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Conformation and Orientation of an Oligopeptide in a Lyotropic Mesophase by ^{1}H NMR

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The analysis of ^{1}H residual dipolar couplings of zwitterionic tetraalanine in the lyotropic system cesium pentadecafluorooctanoate in water (CsPFO/D_2O) showed that the internal peptide residues adopt a polyproline II helix conformation. Moreover, the data allowed an estimate of the dihedral angles of the external residues and suggested that the long molecular axis is tilted by an angle of 56° with respect to the surface of the micelles formed by CsPFO.

Keywords: conformation; liquid crystal NMR; orientation; residual dipolar couplings; zwitterionic tetraalanine

INTRODUCTION

The conformation of short alanine-based peptides in aqueous solution has been studied in the last few years by means of a variety of spectroscopic techniques [1–8]. All these studies revealed the presence of a limited number of conformers, in contrast to a previously accepted view of oligopeptides as flexible entities fluctuating between different conformational minima on a very short time scale [9,10]. In particular, the combination of Polarized Raman, Fourier transform-IR and vibrational circular dichroism indicated that the dihedral angles of the two internal residues of cationic tetraalanine are close to the Ramachandran coordinates of the polyproline II helix (PPII). Further evidence supporting these results was given by us in a previous paper [11], where we

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presented a liquid-crystal ^{1}H NMR study on the conformation and orientation of tetraalanine in the lyotropic solvent cesium pentadecafluorooctanoate/water (CsPFO/D_2O). This system is known to form bilayer-like disk-shaped micelles with average alignment of the disks perpendicular to an external magnetic field [12], which induces alignment also on solute molecules.

In the previous article the analysis of the data was simplified by the use of partially deuterated tetraalanine isotopomers. We present here a ^{1}H NMR study of the conformation and orientational order of fully protonated zwitterionic tetraalanine in CsPFO/D_2O, exploiting the previous findings for spectral analysis, otherwise difficult due to extensive signal overlap. In fact, the investigation of the fully protonated molecule guarantees the measurement of all the dipolar couplings in the same experimental conditions, i.e., concentration and temperature, which are critical in lyotropic phases.

MATERIALS AND METHODS

Preparation of the NMR Samples

L-Alanyl-L-alanyl-L-alanyl-L-alanine (A_4) was purchased from Bachem Bioscience Inc. ($>98\%$ purity). CsPFO was prepared according to the procedure reported in the literature [8]. A_4 was dissolved in the CsPFO/D_2O solution, prepared as reported in the literature [11], at a concentration of 0.3 wt%. The isotropic-nematic phase transition occurs at 308 K and the nematic range of stability is approximately 10 K.

NMR Measurements

^{1}H and ^{2}H experiments were carried out on a Bruker AMX-300 WB spectrometer, equipped with a 5 mm reverse probe. The sample temperature was controlled employing a BVT 1000 Eurotherm unit, with a temperature stability of ± 0.1 K. The $\pi/2$ pulse was 5.7 and 10.5 μs on the ^{1}H and ^{2}H channels, respectively.

^{1}H single pulse experiments were carried out on A_4 in CsPFO/D_2O at 309 and 308 K. The relaxation delay was set to 5 s. A ^{2}H single pulse experiment at 308 K was performed in order to check the alignment of the mesophase from the D_2O quadrupolar splitting.

Analysis of Direct Couplings

The intramethyl $D(H^{Mei})$ dipolar couplings and the intraresidue methine-methyl $D(H^{\alpha i}, H^{Mei})$ dipolar couplings were determined from

the anisotropic ^1H spectrum with the aid of the program SpinWorks (by K. Marat, University of Manitoba, Winnipeg, MB, Canada). The fitting of the estimated dipolar couplings was performed using a home-written program within Mathematica 5 environment (Wolfram Research Inc.). The order parameters were optimized using a multiparameter nonlinear least-squares optimization routine based on Levenberg-Marquardt algorithm. The set of conformers analyzed was generated from conformer 1 of Ref. [11] varying the angles ψ_1 and φ_4 in steps of 30° in the range -180°–180°, and in steps of 5° in the regions between -110° and -130° for ψ_1 and between -70° and -85° for φ_4, where the experimental values were best reproduced on the basis of a preliminary screening.

RESULTS AND DISCUSSION

^1H NMR of A_4 in the Isotropic Phase

The ^1H spectrum of A_4 in isotropic CsPFO/D_2O at 309 K is characterized by signals in the region of the methyl protons (1.5–1.7 ppm) and of the α protons (4.2–4.4 ppm). The doublets due to methyl protons resonate at 1.66, 1.53, and 1.46 ppm, the intensity of the signal centered at 1.53 ppm being double than each of the others. On the basis of previous assignment [11], these signals were attributed to the methyl groups of the N-terminal alanyl residue, of the two inner residues, and of the C-terminal residue, respectively. Because the H^N protons readily exchange with D_2O, they are not observed in the ^1H spectrum. The scalar couplings between the methyl and the α protons of the same alanyl residue were found to be 7.2 Hz.

