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Short Communication

2D Relayed $^{15}N,^{1}H$ Correlated NMR Spectroscopy of a Pentadecapeptide at Natural Abundance of ^{15}N

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Summary. Proton-detected H-relayed N,H correlation NMR spectroscopy at natural abundance of ^{15}N has been used to demonstrate the enormous value of heteronuclear NMR spectroscopy for the proton assignment of medium-sized oligopeptides.

Keywords. Peptide; NMR; Nitrogen-15; Reverse Techniques.

Zweidimensionale *relayed* $^{15}N,^{1}H$-korrelierte NMR-Spektroskopie an einem Pentadecapeptid bei natürlicher Häufigkeit von ^{15}N

Zusammenfassung. Anhand ^{1}H-detektierter *relayed*-N,H-korrelierter NMR-Spektroskopie bei natürlicher Häufigkeit von ^{15}N wird die große Bedeutung der heteronuklearen Kernresonanzspektroskopie für die Protonenzuordnung mittlerer Oligopeptide demonstriert.

Recently, we have shown that C,H correlation techniques at natural abundance of ^{13}C are a powerful tool for the sequential NMR spectroscopic assignment of oligopeptides [1], circumventing the problem of the low sensitivity of the ^{13}C nucleus by reverse detection [2]. The utilization of the second important heteronucleus in peptides, ^{15}N, is hampered by its still lower sensitivity. The value of the reverse methods for assigning ^{15}N chemical shifts in peptides has been demonstrated early [3, 4]. However, the routine use of ^{15}N NMR spectroscopy for proton assignment purposes in multidimensional NMR spectroscopy of peptides is still largely restricted to uniformly ^{15}N labelled compounds [5, 6]. In this paper we demonstrate that in the case of medium-sized oligopeptides like **1** ^{15}N NMR spectroscopy is superior to the corresponding C,H techniques for the assignment of the α protons.

1: H_2N-Ile^{1}-Val^{2}-Thr^{3}-Arg^{4}-Pro^{5}-Ile^{6}-Ile^{7}-Thr^{8}-Thr^{9}-Tyr^{10}-Gly^{11}-Pro^{12}-Ser^{13}-Asp^{14}-Met^{15}-NH_2

In Ref. [1] we have presented an idealized scheme for the sequential NMR

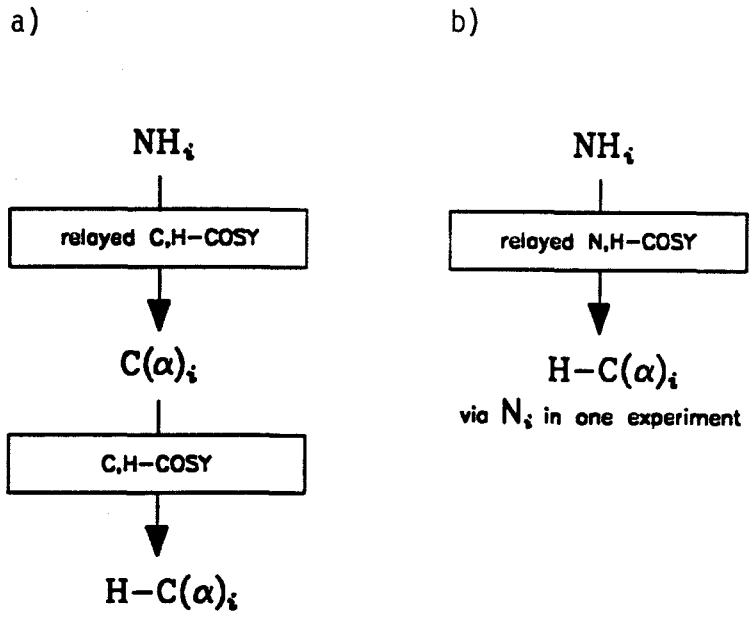

Fig. 1. Assignment of the α-protons of a peptide starting from the amide protons using exclusively heteronuclear connectivities: (a) via carbons (cf. Ref. [1]), (b) via nitrogens

spectroscopic assignment procedure of a peptide backbone using exclusively C,H correlation techniques. The assignment of the α protons was achieved by a two-step sequence, connecting first NH_i with $C(\alpha)_i$ and in a second experiment $C(\alpha)_i$ with $H-C(\alpha)_i$ (Fig. 1a). The detection of the $H-C(\alpha)_i$ from direct C,H connectivities which of course are also present in C,H relayed 2D NMR spectra is difficult because of

Table 1. ^{15}N chemical shifts of **1** in $H_2O:D_2O = 4:1$ (12 m*M*) at 305 K relative to external nitromethane

Amino acid residue	Amide nitrogen shift
Val^2	−257.8
Thr^3	−262.7
Arg^4	−258.1
Ile^6	−261.2
Ile^7	−257.1
Thr^8	−263.5
Thr^9	−266.5
Tyr^{10}	−260.6
Gly^{11}	−272.1
Ser^{13}	−267.4
Asp^{14}	−261.9
Met^{15}	−261.9

