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Conformational Analysis of Aβ-(25–35) Peptide by NMR: Insights into Secondary Structure and Aggregation Propensity

Gulyaz Najafova Zulfu [1,*], Gulshen Agaeva Alakbar [2], Kelton Rodrigues de Souza [3] and Philippe Bertani [4]

[1] French-Azerbaijani University – (UFAZ) under Azerbaijan State Oil and Industry University, 183 Nizami St, Baku, Azerbaijan

[2] Institute for Physical Problems, Baku State University, AZ-1148, Baku, Z.Khalilov Str.23, Azerbaijan

[3] Laboratoire de RMN et biophysique des membranes, UMR 7177, Institut de Chimie de Strasbourg, Université de Strasbourg CNRS,4Rue Blaise Pascal 67081, Strasbourg, France

Abstract: In Alzheimer's disease, amyloid β (Aβ) is a principal component that accumulates within neuritic plaques. This disease is characterized by the aggregation of the protein Aβ (amyloid β) in the brain. Sequence GSNKGAIIGLM (Abeta 25-35) is the shortest toxic fragment of the protein Aβ-peptide, able to reproduce the aggregation process. NMR spectroscopy was utilized to investigate the spatial arrangement of the β-amyloid (25–35) peptide, which had been solubilized in water and HFIP at 25°C and prepared with an 80:20 D_2O/H_2O solvent mixture (900 µL). The XPLOR-NIH software (version 2.27) was used for the calculation and refinement of the three-dimensional structures. Using MOLMOL, the lowest-energy ten structural models were depicted and evaluated. NMR analysis of the Aβ-(25–35) peptide dissolved in the 20/80 HFIP and water mixture revealed that the C-terminal area tends to form more organized conformations, in contrast to the predominantly disordered nature of the N-terminal region observed in the structural overlays. Such conformational properties are anticipated to facilitate the design of molecules that can associate with amyloid peptides and act as inhibitors of their aggregation into fibrils.

Keywords: Amyloid β -(25-35) peptide, NMR spectroscopy, conformation, secondary structure.

1. Introduction

Alzheimer's disease (AD) is one of the most common neurodegenerative disorders, characterized by progressive memory loss and cognitive decline [1,2]. A pathological characteristic of AD is the formation of amyloid plaques in the brain, which are primarily composed of aggregated amyloid β protein (Aβ) [1,3,4]. Research has shown that proteins and peptides can transform from their native functional states into amyloid assemblies [5]. Such aggregates, referred to as amyloids, are built from misfolded peptides or proteins aligned in a cross-β arrangement, a property that contributes to their increased resistance to proteolytic enzymes [1–3]. The buildup of these protein aggregates in diverse tissues of animals and humans is connected to the progression of various pathological conditions, among them Alzheimer's disease.

The amyloid β protein (Aβ) plays a key role in AD; its misfolding and aggregation lead to the formation of neurotoxic oligomers and insoluble amyloid fibrils that form the plaques. Understanding the conformational changes of Aβ is crucial for elucidating the molecular basis of AD and for developing therapeutic strategies [6]. Although Aβ aggregation plays a central role in AD pathogenesis, the molecular mechanisms driving amyloid formation in vivo remain incompletely understood. Thus, in vitro models of amyloid formation are valuable tools for studying the aggregation process, as the ability to form amyloid-like structures is a universal property of polypeptide chains [7-9]. These models allow for controlled investigation of factors influencing Aβ aggregation. Several proteins have been identified to form amyloid-like aggregates or fibrils under specific in vitro conditions [10,11].

Among the various fragments of the Aβ peptide, the sequence GSNKGAIIGLM, known as Aβ (25–35), is particularly significant. It represents the shortest toxic fragment of the Aβ peptide and is capable of reproducing the aggregation process observed in the full-length protein [12]. Figure 1 highlights the Aβ (25–35) peptide fragment in the full Aβ42 protein sequence (DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA).

