1-26-2008

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Recommended Citation

Kaur K, Sprules T, Soliman W, Beleid R, Ahmed S. Right-handed 14-helix in beta(3)-peptides from l-aspartic acid monomers. *Biochim. Biophys. Acta*. 2008;1784(4):658-665. doi: 10.1016/j.bbapap.2008.01.009

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Right-handed 14-Helix in β^3-Peptides from L-Aspartic Acid Monomers

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Keywords: β-Peptides; 14-Helical conformation; Solution structure; MD simulations

Abstract

β-Peptides made from L-aspartic acid monomers form a new class of β^3-peptides. Here we report the first three-dimensional NMR solution structure of a β^3-hexapeptide (**1**) from L-aspartic acid monomers in 2,2,2-trifluoroethanol (TFE). We show that **1** forms a *right-handed* 14-helical structure in TFE. α-peptides from naturally occurring L-amino acids adopt a right-handed α-helix whereas β^3-peptides formed from β^3-amino acids derived from naturally occurring L-amino acids form left-handed 14-helices. The right-handed 14-helical conformation of **1** is a better mimic of α-peptide conformations. Using the NMR structure of **1** in TFE, we further study the conformation of **1** in water, as well as two similar β^3-peptides (**2** and **3)** in water and TFE by molecular dynamics (MD) simulations. NMR and MD results suggest loss of secondary structure of **1** in water and show that it forms a fully extended structure. **2** and **3** contain residues with oppositely charged side chains that engage in salt-bridge interactions and dramatically stabilize the 14-helical conformation in aqueous media.

Abbreviations: CD, circular dichroism; MD, molecular dynamics; NMR, nuclear magnetic resonance spectroscopy; NOE, nuclear Overhauser effect; NOESY, two-dimensional NOE spectroscopy; ns, nanosecond; pbc, periodic boundary conditions; ps, picosecond; RMSD, root mean square deviation; TFE, 2,2,2-trifluoroethanol; SPC, simple point charge

1. Introduction

β-peptide foldamers are interesting from a pharmaceutical standpoint as mimics of α-peptides since they are not susceptible to proteolysis and metabolism [1-6]. β-peptides contain one additional methylene group in the backbone compared to the α-peptides. They are subdivided into mainly three types, namely, β^3-, β^2-, and $\beta^{2,3}$-peptides, depending upon the substitution of the backbone carbon. The substitution pattern dictates the solution conformation of these peptidomimetics. In general, monosubstituted β^3- or β^2-peptides form 14-helical structures and alternating β^2/β^3-peptides prefer 10/12 helices. β^3-peptides that fold into stable 14-helical conformations have received particular attention [7-12]. The helical secondary structure is enhanced by the presence of organic solvents, such as methanol and 2,2,2-trifluoroethanol (TFE), and efforts have been made to stabilize this conformation in aqueous solutions [13-17]. Introduction of constraints like cyclic ring systems, salt-bridge formation, or neutralization of the helix macrodipole have been utilized to obtain stable helical structures in aqueous media [13, 18, 19].

β^3-peptides are made from β^3-amino acid monomers using solid-phase synthesis, while the β^3-amino acids themselves are synthesized by homologotion of α-amino acids [12, 20]. We have recently reported the synthesis of novel β^3-peptides from L-aspartic acid monomers [21]. The synthetic strategy employed allowed easy access to a wide variety of side chains by coupling of a free amine to the side chain α-carboxylic group of aspartic acid. This procedure introduces an extra amide bond in the side chain of the β-peptide scaffold making them more polar than the previously reported β-peptides (Figure 1a). However, this extra amide bond provides opportunities for more hydrogen-bonding capabilities and is speculated to give unprecedented

secondary structure in this class of β-peptides. Furthermore, the stereochemistry at the β^3 carbon is opposite in these two classes of β^3-peptides (Figure 1a).

Here, we study the solution conformation of few representative β^3-peptides (Figure 1b) from L-aspartic acid monomers. Using NMR spectroscopy and MD simulations, we have determined the three-dimensional structure of a β^3-hexapeptide **1** in 2,2,2-trifluoroethanol (TFE). We show that **1** forms a *right-handed* 14-helical structure in TFE. In contrast, the reported β^3-peptides formed from β^3-amino acids derived from naturally occurring L-amino acids form left-handed 14 helices [1, 4]. The right-handed 14-helical conformation of **1** is a better mimic of right-handed α-helix of α-peptides (Figure 2). The NMR solution structure of **1** in TFE is further used to study the conformation of **1**, as well as two similar β^3-hexapeptides (**2** and **3)** in water and TFE by molecular dynamics (MD) simulations. Six independent simulations, each of 10 or 20 ns duration, were conducted with β^3-peptides immersed in water or TFE with appropriate salt concentrations. The results from these simulations suggest that a salt-bridge interaction between the side chain residues is required for maintaining the helical conformation in water. The different conformations acquired by these peptides in the two solvent systems are discussed.

Fig. 1. (a) Chemical structure of β^3-peptide from L-aspartic acid monomers (left) and β^3-peptides from β^3-amino acids derived from natural L-amino acids (right). (b) Chemical structure of β^3-peptides from L-aspartic acid monomers (**1**, **2**, and **3**) studied here.

Fig. 2. Backbone conformation of right-handed α-helix in an α-peptide and 14-helix in a β-peptide from L-aspartic acid monomers. N and C stand for N- and C-terminus of the peptides, respectively.

