Calibration of ring-current effects in proteins and nucleic acids

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Summary

Density functional chemical shielding calculations are reported for methane molecules placed in a variety of positions near aromatic rings of the type found in proteins and nucleic acids. The results are compared to empirical formulas that relate these intermolecular shielding effects to magnetic anisotropy ('ring-current') effects and to electrostatic polarization of the C-H bonds. Good agreement is found between the empirical formulas and the quantum chemistry results, allowing a reassessment of the ring-current intensity factors for aromatic amino acids and nucleic acid bases. Electrostatic interactions contribute significantly to the computed chemical shift dispersion. Prospects for using this information in the analysis of chemical shifts in proteins and nucleic acids are discussed.

Introduction

Advances in NMR instrumentation and methodology have now made it possible to determine site-specific proton chemical shift assignments for a large number of proteins and nucleic acids (Wüthrich, 1986; Van de Ven and Hilbers, 1988; Seavey et al., 1991). It has been known for some time that the 'secondary' chemical shifts, i.e., the differences between the resonance positions in a protein and in a 'random coil' polypeptide (Bundi and Wüthrich, 1979; Merutka et al., 1995), carry useful structural information. Two of the most important contributions to secondary shifts are 'ring-current' effects for nuclei near aromatic rings, and electrostatic interactions arising from the arrangement of charges and dipoles in the macromolecule. The basic principles of these interactions can be understood from very simple wave functions (Salem, 1966), but it is only recently that chemical shift calculations approaching chemical accuracy have been feasible for more than the smallest molecules. Here I take advantage of recent advances in density functional theory to explore proton secondary shifts arising from aromatic rings found in proteins and nucleic acids. The results can be fit quite accurately by relatively simple empirical formulas that have been long used to predict ring-current (Haigh and Mallion, 1980) and electrostatic (Buckingham, 1960) interactions, and provide new estimates of

the ring-current intensity and bond polarizability parameters that enter these models. Since the empirical formulas are fairly simple functions of macromolecular geometry, this development points the way to improved use of chemical shift data as a source of structural information.

Methods

Structures examined

The basic model used here to investigate secondary shifts caused by the presence of aromatic rings consists of a methane 'probe' molecule placed successively at various positions in the vicinity of the ring, both in and out of the plane of the ring. Figure 1 shows a typical calculation for the benzene system. Each such geometry yields four secondary shifts, computed for each proton as the isotropic shielding in methane minus that for the methane/ring dimer (both computed using the same method and basis set). Eight to twelve such dimers were computed for benzene, phenol, imidazole, imidazolium, indole, adenine, guanidine, uracil, thymine and cytosine, for a total of 421 computed secondary shifts. The distance of the methane protons to the nearest ring atom varied from 2.9 Å (about a van der Waals contact) to about 5.5 Å; beyond this distance, the effects of the ring are quite small. Ring geometries were taken from the X-ray structure averages
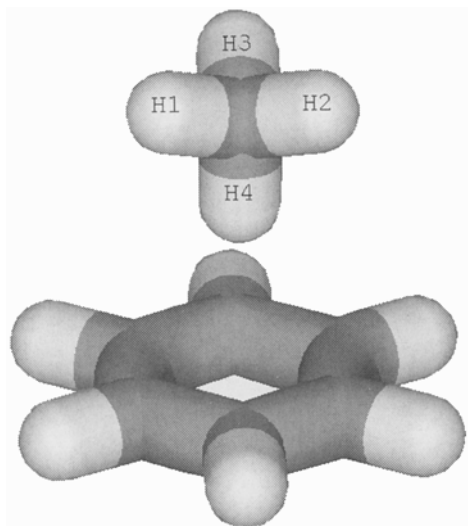
Fig. 1. Sample geometry of methane above a benzene ring. The distance from H4 to the center of the ring is 2.5 Å for geometry 1 and 3.0 Å for geometry 2.

in the AMBER database (Pearlman et al., 1991) to facilitate subsequent empirical calculations.

