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Molecular Mechanics Simulation and Ftir Spectrocopy Optimization of Secondary Structure of Aβ (25-35) Peptide

Abstract

In this study, we probed the secondary structure of a soluble Aβ (25-35) peptide in water solution by applying a molecular mechanics method as well as infrared spectroscopy. 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 shows 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. The simulation approach reveals that the C-terminal octapeptide of this molecule preferably adopts an α-helical structure, while the N-terminal tripeptide may exist as a β-turn and unordered structures.

Keywords: *Alzheimer's amyloid-β (25-35) peptide, molecular mechanics method, Fourier Transformed Infrared Spectroscopy (FTIR), secondary structure, alpha-helix formation*

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Alzheimerin amiloid-β (25-35) peptidi, molekulyar mexanika metodu, Fourier Transformasiyalı İnfraqırmızı Spektroskopiya (FTIR), ikincil struktur, alfa-spiral formalaşması

Xülasə

Bu tədqiqatda biz Aβ (25-35) peptidinin su məhlulunda həll olunan ikincil strukturunu molekulyar mexanika metodu və infraqırmızı spektroskopiya tətbiq edərək araşdırdıq. Peptidin ikincil strukturunu öyrənmək üçün Fourier Transformasiyalı İnfraqırmızı Spektroskopiyadan (FTIR) istifadə etdik. Transmissiya-FTIR spektrlərindən əldə edilən Aβ (25-35) peptidinin amidi I zolaqları hər iki istifadə olunan konsentrasiyada (200 μM və 1 mM) 1658 sm^{-1}-də yerləşən bir əsas zolaqdan ibarətdir. Bu zolaq bizə göstərir ki, Aβ (25-35) su məhlulunda əsasən α-spiral strukturda təşkil olunmuşdur (48 %). Sərbəst strukturun payı təxminən 12 % olaraq müəyyən edilmişdir. Həm β-vərəq, həm də β-burulma strukturlarının nisbəti hər iki konsentrasiya üçün müvafiq olaraq bir qədər aşağıdır, 12-15 % və 13-14 %. 12 saatlıq inkubasiyadan sonra su məhlulunda (100 μM) olan peptidin FTIR spektrləri göstərdi ki, Aβ peptidinin konformasiyası istifadə olunan ətraf mühit şəraitindən kritik dərəcədə asılıdır. Simulyasiya yanaşması göstərir ki, bu molekulun C-terminal oktapeptidi α-spiral strukturunu üstünlük təşkil edir, halbuki N-terminal tripeptidi β-burulma və sərbəst strukturlar kimi mövcud ola bilər.

Açar sözlər: *Alzheimerin amiloid-β (25-35) peptidi, molekulyar mexanika metodu, Fourier Transformasiyalı İnfraqırmızı Spektroskopiya (FTIR), ikincil struktur, alfa-spiral formalaşması*

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, 2014). The secondary structure of the aggregated Aβ peptides show a β-sheet character (Cerf, 2009).

Research

The Alzheimer's amyloid-β (25-35) peptide possesses many of the characteristics of the full-length Aβ (1-40/42). The Aβ (25-35) peptide (with sequence GSNKGAIIGLM) retains many of the characteristics of the full-length Aβ (1-40/42), including its amphiphilic nature, toxicity and the tendency to aggregate. 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 (Zhao, 2014; Cerf, 2009; Sultana, 2006; Limon, 2008). Despite significant progress in understanding soluble Aβ peptides, many critical details about their structures remain unclear or contradictory in the existing literature. This lack of clarity necessitates further investigation into the conformational properties of Aβ (25-35) peptide in its soluble monomeric form, as such studies can provide crucial insights into the nature of early peptide species before the oligomerization process begins. Understanding these initial conformations is vital for comprehending the molecular mechanisms underlying the aggregation and toxicity of amyloid peptides in Alzheimer's disease.

