A Basis Size Dependence Study of Carbon-13 Nuclear Magnetic Resonance Spectroscopic Shielding in Alanyl and Valyl Fragments: Toward Protein Shielding Hypersurfaces

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Abstract: We have investigated the effects of basis set quality on the accuracy and speed of *ab initio* calculations of C^{α}, C^{β} chemical shifts (or shieldings) of alanine and valine residues in proteins. Five basis set combinations were studied: a uniform 3-21G and a 4-31G/3-21G "locally dense" basis, both producing 88 contracted functions; a 6-311G+(2d)/3-21G and a 6-311G+(2d)/6-31G basis, each giving a total of 108 contracted functions; and a uniform STO-3G basis, giving 48 contracted functions. We find that results obtained by using small Gaussian basis sets correlate well with calculations performed using very large (6-311G++(2d,2p)/6-31G) basis sets, with only changes in slope and offset being required in order to bring results into excellent agreement, while the time necessary for the calculations is significantly reduced. This marked decrease in computational time using small, locally dense basis sets has permitted the first calculation of a three-dimensional chemical shielding hypersurface for valine. Chemical shifts predicted by using the ϕ,ψ,χ^1 shielding hypersurface are in good accord with chemical shift values determined experimentally. These results open up new opportunities for the relatively rapid evaluation of amino acid shielding hypersurfaces for future use in protein structure prediction and refinement.

Introduction

The origins of the chemical shift nonequivalencies seen in native proteins have long been the topic of debate.[1,2] For atoms such as ^{13}C, ^{15}N, and ^{19}F, the range of chemical shifts is considerable—up to about 30 ppm for ^{15}N, while for heavier elements, such as ^{57}Fe, the chemical shifts observed in different proteins can be even larger, as seen for example in the ~3000 ppm chemical shift difference between $^{57}Fe^{II}$ in myoglobin and cytochrome *c*.[3] Until recently, the *ab initio* computation of chemical shifts in molecules as large as proteins was generally thought to be intractable because of the prohibitive size of the calculation. Fortunately, however, the chemical shift is a rather "local" property, with only a limited number of atomic interactions governing shielding. For example, using single processor workstations and modern codes,[4,5] we and others have been able to successfully compute the ^{13}C, ^{15}N, and ^{19}F shieldings of individual sites in proteins by using small atomic clusters to mimic individual protein sites,[6-11] and equally promising results have been obtained for much more complex systems, such as CO and O_2 ligands bound to model heme systems.[12]

One immediate and useful outcome of this work is that chemical shift assignments can be verified; but more importantly, NMR chemical shifts appear to have promise in both structure prediction[13] and structure refinement.[14-16] This is because many chemical shifts, especially those of ^{13}C, are strongly related to the protein torsion angles ϕ, ψ, and χ. We have already reported ^{13}C chemical shift surfaces where the shift (δ) is a function of ϕ,ψ (alanine) or ϕ,ψ,χ^1 (valine), but these calculations were exceptionally lengthy because large basis sets were used in order to obtain the best possible absolute shieldings. For example, the time necessary to calculate a complete ϕ,ψ shielding surface for alanine when using a very large basis was about 3 CPU months (at 15 Mflop), rendering evaluation of complete shielding surfaces for residues with larger side chains impractical. In a typical amino acid such as isoleucine there are four dihedral angles which can influence C^{α} and C^{β} shielding, requiring a four-dimensional shielding hypersurface, $\sigma = f(\phi,\psi,\chi^1,\chi^2)$. Utilizing very large basis sets makes such a calculation (nearly 21 000 points) quite difficult in any reasonable length of time. Thus, the need to minimize the length of chemical shift calculations while maintaining the accuracy of the results is apparent, especially when considering the need for such surfaces in structure refinement.[14-16]

The amount of time necessary to complete a self-consistent-field gauge-including-atomic-orbital (SCF-GIAO) chemical shielding calculation scales between $(N \log N)^2$ and N^4, where

