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Spatial structures of tripeptides glycylglycyl-L-histidine and glycylglycyl-L-tyrosine based on residual dipolar couplings and quantum-chemical computations

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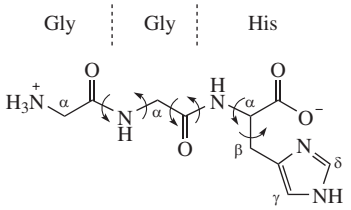
A novel approach to the determination of the spatial structure of oligopeptides on the basis of an analysis of the residual dipolar couplings ^1H–^{13}C assisted by quantum-chemical computations with considering solvent effects is proposed to characterize the conformations of the tripeptides GlyGlyHis and GlyGlyTyr with significant folding of the latter to left-handed helix.

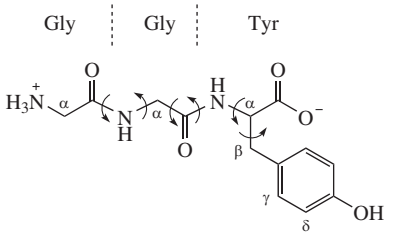
The investigation of oligopeptide conformations is important because oligopeptides can be considered as building blocks for protein structures, and the knowledge of their three-dimensional structures can be used to predict polypeptide chains and the design of proteins *de novo*.[1] Tripeptides with a terminal histidine residue in complexes with copper(II) are good models for blood copper transport form in the composition of human serum albumin.[2,3] Tyrosine in a terminal position is a constituent of many neuropeptides[4,5] and the subject of phosphorylation by kinases operating important regulatory functions in living cells.[6,7]

In continuation of our research,[8–10] we determined the spatial structure of the tripeptides glycylglycyl-L-histidine (GlyGlyHis) and glycylglycyl-L-tyrosine (GlyGlyTyr) partially aligned in a lyotropic liquid crystalline medium on the basis of an analysis of the residual dipolar couplings[11,12] combined with quantum-chemical density functional theory (DFT) calculations performed at the high level of theory with considering solvent effects.

To elucidate the spatial structures of GlyGlyHis and GlyGlyTyr tripeptides, the residual dipolar couplings between the magnetic nuclei ^{13}C and ^1H separated by one chemical bond ($^1D_{CH}$) were used. The ^{13}C NMR spectra of GlyGlyHis and GlyGlyTyr (in D_2O and lyotropic medium) contain six signals (we considered CH and CH_2 carbons only) with the chemical shifts collected in Tables 1 and 2.† The assignment of signals has been carried out in accordance with literature data and 2D COSY NMR experiments.[13]



GlyGlyHis



GlyGlyTyr

The direct spin–spin couplings ($^1J_{CH}$ + $^1D_{CH}$) for both tripeptides obtained from ^{13}C NMR spectra without broad-band proton decoupling are shown in Tables 1 and 2.

Table 1 ^{13}C NMR chemical shifts of carbons (δ_C/ppm relative to TMS) and direct spin–spin couplings ($^1J_{CH}$ + $^1D_{CH}$/Hz, bottom row) of the tripeptide GlyGlyHis dissolved in an isotropic solvent and lyotropic liquid crystalline medium.

Medium	αCH$_2$ Gly1	αCH$_2$ Gly2	αCH His	βCH$_2$ His	γCH His	δCH His
D_2O	40.4	41.5	54.1	27.9	116.7	134.3
	142.8	141.6	142.8	131.0	190.4	211.2
($C_{12}E_5$)/*n*-hexanol	40.4	41.6	54.2	27.9	116.8	134.4
	147.7; 146.5	147.7; 147.8	142.7	126.9; 124.5	183.1	206.3

Table 2 ^{13}C NMR chemical shifts of carbons (δ_C/ppm relative to TMS) and direct spin–spin couplings ($^1J_{CH}$ + $^1D_{CH}$/Hz, bottom row) of the tripeptide GlyGlyTyr dissolved in an isotropic solvent and lyotropic liquid crystalline medium.

Medium	αCH$_2$ Gly1	αCH$_2$ Gly2	αCH Tyr	βCH$_2$ Tyr	γCH Tyr	δCH Tyr
D_2O	40.4	42.2	56.4	36.7	115.2	130.6
	144.0	140.4	142.8	132.0	157.5	162.3
($C_{12}E_5$)/*n*-hexanol	40.4	42.2	56.3	36.7	115.3	130.7
	142.7; 142.8	152.6; 156.3	113.5	94.0; 89.1	128.2	124.0

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 ($^1J_{CH}$) in the isotropic solvent are the following:

(*a*) GlyGlyHis –4.9 and –3.7 Hz for αCH_2 Gly1; –6.1 and –6.2 Hz for αCH_2 Gly 2; +0.1 Hz for αCH His; +4.1 and +6.5 Hz for βCH_2 His; +7.3 Hz for γCH His; +4.9 Hz for δCH His;

(*b*) GlyGlyTyr +1.2 and +1.3 Hz for αCH_2 Gly 1; –12.2 and –15.9 Hz for αCH_2 Gly 2; +29.3 Hz for αCH Tyr; +38.0 and +42.9 Hz for βCH_2 Tyr; +29.3 Hz for γCH Tyr; +38.3 Hz for δCH Tyr.

