Longitudinal three-spin order may be regarded as a *fingerprint* of cross-correlation effects, so that the signals observed in triple-quantum-filtered NOESY spectra provide a direct measure of terms that are usually neglected in routine Overhauser studies. It would be attractive to compare the TQF-NOESY with a normal NOESY spectrum. Unfortunately, the correlation time of cyclosporine in our sample is nearly critical ($\omega_0\tau_c \approx 1.1$), and the cross-relaxation rates σ_{kl} (and hence the NOESY cross-peaks) are very small indeed. It would be necessary to change the experimental conditions (viscosity, temperature) or to obtain cross-relaxation rates in the rotating frame with the CAMELSPIN or ROESY technique,[27,28] but these changes would make a direct comparison more hazardous. Note that the rates δ_{klkm} do *not* vanish for critical correlation times (see eq 1), but that they are negligible for long correlation times, where they cannot compete with the cross-relaxation rates σ_{kl}. Thus in the slow-motion limit relevant to macromolecules, the longitudinal three-spin order terms can be neglected, and N spin systems can safely be described by N-dimensional Solomon equations. This preliminary report suggests that triple-quantum-filtered NOESY experiments may yield valuable information on angles subtended by internuclear vectors in small- and medium-sized molecules. Such measurements hold the promise of broadening the scope of conformational studies.

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Long-Range Heteronuclear Correlation: A Powerful Tool for the NMR Analysis of Medium-Size Proteins

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The introduction of so-called reverse correlation techniques permits the recording of heteronuclear one-bond $^1H-^{13}C$ and $^1H-^{15}N$ chemical shift correlation spectra of macromolecules at natural abundance.[1-3] Recording of heteronuclear chemical shift correlation spectra via the much smaller two- and three-bond couplings necessarily is much lower in sensitivity because the heteronuclear couplings are comparable to the natural line widths and the homonuclear couplings of the protons. Here, we demonstrate that heteronuclear long range correlations can be observed in a medium-sized protein, provided that isotopic labeling is possible, as it generally is for bacterially overexpressed proteins. The heteronuclear multiple bond correlation (HMBC)[4] spectra provide assignment information as well as qualitative structural information regarding the ϕ, ψ, and χ angles,[5-8] which complement information obtainable from the ^1H spectra.

The HMBC pulse scheme is

$$\begin{array}{lccccc} ^1H: & 90_x\text{-}\Delta\text{-} & & -t_1/2\text{-}180_x\text{-}t_1/2\text{-} & & -\text{Acq.}(t_2) \\ X: & & 90_\phi & & 90_x & \end{array}$$

with the phase cycling $\phi = x, -x$ and Acq. $= x, -x$. The phase ϕ is incremented by 90° for successive t_1 increments (TPPI).[9] To compromise for the effects of the short ^1H transverse relaxation time, the delay, Δ, is set to a value significantly shorter than $1/(2J_{XH})$, typically 40 ms. To optimize sensitivity and resolution, the spectrum is recorded in a mixed-mode absorption in the X chemical shift dimension (F_1) and absolute value mode in the ^1H dimension (F_2).[10] The method has been applied to 1.5 mM samples of a staphylococcal nuclease (S. Nase)/pdTp/calcium complex (18 kD) in D_2O, $p^2H = 7.4$, 100 mM NaCl. Sample I has all Leu, Ile, and His residues labeled with $^{15}N\alpha$; sample II has all Thr residues labeled with ^{13}C in the carbonyl position.

The $^1H-^{15}N$ and $^1H-^{13}C$ HMBC spectra, obtained for the two samples, are shown in Figure 1. The assignments indicated are based on a large number of isotopic labeling and double labeling experiments[11] and will be published elsewhere. As can be seen in Figure 1, both two-bond and three-bond correlations are observed, but either is present for all labeled residues. The intensity of the observed correlation depends on the size of the long range coupling and on the width of the ^1H multiplet. Because of short transverse relaxation times and because of homonuclear J modulation during the relatively long delay, Δ, and during the t_1 and t_2 periods, the intensity of the typically unresolved ^1H multiplet rapidly decreases at a rate proportional to the reciprocal of its width (which approximately equals the sum of all homonuclear ^1H couplings). Nevertheless, three-bond couplings involving the $C^\alpha H$ proton that are expected to be larger than about 5 Hz (based on the X-ray crystal structure and suitable Karplus equations[5]) invariably give rise to observable correlations; correlations are not observed when the couplings are smaller than about 2.5 Hz.

