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Calculation of Dipole-Shielding Polarizabilities ($\sigma_{\alpha\beta\gamma}^{I}$): The Influence of Uniform Electric Field Effects on the Shielding of Backbone Nuclei in Proteins

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Of all the NMR parameters, the chemical shielding is the most precisely measured; yet its quantitative use in studies of proteins still remains difficult. The ability to establish the extent that a secondary shielding mechanism contributes to a protein shielding tensor is useful,[1a−c] especially as accurate shielding anisotropy data can lead to structural and site-specific dynamic information.[2a−d]

The interaction of a nuclear magnetic moment, μ_I, of a nucleus I in a molecule occupying a fixed position and orientation relative to an external static homogeneous magnetic field, $\mathbf{B}_0$, with the magnetic field induced by the electrons' motion, $\mathbf{B}_I^{(ind)}$, is given by $-\mu_I \cdot \mathbf{B}_I^{(ind)} = \mu_{I\alpha}\sigma_{\alpha\beta}{}^{I}B_{0\beta}$, where $\sigma_{\alpha\beta}{}^{I}$ is a second rank magnetic shielding tensor. The presence of a weak static uniform electric field, $\mathbf{E}$, acts to polarize the electron cloud changing $\mathbf{B}_I^{(ind)}$.[3,4a−g] According to Buckingham,[3] the nuclear magnetic shielding tensor $\sigma_{\alpha\beta}{}^{I}$ of a nucleus in the presence of an external weak static uniform electric field $\mathbf{E}$ may be expanded using

$$\sigma_{\alpha\beta}{}^{I}(\mathbf{E}) = \sigma_{\alpha\beta}{}^{I} + \sigma_{\alpha\beta\gamma}{}^{I}E_\gamma + {}^1/_2\sigma_{\alpha\beta\gamma\delta}{}^{I}E_\gamma E_\delta + \sigma_{\alpha\beta,\gamma\delta}{}^{I}E_{\gamma\delta} + \ldots \quad (1)$$

The third and fourth rank tensors are referred to as the dipole-shielding polarizability, dipole−dipole-shielding hyperpolarizability, and quadrupole-shielding polarizability,[6c] respectively. $\sigma_{\alpha\beta\gamma}{}^{I}$ describes the nonlinear response of the electron cloud to first order in $\mathbf{E}$, μ_I, and $\mathbf{B}_0$, and the term containing $\sigma_{\alpha\beta\gamma\delta}{}^{I}$, which accounts for the quadratic response in $\mathbf{E}$, is possibly relatively small.[5] Augspurger et al.[5] reported some of the averaged quantities $A_\gamma(\mathrm{I})$ ($A_\gamma(\mathrm{I}) = ({}^1/_3)\sigma_{\alpha\alpha\gamma}{}^{I}$) and $2B_{\gamma\delta}(\mathrm{I})$ ($B_{\gamma\delta}(\mathrm{I}) = ({}^1/_6)\sigma_{\alpha\alpha\gamma\delta}{}^{I}$), for N, H, and C in a number of small molecules, concluding that the role of uniform electric fields to isotropic shielding in proteins, mostly mediated by $\sigma_{\alpha\beta\gamma}{}^{I}$, is probably significant. In contrast, an analysis using ^{1}H isotropic shielding data from proteins[7] concluded that, as compared to other secondary shielding mechanisms, uniform electric field effects for protons are small. The contribution to $\sigma_{\alpha\beta}{}^{I}(\mathbf{E})$ from $\sigma_{\alpha\beta,\gamma\delta}{}^{I}$ and a uniform electric field gradient, $E_{\gamma\delta}$ (efg),[4c,5,6] has rarely been considered; however, it could be significant.[5,6a,b] Here, we report calculations of $\sigma_{\alpha\beta\gamma}{}^{I}$ and show that they can provide considerable insight into the behavior of uniform electric fields upon the shielding of backbone nuclei in proteins.

