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Chemical-Shift Ranges in Proteins*

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The results of ab initio derivative Hartree–Fock calculations of the dipole and quadrupole shielding polarizabilities and hyperpolarizabilities of a number of small molecules are reported, together with estimates of the electric fields and field gradients present in proteins. It is argued that weak electrical interactions, mediated via these shielding polarizabilities, make major contributions to the chemical-shift nonequivalencies observed in proteins due to folding into their native conformations. The electric-field-induced shifts may be very large ($\approx$5 ppm for ^{13}C, $\approx$10 ppm for ^{17}O and ^{19}F), due to the low dielectric constants found in proteins, and in some cases they may dominate the experimentally observed spectral shifts.

The origins of the chemical-shift nonequivalences due to folding a protein (or nucleic acid) into its native conformation are poorly understood. Early workers noted in ^{1}H NMR that, e.g., the four His residues in ribonuclease A (EC 3.1.27.5) could be resolved (*1*), and in 1973, Allerhand *et al.* (*2*) observed very large chemical-shift nonequivalences, due to folding, in the ^{13}C NMR spectra of hen egg white lysozyme (EC 3.2.1.17). For C^{γ} of the six Trp residues in lysozyme, folding produced an ~6 ppm range of chemical shifts, which was almost completely removed upon protein denaturation. More recently, other workers have observed very large chemical-shift ranges for other nuclei in proteins, about 10 ppm for ^{19}F (*3–5*) and up to ~25 ppm for ^{15}N (*6*). Similarly, we have observed about a 10 ppm chemical-shift range for ^{17}O in $C^{17}O$-labeled heme proteins (*7*).

Over the past few years, there have been a number of attempts to rationalize the chemical shifts observed in proteins in structural terms, with most emphasis being placed on analyzing ^{1}H shifts (*8, 9*), and a good review of this topic is given in the thesis by Hoch (*10*). For ^{1}H NMR, moderately good agreement between experimental and predicted chemical shifts based on known structures can be obtained by using random-coil shifts and computed ring-current effects (*8*). The residual "structural" shifts have been interpreted by a number of workers in terms of an "oxygen" effect (*9, 11, 12*), which more recently has been attributed to a combined electric-field and

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magnetic-anisotropy effect (*13–17*). However, there has been much less progress in interpreting ^{13}C, ^{15}N, and ^{19}F shifts, which, paradoxically, are very much larger and thus less sensitive to the smaller "ring-current" and other susceptibility effects which are important ^{1}H NMR. It is also particularly surprising that no clear-cut structural correlations have ever been observed for these heavier elements, where the chemical-shift range of, e.g., ^{15}N is particularly large. Thus, it appears that these large shielding nonequivalences must originate from an "invisible" interaction, that is, one that is not readily observable upon inspection of the three-dimensional structure.

In recent work on CO-labeled heme proteins, we observed ^{13}C and ^{17}O chemical shifts for over a dozen proteins, and we were also able to determine ^{17}O nuclear quadrupole coupling constants for the bound CO, and we compared each of these three NMR parameters with the CO infrared vibrational frequency, ν_{CO}. We found excellent correlations between $\delta_i(^{13}C)$, $\delta_i(^{17}O)$, $e^2qQ/h(^{17}O)$ and ν_{CO} and explained our results in terms of a weak electrical interaction model (*18*) in which changes in the vibrational frequency of CO, the ^{13}C and ^{17}O chemical shifts, as well as the ^{17}O nuclear quadrupole coupling constants, were all interpreted as due to changes in polarization in CO due to large electric fields from the protein (*18*). This model is based on our demonstration that the primary electronic structure change upon weak interaction is electrical polarization (*19–21*). An empirical application of this basic model was then used to interpret over 130 CO vibrational frequencies in heme proteins as being due to weak electrical interactions (*22*), and this led to a molecular model of the four major "conformational substates" seen in heme proteins as due to the electrical influence of the two ring-flip isomers of the $H^{\delta 1}$ and $H^{\epsilon 2}$ forms of the distal histidine residue, which, depending on orientation, can generate fields of up to $\approx 4 \times 10^7$ V cm^{-1} at CO. Such large fields are expected to cause major changes in the shielding tensors for all nuclei, and we have now begun to apply some of these ideas to interpreting the chemical-shift ranges (of ^{1}H, ^{13}C, ^{15}N, ^{17}O, and ^{19}F in amino acids) seen in proteins due to folding; in this paper we outline the approach that we have used.

Our results indicate that weak electrical interactions must make a major contribution to chemical-shift nonequivalencies observed in proteins. In particular, we show that the overall *ranges* of chemical shifts observed in proteins are quite well described by using computed electric-field and field-gradient ranges in proteins, together with the response properties which describe the effects on chemical shifts of electrical perturbation, properties which we term the shielding polarizabilities. We have computed these properties for a number of small molecules by using ab initio derivative Hartree–Fock (DHF) (*23*) methods and in addition have compiled a wide range of approximate experimental and theoretical values already in the literature. Interpretation of the known ranges of chemical shifts in proteins is, we believe, an essential first step toward the much more difficult goal of analyzing *individual* chemical shifts in proteins, which will involve the future development of extremely accurate representations of the potential surfaces in these systems. Nevertheless, existing literature values of electric fields in proteins, together with our quantum-mechanical results, already appear to give a good explanation of the more general question of the shift ranges for most nuclei, as discussed below in detail.

Preliminary accounts of our results have been given (*13, 18*), and more recently, Williamson *et al.* (*14, 15*) have obtained results in ^{1}H NMR which support the claims

made earlier (*13, 18*) of the importance of weak electrical interactions in influencing the chemical shifts observed in proteins. However, the use of at least 10 adjustable parameters to fit the ^{1}H NMR results (*15, 17*) allows for considerable uncertainty in deciding which interactions actually dominate the observed shifts. Moreover, electronic structural changes, due, e.g., to helix/sheet electronic structural differences, have not been incorporated in the recent studies (*15, 17*), and these are also likely to be of importance in interpreting the experimental ^{1}H NMR results. Our method, which is fundamentally different from these empirical approaches, is to employ ab initio calculations to determine the electrostatic influence on chemical shifts and with suitable electric-field models reduce the number of adjustable parameters and allow the use of NMR chemical shifts to test models of protein structure and electrostatics. In this paper we discuss our preliminary results, which show that weak electrical interactions are a potentially major cause of chemical-shift nonequivalence for *most* NMR observable nuclei (^{1}H, ^{13}C, ^{17}O, ^{19}F, and possibly ^{15}N), due to folding in proteins and, by analogy, nucleic acids.

The idea that electric-field effects may be important in explaining protein and peptide chemical shifts is certainly not new, having been discussed in some detail in early work by Horsley, Sternlicht, and Wilson (*24, 25*), but only recently have there been developments in quantum chemistry and protein electrostatics which make application of these earlier ideas feasible.

