CARBON-13 NMR SPECTRA OF cyclo-Glycyl–L-prolyl–glycyl–glycyl–L-prolyl–glycyl: ASSIGNMENT OF THE CARBONYL RESONANCES

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1. Introduction

The carbonyl carbon-13 nuclear magnetic resonances in peptides and depsipeptides are particularly sensitive to charge effects which arise during pH-titration [1, 2] or upon complex formation with metal ions [3, 4]. There is also some evidence that certain conformational changes in uncharged peptides might be manifested in the carbonyl resonances [5]. On the other hand the assignment of the carbonyl resonances to specific amino acid residues is generally rather difficult even in small peptides, and hence the potential of the use of the carbonyl resonances for studies of the molecular conformations has as yet been little explored. This paper describes some proton-carbon-13 heteronuclear double resonance experiments which led to the identification of the carbonyl resonances in cyclo-glycyl–L-prolyl–glycyl–glycyl–L-prolyl–glycyl. On the basis of these data the effect of intramolecular hydrogen bonding on the carbonyl carbon resonance positions can be qualitatively assessed.

c-(–Gly–L-Pro–Gly–)$_2$ was particularly suitable for these studies because its molecular conformation had previously been extensively investigated. Already several years ago it was proposed on the basis of proton NMR data that one of two favorable β-type conformations with two transannular hydrogen bonds is present in solutions of this peptide [6, 7]. More recently the investigation of an α-carbon-deuterated analogue, c-(–Gly–L-Pro–Gly(d_2)–)$_2$, showed that of the two likely β-structures the one with the two intramolecular hydrogen bonds involving the amide protons and the carbonyl groups of the glycyl residues in positions 1 and 4 (structure type IIIb of fig. 2 in [7] and fig. 1 in [8]) is preferred [8, 9].

2. Materials and methods

The synthesis of c-(–Gly–L-Pro–Gly–)$_2$ [6] and c-(–Gly–L-Pro–Gly(d_2)–)$_2$ [8] had been described. For the ^{13}C-NMR studies ca. 0.05 M peptide solutions in d_6-DMSO were prepared. For some measurements the amide protons were exchanged with ^{2}D through the addition of ca. 4% D_2O to the solution in DMSO. There was no indication of a change of the peptide conformation upon addition of the D_2O, neither in the ^{1}H- nor in the ^{13}C-spectra (table 1).

^{1}H-NMR spectra at 100 MHz and ^{13}C-NMR spectra at 25.14 MHz were obtained on a Varian XL-100 spectrometer. For the carbon-13 studies a sample tube of 12 mm outer diameter was used, and the spectra were recorded with the Fourier Transform technique.The sample temperature was approx. 35°. Chemical shifts are relative to internal tetramethylsilane. The system was locked on the ^{2}D signal of d_6-DMSO.

3. Results

Fig. 1 shows four different carbon-13 NMR spectra of c-(–Gly–L-Pro–Gly–)$_2$ and c-(–Gly–L-Pro–Gly(d_2)–)$_2$. The resonance assignment given in table 1 are based on a comparison of these four spectra, and on the double resonance experiments presented in fig. 2, where only the carbonyl resonances are considered. To better illustrate the procedures used, the proton NMR spectra of the two peptides with the resonance assignments determined previously [8] are also given in fig. 3.

From a comparison of the proton noise-decoupled ^{13}C spectrum of c-(–Gly–L-Pro–Gly–)$_2$ with the

Table 1

^{13}C chemical shifts (ppm from internal TMS) of c-(−Gly−L-Pro−Gly−)$_2$, I in d_6-DMSO, I′ in d_6-DMSO + 4% D_2O, and c-(−Gly−L-Pro−Gly(d_2)−)$_2$, II in d_6-DMSO, II′ in d_6-DMSO + 4% D_2O.

Carbon assignment			I	I′	II	II′
CH_2	Pro C^β, C^γ		− 24.7 − 28.8	− 24.8 − 28.8	− 24.8 − 28.9	− 24.7 − 28.7
	Gly (1) and (4) Gly (3) and (6)	C^α	− 42.5	− 42.3	− 42.5	− 42.2
	Pro	C^δ	− 45.8	− 45.8	− 45.8	− 45.7
CH	Pro	C^α	− 60.9	− 60.9	− 60.9	− 60.7
C = 0	Gly (1) and (4) Gly (3) and (6) Pro		−166.7 −169.2 −172.4	−166.5 −169.2 −172.4	−166.5 −169.0 −172.2	−166.6 −169.1 −172.4