The conformations adopted by the Aβ (25-35) peptide in solution are highly sensitive to the experimental techniques and conditions employed, which underscores the need for precise and reproducible methodologies. In this study, we employed Fourier Transformed Infrared Spectroscopy (FTIR) to elucidate the secondary structure of the Aβ (25-35) peptide. Additionally, we utilized molecular mechanics (MM) simulations to gain detailed information about the spatial organization and conformational dynamics of the peptide. MM simulations offer a distinct advantage over other theoretical approaches by allowing a comprehensive treatment of energy contributions from all types of intra-and intermolecular interactions, thereby facilitating the identification of the most stable conformations of the peptide and its fragments.

Our findings represent a significant advancement in the field, revealing for the first time that the Aβ (25-35) peptide predominantly adopts an α-helical conformation in aqueous solution at physiological pH 7.4. This discovery not only enhances our understanding of the peptide's behavior in different environments but also provides a foundation for further studies into the role of specific conformational states in the pathogenesis of Alzheimer's disease.

Methods

Fourier Transformed Infrared Spectroscopy (FTIR)

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 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. 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 Andreas Barth et al (D'Errico, 2008; Barth & Zscherp, 2002).

Theoretical Calculations

The method of molecular mechanics was applied to study the three-dimensional structure and conformational particularities of the beta-amyloid (25-35) undecapeptide and its fragments. Figure 2 shows the amino acid sequence of the beta-amyloid (25-35) (A), its calculated atomic model and variable dihedral angles (B). The application of this method of molecular mechanics permits the determination of a set of possible stable spatial forms of the peptide and its fragments. The molecular geometry of the L-peptide was investigated by conformational analysis by using the program written by Ref. (Barth & Zscherp, 2002), benefiting from the Ramachandran maps (Maksumov, 1983; Ramachandran & Sasisekharan, 1968).

The lowest energy conformations of the undecapeptide and its fragments obtained after the conformational analyses were optimized using the Amber force field molecular mechanics method (Ramachandran, 1968) available in the Gaussian16 program package (Cornell et al., 1995). The energies of the obtained structures were obtained by PM3MM calculations. The calculations were performed using the technique, step by step approach. The step by step procedure of analyzing an amino acid sequence with ever increasing lengths of monopeptide as starting unit reflects adequately the spontaneous process of polypeptide folding (Frisch et al., 2016; Popov, 1979).

Results

The experiments with the sample at 1 mM were performed to exclude an effect of the concentration on the peptide structure (Fig. 1B). The FTIR spectra in the amide I range could be fitted with 4 components at 1623, 1640, 1658 and 1676 cm^{-1} (Fig. 1 A-B). The assignments of each component and the relative contribution of each structural element in Aβ (25-35) peptide is presented in Table 1. The resulting amide I bands were analyzed with an algorithm based on a second-derivative function 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 absolute error due to the deconvolution variability and the baseline correction was estimated to be about ±5 %. 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) (Fig. 1 A and B, respectively). 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 % (Table 1). The

proportion of β-sheet and β-turn structures are slightly lower (12-15 % and 13-14 %, respectively for both concentrations) (Table 1).

A calculation atomic model of the Alzheimer's amyloid-β (25-35) peptide containing 157 atoms and 59 rotational angles is shown in Fig. 2A. Conformational analysis of this molecule was carried out on the base of the calculation scheme in Fig. 2B. The conformation of the peptide Aβ (25-35) was analyzed using the step-by-step approach to compare the conformational properties of the fragment. Each step was divided into a few sequentially solved structural problems.

The conformational analysis of the entire Aβ (25-35) peptide molecule revealed a limited number of the low-energy structures. Therefore, as starting conformations of Aβ (25-35) peptide, we considered 600 conformations belonging to different shapes and forms of backbone skeleton and their geometry was optimized through energy minimization. Our calculation showed that the peptide in the free state adopts conformations with an α-helical structure $RR_1R_{32}RR_1R_{32}R_{32}RR_{21}R_{32}$ at the C-terminal (Fig. 3). The calculations determined the ranges of dihedral angles that are preferable for low energy conformations and it was found that the relative positions of the residues have a tendency to form a regular α-helical structure in low-energy conformations of the Aβ (25-35) peptide.

