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Conformational Studies of [11Ψ12(CN4)]ScyII and [15Ψ16(CN4)]ScyII – Two Scyliorhinin II Analogues by means of 2D NMR Spectroscopy and Theoretical Methods

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Abstract

A conformational analysis of two analogues of scyliorhinin II [$^{11}\Psi^{12}(CN_4)$]]ScyII and [$^{15}\Psi^{16}(CN_4)$]]ScyII was performed in DMSO-d_6. 2D NMR techniques and restrained molecular dynamics were applied. Our previous studies had shown Scyliorhinin II adopts three *cis* peptide bonds in DMSO-d_6 solution. Moreover, in its two analogues [Aib[16]] ScyII and [Sar[16]]ScyII, we also found *cis* peptide bond geometries. Taking above into consideration, we decided to perform extensive conformational studies of restrained ScyII analogues. To do so, we introduced tetrazole groups into either of peptides studied. These peptides were synthesized by the solid-phase method using the Fmoc chemistry. In the case of two analogues, the following spectra were recorded: TOCSY, NOESY, ROESY, DQF-COSY and set of temperature ones. To obtain final structures, we performed restrained molecular dynamics simulations carried out using CHARMM force field as implemented in XPLOR 3.11 programm. Our calculations resulted in two ensembles of 10 conformations each. Comparing the obtained structures, we found that introduction of a 1,5-substituted tetrazole ring influences the three dimensional structure both locally and globally.

Keywords: Conformational studies; Tachykinins; Scyliorhinin II; NMR spectroscopy; Conformational analysis; Molecular dynamics

Introduction

Conformationally constrained peptides are very good subjects for investigations, since the provided modifications make the structure more rigid. It helps in studies of active conformations structures in solution. Widely used *cis* peptide bond constraints include: N-metylated residues [1], double bonds [2], or 1,2,3 triazole [3]. But the most common *cis* amide bond surrogate is 1,5-disubstituted tetrazole [4,5]. This mimic was successfully introduced to among others bradykinin [6], CCK-B receptor ligands [7], somatostatin [8], enkephalins [9], TRH analogues [10] or scyliorhinin I [11], allowing structural studies of bioactive conformations.

The object of this study Scyliorhinin II (ScyII) was isolated from the dogfish gut in 1986 by Conlon et al. [12]. It is a tachykinin peptide which displays selective agonistic activity towards the NK-3 tachykinin receptor [13]. All tachykinin receptors are of similar sequence and belong to the family of G-protein coupled receptors. Their structure is based on heptahelical structure of rhodopsin [14]. The wide range of physiological activity of tachykinin peptides is caused by their short backbone and linearity [15]. Because of these features, they can easily adopt bioactive conformation in contact with the receptor. Scyliorhinin II is one of the biggest tachykinin peptides. Furthermore, there is a disulfide bridge which is rare structural element among all naturally occuring tachykinins. The amino acid sequence of this peptide is as follows:

Ser[1]-Pro-Ser-Asn-Ser-Lys-Cys(&)-Pro-Asp-Gly-Pro-Asp-Cys(&)-Phe-Val-Gly-Leu-Met[18]

Literature data [1,16-18] describes selective agonists for NK-3 tachykinin receptor as ones which prefer to adopt α-helical conformation. Our previous studies showed that ScyII does not adopt any particular secondary structure in the solution. Moreover, we detected the existence of *cis/trans* equilibrium involving three residues of ScyII [19].

Additionally, as reported [1,20,21], Gly16 plays an important role in biological activity and three-dimensional structure of this peptide. Taking above into account, we decided to synthesize two restrained analogues of ScyII. We introduced a tetrazole ring as a surrogate for the *cis* peptide bond between positions 11 and 12 ([$^{11}\Psi^{12}(CN_4)$])ScyII) and 15 and 16 ([$^{15}\Psi^{16}(CN_4)$])ScyII). In this paper, we describe total conformational analysis of [$^{11}\Psi^{12}(CN_4)$]ScyII and [$^{115}\Psi^{16}(CN_4)$]ScyII molecules in DMSO-d_6 using NMR spectroscopy in conjunction with restrained molecular dynamics calculations. We present our results as a set of low energy conformations and discuss them in terms of structural features in comparison to ScyII and its other analogues.

Materials and Methods

Peptide synthesis

Both peptides were synthesized according to protocol described previously [11].

