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Spatial Structure of Triglycine Determined by the Residual Dipolar Coupling Analysis

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Abstract. The possibility to determine the relatively small organic compound conformations by the approach on the basis of the analysis of the residual dipolar couplings ^{1}H-^{13}C in the molecules partially aligned in lyotropic liquid crystalline media has been considered. This approach has been used in the nuclear magnetic resonance investigation of the triglycine structure in lyotropic medium (cetylpyridinium bromide/*n*-hexanol). The conformation of triglycine in solution has been established as *trans-trans* on the basis of the experimental data of observed couplings.

1 Introduction

Traditionally, the determination of three-dimensional (3-D) structures of the relatively small organic compounds in solution is based on both 1-D nuclear magnetic resonance (NMR) spectroscopy and modern NMR methods such as dynamic NMR spectroscopy [1, 2] and 2-D NMR spectroscopy [3, 4]. Note that nuclear Overhauser effect spectroscopy (NOESY) experiments allow one to determine the distances between magnetic nuclei of up to 0.5 nm and, hereby, to establish the 3-D structure of organic compounds in solution [3, 4]. The increase in the number of atoms in a molecule results in the NMR spectral parameters which do not allow one to describe adequately the 3-D structure of organic compounds in solution.

In this work an application of the approach for determination of the conformation of triglycine, a relatively small molecule, partially aligned in lyotropic liquid crystalline medium on the basis of an analysis of the residual dipolar couplings between magnetic nuclei ^{13}C and ^{1}H separated by one chemical bond (^{1}D) is demonstrated. Since recently, this approach is actively used in the NMR investigations of biochemical objects under the condition of slow motion ($\omega_0\tau_c \gg 1$, τ_c is the correlation time, ω_0 is the angular precession rate of magnetic nuclei) [5, 6]. It allows one to obtain independent information about the structure of

these compounds in solution. Applications of this approach to the determination of conformations of small organic compounds has not been described in the literature as proved by review [6, 7]. The significance of the approach itself becomes even higher if one takes into consideration that the application of NOESY to relatively small molecules (under the condition of fast motion, $\omega_0 \tau_c \ll 1$) is not always effective [3, 4]. This is connected with the small correlation time τ_c for these molecules in solution and the fulfilment of the condition of fast motion leading to weak cross-peak intensities in NOESY spectra and making it difficult to obtain quantitative information about the interproton distances in these molecular systems.

2 Experimental

NMR ^{1}H (300 MHz) and ^{13}C (75.43 MHz) spectra of triglycine in isotropic solvent and lyotropic liquid crystalline medium were recorded on an NMR Unity-300 spectrometer (Varian). The 10–15° pulses, relaxation delay of 1–2 s, spectral width of 10 ppm, number of transitions from 10 to 100 were used to obtain ^{1}H NMR spectra. The 20–30° pulses with or without broad-band proton decoupling, relaxation delay of 1–2 s, spectral width of 200 ppm, number of transitions from 400 to 1000, digital exponential filtration with 2–4 Hz were used to obtain ^{13}C NMR spectra. The samples were solutions of compounds in corresponding media with concentrations of 5–10% (w/w). References of chemical shifts were made from the signal of standard liquid tetramethylsilane (TMS). For 2-D NOESY experiments the delay between pulse sequences was chosen to be at least three times the averaged longitudinal relaxation time of triglycine protons. Spectra were recorded in a phase-sensitive mode with 1024 points in the F2 axis and 256 points in the F1 axis. Exponential filtration was applied in both directions. The mixing time parameter τ_m was chosen equal to 0.2, 0.4, 0.6, and 0.8 s.

A liquid crystalline medium composed of cetylpyridinium bromide (CPB) and *n*-hexanol has been prepared as described in ref. 8. The final mixture was characterized by the weight percent of CPB to the water.

3 Results and Discussion

It is known that in solution the dipole-dipole interaction between nuclei inside the molecule is completely averaged as a result of random tumbling of molecules. If the molecular system is dissolved in lyotropic liquid crystalline medium, then the translational and rotational movements of molecules cease to be isotropic owing to striking on the magnetic oriented molecular formations. This anisotropy in the molecular motion results in the appearance of the weak dipole-dipole interaction between nuclei revealed in NMR spectra as residual dipolar couplings without broadening NMR signals [5, 6].