The expression for the residual dipolar coupling $D_{IJ(\theta,\varphi)}$ between two directly coupled nuclei can be presented in the form[12]

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

where $D_a^{IJ} = -(\mu_0 h/16\pi^3) S\gamma_I\gamma_J\{r_{IJ}^{-3}\}$; $A_a = 1/3[A_{zz} - (A_{xx} + A_{yy})/2]$ 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 axis 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 internal dynamic mobility of the internuclear vector; γ_I and γ_J are the gyromagnetic ratios of nuclei I and J; and r_{IJ} is the distance between nuclei.

The analysis of the obtained residual dipolar couplings ($^1D_{CH}$) was carried out by the MODULE program.[14] In this program, the atom coordinates and the experimental values of the residual dipolar couplings are used as the input data (the generalized order parameter *S* has an uniform value for all internuclear vectors C–H[8,12]). A linear correlation between the observed and calculated residual dipolar couplings, on the basis of the given spatial structure of the test compound, is the criterion if the calculated structure agrees with the real one.

To determine one of the possible conformers of the tripeptides GlyGlyHis and GlyGlyTyr, we have proposed a new approach including two stages. At the first stage, the molecular modeling program DYNAMO, a part of the NMRPipe spectral processing system,[15] was used to obtain the spatial structure of compounds. This program allows for NMR-derived experimental constraints during a 'simulated annealing' protocol in addition to *a priori* information on covalent bond lengths, the planarity of peptide bonds, *etc*. Results of simple molecular dynamics modeling did not satisfy experimental $^1D_{CH}$ values, but the structures generated allowing for experimental constraints agreed with them when checked afterwards in the MODULE program.

At the second stage, the structures of zwitter-ionic forms of two tripeptides have been optimized by the GAMESS program package[16] using the DFT method[17] with three parameter Becke exchange functional[18] and the Lee–Yang–Parr correlation functional[19] (B3LYP) and the 6-31+G(d,p) basis set. To best account for solvent effects polarizable continuum model (PCM)[20] and effective fragment potential (EFP) solvent model[21,22] were used in combination. The tripeptide structures calculated at the first stage by the DYNAMO program were taken at the second stage as initial and each one was covered by 60 (GlyGlyHis) or 56 (GlyGlyTyr) water molecules simulating hydrate shells of these tripeptides, which were inserted into the solvent dielectric continuum. In local minima, water molecules were bounded with tripeptides and to one another by hydrogen bonds forming rings with five, six and more members. Local minima were defined with 0.0001 hartree (a.u.) optimization tolerance. The optimized structures of two tripeptides with their hydrate shells are presented in Online Supplementary Materials (Figure 1S).

The tripeptide structures optimized by the GAMESS program are compared with corresponding spatial structures calculated by the DYNAMO program in Figure 1 (at calculation of the GlyGlyTyr conformation with the DYNAMO program the $^1D_{CH}$ values for the phenoxyl ring atoms have been ignored). Molecular images in Figure 1 were produced using the UCSF Chimera package.[23] As can be seen in Figure 1, both calculation methods result in closely related conformations of each tripeptide. Relations between the $^1D_{CH}$ values observed for both tripeptides dissolved in lyotropic liquid crystalline medium and ones calculated for the conformations optimized by the GAMESS program (Figure 1) are presented in Figure 2. Figure 2 shows a good agreement between observed and calculated $^1D_{CH}$ constants with small values of the mean square deviation from linearity ($\chi^2 = 45.3$ for GlyGlyHis and $\chi^2 = 49.7$ for GlyGlyTyr) and satisfactory parameters of the linear regression, $y = Ax + B$ (where *x* and *y* are the calculated and experimental $^1D_{CH}$ values, respectively): $A = 0.04\pm0.85$, $B = 1.00\pm0.18$, $S_y = 2.54$, $R = 0.905$ ($n = 9$) for GlyGlyHis [Figure 2(*a*)] and $A = -0.48\pm1.35$, $B = 1.01\pm0.05$, $S_y = 3.11$, $R = 0.993$ ($n = 7$) for GlyGlyTyr [Figure 2(*b*)].