2. Methods

2.1. NMR Structure Calculation

The NMR sample of previously synthesized **1** [21] was prepared in 100% TFE-d2 (CF_3CD_2OH) (Cambridge Isotope Laboratories, Inc.). Sequence specific assignments were established by following the procedures used for α-amino acid peptides [22]. The complete chemical shift assignments for **1** are listed in Table 1. 2D TOCSY (60 ms mixing time), COSY and NOESY (mixing time 100, 200 and 300 ms) spectra were recorded at 10°C on a Varian INOVA 500 MHz spectrometer equipped with an HCN cold probe and pulsed-field gradients. 153 upper distance restraints for structure calculations were obtained from a 300 ms mixing time 2D-[^{1}H,^{1}H]-NOESY recorded at 10°C on a Varian INOVA 800 MHz NMR spectrometer, equipped with an HCN cold probe and pulsed-field gradients. Calculation of the complete three-dimensional structure was performed with the program CYANA v 2.1 [23]. Input data and structure calculation statistics are summarized in Table 2. The final calculation was started with 200 randomized conformers all of which converged to the helical conformation (average backbone RMSD to mean for 200 structures: 0.61 ± 0.21 Å). A bundle of the 10 lowest energy CYANA conformers were used to represent the NMR structure. The NMR structure of **1** showed a well-defined 14-helical conformation that was further optimized by 10 ns MD simulation in TFE as described below.

Table 1: Chemical shift assignments for β^3-hexapeptide **1**.

Table 2. Structure calculation statistics for β^3-hexapeptide **1**.

2.2. Molecular Dynamics Simulations

The NMR structure obtained for **1** using CYANA was further subjected to molecular dynamics (MD) simulations using GROMACS [24, 25] as described previously [26, 27]. Simulation systems were generated by placing **1** (pdb coordinates from CYANA) in a cubic box with an edge length of ca. 4 nm, followed by solvation of the peptide using 2,2,2-trifluoroethanol (TFE). Sufficient counterions were added to make the system electroneutral and to provide a final concentration of ~25 μM NaCl. All the amines including the N-terminal amine were positively charged giving the peptide a net charge of +3. The details of the periodic cell for simulation system are listed in Table 3. The system was initially subjected to 1000 steps of the steepest descent energy minimization. The peptide coordinates of the resulting system were then restrained, while allowing solvent molecules to relax their positions and optimize interactions with the peptide during a 200 ps positional-restraint simulation. After initial equilibration, 10 ns MD simulation was run. Simulation was analyzed using GROMACS routines. SwissPDB Viewer [28] and WebLab ViewerLite [29] were used to visualize structures. The root-mean-square deviation (RMSD) analysis of the peptide (all residues) showed that the MD structure of **1** is very similar to the initial NMR structure. Visualization of the snapshots from the simulation also showed no difference in NMR structure and the MD structures in TFE. Thus the average structure obtained from the last 1 ns of the MD simulation was used as the final structure of **1** and was used for all subsequent MD simulations.

Table 3: Details of MD simulations in the NPT ensemble.

Structure of **1** in TFE was used as the initial structure for water simulation. The system was setup by placing **1** in a cubic box and the box was filled with SPC water molecules and a 20 ns simulation was conducted as described above.

Initial structures for peptides **2** and **3** were generated by replacing the side chains in **1** (TFE structure) using builder module of Insight II (Accelrys Software Inc.). **1** and **2** differ by one residue. **1** consists of two basic lysine mimicking side chains at positions 2 and 5, whereas **2** contains one lysine mimicking side chain at position 2 and a negatively charged carboxylate side chain at position 5. The length of the side chains (4 CH_2 at position 2 and 3 CH_2 at position 5) was such that it maximizes the salt-bridge interaction. **3** contains two pairs of oppositely charged side chains, ornithine mimicking side chains at positions 1 and 2, and glutamate mimics at position 4 and 5. The side chain length was reduced (by one CH_2 as compared to **1** and **2**) as it has been suggested that shorter side chains are better for 14-helix stability [15].

Simulations for **2** and **3** in TFE and water were conducted as mentioned above. All the amines were protonated and carboxylate(s) unprotonated giving both the peptides a net charge of +1. The details of the periodic cell for each system, including the number of water or TFE molecules, the initial box size, and, the duration of simulations are listed in Table 3.

3. Results and Discussion

Our primary goal is to investigate the solution conformation of β^3-peptides from L-aspartic acid monomers. To probe the secondary structure of these β^3-peptides, we have elucidated the conformation of the previously synthesized hexapeptide **1** [21] in TFE using NMR spectroscopy. Analysis of CD spectra of **1** in TFE previously showed the appearance of characteristic peaks for the 14-helical conformation in β-peptides [21]. A maximum near 195 nm and minimum at 214 nm was observed. The NMR spectrum of **1** in TFE further confirms the presence of a stable secondary structure. The well dispersed chemical shifts in the amide region allowed assignment of all the backbone and side chain protons (Figure 3 top). A two-dimensional NOESY

experiment performed at 10°C in CF_3CD_2OH showed multiple (five out of six possible) long-range $C_\alpha H(i) \rightarrow C_\beta H(i+3)$ NOEs (protons at β-carbon are not stereospecifically assigned) (Figure 4). These peaks are a hallmark of 14-helical secondary structure [10, 15, 30]. A three-dimensional structure of **1** was obtained using the NOE data and the program CYANA [23] as described in the Methods section. The pdb coordinates of the structure (average of 10 structures, rmsd of all backbone atoms 0.47 ± 0.11 Å) obtained from CYANA were subjected to molecular dynamics simulations in TFE using GROMACS software [24, 25]. MD simulations in explicit solvent systems complement the NMR data and are a valuable tool for obtaining the final solution structure that is representative of the ensemble of solution structures [31-33]. Figure 5 shows the final structure of **1** in TFE after 10 ns MD simulation. **1** forms a *right-handed* 14-helical structure with a radius of 2.6 Å and 4.69 Å rise/turn, similar to the reported left-handed β^3-peptides [1, 4]. The helical structure was maintained throughout the 10 ns simulation in TFE.