Thus, it is seen in Figure 1A that for all residues in α-helical regions of the protein[12] a correlation is observed between $C^\alpha H(i)$ and $^{15}N(i+1)$ ($\psi \approx -50°$; $^3J_{NH} \approx 6$ Hz). No such correlation is observed for any of the β-strand residues ($\psi \approx 130°$; $^3J_{NH} <$ 1.5 Hz). The short transverse relaxation times of nonmobile C^β methylene protons (≈15 ms for S. Nase) is the likely reason why very few intraresidue $C^\beta H-^{15}N$ correlations are observed. Two intense correlations observed for the $C^\beta H$ protons of His-8 (complemented by two intense $C^\alpha H-C^\beta H$ correlations in the COSY and HOHAHA spectra, data not shown) suggest that the side chain of this residue has significant conformational flexibility. Similarly, the mobile residues Val-5′, Ala-1, and Ala-145 show intense correlations despite low levels of ^{15}N cross-labeling (Val 5%, Ala 1.5%).

$^1H-^{13}C_1$ correlations (Figure 1B) contain information about the ϕ and χ angles, and, as previously demonstrated for peptides,[6,8,13] they can also provide sequential connectivity information

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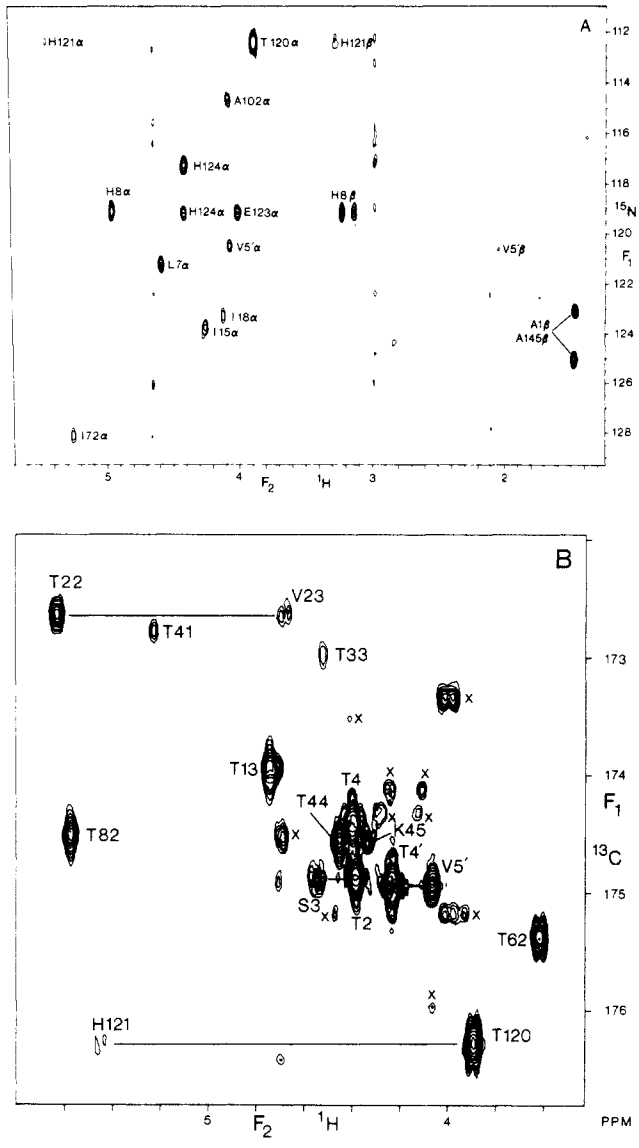

