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Parameter Optimization for Calculation of Proton Chemical Shift in Protein

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Abstract: The magnetic anisotropy effects of peptide group in structured protein on proton chemical shift have been investigated using trialanine modeling. The structure dependent part of chemical shift of $C_\alpha H$ of the second amino acid residue was assumed to come purely from the magnetic anisotropy effects of C=O and C-N bonds of peptide in the direct neighborhood and thus to be dependent on ϕ and ψ angle of this dipeptide. A set of dipeptide models with different ϕ and ψ angles were generated and from these models the chemical shift values were calculated using known algorithm to emphasize the role of parameters used in the equation. Comparison of sets of different parameters resulted in an optimized parameters which could reproduce the statistical chemical shift values observed in proteins with respect to the secondary conformation.

INTRODUCTION

The chemical shift of each nucleus of protein contains a valuable information about the conformation and secondary structure. In fact, the chemical shift is the primary observable from the NMR experiment, which has less interpretational ambiguity than NOE distance data in the measurement process and recently the number of works utilizing the chemical shift data for the structural problem are

steadily increasing in the literature.

The most important factors contributing to conformation dependent part of chemical shift of a nucleus can be assumed to be ring current effects from aromatic rings of Phenylalanine, Tyrosine, Tryptophan and Histidine and magnetic anisotropy effects from C=O and C-N bonds of peptide groups and side chains in the spatial proximity. The ring current effect is basically a occasional one, which occurs only when the aromatic amino acid residues exist in the protein, whereas the peptide bonds are the basic elements in protein. The fundamental theory of these anisotropy effects have long been established and the most of the studies concerning the protein conformation are based on this method. For the parameters governing the magnetic anisotropy effects we can find a wide spectrum of different values in the literature, most of which are empirically estimated or fitted to the crystal structures of proteins, but unfortunately there is no plausible criterion to prefer one set of parameters than the others.

The aim of this work was to find out what is the immediate consequence of these different parameter values when they are used for the calculation of anisotropy effects. An artificial trialanine model, where the magnetic anisotropy effects from two peptide groups are the only source for structural chemical shift, will give the physically reasonable answer what values of parameters should be used for the prediction of structure dependent chemical shift.

METHODS

The structural chemical shift arising purely from magnetic anisotropy effects of C-N and C=O bonds of peptide, δ_{ma}, can be calculated using known equation by ApSimon, et al.[1],

$$\delta_{ma} = \frac{1}{3r^3}\left[\Delta\chi_1\left(1 - 3\cos^2\theta_y\right) + \Delta\chi_2\left(1 - 3\cos^2\theta_x\right)\right] \qquad [1]$$

where the distance r and angle θ are defined in angular coordinate system, which can be transformed from the normal Cartesian coordinates as shown in Fig. 1. $\Delta\chi_1$ and $\Delta\chi_2$ are magnetic anisotropies between the y and z and between the x and y axes respectively. For the parameters $\Delta\chi_1$ and $\Delta\chi_2$ governing the magnetic anisotropy effects, Zürcher[2] has investigated a series of ketosteroid compounds and proposed $\Delta\chi_1$ and $\Delta\chi_2$ values of -25.7 and $-12.2 \times 10^{-30}\text{cm}^3$ for carbonyl group effects and Asakura, et al.[3] gave estimated values of -20.6 and

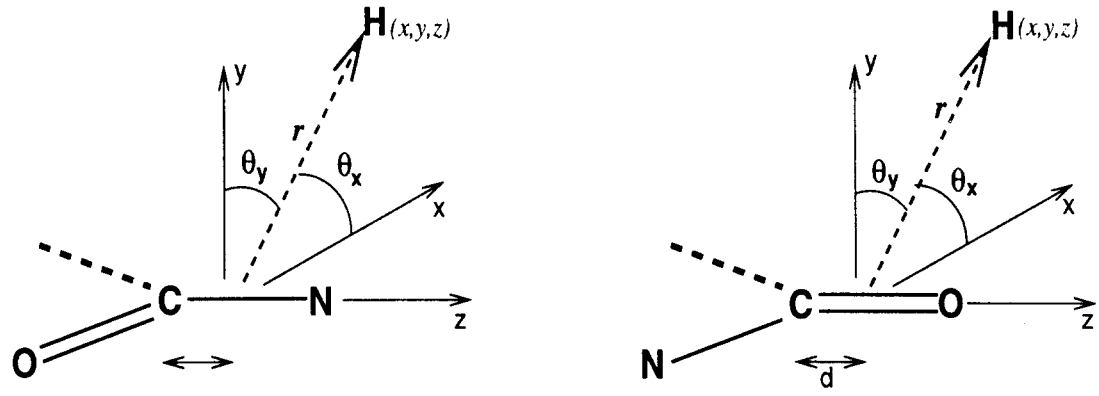

Fig. 1. The geometrical parameters for the calculation of magnetic anisotropy from C-N(a) and C=O bonds(b) of peptide