Fig. 3. 1D NMR of β^3-peptide **1** showing the amide H-atoms in 100% TFE-d2 (top) and 90% H_2O/10% D_2O (bottom). HN refers to the backbone amide proton and HS refers to the one in the side chain. The number in the parentheses is for the number of overlapping peaks and impurities are marked with *.

Fig. 4. 2D NOESY of β^3-peptide **1** in TFE showing the long range NOEs, $C_\beta H(i)$-$C_\alpha H(i+3)$ characteristic of 14-helix conformation.

Fig. 5. Solution structure of β^3-hexapeptide **1** in TFE obtained by NMR and MD simulation.

1 lost all the secondary structure in water as evidenced by the limited dispersion of amide chemical shifts in NMR (Figure 3 bottom). Asp-derived β^3-peptides are expected to be more soluble than the "normal" β^3-peptides. Therefore, **1** should be less prone to self-associate in water compared to analogous sequences with similar distribution of hydrophobic side chains as shown by Gellman and coworkers [34]. The CD spectrum of **1** in water previously indicated the presence of no secondary structure [21]. A 20 ns MD simulation of **1** in water (starting with the helical structure) showed dramatic conformational changes. The overall peptide stability was measured by plotting the RMSD of the peptide structure fitted onto its initial structure as a function of time (Figure 6). The RMSD values suggested stabilization of the peptide conformation (**1**) in TFE. However, simulation in water showed destabilization of the peptide conformation during most of the simulation. Further analysis of the trajectories showed maximum conformational changes of **1** in water in the first 12 ns of the simulation with stabilization of the fully extended structure towards the end. Figure 7 depicts snapshots of **1** at five instants (0, 2, 10, 16, and 20 ns) during simulation in water.

Fig. 6. RMSD of β^3-peptide **1** during MD simulation in TFE (a) and water (b). RMSD was calculated with respect to the initial NMR structure in TFE obtained from CYANA.

Fig. 7. Snapshots from MD simulation of β^3-peptide **1** in water.

We next explored the conformation of **2** and **3** in TFE and water using MD simulations. **2** and **3** contain oppositely charged (amine and carboxylate) side chains at the *i* and *i*+3 positions to enhance intramolecular salt bridge formation, thereby stabilizing the helical conformation. **2** and **3** consist of one and two pairs of oppositely charged side chains, respectively. Both the peptides

displayed a well-defined helical structure in TFE throughout the simulation. In water, the overall structure was less rigid compared to that observed in TFE.

Figure 8 shows the average structures of β^3-peptides **2** and **3** in TFE and water obtained from the last 1 ns of the respective MD simulations. The distance between the backbone amide proton of residue *i* and amide oxygen of residue *i*+3 was used to judge the presence of the 14-helical conformation. A distance of about 2.5 Å was considered to be a C=O···H─N hydrogen bond [32]. For all the peptides (**1**, **2**, and **3**), at least three of these hydrogen bonds were observed in TFE throughout the simulation. These distances are listed in Table 2 averaged over the final 1 ns of the simulations in TFE. During water simulations, helical structure was only maintained by peptide **3**, and to some extent by peptide **2** as shown by the hydrogen bond distance between *i* and *i*+3 backbone amide atoms (Table 3).

Fig. 8. Structures of **2** in TFE (a) and water (b), and **3** in TFE (c) and water (d) obtained by MD simulations.

The helical conformation of **2** and **3** was better maintained compared to **1**. A cluster analysis of the simulations in water showed that there were 653, 422, and 189 clusters present during the 20 ns simulations in water of peptides **1**, **2**, and **3**, respectively (Table 3). Each cluster represents a discrete structure that is different from the other cluster and is created based on the RMS deviation of all atoms of the peptide. The number of clusters obtained during simulation in water suggests that although the presence of two salt-bridges in **3** stabilizes the helical conformation dramatically, the overall structure is still flexible in water (189 clusters). In comparison, only one or two clusters were present for **1**, **2** or **3** during simulations in TFE. β^3-peptides with oppositely charged residues at *i* and *i*+3 positions are known to form helix stabilizing intramolecular salt

bridges [13-16], and a similar strategy for enhancing helical stability seem to work for these novel β^3-peptides (with an amide bond in the side chain) as well. Interestingly, the side chain amide protons were not found to engage in any permanent (or long-lasting) hydrogen bond interaction in the helical or other conformations in any of the peptides in either TFE or water.

