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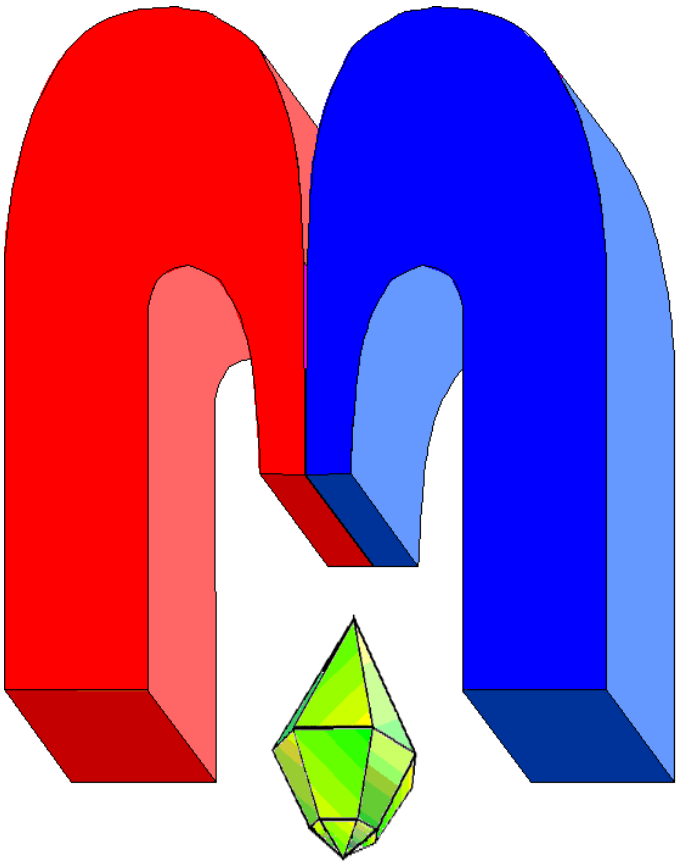

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* In Kazan University the Electron Paramagnetic Resonance (EPR) was discovered by Zavoisky E.K. in 1944.

Spatial structure of tetrapeptide N-AC-Ser-Phe-Val-Gly-OMe in "protein-micelle of sodium dodecyl sulfate" complex and in solid state by NMR spectroscopy

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In the present paper the applicability of a structure determination for a four amino acid residues containing oligopeptide *N*-Ac-Ser-Phe-Val-Gly-OMe was investigated. The spatial structure of the "tetrapeptide (*N*-Ac-Ser-Phe-Val-Gly-OMe) - sodium dodecyl sulfate micelle" complex in aqueous solution was studied by 2D nuclear magnetic resonance (NMR) spectroscopy. The complexation was confirmed NOEs signs and values in the presence of sodium dodecyl sulfate. The spatial structure of the tetrapeptide in the complex was determined by 2D NOESY NMR spectroscopy. In present paper by the comparison ^{13}C NMR chemical shifts was shown that tetra peptide's spatial structure in solid state and tetrapeptide structure in "peptide-micelle" complex are identical.

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Keywords: oligopeptides, micelles, 2D NMR spectroscopy (TOCSY, NOESY), sodium dodecyl sulfate

1. Introduction

Proteins are extremely complex organic molecules - biopolymers (polypeptides) with amino acids as the structural units. It is well known that the biological activity of proteins is related to their spatial structure [1-4]. The study of oligopeptides conformations containing of two or more amino acid residues is also important because they can be considered as building blocks of proteins, and knowledge of their structure can be used to predict the polypeptide chain configuration. We also know that some of the short peptide sequences synthesized by the cell are parts of the immune system of a living organism [1-4].

Traditionally, the structural study of the relatively small organic compounds in solutions is based on 1D NMR (^1H, ^{13}C) spectroscopy, and some modern NMR spectroscopy approaches such as 2D COSY, TOCSY, HSQC, HMBC and NOESY NMR experiments [5, 6]. Note that (^1H - ^1H) NOESY NMR spectroscopy allows to determine the interatomic distances up to 5 Å, and thus, to establish the spatial structure of organic compounds in the solution [5]. However, a method based on 2D NOESY NMR spectroscopy is not always effective for relative small molecules [5, 6]. This is due to the rather small correlation time τ_c of these molecules in solution, which leads to a weak cross-peaks intensity in the NOESY NMR spectra and to some difficulty in obtaining quantitative information about interproton distances in the molecular systems.