Neglecting the $^1D_{CH}$ values for the GlyGlyTyr phenoxyl ring atoms (γCH Tyr and δCH Tyr) in the above analysis appears justified in view of possible hydrophobic interactions between the GlyGlyTyr aromatic cycle and surface of the pentaethylene glycol monododecyl ether or *n*-hexanol in liquid crystalline medium – such an interaction is absent in pure water for which the tripeptide conformations were optimized by the GAMESS program. Hence, the proposed approach allows one to determine the basic

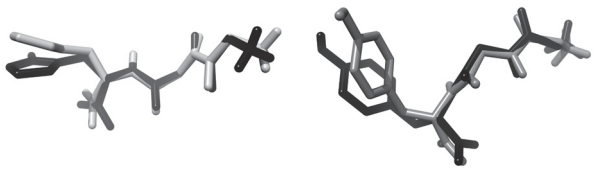


Figure 1 Comparison of the GlyGlyHis and GlyGlyTyr conformations optimized by the GAMESS program (gray) and calculated by the DYNAMO program (black).

† The ^1H (300 MHz) and ^{13}C (75.43 MHz) NMR spectra of the tripeptides GlyGlyHis and GlyGlyTyr in isotropic solvent and lyotropic liquid crystalline medium were recorded on a Unity-300 NMR spectrometer (Varian). 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 4000 to 10000, digital exponential filtration with 2–4 Hz were used to obtain the ^{13}C NMR spectra. References of chemical shifts were made from the signal of the TMS standard. The error in the values of residual dipolar couplings did not exceed 1.0 Hz.

The samples were the solutions of the tripeptides glycylglycyl-L-histidine and glycylglycyl-L-tyrosine (ICN Biomedicals) in corresponding media with concentrations of 0.5–2 wt% (0.02–0.08 mol dm^{-3}). To determine the residual dipolar couplings, the mixture of the pentaethylene glycol monododecyl ether/*n*-hexanol in water (D_2O) (4.1 wt% for GlyGlyHis and 5.8 wt% for GlyGlyTyr) was used; the molar ratio (*r*) of the pentaethylene glycol monododecyl ether to *n*-hexanol was 0.97. Pentaethylene glycol monododecyl ether [$C_{12}E_5$, where 12 is the number of carbons in the *n*-alkyl group and 5 is the number of glycol units in the poly(ethylene glycol)] (⩾98% purity, Sigma), *n*-hexanol (⩾98% purity, Sigma), D_2O (99.9 at% D, Astrachem), and DMSO-d_6 (99.8 at% D, Astrachem) were used without further purification. The presence of the ordered lamellar L_α phase was monitored by the observation of quadrupolar splitting of the ^2H NMR signal of the solvent (D_2O) in dilute liquid crystalline system.[11,12] Liquid crystalline medium for the $C_{12}E_5$/*n*-hexanol system has been prepared as described previously.[26]

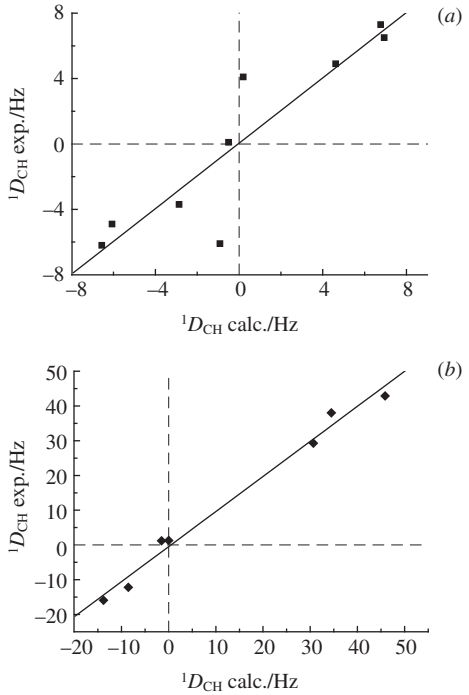
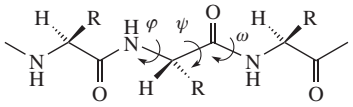


Figure 2 The observed residual dipolar couplings values ($^1D_{CH}$) *vs*. calculated ones in the conformations (gray) represented in Figure 1 for (*a*) GlyGlyHis and (*b*) GlyGlyTyr dissolved in lyotropic liquid crystalline medium.

characteristics of the spatial structure of the chosen tripeptides in aqueous solutions.

Note that the conformations found for GlyGlyHis and GlyGlyTyr are different from one another. Dihedral angles are φ_2(1C–2N–2CA–2C) = 67.27°; ψ_2(2N–2CA–2C–3N) = 80.52° for GlyGlyHis and φ_2 = 177.07°; ψ_2 = –115.55° for GlyGlyTyr. These (φ_2, ψ_2) values represent only slight folding structure of GlyGlyHis but significant folding structure of GlyGlyTyr, which is assigned for left-handed helix, in particular found for polyglycine II[24] and L-alanylglycylglycine.[25]



We believe that the proposed new approach, which combines an analysis of the residual dipolar couplings ^{1}H–^{13}C with quantum-chemical calculations, permits the spatial structure of other oligopeptides to be established.

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Online Supplementary Materials

Supplementary data (the optimized structures of two tripeptides with their hydrate shells and the GlyGlyHis and GlyGlyTyr conformation's atom coordinates) can be found in the online version at doi:10.1016/j.mencom.2011.03.003.

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