4. Conclusion

We have studied the solution conformation of a novel class of β^3-peptides derived from L-aspartic acid monomers using NMR spectroscopy and MD simulations. These β^3-peptides contain an additional amide bond in the side chain that provides opportunities for additional hydrogen bonds and is speculated to give unprecedented secondary structures in this class of β-peptides. The NMR solution structure of a representative β^3-hexapeptide **1** shows that it forms a right-handed 14-helical structure in TFE. This helical conformation is same as the 14-helical conformation for the β^3-peptides which do not have an additional amide bond in the side chain. However, **1** forms a right-handed helix and the β^3-peptides obtained from the β^3-amino acids derived from L-amino acids form left-handed helices, most likely due to the opposite stereochemistry at the β^3 carbon in the peptide backbone. Furthermore, using MD simulations it was found that the helical conformation in **1** can be stabilized in water by introduction of side chain salt-bridges, such as in peptides **2** and **3**. Using this structural information, β^3-peptides that mimic biologically active α-peptides are being currently designed and optimized.

5. Acknowledgements

This work was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC). The infrastructure support from the Canada Foundation for Innovation (CFI) is also acknowledged.

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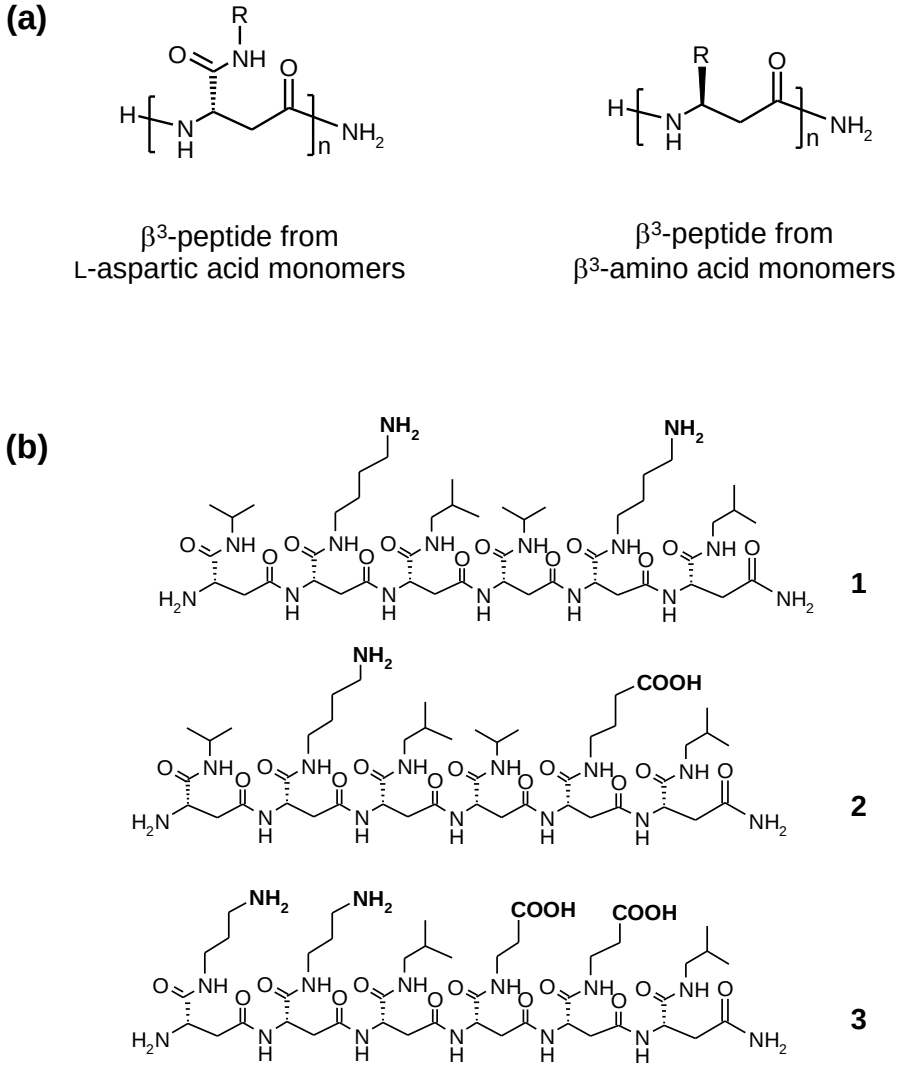

Fig. 1. (a) Chemical structure of β^3-peptide from L-aspartic acid monomers (left) and β^3-peptides from β^3-amino acids derived from natural L-amino acids (right). (b) Chemical structure of β^3-peptides from L-aspartic acid monomers (**1**, **2**, and **3**) studied here.

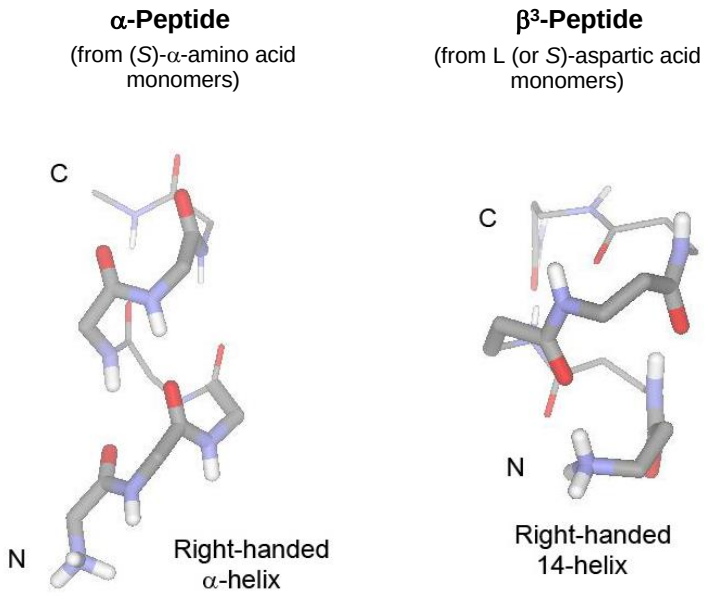

Fig. 2. Backbone conformation of right-handed α-helix in an α-peptide and 14-helix in a β-peptide from L-aspartic acid monomers. N and C stand for N- and C-terminus of the peptides, respectively.

