^{17}O N.M.R. Spectroscopy as a Novel Tool for Investigating β-Turns in Model Peptides

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Chemical shifts of the amide resonances in model dipeptides at dilute concentrations are shown to be strongly dependent on the formation of β-turns.

β-Turns are frequent conformational units in proteins and peptides and their role in structure–activity relationships is emphasized throughout the literature.[1,2] Turns are in general stabilized by an intramolecular hydrogen bond between the C=O of residue *i* and the NH of residue *i* + 3, particularly in cases involving heterochiral sequences (II or II′ β-turns).

N.m.r. spectroscopy is probably the most widely used method for determining the presence of β-turns in peptides in solution, although n.m.r. data must be interpreted with great caution.[3] ^{17}O N.m.r. spectroscopy is a very sensitive tool in studying inter- and intra-molecular hydrogen bonding interactions;[4,6] however, to date no clear evidence has been given on the effect of β-turns on the ^{17}O nuclear screening constants. We report here the ^{17}O n.m.r. spectra of [^{17}O]Ac-L-Pro-D-Ala–NHMe and [^{17}O]Ac-L-Pro–OMe, 40% ^{17}O enriched at the acetyl oxygen atom, and demonstrate for the first time the sensitivity of ^{17}O chemical shifts to β-turn structures bearing hydrogen bonds of medium to weak strength.

In organic solvents, both derivatives exhibit two ^{17}O resonances, which can be attributed to the *cis–trans* isomerization of the Ac–Pro amide bond (Table 1). On the basis of the presence of major *trans* conformer,[7] the smaller high-frequency component can be attributed to the *cis* conformer, and its intensity corresponds to a population of *ca.* 6 and 22% for Ac-L-Pro-D-Ala–NHMe and Ac–Pro–OMe in CH_2Cl_2, respectively.†

† These are tentative values since Gaussian-exponential filtering alters the original integrals and lineshapes of the resonances.[6]

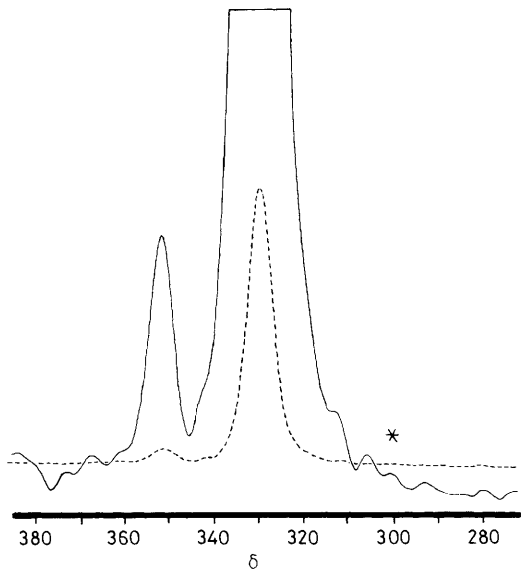

Figure 1. 54.48 MHz ^{17}O N.m.r. spectra of [^{17}O]Ac-L-Pro-D-Ala–NHMe measured on a Bruker AM-400 instrument (10 mm sample tubes, quadrature detection) at 30 °C in CH_2Cl_2 solution. Data acquisition time 7.2 ms, number of scans 450 000. Prior to transformation the free induction decay was multiplied with a Gaussian-exponential function[6] (line broadening = −200 Hz; Gaussian broadening 0.18). The asterisk marks the position of the composite resonance in aqueous solution.

Table 1. ^{17}O N.m.r. chemical shifts of [^{17}O]Ac-L-Pro-D-Ala–NHMe and [^{17}O]Ac-L-Pro–OMe in aqueous and organic solutions measured at 30 °C.

Compound	Solvent	Concn. /M	δ_{cis}[a] /p.p.m.	δ_{trans}[a] /p.p.m.	Δ[b]
[^{17}O]Ac-L-Pro-D-Ala–NHMe	H_2O	10^{-2}	297.7[c]		—[c]
	MeCN	5×10^{-3}	350.6	332.8	17.8
	CH_2Cl_2	5×10^{-3}	351.4	330.1	21.3
[^{17}O]Ac-L-Pro–OMe	H_2O	10^{-2}	296.9[c]		—[c]
	MeCN	5×10^{-3}	348.9	345.1	3.8
	CH_2Cl_2	5×10^{-3}	345.0	342.0	3.0

[a] Measured relative to 1,4-dioxane as an external reference, +0.2 p.p.m. relative to H_2O.[6] The errors were estimated to be ± 0.5 p.p.m. [b] Δ values are the chemical shift differences of the two isomers. [c] The *cis* and *trans* isomers appear as a composite resonance.