**Corresponding author:* Gulyaz Najafova
Email address: gulyaz.najafova@ufaz.az
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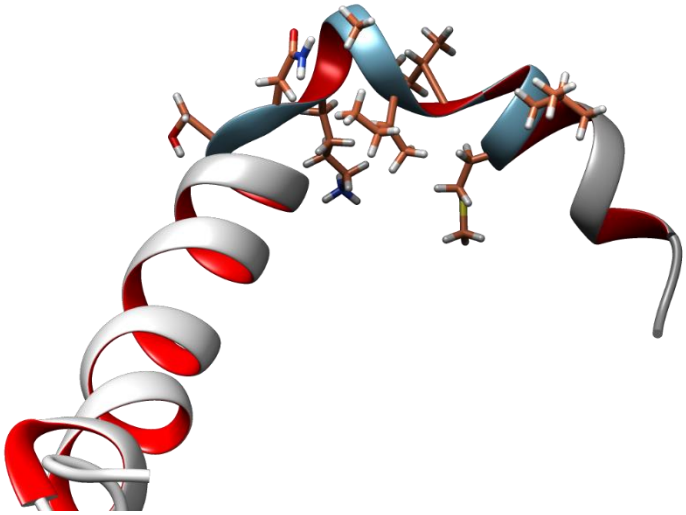

Figure 1. Structure of Aβ monomer protein (Aβ42). The structure highlights the position of the Aβ (25–35) fragment (sky blue color in the external part, ribbon helix interior in red color) within the full-length Aβ sequence. PDB 1IYT.

Previous studies have investigated the conformational behavior of Aβ (25–35) and its fragments under different conditions, suggesting that their secondary structure content is highly dependent on solution conditions [13,14]. For instance, NMR experiments performed on Aβ (25–35) in a 50% aqueous solution of 2,2,2-trifluoroethanol (TFE) revealed that the peptide adopts an α-helical conformation and does not aggregate in the presence of TFE [15]. Other studies have further investigated the Aβ (25–35) peptide [16–18] using NMR spectroscopy in different TFE/water solutions, showing distinct conformational behaviors. As reported, TFE is known to act as a secondary structure-inducing agent [18].

Nuclear Magnetic Resonance (NMR) spectroscopy is a powerful technique for determining the spatial and secondary structures of peptides and proteins in solution, providing atomic-level insights into their conformational dynamics. Studies using such powerful spectroscopies are vital for understanding how environmental factors influence peptide folding and aggregation. Given the critical role of secondary structure in amyloid formation, NMR can provide key insights into the structural transitions of Aβ peptides. In addition, understanding the conformational structure of the Aβ peptide, particularly fragments like Aβ (25–35), may provide important insights into the pathogenesis of Alzheimer's disease. By characterizing the structural features that promote or inhibit aggregation, the researchers can identify potential targets for therapeutic intervention.

Similar to TFE, 1,1,1,3,3,3-Hexafluoro-propan-2-ol (HFIP) is one of the most common and effective cosolvents for the structural stabilization of peptides that form secondary structures [19]. HFIP is a polar organic compound with strong hydrogen bond-donating properties widely used in organic synthesis. However, since the influence of hexafluoroisopropanol (HFIP) on peptide conformation is not well understood, we investigated the secondary structure of the Aβ (25–35) fragment in a water/HFIP solution using NMR spectroscopy. Understanding the effects of solvents like HFIP is vital for gaining insights into protein folding, and consequently, for designing molecules capable of interacting with amyloid peptides as inhibitors of fibril formation, and thus contributing to the development of new strategies to combat Alzheimer's disease.

2. Materials and methods

TOCSY, NOESY, and HSQC experiments were performed on a Bruker Avance III HD 500 MHz spectrometer equipped with a Cryo-Sonde probe (Helium X-1H, 5 mm, 31P < X < 15N). The data were analyzed using CCPNMR and visualized with JRAMA+ software. The assignments were then submitted to TALOS+ online for chemical shift analysis.

