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A slow rate exchange process allows the detection of both carbonyl (C=O) and hydroxyl (–OH) ^{17}O nuclear magnetic resonances of the carboxylic group

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Abstract

In this study we report, for the first time, on the detection of both the carbonyl (–C=O) and the hydroxyl (–OH) ^{17}O resonances of the carboxylic group by ^{17}O NMR spectroscopy. Two well separated peaks, at 340.3 and 175 ppm, were detected for the –C=O and –OH groups, respectively, of Boc-[^{17}O]Tyr(2,6-diClBzl)-OH in DMSO-d_6. This finding is attributed to the participation of the carboxylic group in a rather strong hydrogen-bonded interaction. These resonances disappeared in the presence of a small quantity of TFA (trifluoroacetic acid), which, however, did not cleave the Boc-group and a broad resonance was detected for both oxygens. In fact, this result indicates that the proposed hydrogen-bonded interaction is not stabilized in the presence of TFA, which is a very strong hydrogen bond breaker. Only one broad signal was observed for both carboxylic oxygens at 251.8 ppm for the HCl[^{17}O]Tyr(2,6-diClBzl)-OH in DMSO-d_6 solution, suggesting that the carbonyl oxygen of the Boc-group is probably the proton acceptor group of the carboxylic hydrogen, stabilizing a γ-turn like structure. A single relatively sharp resonance appearing in chloroform, (247.5 ppm) upfield shifted by ~14.5 ppm, compared to the resonance found in DMSO-d_6 for Boc-[^{17}O]Tyr(2,6-ClBzl)-OH in the presence of TFA, is attributed to a γ-turn fraction being in a fast exchange with the open form.

In carboxylic acids only a single resonance is detected in the ^{17}O NMR spectrum for both oxygens (–C=O, –OH), and this is attributed to the tautomerism of the carboxylic group and/or to the proton exchange of the dimeric forms.[1–4] These phenomena are thought to be very fast exchange processes in the NMR time scale. The average ^{17}O resonance is usually observed at 250–260 ppm, which approximately corresponds to the average value of the –C=O and –O–R of carboxylic esters[3,5] (found at 358 and 170 ppm, respectively). On the contrary, the –C=O

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stretching (1725–1700 cm^{-1}), the O–H stretching (3300–2500 cm^{-1}) and the combination of the C–O stretching and the O–H deformation vibrations, in the IR spectrum, are rather easily detected, indicating that the exchange rate is very slow on the IR time scale.

In this study we report now, for the first time, on the detection of both the carbonyl (–C=O) and the hydroxyl (–OH) ^{17}O resonances of the carboxylic group of Boc-[^{17}O]Tyr(2,6-di-Cl-Bzl)-OH, by ^{17}O NMR spectroscopy, in DMSO-d_6 solutions.

The carboxyl enrichment with ^{17}O of the Boc-[^{17}O]Tyr(2,6-diClBzl)-OH was performed by hydrolyzing the Boc-Tyr-(2,6-di-Cl-Bzl)-OMe in CH_3ONa/CH_3OH in the presence of an appropriate quantity of $H_2{}^{17}O$ (46.3% atom ^{17}O). For the NMR (^{17}O and ^{1}H) experiments, ~4 mg were dissolved in 0.5 ml DMSO-d_6 and then the spectra were recorded on a Bruker AMX 400 spectrometer operating at 400 MHz for ^{1}H and at 54.2 MHz for ^{17}O. ^{17}O chemical shifts are referenced to the resonance of the solvent (DMSO, 15.3 ppm).