Conformation and Orientation of A_4 in the Anisotropic Phase

Since the methine protons signals are partially overlapped to the water peak, we focused our analysis on the methyl region. The region of the ^1H spectrum of A_4 due to the methyl protons in the anisotropic phase at 308 K is shown in Figure 1a.

On the basis of distance considerations, the spectrum is expected to be dominated by the dipolar interactions between the protons of each methyl group $D(H^{Mei})$ and those of the intraresidue methine-methyl protons $D(H^{\alpha i}, H^{Mei})$. Therefore, if interresidue couplings can be neglected, the pattern expected for each methyl is a triplet due to the intramethyl interactions, each peak being further split by the smaller methyl-methine proton interaction. In order to obtain

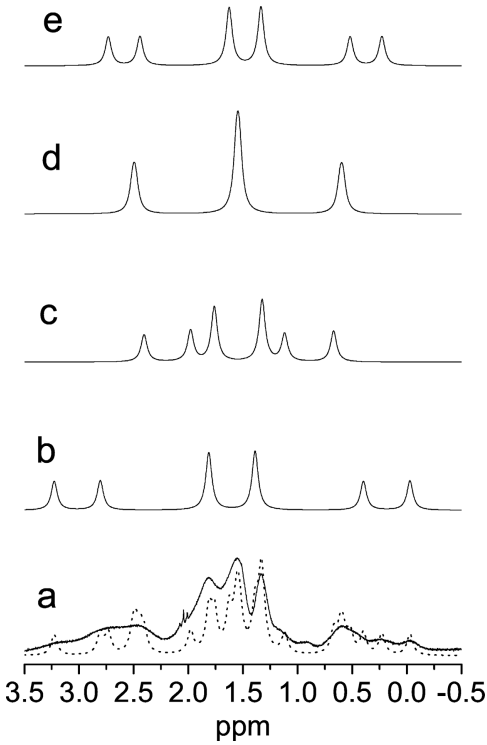

FIGURE 1 (a) ^{1}H spectrum of A_4 in the anisotropic phase (solid line) and its deconvolution (dashed line). (b)–(e) Patterns due to each methyl group contributing to the deconvolution.

a rough estimate of the stronger dipolar couplings, we deconvolved the spectrum as a combination of the four patterns expected (Fig. 1a). The patterns shown in traces **b** and **e** are centered at 1.60 and 1.48 ppm, respectively, while those shown in traces **c** and **d** are both centered at 1.54 ppm. The values of the dipolar couplings used in the simulation are shown in Table 1. Since the deconvolution is insensitive to the relative signs of the dipolar couplings, only the absolute values are given. Moreover, since the linewidth of each peak is more than 20 Hz, all the scalar couplings were neglected.

On the basis of the relative chemical shifts and of the signal assignment in the isotropic solution, patterns **b** and **e** were attributed to Me^1 and Me^4, while **c** and **d** to the internal methyl groups. In fact, due to the negligible values of the proton chemical shift anisotropy [13], the chemical shifts are not expected to differ much in the isotropic and anisotropic phases.

TABLE 1 Experimental ^{1}H Chemical Shifts and Dipolar Couplings in the Anisotropic Phase. The Dipolar Couplings Calculated in the Fitting Procedure for the Conformer Characterized by ψ_1 and φ_4 of -130° and -80°, Respectively (see text), are also Shown

Pattern	Chemical shift (ppm)[a]	D(H^{Mei}) (Hz)[b]	D(H^{Mei})^{calc} (Hz)[b]	D($H^{\alpha i}$,H^{Mei}) (Hz)[b]	D($H^{\alpha i}$,H^{Mei})^{calc} (Hz)[b]
b	1.60	140	139	60	– 51
c	1.54	65	62	62	61
d	1.54	95	– 102	0	– 17
e	1.48	110	– 105	40	45

[a]The proton chemical shifts are referenced to the water signal, which was set at 4.75 ppm.
[b]Patterns **b**,**c**,**d**,**e** are assigned to residues 1,3,2,4 respectively, with the numbering shown in Fig. 2a.