NMR experiment

The sample concentrations were approximately 5 mM in DMSO-d_6

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for [$^{11}\Psi^{12}(CN_4)$]ScyII and [$^{15}\Psi^{16}(CN_4)$]ScyII. All experiments were carried out on a Varian Unity 500 Plus spectrometer (Varian Instruments USA), operating at 500 MHz resonance frequency at 305 K except for temperature ones, which were measured throughout the temperature range of 295-313 K. The assignment of the proton shifts was made by means of one dimensional proton spectra and two dimensional TOCSY (90 ms) [22], NOESY (400 ms) [23], ROESY (200 ms) [24], and DQF-COSY [25,26]. All NMR data was processed using VNMR 6.1B [27], XEASY 3.1 [28] and CARA 1.2 [29] software.

Vicinal coupling constants

The $^3J_{NH\alpha}$ coupling constants were extracted from 1D ^{1}HNMR and 2D DQF-COSY spectra. Due to a great number of overlaping signals in NH region, collecting of $^3J_{HN\text{-}H\alpha}$ constants was possible in the case of [$^{15}\Psi^{16}(CN_4)$]ScyII only.

NOE effects

All NOE cross-peaks, for peptides studied were picked up in the NOESY spectra. The integration was performed in CARA 1.2.

Conformational calculations

Parameterization of tetrazole groups: Two residues including tetrazole ring were build as Pro[ΨCN_4]Asp and Val[ΨCN_4]Gly. They were modelled using bond lengths, the valence and torsional angles of appropriate residues and compatible molecular segments taken from CSDS database [30]. The partial atomic charges were optimized by fitting the point-charge Coulombic potential to the molecular electrostatic potential calculated using GAMESS program and RHF 6-31 G* wave function [31].

Calculations were performed for two different conformations of every non-standard residue, followed by consecutive averaging the charges over all conformations, as recommended by the RESP protocol [32,33].

Molecular Dynamics Calculations

Calculations were carried out in CHARMM force field implemented in XPLOR 3.1 package [34]. The starting conformation was set to random. Additionally, NMR-derived constraints for interproton distances, dihedral angles and ω angles of the peptide groups (to keep them in a trans configuration) were added to the target function with force constants: f=50 kcal/mol×Å^2, f=50 kcal/mol×rad^2 and f=500 kcal/mol×rad^2, respectively. The chirality of C^α atoms (except for Gly) was fixed to L by imposing a three-fold potential on the improper N-CO-C^α-C^β torsion angles with force constant f=500 kcal/mol×rad^2.

Results and Discussion

Assignment of the proton chemical shifts of both peptides was completed using DQF-COSY, TOCSY (Figure 1a and 1b) and NOESY spectra. Spin systems of Val, Leu and Met were identified based on the position of their β, δ and γ protons. Signals of protons of aminoacids joined with a tetrazole group were recognized by the cross-peak between H^α atoms of these residues. Asn 4 protons were possible to identify by means of couplings between H^β and HN^δ. All Gly residues were unambiguously identified by their H^α positions. The rest of H^α protons were identified by sequential couplings visible in fingerprint region of NOESY spectra (Figure 2a and 2b). Next using TOCSY spectra, the rest of protons were assigned. Correctness of this assignment was proved by means of DQF-COSY and NOESY. For two residues of [$^{15}\Psi^{16}(CN_4)$]ScyII, we found more than one set of residual proton