In order to use the 2D NOESY NMR spectroscopy for the spatial structure determination of small oligopeptides, a method was introduced by authors [7]. This method is based on the mechanism of "oligopeptide – micelle" complex formation. Under this process the protein is transferred from the domain of small molecules (for which the condition of rapid tumbling is valid) to the category of large molecules (for which the condition of slow tumbling is effective) [5, 6]. The "efficiency" of the proposed method was confirmed by a number of structural studies for peptides with 7, 10 and 41 amino acid residues in length [7-10].

The aim of this study was to determine the applicability of 2D NOESY NMR spectroscopy to the spatial structure determination of oligopeptides in a complex with sodium dodecyl sulfate (SDS)

micelles and to establish the spatial structure of the tetrapeptide *N*-Ac-Ser-Phe-Val-Gly-OMe in a complex with SDS micelles in aqueous solution. The tetrapeptide *N*-Ac-Ser-Phe-Val-Gly-OMe was taken as a model. This oligopeptide was previously studied [11] and the inefficiencies of the NOESY spectroscopy to study the conformation of the tetrapeptide in aqueous solution has been shown.

2. Experimental

^{1}H NMR (500 MHz) spectra of the tetrapeptide *N*-Ac-Ser-Phe-Val-Gly-OMe in complex with SDS in water solution were recorded on an ''AVANCE II-500'' spectrometer (Bruker). ^{1}H NMR spectra were recorded using 90° pulses, with a spectral width of SW = 9.40 ppm, using 10 scans. 2D TOCSY [12] was used to assign signals in the ^{1}H NMR spectra of the tetrapeptide *N*-Ac-Ser-Phe-Val-Gly-OMe.

The relaxation delay between subsequent runs of the NOESY sequence was 1.5 s. The spectra were recorded in a phase-sensitive mode with 1,024 points in the F2-direction and 256 points in the F1-direction. Exponential filtration was applied in both directions. Mixing time values, τ_m, were 0.075, 0.10, 0.15, 0.20 and 0.25 s.

3. Results and discussion.

In this paper interproton distances which directly characterize the spatial geometry of the tetrapeptide *N*-Ac-Ser-Phe-Val-Gly-OMe (fig. 1) were defined by 2D NOESY NMR spectroscopy. On the basis of these experimental data the structure of a "protein - surface model of the cell membrane" (micellar systems of sodium dodecyl sulfate) complex was obtained. As the tetrapeptide *N*-Ac-Ser-Phe-Val-Gly-OMe is a short peptide with several aromatic and aliphatic components and polar groups, it may be of interest as a model system for peptide-solvent molecular interactions studies (for example water and trifluoroethanol).

The ^{1}H NMR spectrum assignment of the tetrapeptide *N*-Ac-Ser-Phe-Val-Gly-OMe in solution $H_2O + D_2O$ with SDS was done on the basis of previous data [13] and 2D TOCSY NMR experiments [12], and on the basis of the ^{1}H NMR chemical shifts of the tetrapeptide, dissolved in water [11, 14] (tab. 1).

Previously [11] the spatial structure of the tetrapeptide *N*-Ac-Ser-Phe-Val-Gly-OMe was determined by an approach based on the value of residual dipolar coupling constants [15] between the magnetic nuclei ^{13}C and ^{1}H, separated by one chemical bond (^{1}D). In this article we tried to use 2D NOESY NMR for the determination of the interproton distances that directly characterize the spatial geometry of the tetrapeptide *N*-Ac-Ser-Phe-Val-Gly-OMe in D_2O solution, but there were not any intraresidue NOE's in the 2D NOESY NMR spectra (fig. 2a). So in this case the 2D NOESY NMR spectroscopy method is ineffective for the structural studies of relatively small molecules.

