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Chemical Shifts in Biochemical Systems

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1 INTRODUCTION

The purpose of this article is to outline briefly recent progress in understanding chemical shifts in biochemical systems –proteins, nucleic acids, carbohydrates, and lipids. The chemical shift is probably the most sensitive spectroscopic probe of local structure and environment, but until quite recently it has been quite refractory to detailed analysis, at least in macromolecules. In small molecules, this sensitivity manifests itself in isotope shifts, temperature dependence, gas-to-liquid shifts, and solvent shifts. In macromolecules, sensitivity is manifested as chemical shift nonequivalencies. The NMR chemical shift in biological systems contains a wealth of encoded structural information, and in order to decode this information it is first necessary to be able to predict chemical shifts from known structures.

From a theoretical standpoint, insights gained in studying chemical shifts in biological systems should also greatly help in testing, modifying, and improving present theories of the chemical shift. For example, proteins provide a unique test case for investigating the dependencies of the chemical shift on factors such as torsion angles, hydrogen bonding, and electrostatics. In sharp contrast, gas phase studies of small molecules would not be capable of yielding the torsional dependencies of the chemical shift. This is because variable-temperature measurements, in which the torsional effects would be visible, also include significant contributions from centrifugal distortion, and, in addition, a change in one torsion angle may significantly change the overall shape of a small molecule, and consequently the way it interacts with its environment. Proteins, on the other hand, do not require variable-temperature studies, since sites which differ only in torsion angles are already present. Hence, the ability to interpret and reproduce chemical shift nonequivalencies in biological systems should not only be useful for structure elucidation, but is also of fundamental importance for the evaluation, development, and design of methods for chemical shift computation in general.

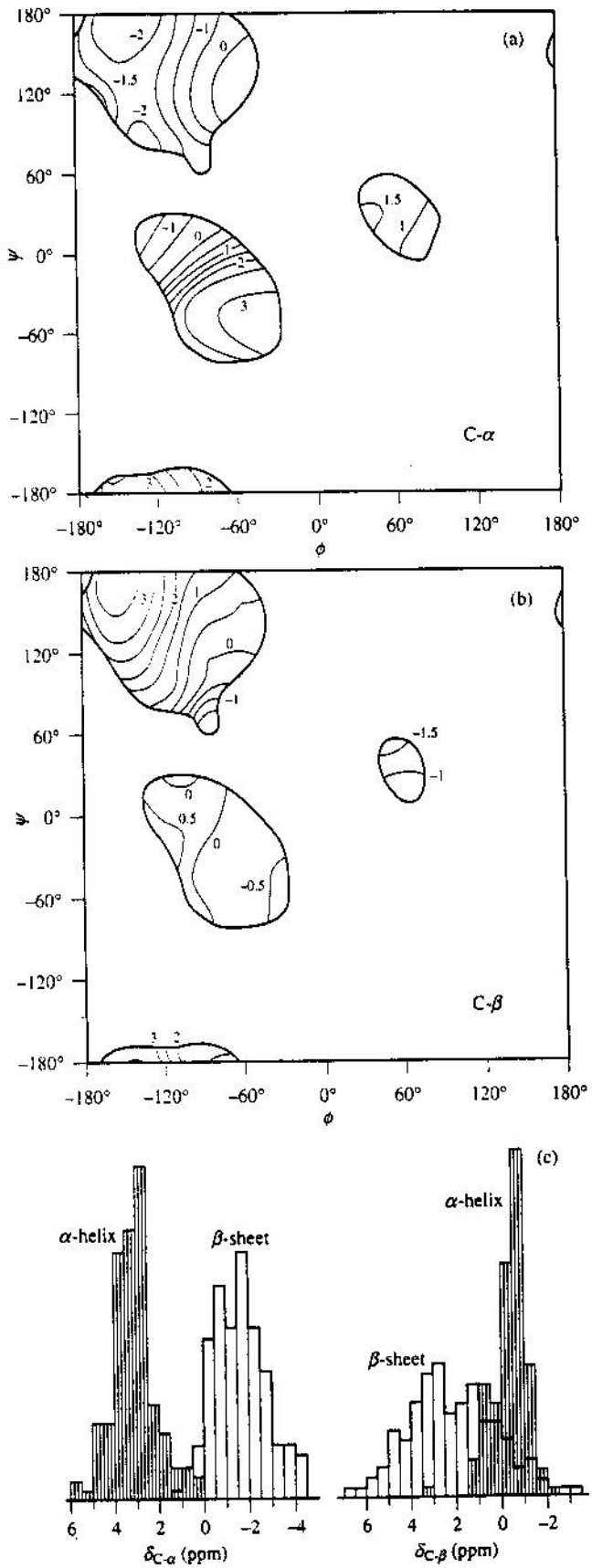
Chemical shift nonequivalencies due to folding have been known in proteins for more than two decades. McDonald and Phillips[1] first observed that several residues in hen egg white lysozyme could be resolved in ^{1}H NMR, and this was then followed by Allerhand et al. who observed a ~6 ppm range for the ^{13}C chemical shift of C-γ of the six tryptophan residues in hen egg white lysozyme.[2] Although such chemical shift inequivalencies have made multidimensional NMR studies of proteins possible,[3,4] understanding of the origins of such chemical shift nonequivalencies remained a very major challenge until recently, when a first-principles reproduction of the NMR spectra of the heavier nuclei (^{13}C, ^{15}N, and ^{19}F) in proteins was demonstrated to be feasible.[5] Here, we trace the development of NMR chemical shift interpretations in biological systems, starting from the discovery of empirical correlations between shift and structure in proteins, up to the present ab initio interpretation of protein, nucleic acid, and carbohydrate chemical shifts, and the derivation of structure from shielding.

