Structural dependencies of protein backbone $^2J_{\text{NC}'}$ couplings

NENAD JURANIĆ,¹ J.J. DANNENBERG,² GABRIEL CORNILESCU,³ PEDRO SALVADOR,⁴
ELENA ATANASOVA,⁵ HEE-CHUL AHN,^{3,6} SLOBODAN MACURA,¹
JOHN L. MARKLEY,³ AND FRANKLYN G. PRENDERGAST^{1,5}

¹Department of Biochemistry and Molecular Biology, Mayo Clinic and Foundation, Rochester, Minnesota 55905, USA

²Department of Chemistry, City University of New York, Hunter College and the Graduate School, New York, New York 10021, USA

³National Magnetic Resonance Facility at Madison, Department of Biochemistry, University of Wisconsin–Madison, Madison, Wisconsin 53705, USA

⁴Institute of Computational Chemistry and Department of Chemistry, University of Girona, 17071 Girona (Catalonia), Spain

⁵Department of Molecular Pharmacology and Experimental Therapeutics, Mayo Clinic and Foundation, Rochester, Minnesota 55905 USA

(RECEIVED November 2, 2007; FINAL REVISION December 26, 2007; ACCEPTED December 27, 2007)

Abstract

Protein folding can introduce strain in peptide covalent geometry, including deviations from planarity that are difficult to detect, especially for a protein in solution. We have found dependencies in protein backbone $^2J_{\text{NC}'}$ couplings on the planarity and the relative orientation of the sequential peptide planes. These dependences were observed in experimental $^2J_{\text{NC}'}$ couplings from seven proteins, and also were supported by DFT calculations for a model tripeptide. Findings indicate that elevated $^2J_{\text{NC}'}$ couplings may serve as reporters of structural strain in the protein backbone imposed by protein folds. Such information, supplemented with the H-bond strengths derived from $^{\text{h}3}J_{\text{NC}'}$ couplings, provides useful insight into the overall energy profile of the protein backbone in solution.

Keywords: DFT; H-bond; $^2J_{\text{NC}'}$ scalar coupling; NMR; protein structure

Supplemental material: see www.proteinscience.org

Measurements of small spin–spin couplings (<1 Hz) in proteins by multidimensional-multinuclear NMR spectroscopy have enabled the direct detection of weak interactions that contribute to protein conformation and stability. The

most important example has been the discovery of spin–spin couplings through hydrogen bonds (H-bonds) within the protein backbone (Cordier and Grzesiek 1999; Cordier et al. 1999, 2003; Cornilescu et al. 1999a). However, other weak perturbations arising from a “hidden” strain (Karplus 1996) are potentially detectable. Such a strain may be manifested in deviations of peptide covalent geometry from planarity (Karplus 1996; MacArthur and Thornton 1996; Edison 2001; Esposito et al. 2005), and can have important consequences for protein structure.

Heteronuclear ^{15}N – ^{13}C couplings between protein backbone nuclei have proven to be especially useful, because the relatively slow ^{15}N -relaxation permits the measurement of couplings in proteins of small to medium

⁶Present address: Advanced Analysis Center, KIST, Seoul, South Korea.

Reprint requests to: Nenad Juranić, Mayo Foundation, 200 First Street Southwest, Rochester, MN 55905, USA; e-mail: juranic.nenad@mayo.edu; fax: (507) 284-8433; or J.J. Dannenberg, Department of Chemistry, City University of New York, Hunter College and the Graduate School, 695 Park Avenue, New York, NY 10021, USA; e-mail: jdannenberg@gc.cuny.edu; fax: (212) 772-5332.

Article published online ahead of print. Article and publication date are at <http://www.proteinscience.org/cgi/doi/10.1110/ps.073331608>.

size (Delaglio et al. 1991). Particularly important are the $^{\text{h}3}J_{\text{NC}'}$ couplings across H-bonds that enable the direct detection of protein secondary structure in solution (Cordier and Grzesiek 1999; Cornilescu et al. 1999a). These couplings have been shown to depend on H-bond distances and angles (Cornilescu et al. 1999b; Bagno 2000; Alexandrescu et al. 2001; Barfield 2002; Cordier and Grzesiek 2002), on electronic polarization in H-bond networks (Juranić and Macura 2001; Juranić et al. 2003, 2007; Salvador et al. 2004, 2007), and on protein dynamics (Markwick et al. 2003; Bouvignies et al. 2005). The protocols used in measuring $^{\text{h}3}J_{\text{NC}'}$ couplings also afford the detection of ^{15}N - ^{13}C couplings through three different classes of covalent bonds (Fig. 1): peptide-bond $^1J_{\text{NC}'}$ couplings (Delaglio et al. 1991), intraresidue $^2J_{\text{NC}'}$ couplings (Cornilescu et al. 1999a; Cordier et al. 2003), and intra-residue $^3J_{\text{NC}'\gamma}$ couplings (Delaglio et al. 1991). The peptide-bond $^1J_{\text{NC}'}$ couplings have been shown to correlate with H-bonding (Walter and Wright 1979; Juranić et al. 1995), and the intra-residue $^3J_{\text{NC}'\gamma}$ couplings have been shown to depend on protein side-chain conformation (Perez et al. 2001; Juranić et al. 2005).

However, $^2J_{\text{NC}'}$ couplings in proteins have yet to be interpreted in terms of peptide backbone structure. These two-bond couplings are expected to be sensitive to the relative orientations of adjacent peptide groups. For example, the backbone $^2J_{\text{HH}}$ couplings of glycine in peptides (Barfield et al. 1976) have been interpreted in terms of the electronic hyperconjugation involving the π -orbitals (Barfield and Grant 1962) of adjacent peptide groups, and $^2J_{\text{NC}'}$ couplings in twisted amides (Yamada et al. 1997) and oximes (Gopinathan and Narasimhan 1971) have been correlated with the orientation of the nitrogen lone-pair electrons. Because the coupled nuclei reside within sequential peptide groups (Fig. 2), intra-residue $^2J_{\text{NC}'}$ couplings should be sensitive both to the local π -orbital delocalization (peptide-bond twist angles ω_{-1} , ω) and to orientation-dependent hyperconjugation involving π -orbitals of the sequential peptide groups (backbone dihedral angles ϕ and ψ). Measurable parameters sensitive to

Figure 1. Schematic representation of the four classes of ^{15}N - ^{13}C couplings that can be measured from data acquired in the J -HNCO experiment used for the direct detection of H-bonds.