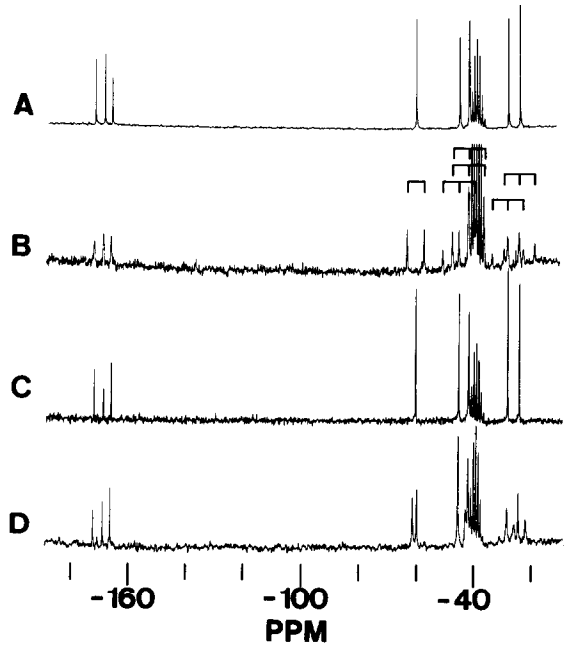


Fig. 1. Natural abundance ^{13}C Fournier transform NMR spectra at 25.14 MHz, T = 35°, d_6-DMSO solvent resonance at −39.8 ppm. A and B: c-(−Gly−L-Pro−Gly−)$_2$; A, proton noise-decoupled; B, no proton irradiation. C and D: c-(−Gly−L-Pro − Gly(d_2)−)$_2$;C, proton noise-decoupled; D, double resonance irradiation at a position approx. 20 Hz upfield from the δ-methylene proton resonances of proline (b in fig. 3B).

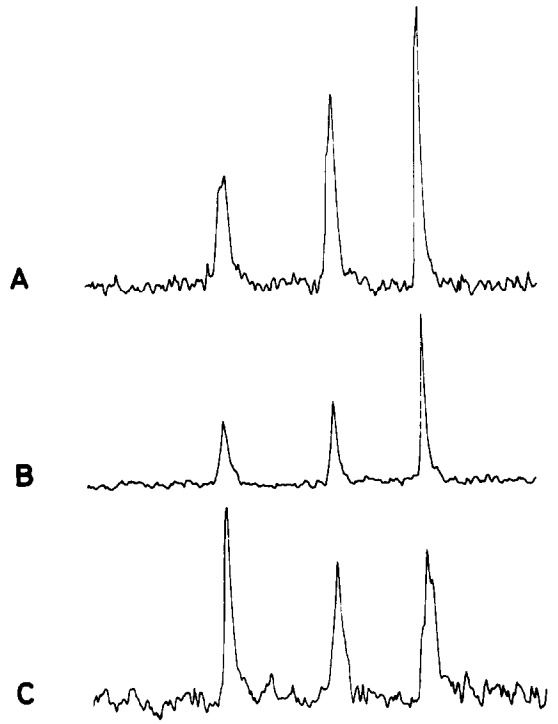
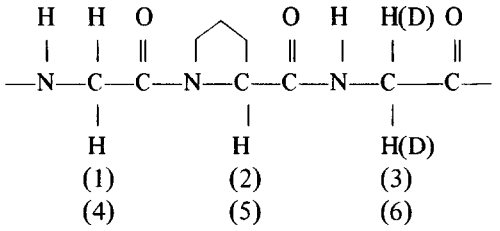


Fig. 2. Natural abundance ^{13}C resonances of the carbonyl groups between −166 and −173 ppm (fig. 1). A: c-(−Gly−L-Pro−Gly−)$_2$ in d_6-DMSO, double resonance irradiation of the C^α-proton resonances of Gly(1) and (4) (resonance d in fig. 3A). B and C: c-(−Gly−L-Pro−Gly(d_2)−)$_2$ in d_6-DMSO + 4% D_2O; B, same conditions as A; C, double resonance irradiation of the β- and γ-methylene protons of proline (resonance a in fig. 3).

undecoupled spectrum (fig. 1, A and B), and considering the covalent structure of the peptide



the C^{α} resonance of Pro can be identified. The assignment of the C^{β} and C^{γ} is further based on a comparison with the corresponding resonance positions in other peptides [5]. The observation that the intensity of the resonance line at −42.5 ppm decreases relative to that of the line at −45.8 ppm when going from spectrum A to C indicates that the C^{α} resonance of Gly (3) and (6) is at −42.5 ppm. Double resonance irradiation at a position approx. 20 Hz upfield from the δ methylene proton resonances of Pro (b in fig. 3B) yields a single resonance line at −45.8, whereas part of the line at −42.5 splits into a multiplet (fig. 1D). Hence we have the assignments for all the methylene carbon atoms as given in the table. The ^{13}C-^{2}D spin–spin couplings seem not to be manifested in the C^{α} resonance of Gly(3) in c-[–Gly–L-Pro–Gly(d_2)–]$_2$. A possible explanation appears to be that the longitudinal relaxation of ^{2}D is rather rapid. Also there is no appreciable isotope shift of the C^{α} resonance position of Gly(3).

