Correlations among $^{1}J_{NC'}$ and $^{h3}J_{NC'}$ Coupling Constants in the Hydrogen-Bonding Network of Human Ubiquitin

Nenad Juranić* and Slobodan Macura

Department of Biochemistry and Molecular Biology
Mayo Graduate School
Mayo Clinic and Mayo Foundation
Rochester, Minnesota, 55905

Received February 8, 2001
Revised Manuscript Received March 26, 2001

Hydrogen bonds (H-bonds) are an essential part of the protein structure.[1] Cooperative formation of H-bonds creates networks whose collective properties may be important for biological functions. The recent discovery of nuclear spin−spin coupling constants across H-bonds in biological macromolecules[2,3] provides a direct tool for studying these H-bond networks.[4−6] In proteins, an extensive H-bond network may be formed by the backbone amide (peptide) groups acting simultaneously as H-bond proton donors at the amide end and proton acceptors at the carbonyl end. The extent of the H-bond networking depends primarily on the peptide group electronic polarization. Therefore, it is very important to have a means of detecting the intrapeptide response to H-bonding.

We have shown that the protein main chain $^{1}J_{NC'}$ coupling constant is sensitive to the H-bonding of peptide groups;[7,8] others have shown that across H-bond $^{h3}J_{NC'}$ coupling constants correlate well with the H-bond distances.[6] Therefore, one may expect the $^{1}J_{NC'}$ coupling constants to correlate with the $^{h3}J_{NC'}$ coupling constants at two ends of the peptide group (Scheme 1). The existence of this correlation may provide a new insight into H-bond networks in proteins.

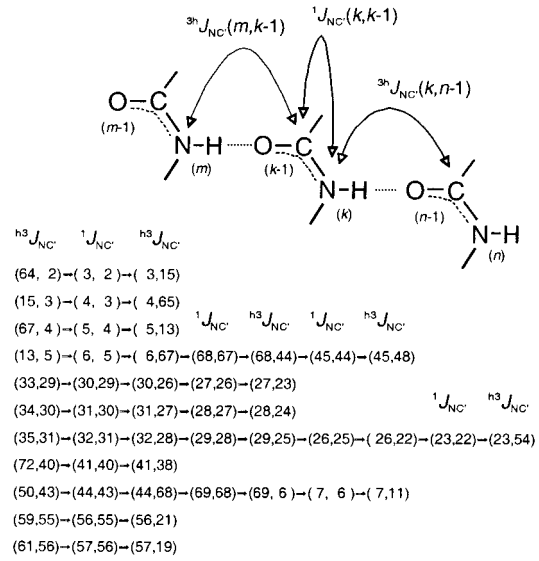
We explored the anticipated correlation in aqueous solution of the small protein human ubiquitin, (MW 6800). The majority of H-bonds identified in the crystal structure of human ubiquitin[9] have been detected by $^{h3}J_{NC'}$ coupling constants.[4,5] We re-determined the coupling constants and from the collected data were able to reconstruct the coupling network as depicted in Scheme 1. Applying the multiple linear regression analysis we found that the three coupling constants are best correlated by:

$$^{1}J_{NC'}(k, k-1) + 2.74\ ^{h3}J_{NC'}(k, n-1) - 0.88\ ^{h3}J_{NC'}(m, k-1) - 15.6 = 0 \quad (1)$$

To calculate the coefficient of correlation one has to declare a dependent variable in eq 1. The largest coefficient of correlation, $r^2 = 0.799$, was found for the $^{h3}J_{NC'}$ (k, $n-1$), Figure 1 (solid line).

This correlation is consistent with our earlier finding that H-bonding at NH sites decreases and H-bonding at CO sites

Scheme 1



increases $^{1}J_{NC'}$ coupling constants.[7,8] The major contribution to the correlation comes from the relationship between $^{h3}J_{NC'}$ (k, $n-1$) and $^{1}J_{NC'}$(k, $k-1$). That relationship has a negative slope ($r^2 = 0.661$) which suggest that the amide H-bond competes with the peptide bond for the nitrogen s-electron density. Namely, theoretical calculations of the intra-amide and inter-amide NC′ couplings suggest that the J constants mainly depend on the Fermi contact term.[10,11]

There is also significant correlation ($r^2 = 0.202$) between the across-H-bond coupling constants $^{h3}J_{NC'}$ (m, $k-1$) and $^{h3}J_{NC'}$ (k, $n-1$). The slope of that correlation is positive, indicating that H-bonds in the network enhance each other, which is experimental evidence for the positive H-bond cooperativity across the peptide groups. The individual chains of the H-bond network may show different collective behavior. In view of the limited set of data considered here, suffice to say that correlation among the H-bond couplings in the β-sheets is higher than in the α-helix.