In 2D ^{1}H-^{1}H NOESY NMR spectrum of the tetrapeptide in a mixture of $H_2O + D_2O$ with SDS in micellar state cross-peaks of the same sign as the diagonal signals were observed (fig. 2b). This is typical of the large molecules. This fact confirm the tetrapeptide - micelles of sodium dodecyl sulfate complex formation.

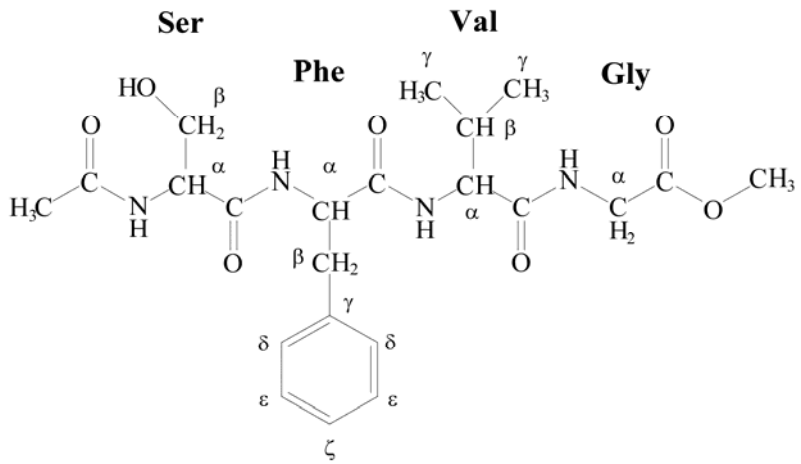

Figure 1. The structural formula of the tetrapeptide *N*-Ac-Ser-Phe-Val-Gly-OMe.