The expression for the residual dipolar coupling $D_{IJ(\theta,\varphi)}$ between two directly coupled nuclei can be simplified to the form [6]

$$D_{IJ(\theta,\varphi)} = D_a^{IJ}(A_a(3\cos^2\theta - 1) + 3/2A_r\sin^2\theta\cos 2\varphi), \quad (1)$$

where $D_a^{IJ} = -(\mu_0 h/16\pi^3)S\gamma_I\gamma_J(r_{IJ}^{-3})$. Here $A_a = 1/3(A_{zz} - (A_{xx} + A_{yy})/2)$ is the axial component of the molecular alignment tensor **A** characterizing the preferential orientation of the molecule relative to the static field direction; $A_r = 1/3(A_{xx} - A_{yy})$ is the rhombic component; A_{xx}, A_{yy}, and A_{zz} are the projections of molecular alignment tensor **A** on x, y, and z directions of the orthogonal axes system connected with the molecule; θ and φ define the polar coordinates of the internuclear vector (between I and J nuclei) connecting the principal axes of the molecular alignment tensor with the static field direction; S is the generalized order parameter describing the internal dynamic mobility of the internuclear vector; γ_I and γ_J are the gyromagnetic ratios of nuclei I and J; r_{IJ} is the distance between nuclei.

Different liquid crystalline media are used to provide partial alignment of the biochemical objects in magnetic field, e.g., phospholipide bicelles [5], bacteria [9], or membrane fragments [10]. The nematic phases may be made on the basis of *N*-cetyl-*N*,*N*,*N*-trimethylammonium bromide [6], CPB or cetylpyridinium chloride and *n*-hexanol mixtures [8, 11]; *n*-alkyl-poly(ethylene glycol) and *n*-alkyl alcohol mixtures in water [12]. These systems form a lyotropic liquid crystalline phase referred to as L_α under any conditions. In a magnetic field the bilayer surfaces are oriented parallel to its direction [6].

To determine the residual dipolar couplings in triglycine a mixture of CPB in water (5.5% [w/w]) was used; the molar ratio (r) of the CPB to *n*-hexanol was 0.203 at the content of 30 mM of the NaBr salt in water. The presence of the ordered lamellar L_α phase was monitored by the observation of the quadrupolar splitting of the ^2H NMR signal of the solvent (D_2O) in the diluted liquid crystalline system [5, 12].

The approach described above was used to determine the spatial structure of triglycine in water presenting unceasing interest over thirty years [13, 14]:

$$H_2N\text{-}CH_2\text{-}C(O)\text{-}HN\text{-}CH_2\text{-}C(O)\text{-}HN\text{-}CH_2\text{-}C(O)\text{-}OH$$
$$\text{— 1 ac —} \quad \text{— 2 ac —} \quad \text{— 3 ac —}$$

The study of oligopeptide and, particularly, tripeptide conformations is important in many respects. First of all, tripeptides may be considered as the building blocks for the protein structure, and knowledge of their 3-D structure may be used to predict the polypeptide chains and the design of protein de novo [15]. Some peptides themselves fulfil the mediator's functions. Their interactions with receptors are conformation-dependent.

The ^1H NMR spectrum of triglycine dissolved in water (D_2O) consists of four singlet signals: three signals of the methylene groups (1 ac CH_2, $\delta_H = 3.68$ ppm; 2 ac CH_2, $\delta_H = 3.81$ ppm; 3 ac CH_2, $\delta_H = 3.93$ ppm, notations are made as in

Table 1. The ^{13}C NMR chemical shifts (δ_C, ppm relative to TMS) and direct spin-spin couplings ($^1J_{CH}$, Hz, bottom row) for triglycine dissolved in isotropic solvent and lyotropic liquid crystalline medium.

