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CONFORMATIONAL PARTICULARITIES OF Aβ(25-35) PEPTIDE DETERMINED BY INFRARED SPECTROSCOPY

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Abstract. The Alzheimer's amyloid-β (25-35) peptide retains the toxicity and ability to aggregate of the amyloid-β(1-40) peptide. The study of the conformational properties of Aβ (25-35) peptide in its soluble monomeric form can play an essential role in determining the mechanism of oligomerization. In this study, we probed the secondary structure of a soluble Aβ (25-35) peptide in water solution at physiological conditions (pH 7.4). We have used Fourier Transformed Infrared Spectroscopy (FTIR) to examine the secondary structure of this peptide. The amide I bands of Aβ (25-35) obtained from transmission-FTIR spectra consists of one main band at 1658 cm^{-1}, at both concentrations used (200 μM and 1 mM). This band show us that Aβ (25-35) in aqueous solution was mostly organized into a α-helical structure (48%). The contribution of the unordered structure was found to be about 12%. The proportion of β-sheet and β-turn structures are slightly lower, 12-15% and 13-14%, respectively for both concentrations. FTIR spectra of the peptide in water solution (100 μM) after incubation for 12h showed Aβ peptide conformation is critically dependent on the environmental conditions used.

Keywords: *Alzheimer's amyloid-β (25-35) peptide, FTIR spectroscopy, secondary structure, conformation.*

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1. Introduction

Alzheimer's disease (AD) is one of the most common neurodegenerative disorders and the cause of dementia. The disease is pathologically characterized by the aggregation of two proteins in the brain tissues, namely the amyloid-β (Aβ) and the brain-specific tau protein. There is cumulative evidence that Aβ peptides self-assembly into soluble oligomers and insoluble fibrils (Zhao *et al*., 2014).

The truncated variants of Aβ (1-42) can be directly neurotoxic, inducing oxidative stress and an inflammatory response (Sultana *et al*., 2006). These events might be

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mediated by the direct interaction of Aβ aggregates with cellular membranes or by the binding of Aβ to neuronal cellular receptors. A particular fragment, Aβ (25-35) (with sequence GSNKGAIIGLM) is considered to be the functional domain of Aβ, responsible for its neurotoxic properties (Limón *et al*., 2009) and the biologically active region of Aβ (D'Errico *et al*., 2008). The Aβ (25-35) peptide possesses many of the characteristics of the full-length Aβ (1-40/42), including its amphiphilic nature and the tendency to aggregate. Its presence in vivo was only recently proven, but the toxicity of fibrillary Aβ (25-35) towards neuronal cells in vitro was previously demonstrated (Resende *et al*., 2007). There is also evidence that the monomeric form of this peptide may itself be cytotoxic (Clementieta *et al*., 2005).

The mechanism of the amyloid peptide's toxicity remains unexplained. Several studies demonstrated that the extracellular soluble species of Aβ-peptides (monomers and oligomers) as well as fibrils play a major role in cytotoxicity (Resende *et al*., 2007; Clementieta *et al*., 2005). Nevertheless, many important details about soluble Aβ including its structures are missing or contradicting each other. Hence, the study of the conformational properties of Aβ (25-35) peptide in its soluble monomeric form can play an essential role in determining the nature of the earlier species before oligomerization.