Figure 2. Backbone geometry of two residues in a protein chain. The relative orientation of adjacent peptide planes is defined by the backbone dihedral angles ϕ and ψ . The planarity of the peptide groups, which depends on the delocalization of π -electrons, is defined by dihedral angles (atoms defining the dihedral planes): $\omega_{-1}(C_{i-1}^{\alpha} - C_{i-1}' - N_i - C_i^{\alpha})$ and $\omega(C_i^{\alpha} - C_i' - N_{i+1} - C_{i+1}^{\alpha})$.

peptide-bond twist angles are of particular interest because small deviations of peptide geometry from planarity are difficult to detect, especially for proteins in solution (Ulmer et al. 2003).

Whereas the backbone dihedral angles of a protein usually can be measured with good relative accuracy from an X-ray or NMR structure, the peptide-bond twist angles, being rather small, are reliably determined only in X-ray structures of ultrahigh resolution (Esposito et al. 2005). For this reason, we have measured $^2J_{\text{NC}'}$ couplings in crambin, a protein for which an X-ray structure of exceptionally high resolution (0.54 Å) has been reported (Jelsch et al. 2000) and with an experimental NMR solution structure identical within 0.77 Å RMSD (Ahn et al. 2006). By interpreting the experimental results in the context of DFT calculations for a model system, we have identified correlations between $^2J_{\text{NC}'}$ and peptide backbone structure. This has enabled the interpretation of experimental $^2J_{\text{NC}'}$ couplings determined here (crambin, GB3, *holo*-, and *apo*-calmodulin) or available from the literature for proteins of known structure.

Results and Discussion

Set of couplings analyzed

We assembled a set of intra-residue $^2J_{\text{NC}'}$ coupling constants for seven proteins of known structure (Supplemental Table S1): four measured here (crambin, GB3, *holo*-, and *apo*-calmodulin), and three from the literature (ubiquitin) (Cordier and Grzesiek 1999; Cornilescu et al. 1999a; Juranić and Macura 2001) *holo*-parvalbumin, (Juranić et al. 2002), and *apo*-intestinal-fatty-acid-binding-protein (IFABP) (Juranić et al. 2002). For *holo*-parvalbumin and *apo*-IFABP a few of the reported values were corrected upon careful reexamination of spectra. We also redetermined some of the $^2J_{\text{NC}'}$ couplings for

ubiquitin, because of inconsistencies in the data from the literature (Cordier and Grzesiek 1999; Cornilescu et al. 1999a; Juranić and Macura 2001). Only $\sim 30\%$ of the residues in these proteins yielded intra-residue $^2J_{\text{NC}'}$ couplings larger than the detection threshold, which we estimated to be at 0.1–0.2 Hz. For the majority of the residues, spectral peaks were undetectable at the position expected for $^2J_{\text{NC}'}$ couplings; these couplings have $^2J_{\text{NC}'} < 0.2$ Hz, and we set the value arbitrarily to $^2J_{\text{NC}'} = 0$ Hz. Couplings could not be determined from a smaller number of residues whose expected peaks were overlapped with other signals so as to prevent coupling measurement; in these cases we report no values for $^2J_{\text{NC}'}$. For each protein, we report three numbers: (1) the number of couplings with measurable nonzero values, (2) the number of couplings determined to be below detection threshold and reported as zero values, and (3) the total possible number of observable peaks (number of residues, excluding prolines): crambin 17/17/40, ubiquitin 24/31/71, GB3 23/31/59, *holo*-parvalbumin 24/75/107, *holo*-calmodulin 27/98/142, *apo*-calmodulin 28/95/142, and *apo*-intestinal-fatty-acid-binding-protein (*apo*-IFABP) 21/91/130. The $^2J_{\text{NC}'}$ couplings are reported here as absolute values, because the method used for their experimental determination (Cordier and Grzesiek 1999) does not indicate the sign; however, $^2J_{\text{NC}'}$ coupling constants have been found to be negative (Cornilescu et al. 1999b).

Association of observed $^2J_{\text{NC}'}$ couplings with uncompensated H-bond acceptors

In all seven proteins studied, the majority of the larger (> 0.5 Hz) $^2J_{\text{NC}'}$ couplings occurred in turns or loops or at the O-capping end of α -helices (Fig. 3). These are cases where the backbone carbonyl oxygen lacks an intra-protein H-bond. The largest $^2J_{\text{NC}'}$ couplings observed (e.g., 1.2 Hz for residue 30 of crambin and 1.0 Hz for residue 34 of ubiquitin) appeared in the O-capping ends of α -helices.

In the proteins studied, the tight reverse turn (3-turn, or structurally similar turns) was the structural motif with the highest frequency of detected $^2J_{\text{NC}'}$ couplings (Fig. 4). Within the reverse turn, the highest frequency of observed $^2J_{\text{NC}'}$ couplings (80%) was at position 3, with the average $|^2J_{\text{NC}'}| \sim 0.5$ Hz. Notably, position 3 regularly lacks an intramolecular H-bond at the $\text{O}=\text{C}<$ acceptor.

The association of larger $^2J_{\text{NC}'}$ values with uncompensated H-bond acceptors may signify a dependence on peptide bond nonplanarity. The peptide bond planarity has been explained by its partial double-bond character (Corey and Pauling 1953; Edison 2001). H-bonding at the peptide $\text{O}=\text{C}<$ acceptor enhances the double-bond character, and hence the planarity, of the peptide bond

Figure 3. Sequential disposition of the intraresidue $^2J_{\text{NC}'}$ couplings in crambin (top) and in GB3 (bottom). Boxes at the bottom indicate the detection threshold of 0.1 Hz; breaks indicate prolyl residues or residues for which spectral overlaps prevented sampling of the couplings. Data are also tabulated in Supplemental Table S1.