Table 1. Chemical shift assignments for β^3-hexapeptide **1**.

Residue	Backbone shifts	Side chain shifts
1	7.84 (HN), 4.25 (HA), 2.84, 3.61 (HBs)	7.46 (HS), 4.03 (HG), 1.17, 1.22 (HDs)
2	8.78 (HN), 5.04 (HA), 2.61, 3.20 (HBs)	7.43 (HS), 3.22, 3.28 (HGs), 1.61 (HDs), 1.70 (HEs), 3.01 (HEs), 7.32 (HR)
3	9.10 (HN), 4.86 (HA), 2.57, 3.08 (HBs)	6.90 (HS), 3.06 (HGs), 1.76 (HD), 0.90 (HEs)
4	8.58 (HN), 5.03 (HA), 2.54, 2.73 (HBs)	8.24 (HS), 4.01 (HG), 1.20 (HDs)
5	7.32 (HN), 5.22 (HA), 2.64, 2.73 (HBs)	8.26 (HS), 3.20, 3.28 (HGs), 1.61 (HDs), 1.70 (HEs), 3.01 (HEs), 7.20 (HR)
6	7.24 (HN), 5.22 (HA), 2.71 (HBs)	8.28 (HS), 2.98, 3.08 (HGs), 1.81 (HD), 0.90 (HEs)
C-terminus	6.73, 6.92 (NH_2)	

The final concentration of **1** in TFE was 1.5 mM. The chemical shifts were referenced to the TFE methylene protons at 2.88 ppm

Table 2. Structure calculation statistics for β^3-hexapeptide **1**.

NOE upper distance limits[a]	153
Intra-residue	102
Sequential	21
Medium range (*i* to *i*+2 or *i*+3)	30
Final CYANA structures[b]	
CYANA target function	0.17 ± 0.002 Å^2
Average backbone RMSD to mean	0.47 ± 0.11 Å
Average heavy atom RMSD to mean	1.31 ± 0.23 Å
Distance restraint violations	0

[a]148 unambiguous NOEs and 5 ambiguous NOEs used for the structure calculation. [b]10 lowest energy structures of the 200 calculated.

Table 3. Details of MD simulations in the NPT ensemble.

β-peptide[a]	Total number of solvent molecules	Duration (ns)	No. of Atoms	Approx. box size (nm)	Distance between *i* and *i*+3 NH-CO (Å)[b]			Number of clusters
1	420 TFE	10	3040	4 x 4 x 4	2.01	2.04	2.77	1
1	2124 H_2O	20	6472	4 x 4 x 4	-			653
2	417 TFE	10	3015	4 x 4 x 4	2.01	2.04	2.70	2
2	2121 H_2O	20	6459	4 x 4 x 4	4.03	2.19	2.08 (15 ns)	422
					3.80	2.93	2.98 (16 ns)	
					5.60	2.47	5.02 (19-20 ns)	
3	416 TFE	10	3012	4 x 4 x 4	2.15	2.10	2.18	2
3	2121 H_2O	20	6463	4 x 4 x 4	1.85	1.92	2.20	189

[a]A simulation box consists of a β-peptide soaked in TFE or water molecules along with ions to maintain electroneutrality and a final concentration of 25 μM sodium chloride. [b]The distance between *i* and *i*+3 NH-CO refers to hydrogen bond distance between the hydrogen and the oxygen atoms (averaged over the last 1 ns of the simulation) of the backbone amide for a 14-helical conformation.