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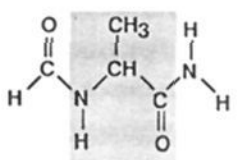
N is the number of contracted basis functions used.[17] So, one logical strategy for decreasing computational time is to reduce the number of contracted functions, as noted by Chesnut and Moore.[18] These workers previously pointed out that the highly local nature of the chemical shift makes it possible to obtain theoretical shielding values of good quality by using large basis sets located only on the atom whose shift is of interest, while the rest of the atoms in the molecule are given more modest bases—the "locally dense" basis approach. In addition, Fowler et al.[19] have demonstrated that there exists a monotonic basis-shift dependence in shielding calculations, and by establishing the basis dependence of shielding in a small system, a means for extrapolating from small to large basis sets was devised. The usefulness of this scaling has been shown in the case of fullerenes, in which the basis dependence was obtained from shielding calculations for the benzene molecule. These computations were, however, performed using a common gauge origin method, raising the question as to whether the same simple basis set dependence is applicable to local origin methods. In addition, the extent to which smaller bases might be used in evaluating the shielding of species as complex as amino acids remains to be determined. We report in this paper results for local origin shielding calculations using a series of basis sets/basis set combinations of varying complexity, on alanine as well as valine fragments. Complete alanine $\delta(\phi,\psi)$ chemical shift surfaces were constructed for three small basis sets, and comparisons were made against experimental results, as well as against theoretical surfaces created using a much larger basis set. In addition, a complete valine $\delta(\phi,\psi,\chi^1)$ hypersurface was also constructed and tested in the same manner. Our results indicate that full three-dimensional amino acid shielding hypersurfaces which compare well with large basis calculations and with experiment are now accessible in reasonable periods of time, opening the way to their use in structure prediction and refinement.

Computational Aspects

All shielding calculations were carried out using the TEXAS-90 program of Pulay, Wolinski, and Hinton[4,5] which employs an efficient version of the gauge-including-atomic-orbital (GIAO) method originally proposed by Ditchfield[20] and, in a different context, by London.[21] Five basis sets were examined in various combinations: a uniform STO-3G basis (basis set 1); a uniform 3-21G basis (basis set 2);[22] a combined "locally dense" or "attenuated" basis[18] (basis set 3) consisting of 4-31G basis functions[22] on selected atoms with a 3-21G basis covering the remaining atoms, shown below with the atoms carrying 4-31G basis functions shaded;



a 3-21G basis with 6-311G+(2d) basis functions[7] on C^α and C^β (basis set 4); and a 6-31G basis with 6-311G+(2d) basis functions on C^α and C^β (basis set 5). Computations were performed on IBM RISC/6000 workstations, Models 340, 350, and 360 (IBM Corporation, Austin, TX). The amino acid fragments consisted of *N*-formyl-L-alanine amide or *N*-formyl-L-valine amide molecules which had been extensively minimized at the helix geometry via a steepest descent method (15 000 steps using the Discover Program; Biosym Technologies, Inc., San Diego, CA). The 2-D shielding surfaces were constructed by choosing 358 ϕ,ψ points over the entire Ramachandran space (360° by 360°) with a more dense placement of points in the frequently occupied regions of ϕ,ψ space. These surfaces were approximated by a Fourier series in ϕ and ψ up to the $n = 3$ term, including all of the cross-terms, giving a total of 66 independent functions, plus a constant offset. The 2-D surfaces were then scaled to the large basis results by using 25 randomly chosen ϕ,ψ points. The 3-D valine hypersurface was constructed by calculating a uniform grid of 1728 points (12^3 points, 30° intervals) encompassing the entire ϕ,ψ,χ^1 Ramachandran space, then fitting these calculated values to a "best fit" function (Matlab, The Mathworks, Boston, MA).

