J|A|C|S
COMMUNICATIONS

Published on Web 08/25/2004

Site-Specific Variations of Carbonyl Chemical Shift Anisotropies in Proteins

Phineus R. L. Markwick* and Michael Sattler*

EMBL, Meyerhofstrasse 1, 69117 Heidelberg, Germany

Received April 14, 2004; E-mail: sattler@embl.de; markwick@embl.de

Chemical shift anisotropies (CSA) potentially contain a wealth of information concerning molecular structure and dynamics of biomacromolecules. Knowledge of the magnitude and orientation of the $^{13}C'$ chemical shift tensor and its variation from one site to another is not only essential for the accurate analysis and interpretation of NMR relaxation data used to probe anisotropic backbone dynamics, but also offers the potential for improving the quality of biomolecular structures. Information concerning the orientation and magnitude of the C′ shift tensor has primarily been obtained from solid-state NMR magic-angle spinning (MAS) measurements.[1] In solution-state NMR, the observation of residual chemical shift anisotropy in partially aligned media has been exploited to investigate the magnitude of the C′ CSA,[2] while in isotropic solution, a complementary set of C′ CSA/DD cross-correlated relaxation data has been used to estimate the C′ CSA.[3] A common problem associated with all these methods is the treatment of internal molecular dynamics and the effect that this has on the measured observables. As a result, reported values for the absolute magnitude of the C′ CSA vary from 120 to 180 ppm (see Supporting Information). Quantum chemistry studies using model systems have shown that the C′ CSA depends on numerous parameters, including backbone geometry and hydrogen bonding.[4] However, the accurate calculation of peptide backbone chemical shift tensors is very challenging: The CSA is extremely sensitive to the local molecular environment and an accurate calculation of the C′ CSA requires the inclusion of all residues in or partially within a 5.0 Å radius of the carbonyl group of interest. Furthermore, there are considerable variations of the CSA due to local molecular dynamics, and a large number of calculations are required to accurately sample the local conformational space.[4] The absolute value of the CSA is also strongly dependent on the size of the basis set used in the quantum chemistry calculations.

In this work, we combine classical molecular dynamics (MD) simulation with a novel method combining density functional theory (DFT) gauge including atomic orbital (GIAO),[5] two-layer ONIOM,[6] and complete basis set (CBS)[7] extrapolation for the prediction of carbonyl chemical shift tensors. We studied 12 carbonyl groups in the SMN Tudor domain, a 55-residue β-barrel fold, for which well-defined NMR structures are available.[8] Our initial objectives were to ascertain the absolute magnitude of the C′ CSA and to investigate how "site-specific" the C′ chemical shift tensor is. We then formulated a model based on the results of our calculations and estimated all carbonyl chemical shift tensors in the SMN Tudor domain. Finally, we combined our C′ CSA model with the MD simulation to show that an accurate prediction of transverse C′/N−H cross-correlated relaxation (CCR) rates can be obtained.

Starting from the NMR structures,[8] three classical molecular dynamics simulations in explicit water solvent were performed using the AMBER program suite.[9] After an initial equilibration procedure, a 10.0 ns MD run was performed at 300 K, 1 bar, using periodic boundary conditions with weak temperature and pressure coupling. Atomic coordinates across the trajectory were saved every 1.0 ps. The MD simulations were used to sample the conformational space and later to extract the relevant spectral density functions required in the calculation of CSA/DD cross-correlated relaxation rates. In total, 12 carbonyl shielding tensors were calculated for residues in β-sheet, loop, and the 3_{10} helix regions of the Tudor domain. For each carbonyl carbon, 100 protein structures were extracted to sample the local molecular geometry about the carbonyl of interest, including intramolecular H-bonds and hydrogen-bonding interactions to solvent water molecules. A molecular fragment containing all residues and water molecules in a 5.0 Å radius sphere about the carbonyl carbon was extracted, and broken bonds were saturated.