Determination of the Preferred Conformation

Within the assumption of slow molecular reorientation compared to internal motions, the dipolar coupling D_{ij} between two nuclei i and j in a liquid crystalline medium can be expressed as [13,14]:

$$D_{ij} = -K_{ij}\left\langle \frac{1}{r_{ij}^3} \right\rangle [S_{zz}\cos^2\theta_z^{ij} + S_{yy}\cos^2\theta_y^{ij} + (-S_{zz} - S_{yy})\cos^2\theta_x^{ij} + 2\cos\theta_z^{ij}\cos\theta_x^{ij}S_{zx} + 2\cos\theta_z^{ij}\cos\theta_y^{ij}S_{zy} + 2\cos\theta_x^{ij}\cos\theta_y^{ij}S_{xy}] \quad (1)$$

where $K_{ij} = \gamma_i\gamma_j/4\pi^2h$, with γ_i and γ_j the gyromagnetic ratios of nucleus i and j, r_{ij} is the internuclear distance, S_{pq} (p, $q = x$, y, z) are the elements of the order matrix in an arbitrary molecular frame (x, y, z), and θ_x^{ij}, θ_y^{ij} and θ_z^{ij} the angles between the internuclear vector and the molecular frame axes. The angular brackets indicate vibrational averaging. If vibrational averaging is mimicked well by a single effective geometry, $\langle 1/r_{ij}^3\rangle$ can be replaced with the inverse of the cube of the internuclear distance in that geometry. The order matrix is a symmetric and traceless tensor and therefore is in general defined by the five independent elements S_{xx}, S_{zz}, S_{xy}, S_{xz} and S_{yz}. The axes system (x', y', z') in which this matrix is diagonal, i.e., the order Principal Axes System (PAS), is usually chosen with z' as the axis of maximum orientation. The molecular system and the PAS are related by an orthogonal transformation defined by the three Euler angles α, β and γ [15]. Therefore at least five couplings for a given rigid conformer are required to determine the order matrix in the molecular fixed frame or in the PAS as well as the orientation of the PAS with respect to the molecular frame.

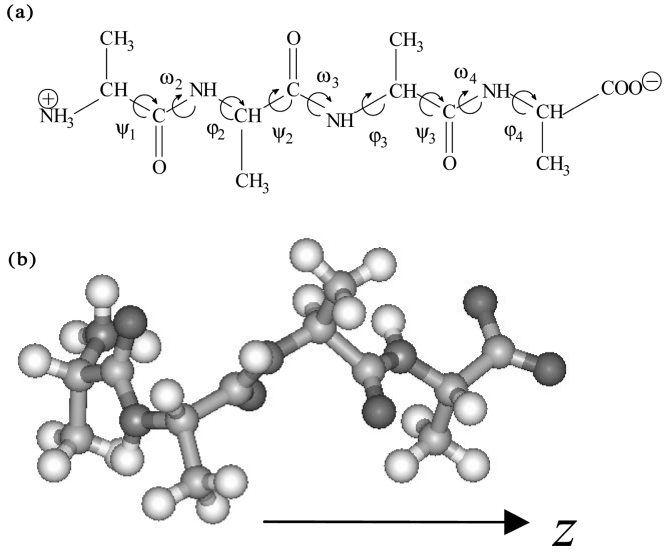

FIGURE 2 (a) Zwitterionic tetraalanine. (b) Example of a conformer of tetraalanine with the z axis of the inertial PAS.

In the molecule here investigated, the molecular frame was set in the PAS of the inertial tensor, with the z axis chosen as the one with the smallest value of the inertial moment, as shown in Figure 2 for a fixed conformation. The estimate of five or more direct couplings for a given rigid conformer allows the five elements of its order matrix to be determined and therefore also the order matrix in the PAS and the orientation of the PAS with respect to a molecular fixed frame. Moreover, free rotation of each methyl group about its C_3 symmetry axis was assumed, using for the intramethyl coupling $D(H^{Mei})$ and the intraresidue methine-methyl coupling $D(H^{\alpha i}, H^{Mei})$ values averaged over the three methyl protons.

The experimental evidence so far acquired in the literature [6,11] gives strong indication that in aqueous environment the inner residues adopt a PPII conformation. As far as the dihedral angles of the external residues are concerned, no clear indication of preferred values are reported. According to a theoretical study on the probability distribution of the diehedral angles of tetraalanine in different protonation states [16], broad regions of ψ_1 and φ_4 are significantly populated, differently from the angles of the internal residues for which distinct stability regions are present. Given the limited number of experimental data available, it is not possible to consider more than

one conformer, unless drastic assumptions are made on the order matrices of all the possible conformers, as well as on the ψ_1 and φ_4 distribution functions.

All this considered, we made the assumption that a single conformer dominates. In order to verify if our data are compatible with this hypothesis, we fixed the geometry of the two inner residues to that of PPII, i.e., -57.6, 145.0, -68.8, 138.4° for φ_2, ψ_2, φ_3, and ψ_3, respectively, according to Ref. [11]. A set of conformers was then generated by systematically varying the dihedral angles of the external residues ψ_1 and φ_4 on a grid extending between $-180°$ and 180°. Among these we searched for the one yielding the best reproduction of the experimental data.

