MANIFESTATION IN THE ^{13}C-NMR SPECTRA OF TWO DIFFERENT MOLECULAR CONFORMATIONS OF A CYCLIC PENTAPEPTIDE

K. WÜTHRICH, A. TUN-KYI and R. SCHWYZER
Institut für Molekularbiologie und Biophysik,
Eidgenössische Technische Hochschule,
CH-8049 Zürich, Switzerland

Received 9 June 1972

1. Introduction

In spite of the limited spectral resolution even at the highest presently available magnetic fields high resolution proton nuclear magnetic resonance (NMR) spectroscopy occupies a prominent position among the physicochemical methods used for the elucidation of the molecular conformations in peptides and proteins [1–6]. It is to be expected that NMR spectroscopy of less abundant and less sensitive nuclei, e.g. ^{13}C, ^{14}N, ^{15}N, ^{17}O, ^{31}P, in biopolymers by the recently introduced Fourier Transform technique [7] will open new possibilities for the application of NMR in biological research. In as far as carbon-13 NMR is concerned these hopes have been partially substantiated. The presently available data show that a greater number of spectral features can be resolved in the ^{13}C-NMR spectrum than in the proton NMR spectrum of a given biopolymer [8–11]. On the other hand it appears, however, that too little knowledge has as yet been accumulated on the correlations between non-bonded structural features and the ^{13}C-NMR parameters for the method to be readily applicable to conformational studies in biopolymers. Thus the interpretations of the ^{13}C-NMR spectra of peptides and depsipeptides have so far mainly yielded information on molecular symmetries [12, 13], and on charge effects during pH-titration [10, 11, 14] and complexation with alkaline metal ions [15, 16]. Some evidence for a manifestation of conformational properties in the ^{13}C-resonances was obtained from studies of valinomycin and nonactin. A comparison of the free and the alkaline metal-complexed forms of these compounds indicated that all the spectral differences could not arise from ion–dipole interactions, and hence some spectral changes were probably due to conformational rearrangements of the ligands upon complex formation [15, 16]. In the present paper we describe the ^{13}C-NMR spectrum of a synthetic cycle pentapeptide, *cyclo*-glycyl–L-alanyl–glycyl–glycyl–L-propyl. In a solution in DMSO two different monomeric conformations of this peptide are simultaneously present in comparable concentrations [17], and these two species can readily be distinguished in the ^{13}C-NMR spectra.

2. Materials and methods

The synthesis of *cyclo*-glycyl–L-alanyl–glycyl–glycyl–L-prolyl had been described [17,18]. For the ^{13}C-NMR studies the peptide was dissolved in d_6-DMSO. The peptide concentration in the two samples used for carbon-13 work was 0.15 and 0.30 M respectively. Identical ^{13}C-resonances were observed in the two samples, and the proton NMR spectra were identical to those in more dilute solutions [17].

^{13}C-NMR spectra at 25.14 MHz were obtained on a Varian XL-100 spectrometer using the Fourier Trans-