A detailed description of the GIAO/ONIOM2/CBS chemical shift calculation is provided in the Supporting Information. In brief, for each molecular fragment an initial partial geometry optimization was performed. DFT NMR shielding tensor calculations were performed using a two-layer ONIOM approach with the B3PW91 functional. The upper layer included just the saturated backbone atoms about the carbonyl carbon: $N^{(i)}(H_2)-C\alpha^{(i)}(H_2)-C^{(i)}(O)-N^{(i+1)}(H)-C\alpha^{(i+1)}(H_3)$. Using standard ONIOM methodology, we calculated the GIAO ONIOM2(cc-PVTZ:6-31G*) and ONIOM2-(cc-PVQZ:6-31G*) shielding tensor components. Finally, we performed a two-point CBS extrapolation from these ONIOM shielding tensors. The resulting shielding tensor components were then averaged over the 100 sample structures. All calculations were performed using the Gaussian 98 program suite.[10]

Results for the 12 shift tensors are shown in Figure 1. As can be seen, the δ_{11} and δ_{33} components are ostensibly fixed, while the δ_{22} component varies linearly with the isotropic chemical shift. The C′ CSA is given by:

$$\mathrm{CSA} = \Delta\delta\,(1 + \Delta\eta^2/3)^{1/2}$$

where

$$\Delta\delta = \delta_{11} - (\delta_{22} + \delta_{33})/2, \quad \Delta\eta = (\delta_{22} - \delta_{33})/(\delta_{11} - \delta_{\mathrm{iso}})$$

The C′ CSA varies from 140 to 146 ppm, which is in excellent agreement with the results of Cornilescu and Bax.[2] A simple model for the carbonyl CSA can be formulated: In this model, $\delta_{11} = 247$ ppm, $\delta_{33} = 85$ ppm, and δ_{22} is given by:

$$\delta_{22} = 3\,\delta_{\mathrm{iso}} - 332\ \mathrm{ppm}$$

The relationship between the magnitude of the δ_{22} component and the isotropic chemical shift is in agreement with previous observations from Oas[1b] and Asakawa.[1f]

On closer inspection of the results, we see that the δ_{11} and δ_{22} components deviate more from this model than the δ_{33} component. We consider that this is due to the effect of direct hydrogen bonding to the carbonyl group. The absolute magnitude of the $^{3h}J_{NC'}$ scalar coupling provides a good measure for the strength of the associated hydrogen bond.[12] Therefore, we calculated the relative change in the shift tensor components as a function of $^{3h}J_{NC'}$ (Supporting

10.1021/ja047859r CCC: $27.50

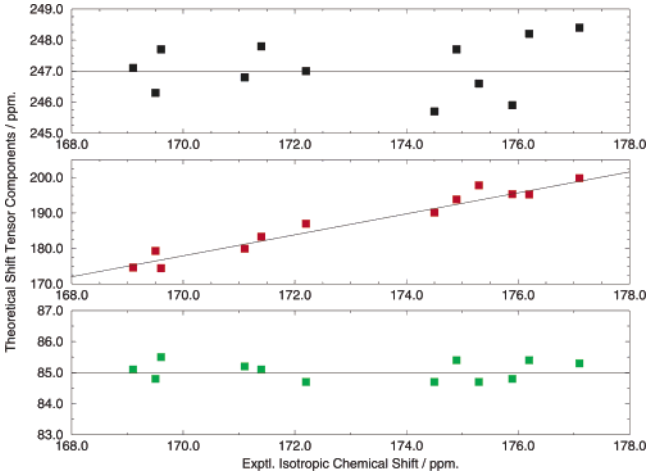

Figure 1. Extended DFT calculated carbonyl chemical shift tensors. The δ_{11} (black squares) and δ_{33} (green squares) components are ostensibly invariant from one residue to the next. The δ_{22} component (red squares) varies linearly with the experimental isotropic chemical shift.