THEORETICAL ASPECTS

The basic approach that we use is that outlined in 1957 by Stephen (*26*), who gave the following description of electric-field effects upon chemical shifts,

$$\Sigma^{(i)} = \alpha\kappa + \sigma^{(i)} + \frac{1}{3}\left\langle \sum_{j\neq i} \epsilon^{(ij)}_{\alpha\beta}\chi^{(j)}_{\alpha\beta}\right\rangle - \frac{1}{3}\zeta^{(i)}_{\alpha\alpha\gamma}\left\langle F^{(i)}_{\gamma}\right\rangle - \frac{1}{3}\phi^{(i)}_{\alpha\alpha\gamma\delta}\left\langle F^{(i)}_{\gamma}F^{(j)}_{\delta}\right\rangle, \quad [1]$$

where $\sigma^{(i)} = \frac{1}{3}\sigma_{\alpha\alpha}$, $\Sigma^{(i)}$ is the isotropic chemical shift in the presence of a field, $\alpha\kappa$ is a volume susceptibility correction, $\sigma^{(i)}$ is the isotropic chemical shift in the absence of an electric field, the third term contains a diamagnetic susceptibility/point dipole correction due to neighboring groups, while the fourth and fifth terms are the linear and second-order electric-field effect terms studied in detail by several groups (*27, 28*).

Precise calculation of the *absolute* shieldings in a protein is probably not possible at present. However, the susceptibility contributions due to ring currents, carbonyl groups, etc., are relatively well understood [see, e.g., Refs. (*15, 17*)], but alone they do not explain the observed chemical-shift ranges in native proteins, especially for higher-Z nuclei. Thus, by a process of elimination, and based on our earlier work with CO-labeled proteins (*18, 22*), we conclude that the only possible source of the invisible interactions remaining are electronic and electric-field effects. In a conventional description these latter effects might be attributed to "hydrogen bonding," "van der Waals" interactions, etc. The electronic effects may be significant for, e.g., backbone (helix, sheet) ^{1}H, ^{13}C, and ^{15}N sites and can be calculated using DHF theory. The other, weak electrical interactions, which in our picture arise from charge polarization (*19, 20*), can likewise be computed by using a combination of quantum-chemical and semiclassical methods.

The first step is to characterize the effect of electric fields on chemical shifts through the shielding polarizability, which Stephen included through the elements of his ζ and ϕ tensors. However, the influence of nonuniform fields (field gradients), not just uniform fields, must be addressed as well. This information, in conjunction with the knowledge of the actual electric field/field gradient that the particular nucleus experiences, allows one to calculate the electrical influence on the chemical shift. We shall thus give a brief overview of the way that we compute the shielding-polarizability tensors and thus the response of the chemical shift to uniform and nonuniform electric fields. More details are to be found in Refs. (*23, 29*).

Let us first consider an external electrical potential which may be represented by a Taylor series expansion about a point, such that

$$\mathbf{V} = V_0 + xV_x + yV_y + zV_z + \tfrac{1}{2}x^2V_{xx} + xyV_{xy} + \cdots, \qquad [2]$$

where V_x is the first derivative of the electrical potential at that point with respect to x, V_{xx} is the second derivative with respect to x at that point, and so on. Then, the electrical potential can be represented as a column vector, whose elements are these derivatives (*30*). The three elements, V_x, V_y, and V_z, are the negatives of the components of the electric field, and V_{xx}, V_{xy}, V_{xz}, V_{yy}, V_{yz}, and V_{zz} are the negatives of the electric-field-gradient tensor elements. If the moments of a distribution of electrical charges are defined as

$$M_x = \sum q_i x_i \qquad [3]$$

and

$$M_{xx} = \frac{1}{2}\sum q_i x_i^2 \qquad [4]$$

and the moments arranged in a vector, the classical interaction energy of the charge distribution and the external field can be written as

$$E^{\text{int}} = \mathbf{M}^{\mathrm{T}} \cdot \mathbf{V} \qquad [5]$$

From this relationship, it is clear that the permanent moments can also be defined as the first derivative of the classical electrical interaction energy with respect to a particular element of the potential vector. If we consider a polarizable charge distribution, such as the electrons in a molecule, the polarizabilities of the charge distribution can be defined as higher-order derivatives of the energy. For example, the dipole polarizability is a second derivative:

$$P_{x,x} = \frac{\partial^2 E^{\text{int}}}{\partial V_x^2} \qquad [6]$$

(using this convention, $P_{x,x} = -\alpha_{x,x}$). So, the polarizability is a second-rank tensor, the hyperpolarizabilities are a third-rank tensor, the second hyperpolarizabilities are a fourth-rank tensor, etc. By analogy, the chemical shift can also be defined as a second derivative,

$$\sigma_{xy} = \frac{\partial^2 E}{\partial \mu_x \partial H_y}, \qquad [7]$$

and the energy of interaction due to the chemical shift can then be written

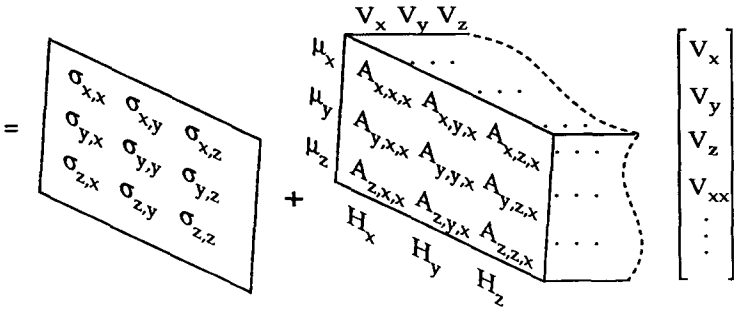
$$E = \boldsymbol{\mu} \cdot \boldsymbol{\sigma} \cdot \mathbf{H} = [\mu_x, \mu_y, \mu_z] \begin{bmatrix} \sigma_{xx} & \sigma_{xy} & \sigma_{xz} \\ \sigma_{yx} & \sigma_{yy} & \sigma_{yz} \\ \sigma_{zx} & \sigma_{zy} & \sigma_{zz} \end{bmatrix} \begin{bmatrix} H_x \\ H_y \\ H_z \end{bmatrix}. \qquad [8]$$

To consider the effects of electric fields on the chemical shifts, we define derivatives of the elements of the chemical shielding tensor with respect to external electrical fields, which we term shielding polarizabilities, as

$$A_{\alpha\beta,\gamma} = \frac{\partial \sigma_{\alpha\beta}}{\partial V_\gamma}. \qquad [9]$$

The shielding polarizability, like the electrical hyperpolarizability, is a third-rank tensor, so that to first order, the chemical shielding tensor in the presence of an electrical potential is

$$\sigma = \sigma_0 + \mathbf{A} \cdot \vec{\mathbf{V}}$$



[10]