2.2. Peptide sample preparation

The Aβ (25-35) peptide (GSNKGAIIGLM) was purchased from GeneCust peptides synthesis. We used a commercially available HCl salt form of the 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 [20]. For solution NMR, we first prepared a 10 mM peptide solution by dissolving 10.6 mg of the peptide in 1 mL of HFIP (Sigma Aldrich, France). Then, we mixed 100 µL of the 10 mM peptide solution with 900 µL of D_2O/H_2O (80/20) (deuterium oxide in water) to achieve a final peptide concentration of 1 mM in a 1 mL solution. *(Regions of collagen triple helix structure)*

2.2. NMR solution structure calculation

Two-dimensional NMR solution experiments (NOESY, TOCSY, and $^{1}H–^{13}C$ HSQC) were recorded on a Bruker Avance spectrometer (Bruker, Germany) operating at a frequency of 500.13 MHz for protons. The experiments were carried out to determine the three-dimensional structure of the Aβ (25–35) peptide. The samples consisted of an aqueous solution of 1 mM Aβ (25–35), containing 10% (v/v) D_2O/H_2O and 1% (v/v) 2,2-dimethyl-2-silapentane-5-sulfonate-d_6 (DSS-d_6) as the internal reference. A 4 mm triple resonance NMR cryoprobe was used for all experiments. Water suppression was achieved using a WATERGATE pulse sequence [21].

Two-dimensional homonuclear Total Correlation Spectroscopy (TOCSY) spectra were acquired using a pulse sequence with a mixing time of 80 ms, 576 t_1 increments, and 32 transients of 6008 points for a spectral width of 6009.6 Hz [22]. Nuclear Overhauser Effect Spectroscopy (NOESY) spectra were obtained using mixing times of 120, 150, and 200 ms to check for spin diffusion, with 576 t_1 increments and 32 transients of 6008 points for a spectral width of 6009.1 Hz [22].

Heteronuclear Single Quantum Coherence ($^{1}H–^{13}C$ HSQC) experiments were performed in edited mode, where CH_2 correlations appear with a negative phase, and CH and CH_3 correlations appear with a positive phase [23]. The data were recorded with F1 and F2 spectral widths of 22,640.47 Hz and 7042.25 Hz, respectively. A total of 640 t_1 increments were accumulated with 16 transients of 3520 points.

Proton resonances were assigned through simultaneous analysis of homonuclear $^{1}H,^{1}H$-TOCSY, and $^{1}H,^{1}H$-NOESY spectra using the Wüthrich method [24]. Heteronuclear spectra were used as additional confirmation. The NMR assignments were performed manually using the NMRViewJ software (version 9.2.0-b20) [25]. NOE intensities were converted into semi-quantitative distance restraints with upper limits of 2.8, 3.4, and 5.0 Å [26]. Dihedral angle restraints were obtained from the chemical shifts of HN, Hα, Hβ, Cα, Cβ, and N using the TALOS+ program [27] within the NMRPipe® computational package [28] (Table 1). The $^{13}C\alpha$ chemical shift index was calculated according to the Wishart method [29]. Geometric restraints were validated for consistency and contribution using the QUEEN program (Quantitative Evaluation of Experimental NMR Restraints) [30].

Table 1. In red, we can see the dihedral angles of the ab peptide. This result can be seen in JRAMA+ viewer and in the phi, psi-dihedral angles – abpeptide.txt file.

VARS	RESID	RESNAME	PHI	PSI	DPHI	DPSI	DIST	S2	COUNT	CS_COUNT	CLASS
FORMAT	%4d	%s	%8.3f	%8.3f	%8.3f	%8.3f	%8.3f	%5.3f	%2d	%2d	%s
2	S	0.000	0.000	0.000	0.000	0.000	0.000	0	9	None	
3	N	-68.092	-40.687	13.545	16.455	48.768	0.699	10	13	Good	
4	K	-62.940	-32.888	5.122	12.208	46.505	0.707	10	12	Good	
5	G	-65.520	-38.722	10.005	14.563	47.039	0.732	10	12	Good	
6	A	-63.676	-41.176	5.685	6.146	46.265	0.752	10	13	Good	
7	I	-63.029	-43.836	9.191	4.562	35.617	0.794	10	14	Good	
8	I	-62.727	-43.605	8.705	4.120	40.315	0.807	10	14	Good	
9	G	-61.344	-37.580	7.294	24.158	44.763	0.791	10	14	Good	
10	L	-71.214	-35.302	11.589	13.207	78.754	0.728	10	13	Good	
11	M	9999.00	9999.00	0.00	0.00	0.00	0.00	0	9	None	

