aβ in the plasma and cerebrospinal fluid (CSF) of AD patients [1,14]. Biosensors are biofunctionalized devices that enable highly specific interactions between a free analyte (ligand) of interest and a receptor which is tightly bound to the sensor. Detection of the ligand can be obtained using different surface-sensitive techniques, such as fluorescence microscopy, UV-visible spectroscopy, light scattering and surface plasmon resonance (SPR) spectroscopy [10]. These methods yield very sensitive measurements but so far few of them have focused on the detection of the Aβ oligomers, in part owing to their inability to provide any physicochemical information neither about the ligand–receptor interaction nor the conformational properties of the molecule of interest [5].

Recently, Voüé et al. have designed a new type of biosensor based on the “BIA-ATR” technique [15]. Detection of the ligand–receptor interaction is achieved using ATR-FTIR spectroscopy and by the immobilization either of the ligand or of the receptor on the surface of a chemically-treated ATR element, often a germanium (Ge) crystal [7]. The functionalization steps by wet chemistry leading to the modification of the surface properties enable the covalent binding of any kind of molecule containing free amino groups, typically proteins. In our case, four antibodies against Aβ were successfully grafted on the surface of the Ge crystal (6E10 against the N-terminal part of Aβ, 4G8 against residues 17–24 and two conformational nanobodies in the region of aa 18–25). Then solutions containing Aβ at a known concentration were passed through the device under semi-continuous flow. The IR beam that reflects inside the crystal allows detecting molecules in solution in the close vicinity of the surface [8,9] – in this case, the Aβ peptides recognized by the antibodies.

A major advantage of this technique is that it provides a quantitative and qualitative measurement, as well as the possibility to determine conformational changes occurring in the target molecule. In this paper, we use the BIA-ATR approach on a functionalized Ge crystal to address the detection of the Aβ peptide in circulating solutions.
2. Material and methods

2.1. Reagents

Synthetic Aβ(1-40) were purchased from American Peptide (Sunnyvale, CA, USA). The monoclonal antibodies 6E10 and 4G8 were purchased from Covance and the nanobodies Abeta3 and Abeta9 were a generous gift of Professor S. Muyldermans from the Vrije Universiteit Brussel. Deuterated water was purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA).

2.2. Peptide preparation

Aβ(1-40) was dissolved in milliQ water at a 2 mg/ml concentration. The peptides were sonicated 3 times 1 min at room temperature (25°C). The dissolved peptides were then divided into aliquots of 25 µl. Water was evaporated under nitrogen flow and the residual solvent was removed under vacuum using a SpeedVac (ThermoSavant). The resulting Aβ films were stored at −20°C until further manipulation.

2.3. IR measurements

Attenuated total reflection Fourier transform infrared (ATR-FTIR) spectra were recorded on an Equinox 55 infrared spectrophotometer (Bruker Optics) equipped with a MCT detector at a resolution of 8 cm⁻¹. The spectrometer was continuously purged with dry air. The internal reflection element is a triangular-shaped germanium crystal (4.8 mm × 4.8 mm × 45 mm) purchased from Biosentech (Naninne, Belgium). A top plate with a groove fitting the crystal was used in replacement of the diamond-bearing plate (WOW Company, Belgium). With this geometry, a single reflection occurs and ten lanes can be used on a crystal. 460 accumulations per spectrum were performed to improve the signal/noise ratio. The software used for data processing was written under MatLab 7.1 (Mathworks Inc., Natick, MA, USA).

2.4. ATR-FTIR experiments

Each experiment was performed on a single lane under a semi-continuous flow. After grafting of the specific antibodies on the crystal surface, 50 µg of Aβ(1-40) were freshly resuspended in milliQ water or D₂O at a concentration of 50 µg/ml. Using a peristaltic pump, the Aβ peptides were successively put in contact with the antibodies on the biosensor at a flow rate of 5 µl/min and further incubated for 15 min. This operation was repeated at least 4 times before performing a wash out of the lane with milliQ water or D₂O at a flow rate of 10 µl/min in order to remove unbound peptides. At each of these steps, 5 IR spectra were recorded. All measurements were made at 21°C.

3. Results and discussion

Detection of Aβ(1-40) in solution was investigated on a new type of sensing device using the BIA-ATR technique. The experimental setup allowed us to follow the fixation of the Aβ peptides to antibodies covalently bound to the Ge crystal by recording ATR-FTIR spectra at different time points of the experiment [7,8].

In order to evaluate the ability of the biosensor to detect Aβ, solutions of Aβ(1-40) were passed through the device according to a protocol described in Section 2. It was first decided to work with a
concentration of 50 µg/ml to ensure that the peptide would indisputably be visible if recognized. Spectra were recorded every 15 min during the fixation and the washing. As measurements occur in solution, the main contributions in the resulting spectra are coming from the solvent. Several treatments have been established and applied to the spectra to eliminate respectively the contributions of water vapour, solvent, baseline deviation and noise [9].