(Jeffrey et al. 1980; Juranić et al. 2002). The absence of such an H-bond would facilitate peptide-bond twisting under strain imposed, for example, by a tight reverse turn.

Dependence of $^2J_{\text{NC}'}$ couplings on the backbone conformation

According to Figure 2, the relative orientation of π -orbitals of the sequential peptide planes is determined by the backbone dihedral angles (ϕ , ψ). However, the relative orientation of π -orbitals as a function of ϕ and ψ has redundancies because the same relative orientation can be obtained with different choices of backbone dihedral angles (for planar peptide groups, $\cos \Theta = -\cos \phi \cos \psi - \sin \phi \sin \psi \cos \alpha$, where α is bond angle $\text{N}-\text{C}^\alpha-\text{C}'$). If the relative orientation of the π -orbitals is a major determinant of $^2J_{\text{NC}'}$, a better descriptor would be the dihedral angle between the sequential peptide planes. Indeed, rather than showing specific dependence on the individual backbone dihedral angles, the $^2J_{\text{NC}'}$ couplings can be represented by a Karplus-type equation (Karplus 1963) in which the dihedral angle Θ is that between sequential peptide planes (Fig. 5).

Figure 4. Compilation of $^2J_{NC'}$ couplings from the tight reverse turns of the seven proteins investigated here, classified according to an idealized 3-turn (data are tabulated in Supplemental Table S2). Average values of the couplings are represented by the solid circles and standard deviations by the error bars. The fractional frequency of observed couplings at each reverse turn position is represented by the height of the rectangular bar.

The line in Figure 5 corresponds to the equation:

$$|^2J_{NC'}| \sim F_{2JNC}(\Theta) = 1.1 \cos^2 \frac{\Theta}{2} + 0.8 \cos^2 \Theta - 0.2 \pm 0.25 \text{ Hz} \quad (1)$$

The coefficients in this equation were determined by a grid search for a minimal standard deviation from the experimental data.

Equation 1 can be interpreted in terms of the relative orientation of the π -orbitals of the peptide groups at the coupled nuclei: The $\cos^2 \frac{\Theta}{2}$ term is consistent with parallel orientation of π -orbitals enhancing the coupling, and the $\cos^2 \Theta$ term is consistent with perpendicular orientation of π -orbitals disfavoring $^2J_{NC'}$ coupling. The scattering of data around the proposed dependence (Fig. 5), which is relatively large compared to the experimental error of the coupling constant measurements (~ 0.1 Hz), suggests that other parameters likely are important, such as peptide-bond twist angles.

In 90% of the cases where the $^2J_{NC'}$ couplings were below the detection threshold, Θ was in the range $85^\circ < |\Theta| < 125^\circ$; this corresponds to the region of α -helix ($|\Theta| \sim 100^\circ$) and parallel β -sheet ($|\Theta| \sim 120^\circ$), and also borders the region of the antiparallel β -sheet ($|\Theta| \sim 135^\circ$).

Dependence of $^2J_{NC'}$ couplings on planarity of the peptide-bond covalent geometry

It has been suggested that $^2J_{NC'}$ coupling constants should depend on the peptide-bond twist angle (Solkan and Bystrov 1975); however, no formulation of this has been put forward. Deviations of peptide-bond covalent geometry from planarity generally are small; consequently, the associated peptide-bond twist angle has to be known at a precision of a fraction of a degree to test for any meaningful relationship with $^2J_{NC'}$ coupling. Because this level of precision is practically unobtainable in experimentally determined protein structures, we calculated $^2J_{NC'}$ coupling constants in the tripeptide model system Ala₃, by applying the density function theory (DFT) procedure described in our earlier work (Salvador et al. 2004). We explored variations of both the ω_{-1} and ω twist angles within 170° – 190° , while fully optimizing the remaining geometrical parameters of the model system. These variations produced changes as large as -1.2 Hz in the calculated coupling constants. The changes depended almost exclusively on the twist angle ω_{-1} , with the calculated $^2J_{NC'}$ couplings exhibiting a linear dependence on ω_{-1} (Fig. 6). The gas-phase optimized structures of Ala₃ preferentially adopt an extended conformation with Θ angle values in the range of 140° – 170° ; therefore, according to Equation 1, the calculated $^2J_{NC'}$ is expected to have a rather small dependence on Θ . Notably, the

Figure 5. Dependence of $^2J_{NC'}$ couplings on Θ , the angle formed between the planes of sequentially adjacent peptide groups (planes are defined by OC'N atoms). Dihedral angles are from the X-ray crystal structures of the six proteins, 1ejg-model A (Jelsch et al. 2000), 1lfg (Derrick and Wigley 1994), 1ubq (Vijay-Kumar et al. 1987), 5cpv (Swain et al. 1989), 1cll (Chattopadhyaya et al. 1992), 1lfc (Scapin et al. 1992), respectively, at resolutions of 0.54 Å, 1.10 Å, 1.8 Å, 1.6 Å, 1.7 Å, and 1.19 Å. Data for apo-calmodulin were omitted, because its structure in solution differs from that in the crystal (Schumacher et al. 2004). The regions of different secondary structure are indicated above the Θ -axis.

Figure 6. Theoretical (DFT) ${}^2J_{NC'}$ coupling constants (squares) in the tripeptide model system Ala₃ as a function of the twist angle ω_{-1} ($C^{\alpha}_{i-1} - C'_{i-1} - N_i - C^{\alpha}_i$). The line approximating these data is given by: $-0.014\Delta\omega_{-1} - 0.62$, where $\Delta\omega_{-1} = \omega_{-1} - 180^\circ$. Sets of the overlapping data for each ω_{-1} value are due to arrayed values of ω . A rather small dispersion signifies a very small influence of ω .

calculations showed almost no correlation between ${}^2J_{NC'}$ values and ω .