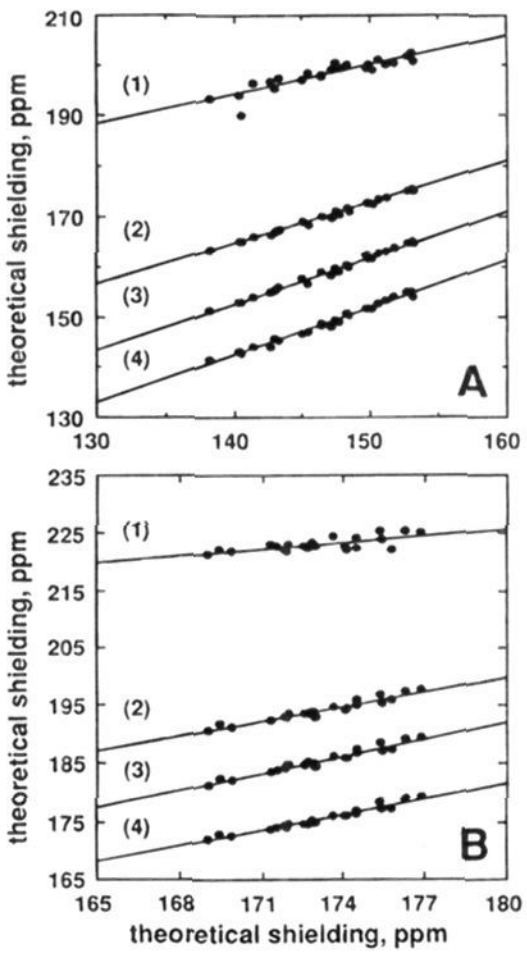


Figure 1. Theoretical C^α shieldings calculated in an *N*-formyl-L-alanine amide fragment at 25 ϕ,ψ torsions for various basis set sizes compared with a large basis calculation for (A) C^α and (B) C^β. The basis sets used were the following: (1) a uniform STO-3G basis; (2) a uniform 3-21G basis; (3) a 4-31G/3-21G locally dense basis; and (4) a uniform 3-21G basis with 6-311G+(2d) functions placed on C^α and C^β.

Results and Discussion

To obtain a general picture of how well the four small basis sets (1−4) would reproduce results obtained from a large basis calculation (using a 6-311G++(2d,2p)/6-31G locally dense basis, which we will refer to as basis set 6), a set of 25 ϕ,ψ pairs was chosen, designed to reproduce the maximum ~14 ppm range of alanine C^α shieldings seen on the ϕ,ψ surface. Chemical shieldings for this set of structures using basis sets 1−4 were computed, and compared with the shieldings produced with the large basis set, 6; results for C^α, C^β are shown in Figure 1, parts A and B, respectively. Slopes, y-intercepts, correlations, rmsd's, and mean computational times are given in Table 1. It is apparent from these results that the STO basis set calculations (set 1) do not accurately reproduce the results from the larger basis set calculation, indicating that basis set 1 is inadequate for constructing shielding surfaces. This is particularly clear from the large rmsd, 2.20 ppm, Table 1. However, results obtained by using the other three basis sets (2−4) are more attractive. All three were able to satisfactorily reproduce the large basis set results, with relatively good correlations and