Arg^4–*N*H–C(δ): −298.4 ppm
Arg^4–C(NH)–*N*H_2: −275.5 ppm
Met^{15}–CO–*N*H_2: −275.5 ppm

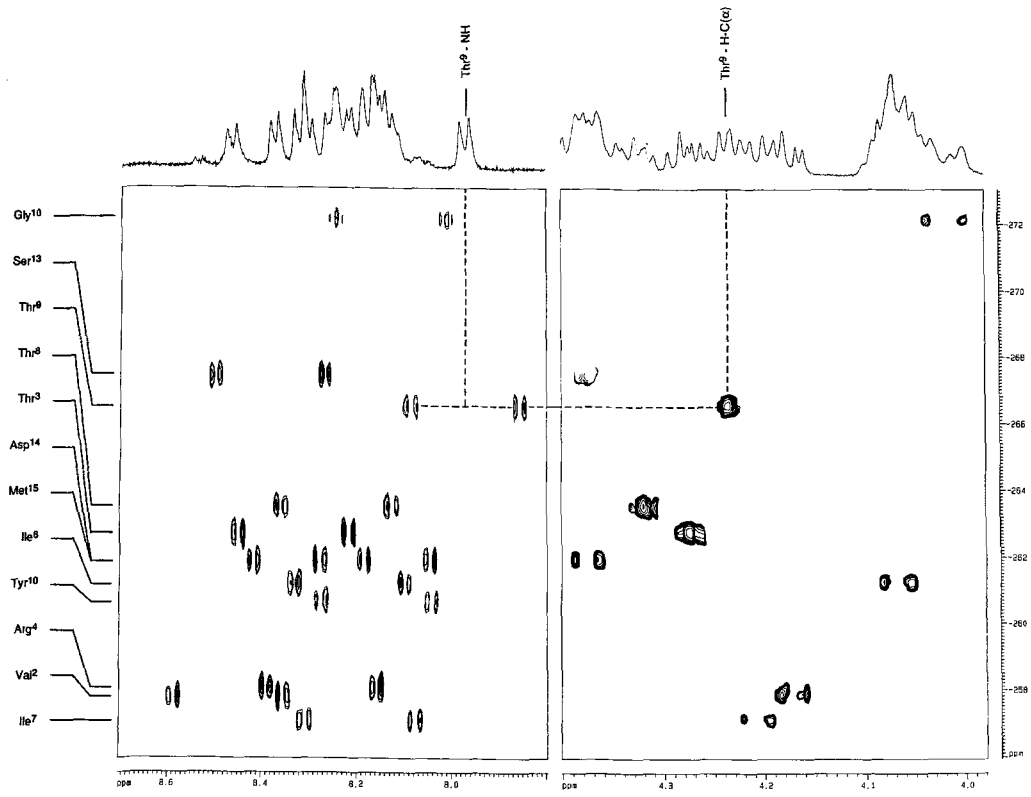

Fig. 2. Amide and α-proton regions of a relayed N,H correlated 2D NMR spectrum. For details, see Exp. Part; the connectivity Thr^9–NH → Thr^9–N → Thr^9–H–C(α) has been indicated as an example

the splitting of the cross peaks in question due to $^{1}J_{C,H}$; furthermore, this splitting frequently causes one of the two cross peaks belonging to each C(α) to be hidden by the H_2O resonance, even if the corresponding H–C(α) itself is located in an undisturbed spectral region.

Using ^{15}N as the heteronucleus, the same goal can be accomplished with a single relayed $^{15}N,^{1}H$ correlated experiment (Fig. 1b) which is much easier to interpret than the corresponding C,H spectrum according to the arguments given above. As a bonus, the experiment yields the chemical shifts of all protonated nitrogen atoms in the peptide. The ^{15}N shifts of the amide nitrogen atoms of **1** are in accordance with values given in the literature for the respective amino acids in small peptides [7] (Table 1).

Figure 2 shows the NH and H–C(α) regions of a relayed N,H correlated 2D NMR spectrum of **1**. As the relative intensity gain associated with reverse detection increases with decreasing sensitivity of the insensitive nucleus [8], measurement times are of about the same magnitude as for the corresponding C,H experiments (cf. [1]).

A reverse N,H COSY experiment devoted solely to the task of assigning the amide nitrogen atoms and using the sample concentration given in the experimental part can be performed in about 12 hours and with high digital resolution in both frequency domains if the spectral widths in ω_1 and ω_2 are

adapted to the relatively narrow spectral regions of amide protons and amide nitrogens, respectively. This requires a spectrometer with a fast switching decoupler which allows the proton offset being changed between the H_2O resonance frequency during the relaxation delay (presaturation) and the center of the amide proton region (acquisition) for each repetition of the pulse sequence. ^{15}N decoupling during the acquisition period yields a further factor of 2 in sensitivity.

