Dynamic processes in the NMR spectra of spiran 3

In the ^{1}H NMR spectrum of compound 3 in the $CDCl_3$ solvent at room temperature an anomalous broadening of signals of protons of quinone cycle δ_H 6.66 and 6.96 ppm, of *tert*-butyl groups at δ_H 1.06 and 1.36 ppm, and methyl groups of the indoline fragment at δ_H 1.59 and 1.98 ppm is observed. At temperature increase to 57°C the abovementioned signal pairs coalescent (Fig. 3).

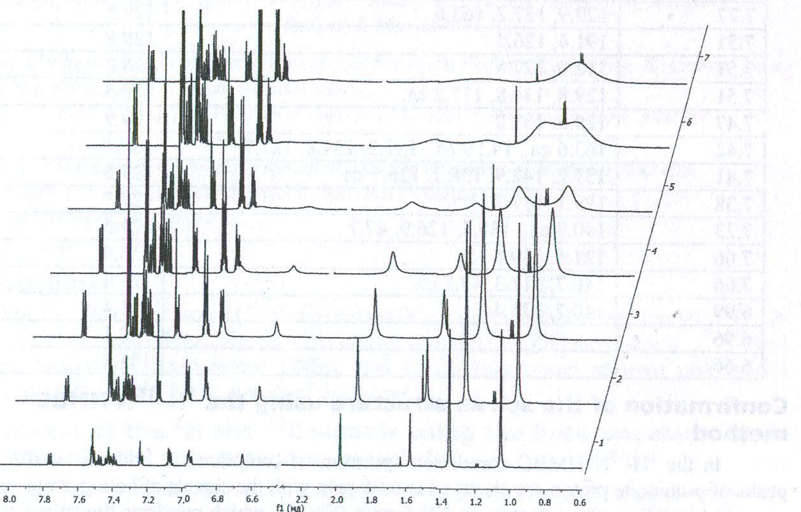

Figure 3. Coalescence of signals at increasing temperature in the NMR ^{1}H spectrum of compound 3 [from 0°C (1) to 60°C (7) with 10°C step]

The dynamics is observed only for paired groups, whose magnetic nonequivalence is due to their spatial position with respect to the noncoplanar molecular structure, i.e., the absence of symmetry plane of the molecule leads to divergence of the signals of protons of the quinone cycle, *tret*-butyl groups, and methyls of the pyrrole cycle. Thus, the most probable reason for the coalescence of NMR spectra signals is the inversion of the molecule by inversion of the pyramidal pyrrole nitrogen.

Acknowledgements

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Structural studies of the antimicrobial peptide protegrin-5 in membrane mimicking environment by high-resolution NMR spectroscopy

O. A. Kolosova, K. S. Usachev, V. V. Klochkov
Institute of Physics, Kazan Federal University
E-mail: kolosova.olga11@gmail.com

Introduction

Antimicrobial peptides (AMPs) are small peptides with a strong antibiotic activity which play an important role in the immune system of many different animals. Naturally occurring AMPs probably represent one of the very first evolved forms of chemical defense of living eukaryotic cells against invasion by bacteria, protozoa, fungi, and virus. AMPs are less susceptible to the development of bacterial resistance because they disrupt the membrane of bacteria through nonspecific peptide–lipid interactions. Natural AMPs have such properties as the broad-spectrum antibacterial activity, high selectivity, and the disruption of bacterial cell membranes, which allow to suggest that these molecules are potentially useful as antibiotics. So, it is important to investigate their structure and function at high resolution in order to increase their potency and selectivity.

There are five known naturally occurring porcine protegrins: PG-1 (RGGRL^{5}CYCRR10RFCVC^{15}VGR^{18}), PG-2 (RGGRL^{5}CYCRR10RFCIC^{15}V^{16}), PG-3 (RGGGL^{5}CYCRR^{10}RFCVC^{15}VGR^{18}), PG-4 (RGGRL^{5}CYCRG^{10}WICFC^{15}VGR^{18}), and PG-5 (RGGRL^{5}CYCRP^{10}RFCVC^{15}VGR^{18}), with a high content of cysteine (Cys) and several positively charged arginine (Arg) residues. In this study we represent PG-5(Fig.1).

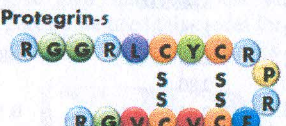

Figure 1. Amino acid sequence of PG5

Materials and method

The protegrin peptides were synthesized by solid-phase peptide synthesis by Dr. Andrey Filippov in the Chemistry of Interfaces Laboratory at the Luleå University of Technology.

The NMR investigation was done on a Bruker Avance 700 spectrometer equipped with a cryoprobe. The peptide (4 mg) was solubilized in an aqueous solution (H_2O or $^{2}H_2O$, 500 µl) containing 20 mg perdeuterated dodecylphosphocholine (DPC) (molar ratio ~1:12). 3-(Trimethylsilyl)-propionic-2,2,3,3–$^{2}H_4$ acid (TMSP-2,2,3,3-$^{2}H_4$) (98 % atom ^{2}H, Aldrich) was added as an internal chemical shift standard for ^{1}H NMR spectroscopy. Perdeuterated d_{38} DPC (98 % ^{2}H) and TSP-d_4 were purchased from Aldrich. Chemical shift assignments of PG-5 in DPC micelles were obtained using standard methods of protein NMR spectroscopy by 2D NMR 1H–1H TOCSY and NOESY (Nuclear Overhauser Effect SpectroscopY) experiments. Interproton NOE distance constraints determined from NOESY experiments were used for the structural calculations by molecular dynamic method calculations of the Xplor-NIH program.