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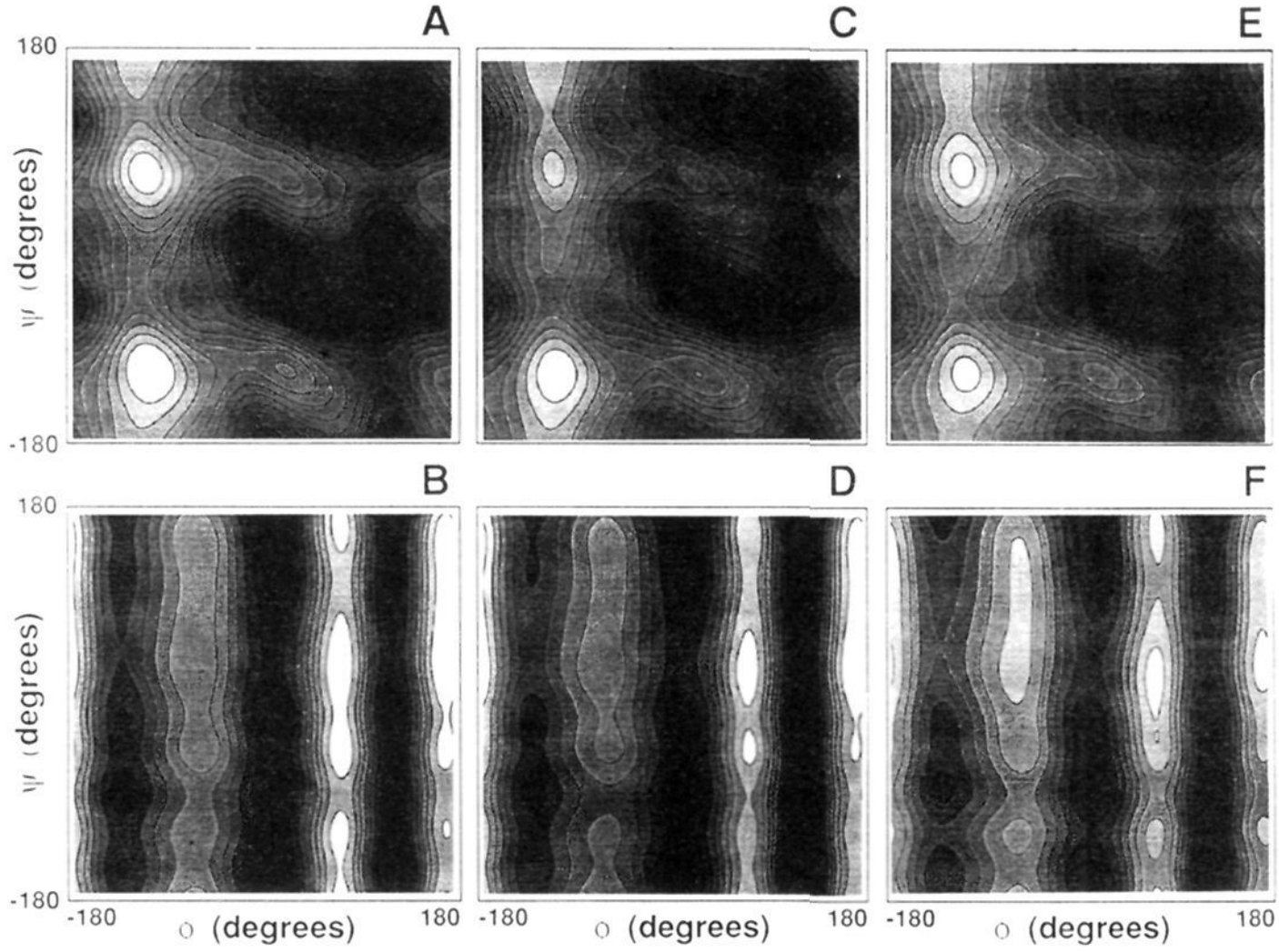

Figure 2. Alanine Ramachandran shielding surfaces calculated for C^{α} and C^{β} sites in *N*-formyl-L-alanine amide. (A) C^{α} surface using a uniform 3-21G basis with 4-31G basis functions on selected atoms (basis set 3); (B) same as part A but for C^{β}; (C) C^{α} using a uniform 3-21G basis witn 6-31G+(2d) functions placed on C^{α} and C^{β} (basis set 4); (D) as in part C but for C^{β}; (E) C^{α} using a 6-311G++(2d,2p)/6-31G locally dense basis (basis set 6); (F) as in part E but for C^{β}.

Table 1. Comparison between 25 Small and Large Basis Set Calculations of C^{α}, C^{β} Shieldings in *N*-Formyl-L-Alanine Amide

basis set[a]	slope[b]	intercept (ppm)[c]	R^2 [d]	rmsd (ppm)[e]	time (min)[f]
C^{α} 1	0.59	111.69	0.801	2.20	6.7
2	0.81	51.94	0.992	0.46	10.5
3	0.94	23.62	0.991	0.42	14.2
4	0.95	12.78	0.987	0.51	24.2
C^{β} 1	0.38	157.08	0.480	2.13	6.7
2	0.84	48.45	0.917	0.69	10.5
3	0.97	17.28	0.942	0.48	14.2
4	0.90	20.10	0.954	0.47	24.2

[a] Basis set designations are those noted in the text under computational aspects. [b] The slope indicated is that obtained by plotting theoretical shieldings using the basis set indicated versus shieldings for a very large basis calculation (basis set 6). [c] Absolute shielding error from the large basis prediction (set 6) for the basis indicated. [d] Correlation coefficient, small to large basis. [e] Root-mean-square deviation from the linear relation scaled by the slope between the large and small basis set indicated. [f] Time taken for a single chemical shielding calculation in CPU minutes on an IBM RISC/6000 Model 360 (at a ~100 Mflop peak speed).