$-13.2 \times 10^{-30}\text{cm}^3$ for peptide C-N bond. Williamson group[4] has fitted these parameters to the crystal structures of a number of proteins and the resulting $\Delta\chi_1$ and $\Delta\chi_2$ values were -18.0 and $-8.0 \times 10^{-30}\text{cm}^3$ for peptide C=O bond and -12.0 and $1.3 \times 10^{-30}\text{cm}^3$ for peptide C-N bond, respectively. They reported afterwards another set of slightly different data of -13.0, -4.0, -11.0 and $1.4 \times 10^{-30}\text{cm}^3$ in a model study[5]. Ösapay and Case[6] assumed the peptide group to be axially symmetric and approximated the whole peptide bond with a single anisotropy, $\Delta\chi = -7.3 \times 10^{-30}\text{cm}^3$.

For the detailed study of these parameters a structured peptide was modeled by a trialanine molecule which contains only two peptide groups defining one set of ϕ and ψ angles. Fig. 2 shows the atomic arrangement in this model and the ϕ and ψ are defined by dihedral angles $\phi(C_1 - N_2 - C_2^\alpha - C_2)$ and $\psi(N_2 - C_2^\alpha - C_2 - N_3)$ respectively. The $C^\alpha H$ of the second alanine residue should be therefore the probe for the estimation of magnetic anisotropy effects with respect to different ϕ and ψ angle sets. With variation of ϕ and ψ between -180° and +180° with interval 15°, 576 conformers were generated in cartesian coordinate system using GROMOS96 molecular modeling package[7]. The transformation of coordinate system and anisotropy equation (1) were programmed[8] in FORTRAN77 code and implemented into GROMOS96 program. Four different calculations were performed under UNIX environment. In calculation **A** we used the values by Zürcher[2] for peptide C=O bond and by Asakura, et al.[3] for

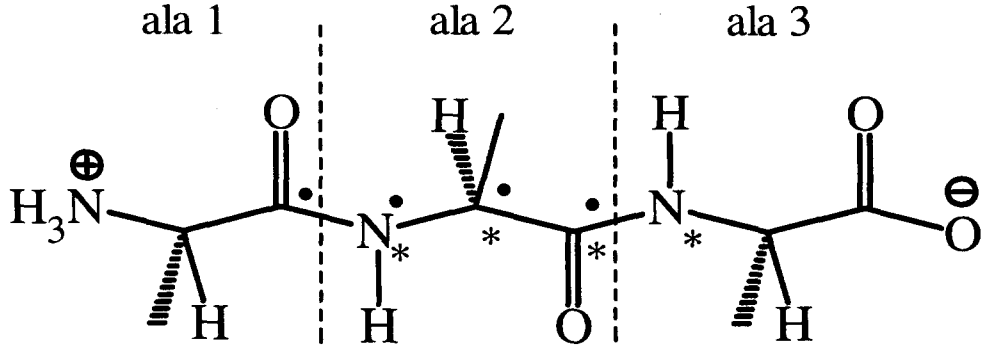

Fig. 2. Trialanine model. The ϕ and ψ are defined by dihedral angle from $C_1 - N_2 - C_2^{\alpha} - C_2(\bullet)$ and $N_2 - C_2^{\alpha} - C_2 - N_3(*)$ respectively.

peptide C-N bond, in **B** by Williamson, et al.(1992)[4] and in **C** by Williamson and Asakura(1993)[5] for peptide C=O and C-N bonds.

In order to measure the goodness of one set of parameters compared to the other and to optimize them we adopted the statistical observation by Wishart, et al.[9]. They investigated experimental NMR data of over 70 proteins and analyzed them in terms of secondary structure and found that the average chemical shift of $C_{\alpha}H$ in a helical and β-sheet conformation is upfield and downfield shifted by an average of 0.39 and 0.37 ppm, respectively, compared to random coil shift[9], which was measured experimentally from the unstructured tetrapeptide. And thus the parameters were changed so, that the difference between the $C_{\alpha}H$ shifts in α-helix and β-strand fit to the difference 0.76 ppm. The new optimized set was used in calculation **D**.

