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Protein dynamics and NMR relaxation: comparison of simulations with experiment

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Comparisons between molecular dynamics simulations of proteins and experiment have been based on temperature factors[1-5] derived from X-ray spectra and on the stability of hydrogen bonds[6]. Here we present a novel method of testing molecular dynamics simulations against nuclear magnetic resonance (NMR) relaxation measurements, based on the recently developed model-free approach[7] to the interpretation of NMR data. As NMR relaxation in proteins is determined by dynamics on the picosecond–nanosecond time scale, a comparison of NMR experiments and molecular dynamics simulations that last for less than ~100 ps is more meaningful than previous ones[3-6] involving much longer time scales. We make contact between a molecular dynamics simulation[8] and a ^{13}C-NMR relaxation study[9] of pancreatic trypsin inhibitor (PTI) by comparing generalized order parameters (which are measures of the extent of angular motion of the bonds) extracted from the relaxation data[9] with those calculated[10] from a 96-ps trajectory[8]. We show that the theoretical order parameters indicate less motion than their experimental counterparts. The relative flexibility of the residues studied, however, is reasonably well described by the simulation.

At presently available spectrometer frequencies, ^{13}C-NMR relaxation of protonated carbons is determined by the reorientation of the ^{13}C—H bond vectors relative to the external magnetic field. For a protein in solution both the motion of the macromolecule as a whole and internal motions in a macromolecule-fixed frame contribute to the relaxation. It has been shown recently[7] that the unique information on fast internal motions contained in NMR relaxation experiments can be specified by two model-independent quantities: an effective correlation time, τ_e, and a generalized order parameter, $\mathcal{S}$. These quantities can be extracted from relaxation data using a simple fitting procedure. τ_e is proportional to the area of the time correlation function for internal motions; the time correlation function can have components which decay with correlation times faster or slower than τ_e. For ^{13}C-NMR of protonated carbons, $\mathcal{S}$ is a measure of the angular restriction of the motion of a ^{13}C— H vector and is defined as

$$\mathcal{S}^2 = \frac{4\pi}{5}\sum_{m=-2}^{2}|\langle Y_{2m}(\Omega)\rangle|^2 \qquad (1)$$

where Y_{2m} are spherical harmonics, Ω specifies the orientation of a ^{13}C—H bond in a macromolecule-fixed frame and the angular brackets denote an average over all possible orientations accessible on a time scale shorter than the correlation time for the overall reorientation of the macromolecule (~4 ns for PTI). It follows from equation (1) that $0 \leq \mathcal{S}^2 \leq 1$ and that $\mathcal{S} = 0$ when the internal motion is isotropic, while $\mathcal{S} = 1$ when the motion is completely restricted.

Values of $\mathcal{S}$ and τ_e of ^{13}C—H bonds for 12 methyl groups have been extracted[7] from the relaxation data of Richarz *et al.*[9]. The τ_e values which contain contributions due to motions of and about the symmetry axis of the methyl groups, were in the range 19–70 ps.

As in the molecular dynamics simulation of Karplus and McCammon[8] the hydrogens are incorporated into the heavy atoms to which they are bound, it is impossible to calculate $\mathcal{S}$ and τ_e for methyl groups directly from their trajectory. However, a comparison with experimental order paramcters becomes possible if it is assumed that the motion of the methyl symmetry axis (for example, the $C^\alpha - C^\beta$ bond in alanine) and the motion of the protons about this axis are uncoupled. In this case, independent of the nature of the motion of the symmetry axis, one has[7]

$$\mathcal{S}^2 = \mathcal{S}^2_{\text{axis}}\left(\tfrac{1}{2}(3\cos^2\beta - 1)\right)^2 \qquad (2)$$

where β is the angle between the ^{13}C– H bond and the symmetry axis and $\mathcal{S}_{\text{axis}}$ is the generalized order parameter of the bond joining a methyl carbon and its nearest-neighbour heavy atom. $\mathcal{S}^2_{\text{axis}}$ can be calculated from a trajectory by replacing the average in equation (1) by a time average.

Figure 1*A* shows values of $\mathcal{S}^2_{\text{axis}}$ obtained from the values of $\mathcal{S}^2$ extracted[7] from the experimental relaxation data[9] using equation (2) with the value of β (69.8°) determined from a neutron diffraction study[11] of alanine. The experimental relaxation parameters for Ala 16 and Ala 40 are average values because these resonances were not resolved (we used the same order parameters for these two residues). The δ-carbon resonances of Ile 18 and Ile 19 could be identified (resonances *a* and *b*) but were not assigned[9]. In constructing Fig. 1*A* we assigned resonance *a* to the Ile 18 δ-carbon so that the relative ordering of $\mathcal{S}^2_{\text{axis}}$ of the δ-carbons of Ile 18 and Ile 19 agrees with the simulation (Fig. 1*B*). Figure 1*B* shows the values of $\mathcal{S}^2_{\text{axis}}$ calculated from a 96-ps trajectory of PTI[8]. With the exceptions of Met 52 and Leu 6, the theoretical order parameters are larger than the experimental ones, indicating that there is usually less motional averaging in the 96-ps simulation than detected in the experiment. Such behaviour will occur when the length of the trajectory is too short for a bond to assume all accessible orientations. If there are activation barriers between various

