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Direct Detection of Hydrogen Bonds in Biopolymers by NMR Spectroscopy

Gerd Gemmecker*

Dedicated to Professor Horst Kessler on the occasion of his 60th birthday

Today every chemist knows about the existence and importance of hydrogen bonds. The concept of hydrogen bonding plays a major role in the understanding of the physicochemical properties of water, inorganic and organic acids, as well as acid – base catalysis. Hydrogen bonds influence the conformational preferences and reactivity of many classes of organic molecules.

In the field of biochemistry, hydrogen bonds are responsible for the selectivity of base pairing in nucleic acids—the basis for the preservation and propagation of genetic information. Furthermore, hydrogen bonds play a crucial role in the three-dimensional folding of proteins: the most prominent structural features of proteins—helical and β-sheet regions—are defined and stabilized by their unique pattern of hydrogen bonds.

Finally, intermolecular hydrogen bonds contribute to the affinity and selectivity of molecular recognition, from simple host – guest systems all the way up to the multi-component complexes of proteins, nucleic acids, etc., which are at the heart of so many biological functions.

However, in the course of structure determinations by X-ray diffraction or NMR spectroscopy, the existence of hydrogen bonds is usually only inferred in a rather indirect way from an appropriate spatial arrangement of possible hydrogen donors and acceptors that is already known to exist in high-resolution molecular structures. Particularly in the field of NMR spectroscopy, several approaches have been proposed to deduce the existence and localization of hydrogen bonds indirectly from parameters such as hydrogen-exchange rates, isotropic and anisotropic chemical shifts, ^{2}H quadrupolar splittings, and ^{1}H/^{2}H isotope shifts.

At the XVIIIth International Conference on Magnetic Resonance in Biological Systems (ICMRBS) in Tokyo in August 1998 Andrew Dingley and Stephan Grzesiek reported the observation of scalar couplings across the hydrogen bonds in the Watson – Crick base pairs of a ^{15}N-labeled 69 nucleotide RNA(their paper appeared in print the same month).[1] In an experiment set up for the detection of scalar couplings between two ^{15}N spins (a type of ^{15}N-COSY spectrum), several fairly intense cross-peaks had been observed (Figure 1) that could only be assigned to correlations between N3 of a uridine and N1 of an adenosine group, and N1 of a guanosine and N3 of a cytosine group, each across the hydrogen bonds within a U-A or G-C Watson – Crick base pair, respectively (Figure 2).

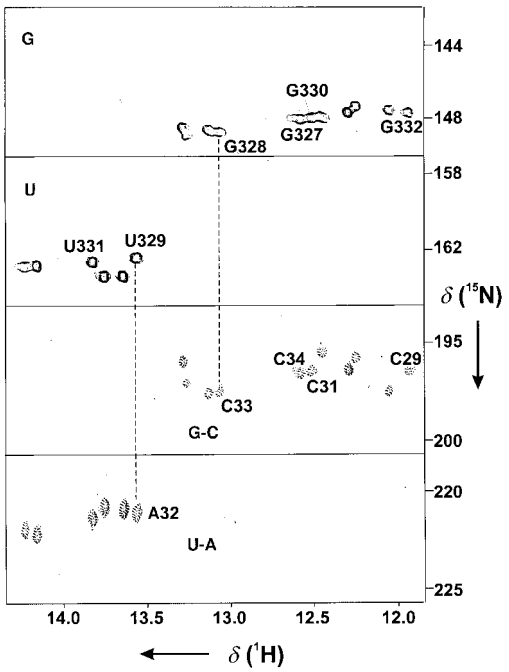

Figure 1. Strips from an HNN-COSY spectrum of a ^{13}C,^{15}N-labeled 69-nucleotide RNA domain.[1] The upper two panels show the COSY "diagonal peaks", that is, the ^{15}N spins (in F1) correlated with their directly bound proton (F2). The cross-peaks in the lower two panels are caused by ^{15}N-^{15}N correlations through a $^{2h}J_{^{15}N,^{15}N}$ coupling across the hydrogen bonds within Watson – Crick base pairs; the correlations G328-N1/C33-N3 and U329-N3/A32-N1 are indicated by dashed lines. (Reproduced with permission from the American Chemical Society 1998.)