rmsd's, Table 1. What the smaller basis sets are not able to do, however, is reproduce the relatively good absolute shielding that basis set 6 produces, as demonstrated by the large *y*-intercepts shown in Table 1. Nevertheless, the good correlations seen suggest that an accurate NMR chemical shielding surface might be produced by using a smaller basis set to construct the correct *shape* of a shielding surface, then offsetting and, as necessary, scaling the absolute shieldings using results obtained from computations employing large, saturated basis sets (or possibly from experiment). Since the R^2 values shown in Table 1 are not unity, several points need to be obtained from large basis set calculations in order to calibrate both slope and intercept. A comparison between the results obtained using basis sets 3 and 4 for C^{α} and C^{β} in alanine seems to indicate

Table 2. Comparisons between Small and Large Basis Set Calculations for Complete C^{α}, C^{β} Chemical Shift Surfaces for *N*-Formyl-L-alanine Amide[a]

basis set	slope	intercept (ppm)	R^2	rmsd (ppm)
C^{α} 2	0.78	55.46	0.979	0.51
3	0.89	28.93	0.976	0.49
4	0.93	11.98	0.964	0.58
C^{β} 2	0.83	49.83	0.913	0.73
3	0.98	16.20	0.938	0.46
4	0.90	19.81	0.951	0.47

[a] See footnotes in Table 1 for further details.

that the C^{α} site is more susceptible to basis set deficiencies, primarily on the amide nitrogen,[23] although the overall agreement is still quite good.

To further test the accuracy of calculations done with smaller basis sets, complete chemical shielding surfaces were produced for alanine using 358 points in ϕ,ψ space, and results are shown in Figure 2. The surfaces shown in Figures 2A and 2B (C^{α} and C^{β}) were constructed using shieldings calculated with basis set 3. Figures 2C and 2D were produced using basis set 4, and the surfaces shown in Figures 2E,F were constructed from basis set 6. (A basis set 2 surface was also calculated; data not shown.) As can be seen from Figure 2, both smaller basis set calculations reproduce, after scaling, the general features of the larger basis set (6) surface, especially in the conformationally allowed regions corresponding to α-helices and β-sheets in proteins. Comparisons between the chemical shifts for each of the three small basis set surfaces (without scaling) and those of the larger basis set (slope, intercept, rmsd, and correlation) are shown in Table 2. As can be seen from the results given in Table 2, as the number of basis functions on the atom being calculated is increased, the overall accuracy of the absolute

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Table 3. Comparisons between Experimental Chemical Shifts and Small and Large Basis Set Calculations of C^α, C^β Shieldings Using the *N*-Formyl-L-alanine Amide Shielding Surface[a]

basis set	slope	intercept (ppm)	R^2	rmsd (ppm)
C^α 2	−0.57	200.0	0.902	1.21
3	−0.60	191.3	0.908	0.68
4	−0.69	186.0	0.890	0.77
6	−0.79	189.3	0.928	0.61
C^β 2	−0.48	204.0	0.686	1.33
3	−0.55	196.8	0.717	1.24
4	−0.44	184.6	0.727	1.20
6	−0.59	185.4	0.779	1.03

[a] See footnotes in Table 1 for further details.

shielding improves dramatically. Yet it is the smaller basis set (set 3) which affords the best correlation and rmsd when considering both C^α and C^β, due presumably to the lone pair on the nitrogen requiring a larger number of valence basis functions to be described adequately.[24]

We can also gauge the quality of the small basis set surfaces for the alanine C^α and C^β sites by making comparisons with known experimental NMR chemical shifts. Table 3 shows statistical results from comparisons between experimental C^α, C^β NMR chemical shifts for two proteins and the chemical shieldings predicted using the surfaces shown in Figure 2 with torsion angles obtained from their X-ray structures. The two proteins used were *Staphylococcal* nuclease[25,26] and an invertebrate calmodulin.[27,28] As in the previous comparisons, relatively good agreement between results produced from the large basis set (6) and those from the smaller basis sets (2−4) exists, validating the utility of the small basis method. The observation that the slopes for C^β do not get very close to −1, even for basis set 6, shows that C^β chemical shift non-equivalencies in proteins are not entirely determined by dihedral angles and has been discussed previously.[13] However, small basis results show considerable promise as a means of drastically speeding up surface construction without significantly degrading the quality of the calculations.

