COMMENTARY

NMR provides evidence for dynamic hydrogen bonding in proteins

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Despite the importance of hydrogen bonds for protein folding and stabilization of secondary and tertiary structure, as well as processes governing intermolecular recognition and molecular signaling, the basic structural and dynamic factors dictating their in situ stability and energetics remain poorly characterized. This is in large part due to the lack of information from most crystallographic structures concerning the positions of protons, and therefore the geometry of the hydrogen bond. Over the last decade the discovery and subsequent development of new experimental methods for the detection of scalar couplings across hydrogen bonds (Cordier and Grzesiek 1999; Cornilescu et al. 1999) have provided essential tools for studying the conformational behavior of hydrogen bonds in proteins in solution. *Trans*-hydrogen bond scalar couplings (HBSCs) between the donor amide nitrogen and the acceptor carbonyl carbon have been shown to be commonly measurable via hydrogen bonds present in protein secondary structural elements. In addition to the orientational information common to covalent ^{3}J scalar couplings, often used to define dihedral angles in peptide chains, the value of the measured HBSC is of course determined by the length of the hydrogen bond. Due to this strong dependence on the relative geometry of the hydrogen bonding partners (Grzesiek et al. 2004) HBSCs offer precise structural information with which to study hydrogen bonded pairs in proteins.

Equally importantly for the understanding of protein function and stability, HBSCs offer a fascinating insight into the dynamic nature of hydrogen bonding interactions. Experimentally measured HBSCs average over all conformations sampled within the characteristic timescale of the measured coupling (defined by the inverse of this value), and are therefore sensitive to dynamic events occurring up to the 100-msec range. Protein motions occurring on these slower timescales are of particular interest because many biologically important processes, such as enzymatic catalysis, signal transduction, ligand binding, allosteric regulation, and early folding events are expected to occur in this range.

Residual dipolar couplings (RDCs), that become measurable under conditions of partial molecular alignment, report on bond–vector orientation relative to a common molecular frame, and are therefore highly complementary to HBSCs that have a local geometric dependence. RDCs are also sensitive to motions up to the same time range as HBSCs, and have recently been widely used to probe the extent and nature of slow motions in proteins (Prestegard et al. 2000). The orientational complementarity of RDCs and HBSCs was exploited in a recent study of the small protein G, where RDCs were used to reveal a complex and heterogeneous distribution of slow motions along the peptide chain. Large strands of the protein, including the α-helix and some loop regions, exhibited ostensibly identical backbone motions to those determined using spin relaxation, sensitive to fast motions on the picosecond–nanosecond timescale, indicating that negligible additional motions in the nanosecond–millisecond time range were occurring. In other parts of the molecule however, significant slower motions were detected, in particular in one loop and the β-sheet, where a complex network of dynamics was revealed, with similarly positioned amino acids in adjacent strands apparently experiencing similar modes and amplitudes. HBSCs were used in this case to provide strong evidence that the observed dynamics in the β-sheet involved correlated motions between hydrogen bonded peptide planes (Bouvignies et al. 2005). Interestingly an alternance of dynamic modes was observed along the β-strands in this study, correlating with alternating exposure of amino acid side chains along the strands, and leading to speculation that the hydrophobic core somehow stabilizes the correlated dynamics across the sheet. A similar observation has been made in β-strands in the protein ubiquitin on the basis of analysis of a very large number of NH RDCs (Lakomek et al. 2005). This kind of motion may also be related to the

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so-called ''backrub motion'' observed by Richardson and coworkers, who performed a detailed analysis of observable conformational degrees of freedom in high-resolution crystal structures and found a similar alternating mobility of peptides to be present in β-strands throughout the structural database (Davis et al. 2006).

In a recent study, Juranić and coworkers (this issue) have made related observations, correlating measured HBSCs in *apo* and *holo* Calmodulin with differential local dynamics and solvent exposure. Prediction of HBSCs on the basis of solid state crystal and solution state NMR structures was found to accurately reproduce only half of the experimentally measured values. The remaining values that were predicted to be above the detection threshhold on the basis of local conformation were not detected, including HBSCs between partners in the α-helices. Further inspection revealed that only HBSCs in the vicinity of amino acids containing anchoring side chains involved in hydrophobic interactions were detected. Sites that were susceptible to solvent accessibility were also often found to coincide with undetected HBSCs. Indeed, in the case of interaction between two helices in calmodulin, detected HBSCs are clearly localized on the side of both helices, that is, facing the hydrophobic contact.

These observations lead the authors to analyze HBSCs from six other proteins, and to divide the couplings into two ranges based on the amplitude of the measured coupling. Low-valued couplings (absolute values of <0.1 Hz) are found to show no correlation with the hydrogen bonding parameters, while the higher values couplings (absolute value >0.2 Hz) show clear correlations. In addition, in the case of calmodulin, the authors compare experimental RDCs with the NH vectors extracted from the X-ray crystallographic structure. They are able to show a clear improvement in the reproduction of RDCs when using only those sites that belong to the class exhibiting higher HBSCs, compared to the RDCs corresponding to the class exhibiting lower values HBSCs. This observation very nicely illustrates the correlation between dynamically averaged HBSCs and the accuracy of the static structural model as a valid representation of the average conformation in solution.

More generally, these results underline the importance of addressing the crucial question of dynamic averaging of NMR parameters, and in particular, of whether static conformations represented by X-ray crystallographic, or high-resolution NMR studies, are actually representative of an average solution conformation, and if so, under which circumstances these coordinates can be used to better understand conformationally averaged experimental data. In the case of *trans*-hydrogen bond scalar couplings the authors of this study have exploited a promising and sensitive probe of molecular conformation and dynamics.

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