2 EMPIRICAL SHIFT–STRUCTURE CORRELATIONS IN PROTEINS

The earliest attempts at unraveling the relationship between secondary structure and chemical shifts were focused on H-α.[6–8] Due to the small chemical shift range and the sensitivity of ^{1}H shielding to not only electrostatic effects but also magnetic factors, such as ring currents and the anisotropy of the diamagnetic susceptibility of peptide groups, only general trends were found between structure and shielding.[9,10] In contrast to ^{1}H NMR, the heavier nuclei exhibit a much wider chemical shift range, rendering such magnetic effects of minor importance. Hence, ^{13}C and ^{15}N nuclei offer more promise, and considerable efforts were made in interpreting these shifts as soon as they became available.

In early work, Kricheldorf et al.[11] and Saito et al.[12] showed that ^{13}C chemical shifts in peptides were mainly influenced by the torsion angles ϕ and ψ. Theoretical attempts involving a semiempirical finite perturbation–incomplete neglect of differential overlap (FPT–INDO) method aimed at reproducing these observations were somewhat successful in demonstrating the nonequivalency between helical and sheet residues,[13] but the calculated differences were much smaller than the range observed experimentally. With an increasing number of assigned C-α and C-β resonances in various proteins—bovine pancreatic trypsin inhibitor (BPTI), calmodulin, interleukin-1β

For list of General Abbreviations see end-papers



and staphylococcal nuclease—Spera and Bax introduced the first empirical ϕ, ψ chemical shift surface for C-α and C-β using known X-ray data,[14] shown in Figures 1(a) and (b). Figures 1(c) shows histograms of the secondary shift distribution in α-helical and β-sheet residues. From the presently available database of ^{13}C chemical shifts it is clear that C-α resonances of helical sites are deshielded compared with those residues which occur in sheet regions. The opposite trend is evident for C-β, where helical sites are more shielded.[14] Such empirical observations have found use in the chemical shift index,[15] which permits prediction of helical and sheet structure, based on C-α C-β, C°, and H-α shifts.

Considerable attention was also given early on to ^{15}N shifts in proteins.[16,17] However, the problem of whether or not ^{15}N chemical shifts can provide information regarding local structure remained unanswered. Glushka et al. then noticed a correlation between ^{15}N chemical shifts and the torsion angle ψ_{i-1} for residues in sheet regions in BPTI and apamin, but no other structural or 'interaction' features were found.[18] More recently, using a much larger database (consisting of 14 proteins and 1477 ^{15}N chemical shifts), Le and Oldfield[19] found a better empirical correlation between the peptide ^{15}N chemical shift and the torsion angles ϕ_i and ψ_{i-1}, indicating that there is indeed a significant relationship between ^{15}N chemical shifts and secondary structure. The larger number of points permitted study of individual amino acid shift surfaces. Figures 2(a)–(c) show the agreement obtained between experimentally obtained ^{15}N chemical shifts and those derived from the empirically fitted individual shift surfaces, for aspartic acid, lysine, and valine. The presence of an intrinsic relationship between ^{15}N chemical shifts and secondary structure becomes evident from these studies. However, it can also be inferred that there are other factors, such as electrostatic interactions, side-chain conformation, and hydrogen bonding, which all potentially influence ^{15}N shielding. The side chain of valine, for example, introduces an additional angle χ^1, which can modify the ^{15}N chemical shift behavior, since the presence of a substituent at the β-carbon site introduces a *γ-gauche* effect, as noted earlier by Tonelli.[20] In order to take this into account, a much larger database would be needed in order to create separate shift surfaces for each of the three common χ^1 conformers. Empirical database approaches are thus useful in indicating trends, but it would clearly be desirable to be able to predict shifts accurately using first principles or ab initio approaches. For example, the ^{15}N-valine χ^1-torsional problem is one which can easily be solved if the torsional dependencies can be derived from first principles. As demonstrated recently, this is now possible.[15] In addition, sensitivity to side-chain conformation is not exclusive to ^{15}N, since ^{13}C shifts for C-α and C-β sites are also expected to exhibit a χ^1 dependence. This, however,

Figure 1 Secondary chemical shift surfaces $\Delta\delta(\phi,\psi)$ and secondary chemical shift histograms for C-α and C-β sites in proteins. (a) Empirical secondary chemical shift surface for C-α nuclei in proteins; (b) as in part (a) but for C-β; (c) histogram showing the distribution of observed secondary shifts for C-α and C-β. (Reproduced by permission of the American Chemical Society from S. Spera and A. Bax, *J. Am. Chem. Soc.*, 1991, **113**, 5490)