(where the values of the indices of the individual **A** tensor elements are identified). The second-order effect of the electrical potential will be a fourth derivative, and therefore a fourth-rank tensor, designated **B**. To illustrate, consider a specific element of the chemical-shift tensor, $\sigma_{\alpha\beta}$:

$$\sigma_{\alpha\beta} = \sigma^0_{\alpha\beta} + [A_{\alpha\beta,x} \quad A_{\alpha\beta,y} \quad A_{\alpha\beta,z} \quad A_{\alpha\beta,xx} \quad A_{\alpha\beta,xy} \quad A_{\alpha\beta,xz} \cdots] \cdot \begin{bmatrix} V_x \\ V_y \\ V_z \\ V_{xx} \\ V_{xy} \\ \vdots \end{bmatrix}$$

$$+\frac{1}{2}[V_x \cdots V_{xy} \cdots]\cdot\left[\begin{array}{ccc|cc|c} B_{\alpha\beta,x,x} & \beta_{\alpha\beta,x,y} & B_{\alpha\beta,x,z} & B_{\alpha\beta,x,xx} & B_{\alpha\beta,x,xy} & \cdots \\ B_{\alpha\beta,y,x} & B_{\alpha\beta,y,y} & B_{\alpha\beta,y,z} & B_{\alpha\beta,y,xx} & B_{\alpha\beta,y,xy} & \cdots \\ B_{\alpha\beta,z,x} & B_{\alpha\beta,z,y} & B_{\alpha\beta,z,z} & B_{\alpha\beta,z,xx} & B_{\alpha\beta,z,xy} & \cdots \\ \hline B_{\alpha\beta,xx,x} & B_{\alpha\beta,xx,y} & B_{\alpha\beta,xx,z} & B_{\alpha\beta,xx,xx} & B_{\alpha\beta,xx,xy} & \cdots \\ \vdots & \vdots & \vdots & \vdots & \vdots & \end{array}\right]\cdot\left[\begin{array}{c} V_x \\ V_y \\ V_z \\ V_{xx} \\ V_{xy} \\ \vdots \end{array}\right] \quad [11]$$

The second term describes the linear electric-field- and field-gradient-induced changes in $\sigma_{\alpha\beta}$ due to the dipole and quadrupole shielding polarizabilities, and the third term is the second-order electrical influence through the shielding hyperpolarizability, **B**. The upper left box of the **B** tensor represents the dipole–dipole shielding hyperpolarizability, the lower right the quadrupole–quadrupole shielding hyperpolarizability, and the lower left and upper right the dipole–quadrupole shielding hyperpolarizability.

Each of these tensors can be calculated analytically by using derivative Hartree–Fock (DHF) theory, as described elsewhere (*23*), from the total molecular energy, E, since

$$\begin{aligned} E = {} & E(0) + V_0\frac{\partial E}{\partial V_0} + V_x\frac{\partial E}{\partial V_x} + \cdots + \frac{1}{2}V_x^2\left(\frac{\partial^2 E}{\partial V_x^2}\right) + \cdots + H_x\mu_x^m\left(\frac{\partial^2 E}{\partial H_x \partial \mu_x^m}\right) \\ & + H_x\mu_x^m V_x\left(\frac{\partial^3 E}{\partial H_x \partial \mu_x^m \partial V_x}\right) + \cdots + H_x\mu_x^m V_{xx}\left(\frac{\partial^3 E}{\partial H_x \partial \mu_x^m \partial V_{xx}}\right) \\ & + H_x\mu_x^m V_x^2\left(\frac{\partial^4 E}{\partial H_x \partial \mu_x^m \partial V_x^2}\right) + \cdots + H_x\mu_x^m V_{xx}^2\left(\frac{\partial^4 E}{\partial H_x \partial \mu_x^m \partial V_{xx}^2}\right) + \cdots \qquad [12] \\ = {} & E(0) + V_0\frac{\partial E}{\partial V_0} + V_x\mu_x + \cdots + \frac{1}{2}V_x^2 P_{x,x} + \cdots + H_x\mu_x^m\sigma_{xx} + \cdots \\ & + H_x\mu_x^m V_x A_{xx,x} + \cdots + H_x\mu_x^m V_{xx}A_{xx,xx} + \cdots \\ & + H_x\mu_x^m V_x^2 B_{xx,x,x} + \cdots + H_x\mu_x^m V_{xx}^2 B_{xx,xx,xx} + \cdots, \end{aligned}$$

where $\mu_x\mathbf{i} + \mu_y\mathbf{j} + \mu_z\mathbf{k} = \boldsymbol{\mu}$ = electric dipole moment; $\frac{1}{3}(P_{x,x} + P_{y,y} + P_{z,z}) = P^{(i)} = -\alpha^{(i)}$ = polarizability; $\frac{1}{3}(\sigma_{xx} + \sigma_{yy} + \sigma_{zz}) = \sigma_0^{(i)}$ = isotropic field-free chemical shielding; $\frac{1}{3}(A_{xx,x} + A_{yy,x} + A_{zz,x}) = \bar{A}_x$ = dipole shielding polarizability along x; $\frac{1}{3}(B_{xx,x,x} + B_{yy,x,x} + B_{zz,x,x}) = \bar{B}_x$ = dipole shielding hyperpolarizability along x; $\frac{1}{3}(A_{xx,xx} + A_{yy,xx} + A_{zz,xx}) = \bar{C}_{xx}$ = quadrupole shielding polarizability along xx; and $\frac{1}{3}(B_{xx,xx,xx} + B_{yy,xx,xx} + \mathrm{B}_{zz,xx,xx}) = \bar{D}_{xx}$ = quadrupole shielding hyperpolarizability along xx, plus additional cross terms (dipole–quadrupole polarizability, etc.) which are not enumerated.

The symmetry properties of the two dipole shielding-polarizability tensors have been described fully by Raynes and Ratcliffe (*31*) and represent the "A" and "B"

terms of the so-called Buckingham equation (*27*). For symmetric systems, e.g., ^{19}F in fluorobenzene (or *p*-fluorophenylalanine), as well as many C–H groups, most of these tensor elements are identically zero (*31*). For example, for fluorobenzene, the only nonzero elements of the dipole polarizability tensor of ^{19}F are $A_{xx,x}$, $A_{xy,x}$, $A_{xz,x}$, $A_{yx,x}$, $A_{yy,x}$, $A_{yz,x}$, $A_{zx,x}$, $A_{zy,x}$, and $A_{zz,x}$. So, if we consider the case where the electric field is fixed with respect to the molecular axis, while the molecule still tumbles freely in the magnetic field, as in a protein, then the isotropic chemical shielding is

$$\bar{\sigma} = \bar{\sigma}_0 + \bar{A}_x V_x, \qquad [13]$$

where

$$\bar{A}_x = \tfrac{1}{3}(A_{xx,x} + A_{yy,x} + A_{zz,x}) \qquad [14]$$

and similar relations hold for the *B* and *C* terms, as described in expression [12].

We have already reported results for the dipole and quadrupole shielding polarizabilities, as well as the chemical shielding tensors, for a number of small molecules (*18, 23*). Also, we have carried out a detailed examination of basis-set and gauge dependence of these molecular properties for the case of CO (*29*). From this, it is clear that the *A* and *B* properties can be accurately calculated with moderately sized basis sets, specifically an augmented triple-zeta valence set, doubly polarized [TZ'2P in Ref. (*29*)]. The additional errors from using the smaller basis, TZ'P (see footnote *a* to Table 2), as in some of the results reported here, should be less than 5% (*29*). In what follows, we will use the results from our DHF calculations, as well as experimental and theoretical results determined by others, to estimate the ranges of chemical shifts in proteins due to weak electrical interactions. Conversely, we show that comparing the known chemical-shift ranges for various nuclei with their (dipole) shielding polarizabilities gives a value for the range of electric fields found in proteins in good accord with values determined by others using a variety of electrostatic models.