Several studies aimed at probing the membrane mediated structure of amyloid peptide Aβ (25-35) to clarify the structure-function relationships of the peptide (Kohno *et al*., 1996; Coles *et al*., 1998). Several studies identified the solution conformation of the Aβ (25-35) peptide using hydrophobic solvents such as trifluoroethanol (TFE) and hexafluoroisopropanol (HFIP). For example, CD (circular dichroism) studies found that Aβ (25-35) adopts an ordered conformation including some α-helix, with the increase of the trifluoroethanol concentration (El-Agnaf *et al*., 1998) or unordered structures in methanol (Shanmugam & Jayakumar, 2004). In another study using vibrational circular dichroism spectroscopy, Aβ (25-35) in DMSO solution was shown to adopt a β-turn structure (Shanmugam & Polavarapu, 2004). In addition, the three dimensional structure of Aβ (25-35) was determined by NMR (nuclear magnetic resonance) and it was shown that in a solution containing HFIP/water (20/80 v/v) the peptide has a less regular folding in the C-terminal region of the peptide with a β-turns in the 25-28 segment (D'Ursi *et al*., 2004). A different NMR study have been shown that Aβ (25-35) *adopts* a turn-*helical structure* in solution containing at least 50% HFIP. This *structure* has a partially ordered turn in the N-terminus (residues 26-28), followed by an *α-helix* from residues 28-31 and a 3_{10}-*helix* spanning the C-terminus residues 32-34 (Wei & Shea, 2006). While in a solution containing 50% TFE was found that Ala^{30} to Met^{35} in the Aβ (25-35) structure adopts an α-helical conformation (Lee & Kim, 2004).

Efforts at characterizing the Aβ (25-35) conformation in aqueous solvents have been performed (Wei & Shea, 2006; Millucci *et al*., 2010; Ma & Nussinov, 2006; Terzi *et al*., 1994; Song *et al*., 2018). Simulation using replica exchange molecular dynamics approach revealed that in explicit water the peptide adopts a collapsed coil and to a lesser extent *β*-hairpin conformation (Wei & Shea, 2006). While the energy landscape calculations show that the extended conformation, together with the α-helix are important intermediates for Aβ (25-35) peptide oligomerization leading to amyloid formation (Ma & Nussinov, 2006). In addition, the secondary structure of the peptide in buffer solution at pH 4.0 or 5.5 determined by circular dichroism spectroscopy, showed predominantly random coil structure at low peptide concentration (< 125 uM) and a β-

sheet structure at higher concentrations (Terzi *et al*., 1994). However, at pH 7.4 only a β-sheet structure was observed for the peptide even at low concentration (Terzi *et al*., 1994). Another CD study of the peptide in aqueous solutions (100 μM) suggested the presence of unordered structure in water solution and β-sheet structure in phosphate buffer at pH 7.15 (Song *et al*., 2018). In contrast, when the measurements were performed by ATR-FTIR spectroscopy with the same diluted peptide sample in pure water or buffer solution, aggregated β-sheet structure was observed (Song *et al*., 2018). Hence, the conformations adopted by the Aβ (25-35) peptide in solution are extremely sensitive to the techniques and the experimental conditions involved.

In this study, we probed the structure of a soluble Aβ (25-35) peptide in water solution at physiological conditions (pH 7.4). We also have used Fourier Transformed Infrared Spectroscopy (FTIR) to examine the secondary structure of the peptide. Our results reveal for the first time that Aβ (25-35) adopts α-helical conformation in water at pH 7.4. This finding is consistent with the hypothesis that the helix-containing conformer is an important intermediate in Aβ fibril assembly (Fezoui & Teplow, 2002; Kirkitadze *et al*., 2001; Vivekanandan *et al*., 2011). The possible role of α-helical structure in initiating aggregation of the Aβ peptide will be discussed.

2. Materials and Methods

2.1. Peptide sample preparation

The Aβ (25-35) peptide (GSNKGAIIGLM) was purchased from GeneCust peptides synthesis. We used here a commercially available HCl salt form of Aβ (25-35) peptide (without TFA). The purity of the peptide was estimated to be higher than 95% according to mass spectrometry and HPLC control data provided by GeneCust.

The solubilization procedure for the Aβ (25-35) peptide was adapted from (Song *et al*., 2018). The peptide (0.5 mg) was dissolved in 500 μL of 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP, Sigma Aldrich, France) and subsequently sonicated and vortexed three times for 10 seconds. To ensure complete solubilization of the peptide, the solution was kept in a thermomixer (IsoTherm-System 3880, Eppendorf, Germany) for 24 h at 350 rpm min^{-1} at 25°C. After that, the HFIP solvent was removed by a stream of argon and the peptide film was stored in a vacuum drying lyophilizateur (Cosmos model, Cryotec, France) for 24 h. The obtained peptide film was dissolved in deuterium oxide (D_2O solution with 100 atoms % D, Acros Organics, USA) for a final concentration of 200 μM and 1 mM at pD 7.4. Finally, the fresh peptide solutions were stirred 5 min before the FTIR measurements.