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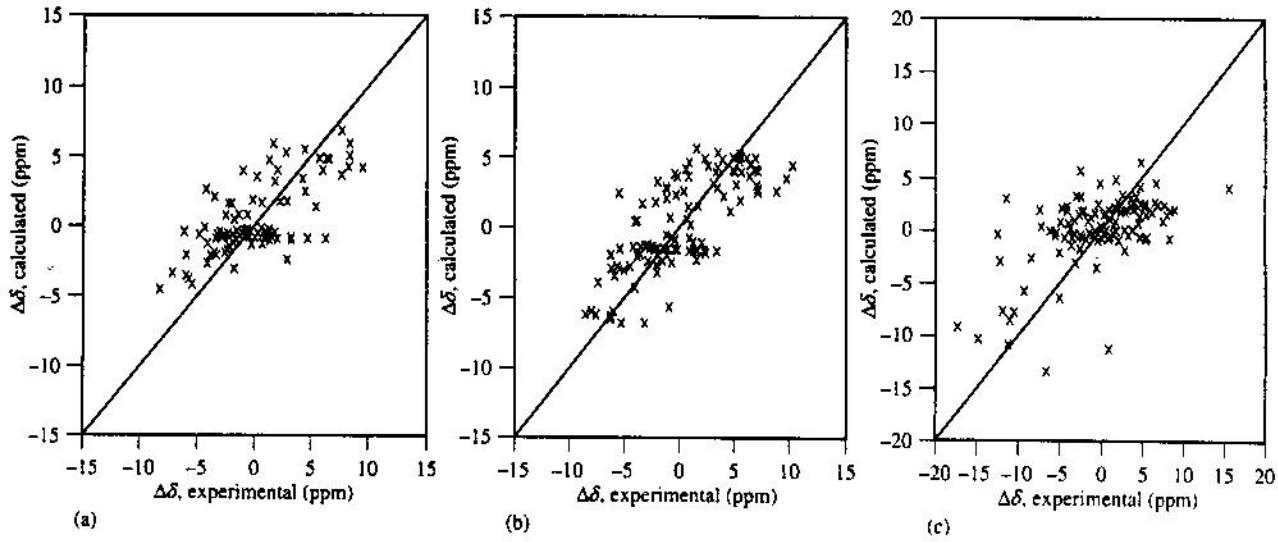

Figure 2 Correlation between experimental secondary ^{15}N chemical shifts and those computed from empirically fitted individual secondary chemical shift surfaces. (a) Aspartic acid; (b) lysine, and (c) valine. (Reproduced by permission of ESCOM Science Publishers BV from H. Le and E. Oldfield, *J. Biomol. NMR*, 1994, 4, 341)

would be difficult to see with only a small database of assigned ^{13}C shifts. Ab initio methods therefore clearly have an advantage over empirical approaches since chemical shift surfaces for individual amino acids, as well as for various χ^1 conformers, can in principle be evaluated.

With the rapidly growing database of ^1H chemical shifts,[21] there has been renewed interest in using ^1H NMR in protein structure studies.[22–27] These studies have taken advantage of the availability of large numbers of ^1H chemical shifts to relate chemical shifts to structure. In addition to the correlation found between secondary structure and the chemical shifts of H-α and H-N, the large amount of spectroscopic information available has also permitted a breakdown of ^1H shielding into electrostatic and peptide magnetic anisotropy effects. Thus, the coefficients needed in order to evaluate electrostatic contributions from nearby polar groups, as well as the anisotropy effects arising from peptide bonds, have been derived empirically. The complexity and number of factors that affect ^1H chemical shifts, however, make a direct chemical shift to structure route very difficult, although structure refinement using H-α shifts has promise.[30]

3 THE AB INITIO APPROACH TO PROTEIN SHIELDING

Although empirical and semiempirical approaches for ^1H NMR are useful for analyzing secondary structures, these approaches are more limited for the heavier nuclei. The reason for this is that long range magnetic anisotropy effects will be very small for ^{13}C and ^{15}N, due to the large ^{13}C and ^{15}N shift ranges (~8–30 ppm). Moreover, C-α and C-β, being both sp^3-hybridized carbons, are not expected to have their chemical shift greatly influenced by electrostatic factors. Thus, in order successfully to predict and interpret the chemical shift of heavy nuclei in proteins, it is desirable to apply ab initio methods. These, in general, successfully predict shielding for such heavy elements—at least for ^{13}C, ^{15}N, and ^{19}F. The system, however, consists of so many atoms that a computation which includes the whole protein would be prohibitive, even with supercomputers. Fortunately, however, since the chemical shift is inherently a localized property, it is wrong to suppose that the effects of all atoms in a protein need to be incorporated into the computation. One approach is to separate the total shielding, σ_t, into three parts[31]

$$\sigma_t = \sigma_s + \sigma_l + \sigma_0 \quad (1)$$

where σ_s refers to the short-range contribution, σ_l corresponds to long range electrostatic effects, and σ_0 contains the magnetic effects. Although orthogonality may not be achieved, such a partitioning of the various chemical shift contributions helps develop computational strategies for solving each contribution.