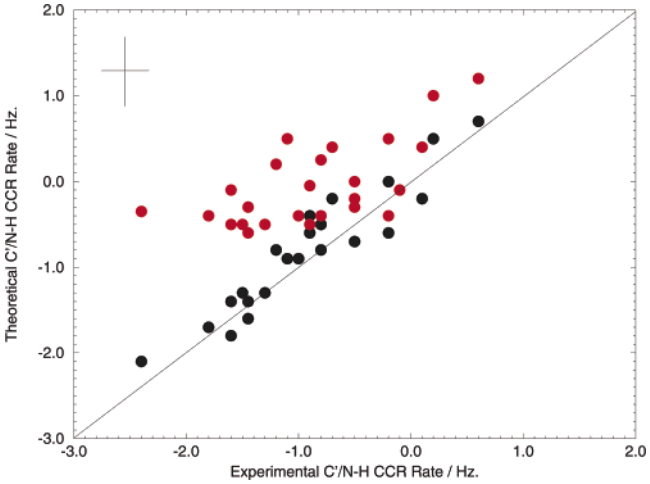

Figure 2. Predicted transverse C′/N−H CSA/DD cross-correlated relaxation rates using our site-specific CSA model (black circles) and a fixed CSA from Teng and Cross[1a] (red circles). Experimental relaxation rates were measured as described previously.[11]

Information). We observe that the effect of hydrogen bonding decreases the magnitude of δ_{11} and increases the magnitude of δ_{22} by up to 3 ppm and is linearly proportional to the magnitude of the $^{3h}J_{NC'}$ coupling. No effect is seen for the magnitude of the δ_{33} component. As a result, hydrogen bonding causes a small change ($\leq$3 ppm) in the C′ CSA but, notably, has a negligible effect on the isotropic C′ chemical shift. For solvent-exposed carbonyls located on the surface of the protein, we observe that transient hydrogen bonding to water molecules results in a similar but small (1 ppm) average change in the δ_{11} and δ_{22} components.

Using the CSA model defined above, we calculated transverse C′/N−H CCR rates, which are extremely sensitive to variations in the δ_{22} component. The effect of local anisotropic dynamics is included in the spectral density functions, which were calculated from cross-correlated correlation functions derived from the MD simulation. In Figure 2 we show the results for the transverse C′/N−H CCR rate using our "site-specific" CSA model, and the CSA determined by Teng and Cross.[1a] A dramatic improvement in the correlation between the theoretical and experimental rates is observed. Similar improvements are also obtained for ubiquitin (Supporting Information).

In conclusion, we have combined a novel GIAO/ONIOM2/CBS method with MD simulation to calculate carbonyl chemical shift tensors. We observe site-specific variations of the C′ CSA with values ranging between 140 and 146 ppm. This small range in the C′ CSA is deceptive, as we notice that in particular the δ_{22} component of the shift tensor varies significantly (up to 30 ppm) and is linearly proportional to the isotropic chemical shift. Direct hydrogen bonding to the carbonyl group causes a small change in the C′ CSA and has a negligible effect on the isotropic chemical shift. A simple model for the prediction of the C′ CSA based solely on the isotropic chemical shift is presented. The validity of the C′ CSA model is demonstrated by the accurate prediction of the C′/N−H cross-correlated relaxation rate.

Acknowledgment. This work was supported by the DFG and the EU. P.M. acknowledges an EMBO Fellowship. We thank B. Brutscher for providing experimental CCR rates for ubiquitin.

Supporting Information Available: C′ CSA data, MD protocol, DFT shift tensor calculation, and CCR data for ubiquitin. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Teng, Q.; Iqbal, M.; Cross, T. A. *J. Am. Chem. Soc.* **1992**, *114*, 5312−5321. (b) Oas, T. G.; Hartzell, C. J.; McMahon, T. J.; Drobny, G. P.; Dahlquist, F. W. *J. Am. Chem. Soc.* **1987**, *109*, 5956−5962. (c) Lumsden, M. D.; Wasylishen, R. E.; Eichele, K.; Schindler, M.; Penner, G. H.; Power, W. P.; Curtis, R. D. *J. Am. Chem. Soc.* **1994**, *116*, 1403−1413. (d) Wei, Y.; Lee, D.-K.; Ramamoorthy, A. *J. Am. Chem. Soc.* **2001**, *123*, 6118−6126. (e) Duncan, T. M. *A Compilation of Chemical Shift Anisotropies*; The Farragut Press: Chicago, 1990. (f) Asakawa, N.; Kuroki, S.; Kurosu, H.; Ando, I.; Shoji, A.; Ozaki, T. *J. Am. Chem. Soc.* **1992**, *114*, 3261−3265.