The XPLOR-NIH software (version 2.27) was used for the calculation and refinement of the three-dimensional structures [31,32]. A total of 100 structures, starting from an extended conformation, were generated using a simulated annealing protocol in torsional angle dynamics. This included 20,000 steps of simulated annealing at 1000 K, followed by a temperature decrease over 15,000 steps in the initial slow-cooling stage. The resulting structures were then refined in a subsequent calculation using more stringent topology parameters (ref_sa_new.php) [33]. The ten lowest-energy structures were visualized and analyzed using MOLMOL[20] and Chimera [34]. Structural quality was assessed using Ramachandran plots and root-mean-square deviation (RMSD) values, both obtained via the PSVS online platform [35] (Figure 2).

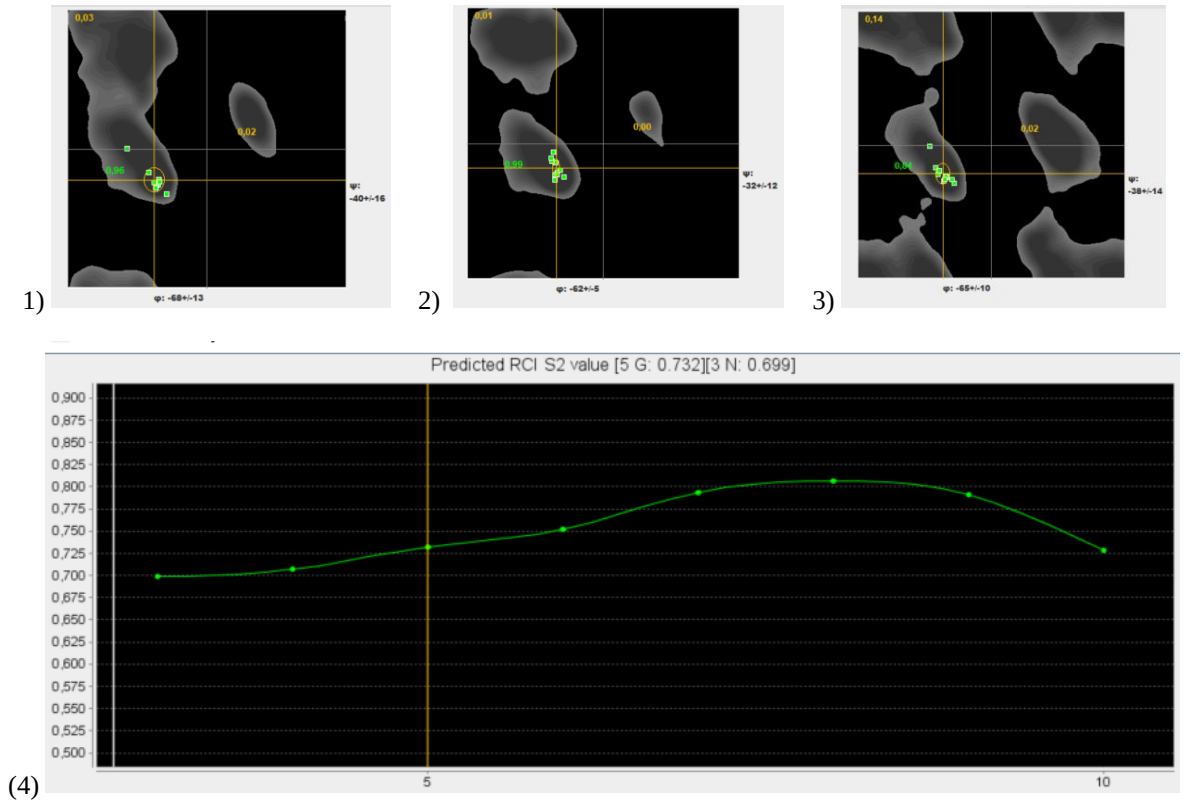

Figure 2. 1) Dihedral angles of residue N3, 2) K4, and 3) G5. 4) Predict RCI – Secondary structures and Motifs.