The ^{17}O resonance of the *trans* isomer of [^{17}O]Ac-L-Pro-D-Ala–NHMe is shifted to low frequency relative to the *cis* by 21.3 p.p.m. Since model dipeptides which contain the heterochiral L-Pro-D-Ala sequence are known to favour strongly a βII-turn structure,[8] this large chemical shift can be correlated with the existence of a folded β-turn structure, stabilized by an intra-molecular $i + 3 \rightarrow i$ interaction involving the acetyl oxygen. In fact, open and folded *trans* conformers are in rapid exchange, and the observed chemical shift $\delta_{obs.}$ is linearly related to the two contributions δ_{turn} for the IIβ-turn and δ_{open} for the open conformers by equation (1), where f is the fraction of βII-folded *trans* isomers. In a good approximation, the difference in the ^{17}O chemical shifts of the *cis* and *trans* isomers of [^{17}O]Ac-L-Pro–OMe, Δ = 3.0 p.p.m. (Table 1), can be considered equal to that for [^{17}O]Ac-L-Pro-D-Ala–NHMe if no β-turn exists.‡ The estimation of $\delta_{open} - \delta_{turn}$ is easy, providing that the fraction f is known. Boussard and Marraud[9] have shown that for dilute solutions of Bu^{t}CO-L-Pro-D-Ala–NHMe in CH_2Cl_2, *ca.* 90% of the molecules exist in the intra-molecular β-turn hydrogen bonded form. If we assume a similar large β-turn probability for Ac-L-Pro-D-Ala–NHMe,[10] the difference in the chemical shift between the open *trans* isomer and the folded *trans* isomer, $\delta_{open} - \delta_{turn}$, at low concentration in CH_2Cl_2, is 20.3 p.p.m.

$$\delta_{obs} = f\,\delta_{turn} + (1 - f)\,\delta_{open} \quad (1)$$

It is therefore clear that the existence of β-turn structures induces significant modification of the ^{17}O screening constants, although *X*-ray structural data indicate medium to weak hydrogen bonding interactions (N · · · O distances are 2.97 to 3.10 Å).[2,11]

Assuming the same $\delta_{open} - \delta_{turn}$ value in MeCN as in CH_2Cl_2, a similar analysis to that above results in a β-turn amount, f, of 69%. The destabilizing effect of MeCN on β-turns is confirmed by the concomitant increase of the amount of *cis* conformers (*ca.* 15%).

In aqueous solution the *cis* and *trans* amide resonances for both [^{17}O]Ac-L-Pro-D-Ala–NHMe and [^{17}O]Ac-L-Pro–OMe compounds could not be resolved despite an appreciable population of the *cis* isomer as derived from ^{13}C n.m.r. spectral data. From CH_2Cl_2 to water, the *cis* contribution is shifted to low frequencies by 48.1 p.p.m. for Ac–Pro–OMe and 53.7 p.p.m. for Ac-L-Pro-D-Ala–NHMe. These values are similar to those found for simpler amides (52—56 p.p.m.) in the same solvents,[4,5] and are associated with the solvation of the carbonyl group by two water molecules.[4,12] This implies that the *cis* and *trans* acetylamide oxygens of [^{17}O]Ac-L-Pro-D-Ala–NHMe are fully exposed to the solvent and are equally solvated by two molecules of water. We therefore conclude that the β-turn structure does not exist in aqueous solution.

From the above it is clear that ^{17}O n.m.r. spectroscopy is a new tool for studying β-turn structures, particularly in the case of small or medium size peptides where the problems of selective enrichment and large ^{17}O-linewidths are relatively minor.

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‡ The *cis*-acetylamide resonance of [^{17}O]Ac-L-Pro–OMe appears at a lower frequency relative to that of [^{17}O]Ac-L-Pro-D-Ala–NHMe. This may be due to the difference between the inductive effects of the amide and ester groups.

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10 This assumption is probably valid since *X*-ray, n.m.r., and i.r. structural data on Bu^{t}CO-L-Pro–Me-D-Ala–NHMe and Pr^{i}CO-L-Pro-D-Ala–NHPr^i have shown that both compounds accommodate very similar conformations in both solid state and solution (ref. 8; A. Aubry, J. Protas, G. Boussard, and M. Marraud, *Acta Crystallogr., Sect. B*, 1977, **33**, 2399; A. Aubry, B. Vitoux, G. Boussard, and M. Marraud, *Int. J. Pept. Protein Res.*, 1981, **18**, 195).
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