Fig. 1 shows the ^{17}O NMR spectra of the Boc-[^{17}O]Tyr (2,6-diClBzl)-OH in DMSO-d_6 at various temperatures. Two resonances are observed for the carboxylic oxygens (Fig. 1A): (i) the carbonyl resonance at 340.3 ppm, which is similar to that found for the C=O of the methyl ester (~337 ppm) of *Z*-Pro-Leu-[^{17}O]Gly-OMe in DMSO solution[5] and (ii) the hydroxyl resonance at 175 ppm shifted slightly downfield (~4 ppm), when compared to the methoxy oxygen of methyl acetate.[2] This finding can be explained if we assume, that the carboxylic group participates in a rather strong hydrogen bonded interaction. The spectra at higher temperatures provide evidence for this hypothesis, since, as the temperature is raised, the two lines broaden and move towards each other (Fig. 1B, C). A sharp signal detected for the carboxylic hydrogen at 12.34 ppm, in the ^{1}H NMR spectrum, further supports this assumption.

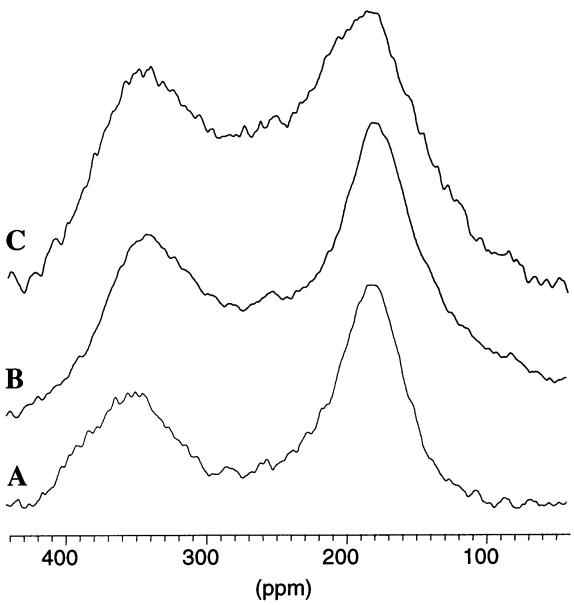

Figure 1. 54.2 MHz ^{17}O NMR spectra of Boc-[^{17}O]Tyr (2,6-di-Cl-Bzl)-OH in DMSO-d_6 solution at 313 K (A), 333 K (B) and 343 K (C). The spectra were processed using exponential line broadening (LB = 200 Hz)

Nevertheless, with the aim of elucidating the origin of this surprising finding the following experiments were performed:

(a) Trifluoroacetic acid (TFA) was added to the same NMR sample, previously used, in a ratio of TFA/amino acid (35/1) and then the ^{17}O NMR spectrum was recorded. Under these experimental conditions, and during the period of the NMR experiment, the α-amino protective group, N^{α}-*t*-Boc-, was not cleaved as evidenced by the ^{1}H NMR spectrum. The ^{17}O NMR spectrum of Boc-Tyr(2,6-di-Cl-Bzl)-OH in the presence of TFA is given in Fig. 2A. Broad and narrow overlapping signals appeared at 262 ppm, while the resonances at 340.3 and 175 ppm (Fig. 2A) disappeared. The broad resonance, in Fig. 2A, results from the contribution of the carboxylic oxygen of Boc-Tyr(2,6-di-Cl-Bzl)-OH, while the sharp one comes from the carboxylic group of TFA, confirmed by recording the ^{17}O NMR spectrum of a TFA solution in DMSO-d_6 (Fig. 2B). In fact, the proposed hydrogen bonded interaction is not stabilized under these experimental conditions showing that TFA is a very strong hydrogen bond breaker.