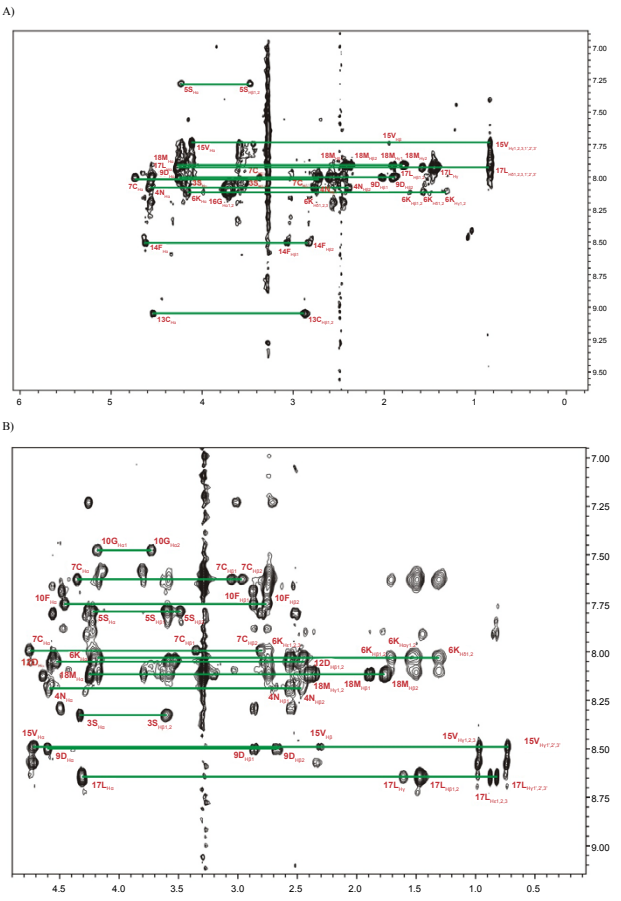

Figure 1: Fingerprint region of TOCSY spectra of Scyliorhinin II analogues in dmso-d_6: a) [$^{11}\Psi^{12}CN_4{}^{12}$]ScyII b) [$^{15}\Psi^{16}CN_4$]ScyII.

resonances (Lys6, Val15). It could be connected with either the presence of *cis/trans* isomerization or flexibility of peptide's fragments containing these residues. All the chemical shifts are summarized in Table 1a and 1b. In both cases, all peptide bonds were in *trans* configuration. To obtain interproton distances, 67 and 124 NOE effects were picked for [$^{11}\Psi^{12}(CN_4)$]ScyII and [$^{15}\Psi^{16}(CN_4)$]ScyII, respectively. Obtained NOE pattern for [$^{11}\Psi^{12}(CN_4)$]ScyII and temperature coefficients (Figure 3a) suggested lack of any particular dominant secondary structure element. However $d_{NN}(i,i+2)$ and $d_{\alpha N}(i,i+2)$ NOEs of Cys13 and Val15 pointed the existence of two β-turns in regions involving these residues. Moreover, Δδ/ΔT values obtained for these residues indicated involvement of their HN protons in the formation of strong hydrogen bonds. The second peptide [$^{15}\Psi^{16}(CN_4)$]ScyII showed also a rigid structure at the *C*-terminus. NOE pattern (Figure 3b) suggested existence of two overlapping β-turns in the region Phe14-Met18. One of their determinants was the tetrazole ring between Val15 and Gly16.

The vicinal coupling constants indicated extended structure of the peptide's backbone (most of obtained values are above 8 Hz). Additionally, when comparing values of temperature coefficients, we deducted that the second peptide studied characterized more packed arrangement of the backbone.

Conformational calculations were carried out only for major species because there was too little data to determine minor ones. As a result, we chose ten conformers of the lowest energy from two

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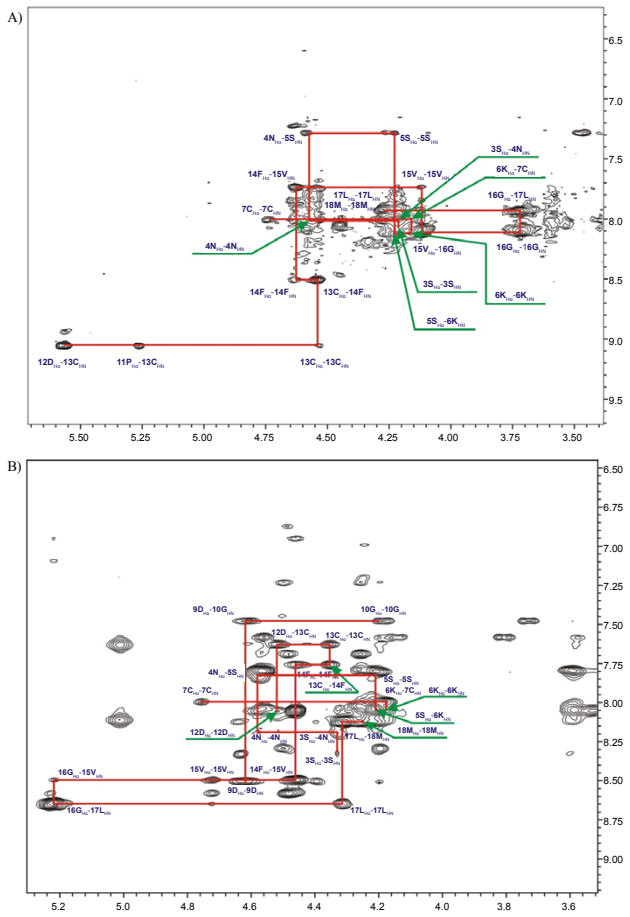

Figure 2: Fingerprint of NOESY spectra of peptides studied in dmso-d_6: a) [$^{11}\Psi^{12}CN_4$]ScyII b) [$^{15}\Psi^{16}CN_4$]ScyII.

ensembles of 100 conformations for each of the peptide studied. For obtained structures, we calculated the positions and types of β-turns (Table 2). They pointed the rigid structure of the peptides and were in good agreement with NMR data indicating [$^{15}\Psi^{16}(CN_4)$]ScyII as more rigid and packed than the other peptide.

