JOURNAL OF MAGNETIC RESONANCE, Series B **112,** 79–81 (1996)
ARTICLE NO. 0113

A Potential Involving Multiple Proton Chemical-Shift Restraints for Nonstereospecifically Assigned Methyl and Methylene Protons

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Received April 18, 1996

The quality of any protein structure determination by NMR is critically dependent on the number of experimental restraints (*1*). While the principal source of geometric information resides in short interproton distance restraints derived from NOE measurement (*1*), information from coupling constants, ^{13}C chemical shifts, and ^{1}H chemical shifts can also be employed (*2–4*). We recently demonstrated that direct refinement against ^{1}H chemical shifts provides a valuable source of additional information for the refinement of protein NMR structures (*4*).

Using modern heteronuclear multidimensional NMR, it is possible, with a significant expansion of effort, to obtain a fair number of stereospecific assignments for β-methylene protons and methyl groups (*1, 5*). Nevertheless, there are always cases where stereoassignments cannot be easily derived from the data at hand. Thus, for example, it may not be feasible to measure all the relevant coupling constants, due either to the poor spectral quality of the sample (e.g., for proteins or protein complexes greater than 25–30 kDa) or to lack of sufficient instrument time. In our original implementation of direct ^{1}H chemical-shift refinement (*4*), we dealt with nonstereospecifically assigned prochiral protons by minimizing the difference between the mean of the observed ^{1}H shifts and the mean of the calculated ^{1}H shifts of the two protons or proton groups. In doing so, it is clear that information is lost. Recently, Constantine *et al.* (*6*) introduced a set of *J*-coupling-restraint potentials involving the sums and differences of the coupling constants in order to automatically handle coupling constants involving prochiral protons without the need for making *a priori* stereoassignments. In this Communication, we derive a similar set of potentials to deal with ^{1}H chemical-shift refinement of nonstereospecifically assigned prochiral protons and demonstrate its application using data derived from the structure determination of a complex of human thioredoxin with a peptide from the redox regulator Ref-1 (*7*).

The functions chosen to define the multiple ^{1}H chemical-shift potential, E_{multprot}, are similar to those described by Constantine *et al.* (*6*) for ^{3}J coupling-constant refinement. Thus, there are four individual potential terms, E_{p}, E_{m1}, E_{m2}, and E_{m3}, defined as

$$E_{\text{p}} = k_{\text{p}}\{(S_{\text{calcA}} + S_{\text{calcB}}) - (S_{\text{obs1}} + S_{\text{obs2}})\}^2 \quad [1]$$

$$E_{\text{m1}} = k_{\text{p}}\{|S_{\text{calcA}} - S_{\text{calcB}}| - |S_{\text{obs1}} - S_{\text{obs2}}|\}^2 \quad [2]$$

$$E_{\text{m2}} = k_{\text{m}}k_{\text{p}}\{|S_{\text{obs1}} - S_{\text{obs2}}| - |S_{\text{calcA}} - S_{\text{calcB}}|\}^2 \quad [3]$$

$$E_{\text{m3}} = k_{\text{m}}k_{\text{p}}\{0.5(S_{\text{obs1}} - S_{\text{obs2}})^2 - (S_{\text{calcA}} - S_{\text{calcB}})^2\}, \quad [4]$$

where S_{obs1} and S_{obs2} are the two observed ^{1}H shifts (in arbitrary order), S_{calcA} and S_{calcB} are the two expected ^{1}H shifts (in arbitrary order) calculated from the structure, and k_{p} and k_{m} are force constants. S_{calcA} and S_{calcB} are calculated using the method of Williamson and Asakura (*8*), as described in (*4*). The multiple ^{1}H chemical-shift potential, E_{multprot}, is then given by

$$\begin{aligned} E_{\text{multprot}} &= E_{\text{p}} + E_{\text{m1}}, \\ &\quad \text{if } |S_{\text{calcA}} - S_{\text{calcB}}| > |S_{\text{obs1}} - S_{\text{obs2}}| \\ &= E_{\text{p}} + E_{\text{m2}}, \\ &\quad \text{if } |S_{\text{obs1}} - S_{\text{obs2}}| \geqslant |S_{\text{calcA}} - S_{\text{calcB}}| \\ &\qquad\qquad \geqslant |S_{\text{obs1}} - S_{\text{obs2}}|/2 \\ &= E_{\text{p}} + E_{\text{m3}}, \\ &\quad \text{if } |S_{\text{calcA}} - S_{\text{calcB}}| < |S_{\text{obs1}} - S_{\text{obs2}}|/2. \end{aligned} \quad [5]$$