Quantum chemistry calculations

The quantum chemistry calculations were carried out using the DeMON program, which implements a sum-over-states perturbation approach to the computation of shielding tensors (Malkin et al., 1994). The method inserts the Kohn–Sham orbitals into a standard formula for chemical shielding (Ramsey, 1950), and energy denominators are approximated by differences in Kohn–Sham orbital energies, corrected for changes in the exchange correlation potential that occur upon excitation. Approximate gauge invariance is obtained using the individual gauge for localized orbitals (IGLO) approach (Kutzelnigg et al., 1990). Full details of the method have been given elsewhere (Malkin et al., 1994). The calculations used the IGLO-III basis set of Kutzelnigg and co-workers (Kutzelnigg et al., 1990); this is a relatively large basis set, with 11 s-type and 7 p-type Gaussians on first row atoms (contracted to 7s/6p) along with two uncontracted polarization functions. For comparison, some calculations were repeated with a smaller IGLO-II basis, which has 5s and 4p contracted basis functions and a single polarization function on first row atoms. All calculations used the Perdew–Wang-91 (PW91) exchange-correlation potential (Perdew and Wang, 1992) and the 'Loc.1' correction for energy denominators (Malkin et al., 1994).

To provide insight into basis-set and correlation effects, some additional shielding calculations were carried out for the methane/benzene system at the Hartree–Fock level using the Gaussian 94 program. These Gaussian calculations used the gauge-including atomic orbitals (GIAO) method to handle gauge dependence, and ex-

plored a variety of basis sets from the groups of Pople and Dunning, as described below.

Empirical ring-current calculations

The basic ideas of ring-current calculations may be found in various textbooks (Salem, 1966; Harris, 1986) and reviews (Haigh and Mallion, 1980). The general form expected for ring-current contributions is:

$$\sigma_{rc} = iBG(\mathbf{r}) \qquad (1)$$

where $\mathbf{r}$ is the vector from the observed proton to the aromatic ring, $G(\mathbf{r})$ is a geometric factor and i and B are constants. It is conventional to incorporate into B those constants that would yield the expected contribution from a benzene ring, and to use i (the 'ring-current intensity' factor) to represent the ratio between the intensity expected for the ring in question and that of a benzene ring. There are two widely used empirical formulas for calculating the geometric factors. The Johnson–Bovey model (Johnson and Bovey, 1958) attributes the shift to current loops above and below the plane of the aromatic ring. If the electrons circulate in loops of radius a, the geometric factor becomes:

$$G(\mathbf{r}) = \frac{1}{[(1+\rho)^2+z^2]^{1/2}} \left[K(k) + \frac{1-\rho^2-z^2}{(1-\rho)^2+z^2} E(k) \right] \qquad (2)$$

where ρ and z are cylindrical coordinates relative to the ring center, measured in units of a, and K and E are complete elliptic integrals of the first and second kind, respectively. The argument k is given by:

$$k = \left[\frac{4\rho}{(1+\rho)^2+z^2} \right] \qquad (3)$$

Various values have been used for the loop radius and z-displacement, but most of the resulting differences can be incorporated into the constant B. I have followed Cross and Wright (1985) in placing the current loops 0.64 Å from the plane of the ring (fit to the expected value of the ring-current contribution of benzene to its own protons) and in using radii of 1.39 and 1.182 Å for six- and five-membered rings, respectively. Then B becomes:

$$B = 3e^2/6\pi mac^2 \qquad (4)$$

A second popular empirical ring-current model is based on Hückel molecular orbital theory; details of the development and history of this model have been given by Haigh and Mallion (1980). Here the geometric factor is:

$$G(\mathbf{r}) = \sum_{ij} s_{ij} \left\{ \frac{1}{r_i^3} + \frac{1}{r_j^3} \right\} \qquad (5)$$

where r_i and r_j are the distances from ring atoms i and j to the proton and s_{ij} is the area of the triangle formed by