2.2. Fourier Transformed Infrared Spectroscopy (FTIR)

The infrared spectra were obtained with a Vertex 70 FTIR spectrometer (Bruker, Germany) equipped with a liquid nitrogen MCT (mercury cadmium telluride) detector. The spectrometer was purged with dry air to avoid the contributions from humidity. The FTIR spectra of the peptide obtained in the transmission and in attenuated total reflection (ATR) mode, were recorded in the spectral range from 4000 to 1000 cm^{-1} with a scan velocity of 40 kHz. Three spectra with a resolution of 4 cm^{-1} (256 co-added scans) were averaged for each peptide sample (200 μM or 1 mM). Also, two infrared experiments (transmission or ATR) were performed for each peptide sample. A

reference spectrum was collected for a pure D_2O and subtracted from the transmission or the ATR-FTIR spectra of the peptide, respectively.

The infrared transmission spectra of the Aβ (25-35) peptide were obtained with a transmission cell made of two calcium fluoride (CaF_2) windows. 0.5 μL of the peptide solution (200 μM or 1 mM) were deposited on a window that was covered with a second window. A defined path (17 μm) was obtained as described by Barth & Zscherp (2002). The windows were placed in the metallic support and then the transmission cell was placed on the optical path of the IR beam. For ATR-FTIR spectra of the peptide, 2.5 μL of the peptide solution (200 μM or 1 mM) were deposited on the diamond crystal (Harrick) and the spectra were recorded before obtaining the dry film peptide.

2.3. Analysis of the infrared data

Before analysis, a pure D_2O spectrum was subtracted from the peptide sample spectrum (transmission or ATR-FTIR) in the spectral range from 1800 to 1400 cm^{-1}. A linear baseline was then subtracted from all spectra. For clarity, the maximum area for the amide I band (1700-1600 cm^{-1}) for all spectra shown has been normalized to the same value. The resulting amide I bands were analyzed with an algorithm based on a second-derivative function (Origin Pro 8.5, OriginLab Corporation) and a self-deconvolution procedure (Origin Pro 8.5, OriginLab Corporation) to determine the number and wavenumber of individual bands within the spectral range 1700-1600 cm^{-1}. The amide I band of each spectrum could be fitted by four bands assigned to the vibration of amide I involved in four different secondary structures.

The Gaussian band profile was used to fit all the individual amide I band components obtained at 1623, 1640, 1658 and 1676 cm^{-1} and have been assigned to the secondary structure elements in D_2O as follows: α-helices (1658 cm^{-1}), unordered structures (1640 cm^{-1}), β-sheet (1623 cm^{-1}) and β-turns (1676 cm^{-1}). The relative contributions of the different bands were determined from the fit results obtained for the amide I bands. The amount of each secondary-structure element is given as a percentage and is calculated by dividing the area of one amide I band component by the total area of all amide I band components. The absolute error due to the deconvolution variability and the baseline correction was estimated to be about ±5%.

3. Results

3.1. Identification of the secondary structure of Aβ (25-35) by FTIR spectroscopy

The Fourier-transform infrared spectroscopy is an established tool for the characterization of the aggregates formed during the aggregation process of the Alzheimer's amyloid-β peptides, Aβ 42 (Cerf *et al*., 2009) and Aβ 40 (McLaurin *et al*., 1999). A full analysis of the protein or the peptide structure requires a complete study of the amide I band (1600-1700 cm^{-1}) by curve-fitting (Cerf *et al*., 2009; Hashioka *et al*., 2005). In this study, we carried out the deconvolutions of the amide I band obtained for the Aβ (25-35) peptide in aqueous D_2O solution (pD 7.4) at both concentrations (200 μM and 1 mM) (procedure described in Materials and Methods part).