RESULTS AND DISCUSSION

Dipole and quadrupole shielding polarizabilities. As noted above, the purpose of this paper is to determine the overall magnitude of the chemical-shift ranges observed in native proteins (and, by analogy, in nucleic acids) due to electric-field effects. In order to make such estimates, we need to determine some of the shielding-polarizability tensors discussed above for suitable model compounds and then estimate typical electric fields (V_x, V_y, V_z) and field gradients (V_{xx}, V_{xy}, . . . , V_{zz}) in proteins. In addition, we can also use the alternative approach, which is to compare known chemical-shift ranges with computed shielding polarizabilities, to estimate the ranges of electric fields expected in proteins. The results obtained may then be compared with literature (or computed) values for *E* fields in proteins. An assumption of this latter approach is that the major contribution to the *E*-field shift for most (heavier) nuclei arises from the dipole shielding-polarizability/uniform field term, which we show below to be a good first approximation. The dipole and quadrupole hyperpolarizability terms appear to be small, although the quadrupole polarizability term is not and will need to be considered in future work aimed at specific chemical-shift assignments. This will require a very accurate description of the potential surface, in order to derive the field-gradient terms, and at present we only have estimates of these values, for proteins. Nevertheless,

all reasonable estimates of the fields, field gradients, and shielding-polarizability tensors indicate that the uniform field effect dominates most of the observed shielding ranges, in general accord with previous estimates by Batchelor (*32*). For ^{1}H, susceptibility effects are also important (*15, 17*), and for nuclei in the peptide backbone, we suggest below that helix/sheet electronic structural differences (*33, 34*) are also significant, and these will need to be explored in more detail in future work. The likely importance of electrical interactions, even in ^{1}H NMR, can also be deduced from the recent work of Wishart *et al.* (*34*).

We show in Table 1 a compilation of literature shielding-polarizability tensor results, and in Table 2 we present additional published and unpublished results from our

TABLE 1

Representative Literature $\bar{A}_x$, $\bar{B}_x$, and $\bar{C}_{xx}$ Shielding Polarizabilities

System	$\bar{A}_x$ (ppm/a.u.)[a]	$\bar{B}_x$ (ppm/a.u.^{2})[b]	System	$\bar{A}_x$ (ppm/a.u.)[a]	$\bar{B}_x$ (ppm/a.u.^{2})[b]	$\bar{C}_{xx}$ (ppm/a.u.)[c]
H—H	38.9,[d] 24.8[d], 77[d]					
	70[d]		C—Br	2060[e]		
H—C	30,[d] 48,[d] 50,[d]					
	50,[d] 65[d]		CH_3—	340[h]		1000[h]
			CH_2	410[h]		940[h]
HCF_3	50[d]	234.9[d]	—CH—	340[h]		870[h]
H_3CCN	51,[d] 58[d]		CH_3—N	240[h]		1000[h]
H in styrenes	53[d]		C—CAr	620[h]		1100[h]
$HOCH_3$	430[d]		H—CAr	270[h]		1200[h]
H_2S	450[d]	190.8[d]	H_2C=C	580[h]		1100[h]
H—F	45,[g] 71.5[f]		C_2C=C	940[h]		980[h]
H—Cl	570,[d] 680[d]	100[d]	C—C≡C	990[h]		610[h]
H—Br	1100,[d] 110.7[g]	469.7[d]	H—C≡C	750[h]		660[h]
HI	157[g]					
H—N(uracil)	90[d]		F—H		1930,[f] 1621[i]	
				704,[f] 605.2[i]		
C—H	510[e]		FCH_3	792[i]		
C—C	870[e]		F in β,β-Difluoro-styrene	510[j]		
C—N	770[e]					
C—Cl	1400[e]					

[a] Dipole shielding polarizability $\bar{A}_x = \frac{1}{3}(A_{xx,x} + A_{yy,x} + A_{zz,x})$, in ppm/a.u. field; 1 a.u. field = 5.14225×10^9 V cm^{-1}.

[b] Dipole shielding hyperpolarizability $\bar{B}_x = \frac{1}{3}(B_{xx,x,x} + B_{yy,x,x} + B_{zz,x,x})$, in ppm/a.u. field^2.

[c] Quadrupole shielding polarizability, $\bar{C}_{xx} = \frac{1}{3}(A_{xx,xx} + A_{yy,xx} + A_{zz,xx})$, in ppm/a.u. electric-field gradient; 1 a.u. efg = 9.717447×10^{17} V cm^{-2}.

[d] Tabulated in (*50*).

[e] Ref. (*25*).

[f] Ref. (*28b*).

[g] Ref. (*28c*).

[h] Ref. (*32*).

[i] Ref. (*35*).

[j] Ref. (*51*).

TABLE 2

Representative DHF $\bar{A}_x$, $\bar{B}_x$, and $\bar{C}_{xx}$ Shielding Polarizabilities

System	$\bar{A}_x$ (ppm/a.u. field)[a]	$\bar{B}_x$ (ppm/a.u. field^2)[a]	$\bar{C}_{xx}$ (ppm/a.u. efg)[a]
$\underline{H}-H$[b]	50.3	187.7	−5.8
$\underline{H}_4C$[b]	45.1	114.4	−2.1
$\underline{H}-CN$[b]	54.1	173.2	−89.5
$\underline{H}-CCH$[b]	67.2	13.6	−109.5
$\underline{H}_2CCH_2$	64.4		
$\underline{H}_6C_6$	87.5		
$\underline{H}_2O$	157.9		
$\underline{H}_3N$[b]	27.7	278.3	−18.1
$\underline{H}F$[b]	81.5	328.5	−61.8
$\underline{C}O$[b]	374.5	535.5	−532.7
$H_2\underline{C}O$[b]	697.4	3,874.9	−746.0
$H_2\underline{C}CH_2$	1144.5		
$H-\underline{C}CH$[b]	733.9	1,106.9	−351.8
$H_6\underline{C}_6$	645.0		
$H\underline{C}N$[b]	422.6	1,502.9	−512.1
$H_2N\underline{C}HO$	215.9		
$\underline{N}H_3$[b]	50.8	1,613.4	−333.3
$HC\underline{N}$[b]	1910.1	11,336.1	−603.6
$H_2\underline{N}CHO$	902.8		
$H_2\underline{O}$	401.1		
$C\underline{O}$[b]	1526.7	5,906.1	−1044.0
$H_2C\underline{O}$[b]	7018.9	70,843.0	−1377.8
$H_2NCH\underline{O}$	3195.7		
$H\underline{F}$[b]	636.5	8,486.1	−741.7
$CH_3\underline{F}$	551.4		
$C_6H_5\underline{F}$	1884.5		−4860.0
1.4-$C_6H_4\underline{F}_2$	1955.5		
para-$LiC_6H_4\underline{F}$	1984.9		

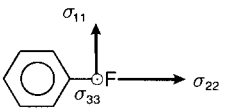
[a] Units are in ppm/a.u. field, (field)^2, or field gradient, as in Table 1. Estimated errors are ±10–30%.
[b] Ref. (*23b*).

laboratory, in units of ppm/a.u. for $A_{\alpha\beta,x}$ (1 ppm/a.u. field $\equiv 5.14225 \times 10^9$ ppm cm V^{-1}). For $B_{\alpha\beta,x,x}$, 1 ppm/(a.u. field)^2 $= 2.64427 \times 10^{19}$ ppm cm^2 V^{-2}. For $A_{\alpha\beta,xx}$, 1 ppm/a.u. electric-field gradient $\equiv 9.71745 \times 10^{17}$ ppm cm^2 V^{-1}.