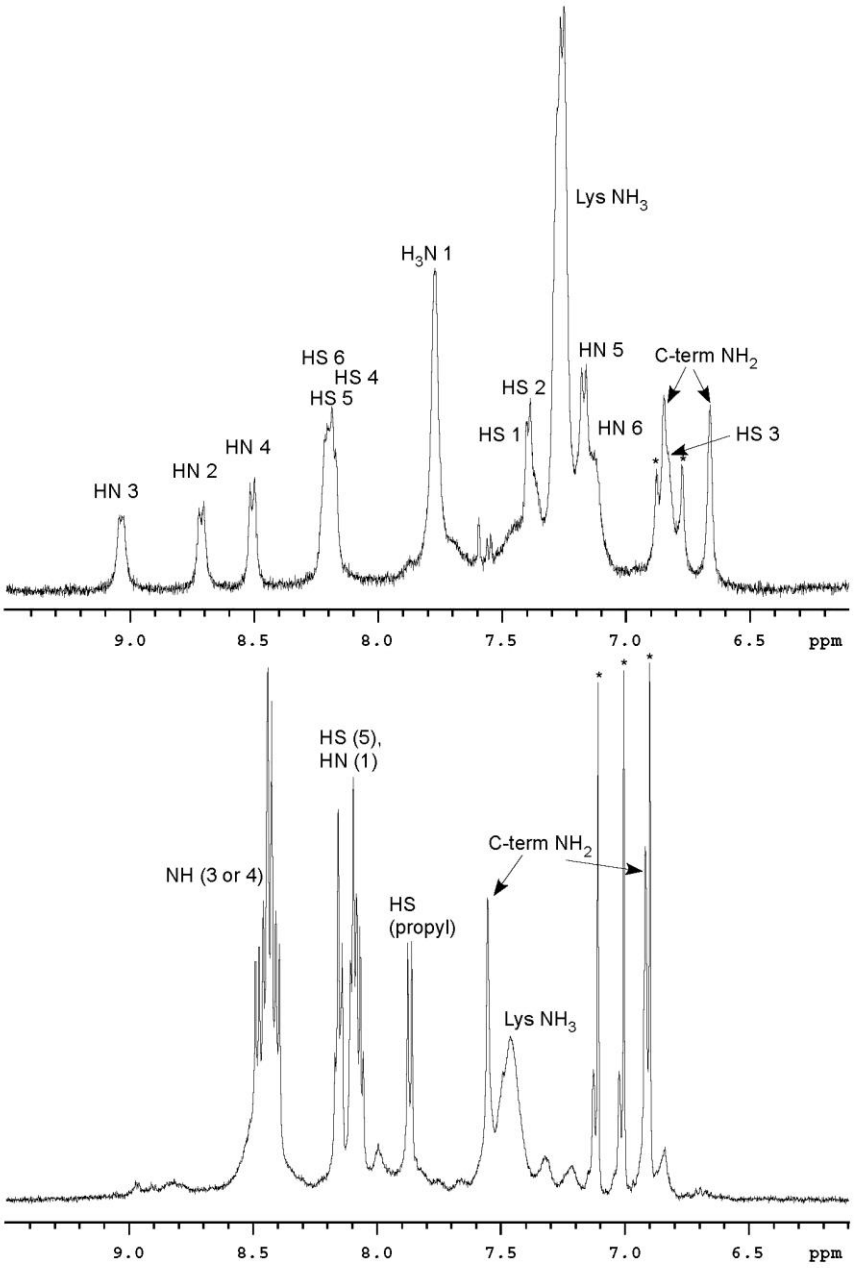

Fig. 3. 1D NMR of β^3-peptide **1** showing the amide H-atoms in 100% TFE-d2 (top) and 90% H_2O/10% D_2O (bottom). HN refers to the backbone amide proton and HS refers to the one in the side chain. The number in the parentheses is for the number of overlapping peaks and impurities are marked with *.

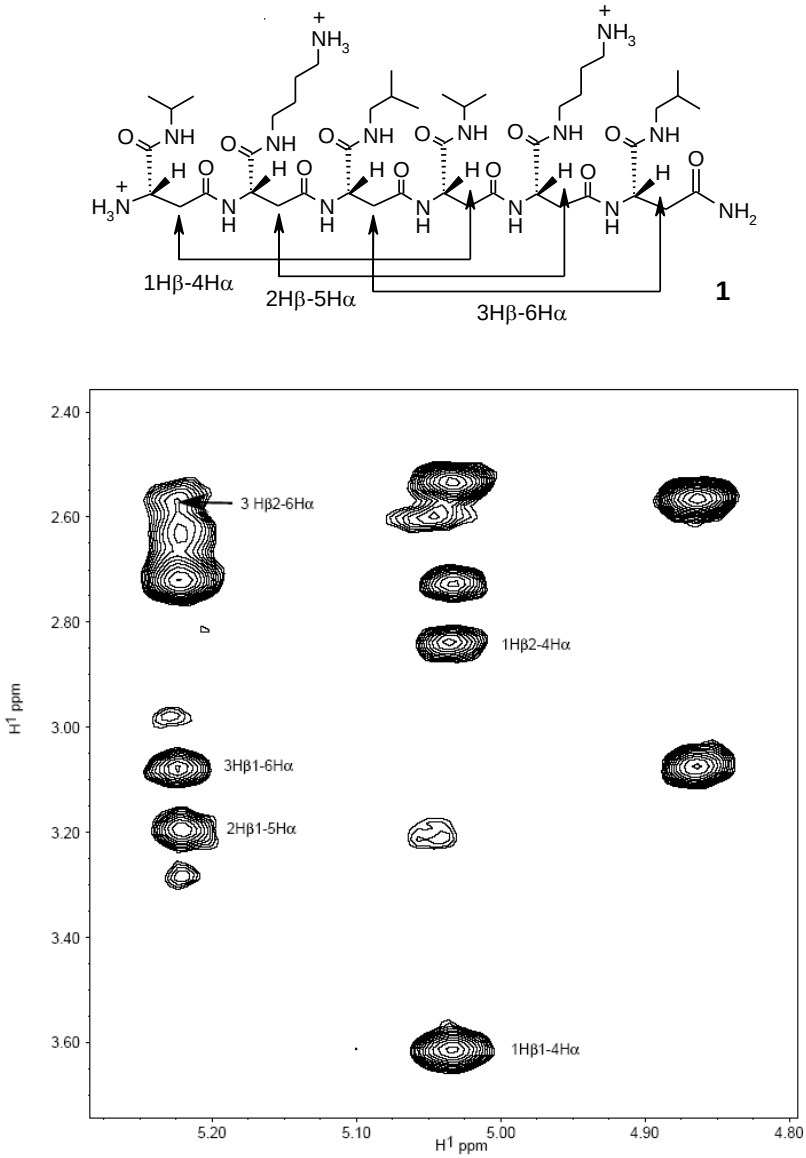

Fig. 4. 2D NOESY of β^3-peptide **1** in TFE showing the long range NOEs, $C_\beta H(i)$-$C_\alpha H(i+3)$ characteristic of 14-helix conformation.

