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CONFORMATIONAL CHARACTERIZATION OF BIOLOGICALLY ACTIVE PEPTIDES, PRO-LEU-GLY-NH_2 AND GRAMICIDIN S, IN THE SOLID AND SOLUTION AS REVEALED BY CONFORMATION-DEPENDENT ^{13}C CHEMICAL SHIFTS

Hazime SAITÔ* and Ryoko TABETA
Biophysics Division, National Cancer Center Research Institute,
Tsukiji 5-chome, Chuo-ku, Tokyo 104

^{13}C NMR spectra of biologically active peptides, Pro-Leu-Gly-NH_2 and gramicidin S, in the solid state were compared with those in solution, to gain insight of stability of the β-turn structure in the former in solution and of conformational feature of the latter in the solid state.

Previously, we showed that the ^{13}C chemical shifts of individual amino acid residues of polypeptides and fibrous proteins in the solid state vary with their local conformations as characterized by the torsion angles (ϕ and ψ) as well as the manner of hydrogen bondings (up to 7 ppm).[1-8] There is no significant contribution from the peptide sequence or the extent of intermolecular packing to the conformation-dependent ^{13}C chemical shifts.[6-8] Thus, conformational feature of polypeptides in the solid state is easily revealed by examining the ^{13}C chemical shifts as compared with those of reference compounds.

By contrast, no such correlation has been established for the ^{13}C chemical shifts observed in solution, because the ^{13}C chemical shifts are, in many instances, observed as time-averaged values among several kinds of rapidly converting conformers for flexible molecules. Clearly, the presence of such a rapid conformational fluctuation in solution is easily evaluated by comparing the ^{13}C chemical shifts in solution with those in the solid. On the contrary, the ^{13}C chemical shifts in solution can be utilized as reference to examine conformational feature in the solid when molecular conformation is well investigated in solution.

Here we applied these two approaches to reveal conformational stability of the β-turn form of Pro-Leu-Gly-NH_2 in solution and to gain insight of conformational feature of gramicidin S in the solid state. High resolution ^{13}C NMR spectra of these peptides in the solid state were recorded on a Bruker CXP-300 spectrometer at 75.46 MHz, by means of the cross polarization-magic angle spinning (CP-MAS) method.

Pro-Leu-Gly-NH_2 is the C-terminal tripeptide of oxytocin and has been postulated as the inhibiting factor of the release of melanocyte-stimulating hormone (MSH).[9] The basic conformational feature of the proposed solution model, a ten-membered β-turn structure,[10] was confirmed by X-ray diffraction.[11] Thus, it is expected that the ^{13}C chemical shifts of this molecule are similar between the solid and solution state if stable β-turn form exists in solution. Figure 1 shows

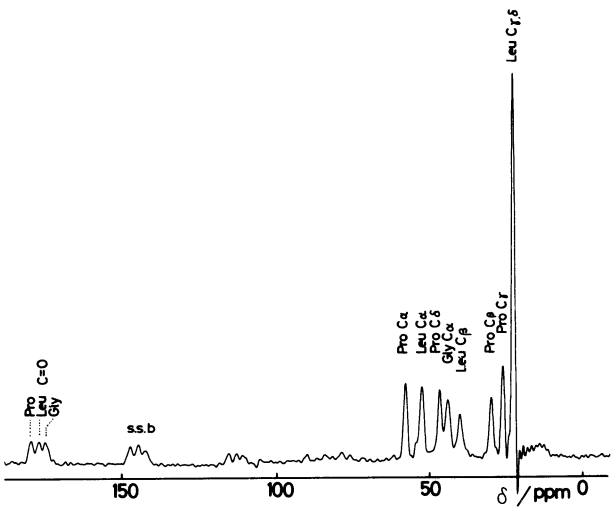

Fig. 1. 75.46 MHz ^{13}C CP-MAS NMR spectrum of Pro-Leu-Gly-NH_2 0.5 H_2O in the solid state.