The experiments with the sample at 1 mM were performed to exclude an effect of the concentration on the peptide structure. The transmission and ATR-FTIR spectra in the amide I range could be fitted with 4 components at 1623, 1640, 1658 and 1676 cm^{-}.

We assigned individual amide I band components to various elements of peptide secondary structures based on a large body of experimental data (Pike *et al*., 1993). The assignments of each component and the relative contribution of each structural element in Aβ (25-35) peptide is presented in Tables 1 and 2.

Table 1. Comparison of secondary structure composition of Aβ (25-35) peptide in solution in D_2O

Secondary Structure (%)	**Experimental (200 μM)**	**Experimental (1 mM)**
α-helices	48	48
Unordered	23	28
β-sheets	15	12
β-turns	14	13

(pD 7.4) at 200 μM and 1 mM obtained from curve fitting of transmission-FTIR spectra

Table 2. Comparison of secondary structure composition of Aβ (25-35) peptide in solution in D_2O

Secondary Structure (%)	**Experimental (200 μM)**	**Experimental (1 mM)**
α-helices	45	48
Unordered	23	24
β-sheets	11	16
β-turns	11	12

(pD 7.4) at 200 μM and 1 mM obtained from curve fitting of ATR-FTIR spectra

The amide I bands of Aβ (25-35) obtained from transmission-FTIR spectra consists of one main band at 1658 cm^{-1}, at both concentrations used (200 μM and 1 mM) (Figure 2 A and B). This band show us that Aβ (25-35) in aqueous solution was mostly organized into a α-helical structure (48%), a value that is consistent with the molecular simulation data presented above (48%) (Table 1). The contribution of the unordered structure was found to be about 12% (Table 1). The proportion of β-sheet and β-turn structures are slightly lower (12-15% and 13-14%, respectively for both concentrations) as compared to the theoretical values (20 % for β-sheet, 20 % for β-turns) (Table 1).

The peptide was measured in both in solution (transmission mode) and before obtaining the dry film (in the ATR mode). This later method allows to distinguish different amyloid aggregated forms (Cerf *et al*., 2009; Sarroukh *et al*., 2011; Berthelot *et al*., 2009). Figure 2 A-B and Table 2 show the direct comparison of 200 μM and 1 mM peptide solutions measured by ATR infrared spectroscopy. In both concentrations,

the contributions from α-helices, unordered, β-sheet and β-turns in the secondary structure of the peptide were highly comparable with the transmission infrared data (Figure 1 and Table 1).