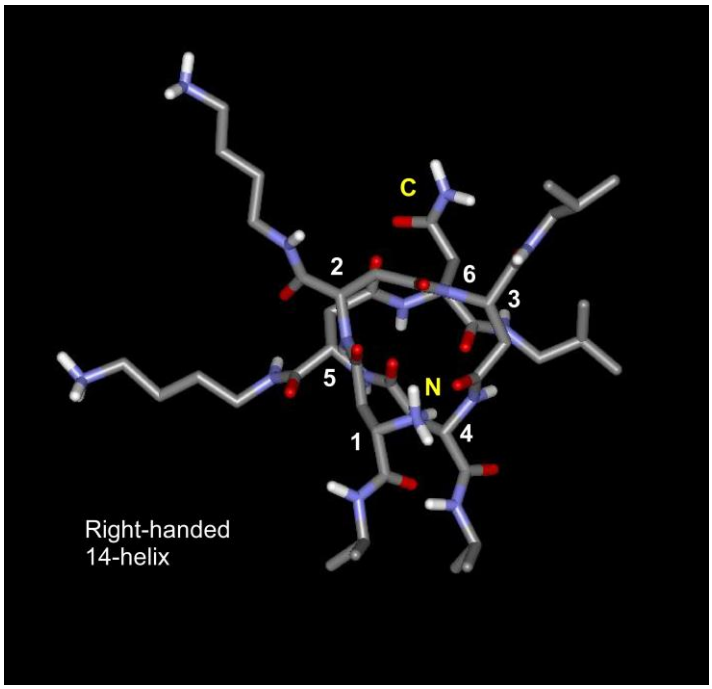

Fig. 5. Solution structure of β^3-hexapeptide **1** in TFE obtained by NMR and MD simulation.

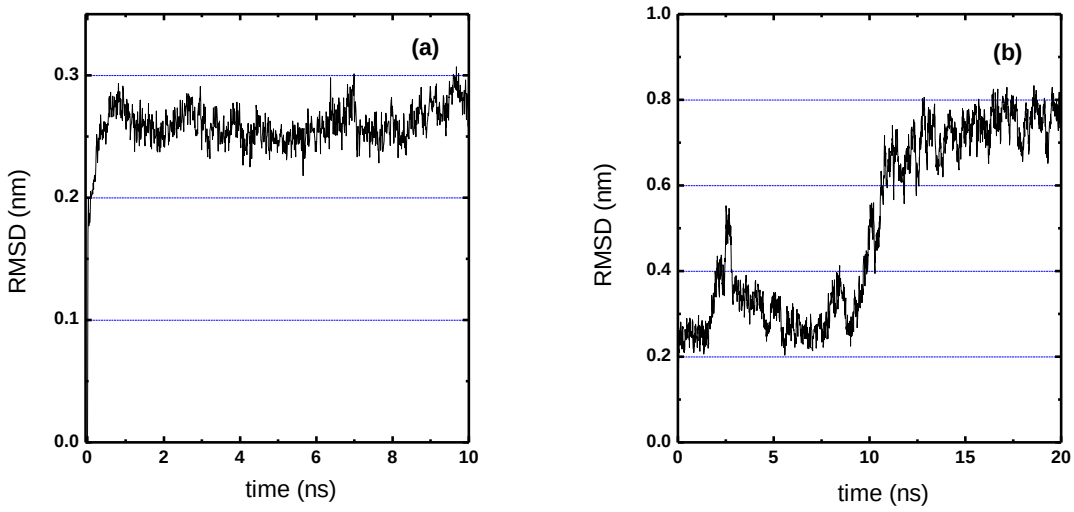

Fig. 6. RMSD of β^3-peptide **1** during MD simulation in TFE (a) and water (b). RMSD was calculated with respect to the initial NMR structure in TFE obtained from CYANA.

