ATR–FTIR, a new tool to analyze the oligomeric content of A β samples in the presence of apolipoprotein E isoforms

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Abstract. Alzheimer's disease (AD) is a neurodegenerative disorder caused by the aggregation of the amyloid-beta peptide (A β), leading to amyloid plaques deposition in the brain. Although A β aggregation pathway still remains unclear, recent studies point out the enhanced toxicity of oligomers compared to fibrils. The E4 isoform of apolipoprotein E (ApoE) is the major risk factor in AD as people carrying one ϵ 4 allele have significantly higher chances to develop the disease. Nevertheless, this phenomenon is still poorly understood.

Our group has shown that attenuated total reflection Fourier-transform infrared spectroscopy (ATR–FTIR) could discriminate between A β 42 oligomers and fibrils. Indeed, oligomers display anti-parallel β -sheet spectral components while fibrils are characterized by a parallel β -sheet organization. Using those spectral features to analyze the oligomeric content of our samples, we studied the influence of ApoE on A β 42 aggregation. Our experiments demonstrated that ApoE3 increased the amount of A β 42 oligomers in the sample.

We can thus determine the proportion of A β oligomers in the presence of another compound in the sample. We plan to use ATR–FTIR to assess the effects of the other isoforms of ApoE on A β aggregation. Moreover, this could be extended to study the influence of other molecules or proteins on A β aggregation.

Keywords: Amyloid-beta, apolipoprotein E, oligomers, ATR–FTIR

1. Introduction

Alzheimer's disease (AD) is the first cause of dementia worldwide. Advances in medicine tend to raise life expectancy but one of the prices to pay is the increasing incidence of this age-related neurodegenerative disorder. Although numerous studies are carried on to understand and cure the disease, it represents a huge challenge and a lot remains to be elucidated.

The main responsible for AD is most probably the amyloid-beta peptide (A β), which aggregates and finally forms amyloid plaques in the brain. Soluble A β oligomers have been shown to be 10 times more toxic to neurons than insoluble fibrils and 40 times more than monomers [5]. Many factors can influence the development of the disease such as age, sex, cholesterol level, obesity, Down syndrome or even brain stimulation . . . but so far, the presence of one or two ϵ 4 alleles of apolipoprotein E (ApoE) represents the major known genetic risk factor in AD [9]. While E3 isoform of ApoE seems to be neutral regarding the disease, E2 carriers seem to be protected against AD. It has been reported that ApoE could influence

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AD development at several levels [1]: role in A β aggregation, production and clearance, cholesterol transport, synaptic plasticity and repair . . . but no clear mechanism has been proposed so far.

Our group has previously shown that ATR-FTIR spectroscopy structurally discriminates between A β 42 oligomers and fibrils. Indeed, oligomers display anti-parallel β -sheet spectral components while fibrils are characterized by a parallel β -sheet organization [2]. The present work focuses on the effect of ApoE on A β 42 aggregation. Therefore, we applied ATR-FTIR to our A β -ApoE samples to detect changes in the aggregation of A β that could be triggered by direct contact with ApoE. We show that the more ApoE3, the more A β oligomers are found in the samples.

2. Materials and methods

2.1. Materials

A β 42 is purchased from American Peptide (Sunnyvale, CA, USA). We express E3 isoform of ApoE in *E. Coli* (ER2566 strain). The purification step is achieved thanks to the affinity of the intein-tagged ApoE for chitin beads (IMPACT-CN System, New England Biolabs). We obtain a pure and native protein, without any additional residues [4].

2.2. *In vitro* A β -ApoE interaction

After initial pre-treatment as previously described [2], the desiccated peptide is resuspended in 2% of DMSO to destroy all pre-existing structures. Then we dilute it to 70 μ M with Tris Buffer Saline (TBS – 10 mM Tris, 150 mM NaCl, pH 7.4) and add E3 isoform in ApoE-A β molar ratios varying from 1:100 to 1:25 (from 700 nM to 3 μ M of ApoE, respectively). An A β control without any ApoE is always tested in the same conditions. The samples are incubated at 37°C for 24 h before performing experiments.

2.3. Dialysis of A β -ApoE samples

For IR experiments, we first need to get rid of salts from TBS, which interfere with the measurement. As we work with quite small volumes, we use Slide-A-Lyzer Mini dialysis units (Pierce) for 10–100 μ l samples. As ApoE and A β weigh 34 and 4 kDa, respectively, a 10 kDa cut-off was chosen: only A β monomers and dimers, if present, will be lost. The dialysis is performed against 2 l of milliQ water during 1 h at room temperature.

2.4. Attenuated total reflection Fourier-transform infrared spectroscopy

IR experiments were performed on an Equinox 55 infrared spectrophotometer (Bruker optics) as detailed in [2]. 2×3 μ l of each dialyzed sample were plated on the diamond surface and dried under nitrogen flow. Spectra were analyzed with Kinetics, a home-written program running under Matlab, and processed as previously described [2].