Results

In this study, a 2D NMR ^1H–^1H COSY, ^1H–^1H TOCSY and ^1H–^1H NOESY methods of protein NMR spectroscopy approaches was used for sequential assignment of the backbone and sidechain resonances for antimicrobial peptide protegrin-5 in the presence of DPC micelles (detergent/protein ratio of ~1:12) as a membrane mimicking environment (Table 1). Final chemical shifts values were deposited in the BioMagResBank database (http://www.bmrb.wisc.edu) under accession number 26009.

Table 1. ^1H chemical shifts in ppm measured in water for PG-5 in the presence of perdeuterated DPC micelles(detergent/peptide molar ratio ~1:12) at 293 K.

Residue	NH	$C_\alpha H$	$C_\beta H$	$C_\gamma H$	$C_\delta H$	$C_\varepsilon H$
R1	–	3.97	1.83	1.58	3.10	7.38
G2	8.98	3.89				
G3	8.46	3.89				
R4	8.65	4.20	1.71	1.53	3.04	7.52
L5	8.54	4.21	1.62	1.51	0.83, 0.77	
C6	8.01	4.28	2.73			
Y7	8.07	4.43	2.97, 2.82		7.05	6.74
C8	7.88	4.35	2.74			
R9	8.40	4.25	1.75, 1.64	1.52	3.03	7.53
P10	–	4.28	1.93	1.64	3.77, 3.46	
R11	8.20	4.07	1.67, 1.53	1.47	3.00	7.42
F12	8.00	4.45	3.06, 3.00		7.22	7.15
C13	8.20	4.28	2.78			
V14	7.91	4.02	2.05	0.85		
C15	8.29	4.35	2.80			
V16	7.95	4.09	2.05	0.79		
G17	8.33	3.87				
R18	8.02	4.17	1.79	1.60, 1.50	3.05	7.42

A total of 164 distance constraints derived from NOE experiment recorded in H_2O and D_2O were used for structure calculations by molecular dynamics method in the Xplor-NIH program. A total of 1000 structures were calculated and 20 with minimal energy were chosen. None of the 20 structures had any violated NOE distances. The final NMR ensemble of 20 structures has been deposited in the Protein Data Bank with the code 2NC7. Superimposed conformations of the minimized structures for the PG-5 in a solution of H_2O + D_2O with DPC micelles are shown in Fig. 2. The overall quality of the structures was assess by the program MolProbity. The Ramachandran analysis of the best 20 structures showed that all ϕ and ψ angles were localized to allowed (100 %) and favored (72.8 %) areas of the Ramachandran plot. The mean value of the overall backbone root-mean-squared deviation (RMSD) for backbone was 0.53 ± 0.21 Å and for heavy atoms 1.34 ± 0.27 Å.

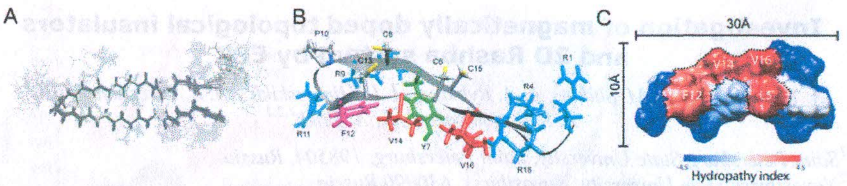

*Figure 2. Spatial structure of protegrin-5. **A** Overlay of the ensemble of 20 final energy-minimized XPLOR structures in stereo (PDB ID: 2NC7). The main and side chains are shown in black and light gray, respectively. **B** Ribbon diagrams of the lowest energy structure. Aliphatic amino acid residues are marked in red (Leu and Val), polar residue (Tyr) are marked in green, phenilalanine residue is marked in magneta and positively charged residues (Arg) are marked in cyan. **C** Kyte–Doolittle hydrophobicity surface structural model for PG-5: from blue for the most hydrophilic, to white, to red for the most hydrophobic (50). An apolar cluster (Leu5, Phe12, Val14 and Val16) marked by dotted line*

Protegrin dimerization in the presence of DPC micelles was observed for PG-1 and PG-3[1] from 2D ^1H–^1H NOESY spectra, where there were observed several additional NOEs between side chains, which appear inconsistent with the β-sheet structure, and it was assumed that these NOEs appear due to formation of an additional antiparallel β-sheet between two monomers. We also observed such "inconsistent" NOEs for PG-5, which are an indication that PG-5 adopts an antiparallel dimer [2]. Several experimental and computational studies shown by potentials of mean force (PMF) calculations and atomic force microscopy that protegrins could associate and insert into an anionic membrane forming an octameric or decameric β-barrels. According to PMF data, octamers were more stable and exhibit a favorable binding energy to the pore so we used this model for our further calculations of pore structure for PG-5 based on the observed intermonomer NOEs (the final structure in Fig. 3).

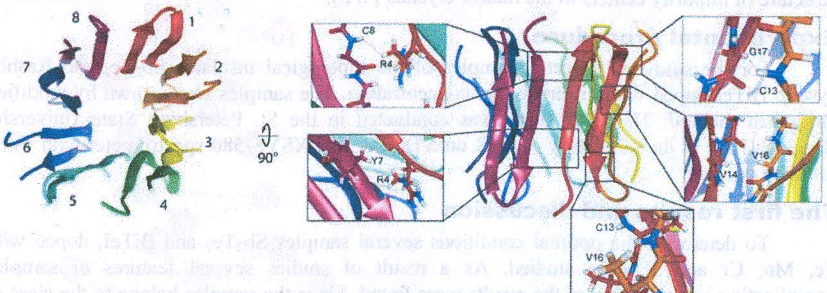

Figure 3. Structure of the transmembrane pore of protegrin-5

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