Refinement by simulated annealing employing molecular dynamics requires knowledge of the forces on the atoms, and these are calculated as

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1064-1866/96 $18.00

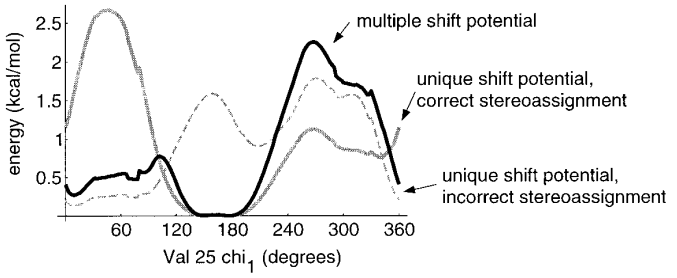

FIG. 1. Illustration of the multiple (Eq. [5]) and unique (Ref. *4*) ^{1}H chemical-shift energy landscapes for the methyl groups of Val 25 in the complex of human thioredoxin with a 13-residue peptide from Ref-1. The χ_1 angle of Val 25 was varied and the energy of the ^{1}H chemical-shift restraints on the two methyl protons was calculated every 2°. The ^{1}H chemical shifts of the two methyl groups are at 0.03 ($\gamma 1$) and 0.87 ($\gamma 2$) ppm. The solid gray line represents the sum of the energies of two unique ^{1}H chemical-shift restraints, one for each methyl group, with the correct stereoassignment. The dotted gray line represents the sum of the energies of two unique ^{1}H chemical-shift restraints, one for each methyl group, with the incorrect (i.e., reversed) stereoassignment. The black line is the energy of the multiple ^{1}H chemical-shift restraint comprising both methyl groups. The force constant for the unique ^{1}H chemical-shift potential is 1.0 kcal·mol^{-1}·ppm^{-2}. The two force constants, k_p and k_m, for the multiple ^{1}H chemical-shift potential are 1.0 and 0.2 kcal·mol^{-1}·ppm^{-2}, respectively. The coordinates for the complex of human thioredoxin and the Ref-1 peptide were taken from (*7*).

$$\begin{aligned}\partial E_{\text{multprot}}/\partial x_i &= \partial E_p/\partial x_i + \partial E_m/\partial x_i \\ &= \partial E_p/\partial S_{\text{calcA}}\,\partial S_{\text{calcA}}/\partial x_i \\ &\quad + \partial E_p/\partial S_{\text{calcB}}\,\partial S_{\text{calcB}}/\partial x_i \\ &\quad + \partial E_m/\partial S_{\text{calcA}}\,\partial S_{\text{calcA}}/\partial x_i \\ &\quad + \partial E_m/\partial S_{\text{calcB}}\,\partial S_{\text{calcB}}/\partial x_i\,, \qquad [6]\end{aligned}$$

where $\partial E_{\text{multprot}}/\partial x_i$ is the partial derivative of the multiple ^{1}H chemical-shift energy with respect to the x coordinate of atom i (for example), and E_m is E_{m1}, E_{m2}, or E_{m3}, whichever of these terms is used in the calculation of the energy. The partial derivatives $\partial S_{\text{calcA}}/\partial x_i$ and $\partial S_{\text{calcB}}/\partial x_i$ are calculated as described in (*4*).

To test this approach, we used experimental restraints derived from the structure determination of a complex of hTRX (105 residues) with a 13-residue peptide from Ref-1 (*7*). The original structure determination was based on 3663 experimental NMR restraints, comprising 2581 interproton distance restraints, 36 distance restraints for 18 backbone–backbone hydrogen bonds, 86 $^3J_{\text{HN}\alpha}$ coupling constant restraints, 321 torsion angle restraints, 197 ^{13}C secondary shift restraints, and 442 ^{1}H chemical-shift restraints. Stereospecific assignments were obtained for 59 out of the 77 β-methylene groups and for the methyl groups of all 7 Leu residues and 10 out of 12 Val residues in the complex. (The resonances for the methyl groups of the remaining 2 Val residues were degenerate.)