In order to explore whether this result might be relevant to a real protein system, we examined correlations between the experimental coupling data for crambin and the backbone coordinates of the ultrahigh-resolution X-ray structure (Jelsch et al. 2000). The 16 experimental ${}^2J_{NC'}$ coupling constants of crambin larger than the 0.1 Hz threshold were found to exhibit correlations with the peptide-bond twist angles of both sequentially connected peptide groups: $r^2(\omega_{-1}) = 0.21$, $r^2(\omega) = 0.45$. Because the two twist angles have low correlation to one another, their joint correlation to the ${}^2J_{NC'}$ coupling is significantly higher ($r^2 = 0.6$) than the individual values. This dependence of ${}^2J_{NC'}$ on the two twist angles is approximately linear (Fig. 7). In order to bring the ${}^2J_{NC'}$ experimental couplings (measured as magnitudes) into the framework of DFT couplings, their inherent negative sign was applied.

The major dependence of ${}^2J_{NC'}$ on ω observed for crambin appears to contradict the DFT results for the model system, where no significant correlation with ω was observed. This discrepancy might be reconciled if the dependence on the twist angles is modulated by the angle Θ . The ${}^2J_{NC'}$ couplings of crambin come mostly from turns and helices ($\Theta < 90^\circ$), whereas the DFT calculated couplings are from extended conformations ($\Theta > 110^\circ$). To introduce an interdependence of Θ and the twist angles, we rewrote Equation 1 as,

$${}^2J_{NC'} \sim F_{2JNC}(\Theta, \omega_{-1}, \omega) = A \cos^2 \frac{\Theta}{2} + B \cos^2 \Theta + C \quad (2)$$

where A , B , and C are linear combinations of the twist angles ω_{-1} and ω . The three parameters determined empirically by jointly fitting the crambin and DFT data were:

$$\begin{aligned} A &= -0.090 - 0.043\Delta\omega_{-1} \\ B &= -0.72 - 0.020\Delta\omega_{-1} + 0.017\Delta\omega \\ C &= +0.07 + 0.008\Delta\omega_{-1} - 0.015\Delta\omega \end{aligned} \quad (2.1)$$

With angles derived from the crambin and DFT data, Equations 2 and 2.1 fit the observed ${}^2J_{NC'}$ coupling constants with a standard deviation of 0.05 Hz ($r^2 = 0.93$; Fig. 8, top).

The experimental crambin couplings correlated more closely with those predicted from Equation 2 (standard deviation = 0.09 Hz) than from Equation 1 (standard deviation = 0.15 Hz). Equation 2 also was partially successful in reproducing the experimental ${}^2J_{NC'}$ couplings in three proteins with lower resolution X-ray structures: ubiquitin, GB3, and parvalbumin. For these proteins, the standard deviation was ~ 0.12 Hz ($r^2 \sim 0.7$) (Fig. 8, bottom). We also compared calculations for crambin based on its ultrahigh-resolution structure (1ejg-model A, 0.54 Å) (Jelsch et al. 2000), and its lower resolution structure (1crn, 1.5 Å) (Teeter 1984). With angles derived from the lower resolution crambin structure, the correlation between predicted and experimental ${}^2J_{NC'}$ couplings had the same level of accuracy obtained with ubiquitin, GB3, and parvalbumin, well below the correlation achieved with angles from the ultrahigh-resolution X-ray structure. Therefore, insufficient structural resolution can explain the larger

Figure 7. Dependence of the experimental ${}^2J_{NC'}$ coupling constants of crambin (taken with negative sign) on the average of the two twist angles $\omega(C^{\alpha}_i - C'_{i-1} - N_{i+1} - C^{\alpha}_{i+1})$ and $\omega_{-1}(C^{\alpha}_{i-1} - C'_{i-1} - N_i - C^{\alpha}_i)$ from sequential peptide groups. The twist angles were calculated from the atomic coordinates in the crambin Protein Data Bank file 1ejg-model A (Jelsch et al. 2000). The line approximating these data represents the function: $-0.043(\Delta\omega_{-1} + \Delta\omega)/2 - 0.4$, where $\Delta\omega = \omega - 180^\circ$.

Figure 8. (Top) Correlation ($r^2 = 0.93$) between empirical (Equation 2) and experimental ($\circ$ crambin) or DFT ($\square$ Ala₃) $^2J_{\text{NC'}}$ coupling constants. A negative sign was assumed for all experimental $^2J_{\text{NC'}}$ coupling constants, which were measured as absolute values. The angles (Θ , ω_{-1} , ω) were calculated from the atomic coordinates of the ultrahigh resolution (0.54 Å) structure of crambin, 1ejg-model A (Jelsch et al. 2000), or from the Ala₃ model. (Bottom) Analogous correlation from protein X-ray structures with resolution >1 Å: ubiquitin ($\times$), 1ubq -1.8 Å, (Vijay-Kumar et al. 1987); GB3 ($+$), 1igd -1.1 Å (Derrick and Wigley 1994); parvalbumin ($\#$), 5cpv -1.6 Å (Swain et al. 1989); and crambin ($\circ$), 1crn -1.5 Å (Teeter 1984).

deviations from Equation 2 for the structures in the bottom panel of Figure 8.

In addition to structural resolution, internal dynamics (mobility) are always of concern in predicting J -couplings. A peculiarity of the $^2J_{\text{NC'}}$ couplings, which may render them less susceptible to dynamics, is that larger magnitude couplings are associated with protein backbone strain. In ubiquitin, residues that have $|{}^2J_{\text{NC'}}| > 0.5$ Hz also have order parameters $S^2 > 0.84$ (Tjandra et al. 1995a), which characterizes them as rather rigid. Indeed, we observed larger standard deviations for $|{}^2J_{\text{NC'}}|$ couplings < 0.5 Hz than for ≥ 0.5 Hz (see Fig. 8).