The main results of the structural variants from the undecapeptide, in a water solution showed to be a family of conformations with relative energies in the range of 0-10 kcal/mol. The global conformation, except for the N-terminal unfolded Gly25 residue, forms a long α-helical segment towards the C-terminal end. Thus, the global conformation of the undecapeptide amide is based on the global α-helical conformation of the C-terminal octapeptide.

Our study showed that the conformations of Aβ (25-35) undecapeptide molecule are characterized by the mobility of its N-terminal tripeptide and, at the same time, with a considerable rigidity of its C-terminal octapeptide fragment. Among the stable structures with a common α-helical conformation at the C-terminal end, there is a considerable variation of different conformations at the N-terminal tetrapeptide. Accordingly, the flexible structures at the N-terminal region of Aβ (25-35) are differently oriented relative to the structures at the C-terminal part in the low-energy conformations.

Based on the theoretical conformational analysis, we present the results of the folding of Aβ (25-35) peptide in the secondary structure elements: α-helix, β-sheet, β-turn and unordered structures. The percentage of each structure in the spatial organization of the molecule is based on the large number of minimized conformers of the whole molecule and its free separate fragments. The relative contribution (as %) of each secondary structure to the overall structure is calculated and given in Table 1. The presence of an α-helical structure in all lowest conformations of C-terminal pentapeptide, octapeptide and whole molecule allows us to attribute a higher percentage for α-helical structure (48 %) (see Table 1). Only 20 % are present as extended β-sheets as observed from the low-energy conformations of the whole peptide (see Table 1). Our calculations also reveal that the β-turn structure was found only at the N-terminal end of the molecule and was estimated to 20 %. A smaller overall percentage of unordered structure (12 %) was found for the Aβ (25-35) peptide. To complete the study, FTIR experimental approach was used to provide quantitative data on the structure of Aβ (25-35) peptide in comparison to the structural composition suggested by theoretical calculations.

In this work, we combined theoretical and 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 theoretical and 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, 1993; Klimov & Thirumalai, 2003). Using conformational landscape analysis and molecular dynamics simulations (Ma & 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).

Conclusion

The originality of this study lies in its novel integration of Fourier Transformed Infrared (FTIR) spectroscopy and molecular mechanics (MM) simulations to analyze the monomeric structure of the Aβ (25-35) peptide in aqueous solution under physiological conditions. By combining these two advanced techniques, we provide a comprehensive examination of the peptide's secondary structure, which has not been previously reported. Our findings support the hypothesis that an α-helix-containing conformer serves as a crucial intermediate in the assembly of Aβ fibrils. The potential role of the α-helical structure in initiating the aggregation of Aβ peptides will be critically examined, considering its implications for the early stages of amyloid fibril formation.

This study extends those findings by suggesting that the folding of the Aβ (25-35) monomer into an α-helical structure may be essential for facilitating intermolecular packing within Aβ oligomers. The formation of an intramolecular α-helix could occur as a natural consequence of the conformational landscape available to the Aβ (25-35) monomer. Once the α-helical structure is established, subsequent intermolecular interactions among these α-helix-containing monomers could drive the oligomerization process. This oligomerization is likely followed by a conformational reorganization, transitioning from α-helical intermediates to the extended β-sheets that constitute the core of mature amyloid fibrils.

Furthermore, understanding the specific conditions that promote the formation of α-helical intermediates could provide insights into therapeutic strategies aimed at modulating peptide aggregation. These findings underscore the importance of exploring the dynamic conformational changes of Aβ peptides and their implications for the pathogenesis of Alzheimer's disease.