RESULTS AND DISCUSSION

The resulting chemical shift values from calculation **A**, **B** and **C** are represented chemical shift surface with respect to ϕ and ψ angle in Ramachandran plot in Fig. 3 and a set of selected chemical shifts are summarized in Table 1 along with the parameters used for calculations.

The overall shape of the chemical shift surface shows a quite different behavior between the first calculation **A** and the other three calculation, **B**, **C** and

D, which can readily be read from the maximum and minimum values, δ_{max} and δ_{min} at corresponding (ϕ, ψ) from the Table 1. Calculation **A** from parameters by Zürcher shows the maximal and minimal chemical shifts at the regions which cannot be found in regular structured protein, while the parameters optimized by Williamson, et al.[4](**B**), Williamson and Asakura[5](**C**) and in this work(**D**) yielded δ_{max} in the typical β-strand region(-120°,120°) and δ_{min} in slightly shifted position(-30° ~-15°,-90°) from α-helical region. These facts in calculation **B**, **C**

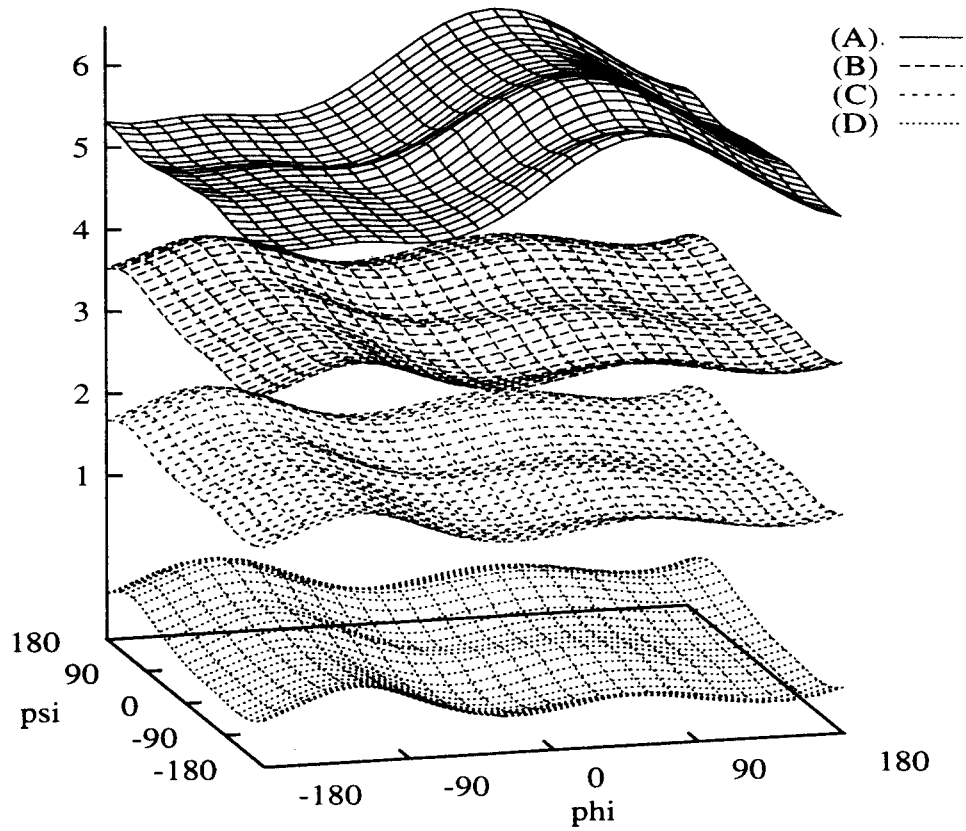

Fig. 3. The chemical shift surface calculated using Eq. 1 with anisotropy parameters by Züricher[2] for C=O and Asakura, et al.[3] for C-N (**A**), by Williamson, et al.[4] (**B**), by Williamson and Asakura[5] (**C**) and from the optimization (**D**). The chemical shift scale is arbitrary.

and **D**, obviously, prove the general trend that the chemical shift of $C_\alpha H$ in β conformation is downfield shifted compared to that in α-helix.