To guard against our results being simply an aberration for the specific case of alanine, calculations of valine chemical shieldings were also performed. Since results with alanine dictated the clear need for a larger number of basis functions on the nitrogen than afforded by a 3-21G basis, we chose to test the affect on time and quality of moving from a 3-21G basis on non-calculated atoms (basis set 4) to the larger 6-31G basis (basis set 5). Although this would add four Gaussian functions per heavy atom and one Gaussian function per hydrogen atom, there would be no increase in either the number of contracted functions or integrals—so the only time increase would come from the greater number of primitive Gaussians used. Thus complete 2-D valine surfaces were constructed using basis set 4, *together with partial surfaces* ($-180° \leq \phi \leq 0°$) using the larger basis set 5, at $\chi^1 = 60$, −60, and 180°. The surfaces corresponding to $\chi^1 = 60°$ are presented in Figure 3 together with a partial valine surface constructed using basis set 6, and as before, both surfaces were tested using a point-by-point comparison to the basis set 6 valine surface. As can be seen, both smaller basis set surfaces 4 and 5 correlate well with the larger calculation, 6. Although the improvement in the correlation from basis set 4 to 5 is noticeable, the same is also true of the computational time. On average, calculations using basis set 5 were 2.3 CPU hours for valine, to be compared with 0.91 CPU hours for basis set 4, an increase of 2.5 in time. Believing that the somewhat modest increase in the quality of calculations done using basis set 5 as opposed to set 4 did not justify such a large increase in computational time, valine shielding calculations using basis set 4 were expanded to encompass the entire ϕ,ψ,χ^1 space to test the final utility of the small basis method: the construction of a full three-dimensional C^α chemical shielding hypersurface for valine, and comparison with experiment.