Medium	1 ac		2 ac		3 ac	
	C=O	CH_2	C=O	CH_2	C=O	CH_2
D_2O	167.7	40.4 144.2	170.8	42.2 140.4	176.4	42.9 139.4
CPB + *n*-hexanol + NaBr + D_2O	167.7	40.5 142.2; 142.0	170.8	42.4 124.5; 124.1	176.4	43.2 133.4; 132.4

ref. 14) and a signal with $\delta_H = 4.72$ ppm relating to amine and amide protons (in zwitter-ionic form), proton of triglycine carboxyl group, and residual protons of deuterated water participating in fast intermolecular hydrogen exchange [16]. The ^{13}C NMR spectrum of triglycine in D_2O consists of six singlet signals, the chemical shifts of which are collected in Table 1. The assignment of signals has been made in accordance with the literature data [16, 17].

NMR spectroscopy allows one to interpret the spatial structure of triglycine in terms of the arrangement of the glycine fragments with respect to the bonds -C(O)-NH- since the rotation about these bonds requires to overcome the high energy barrier (75–80 kJ/mol [1, 2]) resulting in the "frozen", in the NMR scale, spectra of *trans* and *cis* (the orientation of C(O) and NH bonds) conformers as in the case of the benzene-substituted triglycines [14]. As for the studied compound, the single set of the ^1H NMR spectrum signals in D_2O is observed, as well as the single set of the ^{13}C NMR signals, with NMR data it is possible only to conclude that triglycine exists in solution in one of the possible conformers: *trans-trans*, *trans-cis*, *cis-trans*, or *cis-cis*.

2-D NOESY spectra with different mixing times were recorded to determine the interproton distances characterizing directly the 3-D structure of triglycine in solution. It was impossible to fix the cross-peaks in 2-D NOESY spectra of the studied compound dissolved in D_2O due to the remoteness of the methylene protons one from another and to the participation of the amine and amide protons in the intermolecular exchange.

To determine one of the possible conformers of triglycine, *trans-trans*, *trans-cis*, *cis-trans*, or *cis-cis*, the quantum chemical semiempirical calculations of the molecule have been carried out by the MOPAC 93 program (PM3 method), which allowed us to determine unambiguously the *cis-trans* conformation as the most preferable one for triglycine (see Fig. 1a).

To elucidate the triglycine spatial structure, the approach on the basis of the analysis of the residual dipolar couplings between nuclei was used. For this purpose, the one-bond couplings between ^{13}C and ^1H nuclei in molecule, dissolved in isotropic solvent (D_2O) and lyotropic medium, were considered. The carbon chemical shift data (δ_C) and the direct spin-spin couplings ($^1J_{CH}$) for triglycine obtained from ^{13}C NMR spectra without broad-band proton decoupling

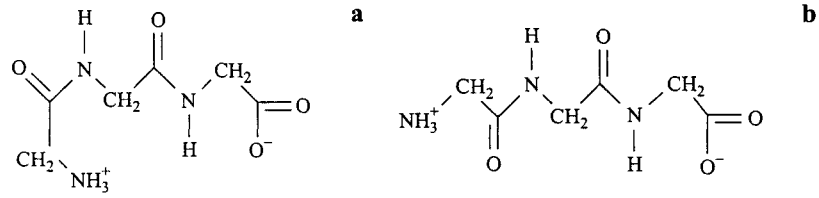

Fig. 1. Schematic representation of the *cis-trans* (**a**) and *trans-trans* (**b**) conformation for triglycine.

are shown in Table 1. The residual dipolar couplings ($^1D_{CH}$) determined from the difference of the observed couplings ($^1J_{CH} + {}^1D_{CH}$) for the magnetic nuclei dissolved in the lyotropic liquid crystalline medium and the ones in the isotropic solvent [6] are the following: 1 ac, $^1D_{CHA} = -2.0$ Hz, $^1D_{CHB} = -2.2$ Hz; 2 ac, $^1D_{CHA} = -15.9$ Hz, $^1D_{CHB} = -16.3$ Hz; 3 ac, $^1D_{CHA} = -6.0$ Hz, $^1D_{CHB} = -7.0$ Hz.

The analysis of the obtained residual dipolar couplings ($^1D_{CH}$) was carried out by the MODULE program [18]. On the basis of Eq. (1), the program allows one to bind the values of observed residual dipolar couplings and the arrangement of internuclear vectors relative to the external magnetic field in the frames of the known conformation of the studied molecule. The linear correlation between the observed and calculated residual dipolar couplings, on the basis of the given spatial structure of studied compound, is the criterion if the calculated structure agrees with the real one. In the case of disparity of the observed and calculated constants, the program allows one to change the initial conformation by rotating the separate fragment relative to others around the chemical bond between them.