3. Results and Discussion

Several studies have established a correlation between soluble Aβ and Alzheimer's disease, with evidence suggesting that soluble Aβ peptides play an essential role in the pathogenesis and advancement of this disease [36]. Therefore, elucidating the soluble monomeric conformation of the Aβ (25-35) peptide is of great importance, as this conformation may profoundly influence the nature of early aggregates and the resulting morphology of the amyloid fibril [37].

To perform multidimensional solution NMR experiments, the Aβ (25–35) peptide was solubilized in a water/HFIP solution at 25 °C. The sequential $d_{\alpha N}$ (*i*, *i*+1) connectivities are summarized in Figure 3.

Complete H, C, and N signals were assigned using a combination of TOCSY, NOESY, and HSQC NMR experiments. The spin systems of the significant amino acid residues and their respective ^{1}H and ^{13}C chemical shifts were identified by analyzing intra-residual correlations in the TOCSY spectrum and the ^{1}H–^{13}C HSQC spectrum. The number of inter-residual cross-peaks detected in the NOESY contour map helped resolve all ambiguities. The NMR data suggest the absence of stable secondary structures, as no medium-range dαN (i, i+n) contacts (with n = 2, 3, or 4) were detected in the NOESY spectra. These chemical shifts were then used for secondary structure and dihedral angle prediction using TALOS+, in which the chemical shift dispersion of alpha and amide protons indicates an unfolded peptide conformation (Figure 4).