North-Holland Publishing Company – Amsterdam

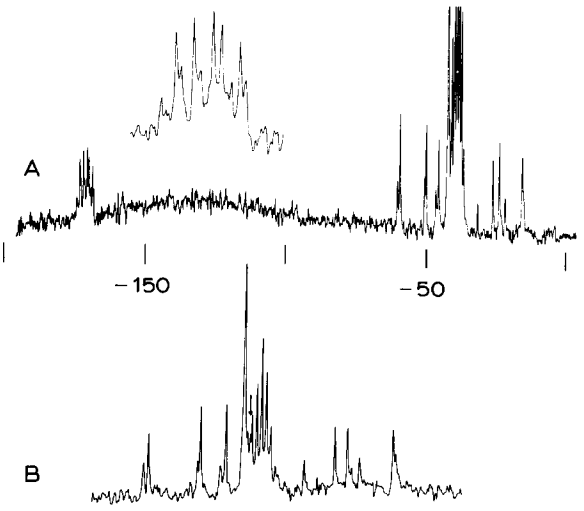

Fig. 1. Proton-decoupled natural abundance ^{13}C Fourier transform NMR spectra at 25.14 MHz of a 0.3 M solution of *c*-(–Gly–L-Ala–Gly–Gly–L-Pro–) in d_6-DMSO, T = 28°. A sample tube of 5 mm outer diameter was used. 45 000 transients were accummulated. The horizontal scale is in ppm from internal TMS, where shifts to low field are assigned negative values. The septet centered at —39.8 ppm corresponds to d_6-DMSO. A) Entire spectrum, recorded with a pulse width of 75 μsec, acquisition time 0.4 sec, pulse delay 1.0 sec. The carbonyl region from —165 to —175 ppm is also shown on an expanded scale. B) Spectral region from —10 to —70 ppm on an expanded horizontal scale with PW = 75 μsec, AT = 0.4 sec, PD = 0. Under these conditions an additional resonance of the peptide could be detected at —40.6 ppm (arrow).

form technique. A sample tube of 5 mm outer diameter was used. The sample temperature was approximately 28°. Chemical shifts are relative to internal tetramethylsilane, the center of the multiplet of d_6-DMSO being at —39.8 ppm. The system was locked on the ^2D signal of the solvent.

3. Results

The ^{13}C-NMR spectrum of *c*-(–Gly–L-Ala–Gly–Gly–L-Pro–) in DMSO is shown in fig.1. From proton NMR studies it had previously been found that two different molecular conformations M and m of the peptide occur simultaneously under the conditions of the present experiments (fig.1), the relative concentrations being 2:1 [17]. The occurrence of these two species is clearly manifested in the ^{13}C-NMR spectra, e.g. the five carbonyl carbon atoms in the peptide give rise to two sets of five lines with relative intensities of approximately 2:1 (fig. 1A). Table 1 gives the ^{13}C chemical shifts corresponding to the two molecular conformations M and m.

Table 1
^{13}C chemical shifts (ppm from internal TMS) of the two molecular conformations M (65%) and m (35%) of *c*-(–Gly–L-Ala–Gly–Gly–L-Pro–) which occur simultaneously in a solution in DMSO at 28°.

Carbon assignment		Conformation M	Conformation m
CH_3	Ala	16.3	15.8
CH_2	Pro C^β, C^γ	24.3 26.7	22.2 32.1
	Gly (1) Gly (3) Gly (4) C^α	42.8	40.6
	Pro C^δ	46.0	46.8
CH	Ala C^α	50.4	51.0
	Pro C^α	59.6	60.5
C=0	Gly (1)	168.6	168.2
	Ala	169.9	169.2
	Gly (3)	170.5	171.4
	Gly (4)	171.9	172.8
	Pro	173.2	174.2

The resonance assignments in table 1 are based on the following considerations. First the carbon atoms carrying three, two, one or no protons were distinguished in off-resonance proton decoupling experimer The assignment of the methyl carbon atom of alanine was then straight-forward. The β-, γ-, and δ-methylene carbon atoms of proline were assigned from comparison with the spectra of the amino acids in small peptides [19, 20]. For the three α-carbon atoms of the glycyl residues only one resonance line was observ in the spectrum of fig. 1A. From its intensity, which was compared with the intensities of the other resonances under different conditions of pulse width and pulse delay, this line can correspond to the methylene carbons of all the three glycyl residues in

the species M. Under the experimental conditions of fig. 1B an additional peptide resonance could be observed at −40.6 ppm which was tentatively assigned to one or several of the α-carbon atoms of glycine in the conformation m. The α-carbon atoms of proline and alanine were again assigned from comparison with the spectra of the individual amino acids [19, 20].

4. Discussion

From proton NMR studies it had been concluded that the two species M and m in solutions in DMSO of *c*-(−Gly− L-Ala−Gly−Gly−L-Pro−) correspond to the two types of conformations in fig.2 [17]. According to these models the species M contains the prolyl residue in the trans–trans′ conformation [21], which allows the formation of two apparently equally favorable transannular hydrogen bonds. The molecular conformation m contains cis–trans′ proline [21], and there is one preferred transannular hydrogen bond. Fig. 1 and table 1 show that all the carbon resonances in the peptide, possibly with the exception of some or all of the α-carbons in the three glycyl residues, are affected by the conformational differences between M and m.