[*] Priv.-Doz. Dr. G. Gemmecker
Institut für Organische Chemie und Biochemie
TU München, Lichtenbergstrasse 4
85747 Garching (Germany)
Fax: (+49) 089-289-13210
E-mail: Gerd.Gemmecker@ch.tum.de

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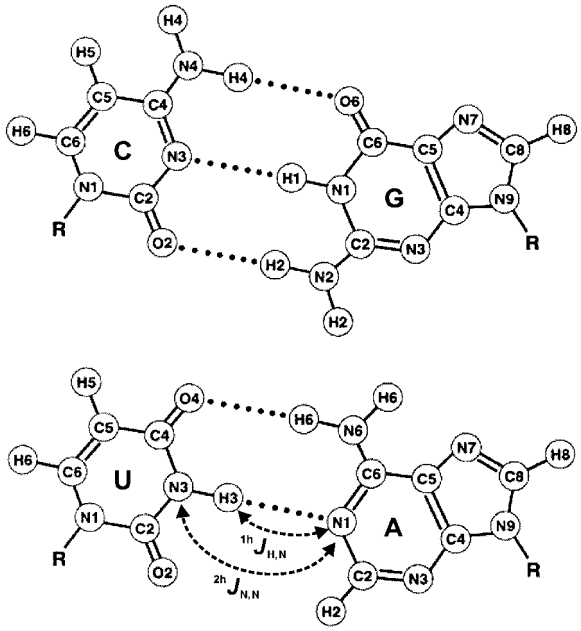

Figure 2. Hydrogen-bonding pattern in the Watson–Crick base pairs uridine–adenosine (U-A) and cytidine–guanosine (C-G).

Grzesiek's revelations caused quite a lively discussion and, of course, several critical questions as to whether the observed correlations could be explained by some other mechanism, for example, dipolar cross-correlation. However, Grzesiek's experimental data were quite thorough and proved that the mysterious correlation across the hydrogen bond behaves exactly like a scalar coupling of 6.5–7.0 Hz.

It is well known that hydrogen bonds are not just purely ionic bonds based only on electrostatic attraction, but can exhibit a considerable covalent character. Hence, the existence of a scalar coupling across the hydrogen bond could, in principle, be expected. What came as a big surprise in Grzesiek's work was the size of the observed coupling: the measured values are comparable to the average vicinal coupling between protons in a H-C-C-H fragment—couplings involving ^{15}N are usually much smaller. The observed coupling could be called a $^{2}J_{^{15}N,^{15}N}$, that is, a two-bond coupling; however, to emphasize the fact that these are not two ordinary bonds, but hydrogen bonds, it was suggested by Kurt Wüthrich (who confirmed the original findings) to use the symbol $^{2h}J_{^{15}N,^{15}N}$.[2]

Considering the size of the $^{2h}J_{N,N}$ coupling, the idea to look for $^{1h}J_{H,N}$ couplings across the hydrogen bonds arose; these should be even easier to detect. However, the Wüthrich group found these values to be in the order of only 2–4 Hz.[2]

Scalar couplings between two spatially close spins that are not transmitted through ordinary covalent bonds have been reported before in the literature (for example, between ^{15}N and ^{19}F atoms)[3, 4] and dubbed with the rather misleading term "through-space *J* couplings", although they are real scalar couplings transmitted by the valence electrons. A few years ago such a scalar coupling had even be detected and measured between the amide proton and ^{113}Cd in an N–H···S–Cd hydrogen bond in a Cd-substituted iron–sulfur protein.[5] Recently, the groups of Limbach and Denisov investigated the ^{19}F–^{1}H···^{15}N hydrogen bond in the complex between hydrogen fluoride and trimethylpyridine at low temperature. They found a similar pattern of (temperature dependent) scalar couplings: $^{1h}J_{H,N} = 39-54$ Hz, $^{1h}J_{F,H} = 15-105$ Hz, and $^{2h}J_{F,N} = 96$ Hz.[6] Again, the *two-bond* coupling was clearly larger than each of the one-bond couplings (on average), which suggested that the molecular orbitals with large s character at ^{19}F and ^{15}N exhibit only a weak s character at the bridging hydrogen atom itself.