Tables and Figures

Table 1.

Comparison of secondary structure of Aβ (25-35) peptide in solution in D2O (pD 7.4) at 200 μM and 1 mM obtained from curve fitting of transmission-FTIR spectra. The corresponding values obtained by the theoretical calculations are given in the last column

Secondary Structure (%)	Experimental (200 μM)	Experimental (1 mM)	Theoretical calculations
α-helices	48	48	48
Unordered	23	28	12
β-sheets	15	12	20
β-turns	14	13	20

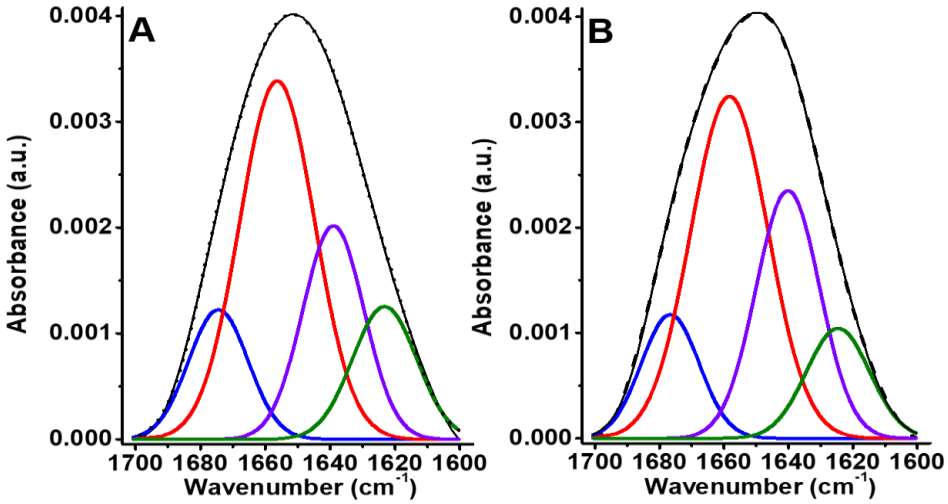
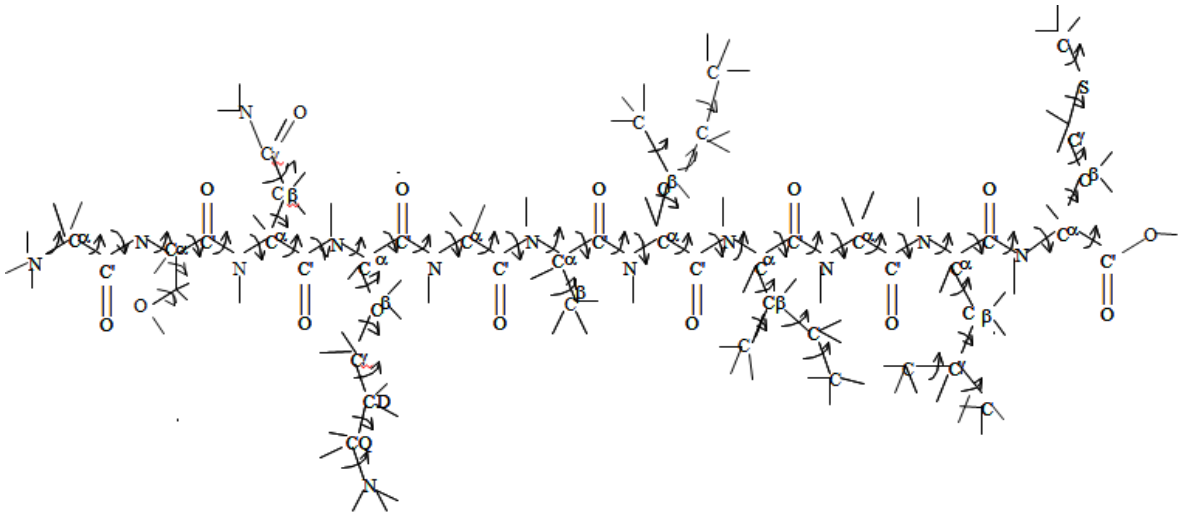