TABLE 1
BASIS-SET AND METHOD DEPENDENCE FOR SHIELD-INGS IN METHANE

Method[a]	Basis set	Carbon shielding	Proton shielding
HF	6-31G*	199.2	32.1
HF	6-31G**	201.2	31.5
HF	6-31++G**	202.5	31.5
HF	6-31++G**	197.9[b]	29.0[b]
HF	AUG-cc-pVTZ	195.8	31.4
PW91	IGLO-II	195.37	31.1
PW91	IGLO-III	191.87	31.2
Exp.		195.1	30.6

[a] HF: Hartree–Fock, using the GIAO option of Gaussian 94. PW91: density function theory using the Perdew–Wang (1991) exchange-correlation potential and the IGLO method for gauge correction; see text.
[b] Using the continuous series of gauge transformations (CSGT), rather than the GIAO method.

atoms i and j and the proton projected onto the plane of the aromatic ring. The sum is over the bonds in the ring. The benzene results can be reproduced by setting $B = 5.455 \times 10^{-6}$ Å.

I have used a modified version of codes originally written by Keith Cross (University of New South Wales, Australia) to compute the Johnson–Bovey and Haigh–Mallion estimates of these contributions. Although the two models give somewhat different predictions for relative amounts of shielding (above the plane of the ring) to deshielding (in the plane of the ring) (Haigh and Mallion, 1980), the differences are minor for protons more than 3 Å away from any ring atom, and can mostly be absorbed into slightly different values for the ring-current intensity i in the two models (see below).

Bond-polarization effects

A significant contribution to chemical shifts can also arise from distant polar groups, which can polarize the C-H bond and thereby increase or decrease the local shielding by electrons. The most significant term is expected to be proportional to the projection of the local electric field onto the C-H bond vector:

$$\sigma_{el} = AE(C-H) \qquad (6)$$

This can be considered as the first term in a Taylor expansion, but contributions from higher order terms are expected to be very small for the proton shifts considered here (Augspurger and Dykstra, 1991). In this work, the field is estimated using Coulomb's law and partial charges are taken from the Amber 4.1 force field (Cornell et al., 1995); these charges, determined by fitting electrostatic potentials generated from Hartree–Fock 6-31G* calculations, are very similar to those obtained from density

functional calculations using the present basis sets (D.A. Case, unpublished data), and were chosen to facilitate use of these formulas in macromolecular calculations.

Many years ago, Buckingham (1960) suggested that an appropriate value for A would be -2×10^{-12} esu^{-1}. It is now possible to obtain the derivative of the proton shielding with respect to an external electric field by modern quantum mechanical methods, and Augspurger and Dykstra (1991) have estimated A for the proton in methane to be -2.6×10^{-12} esu^{-1}. A methane probe is used here, and yields similar values, as shown below. It is worth noting that other C-H bonds may respond differently to external fields, and that fields in condensed phases will also be influenced by solvent effects.

Parameter fitting

In order to fit the ring-current intensity factors and the electrostatic A coefficient, I used a nonlinear optimization program to minimize the parameter $\rho(z)$:

$$\rho(z) = \sum_{shifts} 1 + (1/2)z^2 \qquad (7)$$

where $z = (\sigma_{DFT} - \sigma_{empirical})/c$ with $c = 0.5$ ppm. In comparison with conventional least-squares optimization, this has the effect of reducing the importance of shifts whose errors are much larger than the rms error over the data set. In order to determine the predictive ability of the correlations and the uncertainty in the parameters, the optimizations were repeated 10 times, each time removing 1/10 of the points to be fitted. This 'jackknife' procedure can then be used to develop an estimate of the uncertain-

TABLE 2
BASIS-SET AND METHOD DEPENDENCE FOR METHANE PLUS BENZENE

Geometry[a]	Method[b]	Basis set	Shift (1,2,3)[c]	Shift (4)[c]
1	HF	6-31G*	-1.06	-2.67
1	HF	6-31G**	-1.07	-2.69
1	HF	6-31++G**	-1.04	-2.96
1	PW91	IGLO-II	-1.10	-3.26
1	PW91	IGLO-III	-0.98	-2.90
2	HF	6-31G*	-0.78	-1.69
2	HF	6-31G**	-0.78	-1.70
2	HF	6-31++G**	-0.78	-1.91
2	HF	6-31++G**	-0.81[d]	-1.74[d]
2	PW91	IGLO-II	-0.81	-2.28
2	PW91	IGLO-III	-0.75	-2.09