3. Results and discussion

Beside the fact that ATR-FTIR is a fast and economical technique in terms of protein quantity (less than one microgram/spectrum is enough), working in attenuated total reflection is ideally suited to study

aggregates. As A β fibrils are insoluble, using techniques classically used to investigate soluble material in solution could be misleading. Although recently developed conformational antibodies can specifically detect oligomers from amyloidogenic proteins [8,10], ATR-FTIR gives additional quantitative information. Thanks to the structural differences between A β oligomers and fibrils recently evidenced by our group, the ratio of oligomers to fibrils can be calculated, giving useful information for the characterization of A β samples. Moreover, conformational antibodies are trustworthy as long as the sample is only composed by one type of amyloidogenic protein that possesses the suitable conformational epitope, corresponding to a local conformational feature of the protein. FTIR on the contrary is sensitive to an overall conformational change, independently of any localized structure.

We therefore investigated the possibility to use ATR-FTIR to evaluate the ratio of oligomers formed in the presence of several ApoE3 concentrations compared to A β alone.

After 24 h incubation at 37°C, ApoE and A β 42 interact together to form complexes that are SDS-resistant. The characterization of these samples has been handled by means of SDS-PAGE gels followed by western blot either detected by 6E10, a monoclonal anti-A β antibody, or by A1.4, a monoclonal antibody raised against ApoE (data not shown).

3.1. Apolipoprotein E contribution in the IR spectrum

ApoE folds into almost 100% α -helix as shown in Fig. 1A where the main peak is located at 1653 cm⁻¹ in the Amide I region. On the contrary, typical A β 42 oligomers formed in TBS as detailed in [2] are mainly composed of β -sheet organized in an anti-parallel conformation, as attested by the

Fig. 1. ApoE and A β IR spectra. (A) 2 μ g of ApoE3 (solid line) or A β oligomers (dashed line) have been spread on the diamond surface after dialysis, in order to compare their contributions in the spectrum. ApoE and A β IR spectra do not overlap in their respective most intense spectral contributions. (B) Secondary structure analysis of A β samples containing ApoE3. (a) No ApoE (control). (b–e) ApoE–A β samples incubated in ApoE–A β molar ratios of 1:100 (b), 1:75 (c), 1:50 (d) and 1:25 (e). Spectral intensities were normalized to the intensity of the 1630 cm⁻¹ peaks. All spectra were deconvoluted using a Lorentzian deconvolution factor with a full width at half height of 20 cm⁻¹, a Gaussian apodization factor with a full width at half height of 13.33 cm⁻¹ to obtain a resolution enhancement factor $K = 1.5$ [7].

simultaneous presence of the 1630 and 1695 cm^{-1} peaks (Fig. 1A). A β 42 fibrils only display the major component of β -sheet at 1630 cm^{-1} , corresponding to a parallel organization of the sheets (data not shown) [2]. A β and ApoE have thus clear distinct contributions to the IR spectrum and we can therefore look at changes in the spectrum of A β in the presence of ApoE in the sample. The contribution of ApoE to the spectrum is nevertheless negligible since the ApoE–A β molar ratios contain 25–100 times more A β than ApoE. Even if ApoE had a slight contribution in the β -sheet regions, its relative small amount in the complexes would render it undetectable.

3.2. ApoE increases the A β oligomeric content of ApoE–A β samples

Figure 1B shows the evolution of the secondary structures of A β 42 with increasing amounts of ApoE3. Regarding the β -sheet components of the spectra, we noticed a dramatic increase of the 1695 cm^{-1} peak (vertical mark), characteristic of an anti-parallel β -sheet structure, which is typical of A β oligomers. This result shows that ApoE3 favours oligomers and prevents them from forming fibrils. Since the ApoE3–A β molar ratio is significantly lower than 1, it is difficult to conceive that ApoE3 stabilizes A β oligomers via complex formation, unless several acceptor sites for A β would be simultaneously available on ApoE3. It seems therefore more likely that ApoE3 acts kinetically by slowing down the aggregation process of conversion of oligomers into fibrils, a process that could involve transient association of ApoE3 with oligomers in a kinetic way [6]. However, this hypothesis needs to be further investigated.

As the 1695/1630 intensity ratio has been shown to be proportional to the amount of anti-parallel arrangement of the β -sheet structure in proteins [3], we calculated the 1695/1630 cm^{-1} peaks intensity ratios in the different conditions. ApoE–A β 1:25 and ApoE–A β 1:50 contained, respectively, 6 and 2.3 times more oligomers than the pure A β control as estimated from spectra in Fig. 1B.

To our knowledge, this is the first time that an effect of ApoE on A β aggregation is brought to light using infrared spectroscopy, which has interesting implications and perspectives.

4. Conclusion

We have developed a fast and accurate tool to monitor the effect of apolipoprotein E on A β aggregation. ATR-FTIR will help us to define how the other isoforms of ApoE, especially ApoE4, can influence the formation of A β insoluble fibrils. Our first data suggest a slowing effect of ApoE3 on A β aggregation rate, stabilizing the most toxic species of the peptide. The final goal of this work is to propose a mechanism that could, at least partly, explain the enhanced pathogenicity of ApoE4 compared to the other isoforms in AD.

In this work, we focused on apolipoprotein E as it is the main risk factor in the Alzheimer's disease but another interesting perspective using infrared spectroscopy would be to study whether and how the presence of other proteins, peptides or small organic molecules in the sample would influence the formation of A β aggregates. The identification of novel inhibitors of A β aggregation would be particularly exciting.

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