Many of the results shown in Table 1 were derived by using approximate theoretical methods, or were estimated from experiment. Mostly, theoretical approaches have not used direct, analytical methods to determine the various derivative properties required ($A_{\alpha\beta,\gamma} \cdots$), although in recent work, Packer and Raynes used a finite difference approach to calculate chemical-shift and shielding-polarizability tensors for HF and CH_3F (*35*).

The results in Tables 1 and 2 show comparisons of A and B tensor elements with our DHF results (Table 2), which are in accord with those prior estimates, and where applicable, we find good agreement between experimentally determined chemical

shielding tensors and the DHF calculations (*18, 23*). This is an important point, since the shielding-polarizability tensors that we are interested in are the derivatives of the shielding tensors. By way of example, let us consider our results for fluorobenzene (C_6H_5F), which we believe to be a plausible model for the fluorophenyl group in fluorophenylalanine in proteins, where a large chemical-shift range (~10–12 ppm) has been observed by Gerig and co-workers (*4*) in [F–Phe] hemoglobin, and a similar large shift range has been observed by Luck and Falke for a 5-F–Trp-labeled galactose binding protein (*5*). As shown in Table 3, we find the following values for the principal elements of the ^{19}F chemical shielding tensor, using the following coordinate system (σ_{33} is perpendicular to the plane of the benzene ring):



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The experimental results for fluorobenzene (*33*) are $\sigma_{11} = -58$, $\sigma_{22} = 7.0$, and $\sigma_{33} = 51$ ppm; the theoretical results (in traceless form also) are $\sigma_{11} = -57.0$, $\sigma_{22} = 7.0$, $\sigma_{33} = 51.0$ ppm. Anisotropies ($\Delta\sigma$) are 109 ppm (experiment) and 108 ppm (theory). The calculations are in good agreement with the experimental observations on fluorobenzene reported by Mehring (*36*) and are also in quite good agreement with the results on fluorophenylalanine reported by Hiyama *et al.* (*37*), although less good agreement is expected in this case, since F–Phe contains charged (NH_3^+ and CO_2^-) moieties in the zwitterion, and these can be expected to cause sizable electric-field-induced chemical-shift changes (which will be absent in F–Phe-labeled proteins). Nevertheless, the results in Table 3, and those reported previously (*18, 23*), do show good agreement between theory and experiment, and since the various shielding polarizabilities are calculated *analytically* using DHF theory, the values obtained can be expected to be quite accurate.

As an example of this, we have also computed the shielding-tensor elements, and the dipole shielding polarizability, of 1,4-difluorobenzene (Table 3). Mehring reports

TABLE 3

Chemical Shielding-Tensor Elements for Fluorobenzenes and Fluorophenylalanine[a]

System	σ_{11} (ppm)	σ_{22} (ppm)	σ_{33} (ppm)	$\Delta\sigma$ (ppm)
Fluorobenzene (experimental)[b]	−58	7	51	109
Fluorobenzene (calculated)[c]	−57	7	51	108
Fluorophenylalanine (experimental)[d]	−67	8	58	125
1,4-Difluorobenzene (experimental)[b]	−63	7	56	119
1,4-Difluorobenzene (calculated)[c]	−57.7	1.3	59.1	116.8
1-Fluoro,4-lithiobenzene (calculated)[c]	−35.2	−3.1	38.4	73.6

[a] $\sigma_i = 0$, traceless chemical shielding-tensor elements.
[b] From Mehring (*36*).
[c] Computed by using DHF theory and a TZ^+P basis net.
[d] From Hiyama *et al.* (*37*).

$\sigma_{11} = -63$, $\sigma_{22} = 7$, and $\sigma_{33} = 56$ ppm, with $\Delta\sigma = 119$ ppm, while our DHF results yield $\sigma_{11} = -57.7$, $\sigma_{22} = 1.3$, and $\sigma_{33} = 59.1$ ppm, with $\Delta\sigma = 116.8$ ppm. Again, there is good agreement between theory and experiment, although one should not be overconfident in the level of agreement obtained given the errors anticipated in the experimental measurements, the fact that there are inevitably basis deficiencies, and the fact that intermolecular interactions must influence the experimental results. Notwithstanding these caveats, the agreement appears to be good.

These results on the mono- and difluorobenzenes are also of great interest since they indicate that there are only very small differences between the dipole shielding polarizabilities of both species. For fluorobenzene, $\bar{A}_x = 1885$ ppm/a.u., while for 1,4-difluorobenzene, $\bar{A}_x = 1955$ ppm/a.u. Thus, we believe an $\bar{A}_x$ of ~ 1900 ppm/a.u. is a good estimate for an aromatic fluorine in, e.g., 5-F–Trp (*5*), an amino acid which has been used quite extensively in ^{19}F NMR studies of proteins (*5, 38, 39*) and displays a very large chemical-shift range due to folding, but which is currently somewhat too challenging for an ab initio study.

As a further check of the sensitivity of the ^{19}F $\bar{A}_x$ value in an aromatic system to aromatic substitution, we have also investigated the (hypothetical) species lithium parafluorobenzene (LiC_6H_4F), which contains the highly electropositive Li, as opposed to the highly electronegative fluorine atom in 1,4-difluorobenzene. For lithium fluorobenzene we find $\bar{A}_x = 1985$ ppm/a.u., essentially the same as the other two fluorobenzenes, even though $\Delta\sigma$ is only about 2/3 that found in the fluorobenzenes (Table 3). Thus, a value of ~ 1900 ppm/a.u. should be applicable to most of the fluorinated aromatic amino acids. We believe these results on relatively complex systems indicate that we are now poised to investigate the effects of weak electrical interactions in proteins, and we thus now proceed to determine the strengths of these fields.