For *holo*-calmodulin and *apo*-IFABP, the inclusion of the twist angle dependences (Equation 2) did not significantly improve the predictability of the experimental $^2J_{\text{NC'}}$ couplings over those achieved with Equation 1. This may simply be a consequence of the lower rigidity of

these proteins and the larger difference between X-ray and solution structures (Hodsdon and Cistola 1997; Chou et al. 2001). For example, solvation has been found to change the protein backbone H-bond network in *holo*-calmodulin (Juranić et al. 2007).

Association of $^2J_{\text{NC'}}$ couplings with relative energy of the backbone conformation

Larger magnitude $^2J_{\text{NC'}}$ couplings appeared in tight turns of the peptide backbone, particularly at residues with uncompensated H-bond acceptors or increased twisting of the peptide bond. The unfavorable configurations (larger magnitude $^2J_{\text{NC'}}$ couplings) are likely brought about by the network of secondary structure H-bonds and by gains in H-bond energy elsewhere along the protein backbone. It may be expected that a tightly packed backbone with an extensive H-bond network would support isolated regions of unfavorable backbone geometry, which would not be the case for a less structured and more flexible backbone. This idea can be tested by comparing the $^2J_{\text{NC'}}$ couplings with H-bond $^3J_{\text{NC'}}$ couplings along the protein sequence, which provide a good measure of H-bond strengths (Cornilescu et al. 1999b; Barfield 2002). Thus, we postulate that backbone domains with larger $^2J_{\text{NC'}}$ couplings are also rich in larger $^3J_{\text{NC'}}$ couplings. As a test of this hypothesis, we examined couplings in *apo*-calmodulin (Juranić et al. 2007), which has two lobes of similar fold (Kuboniwa et al. 1995) but with the N-terminal lobe more rigidly and more tightly packed (Tjandra et al. 1995b). The N-terminal lobe is richer in both $^3J_{\text{NC'}}$ and $^2J_{\text{NC'}}$ couplings, signifying a sharper differentiation of the backbone energy than in the C-terminal lobe (Fig. 9). Large $^2J_{\text{NC'}}$ couplings (magnitude > 0.5 Hz) are found almost exclusively in the more rigid N-terminal lobe, in agreement with the inference that such couplings signify a strained (immobile) backbone region.

Figure 9. Sequential profile of $^3J_{\text{NC'}}$ couplings (Juranić et al. 2007) and $^2J_{\text{NC'}}$ couplings in *apo*-calmodulin. Secondary structure elements are indicated by the central cartoon.

Conclusion

We have demonstrated the dependence of $^2J_{\text{NC}'}$ couplings on the planarity and relative orientation of the sequentially adjacent peptide planes at the protein backbone. A possible explanation for these observations is that increased twisting disengages π -orbitals from the peptide bonds and enables their participation in an electronic hyperconjugation of the peptide groups. This also would rationalize why large $^2J_{\text{NC}'}$ couplings are associated with the absence of an H-bond at the carbonyl oxygen; an H-bond at this position would strengthen the planar double-bond character of the peptide bond and thereby reduce peptide-bond twisting. The DFT calculations show (see Materials and Methods: calculations on the model system) that almost all the coupling comes from the Fermi contact (FC) term, and that the other three terms in the Ramsey treatment are small and do not vary much with changes in backbone conformation. The apparent dependence on the π -orbitals therefore arises from their polarizing effect on the s-orbitals.

Elevated $^2J_{\text{NC}'}$ couplings may serve as reporters of structural strain in the protein backbone imposed by protein folds. Such information, supplemented with the H-bond strengths derived from $^3J_{\text{NC}'}$ couplings, may provide useful insight into the overall energy profile of the protein backbone in solution.

Materials and Methods

Sample preparation

Ubiquitin

Uniform ^{13}C , ^{15}N -enriched human ubiquitin was purchased from VLI Research. The 2-mM sample was prepared as described earlier (Juranić and Macura 2001).

Crambin

Expression of recombinant crambin (P22,L25), one of the natural isoforms of crambin, labeling with ^{13}C and ^{15}N , and protein isolation and purification, were as described earlier (Ahn et al. 2006). The lyophilized crambin powder was dissolved in 75% acetone-D6 (Fluka)/15% H_2O /10% D_2O at ~1-mM concentration.

Third IgG-binding domain of protein G (GB3)

$[^2\text{H}, ^{13}\text{C}, ^{15}\text{N}]$ -GB3 (Gronenborn et al. 1991) was a gift from Ad Bax.

Calmodulin

Expression, labeling with ^{13}C and ^{15}N , and protein isolation and purification were as described earlier (Juranić et al. 2007). The 2.0-mM sample of uniformly ^{15}N , ^{13}C -labeled *holo*CaM, was prepared in 20-mM HEPES buffer (pH 7.5) containing

100 mM KCl, 1 mM EDTA, 1 mM EGTA, with addition of 20 mM Ca^{2+} .