(2) Cornilescu, G.; Bax. A. *J. Am. Chem. Soc.* **2000**, *122*, 10143−10145.

(3) (a) Pang. Y.; Zuiderweg, E. R. P. *J. Am. Chem. Soc.* **2000**, *122*, 4841−4842. (b) Dayie, K. T.; Wagner, G. *J. Am. Chem. Soc.* **1997**, *119*, 7797−7806.

(4) (a) Sitkoff, D.; Case, D. A. *Prog. Nucl. Magn. Reson. Spectrosc.* **1998**, *32*, 165−190. (b) Xu, X.-P.; Case, D. A. *Biopolymers* **2002**, *65*, 408−423. (c) Scheurer, C.; Skrynnikov, N. R.; Lienin, S. F.; Straus, S. K.; Brüschweiler, R.; Ernst, R. R. *J. Am. Chem. Soc.* **1999**, *121*, 4242−4251. (d) Lienin, S. F.; Bremi, T.; Brutscher, B.; Brüschweiler, R.; Ernst, R. R. *J. Am. Chem. Soc.* **1998**, *120*, 9870−9879.

(5) (a) Ditchfield, R. *Mol. Phys.* **1974**, *27*, 789−807. (b) Wolinski, K.; Hilton, J. F.; Pulay, P. *J. Am. Chem. Soc.* **1990**, *112*, 8251−8260.

(6) Karadakov, P. B.; Morokuma, K. *Chem. Phys. Lett.* **2000**, *317*, 589−596.

(7) (a) Fellar, D.; Peterson, K. A. *J. Mol. Struct. (Theochem.)* **1997**, *400*, 69−92. (b) Peterson, K. A.; Dunning, T. H., Jr. *J. Mol. Struct. (Theochem.)* **1997**, *400*, 93−117. (c) Kupka, T.; Ruscic, B.; Botto, R. E. *Solid State Nucl. Magn. Reson.* **2003**, *23*, 145−167.

(8) Selenko, P.; Sprangers, R.; Stier, G.; Bühler, D.; Fischer, U.; Sattler, M. *Nat. Struct. Biol.* **2001**, *8*, 27−31.

(9) Case, D. A.; Pearlman, D. A.; Caldwell, T. W.; Cheatham, T. E., III; Ross, W. S.; Simmerling, C. L.; Darden, T. A.; Marz, K. M.; Stanton, R. V.; Cheng, A. L.; Vincent, J. J.; Crowley, M.; Tsui, V.; Radmer, R. J.; Duan, Y.; Pitera, J.; Massova, I.; Seibel, G. L.; Singh, U. C.; Weiner, P. K.; Kollman, P. A. *AMBER 6*; University of California: San Fransisco, CA, 1999.

(10) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A., Jr.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B. G.; Chen, W.; Wong, M. W.; Andres, J. L.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. *Gaussian 98*, revision A.11; Gaussian, Inc.: Pittsburgh, PA, 1998.

(11) (a) Brutscher, R.; Skrynnikov, N. R.; Bremi, T.; Brüschweiler, R.; Ernst, R. R. *J. Magn. Reson.* **1998**, *130*, 346−351. (b) Carlomagno, T.; Griesinger, G. *J. Magn. Reson.* **2000**, *144*, 280−287.

(12) (a) Barfield, M. *J. Am. Chem. Soc.* **2002**, *124*, 4158−4168. (b) Markwick, P. R. L.; Sprangers, R.; Sattler, M. *J. Am. Chem. Soc.* **2003**, *124*, 644−645.

JA047859R