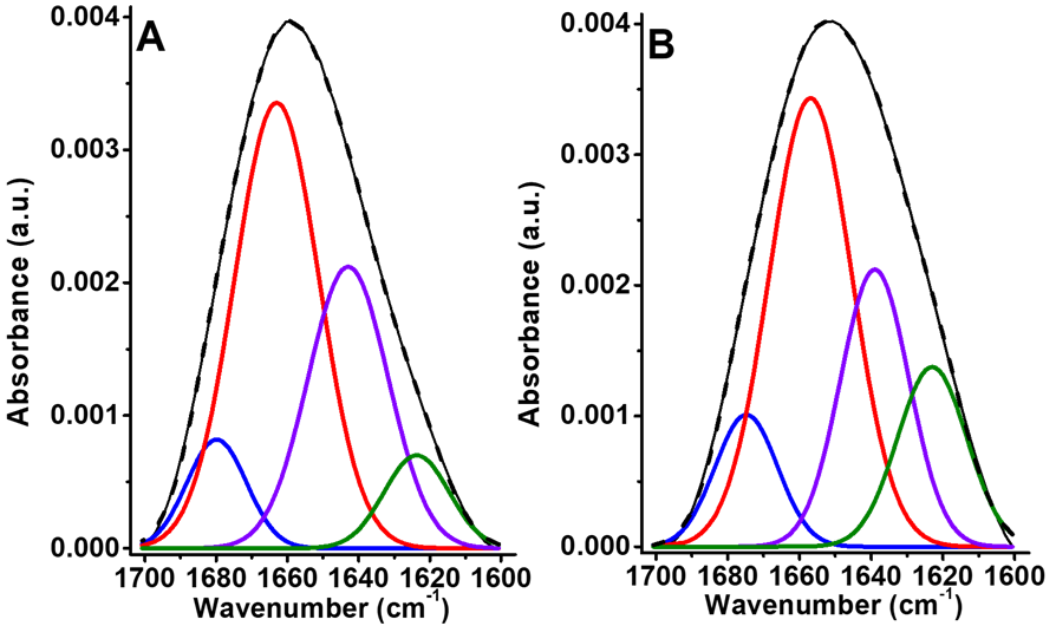

Figure 1. Secondary structure analysis of the peptide Aβ (25-35) solution in D_20 (pD 7.4) at 200μM (A) and 1mM (B). Curve-fitting of the deconvoluted amide I bands (1700-1600 cm^{-1}) obtained from spectra of Transmission-FTIR. The Gaussian curves underneath the experimental curve represent the various secondary structures of the amide I band: α-helix (red curve), unordered structures (violet curve), β-turns (blue curve) and β-sheets (green curve)

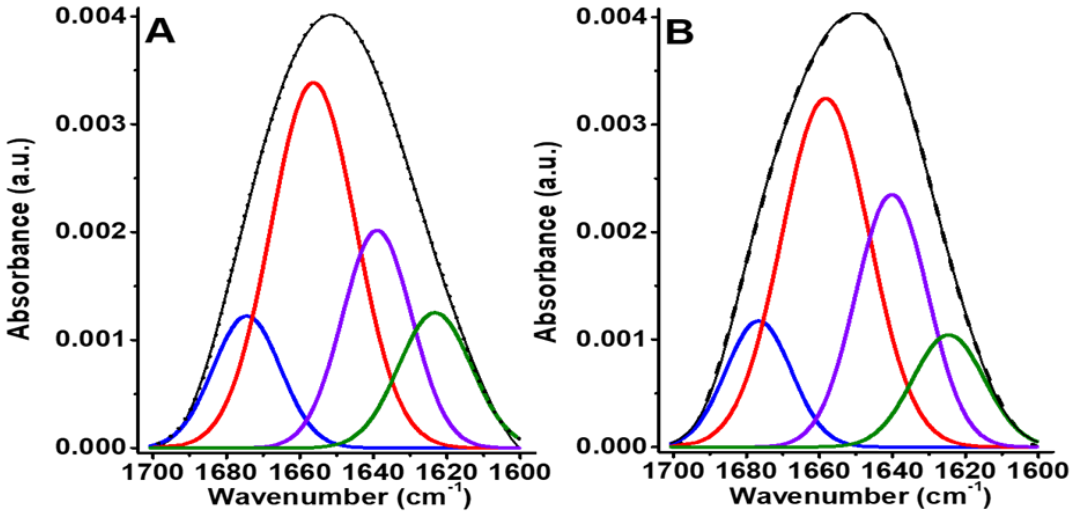

Figure 2. Secondary structure analysis of the peptide Aβ (25-35) solution in D_20 (pD 7.4) at 200μM(A) and 1 mM (B). Curve-fitting of the deconvoluted amide I bands (1700-1600 cm^{-1}) obtained from spectra of ATR-FTIR. The Gaussian curves underneath the experimental curve represent the various secondary structures of the amide I band: α-helix (red curve), unordered structures (violet curve), β-turns (blue curve) and β-sheets (green curve)

4. Discussion

Several studies have shown that the soluble Aβ and Alzheimer's disease are intertwined and that soluble Aβ peptide contributes to disease development and progression (Sarroukh *et al*., 2013). It is thus crucial to determine the soluble monomeric conformation of the Aβ (25-35) peptide as it can play an important role in determining the nature of early aggregates and the resulting morphology of the amyloid fibril (Celej *et al*., 2012). Few works have been performed in studying the conformational properties of Aβ (25-35) in aqueous solution, however no consensus is reached so far to describe the soluble peptide structure (Wei & Shea, 2006; Millucci *et al*., 2010; Ma & Nussinov, 2006; Terzi *et al*., 1994; Song *et al*., 2018).