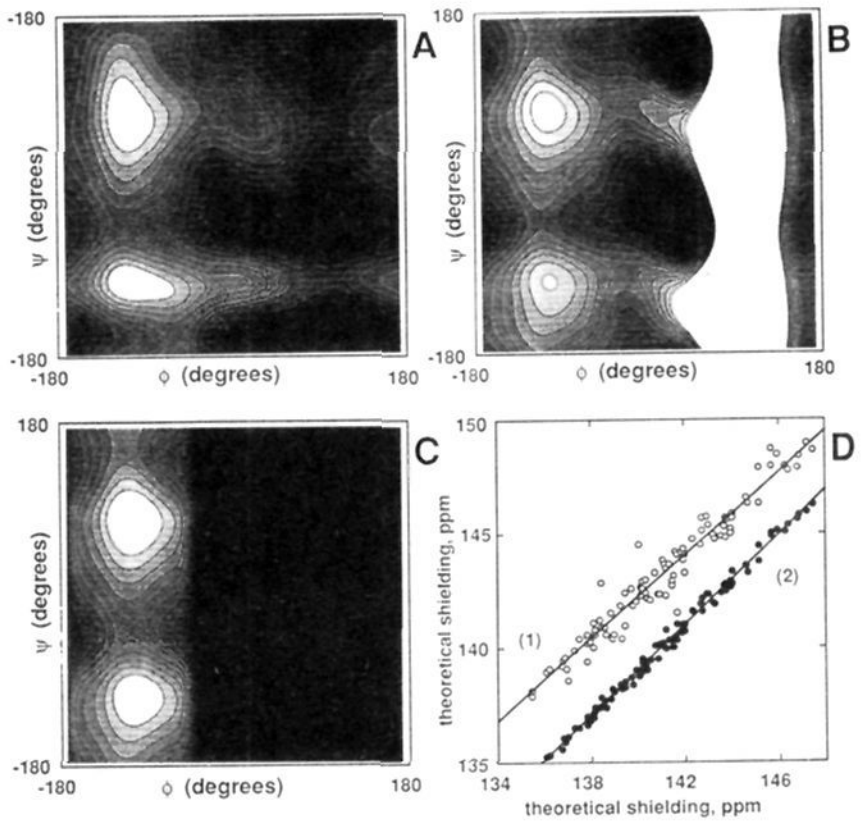

Figure 3. Valine Ramachandran shielding surface calculated for C^α of *N*-formyl-L-valine amide at $\chi^1 = 180°$ using (A) a uniform 3-21G basis with 6-311G+(2d) functions placed on C^α and C^β, (B) a uniform 6-31G basis with 6-311G+(2d) functions placed on C^α and C^β, and (C) a 6-311G++(2d,2p)/6-31G locally dense basis. In part D theoretical chemical shieldings are shown for valine at $\chi^1 = 180°$, 60°, and −60° using (1), shieldings calculated using a uniform 3−21G basis with 6-311G+(2d) functions placed on C^α and C^β ($m = 0.91$, $b = 15.34$, $R^2 = 0.947$, rmsd = 0.674) and (2) shieldings calculated using a uniform 6-31G basis with 6-311G+(2d) functions placed on C^α and C^β ($m = 0.99$, $b = 0.98$, $R^2 = 0.994$, rmsd = 0.227) versus shieldings obtained using a 6-311G++(2d,2p)/6-31G locally dense basis.

The three-dimensional shielding hypersurface for the C^α site in valine is shown in Figure 4. A 12 × 12 × 12 (30° increment) grid of ϕ,ψ,χ^1 points was utilized to map the hypersurface, for a total of 1728 *ab initio* shielding calculations. Figure 4A shows slices through the 3-D hypersurface: the bottom plane of Figure 4A is a normal Ramachandran shielding surface $\delta(\phi,\psi)$ at $\chi^1 = 180°$; the other two surfaces are $\delta(\phi,\chi^1)$ at $\psi = 180°$ and $\delta(\psi,\chi^1)$ at $\phi = 180°$. Perhaps of more immediate use, Figure 4B shows three slices through the hypersurface, representing conventional ϕ,ψ chemical shift surfaces for the three valine χ^1 angles of ±60, 180°. To investigate the accuracy of the hypersurface, valine C^α chemical shieldings predicted using ϕ, ψ, and χ^1 angles from *Staphylococcal* nuclease and an invertebrate calmodulin were plotted versus the experimentally determined chemical shifts, Figure 5. As can be seen, a relatively good correlation between theory and experiment ($R^2 = 0.84$) is obtained. The slope of −0.80, although somewhat less than the correct value of −1.00, still reproduces the experimental 9 ppm chemical shift range to within 2 ppm, and is close to the large basis (set 6) result of −0.86. The rmsds

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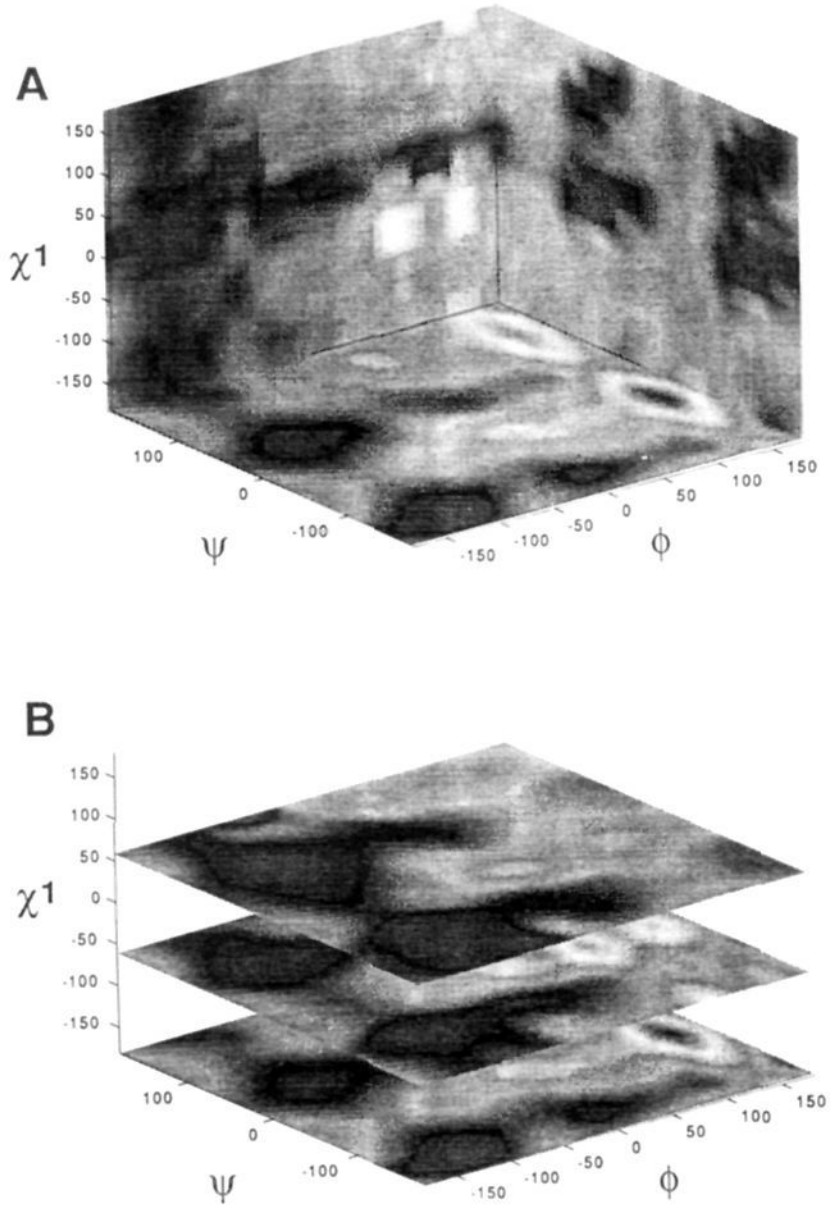