The set of experimental dipolar values was fitted using Eq. (1), the elements of the order matrix being the fitting parameters. Given the higher signal overlap in the spectrum of fully protonated A_4 with respect to that of partially deuterated A_4, in the fitting procedure, the combination of signs and of relative magnitude of the couplings matching that of Ref. [11] was chosen, thus attributing patterns **d** and **c** to the second and third residue, respectively, only $D(H^{\alpha 1},H^{Me1})$, $D(H^{Me2})$ and $D(H^{Me4})$ being characterized by a negative sign.

Of the set of conformers examined, only the one characterized by ψ_1 and φ_4 of $-130°$ and $-80°$, respectively, satisfactorily reproduced the experimental values of the dipolar couplings, as shown in Table 1. The value of φ_4 is very close to the value of $-78.2°$ found previously [11]. On the other hand, the value of ψ_1 is shifted with respect to 165° [11], where the probability distribution is theoretically centered, but still belongs to a significantly populated region [16]. The different ψ_1 value here obtained with respect to the one found using deuterated isotopomers [11] could be ascribed to an isotope effect on the molecular polarity [17] thus affecting the interaction with the micelle. Such interaction could induce a different geometry on the N-terminus, which was found to interact with the micelle surface more strongly than the C-terminus [11].

Order Parameters

Table 2 lists the set of order parameters in the molecular inertial frame derived from the fitting procedure and their values for the conformer in question.

The order parameters are comparable to those already observed on partially deuterated tetraalanine [11], the small deviations being ascribable to slight differences in concentration and temperature. It must be pointed out that limiting values of $+0.06$ and -0.03 for

TABLE 2 Order Parameters in the Inertial PAS (S_{ij}) and in the Order PAS ($S_{i'i'}$)

S_{xx}	−0.014
S_{zz}	0.003
S_{xy}	−0.014
S_{xz}	−0.021
S_{yz}	−0.018
$S_{x'x'}$	0.026
$S_{y'y'}$	0.012
$S_{z'z'}$	−0.038

perfect orientation of the peptide long axis normal and parallel, respectively, to the micelle surface can be estimated within assumptions on the peptide partitioning, micelle order parameters and dimensions, as well as interfacial thickness [8].

The negative sign of the major order parameter $S_{z'z'}$ indicates that the molecule is on average oriented with the principal axis of the order PAS z' making an angle larger than the magic angle with the normal axis of the CsPFO disks, which is parallel to the external magnetic field. In addition, the relatively high absolute value of $S_{z'z'}$ suggests that z' is almost parallel to the disk surface. The Euler angles α, β, γ determining the relative orientation of the PAS of the inertial tensor and that of the order matrix are 30°, 56° and 36°, respectively. The long axis of the molecule (z) is then tilted with respect to z' by 56°, as indicated by β, hence tilted with respect to the micelle surface (Fig. 3). These results favorably compare with those reported in Ref. [11] and can be justified on the basis of the positive charge of the N terminus.

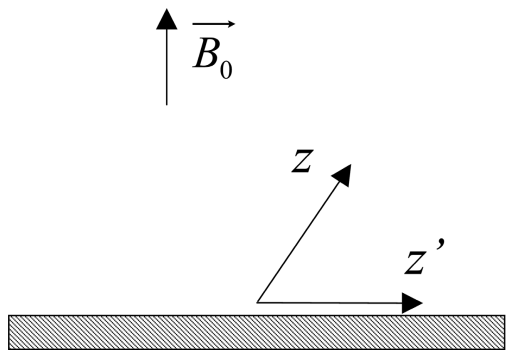

FIGURE 3 Possible orientations of the major axes of the inertial (z) and order (z') PAS for A_4 in CsPFO/D_2O. The rectangle represents the micelle from a lateral view.

CONCLUSIONS

In this study the interpretation of ^{1}H NMR data allowed us to obtain information on the conformation and orientation of tetraalanine in a partially ordered system, where non vanishing dipolar couplings are observed. Although a certain degree of flexibility of the external residues cannot be excluded, our data are compatible with a single conformer characterized by the dihedral angles of external residues ψ_1 and φ_4 equal to -130° and -80°, respectively, while the internal residues are characterized by PPII helix angles. Our results compare well with previous findings reported for partially deuterated tetraalanine molecules, except for a slight difference in the geometry of the N-terminus, ascribable to an isotope effect.

In addition, our results are in agreement with data reported for tetraalanine in aqueous solution, which is evidence for the fact that the liquid crystal medium does not perturb tetraalanine conformational equilibrium, as sometimes argued [18].

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