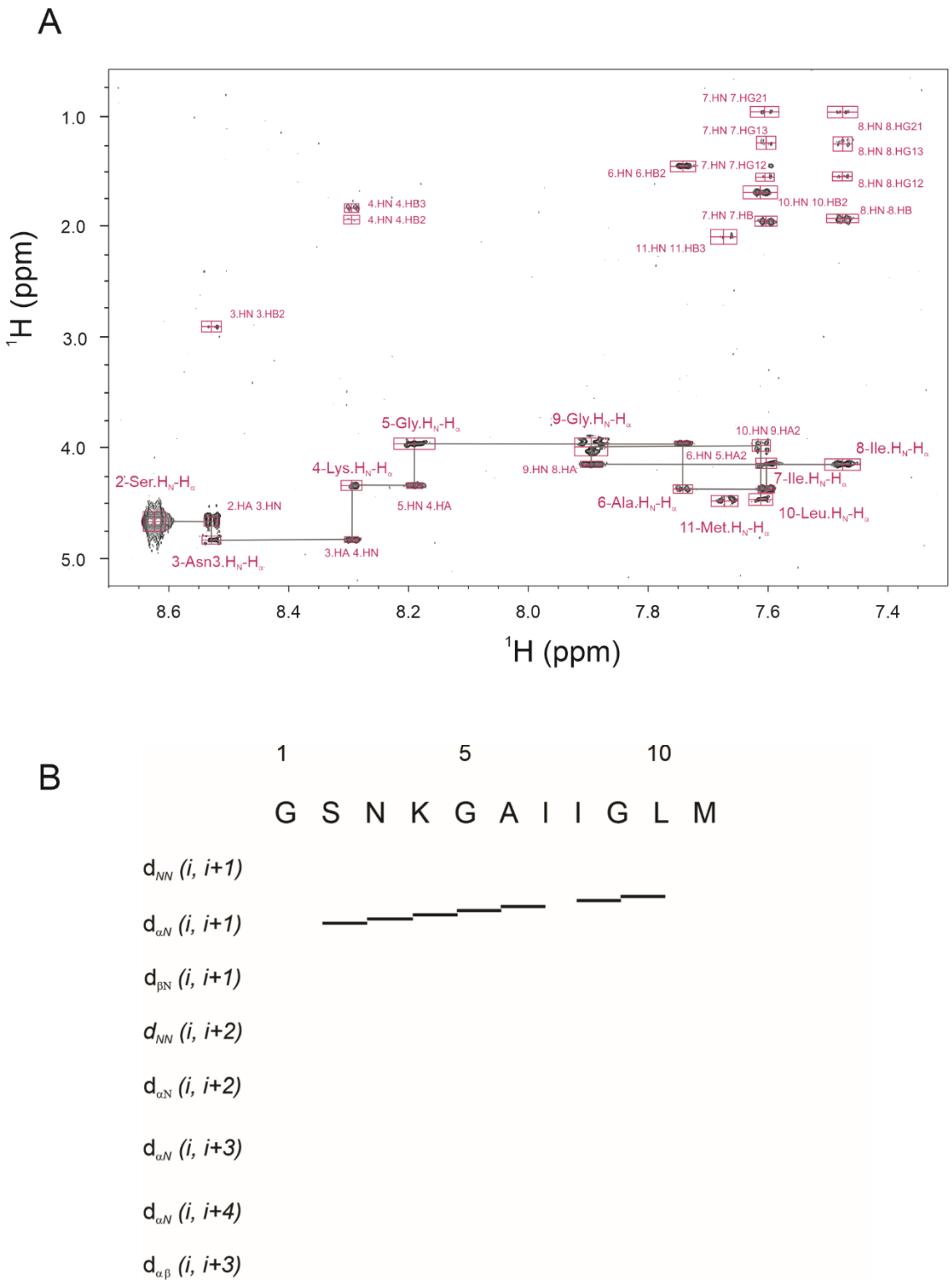

Figure 3. HN-Hα region of NOESY spectrum of Aβ (25-35) peptide at 1 mM in water/HFIP/D2O (A). Spectrum recorded at 25 ºC, 500 MHz. Only the inter-residual HN-Hα (i, i +1) cross peaks are labeled.