The superposition of all C^{α} atoms of [$^{11}\Psi^{12}(CN_4)$]ScyII and [$^{15}\Psi^{16}(CN_4)$]ScyII gave RMSDs of 1.778 and 1.869 Å, respectively. In both ensembles of results, we indicated families of conformations with lower RMSD values. They were: the family of 6 conformations with RMSD of 0.878 Å for [$^{11}\Psi^{12}(CN_4)$]ScyII and two families of 4 conformations for [$^{15}\Psi^{16}(CN_4)$]ScyII with RMDSs of 0.597 and 0.555 Å (Figure 4a-4c). Fragments of studied peptides were better defined what was confirmed by the values of corresponding RMSDs. For 10 conformations of [$^{11}\Psi^{12}(CN_4)$]ScyII, superposition of C^{α} atoms of 7-13 and 12-18 fragments produced RMSDs of 0.792 and 0.546 Å, respectively, whereas for the second peptide, the same fragments gave RMSDs of 1.078 and 1.040 Å. In Figure 5, we showed the comparison of the lowest energy conformations obtained for both ScyII analogues. Analyzing -turns, we could say that IV type β-turn is present in almost all conformations in the regions, which contain tetrazole ring. Positions of other β-turns in each conformational ensemble were similar, but the type.

Conclusions

Studying published data, we have found that 1,5-disubstituted tetrazole is an effective restraint, which allows the peptide to adopt conformation to be recognized by the enzyme [4-6,35-38]. Further, more introduction of this mimic into bradykinin showed that the peptides were able to adopt most conformations of those for native hormone [4-6,35-38].

Aminoacid	Chemical shifts (ppm)					
	HN	α-H	β-H	γ-H	δ-H	Others
Ser1	8.06	4.20	3.55			
Pro2		4.45	2.10 1.84	1.90	3.67 3.53	
Ser3	8.04	4.20	3.55			
Asn4	8.08	4.58	2.75 2.40			NH1 6.93 NH2 7.43
Ser5	7.27	4.23	3.47			
Lys6	8.11	4.16	1.71	1.50	1.32	ε 2.74
Cys7	7.99	4.73	3.37	2.72		
Pro8		4.12	2.11 1.62	1.79	3.45 3.30	
Asp9	8.00	4.26	2.02 1.89			
Gly10						
Pro11		5.28	2.26 1.83	2.00	3.90 3.61	
Asp12		5.58	3.35			
Cys13	9.05	4.57	2.88			
Phe14	8.49	4.63	3.05 2.83			Ar 7.21
Val15	7.74	4.11	1.95	0.84		
Gly16	8.10	3.72				
Leu17	7.92	4.28	1.59	1.45	0.84	
Met18	7.91	4.23	1.91 1.79	2.43 2.37		

Table 1a: The chemical shifts (ppm) of [$^{11}\Psi^{12}(CN_4)$]ScyII in DMSO-d_6 at 305 K.

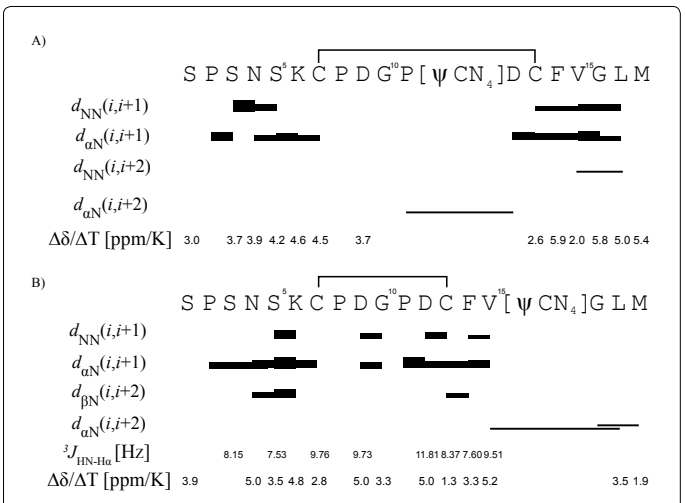

Figure 3: Internal intensities of off-diagonal signals in NOESY spectra, the HN temperature coefficients Δ/ΔT (-p.p.b./K) and vicinal coupling constants $^3J_{HN\text{-}H\alpha}$ of ScyII analogues: a) [$^{11}\Psi^{12}CN_4$]ScyII b) [$^{15}\Psi^{16}CN_4$]ScyII.