Electric-field effects. The topic of electric fields, or electrostatics, in proteins has been of interest for many years, and large electric fields have been shown to be associated with the presence of charged groups, peptide bonds, α-helix dipoles, and other polar species, such as histidine residues. In previous work, we estimated effective fields at CO in CO heme proteins, due to the distal histidine, of up to $\sim 4 \times 10^7$ V cm^{-1} [~ 0.008 a.u.; Ref. (*18, 22*)], and similar large fields have been computed by other workers for groups near α helices (*40*), or in, e.g., the active site of lysozyme (*41*). A number of workers have developed algorithms to compute potentials and electric fields in proteins (*42–44*), and a complete analysis of protein chemical shifts will ultimately rely, for the electrical contributions, on the accuracy of the potential surfaces computed by using such algorithms, i.e., on the validity of the electrical models utilized. Below, we will briefly discuss results obtained by using a simple point-charge Coulomb model, and by using a finite-difference Poisson–Boltzmann approach (*42*), which give plausible estimates of the fields present in proteins. However, we can also investigate this question in an alternative way, by comparing known experimental chemical-shift ranges with computed (or experimental) shielding-polarizability tensor values, as shown in Fig. 1, where we have plotted $\bar{A}_x$ values versus the observed shift ranges for ^{1}H, ^{13}C (aromatic), ^{13}CO, C^{17}O, and ^{19}F. This approach presupposes the dominance of the uniform field contribution, an assumption we explore below in some detail.

For ^{1}H, $A^H \approx 50$ ppm/a.u. and the known range of "secondary shifts" is *about* 1 or 2 ppm, possibly a little more for some unusual sites. For ^{13}C in aromatic compounds,

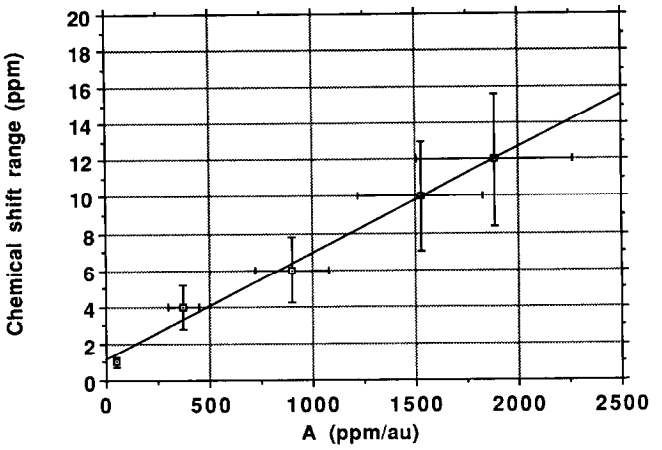

FIG. 1. Graph showing the relationship between the isotropic dipole shielding polarizabilities computed by using derivative Hartree–Fock theory and the observed chemical-shift ranges, for ^{1}H, ^{13}C, ^{17}O, and ^{19}F nuclei in proteins. The slope of $\sim$0.006 a.u. (3×10^{7} V cm^{-1}) approximates the range of electric-field strengths experienced by these nuclei in proteins, as estimated from point-charge ($\epsilon = 2$) and Poisson–Boltzmann methods.

the range of chemical shifts due to folding (after correction for B-field shifts) is $\sim$3–7 ppm, say 5 ppm, $\pm$30% (*2, 45*). The effect seems largest for the rigid Trp-C$^{\gamma}$, almost 6 ppm for a range of CO-hemoglobins. Although such large systems have not yet been computed, preliminary results on benzene yield $\bar{A}_x^C \sim 645$ ppm/a.u. (where two carbon atoms lie on the x axis); for ethylene, $\bar{A}_x^C \sim 1144$ ppm/a.u., for HCN, $\bar{A}_x^C \sim 422$ ppm/a.u.; while, for formaldehyde, $\bar{A}_x^C \sim 697$ ppm/a.u. (Table 2). In addition, in very early work, Horsley and Sternlicht (*25*) reported $A \sim 5 \times 10^{-11}$ esu, or $\sim$830 ppm/a.u. While our value of $\sim$645 ppm/a.u. might be expected to be the most accurate representation for a C$^{\gamma}$ site, Batchelor showed, using a simple bond polarizability model, that $\bar{A}_x^C$ for a C-substituted site was over twice that for an H-substituted site, so we chose a value for $\bar{A}_x^C$ of $\sim$900 ppm/a.u. as the overall most probable value, for C$^{\gamma}$ in aromatic amino acids. In addition, there have been a number of experimental and theoretical estimates of $\bar{A}_x^F$, and we shall use our recent value of 1885 ppm/a.u. (Table 2). $^{13}C^{17}O$ results have been described previously (*18*). All of these results can be described by a linear equation, assuming the dominance of the dipole shielding polarizability, as

$$\Delta\delta\,(\text{ppm}) = 0.00578\bar{A}_x + 1.13, \qquad [15]$$

where the slope, ΔV_x, represents the range of effective fields experienced by the nuclei in question. Although Eq. (*15*) is only strictly applicable for highly symmetric systems, the slope we find, $\Delta V_x = 0.00578$ a.u., appears to be a very reasonable value, based upon previous electrostatic calculations of electric fields in proteins, and is consistent with other literature results, as well as with results obtained by using point-charge or finite-difference nonlinear Poisson–Boltzmann methods, as discussed below. Thus, the effective range of fields in proteins, as determined by this

NMR approach, is $\sim 3 \times 10^7$ V cm^{-1}. However, these fields cannot be expected to be very uniform, and in future work aimed at specific chemical-shift assignments it may be necessary to evaluate much more precisely the nonuniform field contributions to the chemical shift, which will require more accurate information on the potential surfaces than is currently available. Nevertheless, we can make estimates of some of these terms, and as we show below, they are smaller, but nonnegligible. We thus next discuss the various electrical contributions to the chemical shifts for a variety of systems.

For ^{1}H NMR, typical $\bar{A}_x$ values (Tables 1 and 2) are $\sim$50 ppm/a.u., consistent with the early work of Buckingham (*27*). For a uniform electric field of $\sim$0.006 a.u., we obtain a shift of $\approx$0.3 ppm. For a $\bar{B}_x$ term of $\sim$200 ppm/a.u., the second-order shift is $\approx \frac{1}{2} \times 200 \times 0.006 \times 0.006 \approx 0.004$ ppm. The $\bar{C}_{xx}$ term is even more difficult to determine, since there is a considerable range of values for H (Table 2), and the electric-field gradients will require very accurate potential surfaces for computation. For our present purposes, we will use a value of 0.001 a.u. (efg) for ΔV_{xx}, a reasonable estimate based on our previous work on CO adjacent an imidazole ring. An upper bound (HCN, HF, HC≡CH) would then be $\approx 100 \times 0.001 = 0.1$ ppm, comparable to the A term. However, use of the $\bar{C}_{xx}$ values for H_2, CH_4, and H_2CO yields shifts on the order of $\approx 5 \times 0.001 = 0.005$ ppm, and these bonding situations appear to be more realistic for amino acids in proteins. In either case, our results certainly suggest that the secondary structural shift—the "residual oxygen effect", observed previously in many ^{1}H NMR studies—does contain a significant electric-field contribution.