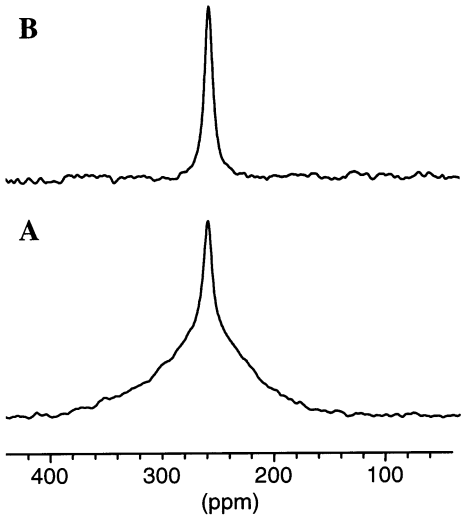

Figure 2. 54.2 MHz ^{17}O NMR spectra at 313 K of (A) Boc-[^{17}O]Tyr(2,6-di-Cl-Bzl)-OH in DMSO-d_6 solution in the presence of TFA (TFA/amino acid ratio 35/1) and (B) TFA solution in DMSO-d_6

(b) The ^{17}O NMR spectrum of HCl[^{17}O]Tyr(2,6-di-Cl-Bzl)-OH in DMSO-d_6 solution was recorded in order to further confirm our concept that the distinction of the carbonyl and the hydroxyl ^{17}O resonances is due to a strong intramolecular hydrogen bonded interaction. Only one broad signal was observed for both carboxylic oxygens at 251.8 ppm (Fig. 3A), confirming our initial attribution; this result provides evidence that the carbonyl oxygen of the *tert*-butoxy-carbonyl group is probably the proton acceptor group of the carboxylic hydrogen, stabilizing a γ-turn like structure.

(c) The ^{17}O NMR spectrum of Boc-[^{17}O]Tyr(2,6-di-Cl-Bzl)-OH in chloroform reveals the existence of a single relatively sharp resonance at 247.5 ppm (Fig. 3B). This carboxyl ^{17}O chemical shift was upfield shifted, $\sim$14.5 ppm in chloroform, compared to the chemical shift resonance observed in the DMSO-d_6 solution of Boc-[^{17}O]Tyr(2,6-di-Cl-Bzl)-OH in the presence of TFA. If we assume that the γ-turn like structure is destroyed in DMSO-d_6 solution in the presence of TFA, then the reported upfield shifting of $\sim$14.5 ppm can be attributed to a γ-turn fraction found in a fast exchange with the open form.[4] The experiments in DMSO (Fig. 1A) and chloroform (Fig. 3B) indicate that the viscosity of the solvent strongly affects the intramolecular conformational exchange rate. A similar slow conformational exchanging phenomenon was also observed for arginine containing peptides in DMSO solution.[6]

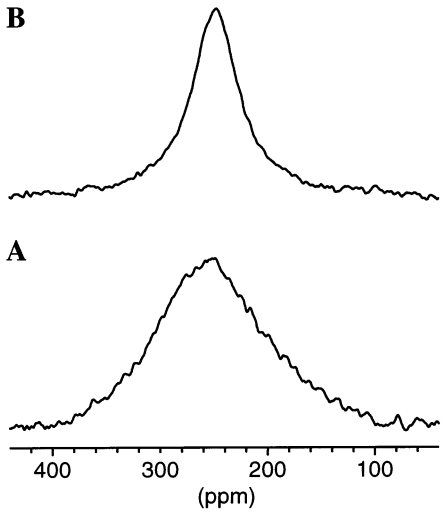

Figure 3. 54.2 MHz ^{17}O NMR spectra at 313 K of (A) HCl[^{17}O]Tyr(2,6-di-Cl-Bzl)-OH in DMSO-d_6 solution and (B) Boc-[^{17}O]Tyr(2,6-di-Cl-Bzl)-OH in $CDCl_3$

In this study, we have been able to detect, for the first time, both the –C=O and –OH ^{17}O resonances of the Boc-[^{17}O]Tyr(2,6-di-Cl-Bzl)-OH carboxylic group, due to the occurrence of an intramolecular interaction. This finding points out the significance of ^{17}O NMR spectroscopy in elucidating the participation of carboxylic groups in the structure stabilization of bioactive compounds. On the other hand, the importance of DMSO as a solvent for conformational studies of biomolecules is shown, due to its propensity to reduce the conformational exchange rate and stabilize the favored conformers.

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