Recently, the phenomenon of scalar couplings across N–H···N hydrogen bonds in nucleic acids and its dependence on bond geometry has been analyzed in detail, and the experimental values could be reproduced and "explained" by density functional calculations.[7] These calculations agreed with the experimental findings that the $^{2h}J_{N,N}$ coupling is much larger, and also much less dependent, on the N-N distance than the $^{1h}J_{H,N}$ coupling (Figure 3).

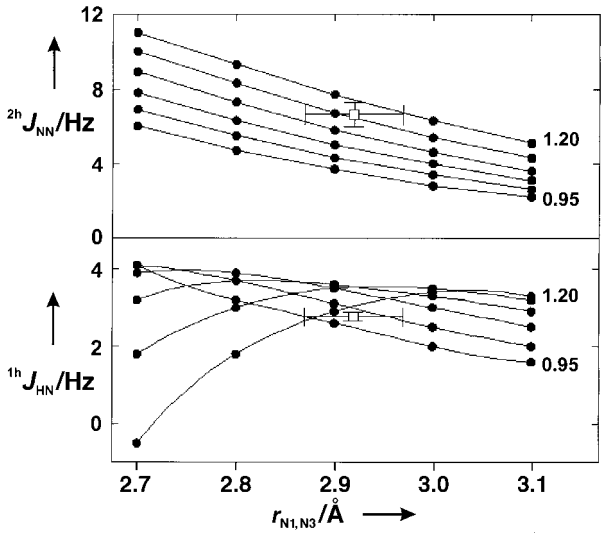

Figure 3. Dependence of $^{2h}J_{^{15}N1,^{15}N3}$ (top) and $^{1h}J_{^{1}H1,^{15}N3}$ (bottom) on the length of the hydrogen bond $r_{N1,N3}$, calculated by density functional theory for a G-C fragment, and $r_{N1,H1}$ distances from 0.95 to 1.20 Å (indicated on the right).[7] The open boxes and error bars represent mean values and deviations for $r_{N1,N3}$ distances as deduced from DNA crystal structures. (Reproduced with permission from the American Chemical Society 1999.)

With the existence and accessibility of the scalar couplings in hydrogen bonds confirmed, several groups immediately went to work to look for similar couplings in proteins. Here, the hydrogen acceptor atom is usually the oxygen atom of a carbonyl group in the peptide backbone, instead of a nitrogen atom in nucleic acids (however, a $^{2h}J_{N,N}$ coupling has recently been detected between two histidine side chains in apomyoglobin[8]). Unfortunately, there is no oxygen isotope that is as suitable for NMR measurements as ^{15}N: the main isotope ^{16}O is magnetically inactive, and the other stable isotopes ^{17}O and ^{18}O are quadrupolar nuclei. The closest suitable spin on the acceptor side of a hydrogen bond in isotopically ^{15}N,^{13}C-enriched proteins (as generally used for NMR studies) is the carbonyl carbon atom ^{13}C′. The hydrogen bond scalar couplings to be expected in an N–H···O=C arrangement are thus the two-bond coupling $^{2h}J_{H,C'}$ and the three-bond coupling $^{3h}J_{N,C'}$ (Figure 4).

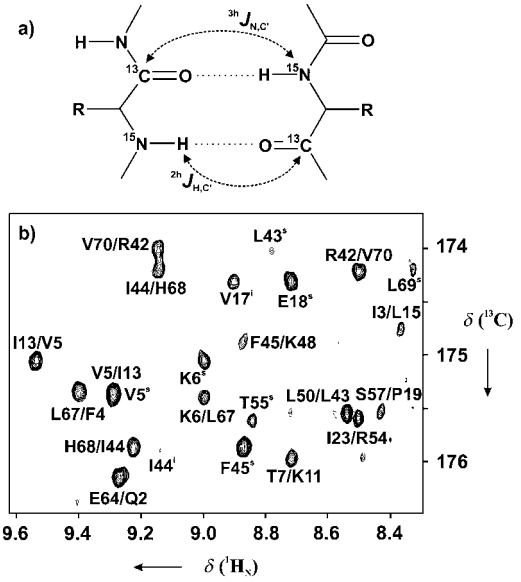

Figure 4. a) Nomenclature for hydrogen-bond couplings in proteins. b) Region from an HNCO spectrum of human ubiquitin tuned for the detection of small $^{15}N,^{13}C$ couplings.[9] Some cross-peaks arise from incomplete suppression of either $^{1}J_{N,C'}$ (superscript s) or $^{2}J_{N,C'}$ couplings (superscript i). However, the majority of peaks correlate two hydrogen-bonded residues through a $^{3h}J_{N,C'}$ scalar coupling ranging from 0.3 to 0.9 Hz. (Reproduced with permission from the American Chemical Society 1999.)