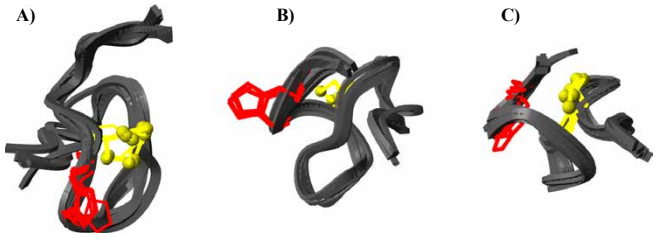

Figure 4: Superposition of Cᵅ atoms of the obtained conformations: a) family of six conformations of [$^{11}\Psi^{12}CN_4^{12}$]ScyII, RMDS=0.878 Å, b) and c) families of four conformations of [$^{15}\Psi^{16}CN_4$]ScyII RMSDs equal 0.597 and 0.555 Å respectively. Disulfide bridge is marked yellow and tetrazole red.

Aminoacid	Chemical shifts (ppm)					
	HN	α-H	β-H	γH	δ-H	Others
Ser1	8.32	4.32	3.60			
Pro2		4.63	2.21 1.80	2.07	3.49 3.42	
Ser3	8.31	4.33	3.60			
Asn4	8.19	4.59	2.56 2.46			NH1 6.95 NH2 7.43
Ser5	7.79	4.22	3.59 3.48			
Lys6	8.03	4.17	1.71	1.53	1.31	ε 2.75
Cys7	7.98	4.76	3.35 2.80			
Pro8		4.20	2.07 1.75	1.82	3.57 3.48	
Asp9	8.49	4.61	2.85 2.68			
Gly10	7.46	4.17 3.74				
Pro11		4.45	2.12 1.84	1.93	3.67 3.53	
Asp12	8.05	4.52	2.54 2.46			
Cys13	7.62	4.35	3.04 2.97			
Phe14	7.75	4.46	2.87 2.77			Ar δ 6.96 Ar ε 7.11
Val15	8.49	4.72	2.31	0.97 0.73		
Gly16		5.22				
Leu17	8.64	4.32	1.46	1.60	0.88 0.82	
Met18	8.11	4.25	1.88 1.77	2.42 2.38		

Table 1b: The chemical shifts (ppm) of [$^{15}\Psi^{16}(CN_4)$]ScyII in DMSO-d_6 at 305 K.

[$^{11}\Psi CN_4^{12}$]ScyII	
Conformation number	Positions (*i*+1 and *i*+2) and types of *β*-turns
[$^{11'}\Psi^{12}$CN4]ScyII	
1	Ser3-Asn4. type I Ser5-Lys6. type II Lys6-Cys7. type II' Pro11-Asp12. type IV Phe14-Val15. type IV
2	Ser3-Asn4. type III' Lys6-Cys7. type IV Pro11-Asp12. type IV Phe14-Val15. type III'
3	Asn4-Ser5. type VII Pro11-Asp12. type IV Phe 14-Val15. type I'
4	Ser3-Asn4. type IV Lys6-Cys7. type IV Phe14-Val15. type IV
5	Asn4-Ser5. type III' Phe14-Val15. type III"
6	Ser5-Lys6. type IV Pro11-Asp12. type IV Phe14-Val15. type II
7	Ser3-Asn4. type III' Ser5-Lys6. type IV Phe14-Val15. type III'
8	Gly10-Pro11. type VI Pro11-Asp12. type IV
9	Ser3-Asn4. type III' Gly10-Pro11. type VI Pro11-Asp12. type IV
10	Asn4-Ser5. type III' Phe14-Val15. type IV
[$^{15}\Psi^{16}CN_4$]ScyII	
1	Ser3-Asn4. type III Ans4-Ser5. type IV Ser5-Lys6. type IV Asp9-Gly10. type II Gly10-Asp11. type III' Phe14-Val15. type III Val15-Gly16. type VI
2	Pro2-Ser3. type I' Asn4-Ser5. type IV Ser5-Lys6. type III' Asp9-Gly10. type II Gly10-Pro11. type III' Phe14-Val15. type I Val15-Gly16. type VI
3	Asn4-Ser5. type II" Ser5-Lys6. type II Asp9-Gly10. type II Gly10-Pro11. type III' Phe14-Val15. type I Val15-Gly16. type VI
4	Asn4-Ser5. type II' Ser5-Lys6. type III' Asp9-Gly10. typeV Gly10-Pro11. type IV Phe14-Val15. type III Val15-Gly16. type VI