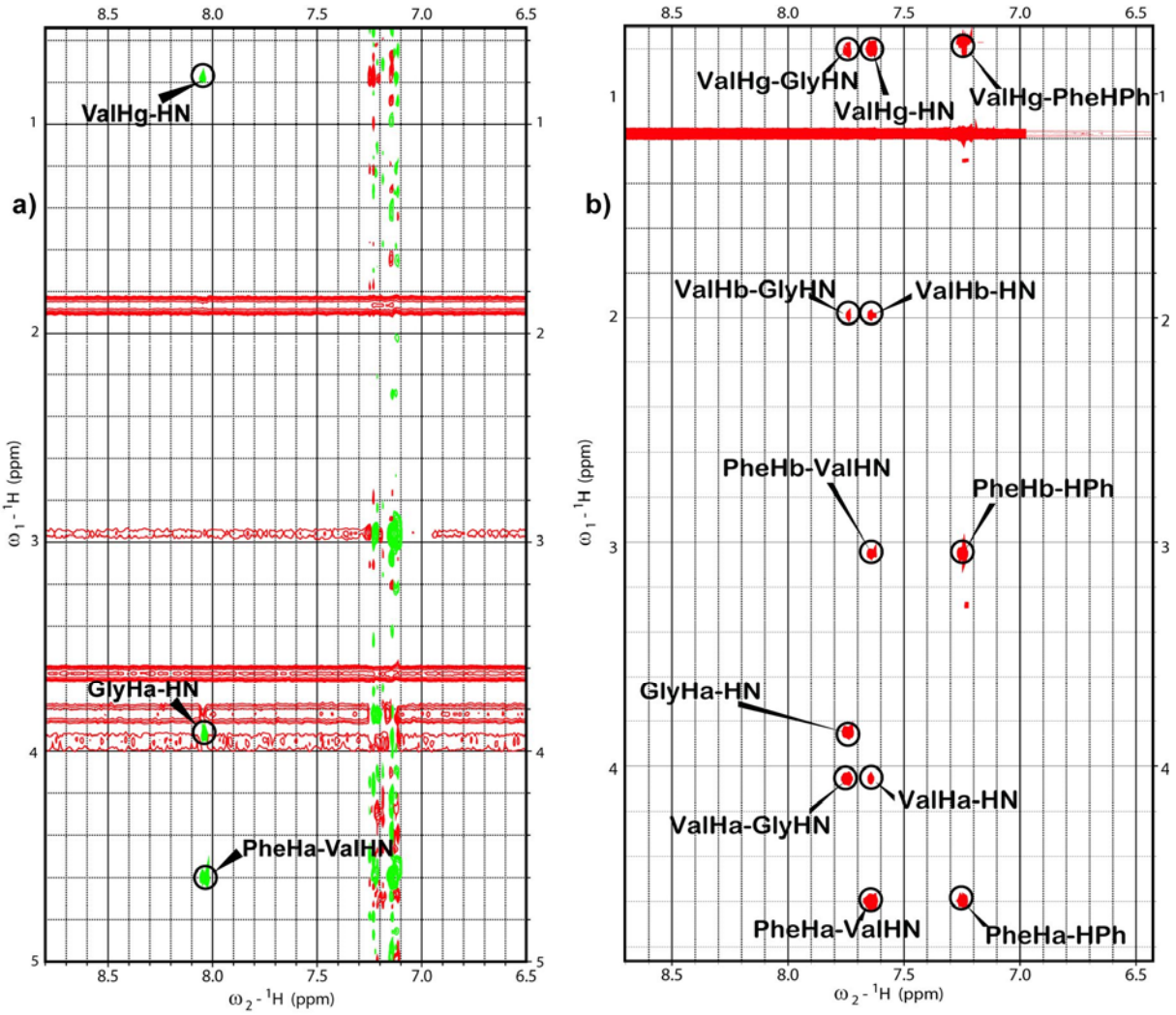

Figure 2. 2D ^{1}H-^{1}H NOESY NMR (500 MHz) spectra of the tetrapeptide *N*-Ac-Ser-Phe-Val-Gly-OMe dissolved: a) in a water, and b) in a mixture of H_2O + D_2O with sodium dodecyl sulfate in micellar state. Mixing time τ_m = 0.250 s. *T* = 298 K.

Table 1. ^{1}H NMR chemical shifts (δ_H, ppm relative DSS) for the tetrapeptide *N*-Ac-Ser-Phe-Val-Gly-OMe dissolved in H_2O + D_2O (90% + 10%) with sodium dodecyl sulfate (5.71 g/l) in the micellar state. *T* = 298 K.

Amino acid residue	Chemical shifts (ppm)			
	HN	Ha	Hb	Others
Ser	7.45	4.30	3.70	
Phe		4.60	3.05	2-6H 7.24
Val	7.63	4.05	1.98	Hg 0.79
Gly	7.73	4.43		

Processing and analyzing the intensity of cross-peaks in the NOESY NMR spectra were obtained as described elsewhere [16]. The dependence of the relative average integral intensities of cross-peaks for the Phe Ha - Val NH proton pairs on the mixing time are shown in figure 3. The slope value corresponds to the cross-relaxation rate constants (σ_{ij}) in the proton pair. The latter one is directly

related to the interproton distance (r_{ij}) [12, 16]. Interproton distances of the tetrapeptide *N*-Ac-Ser-Phe-Val-Gly-OMe in solution $H_2O + D_2O$ with sodium dodecyl sulfate at a micellar state are presented in table 2.

Experimental values of the interproton distances were used as input data for the molecular dynamics method calculations by the XPLOR-NIH program [17]. Following structural calculations, the ensemble was subjected to restrained molecular dynamics using the XPLOR-NIH. Individual structures were minimized, heated to 1000°K for 6000 steps, cooled in 100°K increments to 50°K, each with 3000 steps, and finally minimized with 1000 steps of steepest descent followed by 1000 steps of conjugate gradient minimization. For a starting family of 200 structures, approximately 21–25 ones were kept for following molecular dynamics calculation and finally 8 lowest energy structures (fig. 4) were retained. The backbone RMSD for the extended core (Ser 2–Val 4) is 0.78 ± 0.25 Å. The large value of RMSD is caused by the small size of the oligopeptide and its high mobility. The stereochemical validation of model was carried out with Ramachandran's plot (fig. 6). Psi and Phi dihedral angles is used for the Stereochemical evaluation of backbone of the protein revealing that 60.0, 30.0, 10.0 and 0.0% of residues were falling within the most favoured regions, additionally allowed regions, generously allowed regions and none in disallowed regions respectively of Ramachandran's plot. Coordinates of the atoms for the minimal energy conformation of tetrapeptide are presented in table 4. A model of the "tetrapeptide nAc-Ser-Phe-Val-Gly-OMe - SDS micelle" complex is presented in figure 5. This model was constructed on the basis of tetrapeptide chemical shifts differences in SDS and aqueous solution.