The short range contribution can be further divided into several categories: local structure, repulsive interactions, and strong hydrogen bonding. Local structure includes the dependencies of the chemical shift on bond lengths, bond angles, and torsion angles. For the long range electrostatic contributions, several methods can now be used. One can either incorporate the charge field at the self-consistent field (SCF) level,[32] or use the multipole shielding polarizability (MSP) approach.[33] Lastly, σ_0 can be calculated by a variety of classical methods, basically as discussed above for ^1H NMR. This contribution is, however, still within the error limits of present ab initio methods for calculating σ_s and σ_l, although it can still be expected to help somewhat with shift predictions. For further discussions on intra- and intermolecular factors which influence shielding, several recent reviews are available.[34,35]

Hence, for a protein, atoms belonging to the residue which contains the nucleus of interest can be regarded as contributing to σ_s, while the rest of the protein contributes to σ_l. This partitioning greatly reduces the size of the fragment on which full ab initio calculations need to be performed. When combined with recent improvements in hardware (such as powerful workstations) and a method which avoids gauge errors in calculating the shielding property [the gauge-including atomic

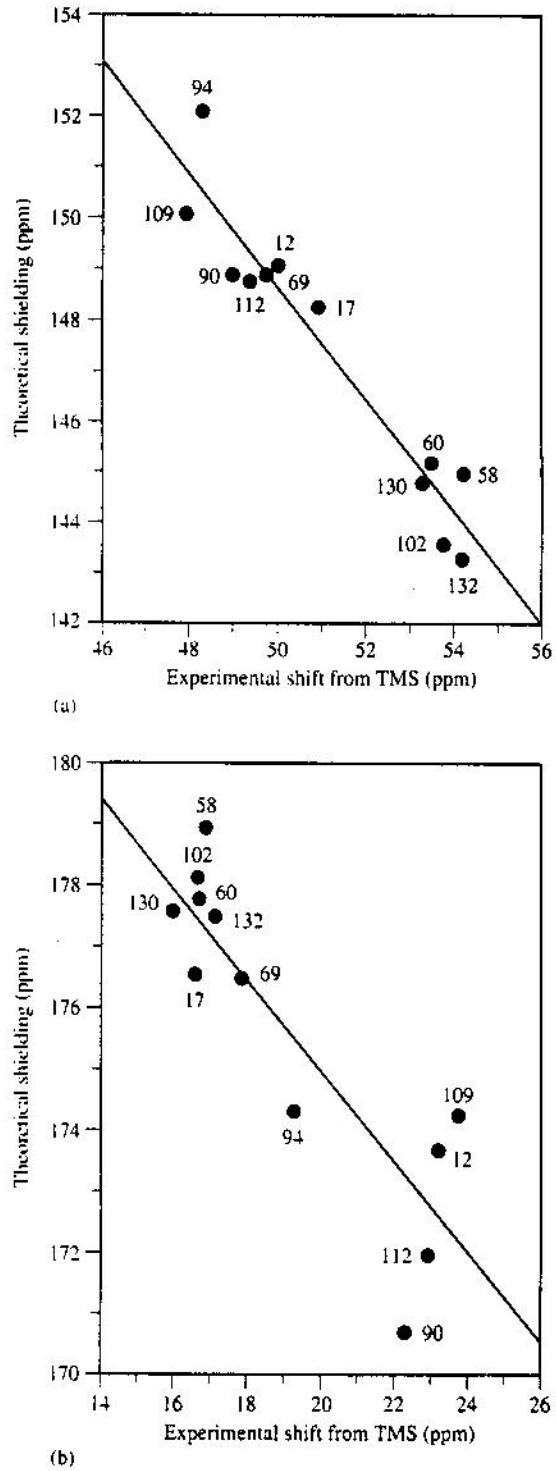

Figure 3 Comparison between calculated ^{13}C NMR shieldings and experimental chemical shifts for alanine residues in staphylococcal nuclease. (a) C-α, (b) C-β. (Reproduced by permission of the American Association for the Advancement of Science from A. C. de Dios, J. G. Pearson, and E. Oldfield, *Science*, 1993, **260**, 1491)

orbital (GIAO) method],[36] together with the application of new algorithms to improve code efficiency,[37] the ab initio reproduction of chemical shifts in proteins is now at hand.

In recent work,[5] it was shown that a small molecular fragment, *N*-formyl-L-alanine amide, interacting with formamide molecules (used to represent hydrogen bond partners) and point charges (representing the rest of the protein) successfully reproduced the experimentally observed ^{13}C chemical shifts for the 12 alanine C-α and C-β sites in the protein staphylococcal nuclease, as shown in Figure 3. The ability to predict the chemical shift opens up a new avenue for protein structure elucidation, especially if the factors which lead to such nonequivalencies can be identified. The partitioning of the shielding as described above greatly facilitated this quest.[5,38] As expected, C-α and C-β chemical shift nonequivalencies were found to be dominated by the local structure, or short-range factors, σ_s.[36] In addition, based upon an examination of the dependencies of C-α and C-β shielding on bond lengths, bond angles, and torsion angles, it was established that torsion angles clearly dominate the experimentally observed trends. In particular, it was also noted that the chemical shifts at these sites were extremely sensitive to bond lengths. In fact, the variations in bond lengths of the same type of residue seen in a single-crystal X-ray structure were shown to lead to a chemical shift range as large as the experimentally measured range, but the predicted shifts were wholly uncorrelated with experiment.[39] Residues of the same type in a protein therefore have bond lengths that are much more similar to each other than X-ray structures would suggest. Nevertheless, from these same studies, ϕ- and ψ-angles were found to be highly correlated with C-α and C-β shifts. Shielding surfaces have therefore been constructed using a model fragment, *N*-formyl-L-alanine amide, in order to map out the effects of ϕ and ψ on C-α and C-β shieldings.[38] With this surface serving as a look-up table, and knowledge of ϕ and ψ, ^{13}C shieldings (or chemical shifts) for any alanine site in a protein can readily be obtained—at least in the absence of large-amplitude motions.