NMR measurements

The $^1J_{\text{NC}'}$ coupling constants for all samples except GB3 were measured at the Mayo Foundation NMR Facility on a Bruker Avance 600 MHz spectrometer equipped with a cryogenic probe by using the constant time 3D J -HNCO experiment (Cordier and Grzesiek 1999; Cornilescu et al. 1999a). The $^1J_{\text{NC}'}$ coupling constants were determined by fitting the time evolution of all observed NC' couplings for a particular peptide nitrogen as described in an earlier publication (Juranić and Macura 2001). The J -HNCO experiments used in collecting data for crambin and ubiquitin utilized a constant time of 70 ms, and were acquired at 10 J -evolution times (16 ms, 20 ms, **33** ms, 40 ms, 50 ms, 54 ms, **65** ms, **66** ms, **66.5** ms, and **69.5** ms). The J -HNCO experiments used in collecting data for calmodulin utilized a constant time of 33 ms and were acquired at seven evolution times (16 ms, 20 ms, 24 ms, 28 ms, **31** ms, **32** ms, and **33** ms). In each data set, the number of transients collected was increased by a factor of four for the evolution times shown in bold type ($^1J_{\text{NC}'}$ couplings quenched). Experimental errors of the coupling constants were estimated from the quality of fitting data at all evolution times. Data for GB3 were collected at 25°C on 600 and 750 MHz Bruker DMX-model spectrometers equipped with triple-resonance, 3-axis pulsed field gradient probe-head. The NMR sample contained 9 mg of GB3 protein in 300 μL of $\text{H}_2\text{O}/\text{D}_2\text{O}$ (90:10), 50 mM sodium phosphate, pH 5.6. Measurements of $^2J_{\text{NC}'}$ were carried out using the quantitative J correlation scheme. Both the reference and the long-range correlation spectra were recorded as 128* 1024* data matrices, with acquisition times of 51 ms (t_1 , $^{13}\text{C}'$) and 52 ms (t_2 , $^1\text{H}^{\text{N}}$), with 16 scans per complex t_1 increment for the reference spectrum (1 h), and 600 scans (36 h) for the long-range correlation spectrum. Both reference and the long-range spectra were recorded using ^{15}N - $^{13}\text{C}'$ dephasing delays of 133.2 ms; but for the reference spectrum, the 180° $^{13}\text{C}'$ pulses were shifted to positions 16.6 ms prior to the midpoint of the dephasing delays, resulting in effective $J_{\text{NC}'}$ dephasing delays of 100 ms.

Parameters describing protein backbone geometry

The dihedral angles, Θ , ω_{-1} , ω were calculated directly from the atomic coordinates of the protein structures retrieved from the Protein Data Bank. In cases of multimodel structures, the model used is indicated in the figure and table captions.

Calculations on the model system

The model system chosen for DFT calculations was an Ala_3 tripeptide. The geometries of the structures were optimized completely in all internal degrees of freedom except for those angles that were specifically fixed for each calculation. We changed these angles in increments as indicated in the figures.

All DFT calculations employed the same procedures used in our previous work (Salvador et al. 2004). We used the Gaussian 03 suite of programs (Gaussian, Inc.) and the B3LYP functional. This method combines Becke's three-parameter functional (Becke 1993) with the nonlocal correlation provided by the correlation functional of Lee et al. (1988). We used B3LYP

alone, because we previously found no difference between the results of UB3LYP (unrestricted) and B3LYP (restricted) calculations for several examples. All calculations employed the D95(d,p) basis set, which we had determined to be adequate for ^{13}C - ^{15}N 3-bond scalar J -couplings in peptides (Salvador and Dannenberg 2004), because it replicated $^3J_{\text{NC}'}$ couplings predicted by other similar and larger basis sets. Nevertheless, for the scalar J -coupling calculations the basis set was completely decontracted to allow for better flexibility, especially for an improved description of the Fermi contact term (Helgaker et al. 1998; Deng et al. 2006). The coupling constants were calculated using the “spin–spin” option in Gaussian 03 and only for selected nuclei. This option calculates scalar couplings including contributions from all four Ramsey terms: the spin–dipole (SD) and both diamagnetic (DSO) and paramagnetic (PSO) spin–orbit terms, in addition to the FC term (Ramsey 1953; Helgaker et al. 2000; Sychrovsky et al. 2000; Barone et al. 2002).

Electronic supplemental material

The electronic supplemental material consists of one table (Table S1) with experimental $^2J_{\text{NC}'}$ coupling constants, and one table (Table S2) detailing experimental $^2J_{\text{NC}'}$ coupling constants in the reverse turns.

Acknowledgments

This research was supported by the Biomedical Research Technology Program, National Center for Research Resources through NIH Grant P41 RR02301 (to J.L.M.). J.J.D. thanks DURSI of the Generalitat de Catalunya for a visiting professorship at the Universitat de Girona. We thank Ad Bax for the GB3 sample.