The ^{13}C chemical shifts of the tetrapeptide in solid state [11] and ^{13}C chemical shifts of tetrapeptide in complex peptide-micelle of SDS in water solution are shown in the table 3. Closeness of the ^{13}C chemical shifts of tetrapeptide in isotropic solutions and solid state ($\Delta\delta \leq 1.5 - 2.5$ ppm, with the exception of the ^{13}C chemical shifts of 2,6 Phe, 3,5 Phe, 4 Phe) allows to conclude about the similarity of the conformations of the chain of the tetrapeptide in water solution with SDS and in the solid state.

Therefore the determined spatial structure of tetrapeptide in "peptide - micelle" complex (fig. 4) is corresponds with ones in solid state.

Table 2. Experimental interproton distances for the tetrapeptide *N*-Ac-Ser-Phe-Val-Gly-OMe in the ($H_2O + D_2O$) solution with SDS micelles. ("*" is the calibration distance).

Pair of protons	Interproton distances r, Å
Gly 5 Ha/Gly 5 HN	2.93 ± 0.5
Val 4 Hg1/Gly 5 HN	3.24 ± 0.5
Val 4 Hg2/Val 4 HN	3.14 ± 0.5
Val 4 Hb/Gly 5 HN	3.01 ± 0.5
Val 4 Hb/Val 4 HN	2.99 ± 0.5
Phe 3 Hb/Val 4 HN	3.30 ± 0.5
Phe 3 Ha/Val 4 HN	2.26 ± 0.5
Phe 3 Ha/Phe3 Hb	2.65*

Table 3. ^{13}C NMR chemical shifts (δ_H, ppm relative DSS) for the tetrapeptide *N*-Ac-Ser-Phe-Val-Gly-OMe dissolved in $H_2O + D_2O$ (90% + 10%) with sodium dodecyl sulfate (5.71 g/l) in the micellar state and tetrapeptide in solid state from Ref. [11]. T = 298 K.

Atom	δ Solid state, ppm	δ In water solution with SDS, ppm
Ser Ca	55.0	55.6
Ser Cb	60.0	60.8
Phe Ca	55.0	55.6
Phe Cb	36.9	37.0
Phe C 2,6	126.9; 126.3	128.6
Phe C 3,5	131.0	129.3
Phe C 4	124.4	126.9
Val Ca	59.4	59.2
Val Cb	29.8	30.2
Gly Ca	40.8	41.0

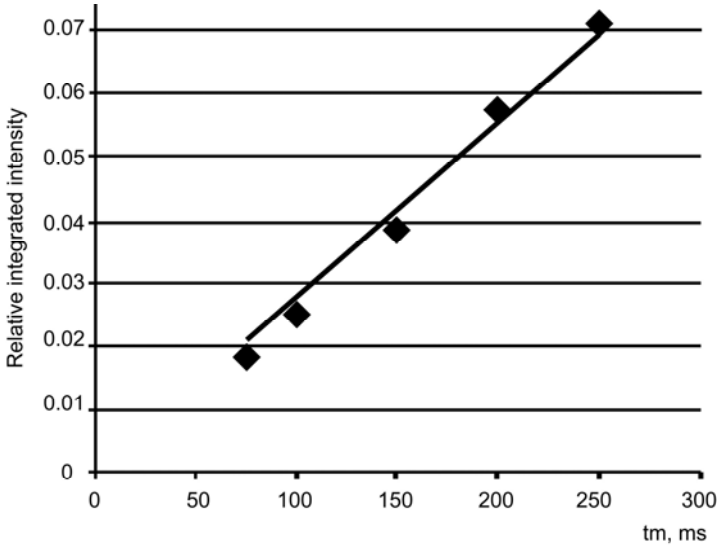

Figure 3. The dependence of cross - peak average relative integrated intensity for pairs of Phe Ha - Val NH protons on the mixing time.