Nitrogen-15 shieldings are expected to be more difficult to predict, since well-defined ϕ and ψ correlations have not been observed, and hydrogen bonding and electrostatic effects could also be important. Recent work,[5] however, has shown that ^{15}N chemical shifts in proteins can now be predicted using the GIAO method, with explicit hydrogen bond partners, and point charges representing the rest of the protein. Figure 4 shows results for valine residues in staphylococcal nuclease.[5] The results in Figure 4(a) were obtained by using a molecular fragment; formyl-L-alanyl-L-valine amide. In Figure 4(b), hydrogen bond partners were explicitly included (as formamide molecules), while Figure 4(c) includes the rest of the protein, represented as point charges. Although the dominance of torsion angles found in ^{13}C chemical shifts is less dramatic with ^{15}N, the generally good accord between calculated and observed values when long range factors are included indicates that ^{15}N NMR is a potentially powerful technique for structure validation. Its sensitivity to long range interactions extends the structural information that can be obtained beyond the local torsion angles, thus complementing ^{13}C NMR.

Although not naturally occurring in most proteins, ^{19}F is a particularly sensitive probe of electrostatic field effects in proteins. Its incorporation, for example, into tryptophan residues of *Escherichia coli* galactose-binding protein (GBP)[40] and hen egg white lysozyme[41] has generated information on how long range factors, such as van der Waals and electrostatic inter-

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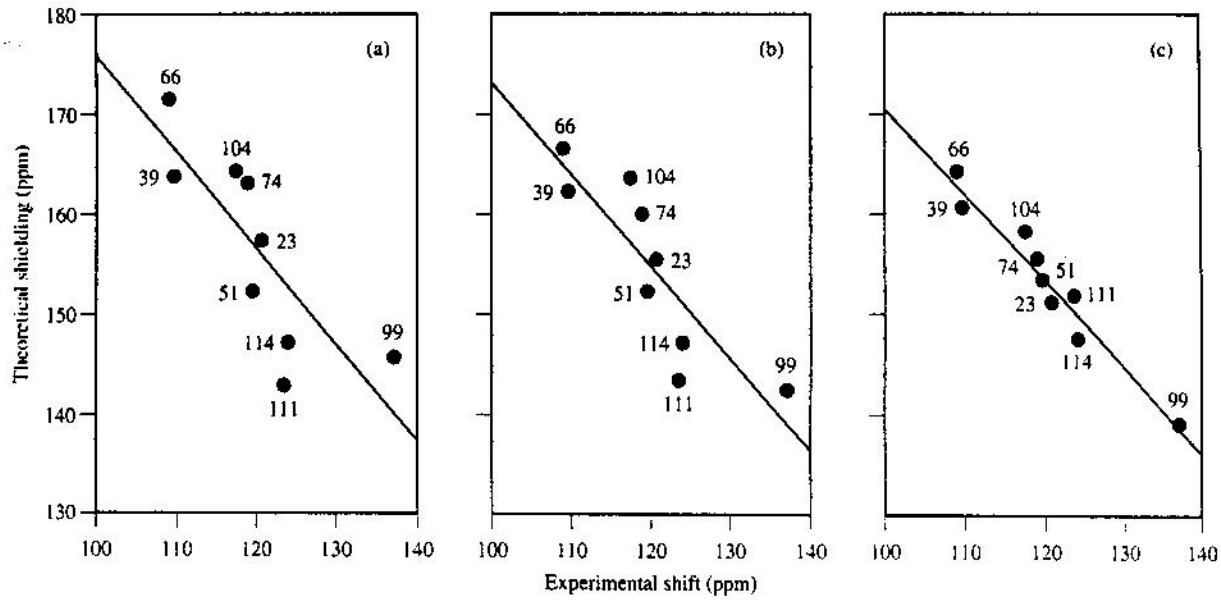

Figure 4 Calculated ^{15}N chemical shieldings versus experimental shifts for the valine sites in staphylococcal nuclease. (a) Using isolated valine fragments; (b) as in part (a) but with inclusion of a hydrogen bond partner; (c) as in part (b) but with incorporation of point charges to represent the rest of the protein. (Reproduced by permission of the American Association for the Advancement of Science from A. C. de Dios, J. G. Pearson, and E. Oldfield. *Science*, 1993, **260**, 1491)

actions, influence the chemical shift. Using point charges, GIAO has been shown[5] to reproduce satisfactorily the observed ^{19}F NMR spectrum of 5-F-Trp-labeled GBP, except for solvent-exposed sites. With prior knowledge of how shielding is affected by a uniform electric field, field gradient, and field hypergradient (the MSP approach), dynamic averaging becomes tractable,[5] and even shielding at sites that are highly mobile and solvent exposed can be satisfactorily reproduced.[39]