Table 1. ^{13}C chemical shifts of Pro-Leu-Gly-NH_2 0.5 H_2O (± 0.5 ppm for the solid)

		Solid	Solution		
			CD_3CN	DMSO-d_6 [a]	D_2O [a]
Pro	C^{α}	58.0	61.3	61.1	61.1
	C^{β}	30.0	31.1	31.9	31.6
	C^{γ}	26.2	26.8	27.3	26.5
	C^{δ}	46.7	47.6	48.2	47.6
	C=O	179.2	176.7	175.9	179.0
Leu	C^{α}	52.7	52.7	52.2	53.6
	C^{β}	41.2	41.2	b	40.8
	C^{γ}		25.5	25.9	25.5
	C^{δ}	22.7	23.1	24.6	23.2
	C^{δ}		21.7	23.2	21.9
	C=O	176.2	173.4	173.7	176.5
Gly	C^{α}	44.2	42.9	43.4	43.3
	C=O	174.5	172.0	172.3	175.2

a) Ref. 12. b) Hidden under solvent peaks.

the 75.46 MHz ^{13}C CP-MAS NMR spectrum of Pro-Leu-Gly-NH_2 0.5 H_2O (Sigma Chemical Company, USA; Lot No. 58c-0260) in the solid state. Well-resolved ^{13}C peaks were straightforwardly assigned on the basis of spectral data taken in solution.[12] As summarized in Table 1, the ^{13}C chemical shifts in the solid state are found to be very similar to those of D_2O solution (<1 ppm), except for Pro C_{α} and C_{β} peaks. By contrast, the carbonyl peaks of this peptide in CD_3CN and DMSO-d_6 solution are displaced upfield (≈2.5 ppm) as compared with those of the solid and D_2O solution.

On the basis of the spin-lattice relaxation times, this peptide can be regarded as a flexible molecule in D_2O but as more rigid form in DMSO solution.[12] In fact, the presence of the β-turn structure in DMSO and CH_3CN solution was previously confirmed by laser Raman[13] and ^{17}O NMR[14] data, respectively. Further, the presence of the β-turn structure can be clarified by examination of the difference of the carbonyl ^{13}C shifts by going from aprotic (CD_3CN) to protic (D_2O) solvent system:[15] this value of Pro residue (2.3 ppm) is significantly smaller than that of Leu (3.1 ppm) and Gly (3.2 ppm) residues, due to formation of the intramolecular hydrogen bond at Pro C=O group. In parallel with these findings, the $\Delta_{\beta\gamma}$ value, which is the difference of chemical shifts between the C_{β} and C_{γ} shifts and varies with the torsion angle ψ ,[16] is 4.3 and 4.6 ppm for CD_3CN and DMSO-d_6 solutions, respectively,and very close to the value of 3.8 ppm in the solid state. However, the $\Delta_{\beta\gamma}$ value in D_2O solution (5.1 ppm) is slightly larger than that of the solid state. Therefore, it is concluded that the local conformation at Pro residue prerequisite to formation of the β-turn structure is retained even in DMSO and CD_3CN solutions. Nevertheless, the β-turn structure in solution is not a rigid structure but rather a preferred conformation in which the hydrogen bond may open and then reform within NMR time scale, as manifested from the existence of the sizable amount of difference in chemical shifts in the Pro C=O signal between the solid and solution. It is also emphasized that the ^{13}C signals of carbonyl carbons not participated in hydrogen bonds in the solid can be displaced in solution, depending

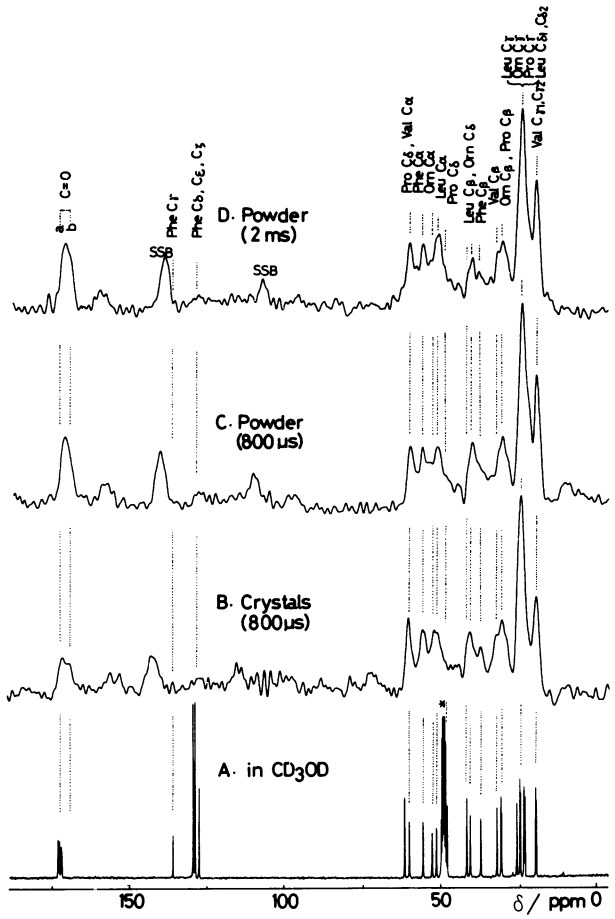

Fig. 2. 75.46 MHz ^{13}C CP-MAS NMR spectra of gramicidin S in the solid state (B-D) and in CD_3OD solution (A).