$\sigma_{\alpha\beta\gamma}{}^{I}$ values for the N, H^N, and C′ of *N*-methyl acetamide (NMA) were calculated using the CTOCD scheme with the diamagnetic contribution set to zero (CTOCD-DZ).[8a] For this method, analytical formulas for $\sigma_{\alpha\beta\gamma}{}^{I}$ are known and were recently used to calculate $\sigma_{\alpha\beta\gamma}{}^{I}$ in a few cases.[8b] The CTOCD-DZ method ensures that all components of $\sigma_{\alpha\beta\gamma}{}^{I}$ are *origin independent* irrespective of basis set size, although the accuracy of the results depends on the size and quality of the basis set. The total magnetic properties of $\sigma_{\alpha\beta\gamma}{}^{I}$ in the CTOCD-DZ scheme are defined as the sum of a paramagnetic, $\sigma_{\alpha\beta\gamma}{}^{pI}$, and a "diamagnetic", $\sigma_{\alpha\beta\gamma}{}^{\Delta I}$, component

$$\sigma_{\alpha\beta\gamma}{}^{I} = \sigma_{\alpha\beta\gamma}{}^{pI} + \sigma_{\alpha\beta\gamma}{}^{\Delta I} \quad (2)$$

The essential properties of $\sigma_{\alpha\beta\gamma}{}^{\Delta I}$ are discussed in detail in ref 8a. Most notably, in the exact Hartree−Fock limit, $\sigma_{\alpha\beta\gamma}{}^{\Delta I}$ is proved to reduce to a symmetric diamagnetic component,[8a] $\sigma_{\alpha\beta\gamma}{}^{dI}$ ($\sigma_{\alpha\beta\gamma}{}^{dI} = \sigma_{\beta\alpha\gamma}{}^{dI}$), when $\sigma_{\alpha\beta\gamma}{}^{\Delta I} = \sigma_{\alpha\beta\gamma}{}^{dI}$; this provides a means to assess the quality of the basis set. The *ab initio* calculations used the SYSMO suite of programs[9] employing a coupled Hartree−Fock level of theory, a basis set[4f,8b] developed by Sadlej[10] for computing molecular electrical properties, and the models shown in Figure 1 (A and B), and neglected the effect of motion.[4d]

The quantities $A_\gamma(\mathrm{I})$ (σ scale, units ppm au) calculated from $\sigma_{\alpha\beta\gamma}{}^{N}$, $\sigma_{\alpha\beta\gamma}{}^{HN}$, and $\sigma_{\alpha\beta\gamma}{}^{C'}$ are shown in Figure 1C, 1D, and 1E, respectively. $A_\gamma(\mathrm{N})$ and $A_\gamma(\mathrm{C}')$ are formed using the NMA molecular frame shown in the figures; however, the $A_\gamma(H^N)$ values were produced after rotation of $\sigma_{\alpha\beta\gamma}{}^{HN}$ by 29.4° around the *z*-axis, so that the N−H^N bond is directed along the *x*-axis, allowing comparison to the value proposed by Buckingham[3] of −34.3 ppm au (-2×10^{-12} esu^{-1}). In this case, $A_x(H^N)$ is slightly larger[1b,4f] at −89.42 ppm au (-5.21×10^{-12} esu^{-1}); however, the results also show that the N−H bond is not axially symmetric with $A_y(H^N)$ found to be −17.89 ppm au. $\sigma_{\alpha\beta\gamma}{}^{N}$ and $\sigma_{\alpha\beta\gamma}{}^{C'}$ are dominated by $\sigma_{\alpha\beta\gamma}{}^{p}$ with $\sigma_{\alpha\beta\gamma}{}^{\Delta}$ having only a small influence. In each case, the $\sigma_{\alpha\beta\gamma}{}^{dI}$ values are symmetric with components of size similar to $\sigma_{\alpha\beta\gamma}{}^{\Delta I}$, indicating the basis set is a reasonable choice for computing $\sigma_{\alpha\beta\gamma}{}^{I}$, although neither $\sigma_{\alpha\beta\gamma}{}^{\Delta I}$ was symmetric ($\sigma_{\alpha\beta\gamma}{}^{\Delta I} \neq \sigma_{\beta\alpha\gamma}{}^{\Delta I}$). For H^N, the components in $\sigma_{\alpha\beta\gamma}{}^{pHN}$ and $\sigma_{\alpha\beta\gamma}{}^{\Delta HN}$ are similar, and the results for $\sigma_{\alpha\beta\gamma}{}^{\Delta HN}$ are close to $\sigma_{\alpha\beta\gamma}{}^{dHN}$, suggesting the basis set is also adequate for accurately computing $\sigma_{\alpha\beta\gamma}{}^{HN}$ at this level of theory. The values for $A_z(\mathrm{I})$ are zero because the dihedral angles have constrained the O, C′, N, and H^N atoms to the *xy* (mirror) plane, leading to C_s symmetry at each site, and in this situation $\sigma_{\alpha\alpha z}{}^{I} = 0$.[4e] Changing the dihedral angle $\omega_4(\mathrm{C_1C'NH^N})$ from 0° rotates the H^N out of the *xy* plane, imposing C_1 symmetry upon the N and H^N sites, and gives the quantities $A_z(\mathrm{N})$ and $A_z(H^N)$ signed values of ∼1.8 and ∼0.6 ppm au/deg, respectively, with the sign changing as the H^N passes through the XY plane. These discussions suggest that uniform electric fields can potentially lead to subtle shielding effects depending upon the direction of $\mathbf{E}$.