References

- Ahn, H.-C., Juranic, N., Macura, S., and Markley, J.L. 2006. Three-dimensional structure of the water-insoluble protein crambin in dodecylphosphocholine micelles and its minimal solvent-exposed surface. *J. Am. Chem. Soc.* **128**: 4398–4404.
- Alexandrescu, A.T., Snyder, D.R., and Abildgaard, F. 2001. NMR of hydrogen bonding in cold-shock protein A and an analysis of the influence of crystallographic resolution on comparisons of hydrogen bond lengths. *Protein Sci.* **10**: 1856–1868.
- Bagno, A. 2000. Quantum chemical modeling of through-hydrogen bond spin–spin coupling in amides and ubiquitin. *Chemistry* **6**: 2925–2930.
- Barfield, M. 2002. Structural dependencies of interresidue scalar coupling $^3J_{\text{NC}'}$, and donor H-1 chemical shifts in the hydrogen bonding regions of proteins. *J. Am. Chem. Soc.* **124**: 4158–4168.
- Barfield, M. and Grant, D.M. 1962. Valence bond calculation of geminal spin–spin coupling constants in substituted methanes. *J. Chem. Phys.* **36**: 2054–2059.
- Barfield, M., Hruby, V.J., and Meraldi, J.P. 1976. The dependence of geminal H–H spin–spin coupling constant on ϕ and ψ angles of peptides in solution. *J. Am. Chem. Soc.* **98**: 1308–1314.
- Barone, V., Peralta, J.E., Contreras, R.H., and Snyder, J.P. 2002. DFT calculation of NMR J(FF) spin–spin coupling constants in fluorinated pyridines. *J. Phys. Chem. A* **106**: 5607–5612.
- Becke, A.D. 1993. Density-functional thermochemistry. III. The role of exact exchange. *J. Chem. Phys.* **98**: 5648–5652.
- Bouvignies, G., Bernado, P., Meier, S., Cho, K., Grzesiek, S., Bruschweiler, R., and Blackledge, M. 2005. Identification of slow correlated motions in proteins using residual dipolar and hydrogen-bond scalar couplings. *Proc. Natl. Acad. Sci.* **102**: 13885–13890.
- Chattopadhyaya, R., Meador, W.E., Means, A.R., and Quirocho, F.A. 1992. Calmodulin structure refined at 1.7 angstrom resolution. *J. Mol. Biol.* **228**: 1177–1192.
- Chou, J.J., Li, S.P., Klee, C.B., and Bax, A. 2001. Solution structure of Ca^{2+} -calmodulin reveals flexible hand-like properties of its domains. *Nat. Struct. Biol.* **8**: 990–997.
- Cordier, E. and Grzesiek, S. 1999. Direct observation of hydrogen bonds in proteins by interresidue $^3J_{\text{NC}'}$ scalar couplings. *J. Am. Chem. Soc.* **121**: 1601–1602.
- Cordier, E. and Grzesiek, S. 2002. Temperature-dependence of protein hydrogen bond properties as studied by high-resolution NMR. *J. Mol. Biol.* **317**: 739–752.
- Cordier, F., Rogowski, M., Grzesiek, S., and Bax, A. 1999. Observation of through-hydrogen-bond $^2J_{\text{HC}'}$ in a perdeuterated protein. *J. Magn. Reson.* **140**: 510–512.
- Cordier, F., Barfield, M., and Grzesiek, S. 2003. Direct observation of C^{α} - $\text{H}^{\alpha} \cdots \text{O}=\text{C}$ hydrogen bonds in proteins by interresidue $^3J_{\text{C}\alpha\text{C}'}$ scalar couplings. *J. Am. Chem. Soc.* **125**: 15750–15751.
- Corey, R.B. and Pauling, L. 1953. Fundamental dimensions of polypeptide chains. *Proc. R. Soc. Lond. B. Biol. Sci.* **141**: 10–20.
- Cornilescu, G., Hu, J.S., and Bax, A. 1999a. Identification of the hydrogen bonding network in a protein by scalar couplings. *J. Am. Chem. Soc.* **121**: 2949–2950.
- Cornilescu, G., Ramirez, B.E., Frank, M.K., Clore, G.M., Gronenborn, A.M., and Bax, A. 1999b. Correlation between $^3J_{\text{NC}'}$ and hydrogen bond length in proteins. *J. Am. Chem. Soc.* **121**: 6275–6279.
- Delaglio, F., Torchia, D., and Bax, A. 1991. Measurements of ^{15}N - ^{13}C J couplings in staphylococcal nuclease. *J. Biomol. NMR* **1**: 439–446.
- Deng, W., Cheeseman, J.R., and Frisch, M.J. 2006. Calculation of nuclear spin–spin coupling constants of molecules with first and second row atoms in study of basis set dependence. *J. Chem. Theory Comput.* **2**: 1028–1037.
- Derrick, J.P. and Wigley, D.B. 1994. The third IgG-binding domain from Streptococcal protein-G - an analysis by X-ray crystallography of the structure alone and in a complex with Fab. *J. Mol. Biol.* **243**: 906–918.
- Edison, A.S. 2001. Linus Pauling and the planar peptide bond. *Nat. Struct. Biol.* **8**: 201–202.
- Esposito, L., De Simone, A., Zagari, A., and Vitagliano, L. 2005. Correlation between ω and ψ dihedral angles in protein structures. *J. Mol. Biol.* **347**: 483–487.
- Gopinathan, M.S. and Narasimhan, P.T. 1971. Effect of lone-pair orientation on nuclear spin couplings. *Mol. Phys.* **22**: 473–481.
- Gronenborn, A.M., Filpula, D.R., Essig, N.Z., Achari, A., Whitlow, M., Wingfield, P.T., and Clore, G.M. 1991. A novel, highly stable fold of the immunoglobulin binding domain of streptococcal protein G. *Science* **253**: 657–661.
- Helgaker, T., Jaszunski, M., Ruud, K., and Gorska, A. 1998. Basis-set dependence of nuclear spin–spin coupling constants. *Theor. Chem. Acc.* **99**: 175–182.
- Helgaker, T., Watson, M., and Handy, N.C. 2000. Analytical calculation of nuclear magnetic resonance indirect spin–spin coupling constants at the generalized gradient approximation and hybrid levels of density-functional theory. *J. Chem. Phys.* **113**: 9402–9409.
- Hodsdon, M.E. and Cistola, D.P. 1997. Ligand binding alters the backbone mobility of intestinal fatty acid-binding protein as monitored by N-15 NMR relaxation and H-1 exchange. *Biochemistry* **36**: 2278–2290.
- Jeffrey, G.A., Ruble, J.R., McMullan, R.K., DeFrees, D.J., Binkley, J.S., and Pople, J.A. 1980. Neutron diffraction at 23 K and *ab initio* molecular-orbital studies of the molecular structure of acetamide. *Acta Crystallogr. B* **B36**: 2292–2299.
- Jelsch, C., Teeter, M.M., Lamzin, V., Pichon-Pesme, V., Blessing, R.H., and Lecomte, C. 2000. Accurate protein crystallography at ultrahigh resolution: Valence electron distribution in crambin. *Proc. Natl. Acad. Sci.* **97**: 3171–3176.
- Juranic, N. and Macura, S. 2001. Correlations among $^1J_{\text{NC}'}$ and $^3J_{\text{NC}'}$ coupling constants in the hydrogen-bonding network of human ubiquitin. *J. Am. Chem. Soc.* **123**: 4099–4100.
- Juranic, N., Ilich, P.K., and Macura, S. 1995. Hydrogen-bonding networks in proteins as revealed by the amide $^1J_{\text{NC}'}$ coupling-constant. *J. Am. Chem. Soc.* **117**: 405–410.
- Juranic, N., Moncrieffe, M.C., Likic, V.A., Prendergast, F.G., and Macura, S. 2002. Structural dependencies of $^3J_{\text{NC}'}$ scalar coupling in protein H-bond chains. *J. Am. Chem. Soc.* **124**: 14221–14226.
- Juranic, N., Macura, S., and Prendergast, F.G. 2003. H-bonding mediates polarization of peptide groups in folded proteins. *Protein Sci.* **12**: 2633–2636.
- Juranic, N., Atanasova, E., Moncrieffe, M.C., Prendergast, F.G., and Macura, S. 2005. Calcium-binding proteins afford calibration of dihedral-angle dependence of $^3J_{\text{NC}'}$ gamma coupling constant in aspartate and asparagine residues. *J. Magn. Reson.* **175**: 222–225.