For ^{13}C NMR, we can make similar determinations of the $\bar{A}_x$, $\bar{B}_x$, and $\bar{C}_{xx}$ tensor contributions to the experimentally determined chemical-shift ranges. For a dipole shielding polarizability $\bar{A}_x \approx 900$ ppm/a.u., there will be an $\sim 900 \times 0.006 = 5.4$ ppm shift range. For $\bar{B}_x \approx 2000$ ppm/a.u., the shift is $\sim \frac{1}{2} \times 2000 \times 0.006 \times 0.006 = 0.04$ ppm. For $\bar{C}_{xx} \approx 500$ ppm/a.u., the shift is $\sim 500 \times 0.001 = 0.5$ ppm. Similarly, for ^{19}F, we can predict an $\bar{A}_x$ shift range of $\sim 1885 \times 0.006 = 11.3$ ppm, a $\bar{B}_x$ shift range of $\sim \frac{1}{2} \times 8000 \times 0.006 \times 0.006 = 0.2$ ppm, and a $\bar{C}_{xx}$ shift range of $\sim 5000 \times 0.001 = 5$ ppm.

We can also predict for C^{17}O-labeled heme proteins an $\bar{A}_x$ term shift of $\sim 1500 \times 0.006 = 9$ ppm, a $\bar{B}_x$ term shift range of $\sim 5900 \times (0.006)^2 = 0.2$ ppm, and a $\bar{C}_{xx}$ term shift range of $\sim 1000 \times 0.001 = 1$ ppm.

For ^{15}N, we have found for formamide (H_2NCHO) that $\bar{A}_x \sim 900$ ppm/a.u., (and $\bar{A}_y = -82$ ppm/a.u.), a rather small value given that the total ^{15}N chemical-shift range for backbone nitrogens is $\sim$25 ppm. This yields an effective field range of 25/900 = 0.028 a.u. or $\sim$5 times larger than that estimated from Fig. 1. However, the effective fields in the peptide group are expected to be *much* larger than those experienced by side chains, dielectric constants have no meaning at such short range, and there are expected to be significant electronic structural changes between, e.g., helical and sheet segments which are not sampled by most side-chain atoms.

The results that we have shown above clearly indicate that a major contribution to the chemical-shift ranges observed in proteins, for many nuclei, will be that due to the dipole shielding polarizability and the uniform component of the field, followed by the quadrupole shielding polarizability and the nonuniform component, the field

gradient—in accord with predictions made some time ago by Batchelor (*32*). The hyperpolarizabilities are expected to be generally smaller, as are the cross terms. For ^{13}C, ^{17}O, and ^{19}F, the large ranges of nonequivalence seen experimentally are almost certainly due to weak electrical interactions. These effects are expected to be largest for ^{19}F, which has $\bar{A}_x \sim 1850$ ppm/a.u., so we will consider this nucleus in some more detail, since it seems likely that the first specific assignments, using electrostatic modeling, can be made with this nucleus. As we have shown above, this A value is relatively unaffected by aromatic substitution and thus appears to be a good value for fluorophenylalanine- or fluorotryphophan-labeled proteins.

Recently, Luck and Falke have reported a 10 ppm chemical-shift range for 5-F–Trp-labeled *Escherichia coli* galactose (glucose) binding protein, a value very close to the ~12 ppm reported by Gerig and co-workers previously for parafluorophenylalanine-labeled rabbit (*46*) and chimpanzee (*4*) [identical to human; Ref. (*47*)] hemoglobins. We have used a finite-difference Poisson–Boltzman algorithm [Delphi, Biosym Technologies; Ref. (*42*)] to estimate the ranges of electric fields observed in these proteins and find generally good agreement between these fields and those computed by using a simple [Amber; Ref. (*48*)] point-charge model with $\epsilon = 2$, in which surface charges are deleted (to model the screening effects of counterions and the water bulk dielectric).

In both proteins, and using both electrostatic models, we obtained overall shift ranges from 5 to 10 ppm, in good general agreement with the experimental shift range of 10–12 ppm (which includes a probable ~1–2 ppm ring-current effect in the case of the heme protein).

Given the overall simplicity of the electrostatic modeling, the agreement between theory and experiment can be considered promising. In addition, in the galactose binding protein, the pattern of most shielded and deshielded (Trp 284, Trp 183) residues appeared in the calculations, while in HbCOA, one highly deshielded resonance which broadened upon spin labeling may arise, on the basis of the E-field calculations and model building, from PheB41. However, much more work needs to be done in order to be able to make firm assignments, based upon the weak electrical interactions that we have described above.

CONCLUSIONS

The results that we have presented above, together with those reported previously (*7, 18, 22*), strongly suggest that weak electrical interactions, mediated via the dipole and quadrupole shielding polarizabilities, make significant contributions to the ^{1}H, ^{13}C, ^{17}O, and ^{19}F chemical shifts observed due to folding in native proteins. Since similar shift ranges and electric fields are present in nucleic acids, it is possible that similar effects are important there also. We find good agreement between E fields computed from IR (*18, 23*) and NMR frequency shifts and those found from electrostatics calculations, and fields of this magnitude (up to ~3–4 $\times$ 10^7 V cm^{-1}) may influence spin–spin couplings also. For ^{19}F in fluorobenzenes, we have computed principal elements of the chemical shielding tensors in accord with experiment, and by using DHF theory, we deduce a dipole shielding polarizability of ~1900 ppm/a.u., enough to explain much of the large (~10–12 ppm) chemical-shift nonequivalencies observed in hemoglobin (*4*) and the galactose binding protein (*5*).

It is to be hoped that over the next few years it will become possible to obtain a much better understanding of electrostatics in proteins by further development of the protein electrostatic and quantum-chemical ideas outlined above. Ideally, it might become possible to routinely use chemical shifts as probes of macromolecular structure, providing potentially new ways of refining protein structures, as well as testing the various models of protein electrostatics. At the very least, it seems clear that electric-field effects on NMR spectra in proteins, mediated via the dipole and quadrupole shielding polarizabilities, seem worthy of further detailed experimental and theoretical study.