A notable exception to the observed correlations may indicate additional interactions. For instance, amide 4 has the $^{1}J_{NC'}$ (4,3) coupling constant larger than suggested by the correlation in Figure 1. Such a high value of $^{1}J_{NC'}$ indicates H-bonding of a carbonyl oxygen to a hydroxyl proton.[8] The hydroxyl group is a strong proton donor that induces shortening of the peptide N−C′ bond with a concomitant increase of the coupling constant.[7] The suggested H-bonding to the hydroxyl group is further corroborated by the couplings in reverse turns where some carbonyl oxygens can H-bond exclusively to water, and only $^{h3}J_{NC'}$ (k, $n-1$) and $^{1}J_{NC'}$ (k, $k-1$) couplings exists (e.g., 56 ← 61 and 62 ← 65). If we attribute zero value to the nonexistent coupling, $^{h3}J_{NC'}$ (m, $k-1$) = 0, and include residues from the reverse turns into the plot, Figure 1, then the peptide (4, 3) coupling constants and the reverse turn coupling constants show the same deviation. They are correlated like the others (dashed line); the only difference is that the $^{1}J_{NC'}$ constants are ∼2 Hz larger. This suggests that the carbonyl oxygen of Ile3 is also H-bonded to hydroxyl proton.

The inferred H-bonding is not observed in the X-ray structure.[9] However, the solution NMR structure of human ubiquitin[12] does open the possibility for H-bonding of the Thr14 hydroxyl group

(1) Jeffrey, G. A.; Seanger, W. *Hydrogen Bonding in Biological Structures*; Springer; New York, 1991.
(2) Dingley, A. J.; Grzesiek, S. *J. Am. Chem. Soc.* **1998**, *120*, 8293−8297.
(3) Pervushin, K.; Ono, A.; Fernandez, C.; Szyperski, T.; Kainosho, M.; Wütrich, K. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 14147−14151.
(4) Cordier, F.; Grzesiek, S. *J. Am. Chem. Soc.* **1999**, *121*, 1601−1602.
(5) Cornilescu, G.; Hu, J.-S.; Bax, A. *J. Am. Chem. Soc.* **1999**, *121*, 2949−2950.
(6) Cornilescu, G.; Benjamin, B. E.; Ramirez, M.; Kirsten, F. G.; Clore, M.; Gronenborn, A. M.; Bax, A. *J. Am. Chem. Soc.* **1999**, *121*, 6275−6279.
(7) Juranić, N.; Ilich, K. P.; Macura, S. *J. Am. Chem. Soc.* **1995**, *117*, 405−410.
(8) Juranić, N.; Likić, V. A.; Prendergast, F. G.; Macura, S. *J. Am. Chem. Soc.* **1996**, *118*, 7859−7860.
(9) Vijay-Kumar, S.; Bugg, C. E.; Cook, W. J. *J. Mol. Biol.* **1987**, *194*, 531−544.
(10) Scheurer, C.; Brüschweller, R. *J. Am. Chem. Soc.* **1999**, *121*, 8661−8662.
(11) Pecul, M.; Leszczynski, J.; Sadlej, J. *J. Phys. Chem. A.* **2000**, 104, 8105−8113
(12) Cornilescu, G.; Marquardt, J. L.; Ottiger, M.; Bax, A. *J. Am. Chem. Soc.* **1998**, *120*, 6836−6837. (PDB structure ID: 1d3z)