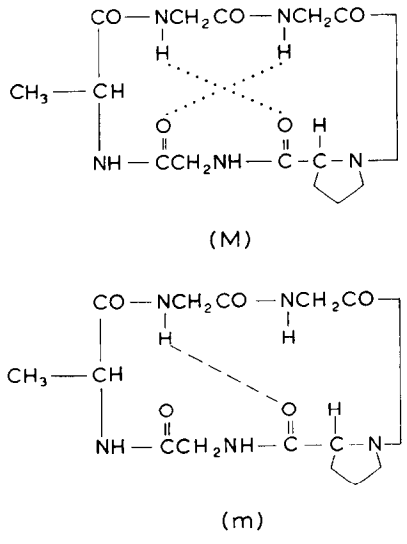

Fig. 2. Molecular conformations in solutions of *c*-(−Gly−L-Ala−Gly−Gly−L-Pro−) in DMSO [17]. M: Two favourable intramolecular hydrogens bonds in a dynamic equilibrium. The prolyl residue would be in a trans–trans′ conformation. m: One preferred transannular hydrogen bond, proline in the cis–trans′ conformation.

An outstanding ^{13}C chemical shift difference between the two conformations of the peptide concerns the β- and γ-carbons of proline. In M these resonances are at −24.3 and −26.7 ppm, in m at −22.2 and −32.1 ppm (table 1). Futhermore the relative shift of 2.4 ppm between the β- and γ-carbon resonances in M is rather small compared to the corresponding values of 9.9 ppm in the species m, and 6.4 ppm in t-BOC-L-Pro-OH* [20]. This pronounced magnetic non-equivalence in M and m of two of the ring carbon atoms of proline would hardly be comprehensible unless it is assumed that the proline ring geometry is different in the two peptide conformations. It had previously been inferred from proton NMR and X-ray studies of various proline compounds that different ring geometries might come about as a consequence of cis–trans isomerism of the peptide bond involving the nitrogen of proline [22]. According to Deber et al. [22] the four carbon atoms are almost exactly coplanar in the cis-prolyl residue, with the amide nitrogen located out of this plane. In trans-proline an alternate ring geometry seems to be more likely, with the α-, β and δ-carbons and the amide nitrogen essentially coplanar and the γ-carbon atom out of the plane [22].

For all the α-carbon atoms, the δ-carbon of proline, and the methyl carbon of alanine the chemical shift differences between corresponding resonances in the two species M and m are of the order of 1 ppm or smaller. Furthermore the chemical shifts between corresponding resonances in M and m on the one hand, and t-BOC-L-Ala-OH, t-BOC-L-Pro-OH, t-BOC-Gly-OH or Z-Gly-OH* in DMSO-solutions [20] on the other hand are smaller than 2 ppm for these carbon atoms. It is quite striking that the α-carbons of the glycyl residues in positions 1, 3 and 4 (fig.2) appear to be magnetically equivalent in M, and that some or all of the glycine α-carbons might also have the same chemical shift in the conformation m.

The carbonyl resonances in spectrum A (fig. 1) extend over a spectral range of 6 ppm. This may be compared to the chemical shift differences of 3 ppm

* t-BOC = tertiary butyloxycarbonyl−, Z = benzyloxycarbonyl−.

between the carbonyl resonances of t-BOC-L-Ala-OH, t-BOC-L-Pro-OH, and t-BOC-Gly-OH in DMSO [20], and one might then take these numbers to indicate that the carbonyl ^{13}C-shifts arising from differences in conformation might be quite sizeable. From the present data it is, however, not possible to assign the individual carbonyl resonances to specific amino acid residues. Hence we can at this point only conclude that chemical shift differences between corresponding carbonyl resonances in M and m cannot be larger than 5.6 ppm. It should be possible in future experiments, e.g. with isotopically labelled peptide analogues of *c*-(–Gly–L-Ala–Gly–Gly–L-Pro–) or similar peptides, to investigate in more detail the influence on the carbonyl resonance positions of the formation of intramolecular hydrogen bonds.

Overall the present data indicate that essentially all the ^{13}C-resonances in *c*-(–Gly–L-Ala–Gly–Gly–L-Pro–) and similar peptides might in principle be useful for studies of the molecular conformations. From the already mentioned ^{13}C-NMR spectral differences between the free and the alkaline ion-complexed forms of nonactin, valinomycin, and related cyclic molecules [15, 16] one arrives at a similar conclusion for these compounds. It has also been observed that the helix-coil transition in poly-(γ-benzyl-L-glutamate) in a mixed solvent of chloroform and trifluoroacetic acid is manifested in at least three ^{13}C resonances, i.e. those of the peptide carbonyl-, α- and β-carbons [23]. All these examples will probably be useful for future systematic investigations of the correlations between ^{13}C-NMR parameters and non-bonded structural features in peptides and proteins. Model systems of the type described in this paper have, however, the particular advantage that all the ^{13}C-NMR spectra differences can be assigned to differences in molecular conformation, and could not conceivably be due in part or entirely to either different total electric charge or different solvent environment of the peptide conformations which are to be compared.