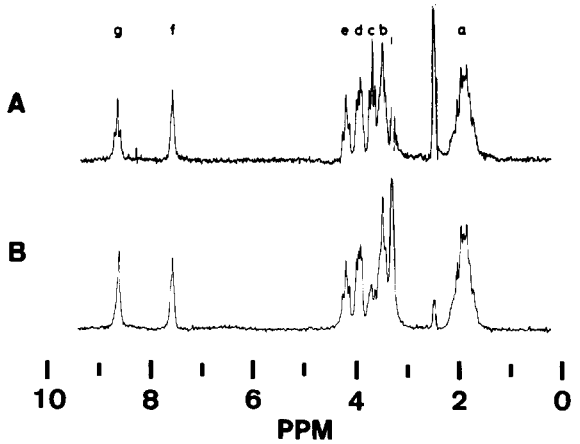


Fig. 3. ^{1}H-NMR spectra at 100 MHz, T = 27°, d_6-DMSO solvent resonance at 2.5 and water resonance at 3.3 ppm. A: c-[–Gly–L-Pro–Gly–]$_2$; B: c-[–Gly–L-Pro–Gly(d_2)–]$_2$. Resonance assignments [8]: a = β- and γ-methylene of Pro; b = δ-methylene of Pro; c = C^{α} protons of Gly(3) and (6); d = C^{α} protons of Gly(1) and (4); e = C^{α} proton of Pro; f = N–H of Gly(1) and (4); g = N–H of Gly(3) and (6).

For the interpretation of the double resonance experiments presented in fig. 2 it is important that $^{13}C-^{1}H$ spin–spin coupling constants between nuclei separated by three covalent bonds appear to be comparable to and in certain instances even larger than those between nuclei separated by two bonds [10,11]. Hence the carbonyl carbon atoms in c-[–Gly–L-Pro–Gly–]$_2$ will in general be subject to appreciable long range coupling with six protons. The resulting multiplets are not well resolved and are mainly manifested in a broadening of the carbonyl resonances when the ^{13}C spectrum is recorded without proton double resonance irradiation (fig. 1B). In the experiments of fig. 2 we have applied a weak field at 100 MHz and selectively removed some of the $^{13}C-^{1}H$ long range spin–spin couplings. In fig. 2A and B the C^{α} proton resonance of Gly(1) (resonance d in fig. 3) was irradiated. Considering the covalent structure of the molecule this should affect mainly the carbonyl resonance of Gly(1), perhaps also that of Gly(3), but not that of Pro. Comparison of spectrum 2A with 1B shows that the peak height of the two upfield resonances is greatly enhanced relative to the line at −172.4 ppm through the proton irradiation. Deuteration at C^{α} of Gly(3) might affect the carbonyl resonances of Gly(3) and Pro, but not of Gly(1). Comparing spectra 2A and 2B shows that the resonance at −169.2 ppm is most strongly affected by the deuteration, and hence we have the assignments given in the table. In fig. 2C double resonance irradiation of the β- and γ-methylene protons of Pro (a in fig. 3B) produces an increase of the peak height of the lowest field and thus confirms the identification of the carbonyl resonance of Pro.

4. Discussion

The carbon-13 data confirm the C_2 symmetry of the molecular conformation of c-[–Gly–L-Pro–Gly–]$_2$ which is also apparent in the proton NMR spectra [6, 7]. On the basis of previous ^{13}C studies of cis/trans-isomerism of proline in cyclic peptides [5] it would also appear that the data in table 1 provide direct evidence that this peptide contains trans-proline, as was postulated earlier [6].

The carbonyl carbon resonance of Gly(1) which is involved in intramolecular hydrogen bonding appears to be shifted to higher field relative to the glycyl carbonyl group which is more freely accessible to the DMSO. This comes rather as a surprise since earlier experiments indicated that intermolecular hydrogen bonding of the carbonyl groups in ketones, and carboxylic acids with protic solvents gives rise to considerable downfield shifts of the carbonyl resonances [12–14].

Blout and coworkers have used a carbon-13 enriched analogue of c-[–Gly–L-Pro–Gly–]$_2$ to distinguish between the two glycyl carbonyl resonances in this molecule, and their results appear to agree with those described here [9]. In principle selective isotope enrichment promises quite generally to be of great help in the identification of nuclear spins with low natural abundance. On the other hand, considering the effort involved in the preparation of suitable isotopically marked analogues, double resonance experiments of the type described here might in certain cases be an attractive alternative for the identification of the carbonyl carbon resonances in peptides.

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