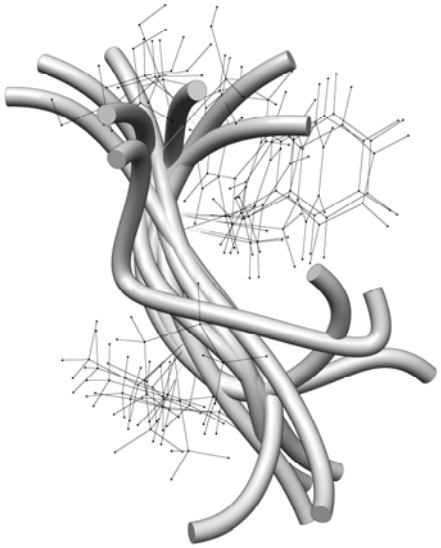

Figure 4. The full ensemble of 8 final structure of the tetrapeptide *N*-Ac-Ser-Phe-Val-Gly-OMe in SDS micelles calculated by the XPLOR-NIH program.

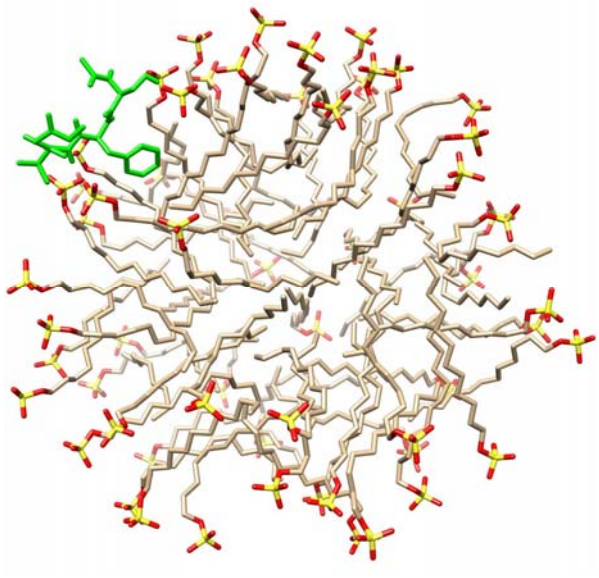

Figure 5. Final structure of tetrapeptide *N*-Ac-Ser-Phe-Val-Gly-OMe binding with an SDS micelle.

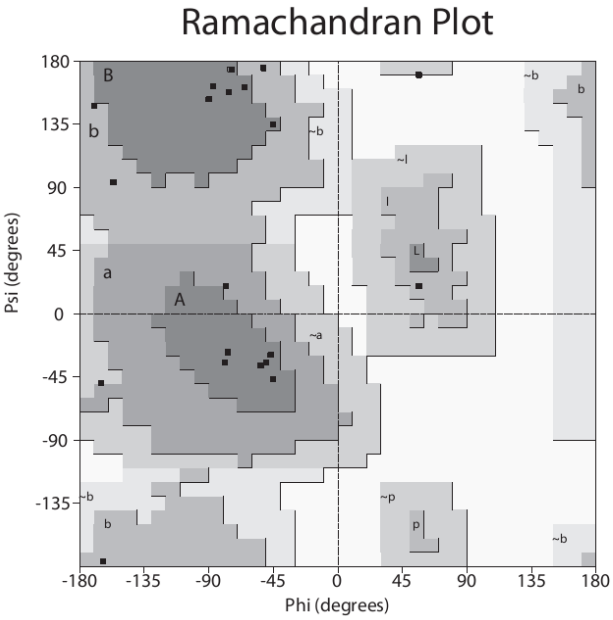

Figure 6. The full ensemble of 8 final structure of the tetrapeptide *N*-Ac-Ser-Phe-Val-Gly-OMe in SDS micelles calculated by the XPLOR-NIH program.

Table 4. Coordinates of the atoms for the minimal energy conformation of tetrapeptide in complex "peptide – micelle of SDS" in pdb-format.