[a] See Fig. 1; $d = 2.5$ Å for geometry 1 and 3.0 Å for geometry 2.
[b] HF: Hartree–Fock, using the GIAO option of Gaussian 94. PW91: density function theory using the Perdew–Wang (1991) exchange-correlation potential and the IGLO method for gauge correction; see text.
[c] Values in ppm, relative to isolated methane. Protons 1, 2, 3 and 4 are identified in Fig. 1.
[d] Using the continuous series of gauge transformations (CSGT), rather than the GIAO method.

TABLE 3
RING-CURRENT AND ELECTROSTATIC PARAMETERS[a]

Ring	Previous[b]	This paper			
		Johnson–Bovey	SD	Haigh–Mallion	SD
Gua-5	0.64	0.81	0.11	1.00	0.13
Gua-6	0.30	0.49	0.07	0.51	0.07
Ade-5	0.66	0.95	0.08	1.14	0.05
Ade-6	0.90	0.83	0.05	0.90	0.04
Cyt	0.28	0.31	0.07	0.37	0.07
Thy	0.11	0.28	0.09	0.35	0.09
Ura	0.11	0.24	0.07	0.30	0.07
A		−2.98	0.26	−3.11	0.23
Phe	1.00	1.27	0.07	1.46	0.04
Tyr	0.94	1.10	0.03	1.24	0.02
His	0.53	1.40	0.02	1.35	0.08
Trp-5	0.56	1.02	0.02	1.32	0.08
Trp-6	1.04	1.27	0.02	1.24	0.06
A		−3.43	0.29	−2.69	0.19

[a] Ring-current intensities are defined in the text; the electrostatic parameter A is given in 10^{-12} esu.
[b] Results from work of Giessner-Prettre and Pullman (1969,1987); see text.

ties in the final parameters (Mosteller and Tukey, 1977), by using the 10 sets of parameter estimates to define pseudo-values for each parameter as

$$y_j^* = k\,y_{all} - (k-1)y_j, \quad j = 1,2,...,k \qquad (8)$$

where y_{all} is the parameter estimate when all of the data is considered, and y_j the estimate when the subset j- is omitted. Then the jackknife estimate for the parameter is the mean of the y_j^* values, and the estimate of its uncertainty is determined by standard formulas for the uncertainty of a mean (Mosteller and Tukey, 1977).

Results

Basis set and method dependence

It has been recognized for some time that fairly extensive basis sets (especially those with diffuse functions) are required to obtain converged results from Hartree–Fock shielding calculations (Webb, 1993). The effects of elec-

tron correlation are less well understood, since correlated shielding calculations using standard methods such as MP2 have only recently become available. Table 1 provides some information about shieldings for methane that are of relevance to the calculations shown here. For GIAO Hartree–Fock calculations, a significant change is seen upon adding polarization functions to hydrogen (going from the 6-31G* to the 6-31G** basis set), but little change is observed upon addition of further diffuse functions or use of a 'correlation-consistent' extended basis set. Both of the IGLO-derived basis sets are rather large, with diffuse and polarization functions included even in the smaller of the two basis sets, and this is reflected in similar proton shieldings. (Absolute carbon shieldings are clearly much more affected by basis-set quality and completeness than are proton shifts, as can be seen by the variation of values reported in Table 1.)