Whereas conformational differences in peptides seem to give rise to sizeable shifts of several ppm for certain ^{13}C- resonances, they appear to be mainly manifested in the proton-decoupled ^{13}C-NMR spectra by chemical shifts of the order of 1 ppm (table 1, [15, 16, 23]). It remains to further investigate the practical aspects of observing and interpreting these rather small chemical shifts. Especially in view of studies with larger peptides and with proteins [8, 9] it would appear from the presently available data that the application of very high magnetic fields might be important for ^{13}C-NMR investigations of peptide and protein conformations as it was for the development of proton NMR studies in the same field [1].

Acknowledgement

Financial support by the Swiss National Science Foundation (Grants Nr 3.374.70 and 3.423.70) is gratefully acknowledged.

References

[1] C.C. McDonald and W.D. Phillips, J. Am. Chem. Soc. 89 (1967) 6332.
[2] G.C.K. Roberts and O. Jardetzky, Adv. Protein Chem. 24 (1970) 447.
[3] R. Schwyzer and U. Ludescher, Biochemistry 7 (1968) 2519.
[4] A. Stern, W.A. Gibbons and L.C. Craig, Proc. Natl. Acad. Sci. U.S. 61 (1968) 734.
[5] K.D. Kopple, M. Onishi and A. Go, Biochemistry 8 (1969) 4087.
[6] K. Wüthrich, Structure and Bonding 8 (1970) 53.
[7] R. Ernst, Adv. Magn. Res. 2 (1966) 1.
[8] A. Allerhand, D.W. Cochran and D. Doddrell, Proc. Natl. Acad. Sci. U.S. 67 (1970) 1093.
[9] F. Conti and M. Paci, FEBS Letters 17 (1971) 149.
[10] M.H. Freedman, J.S. Cohen and I.M. Chaiken, Biochem. Biophys. Res. Commun. 42 (1971) 1148.
[11] G. Jung, E. Breitmaier and W. Voelter, European J. Biochemistry 24 (1972) 438.
[12] W.A. Gibbons, J.A. Sogn, A. Stern, L.C. Craig and L.F. Johnson, Nature 227 (1970) 840.
[13] B. Donzel, B. Kamber, K. Wüthrich and R. Schwyzer, Helv. Chim. Acta 55 (1972) 947.
[14] F.R.N. Gurd, P.J. Lawson, D.W. Cochran and E. Wenkert, J. Biol. Chem. 246 (1971) 3725.
[15] M. Onishi, M.C. Fedarko, J.D. Baldeschwieler and L.F. Johnson, Biochem. Biophys. Res. Commun. 46 (1972) 312.
[16] V.F. Bystrov, V.T. Ivanov, S.A. Koz'min, I.I. Mikhaleva, K.K. Khalilulina and Y.A. Ovchinnikov, FEBS Letters 21 (1972) 34.
[17] J.P. Meraldi, R. Schwyzer, A. Tun-Kyi and K. Wüthrich, Helv. Chim. Acta, in press.
[18] R. Schwyzer, A. Kun-Kyi, M. Caviezel and P. Moser, Helv. Chim. Acta 53 (1970) 15.

[19] W. Horsley, H. Sternlicht and J.S. Cohen, Biochem. Biophys. Res. Commun. 37 (1969) 47.
[20] W. Voelter, G. Jung, E. Breitmaier and E. Bayer, Z. Naturforschung 26b (1971) 213.
[21] J.P. Carver and E.R. Blout, in: Collagen, ed. G.N. Ramachandran (Academic Press, New York, 1967) p. 452.
[22] C.M. Deber, D.A. Torchia and E.R. Blout, J. Am. Chem. Soc. 93 (1971) 4893.
[23] L. Paolillo, T. Tancredi, P.A. Temussi, E. Trivelione, E.M Bradbury and C. Crane-Robinson, Chem. Comm. (1972) 335.