	Atom	Residue	x	y	z		Atom	Residue	x	y	z
1	CA	1ACE	6.348	4.166	4.611	34	CZ	3PHE	7.453	-0.672	4.909
2	HA1	1ACE	6.067	4.801	5.438	35	HZ	3PHE	8.317	-0.423	5.508
3	HA2	1ACE	7.263	4.532	4.170	36	C	3PHE	2.618	-0.964	0.570
4	HA3	1ACE	6.498	3.157	4.967	37	O	3PHE	2.223	-2.115	0.384
5	C	1ACE	5.239	4.175	3.564	38	N	4VAL	1.971	0.106	0.121
6	O	1ACE	4.392	5.069	3.550	39	HN	4VAL	2.335	0.998	0.300
7	N	2SER	5.251	3.176	2.688	40	CA	4VAL	0.729	-0.024	-0.633
8	HN	2SER	5.950	2.492	2.747	41	HA	4VAL	-0.068	-0.285	0.047
9	CA	2SER	4.241	3.079	1.640	42	CB	4VAL	0.387	1.305	-1.308
10	HA	2SER	3.269	3.267	2.072	43	HB	4VAL	-0.410	1.152	-2.021
11	CB	2SER	4.510	4.125	0.558	44	CG1	4VAL	-0.064	2.314	-0.250
12	HB1	2SER	5.381	3.834	-0.014	45	HG11	4VAL	-1.022	2.015	0.148
13	HB2	2SER	4.688	5.083	1.017	46	HG12	4VAL	-0.150	3.293	-0.699
14	OG	2SER	3.377	4.219	-0.296	47	HG13	4VAL	0.662	2.347	0.549
15	HG	2SER	3.413	5.066	-0.745	48	CG2	4VAL	1.625	1.843	-2.029
16	C	2SER	4.246	1.687	1.017	49	HG21	4VAL	2.358	2.157	-1.301
17	O	2SER	4.527	1.529	-0.171	50	HG22	4VAL	1.345	2.686	-2.644
18	N	3PHE	3.933	0.681	1.827	51	HG23	4VAL	2.044	1.067	-2.651
19	HN	3PHE	3.718	0.866	2.765	52	C	4VAL	0.855	-1.115	-1.691
20	CA	3PHE	3.904	-0.695	1.343	53	O	4VAL	-0.130	-1.757	-2.056
21	HA	3PHE	4.747	-0.854	0.688	54	N	5GLY	2.074	-1.320	-2.181
22	CB	3PHE	4.005	-1.665	2.522	55	HN	5GLY	2.822	-0.778	-1.853
23	HB1	3PHE	4.104	-2.674	2.150	56	CA	5GLY	2.316	-2.337	-3.197
24	HB2	3PHE	3.113	-1.590	3.126	57	HA1	5GLY	2.080	-3.308	-2.792
25	CG	3PHE	5.213	-1.317	3.358	58	HA2	5GLY	3.359	-2.313	-3.480
26	CD1	3PHE	5.060	-0.569	4.532	59	C	5GLY	1.455	-2.089	-4.431
27	HD1	3PHE	4.078	-0.240	4.838	60	O	5GLY	1.873	-1.363	-5.333
28	CD2	3PHE	6.486	-1.743	2.961	61	CA	6MEO	-0.444	-2.378	-5.671
29	HD2	3PHE	6.604	-2.321	2.056	62	HA1	6MEO	0.149	-2.705	-6.514
30	CE1	3PHE	6.180	-0.246	5.307	63	HA2	6MEO	-0.583	-1.307	-5.722
31	HE1	3PHE	6.062	0.331	6.212	64	HA3	6MEO	-1.407	-2.866	-5.700
32	CE2	3PHE	7.606	-1.421	3.736	65	O	6MEO	0.221	-2.716	-4.458
33	HE2	3PHE	8.588	-1.749	3.430						

4. Summary

In this paper the spatial structure of the tetrapeptide *N*-Ac-Ser-Phe-Val-Gly-OMe in a complex with SDS micelle in aqueous solution was determined by 2D NOESY NMR spectroscopy. We have shown that the 2D NOESY NMR spectrum of the tetrapeptide in complex with SDS micelles was informative and suitable for the oligopeptide molecular structure determination. The obtained results show that the proposed approach in [7] can be used for small oligopeptides with a few amino acid residues in length.

The identity of the tetrapeptide in solid state and in "peptide-micelle" complex structures was prooved by the invariability of ^{13}C chemical shifts values.

Acknowledgments

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