Figure 1. HMBC spectra of a 1.5 mM staphyloccocal nuclease/pdTp/Ca^{2+} complex in D_2O, recorded at 600 MHz on a Bruker AM-600 spectrometer. (A) ^1H–^{15}N correlation of a sample labeled with ^{15}N for all Leu, Ile, and His residues. Low levels of cross labeling are also found for Val (5%) and Ala (1.5%). (B) ^1H–$^{13}C_1$ correlation of a sample labeled with ^{13}C in the carbonyl position of all Thr residues. Low levels of ^{13}C cross labeling to other amino acids are also observed and give rise to correlations marked "x". Residues labeled with primes correspond to the heptapeptide attached to the N-terminus. Both spectra are presented in the mixed mode, absorption in F_1, and absolute value in F_2 (^1H). Acquisition times for both spectra were 110 (t_2) and 70 ms (t_1). Total measuring times were (A) 14 h and (B) 6.5 h. The delay Δ was set to 40 ms for both experiments; unshifted sine bell filtering was used in t_2 and a 60° shifted sine bell in t_1. A difference of ≈0.7 ppm in ^{15}N chemical shifts relative to values reported earlier[17] is caused by the ^2H isotope effect.

(Thr-4'/Val-5', Thr-2/Ser-3, Thr-22/Val-23, Thr-44/Lys-45, Thr-120/His-121). For α-helical domains, the $C^\alpha H(i)$–$^{13}C_1$ (i − 1) J coupling (<2 Hz) is too small to yield an observable correlation. For β-strands ($J \approx 3.5$ Hz) weak correlations are expected. For ^{13}C it also is possible to record the correlation spectrum with the ^1H detected heteronuclear correlation scheme, originally proposed by Maudsley and Ernst.[14,15] Because this type of spectrum can be obtained in the pure 2D absorption mode, this scheme offers higher resolution albeit at a cost in sensitivity (Supplementary Material).

As demonstrated here, combined use of ^1H-detected heteronuclear long range correlation techniques and isotopic labeling of selected amino acids can yield significant additional information for the study of medium-size proteins. For smaller proteins, complete ^{15}N labeling can be helpful for resolving ambiguities in the sequential assignment of α-helical domains.[16] Intraresidue couplings between C^β protons and C_1 or Nα, combined with qualitative knowledge of the couplings between C^α and C^β protons, can provide information about stereospecific assignment and about the χ angle.[7,8] Two-bond ^1H–^{15}N couplings (combined with one-bond N–H correlation) yield intraresidue $C^\alpha H$–NH connectivities. In the case of small J_{HH} couplings, the intraresidue connectivities cannot always be determined from COSY or HOHAHA spectra because the relatively large line widths of the NH resonances in medium-size proteins makes magnetization transfer inefficient.

Note Added in Proof. In a recent paper, Westler et al. (*J. Am. Chem. Soc.* **1988**, *110*, 4093) reported the observation of sequential $^{13}C_1$–$C^\alpha H$ HMBC connectivity in the streptomyces subtilisin inhibitor.

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Supplementary Material Available: Figure showing ^1H–$^{13}C_1$ correlation recorded with the Maudsley–Ernst sequence (2 pages). Ordering information is given on any current masthead page.

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Double-Strand Cleavage of Genomic DNA at a Single Site by Triple-Helix Formation

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The sequence-specific cleavage of double helical DNA by restriction endonucleases is essential for many techniques in molecular biology, including gene isolation, DNA sequencing, and recombinant DNA manipulations.[1,2] With the advent of pulsed-field gel electrophoresis, the separation of large segments of DNA is now possible.[3,4] However, the recognition sequences of naturally occurring restriction enzymes are in the range of 4–8 base pairs, and hence their sequence specificites may be inadequate for isolating genes from large chromosomes (10^8 base pairs in size) or mapping genomic DNA.[5]

Pyrimidine oligonucleotides bind duplex DNA sequence specifically at homopurine sites to form a triple helix structure.[6,7]

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