10.1021/ja015647d CCC: $20.00
Published on Web 04/10/2001

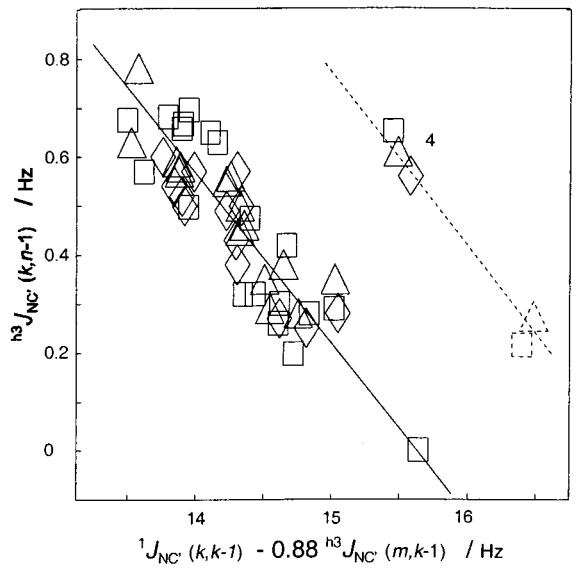


Figure 1. Correlation of $^{1}J_{NC'}$ and $^{h3}J_{NC'}$ coupling constants according to the expression $^{h3}J_{NC'}$ (k, $n-1$) $\approx$ $^{1}J_{NC'}$ (k, $k-1$) $-$ 0.88 $^{h3}J_{NC'}$ (m, $k-1$) derived from eq 1. Multilinear regression analysis (MATLAB, The Math Works) gives a correlation coefficient of $r^2 = 0.799$, excluding amide 4. Designation of residue numbers (k), proton donating (m), and proton accepting ($n-1$) residues in H-bonds, is according to Scheme 1. Two points (dashed simbols) are from the reverse turns, and their placement in the plot is tentative. Experimental data are from our measurements, 2 mM U-^{13}C/ ^{15}N human ubiquitin in 25 mM aqueous acetic acid-d_4 at 303 K and 14 T, (squares) and from other works (triangles,[4] diamonds[5]).

to carbonyl oxygen of Ile3. In the crystal structure, the *gauche*(+) conformation of the Thr14 side chain seems to be steered by H-bonding of the γ1-hydroxyl group to water present in the crystal structure. In the NMR structure the same γ1-hydroxyl is turned around 120° into the *gauche*(−) conformation, which is much closer to the carbonyl oxygen of Ile3. The three bond spin−spin coupling constants relevant for the Thr14 side-chain conformation have been measured and indicate some rotamer averaging that may include the *trans* conformation[13] which is quite favorable for the above inferred H-bonding.

By modeling the Thr14 side-chain conformation to maximize H-bonding of the γ1-hydroxyl group to the Ile3 carbonyl oxygen we obtained a twisted *trans* conformation that reproduces the NOESY spectrum (Figure 2) as good as the *gauche*(−) NMR conformation. Whether this twisted conformation is represented in the rotamer averaging process remains open to speculation, but the existence of the inferred H-bonding would explain the observed change of the Thr14 orientation between the crystal and the liquid structure.

(13) Bax, A. Private communication.

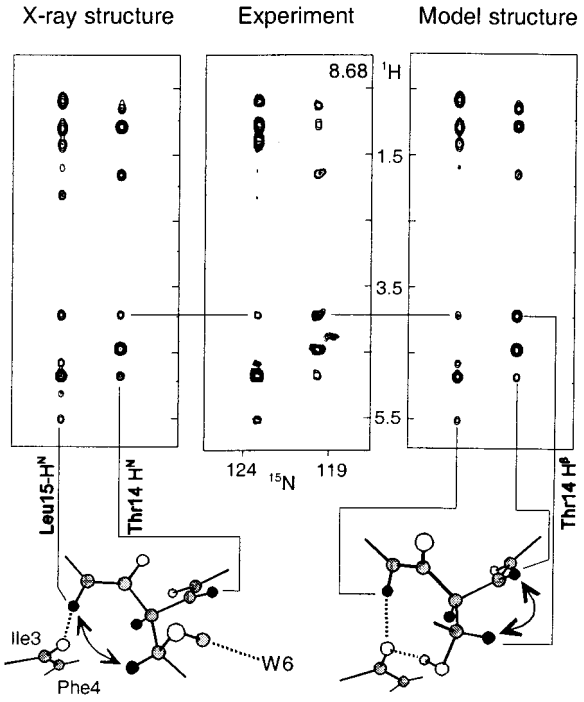


Figure 2. Comparison of the simulated NOESY spectra of the Thr14 side-chain conformation in solution and in the crystal structure with the experimental solution spectrum. The full-matrix simulated spectra assume the rigid body isotropic motion of 4 ns. Shown is the experimental HN spectral plane which is taken from the ^{15}N-filtered 3D NOESY spectrum (mixing time 200 ms) at almost overlapping amide proton resonances of Thr14 H^{N} and Lys15 H^{N}, $\delta = 8.68$ ppm. The Thr14 side chain torsional angle is $\chi 1 = 66°$ for the crystal and $\chi 1 = -130°$ for the modeled structure.

In conclusion, we demonstrated a correlation among protein NC′ coupling constants that provides new insight into protein H-bond networks. Specifically, it gives insight into the cooperative nature of H-bond networks and shows the rearrangement of nitrogen s-orbital density upon H-bonding. Exceptions from the correlation may point to the altered character of respective H-bond, like H-bonding to the solvent, ligand, or side-chain OH groups or the H-bond bifurcation. This may be particularly useful for identifying H-bonding of peptide groups to water which in solution cannot be detected by other means.

Supporting Information Available: One pulse sequence, with experimental details, used for recording 2D H(N)CO; two 2D H(N)CO spectra of human ubiquitin, 1JNC′ optimized ($T = 16$ ms) and ^{h3}JNC′ optimized ($T = 66$ ms); one figure of the coupling constant determination by fitting their time evolution; one table of the measured ^{n}JNC′ coupling constants (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA015647D