Again, the bond over the longer distance, $^{3h}J_{N,C'}$, was the first to be reported by Cordier and Grzesiek,[9] followed shortly afterwards by similar results from the group of Ad Bax.[10] The detection did not even require a completely new pulse scheme, but relied on the HNCO experiment, well known and widely used in protein NMR for correlating the amide proton to the amide nitrogen atom and on to the carbonyl carbon atom of the adjacent $i-1$ amino acid through the one-bond couplings $^{1}J_{^{1}H,^{15}N}$ (ca. 92 Hz) and $^{1}J_{^{15}N,^{13}C'}$ (ca. 15 Hz; Figure 4). The transfer efficieny in these experiments is at a maximum with a delay of $1/2J$ and zero for an integer multiple of $1/J$. Therefore, for the detection of the much smaller coupling $^{3h}J_{^{15}N,^{13}C'}$, only the delay for the transfer from ^{15}N to $^{13}C'$ had to be adjusted from about 33 ms (optimum for $^{1}J_{^{15}N,^{13}C'}$) to the rather lengthy 133 ms (optimum for a 3–4 Hz coupling and zero for $^{1}J_{^{15}N,^{13}C'}$). As a result, the direct ^{1}J correlation between $^{15}N_i$ and $^{13}C'_{i-1}$ was suppressed and another weaker correlation could indeed be seen: from the amide nitrogen atom to the ^{13}C spin of the acceptor carbonyl group of a hydrogen bond!

These first experiments were performed on the small protein ubiquitin, which has a well-known assignment of all ^{15}N and ^{13}C spins and 3D structure. The correlations found in the HNCO spectrum tuned for small $^{3h}J_{^{15}N,^{13}C'}$ couplings proved to agree perfectly with the known pattern of hydrogen bonds. The size of the coupling constant, however, was rather small, only 0.3–0.5 Hz for α-helical regions and 0.5–0.9 Hz for β-sheets—an extremely small coupling for proteins with line widths usually exceeding 10 Hz, and it is not surprising that they had not been discovered by chance before.

Again from extensive experimental data, a quantitative relationship could be established between the length of the hydrogen bond (namely, the ^{15}N-$^{13}C'$ distance) and the size of the $^{3h}J_{^{15}N,^{13}C'}$ value, with the coupling constant dropping below 0.2 Hz for ^{15}N-$^{13}C'$ distances larger than about 3.2 Å.[11]

In the meantime the two-bond coupling $^{2h}J_{^{1}H,^{13}C'}$ had also been observed, with coupling constants slightly smaller than the 0.4–0.6 Hz of the three-bond coupling.[12] However, the $^{2h}J_{^{1}H,^{13}C'}$ coupling is technically much more difficult to detect than $^{3h}J_{^{15}N,^{13}C'}$, as a result of the presence of the larger "ordinary" ^{2}J and ^{3}J couplings between the amide proteins and $^{13}C'$ in the protein backbone. Recently, also the $^{3h}J_{^{1}H,^{13}C}$ coupling has been detected and quantified by Meissner and Sørensen.[13]

It is clear that the direct detection of hydrogen bond donors and acceptors will greatly facilitate the NMR investigation of nucleic acid structures by revealing the presence, localization, and even the length of hydrogen bonds involved in base pairing.[14] The applications to proteins are more problematic because of the small size of the accessible hydrogen-bond couplings. However, it is reasonable to expect that these couplings will also prove useful with much larger proteins by using the TROSY approach. The TROSY (*T*ransverse *R*elaxation *O*ptimized *S*pectroscop*y*) concept was introduced by the Wüthrich group about two years ago and allows for a significant reduction in the line width and relaxation rates, which usually increase with molecule size.[15] For the very long delays required for the detection of small hydrogen-bond couplings, the reduction in relaxation losses is essential, and TROSY versions have been applied almost from the beginning.[1, 2] In combination with protein samples which are perdeuterated (except for the exchangeable protons), it can be expected that the measurement of hydrogen-bond couplings will soon also play an important role in structural studies for medium-sized proteins.

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