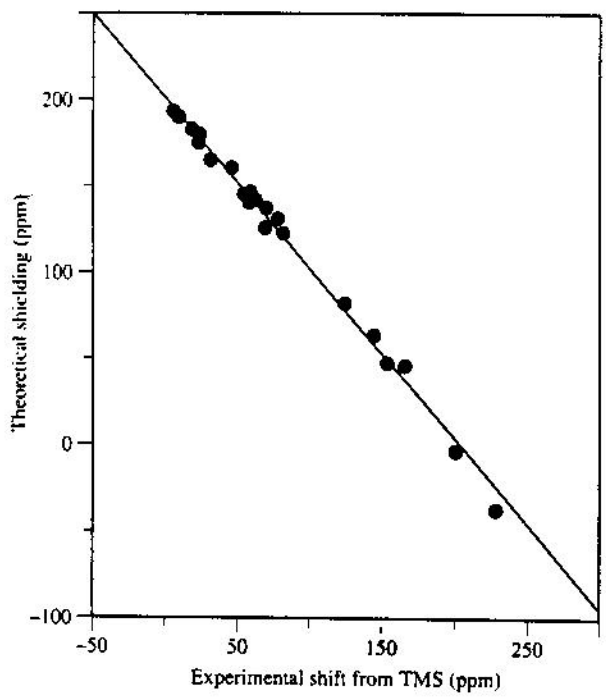

Figure 5 Experimental and theoretical ^{13}C shielding tensor elements for L-threonine in the icosahedral representation. Reprinted with permission from A. C. De Dios et al., *J. Am. Chem. Soc.*, **116**, 5307. Copyright 1994 American Chemical Society

Overall, ^{13}C chemical shifts show great promise in structure elucidation, as noted above for alanine. Successful prediction of ^{13}C chemical shifts has also been shown for glycine and valine residues.[39] Here, the dominance of ϕ and ψ is similarly evident. The correlation between calculated and experimental shieldings for glycine sites is less satisfactory than with alanine and valine, possibly due to glycine mobility. On the other hand, the presence of the side chain in valine introduces an additional angle that can influence both C-α and C-β shieldings. Taking advantage of this additional dependency, crystal-to-solution structural differences became apparent upon close examination of the C-α and C-β shifts of valine residues in calmodulin.[42] Corroborated by coupling constant information, this work demonstrated that five out of the seven valine residues assume a side-chain conformation different from that seen by X-ray crystallography.

Finally, we briefly consider shielding as a tensor quantity. Here, all elements and their directions need to be evaluated—six parameters versus one isotropic shift, representing a serious challenge for theory. For amino acid crystals, the charge field perturbation gauge including atomic orbital (CFP–GIAO) method gives excellent agreement with experiment.[43] Figure 5 shows a comparison between calculated and experimental shielding components for the ^{13}C nuclei in L-threonine[43] in the icosahedral representation[44] of the shielding tensor. The results shown here clearly illustrate the adequacy of present theoretical methods of predicting chemical shieldings, even in complex (zwitterionic, polar amino acid) systems.

For list of General Abbreviations see end-papers

4 NUCLEIC ACIDS

The usefulness of chemical shifts in studying biological systems is naturally not restricted to proteins. Chemical shifts of nucleic acids should similarly encode a wealth of information regarding structure, protonation sites, hydrogen bonding, and electrostatic field effects. The shielding caused by magnetic anisotropy effects on ^{1}H NMR spectra has already been studied using semiempirical methods,[45] and more recently ab initio methods have been used to study the pyrimidine base cytosine.[46] However, these early results were obtained using only a split valence set, insufficiently saturated with basis functions. A more recent study using a larger basis was performed by Schindler[47] using the individual gauge for localized molecular orbitals (IGLO) method.[48] The shielding tensors, as well as the magnetic susceptibilities, were calculated for cytosine, thymine, and uracil, as well as adenine and guanine. The calculated values compared favorably with experiment, but in general the computation of shielding tensors in nucleic acid bases is difficult because definite molecular structures that correspond to the systems on which the NMR measurements were taken are usually not available. In particular, the relative amounts of tautomers depend strongly on the solvent and pH, and the ^{15}N chemical shifts were found to be very sensitive to such environmental effects. Nevertheless, using ab initio optimized structures, it was shown that the calculated ^{15}N shielding trends caused by protonation could indicate which species dominate in solution. However, the lack of a supportive trend from ^{13}C shieldings did not permit a more precise determination of the dominant tautomer.

5 CARBOHYDRATES

With the importance of carbohydrates in lipid and (glyco)protein structure, as well as in, for example, substrate/inhibitor binding (e.g. in lysozyme), and the occurrence of carbohydrates in metabolism, cell surface antigens, etc., it is surprising that so little work has been reported on ^{13}C and ^{17}O NMR shielding, at the ab initio level. An exception to this is the pioneering work of Grant et al.[49] on ^{13}C shieldings in methyl α-D-glycopyranoside and sucrose. Grant et al. showed that a root mean square deviation (rmsd) of ~3 ppm between theory and experiment (the rmsd from the straight line connecting shift and shielding) with R^2 ~0.97 and a slope of −0.99 (methyl glucoside), could be obtained at the 6-31G level, permitting a convincing reassignment of some earlier chemical shift assignments.