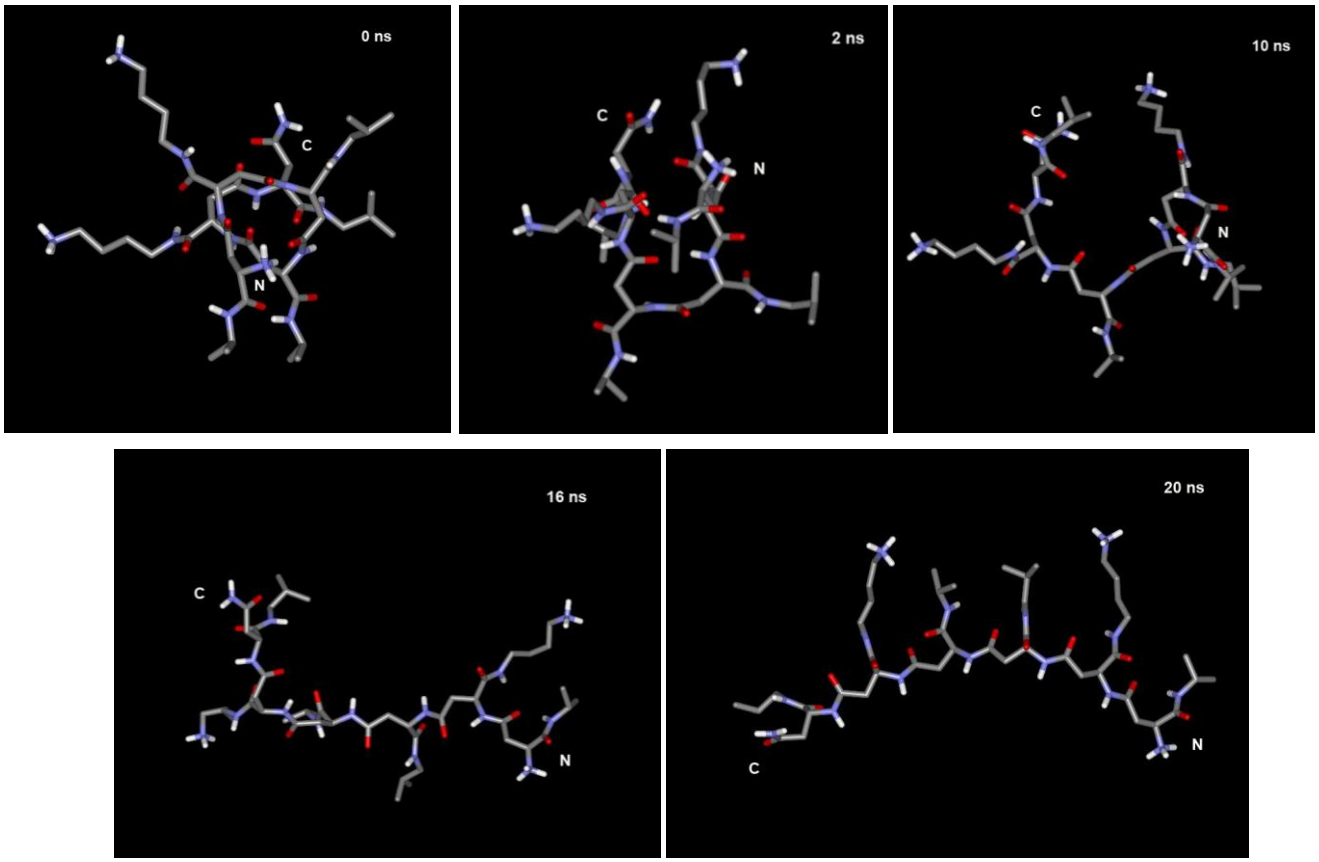

Fig. 7. Snapshots from MD simulation of β^3-peptide **1** in water.

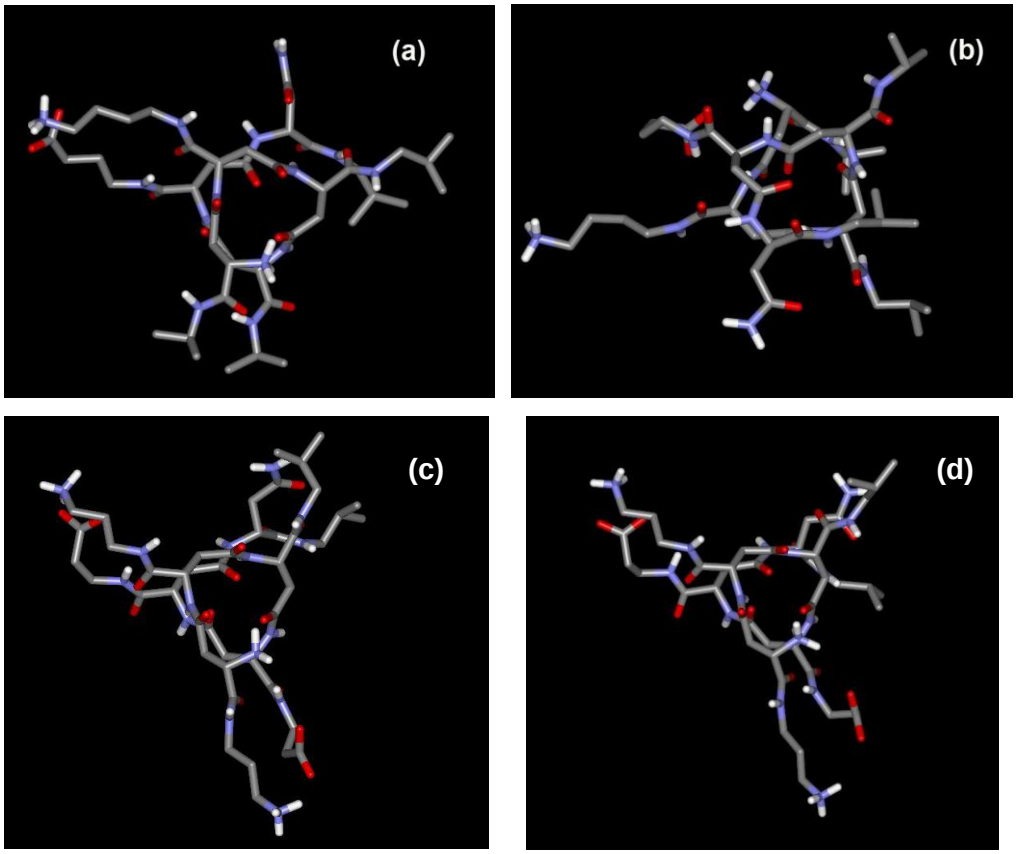

Fig. 8. Structures of **2** in TFE (a) and water (b), and **3** in TFE (c) and water (d) obtained by MD simulations.