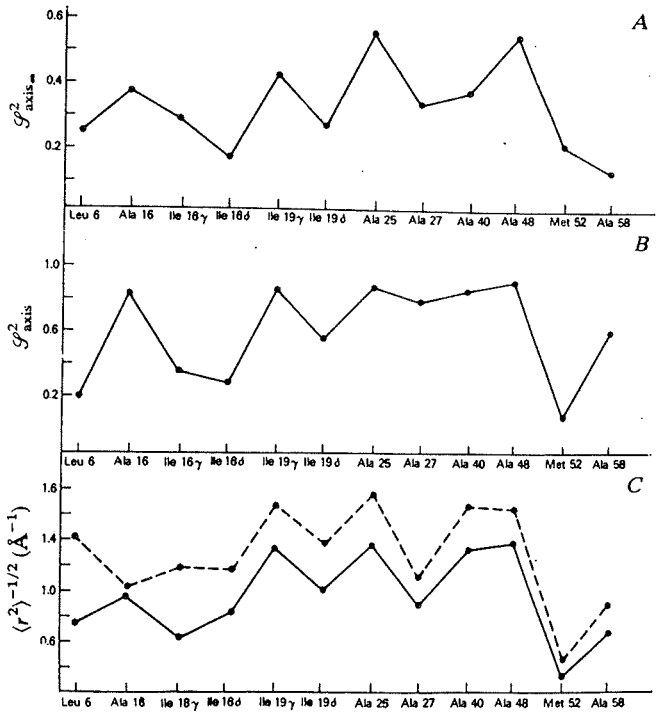

Fig. 1 Comparison of experimental order parameters (*A*) with those calculated from a molecular dynamics simulation (*B*). *C* shows the reciprocals of the theoretical r.m.s. positional fluctuations for the methyl carbons (solid line) and the adjacent heavy atoms (broken line). The experimental order parameters for the δ-carbons of Ile 18 and Ile 19 are based on our assignment of the resonances (see text).

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conformations, the time required for a single computer simulation to develop an equilibrium distribution of bond orientations will probably be longer than 100 ps (that is, the trajectory can be trapped in a local minimum). The very small order parameter (0.069) for the $S-C^{\epsilon}$ bond of Met 52 calculated from the trajectory seems to be due to the neglect of solvent effects in the simulation. Recently, it has been shown[5] that the inclusion of solvent leads to a dramatic reduction of the average of r.m.s. displacements of Met 52. Based on the experimental order parameters, we conclude that the motion of the $C^{\alpha}-C^{\beta}$ bond of Ala 58 is the least restricted of all the residues studied. According to the theoretical order parameters, however, Ala 58 is the most mobile only among the alanines. It is possible that the experimental order parameter of Ala 58 is inaccurate as it is based on only two measured relaxation parameters whereas the other order parameters, with the exception of those of Ala 16 and 40, are based on three. Although differences do exist, it can be seen from Fig. 1*A*, *B* that the relative flexibility of the residues is well described in the simulation. For example, the relative mobilities of all the alanines ($58 > 40 \simeq 27 \simeq 16 > 25 \simeq 48$) is predicted correctly.

Using the model-free approach, an order parameter of 0.87 was extracted from the relaxation data for the α-carbon envelope of PTI. This order parameter, determined by the reorientation of the $C^{\alpha}-H$ vectors, is a measure of the average local flexibility of the backbone. Since to a first approximation the $C^{\alpha}-C^{\beta}$ bonds are expected to move like the $C^{\alpha}-H$ vectors, we evaluated the order parameters of the $C^{\alpha}-C^{\beta}$ axis for all protonated α-carbons in PTI. The average was 0.84, very close to the experimental value. Note, however, that the experiment indicates that the α-carbons of alanines move more than the average of all the α-carbons (that is, $\bar{\mathcal{S}}^2_{\text{axis}} = 0.39$ which is less than 0.87) whereas the molecular dynamics predicts that the motion of the alanines is close to the average (that is, $\bar{\mathcal{S}}^2_{\text{axis}} = 0.81$ as opposed to 0.84).

Finally, it is of interest to compare the pattern of the values of the order parameter to the trends of isotropic r.m.s. displacements from the average atomic positions for a series of residues. As $\mathcal{S}^2_{\text{axis}}$ depends on the concerted motions of two nuclei, in Fig. 1*C* we show the reciprocals of the r.m.s. displacements of both the methyl carbons and their nearest neighbours calculated from the 96-ps trajectory. We have plotted the reciprocals to facilitate a visual comparison with the order parameters. In general, there need not be a simple connection between the ordering of $\mathcal{S}^2$ and the r.m.s. fluctuations as these quantities reflect motional flexibility in different ways. For example, although the calculated order parameters of Ala 16 and 40 are almost the same, the r.m.s. fluctuations of Ala 16 are greater than those of Ala 40. Nevertheless, by comparing Fig. 1*B* and *C* it can be seen that there exist many similarities between the pictures of the relative mobility of residues in a protein derived from order parameters and those derived from r.m.s. fluctuations (for example, Ile 18 is more mobile than Ile 19, and Ala 27 is more mobile than Ala 25 by both criteria).

We have described a novel means of testing molecular dynamics simulations where properties related to motions about individual bonds are compared with their experimental counterparts. To our knowledge, this is the first time that the results of a simulation have been related to observable properties at this level of detail. As more experimental data become available on a variety of protein systems, our approach should prove useful in assessing the range of validity of molecular dynamics simulations.

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