The $N_i/H–C(\alpha)_i$ region of Fig. 2 displays cross peaks for all protons with the exception of Tyr^{10}, Asp^{14} (hidden by the solvent resonance), Ile^1 (N-terminal amino acid), and Arg^4 (reason unknown). The chemical shifts of the nitrogen atoms $Met^{15}–CO–\mathit{N}H_2$, $Arg^4–C(NH)–\mathit{N}H_2$, and $Arg^4–\mathit{N}H–C(\delta)$ can be identified by their cross peaks at the positions of the respective protons (cf. Ref. [1]) in the direct as well as in the relayed $^{15}N,^1H$ correlated spectrum (Table 1). Note especially the identical chemical shifts of $Met^{15}–CO–\mathit{N}H_2$ and $Arg^4–C(NH)–\mathit{N}H_2$ which obviously results from the close structural similarity of the terminal amide group of Met^{15} and its imino analogon at Arg^4. The assignment of $Arg^4–\mathit{N}H–C(\delta)$ is furthermore corroborated by a relayed cross peak connecting $Arg^4–\mathit{N}H–C(\delta)$ and $Arg^4–\mathit{H}–C(\delta)$.

At the present, investigations concerning the routine use of $^{15}N,^1H$ correlated 2D NMR spectroscopy at natural abundance with oligopeptides of approximately 20–25 amino acid residues are in progress. The results will be published elsewhere.

Experimental Part

For the synthesis of compound **1**, cf. Ref. [1]. NMR measurements were performed in neutral aqueous solution ($H_2O:D_2O = 4:1$) at 305 K on a Bruker AM 400 WB NMR spectrometer operating at 9.4 T (^{15}N resonance frequency: 40.56 MHz) in 5 mm tubes at a sample concentration of 12 m*M* using an inverse probe head with a tunable X-nucleus coil. The water resonance was saturated by an appropriate RF field prior to each scan. The spectra were recorded in the phase sensitive mode using the TPPI method [9]. For the acquisition of a ^{15}N decoupled $^{15}N,^1H$ correlated spectrum with selective excitation of the amide proton region, the software supplied by the spectrometer manufacturer had to be modified considerably (cf. text). Data were processed on a satellite station (Bruker Aspect X32) using the UXNMR software [10]. Signal to noise improvement was achieved by submitting the processed data to the AURELIA algorithm [11].

Reverse N,H-COSY with BIRD sequence and GARP1 decoupling [12], modified for solvent presaturation during both relaxation and BIRD delay and for ^1H offset frequency jumping between the solvent resonance (presaturation) and the center of the amide proton region (acquisition): sweep width, 2.5 kHz in ω_1, 1 kHz in ω_2; size, 1 k data points in ω_2, 128 experiments in ω_1 (240 scans); relaxation delay, 0.8 s; BIRD delay, 0.25 s; pulse width ($\pi/2$), 10.9 μs (^1H), 14.7 μs (^{15}N hard), 72 μs (^{15}N soft); zero filling, 1 k × 1 k data points (real); filter function, sine bell squared shifted by $\pi/2$ rad in both dimensions; measurement time, ca. 13 h.

Relayed reverse N,H-COSY with BIRD sequence [13], modified for solvent presaturation during both relaxation and BIRD delay: sweep width, 1.5 kHz in ω_1, 3.5 kHz in ω_2; size, 2 k data points in ω_2, 128 experiments in ω_1 (1024 scans); relaxation delay, 1 s; BIRD delay, 0.25 s; pulse width ($\pi/2$), 10.9 μs (^1H), 14.7 μs (^{15}N); zero filling, 2 k × 1 k data points (real); filter function, sine bell squared shifted by $\pi/2$ rad in both dimensions; measurement time, ca. 60 h.

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References

[1] Kalchhauser H., Kriech M., Ahorn H., Sommergruber W. (1992) Monatsh. Chem. **123**: 757
[2] Kessler H., Gehrke M., Griesinger C. (1988) Angew. Chem. **100**: 507
[3] Bax A., Griffey R. H., Hawkins B. L. (1983) J. Am. Chem. Soc. **105**: 7188
[4] Live D. H., Davis D. G., Agosta W. C., Cowburn D. (1984) J. Am. Chem. Soc. **106**: 6104
[5] Driscoll P. C., Clore G. M., Marion D., Wingfield P. T., Gronenborn A. M. (1990) Biochemistry **29**: 3542
[6] Clore G. M., Bax A. Driscoll P. C., Wingfield P. T., Gronenborn A. M. (1990) Biochemistry **29**: 8172
[7] Witanowski M., Stefaniak L., Webb G. A. (1986) Ann. Rep. NMR Spectr. **18**: 385 ff
[8] Bermel W. (1987) Bruker Report **1/1987**: 30
[9] Marion D., Wüthrich K. (1983) Biochem. Biophys. Res. Commun. **113**: 967; Rance M., Sørensen O. W., Bodenhausen G., Wagner G., Ernst R. R., Wüthrich K. (1983) ibid. **117**: 479
[10] Fa. Bruker Analytische Meßtechnik GmbH, Karlsruhe, FRG; software release 10/90
[11] Neidig K. P., Kalbitzer H. R. (1990) J. Magn. Reson. **88**: 155
[12] Bax A., Subramanian S. (1986) J. Magn. Reson. **67**: 565
[13] Lerner L., Bax A. (1986) J. Magn. Reson. **69**: 375

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