In the first sets of experiments using water as solvent, no relevant data showing the peptides interacting with the antibodies could be extracted, even after spectra processing (especially subtraction of the water spectral contributions). A possible explanation was either that the composition of the buffer somehow prevented efficient fixation of Aβ or that the solvent could only partially be subtracted from the unprocessed data, resulting in uninterpretable spectra. As removal of the salts did not bring any further improvement in the spectral analysis (data not shown), the contribution of water in the spectra was considered. Water covers a broad spectrum in the infrared that might interfere with the absorption of other components like proteins, as shown in Fig. 1(A) between 1700 and 1500 cm\(^{-1}\). This spectral

![Fig. 1. ATR-FTIR spectra obtained by the BIA-ATR biosensor before and after processing. (A) Comparison of the unprocessed FTIR spectra of Aβ(1-40) 50 µg/ml in the presence of H\(_2\)O or D\(_2\)O recorded on the biosensor. (B) Amide I and II spectral range after processing (water subtraction) of Aβ(1-40) 50 µg/ml in the presence of water at different time points of incubation on the biosensor. No clear spectral features are observed. (C) Amide I and II spectral range of Aβ(1-40) 50 µg/ml in the presence of D\(_2\)O at different time points of incubation on the biosensor (0 min → 90 min from bottom to top). The Amide I bands show characteristic features of antiparallel β sheet with a maximum of absorbance at 1625 cm\(^{-1}\) and a shoulder around 1685 cm\(^{-1}\).](image)
range comprises the Amide I and Amide II bands accounting for the main absorption in proteins. The observation of a wide negative peak in this region after spectral processing suggested the significant implication of water in masking the spectrum of the Aβ peptide (Fig. 1(B)).

It is not our purpose to discuss here the reasons why the peptide contribution could not be observed in the presence of water. It demonstrated the general protocol used for other applications of the BIA-ATR technology [7] was not suited to this case and required to be adapted. To avoid spectral interferences due to water, the peptide Aβ(1-40) was alternatively incubated in deuterated water (D2O) at the same concentration. As a consequence of this, an isotopic shift of more than 400 cm\(^{-1}\) towards lower wavenumbers could be observed for the bending band of water and the presence of the Aβ peptide was progressively revealed in the Amide I′/Amide II′ region of the treated ATR-FTIR spectra (Fig. 1(C)).

The Amide I and Amide II bands respectively around 1650 cm\(^{-1}\) and 1550 cm\(^{-1}\) arise mainly from the absorption of proteins. Amide I is the most intense absorption band of the peptide which accounts for the stretching of C=O bounds. The Amide II contribution occurs between 1545 and 1530 cm\(^{-1}\) and is related to the in-plane N–H bending. Moreover the intensity of these absorption bands is related to the quantity of detected peptide and allows monitoring the protein/antibody interaction [8,9].

Notice that because the experiments were performed in D\(_2\)O, the deuterated Aβ(1-40) displayed a different spectrum. In particular the Amide II′ peak usually visible around 1530–1545 cm\(^{-1}\) shifted towards 1450 cm\(^{-1}\). This shift was attributable to the deuteration of the N–H bounds in the backbone of the peptide. Conversely the Amide I′ representing the stretching of C=O bounds moved only slightly to the right. The overall shape of the spectra correlated with the control spectrum of Aβ(1-40) in D\(_2\)O (data not shown), suggesting that recognition of the peptides by the antibodies did not imply any structural changes in both entities. The spectra revealed a characteristic narrow peak at 1625 cm\(^{-1}\) and a slight shoulder around 1685 cm\(^{-1}\) (Fig. 1(C)) which, as demonstrated in previous studies, correspond to the signature of the antiparallel β-sheet structure of Aβ oligomers [3,12]. Another important contribution of the Amide I′ visible around 1660 cm\(^{-1}\) was coming from the presence of random coil in the structural organization of the peptide. This could be easily explained by the fact that Aβ(1-40) was freshly resuspended and is less prone to aggregation compared to Aβ(1-42).

The goal of the present study was to investigate the detection of Aβ(1-40) in circulating solutions in a new type of biosensor using ATR-FTIR spectroscopy. As visualization of the peptide in the spectra was problematic with aqueous solutions, incubation in deuterated water was considered as an original means to reveal the presence of the Aβ peptides when they are recognized by specific antibodies grafted on the surface of a functionalized Ge crystal. In conclusion we demonstrated here that our biosensor based on the BIA-ATR approach is able to detect the Aβ peptide in solution and the spectra obtained contain spectral features characteristic of the conformation adopted by Aβ. First experiments provided promising results in the future discrimination between the toxic oligomeric and fibrillar forms of Aβ(1-40) and Aβ(1-42). This conformation-sensitivity represents a major advantage compared to the current detection methods and offers new perspectives in early-stage diagnosis of Alzheimer’s disease.

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References