However, absolute shieldings are not directly relevant to the current calculations, which should minimize the effects of some absolute errors by comparing computed methane shieldings in the presence and absence of a nearby ring. The most dramatic evidence of this sort of cancelation comes from the CSGT (continuous series of gauge transformations) results in Tables 1 and 2: for the HF/6-31++G** basis, the absolute shieldings of the CSGT method differ from the GIAO approach by 2.5 ppm (Table 1), but the secondary shifts caused by the presence of a benzene ring differ by less than 0.2 ppm (Table 2). Some additional basis-set and method dependencies of these difference calculations are shown in Table 2. Here it appears that the closer the methane proton is to the ring (and hence the larger the secondary shift), the greater is the dependence on basis set and method. Hence, protons 1, 2 and 3 (see Fig. 1), which are further away from the ring, show well-converged behavior, whereas the results vary over about 0.5 ppm for proton 4. In the largest of the basis sets (6-31++G** for Hartree–Fock, IGLO-III for DFT), the Hartree–Fock and DFT results agree to within 0.2 ppm. Although it is impossible at this point to place any confident error bounds on the quantum chemistry results, the IGLO-III DFT results are expected be be the most accurate ones of those investigated here, and they will be compared to empirical formulas in the fol-

TABLE 4
STATISTICS ON THE FITS[a]

System	Model[b]	Number of shifts	Pearson r	Rms error	Slope	Intercept
Nucleic acids	JB	207	0.943	0.097	0.933	0.011
	HM	207	0.953	0.088	0.942	0.007
Protein rings	JB	171	0.994	0.059	1.002	0.012
	HM	171	0.991	0.071	0.988	0.001
His+	HM	43	0.987	0.122	0.974	0.010

[a] The final four columns give the linear correlation coefficient, the rms difference between the DFT and empirical values, and the slope and intercept of the best fit line to predict the DFT estimates from the empirical estimates.
[b] HM = Haigh–Mallion; JB = Johnson–Bovey.

lowing sections. Only small differences in the empirical fits would be found if the large-basis-set Hartree–Fock results were used instead.

Results for aromatic rings

The basic results of the present study are collected in Table 3, which shows optimized ring-current intensity parameters and electrostatic A values for nucleic acid and protein aromatic ring systems. The 'jackknife' uncertainties quoted in Table 3 reflect the likelihood that different parameter values would be obtained from subsets of the current density functional theory data. They do not attempt to include estimates of errors in the DFT model itself, as these are hard to quantitate.

A variety of values have been used in earlier calculations, many based on semiempirical calculations using the PCILO (perturbation configuration interaction with localized orbitals) approach; the values listed in Table 3 come from the work of Giessner-Prettre and Pullman (1969, 1987), and have been widely used in biomolecular ring-current calculations. Other estimates have come from empirical studies of shifts in organic and protein systems (Perkins and Dwek, 1980; Abraham, 1981; Cross and Wright, 1985; Ösapay and Case, 1991). Many of the ring-current intensities determined here are larger than the earlier values, although the general trends in the values are often preserved; for example, both new and old calculations predict pyrimidine bases to have much smaller ring currents than purines, and phenylalanine to have larger currents than tyrosine. The predicted increase in ring-current intensity for histidine and the five-membered ring of tryptophan seen here was also found in the empirical study by Ösapay and Case (1991). If the Haigh–Mallion formula is to be used to compute secondary shifts, it is appropriate to use intensity factors that are
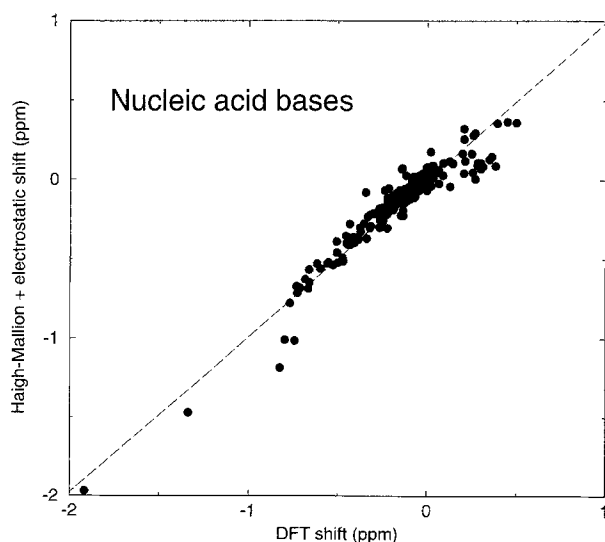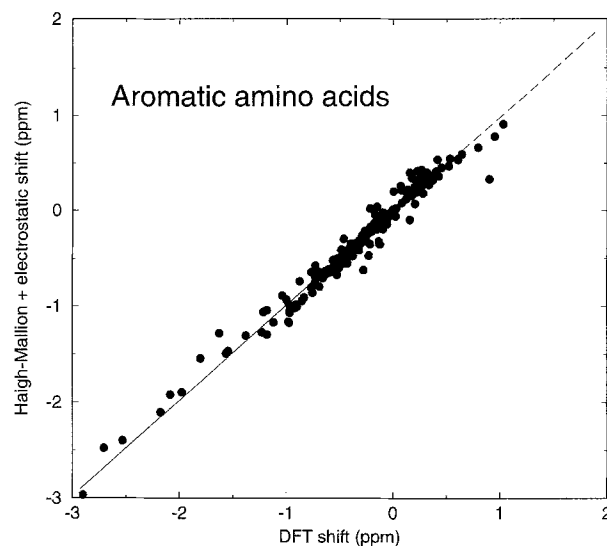