To test that these calculations give reasonable values for the $\sigma_{\alpha\beta\gamma}{}^{I}$ of N, H^N, and C′ nuclei in proteins, a pH titration was performed using Hen Lysozyme (HEWL). E35 in HEWL possesses a pK_a of 6,[12a,b] and to a good approximation its local environment is not perturbed by other groups titrating in the range pH 5−8.[12a] Thus, shielding changes caused by the ionization of E35 can be measured

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Table 1. Experimental (σ^e/ppm) and Predicted Shielding Changes (σ^{c1}/ppm, Method 1 and σ^{c2}/ppm, Method 2) Due to the Ionization of E35[a]

$C_{(i-1)}/N_{(i)}H_{(i)}^N$	$\sigma^e(C_{(i-1)})$	$\sigma^{c1}(C_{(i-1)})$	$\sigma^{c2}(C_{(i-1)})$	$\sigma^e(N_{(i)})$	$\sigma^{c1}(N_{(i)})$	$\sigma^{c2}(N_{(i)})$	$\sigma^e(H_{(i)}^N)$	$\sigma^{c1}(H_{(i)}^N)$	$\sigma^{c2}(H_{(i)}^N)$
A31/A32	+0.45	−0.25	−0.04	+0.51	+0.21	+0.78	+0.10	0.00	+0.07
L56/Q57	+0.10	+0.15	+0.56	+0.04	+0.07	+0.08	+0.02	+0.03	+0.10
Q57/I58	−0.15	−0.05	−0.22	+0.72	+0.25	+1.44	+0.07	+0.02	+0.09
A107/W108	+0.18	+0.14	+0.66	−0.67	−0.67	−1.21	−0.04	+0.02	+0.09
W108/V109	+0.20	−0.13	−0.46	−0.65	+0.63	+0.04	−0.15	+0.13	−0.04
V109/A110	−0.79	−0.21	−0.48	−2.84	−2.13	−3.35	−1.28	−0.84	−1.14
A110/W111	−0.58	−0.41	−0.99	−0.85	+0.17	+0.72	−0.13	−0.14	−0.21
W111/R112	−0.29	−0.21	−0.46	+0.26	+0.16	+0.21	−0.26	−0.04	−0.10

[a] HEWL has a fairly constant electrostatic potential over the pH range 5−8 with the total charge little changed below pH 9.[12a] E35 is in a hydrophobic region[15] and has a reduced solvent accessibility, and its local environment may be modeled using a dielectric constant, ϵ, between 2 and 4[12c] with $\epsilon = 3.5$ used here.[12d] Delphi takes account of the alignment of solvent dipoles and counterions which act to screen the electric field, **E**, inside the protein, hence generally leading to $|\sigma^{c1}| < |\sigma^{c2}|$. Delphi computes **E** in units of (kT/e)/Å $= 10^{10}/38.94130$ V m^{-1} (298 K) and 1 au of **E** $= 5.14221 \times 10^{11}$ V m^{-1}. Delphi calculations used a grid resolution of 4 grid points/Å. − indicates a change to lower shielding with increased pH. 4LZT crystals were formed at pH 4.5.[15]