In this work, we obtained the spectroscopic approaches and clearly showed that Aβ (25-35) adopts mostly an α-helical structure and some smaller contributions of unordered, β-sheet and β-turns in water solution. Our experimental findings correlate well with the α-helical intermediates shown to be important for amyloid formation, especially for the Aβ peptide (Ma & Nussinov, 2006; Murphy & LeVine, 2010; Pike *et al*., 1993; Klimov & Thirumalai, 2003). Using conformational landscape analysis and molecular dynamics simulations, Ma and Nussinov (2006) investigated the monomeric energy landscape and amyloid formation of the Aβ (25-35) peptide in aqueous solution. They found that the stability of the extended conformation, together with the stability of the α-helix, is likely to be important for the peptide oligomerization leading to amyloid formation (Ma & Nussinov, 2006). In a recent study of Song et al. (2018), FTIR spectra of the peptide in water solution (100 μM) after incubation for 12h showed an aggregated β-sheet structure, which is in contrast with our FTIR observations. The incubation time of the peptide sampled in water for 12h may prompt peptides to reach a final equilibrated state at which the aggregated β-sheet structure can appears. In either case, Aβ peptide conformation is critically dependent on the environmental conditions used.

It is pertinent to compare the structure of the aqueous Aβ (25-35) peptide studied here to that of other amyloid peptides. Fonar and Samson (2014) demonstrated by NMR that Aβ (17-34) in water solution adopts an α-helical structure for the residues 19-26 and 28-33. Another study on a larger peptide, Aβ (1-40) studied in aqueous environment at pH 7.3 using NMR spectroscopy (Vivekanandan *et al*., 2011) showed that the peptide adopts a stable α-helical structure in the central hydrophobic core and contacts of the N- and C-termini with the central α-helix.

As cited above, the α-helical conformation was detected previously as an intermediate in Aβ-amyloid fibril assembly (Ma & Nussinov, 2006; Fezoui & Teplow, 2002; Kirkitadze *et al*., 2001; Vivekanandan *et al*., 2011). It is thus likely that the folding of the Aβ (25-35) monomer as an α-helical structure is necessary to facilitate the Aβ intermolecular packing within Aβ oligomers. An intramolecular α-helix formation may occur as a natural consequence of the conformational space by the Aβ (25-35) monomer. Once formed, the intermolecular interactions among these helices containing Aβ monomers could lead to peptide oligomerization followed by a conformational reorganization to form the extended β-sheets that compose the mature amyloid fibril.

This mechanism is supported by previous studies of the fibrillogenesis of Aβ peptides associated with familial forms of cerebral amyloid angiopathy (Kirkitadze *et*

al., 2001) and has been shown to occur in the fibrillogenesis of the model peptide helix-turn-helix (Fezoui *et al*., 2000; McLaurin *et al*., 1999). While the α-helical component in the monomeric structure of the Aβ (25-35) peptide in aqueous solvent seem to play a role in initiating aggregation, the β-turn seen in our experimental and theoretical data may be responsible for the toxicity induced by the soluble monomeric form of this peptide. Indeed, soluble Aβ (25-35) is known to bind to protein receptors on microglia (Doens *et al*., 2014), leading to their activation and to a subsequent neuronal damage. β-turns are a structural motif often involved in binding to receptor proteins and it is possible that the presence of such turns in Aβ (25-35) may be necessary to induce toxicity (Hashioka *et al*., 2005).

5. Conclusion

Our results show us that Aβ (25-35) in aqueous solution was mostly organized into a α-helical structure (48%). The contribution of the unordered structure was found to be about 12%. The proportion of β-sheet and β-turn structures are slightly lower, 12-15% and 13-14%, respectively for both concentrations. FTIR spectra of the peptide in water solution (100 μM) after incubation for 12h showed Aβ peptide conformation is critically dependent on the environmental conditions used. While the mechanism of toxicity of Aβ (25-35) is still unclear, its native folded structure in aqueous environment is expected to play a role in the aggregation cascade.

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Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Availability of data and material

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials.

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