With the triglycine atom coordinates in the *cis-trans* conformation determined by the MOPAC 93 (PM3 method) calculations and the experimental values of the residual dipolar couplings as the input data for the MODULE program (the

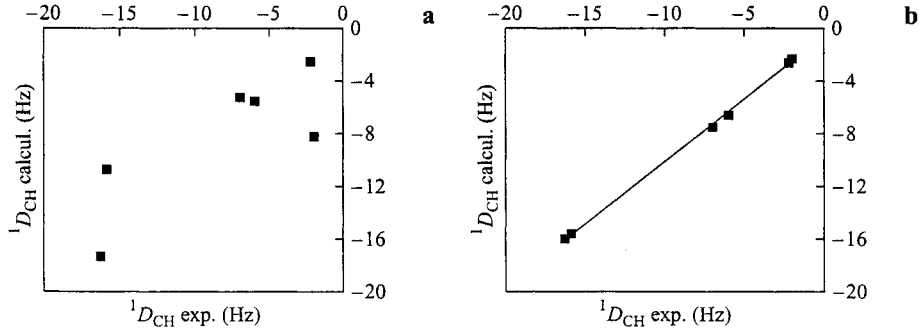

Fig. 2. Dependence between the observed residual dipolar couplings ($^1D_{CH}$) for triglycine dissolved in lyotropic liquid crystalline medium and the calculated ones in *cis-trans* (**a**) and *trans-trans* (**b**) conformations.

Table 2. The coordinates of the triglycine *trans-trans* conformation atoms in the pdb format.

Atom nr.	Atom	Part of triglycine	*x*-Coordinate	*y*-Coordinate	*z*-Coordinate
1	N	1GLY	−3.720	0.677	−0.222
2	CA	1GLY	−2.308	0.569	0.031
3	C	1GLY	−1.820	−0.884	0.030
4	O	1GLY	−1.208	−1.340	1.014
5	1HN	1GLY	−3.979	1.628	−0.383
6	1HA	1GLY	−1.994	0.999	1.030
7	2HA	1GLY	−1.747	1.126	−0.771
8	2HN	1GLY	−4.262	0.300	0.532
10	N	2GLY	−1.489	−0.260	−1.595
11	CA	2GLY	−0.391	−1.165	−1.526
12	C	2GLY	0.928	−0.658	−0.922
13	O	2GLY	1.920	−1.413	−0.951
15	1HA	2GLY	−0.137	−1.504	−2.574
16	2HA	2GLY	−0.671	−2.078	−0.923
18	HN	2GLY	−1.556	0.316	−2.405
9	N	3GLY	1.020	0.631	−0.426
14	CA	3GLY	2.193	1.089	0.251
17	C	3GLY	2.073	1.307	1.744
19	O	3GLY	2.993	1.365	2.562
20	HN	3GLY	0.188	1.147	−0.254
21	1HA	3GLY	2.492	2.093	−0.178
22	2HA	3GLY	3.041	0.357	0.089

generalized order parameter S has a uniform value for all internuclear vectors C-H) showed the absence of correlation between observed and calculated constants (see Fig. 2); the root-mean-square deviation from linearity is $2.7 \cdot 10^2$. To change the initial conformation by rotating around CH_2-C(O) and C(O)-NH bonds of any separate fragment relative to others allows one to choose the single structure *trans-trans* (see Fig. 1b), for which the total agreement between observed and calculated constants is characterized by the root-mean-square deviation from linearity equal to 3.83, and parameters of the axial and rhombic components of molecular alignment tensor are $A_0 = 14.26$ and $A_r = 8.25$. The *trans-trans* triglycine conformation atom coordinates providing the optimal agreement between observed and calculated constants are shown in Table 2.

4 Conclusions

The approach on the basis of the analysis of the residual dipolar couplings ^1H-^{13}C in the molecules partially aligned in liquid crystalline media has been used in the NMR investigation of the triglycine structure in lyotropic medium (CPB/*n*-hexanol). The conformation of triglycine in solution has been established as *trans-trans* on the basis of the experimental data of observed couplings.

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