5	Asn4-Ser5. type II Ser5-Lys6. type III' Lys6-Cys7. type IV Asp9-Gly10. type IV Asp12-Cys13. type IV Phe14-Val15. type II' Val15-Gly16. type VI
6	Asn4-Ser5. type IV Ser5-Lys6. type III' Asp9-Gly10. type IV Gly10-Pro11. type IV Pro11-Asp12. type IV Asp12-Cys13. type IV
7	Asn4-Ser5. type II' Ser5-Lys6. type II Lys6-Cys7. type IV Asp9-Gly10. type IV Asp12-Cys13. type IV Phe14-Val15. type I Val15-Gly16. type VI
8	Asn4-Ser5. type II Ser5-Lys6. type III' Lys6-Cys7. type IV Asp9-Gly10. type IV Phe14-Val15. type I Val15-Gly16. type VI
9	Asn4-Ser5. type II Ser5-Lys6. type III' Lys6-Cys7. type IV Asp9-Gly10. type IV Asp12-Cys13. type IV Phe14-Val15. type I Val15-Gly16. type VI
10	Asn4-Ser5. type IV Ser5-Lys6. type III' Lys6-Cys7. type IV Asp9-Gly10. type IV Asp12-Cys13. type IV Phe14-Val15. type I Val15-Gly16. type VI

Table 2: Position and types of *β*-turns of obtained conformations.

Not contrary to literature [39], obtained conformations for both ScyII analogues do not adopt any particular secondary structure. Studying the positions of β-turns, we assumed that they were similar to those in [Sar^{16}]ScyII and [AiB^{16}]ScyII, but their types were different. Closer analysis of Ramachandran plots obtained for the peptides studied revealed that *C*-terminus of [$^{11}\Psi^{12}$(CN$_4$)]ScyII might tend to adopt helical structure, which additionally could be confirmed by $d_{NN}(i,i+2)$ NOE effect. Such conformation is responsible for biological activity of tachykinin peptides [1], and may be formed in contact with receptor. Introduction of tetrazole between residues 11 and 12 made the *C*-terminus more rigid and helped expose *C*-terminal fragment out of the molecule making it more accessible. We met the opposite situation in the case of [$^{15}\Psi^{16}$(CN$_4$)]ScyII. The IV type β-turn present in the region of tetrazole introduction caused that the Cys13-Met18 fragment resembled the letter U. We assumed that such restriction could disable biological activity of this peptide. Summing up the introduction of tetrazole ring influenced the peptides' backbones not only locally, but also globally. Furthermore, analyzing the obtained conformations, we could also assume that [$^{11}\Psi^{12}$(CN$_4$)]ScyII might exhibit biological activity what was connected with its C-terminal fragment structure, which was similar to one obtained by Dike and Cowsik for scyliorhinin II in DPC micelles [40].

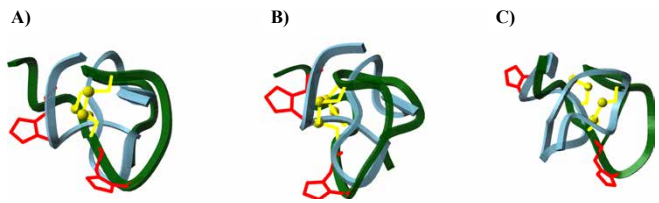

Figure 5: Comparison of the lowest energy conformations of [$^{11}\Psi^{12}$CN$_4$]ScyII (green) and [$^{15}\Psi^{16}$CN$_4$]ScyII (blue): a) superposition of C$^{\alpha}$ of the whole backbones, RMSD=4.895 Å; b) superposition of C$^{\alpha}$ atoms of 3-13 fragments, RMSD=2.934 Å; c) superposition of C$^{\alpha}$ atoms of 13-18 fragments, RMSD=1.948 Å. Disulfide bridge is marked yellow and tetrazole red.

Acknowledgments

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