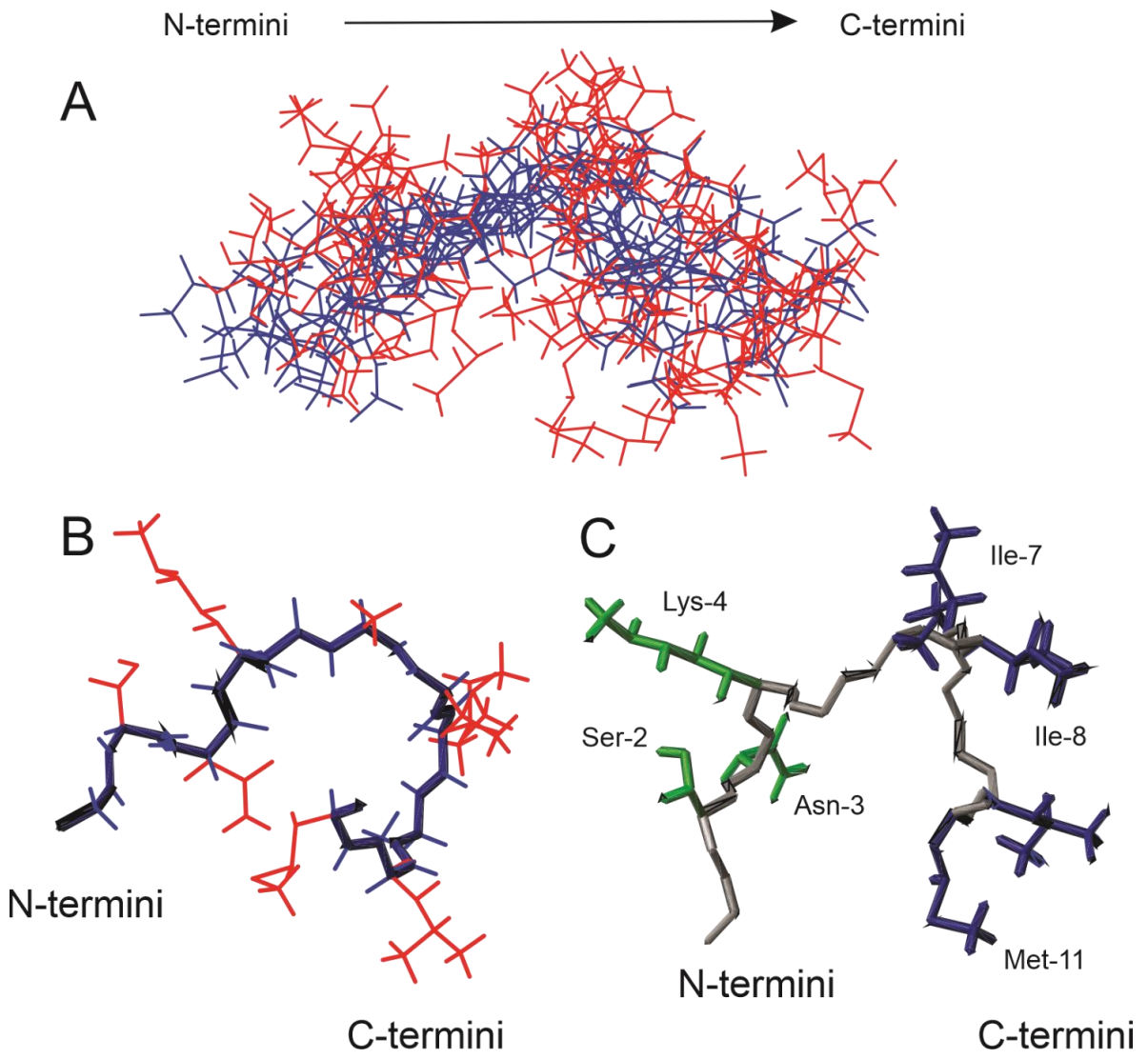

Figure 4. Three-dimensional structures of Aβ (25-35) peptide (1 mM) at 25°C obtained from NMR at 500 MHz. The superposition of the ten lowest-energy structures for ecPis-4s (from G1 to M11) (panel A). Horizontal perspective of the lowest-energy structure (panels B and C). The hydrophobic residues are presented in blue and the hydrophilic residues in green.

The superposition of the ten lowest-energy structures of the Aβ (25–35) peptide is shown in Figure 4, illustrating both backbone and side-chain orientations. The lowest-energy structure is also presented from two different perspectives. As observed, under the conditions of this study (water/HFIP solution), the Aβ (25–35) peptide secondary structure can be described ambiguously.

Overall, our spectroscopic data confirm that the conformational behavior of Aβ (25–35) is strongly environment-dependent. These conclusions have also been reached in many recent structural studies of Aβ (25− 35) peptide [38-42]. Previous studies on Aβ peptide fibrillogenesis associated with familial cerebral amyloid angiopathy support this mechanism [43]. These studies showed that the model peptide can form both unstructured and helix-turn-helix conformations [44,45].

4. Conclusions

The NMR-derived structures of the Aβ (25–35) peptide in a 20/80 HFIP and water mixture indicate that ordered arrangements are concentrated mainly in the C-terminal portion. The mean global backbone RMSD of the Aβ (25–35) peptide is 3.39 ± 0.96 Å. When aligned based on the N-terminal segment (residues 1 to 5), the structures are well-fitted, with a global RMSD of 1.49 ± 0.35 Å. The backbone region spanning residues 25 to 29 exhibited an RMSD of 1.49 Å. Understanding these conformational properties can contribute to creating molecules that target amyloid peptides and block fibril development.

Conflict of interest

The authors declare that they have no conflicts of interest.

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