Table 2. ^{13}C chemical shifts of gramicidin S in the solid and solution (ppm from TMS; ± 0.5 ppm for the solid)

		Solid	Solution[a]	
			CD_3OD	DMSO-d_6
Val	Cγ	18.9	18.5	18.9
	Cγ		18.7	19.9
Leu	Cδ		22.1	23.5
	Cδ		22.5	23.7
Pro	Cγ	23.5	23.6	24.1
Orn	Cγ		23.7	24.1
Leu	Cγ		24.7	25.0
Pro	Cβ	29.8	29.6	30.0
Orn	Cβ		29.8	30.6
Val	Cβ	31.6	31.1	31.6
Phe	Cβ	36.7	36.3	36.7
Orn	Cδ	40.1	39.7	b
Leu	Cβ	40.9	40.8	41.8
Pro	Cδ	48.8	47.1	47.0
Leu	Cα	51.0	50.7	50.6
Orn	Cα	51.7	52.1	52.0
Phe	Cα	55.7	55.1	54.8
Val	Cα	60.3	59.6	57.9
Pro	Cα		61.1	60.9
Pro	C=O[c]	170.1	171.8	170.8
Orn	C=O	170.1	172.0	171.2
Val	C=O	172.2	172.6	171.8
Phe	C=O	170.1	172.8	172.0
Leu	C=O	172.2	173.1	172.7

a) 25 mg/ml, from ext. TMS.
b) Hidden under solvent peaks.
c) Assignment of C=O peaks in DMSO-d_6 based on Ref. 22.

on the extent of hydrogen bonding with solvents.

The cyclic decapeptide antibiotic gramicidin S has the sequence of

$$\left[\begin{matrix}\text{Pro-Val-Orn-Leu-D-Phe}\\ \text{D-Phe-Leu-Orn-Val-Pro}\end{matrix}\right].$$

Despite its relative simplicity, over 12 structures have been proposed based on theoretical and experimental means, because X-ray investigations were unable to define the three-dimensional coordinates.[17,18] The composite application of the solution spectral methods confirmed one of the structure to be a pleated sheet which has been proposed by Hodgkin and Oughton[19] from X-ray studies and by Scwyzer on the basis of chemical data.[17,18] Thus, this is one of examples whose solution structure is defined more clearly than the solid structure.

Figure 2 shows the 75.46 MHz ^{13}C CP-MAS NMR spectra of powder sample (as received) and needle crystals (from ethanol) of gramicidin S (Sigma, Lot. No. G-51 27) together with the solution NMR spectrum recorded in CD_3OD solution. Assignment of peaks was based on previous works.[20,21] Obviously, the ^{13}C peaks from the individual amino acid residues are well resolved to such an extent to permit detailed comparison of peaks between the solid and solution data. Naturally, spectral resolution in the crystalline sample (Fig. 2B) is much improved as compared with that of the powder sample (Fig. 2C). In particular, the C=O peak of

the crystalline sample is split into two peaks. A slight change of the relative peak-intensities is noted at the aliphatic region depending on the contact times.

Clearly, the ^{13}C chemical shifts of the crystalline sample are in good agreement with those of solution (<1 ppm)(see Table 2), except for the peak b of the carbonyl region. This result shows that conformation of gramicidin S in the solid is the same pleated sheet with C_2 symmetry as in solution. Another possibility such as α- or α/β-mixed form is easily ruled out, as compared with the ^{13}C shifts of reference samples.[1-3,7] The peak b is ascribed to the carbonyl carbons of Pro, Orn, and Phe residues which are not involved in the intramolecular hydrogen bonds, because this peak is shifted downfield (≈2 ppm) in CD_3OD due to exposure to solvent molecules. It is also interesting to note that the peak-intensities of D-Phe residues are significantly suppressed in the solid state. This might be caused by the presence of internal motion of the side-chain on the NMR time scale, i.e. comparable to the frequency of decoupling field.[22] The presence of the flipping of phenyl ring in cyclic peptides in the solid was previously described.[23]

In conclusion, it is found that comparison of the ^{13}C chemical shifts between the solid and solution provides an invaluable information as to conformational feature of simple peptides either in the solid or solution.

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