An illustration of the multiple ^{1}H chemical-shift energy landscape for the methyl groups of Val 25 which resonate at 0.03 ($\gamma 1$) and 0.87 ($\gamma 2$) ppm is provided in Fig. 1. It is also compared to the conventional unique ^{1}H chemical-shift potential (*4*) calculated using the sum of the two restraints, one for each methyl group. The landscape of the unique potential is shown both with the correct stereoassignments and the reversed incorrect stereoassignments. The unique ^{1}H chemical-shift potential calculated with the correct stereoassignment has its global minimum in the region $\chi_1 = 145°$ to 190°, while that with the incorrect stereoassignment has its global minimum in the region $\chi_1 = 0°$ to 70°. Not surprisingly, the minimum for the correct stereoassignment is lower than that for the incorrect one. The mutiple ^{1}H chemical-shift potential follows the curve for the unique ^{1}H chemical-shift potential with the correct steroassignment between $\chi_1 = 100°$ and 230°, and the curve for the unique ^{1}H chemical-shift potential with the incorrect stereoassignment outside this range. In this manner, it is capable of distinguishing the two possible stereoassignments.

In the present series of simulated annealing calculations employing the multiple ^{1}H chemical-shift potential, the experimental restraints were modified by removing all side-chain torsion angle restraints (with the exception of the χ_2 angles of Tyr and Phe) and all reference to stereospecifically assigned methylene or methyl protons in the interproton distance restraints. The latter were replaced by a $\Sigma(r^{-6})^{-1/6}$ sum for the β-methylene proton or methyl proton pairs (*9*). All the proton shifts were included in the calculation, but those involving nondegenerate β-methylene and methyl proton pairs were treated using the new multiple ^{1}H chemical-shift potential (Eqs. [1]–[5]). In addition to the experimental NMR restraints, the target function minimized during simulated annealing comprised terms for covalent geometry (bond lengths and angles, planes and chirality), a quartic van der Waals repulsion term, and a conformational database potential term (*10*). A total of 22 simulated annealing structures were calculated and the results are summarized below.

The average RMS differences in ^{1}H chemical shift between the two methyl resonances of Val and Leu residues in the hTRX-Ref-1 complex are 0.46 ± 0.30 and 0.18 ± 0.13 ppm, respectively. The average RMS difference between observed and calculated ^{1}H shifts for the methyl groups is 0.11 ± 0.01 ppm. Consequently, one would expect a reasonably high degree of success in obtaining methyl group stereospecific assignments via ^{1}H chemical-shift refinement. In addition, one would also expect the reliability to be somewhat higher for methyl groups of Val over those of Leu, as the position of the Val methyl groups is only affected by a single side-chain torsion angle (χ_1), whereas that of Leu depends on both χ_1 and χ_2 side-chain torsion angles. This is indeed the case, and the correct stereoassignement for the methyl groups of Val and Leu are obtained on average 81 ± 12 and 85 ± 20% of the time, respectively.

In the case of the β-methylene protons, the average RMS difference between observed and calculated ^{1}H chemical shifts is 0.26 $\pm$ 0.01 ppm. In general, the ^{1}H chemical-shift difference between the two protons of a β-methylene group is less than this. Hence, stereoassignment via ^{1}H chemical-shift refinement alone is likely to be less reliable for β-methylene protons than for methyl groups. In this particular case, however, the presence of a large number of NOE restraints (*7*) in conjunction with the conformational database potential (*10*) results in the correct stereoassignment being obtained at approximately the same frequency as that for the methyl groups. For example, there are 9 Phe residues. Even in the absence of ^{1}H chemical-shift restraints, the correct side-chain χ_1 torsion angle for all the Phe residues is obtained 100% of the time. Hence, inclusion of the new ^{1}H chemical-shift potential term results in the correct stereoassignment of the β-methylene protons to also be obtained 100% of the time.

In summary, the multiple ^{1}H chemical-shift potential function described in this Communication is a useful tool that can produce correct stereoassignments for the methyl groups of Leu and Val about 80–85% of the time and, in combination with the conformational database potential described previously (*10*), also produces correct β-methylene proton stereospecific assignments at approximately the same frequency without human intervention. The real significance, however, of the new multiple ^{1}H chemical-shift potential is that it permits one to make full use of the information content present in the two ^{1}H chemical shifts for each methylene and methyl pair.

ACKNOWLEDGMENT

This work was supported by the AIDS Targeted Antiviral Program of the Office of the Director of the National Institutes of Health (G.M.C. and A.M.G.).

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