Figure 1.
Secondary structure analysis of the peptide Aβ (25-35) solution in D_20 (pD 7.4) at 200μM (A) and 1mM (B). Curve-fitting of the deconvoluted amide I bands (1700-1600 cm^{-1}) obtained from spectra of Transmission-FTIR. The Gaussian curves underneath the experimental curve represent the various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

H-Gly^{25}-Ser^{26}-Asn^{27}-Lys^{28}-Gly^{29}-Ala^{30}-Ile^{31}-Ile^{32}-Gly^{33}-Leu^{34}-Met^{35}-OH

(A)



(B)

Figure 2.
The amino acid sequence of the beta-amyloid (25-35) (A) and its calculated atomic model and variable dihedral angles (B)

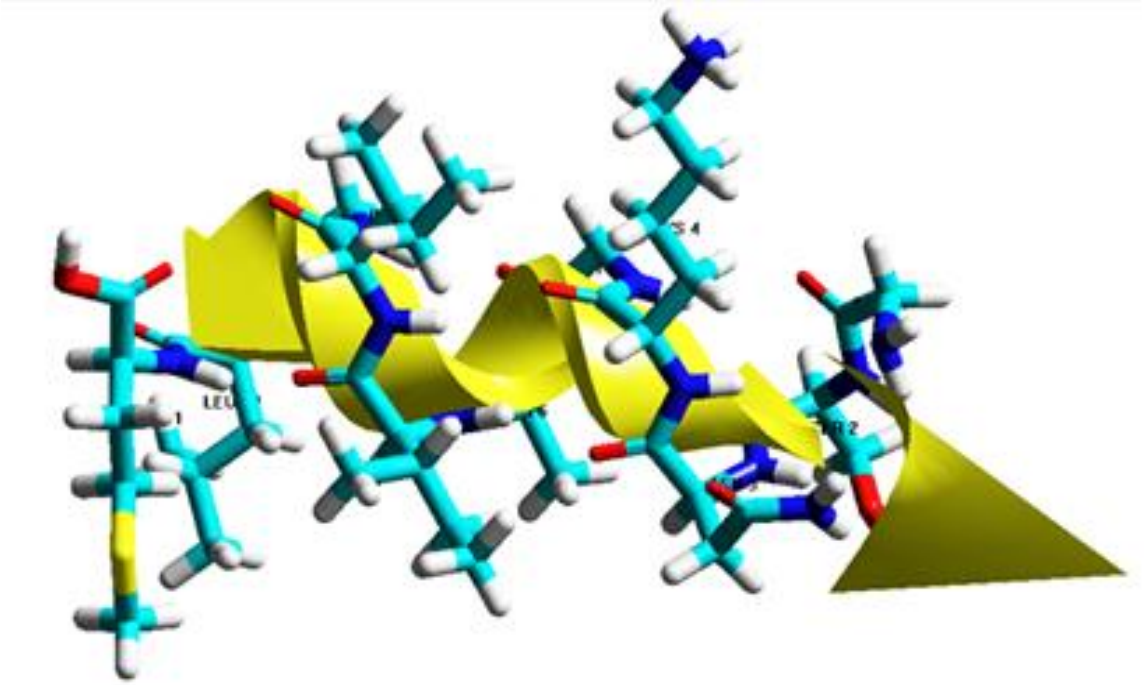


Figure 3.
The molecular model of lowest conformation $RR_1R_1R_{32}RR_1R_{32}R_{32}RR_{21}R_{32}$ (E_{rel} = 0 kcal/mol) of Aβ (25-35) undecapeptide: Secondary structure – α-helix turn shown in bold yellow line

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