Figure 4. (A) Valine C^{α} chemical shielding hypersurface $\delta(\phi,\psi,\chi^1)$ calculated using a uniform 3-21G basis with 6-311G+(2d) functions placed on C^{α} and C^{β}. The entire surface consisted of 1728 points uniformly distributed over ϕ,ψ,χ^1 space. The slices through the surface shown in part B are for the popular χ^1 torsion angles.

versus experiment for the large and small basis set calculations are also fairly close, 1.14 and 1.23 ppm, respectively.

Conclusions

The results we have shown above indicate that good recreations of complete amino acid C^{α} ϕ,ψ shielding surfaces can be obtained using basis sets as small as a 3-21G in as little as 4% of the time that it would take using a conventional large basis set. When one uses the slightly larger 4-31G basis set on selected atoms the results obtained are significantly better than those from the uniform 3-21G basis, and the computation time

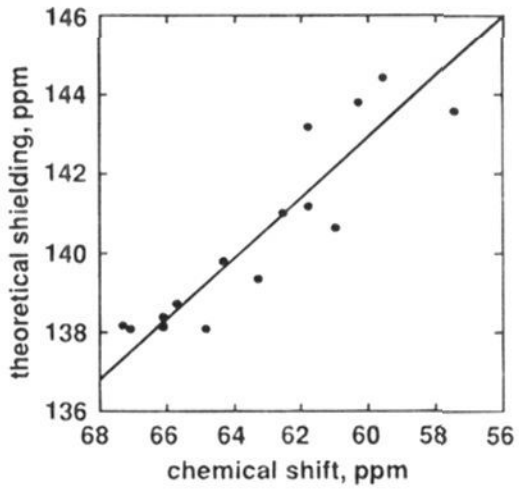

Figure 5. Theoretical chemical shieldings predicted from the X-ray torsion angles using the valine hypersurface versus the experimentally determined NMR chemical shifts for C^{α} in *Staphylococcal* nuclease and an invertebrate calmodulin ($m = 0.80$, $R^2 = 0.842$, rmsd = 1 ppm).

only increases by a factor of ~1.4, making a complete ϕ,ψ surface obtainable in ~85 CPU hours (at 25 MFlops), while previous surfaces took as long as ~2300 CPU hours. Our results also show that it is now possible to calculate full 3-D hypersurfaces in a tolerable time period, and that these hypersurfaces reproduce experimental chemical shift results to a good degree of accuracy (~1.2 ppm rmsd).

Based on the results we have shown above we conclude that (1) basis size primarily affects the absolute shielding, (2) very good correlations can be obtained simply by including slightly larger basis sets on a small number of selected atoms, and (3) 3- and potentially even 4-D NMR chemical shielding hypersurfaces are now within computational grasp. These shielding hypersurfaces are capable of reproducing experimental results within a ~1.2 ppm rmsd error. These observations will greatly aid in the construction of shielding hypersurfaces for the other amino acids, for future use in structure refinement and prediction.

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