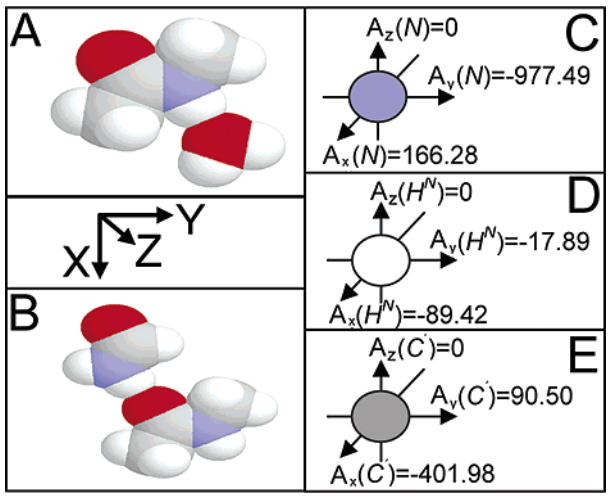

Figure 1. The geometry of the models used to calculate $\sigma_{\alpha\beta\gamma}^I$. The *z*-axis is directed out of the plane. The models in A and B were used to calculate either $\sigma_{\alpha\beta\gamma}^N$ and $\sigma_{\alpha\beta\gamma}^{HN}$ or $\sigma_{\alpha\beta\gamma}^{C'}$, respectively. The NMA is positioned with the C′−N bond parallel to the *y*-axis, the O, C′, N, H^N atoms in the *xy* plane, N−H^N = 1.04 Å, and the bond lengths and angles are consistent with those tabulated by Engh and Huber.[11] The dihedrals $\omega_1(C_1C'NC_2) = \omega_2(OC'NH^N) = 180°$ and $\omega_3(OC'NC_2) = \omega_4(C_1C'NH^N) = 0°$. The N−$H^N$ bond is inclined at 29.4° to the *x*-axis, the H_2O is in the *xy* plane with N··O = 3 Å, the angle N−H^N··O = 180°, the $HCONH_2$ is in the *xy* plane with N··O(C′) = 3 Å, and the angle N−H^N··O(C′) = 180°. The transformation law connecting the components of a third rank tensor in two bases is $\sigma_{\rho\tau\upsilon}^I = R_{\rho\alpha}R_{\tau\beta}R_{\upsilon\gamma}\sigma_{\alpha\beta\gamma}^I$, and the set of numbers $\sigma_{\alpha\alpha\gamma}^I$ transform as the components of a vector. The isotropic shielding contribution from the second rank tensor $\sigma_{\alpha\beta\gamma}E_\gamma^I$ is $A_\gamma(I)\cdot E_\gamma = (^1/_3)\sigma_{\alpha\alpha\gamma}^I E_\gamma$, where the E_γ values are the components of the total electric field vector, **E**, at a nucleus from a set of point charges fixed in the molecular frame. $\sigma_{\alpha\beta\gamma}^I$ has units of ppm au = 1.94469 × 10^{-18} mV^{-1} = 5.83003 × 10^{-14} esu^{-1}.

without significant effects from other titratable groups.[12b] The pH-induced isotropic shielding changes for the C′, N, and H^N nuclei in peptide bonds close to E35[15] (∼ <8 Å) were extracted from fitted titration curves exhibiting a pK_a close to 6. Assuming these experimental shielding changes arise *solely* from uniform electric field effects caused by the deprotonation of E35, without any other pH-induced structural alterations[12a] which might lead to a shielding change, they were compared to the shielding calculated via $A_\gamma(I)\cdot E_\gamma$. The electric field vector, **E**, from the E35 anion was computed either using the electrostatic modeling package Delphi v.4[13] (method 1) or by assuming that **E** a distance r_i from a point charge, Q_i, in the E35 anion immersed in a uniform dielectric, is given by the sum of the gradients of the electric potentials from each charge, $(^1/_{4\pi\epsilon_0\epsilon})\sum_i - \nabla\{Q_i/r_i\}$ (method 2). The partial charges for glutamate proposed by Cornell et al.[14] and the 4LZT X-ray structure[15] (R = 0.95 Å) were used. The local frame of each peptide bond was rotated, prior to the calculation of **E**, so that the O, C, N, and H^N atoms overlaid similar atoms in the NMA molecular frame. The experimental and calculated shielding changes for nuclei in eight peptide bonds near to E35 are reported in Table 1, and in a majority of cases both the sign and the magnitude of the experimental pH induced shielding changes can be predicted reasonably well using uniform electric field effects. Considering that this represents a simplification and that other contributions will be necessary for an adequate description of electric field effects upon shielding, such as $\sigma_{\alpha\beta,\gamma\delta}^I$ and efg's[5,6] electron correlation[4g] and $\sigma_{\alpha\beta\gamma\delta}^I$,[5] the level of agreement seems to be encouraging particularly for those nuclei in the V109/A110 peptide bond which are closest to E35 and experience the largest perturbation. The complete $\sigma_{\alpha\beta\gamma}^I$ tensors will be useful for assessing uniform electric field contributions to shielding in proteins[1a,b,5−7] and protein−ligand interactions.

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Supporting Information Available: Coordinates, $\sigma_{\alpha\beta\gamma}^{dI}$, $\sigma_{\alpha\beta\gamma}^{pI}$, $\sigma_{\alpha\beta\gamma}^{\Delta I}$, and $\sigma_{\alpha\beta\gamma}^I = \sigma_{\alpha\beta\gamma}^{pI} + \sigma_{\alpha\beta\gamma}^{\Delta I}$ for the N, H^N, and C′ of NMA and three additional data sets for Table 1 (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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