- Juranić, N., Atanasova, E., Streiff, J.H., Macura, S., and Prendergast, F.G. 2007. Solvent-induced differentiation of protein backbone hydrogen bonds in calmodulin. *Protein Sci.* **16**: 1329–1337.
- Karplus, M. 1963. Vicinal proton coupling in nuclear magnetic resonance. *J. Am. Chem. Soc.* **85**: 2870–2871.
- Karplus, P.A. 1996. Experimentally observed conformation-dependent geometry and hidden strain in proteins. *Protein Sci.* **5**: 1406–1420.
- Kuboniwa, H., Tjandra, N., Grzesiek, S., Ren, H., Klee, C.B., and Bax, A. 1995. Solution structure of calcium-free calmodulin. *Nat. Struct. Biol.* **2**: 768–776.
- Lee, C., Yang, W., and Parr, R.G. 1988. Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density. *Phys. Rev. B* **37**: 785–789.
- MacArthur, M.W. and Thornton, J.M. 1996. Deviations from planarity of the peptide bond in peptides and proteins. *J. Mol. Biol.* **264**: 1180–1195.
- Markwick, P.R., Sprangers, R., and Sattler, M. 2003. Dynamic effects on J -couplings across hydrogen bonds in proteins. *J. Am. Chem. Soc.* **125**: 644–645.
- Pérez, C., Löhner, F., Rüterjans, H., and Schmidt, J.M. 2001. Self-consistent Karplus parametrization of 3J couplings depending on the polypeptide side-chain torsion χ_1 . *J. Am. Chem. Soc.* **123**: 7081–7093.
- Ramsey, N.F. 1953. Polarizability of the Deuteron. *Phys. Rev.* **91**: 1162–1164.
- Salvador, P. and Dannenberg, J.J. 2004. Dependence upon basis sets of trans hydrogen-bond ^{13}C - ^{15}N 3-bond and other scalar J -couplings in amide dimers used as peptide models. A density functional theory study. *J. Phys. Chem. B* **108**: 15370–15375.
- Salvador, P., Kobko, N., Wieczorek, R., and Dannenberg, J.J. 2004. Calculation of trans-hydrogen-bond ^{13}C - ^{15}N three-bond and other scalar J -couplings in cooperative peptide models. A density functional theory study. *J. Am. Chem. Soc.* **126**: 14190–14197.
- Salvador, P., Wieczorek, R., and Dannenberg, J.J. 2007. Direct calculation of trans-hydrogen -bond ^{13}C - ^{15}N 3-bond J -couplings in entire polyaniline helices. A density functional theory study. *J. Phys. Chem. B* **111**: 2398–2403.
- Scapin, G., Gordon, J.I., and Sacchettini, J.C. 1992. Refinement of the structure of recombinant rat intestinal fatty acid-binding apoprotein at 1.2 Å resolution. *J. Biol. Chem.* **267**: 4253–4269.
- Schumacher, M.A., Crum, M., and Miller, M.C. 2004. Crystal structures of apocalmodulin and an apocalmodulin/SK potassium channel gating domain complex. *Structure* **12**: 849–860.
- Solkan, V.N. and Bystrov, V.F. 1975. Theoretical calculations of spin–spin interaction in the NMR spectra of peptide fragment. *Russ. Chem. Bull.* **23**: 95–100.
- Sychrovsky, V., Grafenstein, J., and Cremer, D. 2000. Nuclear magnetic resonance spin–spin coupling constants from coupled perturbed density functional theory. *J. Chem. Phys.* **113**: 3530–3547.
- Swain, A.L., Kretsinger, R.H., and Amma, E.L. 1989. Restrained least squares refinement of native (calcium) and cadmium-substituted carp parvalbumin using X-ray crystallographic data at 1.6 Å resolution. *J. Biol. Chem.* **264**: 16620–16628.
- Teeter, M.M. 1984. Water structure of a hydrophobic protein at atomic resolution. *Proc. Natl. Acad. Sci.* **81**: 6014–6018.
- Tjandra, N., Feller, S.E., Pastor, R.W., and Bax, A. 1995a. Rotational diffusion anisotropy of human ubiquitin from ^{15}N NMR relaxation. *J. Am. Chem. Soc.* **117**: 12562–12566.
- Tjandra, N., Kuboniwa, H., Ren, H., and Bax, A. 1995b. Rotational-dynamics of calcium-free calmodulin studied by ^{15}N NMR relaxation measurements. *Eur. J. Biochem.* **230**: 1014–1024.
- Ulmer, T.S., Ramirez, B.E., Delaglio, F., and Bax, A. 2003. Evaluation of backbone proton positions and dynamics in a small protein by liquid crystal NMR spectroscopy. *J. Am. Chem. Soc.* **125**: 9179–9191.
- Vijay-Kumar, S., Bugg, C.E., and Cook, W.J. 1987. Structure of ubiquitin refined at 1.8 Å resolution. *J. Mol. Biol.* **194**: 531–544.
- Walter, J.A. and Wright, J.L.C. 1979. ^{13}C - ^{15}N spin-spin coupling constants of amides: H-bonding effects in a cyclohexapeptide. *Tetrahedron Lett.* **41**: 3909–3912.
- Yamada, S., Nakamura, M., and Kawauchi, I. 1997. ^{13}C - ^{15}N coupling constants in a series of twisted amides: Relationships with C(O)-N twist angles. *Chem. Commun.* 885–886.