To be more specific we are now concentrating mainly on the values of δ_β at (-120°,120°) and δ_α at (-60°,-45°). The choice of dihedral angles is somewhat arbitrary but they would be typical representative value for β-sheet conformation (-140° ~-110°,110° ~ 140°) and for α-helix (-60° ~-40°,-70° ~-20°). The absolute values of δ_β and δ_α, however, do not correspond to the statistical result by Wishart, et al.[10] A similar discrepancy from random coil shift was also reported by Williamson, et al.[4] and they subtracted an arbitrary value of 0.76ppm from the calculated values to match to the experimental values. But we found that this discrepancy depends largely on the parameters used in calculations as indicated by $\delta_{max} - \delta_{min}$ values in Table 1.

Our optimization and interpretation on anisotropy parameters are based on the differences of chemical shifts, $\Delta\delta(\delta_\beta - \delta_\alpha)$. Calculation **A** with Zürcher parameters gave practically no difference between δ_β and δ_α, whereas calculation **B** and **C** yielded $\Delta\delta$ of 0.64 and 0.58 ppm respectively. This values are significantly smaller compared to the statistical averages of 0.76 ppm for $\Delta\delta$ observed in proteins. The results of calculations **B** and **C** reflect from the facts that those parameters were originally derived from the crystal structures of proteins and the statistical data were measured for solution states. Although the global secondary

Table 1. Magnetic anisotropy of peptide bonds used for calculations and the secondary chemical shifts of $C_2^\alpha H$ of trialanine model

calculation		**A**	**B**	**C**	**D**
magnetic	$\Delta\chi_1^{C=O}$	-25.7	-18.0	-13.0	-17.0
anisotropy of	$\Delta\chi_2^{C=O}$	-12.2	-8.0	-4.0	-9.0
peptide	$\Delta\chi_1^{C-N}$	-20.6	-12.0	-11.0	-12.0
(10^{30}ppm cm^3)	$\Delta\chi_2^{C-N}$	-13.2	1.3	1.4	3.0
	δ_{max} (ϕ,ψ)	1.48 (60°,−45°)	1.21 (−120°,120°)	1.16 (−120°,120°)	1.31 (−120°,120°)
secondary chemical shift	δ_{min} (ϕ,ψ)	0.15 (−75°,75°)	0.43 (−30°,−90°)	0.43 (−15°,−90°)	0.38 (−15°,−90°)
(ppm)	$\delta_{max} - \delta_{min}$	1.33	0.78	0.73	0.93
	$\delta_{\beta(-120°,120°)}$	0.26	1.21	1.16	1.31
	$\delta_{\alpha(-60°,-45°)}$	0.31	0.57	0.58	0.55
	$\Delta\delta(\delta_\beta - \delta_\alpha)$	-0.05	0.64	0.58	0.76

structure of protein in solution are generally known to be similar to the crystal structure but the individual local dihedral angles ϕ and ψ might differ significantly. Physically consistent fitting should be therefore performed on solution structures, which is also limited in accuracy due to low local resolution of NMR driven solution structures.

Therefore the best thing we could do was to fit the parameters on only two values of δ_β and δ_α, which were measured and statistically categorized for the wide range of proteins in solution. The value of $\Delta\delta$ in calculation **D** was found in this way, where the starting parameters were taken from the calculation **B** and **C** and gradually modified to give the value of 0.76 ppm. In our opinion, this set of fitted parameters is physically more plausible for the prediction of structural chemical shifts arising from the peptide bond contribution. If one resolves the measured and assigned chemical shift values from available proteins with respect to individual backbone dihedral angles and use them for fitting process the parameters can be further refined.

From this investigation we conclude that the original magnetic anisotropy parameters estimated by Zürcher[2] and Asakura, et al.[3] used for calculation **A** should not be used for calculations of structural chemical shift value. As found from the calculations **B** and **C** the parameters empirically fitted by the Williamson group[4,5] can be a good basis for prediction of conformation dependent chemical shift. In this case not only the absolute values of individual terms for C=O and C-N of peptide but the relation between them are playing the key role for chemical shift in α-helix and β-strand conformations. For this reason the assumption by Ösapay and Case[7] that the peptide group can be approximated by an axially symmetric model with one single parameter $\Delta\chi$ cannot be accepted for proper treatment of chemical shift. The optimized magnetic anisotropy parameters in calculation **D** which reproduce exactly the statistical observations in terms of δ_β and δ_α are evidently the most plausible basis for the prediction of secondary chemical shift of protein in solution. We believe that this work has given a definitive answer to the choice of anisotropy parameters and opened the possibility of wide variety of useful applications in investigation of solution structure.

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