Fig. 3. Comparison of empirical and DFT estimates of ring-induced shifts for side chains of aromatic amino acids, using the fit parameters in Table 4.

somewhat larger than those in the Johnson–Bovey formula; this has been known for many years (Haigh and Mallion, 1980), but in practice is often neglected.

As mentioned above, the estimates for the electrostatic parameter A are close to previous theoretical estimates. It should be noted that the fields in Eq. 6 were computed from Coulomb's law, which is appropriate for the gas-phase situation considered in this paper. In condensed phases, a more complex calculation would be required in order to include polarization effects from the solvent, and the best way to handle electrostatic effects in empirical shift calculations in solution has not yet been established.

Table 4 gives statistics on the overall quality of fits, and these are plotted in Figs. 2 and 3. The fits are quite good, with slopes near unity, intercepts near zero, and root-mean-square errors generally less than 0.1 ppm. There is no significant difference between the Johnson–Bovey and Haigh–Mallion models in terms of the quality of fit. For most applications to biomolecules, the use of the empirical functions in Eqs. 1 and 6 would be a good substitute for the DFT calculations to which these were fit.

Conclusions

In recent years, there has been a revival of interest in biomolecular chemical shifts and the structural information that might be gleaned from them (Wishart et al., 1991; Case et al., 1994). Much attention has been paid to ^{13}C shifts (Spera and Bax, 1991; Le et al., 1995; Oldfield, 1995), but proton shifts also appear to contain significant structural information, and a number of empirical studies of proton shifts in proteins have been reported (Ösapay and Case, 1991,1994; Williamson and Asakura, 1993).

Fig. 2. Comparison of empirical and DFT estimates of ring-induced shifts for nucleic acid bases, using the fit parameters in Table 4.

These results have been encouraging enough to inspire some initial studies that used penalty functions based on these empirical formulas in protein structure refinement (Ösapay et al., 1994; Kuszewski et al., 1995), and an improved understanding of the strengths and limitations of such an approach would clearly be helpful. Current parametrizations (Ösapay and Case, 1991,1994; Williamson and Asakura, 1993) are based on analyses of observed protein shifts, but a potentially important new source of information can come from quantum chemistry calculations, which can now be carried out in a practical fashion for protein fragments such as amino acids (Chesnut and Phung, 1993; Jiao et al., 1993; Malkin et al., 1994; Sulzbach et al., 1994,1995; Le et al., 1995; Oldfield, 1995). These results suggest that modern quantum calculations are now sufficiently accurate to offer real insight into chemical shift trends.

One advantage of quantum chemistry investigations is that relatively simple geometries can be established to isolate particular features that influence chemical shifts; I have done that here to focus on ring-current and electrostatic effects that arise from aromatic rings, and the general procedures outlined here have also been used to study peptide and sugar moieties (to be published elsewhere). The new ring-current intensity factors reported here should provide a good starting point for a reinvestigation of these effects in proteins and nucleic acids.

Acknowledgements

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