6 STRUCTURE FROM SHIELDING: PREDICTION, REFINEMENT, AND VALIDATION

The ability to predict the NMR spectra of biologically important molecules via ab initio methods heralds a new era in the use of the easiest NMR parameter to measure, the isotropic chemical shift. For proteins, the intimate relationship found between the torsion angles ϕ and ψ and the ^{13}C chemical shifts of C-α and C-β sites strongly suggests that it should be possible to derive structural information from chemical shifts in ways not founded on empirical relationships, size of database, or on X-ray structures. One such method of extracting torsion angles from spectroscopic parameters has recently been proposed.[48] Referred to simply as the 'Z-surface method', it begins with the assumption that a given spectroscopic parameter, P, can be represented as a function of a torsion angle α

$$P = f(\alpha) \tag{2}$$

and the probability that a given value P_i corresponds to a value of α can be cast as in

$$Z_i = \exp[-(P_i - f(\alpha))^2/W] \tag{3}$$

W is a search width of typically about 1 ppm, and takes into account uncertainties in theoretical and experimental results, e.g. basis deficiencies, referencing errors, etc. Equation (3), of course, assumes a knowledge of $f(\alpha)$, which can be obtained via ab initio methods or from experiment. A plot of Z_i over possible values of α constitutes a probability surface for the angle α. Since the chemical shifts of C-α and C-β are both functions of torsion angles ϕ and ψ, a map of the whole Ramachandran space that shows which pair of ϕ- and ψ-angles closely agree with the observed chemical shifts can be easily constructed. For alanine residues in a variety of proteins, using this approach and the theoretical shielding surfaces for C-α and C-β, plus an experimental surface for H-α, yields values for ϕ and ψ that have only ~10° rmsd values from the X-ray values.[50]

Although ^{15}N chemical shifts are more susceptible to environmental effects, and ^{19}F shift inequivalencies are largely due to electrostatic interactions, this sensitivity of the ^{19}F and ^{15}N shieldings to electrostatic interactions can be utilized in testing present force fields, molecular dynamics programs, and electrostatic field calculations.[51] Moreover, ^{15}N being sensitive to factors other than local structure, it can complement ^{13}C NMR in validating the overall structure of a protein.

7 CONCLUSIONS

Chemical shifts have been reported in biochemical systems for about 30 years. However, only very recently has it been possible to solve the chemical shift problem, and successfully predict chemical shifts in macromolecules, such as proteins.[5] Since chemical shifts can now be intimately related to structure, and vice versa, it seems likely that many new applications to structure prediction, refinement, and validation will stem from these developments. Until recently, it was thought by many to be impossible to predict protein chemical shifts. However, the continuing revolution in computer/disk price/performance ratios now enables study of much larger fragments than heretofore possible. In the future, we believe that many such applications of 'biochemical shifts' and quantum chem-

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istry will contribute greatly to our studies of lipid, protein, nucleic acid, and carbohydrate structure.

8 RELATED ARTICLES

Shielding Calculations: LORG & SOLO Approaches; Chemical Shift Scales on an Absolute Basis; Chemical Shift Tensor Measurement in Solids; Chemical Shift Tensors in Single Crystals; Shielding Calculations: IGLO Method; Shielding Calculations: Perturbation Methods; Shielding: Overview of Theoretical Methods; Shielding in Small Molecules; Shielding Tensor Calculations; Shielding Theory: GIAO Method.