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REFERENCES

1. D. H. MEADOWS, J. L. MARKLEY, J. S. COHEN, AND O. JARDETZKY, *Proc. Natl. Acad. Sci. USA* **58,** 1307 (1967).
2. A. ALLERHAND, R. F. CHILDERS, AND E. OLDFIELD, *Biochemistry* **12,** 1335 (1973).
3. W. E. HULL AND B. D. SYKES, *J. Mol. Biol.* **98,** 121 (1975).
4. M. P. GAMCSIK, J. T. GERIG, AND R. B. SWENSON, *Biochim. Biophys. Acta* **874,** 372 (1986).
5. L. A. LUCK AND J. J. FALKE, *Biochemistry* **30,** 4248 (1991).
6. J. GLUSHKA, M. LEE, S. COFFIN, AND D. COWBURN, *J. Am. Chem. Soc.* **111,** 7716 (1989).
7. K. D. PARK, K. GUO, F. ADEBODUN, M. L. CHIU, S. G. SLIGAR, AND E. OLDFIELD, *Biochemistry* **30,** 2333 (1991).
8. S. J. PERKINS, *Biol. Magn. Reson.* **4,** 193 (1982).
9. G. WAGNER, A. PARDI, AND K. WÜTHRICH, *J. Am. Chem. Soc.* **105,** 5948 (1983).
10. J. C. HOCH, Ph.D. thesis, Harvard University, No. 8322365, University Microfilms International, Ann Arbor, Michigan, 1983.
11. A. PARDI, G. WAGNER, AND K. WÜTHRICH, *Eur. J. Biochem.* **137,** 445 (1983).
12. C. REDFIELD AND C. M. DOBSON, *Biochemistry* **29,** 7201 (1990).
13. E. OLDFIELD, Abstracts of the American Chemical Society Southeastern–Southwestern Meeting, New Orleans, Louisiana, December 7, 1990.
14. M. P. WILLIAMSON AND T. ASAKURA, *J. Magn. Reson.* **94,** 557 (1991).
15. M. P. WILLIAMSON, T. ASAKURA, E. NAKAMURA, AND M. DEMURA, *J. Biomol. NMR* **2,** 83 (1992).
16. T. ASAKURA, E. NAKAMURA, H. ASAKAWA, AND M. DEMURA, *J. Magn. Reson.* **93,** 355 (1991).
17. K. ÖSAPAY AND D. A. CASE, *J. Am. Chem. Soc.* **113,** 9436 (1991).
18. J. A. AUGSPURGER, C. E. DYKSTRA, AND E. OLDFIELD, *J. Am. Chem. Soc.* **113,** 2447 (1991).
19. C. E. DYKSTRA, *J. Am. Chem. Soc.* **111,** 6168 (1989).
20. C. E. DYKSTRA, *J. Phys. Chem.* **91,** 6216 (1987).
21. H. S. GUTOWSKY, T. C. GERMANN, J. D. AUGSPURGER, AND C. E. DYKSTRA, *J. Chem. Phys.* **96,** 5808 (1992).
22. E. OLDFIELD, K. GUO, J. D. AUGSPURGER, AND C. E. DYKSTRA, *J. Am. Chem. Soc.* **113,** 7537 (1991).
23. (*a*) C. E. DYKSTRA AND P. G. JASIEN, *Chem. Phys. Lett.* **109,** 388 (1984); (*b*) J. D. AUGSPURGER AND C. E. DYKSTRA, *J. Phys. Chem.* **95,** 9230 (1991).
24. H. STERNLICHT AND D. WILSON, *Biochemistry* **6,** 2881 (1967).
25. W. J. HORSLEY AND H. STERNLICHT, *J. Am. Chem. Soc.* **90,** 3738 (1968).
26. M. J. STEPHEN, *Mol. Phys.* **1,** 223 (1957).
27. A. D. BUCKINGHAM, *Can. J. Chem.* **38,** 300 (1960).
28. (*a*) B. DAY AND A. D. BUCKINGHAM, *Mol. Phys.* **32,** 343 (1976); (*b*) M. ŽAUCER AND A. AŽMAN, *Z. Naturforsch.* **34a,** 1279 (1979)); (*c*) M. I. VOLODICHEVA AND T. K. REBANE, *Teor. Eksp. Khim.* **19,** 357 (1984).
29. J. D. AUGSPURGER AND C. E. DYKSTRA, *Chem. Phys. Lett.* **183,** 410 (1991).

30. J. APPLEQUIST, *J. Math. Phys.* **24,** 736 (1983); *J. Chem. Phys.* **83,** 809 (1985).
31. W. T. RAYNES AND R. RATCLIFFE, *Mol. Phys.* **37,** 571 (1979).
32. J. G. BATCHELOR, *J. Am. Chem. Soc.* **97,** 3410 (1975).
33. A. SHOJI, T. OZAKI, T. FUJITO, K. DEGUCHI, AND I. ANDO, *Macromolecules* **20,** 2441 (1987); S. KUROKI, N. ASAKAWA, S. ANDO, I. ANDO, A. SHOJI, AND T. OZAKI, *J. Mol. Struct.* **245,** 69 (1991); S. KUROKI, S. ANDO, I. ANDO, A. SHOJI, T. OZAKI, AND G. A. WEBB, *J. Mol. Struct.* **240,** 19 (1990); T. ANDO, H. SAITO, R. TAKETA, A. SHOJI, AND T. OZAKI, *Macromolecules* **17,** 457 (1984).
34. D. S. WISHART, B. D. SYKES, AND F. M. RICHARDS, *J. Mol. Biol.* **222,** 331 (1991).
35. M. J. PACKER AND W. T. RAYNES, *Mol. Phys.* **69,** 391 (1990).
36. M. MEHRING, *in* "NMR Basic Principles and Progress" (P. Diehl, E. Fluck, and R. Kosfeld, Eds.), p. 181, Springer-Verlag, New York/Berlin, 1976.
37. Y. HIYAMA, J. V. SILVERTON, D. A. TORCHIA, J. T. GERIG, AND S. J. HAMMOND, *J. Am. Chem. Soc.* **108,** 2715 (1986).
38. C. HO, E. A. PRATT, AND G. S. RULE, *Biochim. Biophys. Acta* **988,** 173 (1989).
39. B. J. KIMBER, D. V. GRIFFITH, B. BIRDSALL, R. W. KING, P. SCUDDER, J. FEENEY, G. C. K. ROBERTS, AND A. S. V. BURGEN, *Biochemistry* **6,** 3492 (1977).
40. W. G. J. HOL, *Prog. Biophys. Mol. Biol.* **45,** 149 (1985).
41. S. DAO-PING, D. I. LIAO, AND S. J. REMINGTON, *Proc. Natl. Acad. Sci. USA* **86,** 5361 (1989).
42. K. A. SHARP AND B. HONIG, *Annu. Rev. Biophys. Chem.* **19,** 301 (1990).
43. A. WARSHEL AND J. AQUIST, *Annu. Rev. Biophys. Biochem.* **20,** 267 (1991).
44. M. SCHAEFER AND C. FROEMMEL, *J. Mol. Biol.* **216,** 1045 (1990).
45. E. OLDFIELD AND A. ALLERHAND, *J. Biol. Chem.* **250,** 6403 (1975).
46. J. T. GERIG, J. C. KLINKENBORG, AND R. A. NIEMAN, *Biochemistry* **22,** 2076 (1983).
47. D. RIFKIN AND W. KONIGSBERG, *Biochim. Biophys. Acta* **104,** 457 (1965).
48. S. J. WEINGER, P. A. KOLLMAN, D. T. NGUYEN, AND D. A. CASE, *J. Comput. Chem.* **7,** 230 (1986).
49. L. HYGAARD, I. BOJESEN, T. PEDERSON, AND J. RATRUP-ANDERSON, *J. Mol. Struct.* **2,** 209 (1968).
50. W. T. RAYNES, *in* "Specialist Periodical Reports" (R. K. Harris, Ed.), Vol. 1, p. 36, Chemical Society, London, 1972.
51. W. F. REYNOLDS, V. G. GIBB, AND N. PLAVAC, *Can. J. Chem.* **58,** 839 (1980).