9 REFERENCES

1. C. C. McDonald and W. D. Phillips, *J. Am. Chem. Soc.*, 1969, **91**, 1513.
2. A. Allerhand, R. F. Childers, and E. Oldfield, *Biochemistry*, 1973, **12**, 1335.
3. A. Bax, M. Ikura, L. E. Kay, D. A. Torchia, R. Tschudin, *J. Magn. Reson.*, 1991, **86**, 304.
4. G. M. Clore and A. M. Gronenborn, *Prog. Nucl. Magn. Reson. Spectros.*, 1991, **23**, 43.
5. A. C. de Dios, J. G. Pearson, and E. Oldfield, *Science*, 1993, **260**, 1491.
6. J. L. Markley, D. H. Meadows, and O. Jardetzky, *J. Mol. Biol.*, 1967, **27**, 25.
7. H. Sternlicht and D. Wilson, *Biochemistry*, 1967, **6**, 2881.
8. H. L. Tigelaar and W. H. Flygare, *J. Am. Chem. Soc.*, 1972, **94**, 343.
9. N. J. Clayden and R. J. P. Williams, *J. Magn. Reson.*, 1982, **49**, 383.
10. A. Pardi, G. Wagner, and K. Wuthrich, *Eur. J. Biochem.*, 1983, **137**, 445.
11. H. R. Kricheldorf, W. E. Hull, and D. Muller, *Macromolecules*, 1985, **18**, 2135.
12. H. Saito, R. Tabeta, A. Shoji, T. Ozaki, I. Ando, and T. Asakura, in 'Magnetic Resonance in Biology and Medicine', ed G. Govil, C. L. Khetrapal, A. Saran, Tata McGraw-Hill, New Delhi, 1985, pp. 195–215.
13. I. Ando, H. Saito, R. Tabeta, A. Shoji, and T. Ozaki, *Macromolecules*, 1984, **17**, 457.
14. S. Spera and A. Bax, *J. Am. Chem. Soc.*, 1991, **113**, 5490.
15. D. S. Wishart and B. D. Sykes, *J. Biomol. NMR*, 1994, **4**, 171.
16. G. C. Levy and R. L. Lichter, in 'Nitrogen-15 NMR spectroscopy', Wiley-Interscience, New York, 1979.
17. M. Witanowski, L. Stefaniak, G. A. Webb, *Annu. Rev. NMR Spectrosc.*, 1986, **18**, 65.
18. J. Glushka and D. Cowburn, *J. Am. Chem. Soc.*, 1987, **109**, 7879.
19. H. Le and E. Oldfield, *J. Biomol. NMR*, 1994, **4**, 341.
20. A. E. Tonelli, *J. Am. Chem. Soc.*, 1980, **102**, 7635.
21. B. R. Seavey, E. A. Farr, W. M. Westler, and J. L. Markley, *J. Biomol. NMR*, 1991, 217.
22. M. A. Jiménez, J. L. Nieto, J. Herranz, M. Rico, and J. Santoro, *FEBS Lett.*, 1987, **221**, 320.
23. L. Szilágyi and O. Jardetzky, *J. Magn. Reson.*, 1989, **83**, 441.
24. M. P. Williamson, *Biopolymers*, 1990, **29**, 1423.
25. A. Pastore and V. Saudek, *J. Magn. Reson.*, 1990, **90**, 165.
26. D. S. Wishart, B. D. Sykes, and F. M. Richards, *J. Mol. Biol.*, 1991, **222**, 311.
27. I. D. Kuntz, P. A. Kosen, and E. C. Craig, *J. Am. Chem. Soc.*, 1991, **113**, 1406.
28. K. Ösapay and D. A. Case, *J. Am. Chem. Soc.*, 1991, **113**, 9436.
29. M. P. Williamson and T. Asakura, *FEBS Lett.*, 1992, **302**, 185.
30. K. Ösapay and D. A. Case, *NATO Adv. Study Inst.*, ed. J. A. Tossel, Kluwer, Dordrecht, 1993, **572**; D. A. Case and P. E. Wright, in 'NMR of Proteins', ed. G. M. Clore, and A. M. Gronenborn, Macmillan, New York, 1993, pp. 53–91.
31. A. C. de Dios and E. Oldfield, *Chem. Phys. Lett.*, 1993, **205**, 108.
32. A. D. Buckingham and K. P. Lawley, *Mol. Phys.*, 1960, **3**, 219.
33. J. D. Augspurger, C. E. Dykstra, and E. Oldfield, *J. Am. Chem. Soc.*, 1991, **113**, 2447.
34. C. J. Jameson and A. C. de Dios, in 'Nuclear Magnetic Shieldings and Molecular Structure', ed. J. A. Tossell, Kluwer, Dordrecht, 1993, pp. 95–116.
35. A. C. de Dios and C. J. Jameson, *Annu. Rep. NMR Spectrosc.*, 1994, **29**, 1.
36. R. Ditchfield, *J. Chem. Phys.*, 1972, **56**, 5688.
37. K. Wolinski, J. F. Hinton, and P. Pulay, *J. Am. Chem. Soc.*, 1990, **112**, 8251.
38. A. C. de Dios, J. G. Pearson, and E. Oldfield, *J. Am. Chem. Soc.*, 1993, **115**, 9768.
39. D. D. Laws, A. C. de Dios, and E. Oldfield, *J. Biomol. NMR*, 1993, **3**, 607.
40. L. A. Luck and J. J. Falke, *Biochemistry*, 1991, **30**, 4248.
41. C. Lian, H. Le, B. Montez, J. Patterson, S. Harrell, D. D. Laws, I. Matsumura, J. G. Pearson, and E. Oldfield, *Biochemistry*, 1994, **33**, 5238.
42. A. C. de Dios and E. Oldfield, *J. Am. Chem. Soc.*, 1994, 116, 5307.
43. A. C. de Dios, D. D. Laws, and E. Oldfield, *J. Am. Chem. Soc.*, 1994, **116**, 7784.
44. D. W. Alderman, M. H. Sherwood, and D. M. Grant, *J. Magn. Reson.*, 1993, **101**, 188.
45. C. Giessner-Prettre and B. Pullman, *Biochem. Biophys. Res. Commun.*, 1976, **70**, 578.
46. F. R. Prado and C. Giessner-Prettre, *J. Magn. Reson.*, 1982, **47**, 103.
47. M. Schindler, *J. Am. Chem. Soc.*, 1988, **110**, 6623.
48. M. Schindler and W. Kutzelnigg, *J. Chem. Phys.*, 1982, **76**, 1919.
49. D. M. Grant, J. C. Facelli, D. W. Alderman, and M. H. Sherwood, in 'Nuclear Magnetic Shieldings and Molecular Structure', ed. J. A. Tossell, Kluwer, Dordrecht, 1993, pp. 367–384.
50. H. Le, J. G. Pearson, A. C. de Dios, and E. Oldfield, *J. Am. Chem. Soc.*, 1995, **117**, 3800.
51. J. G. Pearson, E. Oldfield, F. S. Lee, and A. Warshel, *J. Am. Chem. Soc.*, 1993, **115**, 6851.

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For list of General Abbreviations see end-papers