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Crankshaft motions of the polypeptide backbone in molecular dynamics simulations of human type-α transforming growth factor

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Summary

Order parameters for the backbone N-H and C^{α}-H bond vectors have been calculated from a 150 ps molecular dynamics (MD) simulation of human type-α transforming growth factor in H_2O solvent. Two kinds of 'crankshaft motions' of the polypeptide backbone are observed in this MD trajectory. The first involves small-amplitude rocking of the rigid peptide bond due to correlated changes in the backbone dihedral angles ψ_{i-1} and ϕ_i. These high-frequency 'librational crankshaft' motions are correlated with systematically smaller values of motional order parameters for backbone N-H bond vectors compared to C^{α}-H bond vectors. In addition, infrequent 'crankshaft flips' of the peptide bond from one local minimum to another are observed for several amino acid residues. These MD simulations demonstrate that comparisons of N-H and C^{α}-H order parameters provide a useful approach for identifying crankshaft librational motions in proteins.

Recent advances in multidimensional NMR spectroscopy have provided the means for determining nuclear relaxation rates and heteronuclear NOE (HNOE) values for a large number of ^{15}N-H and ^{13}C-H bonds in small proteins and nucleic acids. For diamagnetic proteins in isotropic solutions, the primary mechanisms of nuclear relaxation of protonated ^{13}C (at natural abundance) and ^{15}N nuclei are the dipolar interaction with the directly attached proton and chemical shift anisotropy. For such macromolecular systems, relaxation data can be fit to generalized dynamical models (Levy et al., 1981a,b; Lipari and Szabo, 1982a,b; Clore et al., 1990), to specific models of molecular motions (Richarz et al., 1980; Brainard and Szabo, 1981; Levy and Sheridan, 1983) or to the spectral density functions themselves (Peng and Wagner, 1992) in order to provide information about internal molecular dynamics. This dynamical information is complementary to the structural information available from other NMR experiments or X-ray crystallography. For internal motions faster than the overall tumbling time of the macromolecule, relaxation data are often interpreted in terms of parameterized models involving amplitudes and time constants for internal motions (Levy et al., 1981a,b; Lipari and Szabo, 1982a,b). In this formalism, the amplitudes of internal motions relative to a fixed molecular frame are characterized by generalized order parameters, S^2, which can range from zero for internal motion that is isotropic to unity for a probe that is completely restrained. More complex internal motions are sometimes fit to models involving multiple order parameters for internal motions on different time scales (Clore et al., 1990).

While ^{15}N and natural abundance ^{13}C relaxation studies have been carried out for a large number of polypeptide and protein systems, the literature contains few examples in which both ^{15}N and ^{13}C relaxation data are available for the same system. In molecular dynamics simulations on the 25-residue zinc finger peptide xfin31, Palmer and Case (1992) have observed that order parameters for the backbone N-H spin pairs were, on average, 0.06 units smaller than those for backbone C^{α}-H^{α} pairs. This result appears counterintuitive (Palmer and Case, 1992), since the nitrogen atom is involved in a peptide bond with partial double bond character. Dellwo and Wand (1989) have proposed that anticorrelated variations in torsion angles ψ of residue i − 1 (ψ_{i-1}) and φ of residue i (ϕ_i) could account for the smaller order parameters found for N-methyl carbons

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compared with C^{α} carbons in the cyclic undecapeptide cyclosporin A. Similar 'crankshaft librations' and transitions between low-energy structures with anticorrelated ψ_{i-1} and ϕ_i values are often observed in MD trajectories of polypeptides (Gō and Gō, 1976; Levy and Karplus, 1979) and proteins, including trajectories of bovine pancreatic trypsin inhibitor (BPTI) (McCammon et al., 1976; Levitt, 1983), elastin (Wasserman and Salemme, 1990), human interleukin 1β (Il-1β) (Chandrasekhar et al., 1992), and the cyclic decapeptide antamanide (Brunne et al., 1993).

In this communication we present an analysis of a 150 ps unconstrained MD trajectory for the 50 amino acid protein human type-α transforming growth factor (hTGFα) in water. This trajectory predicts smaller motional order parameters for backbone N-H bond vectors compared to C^{α}-H bond vectors. A detailed analysis of the ensemble of structures reveals that this effect can be attributed to crankshaft librational motions of the polypeptide backbone, involving anticorrelated variations of ψ_{i-1} and ϕ_i on the picosecond time scale. The theoretical results, combined with experimental observations in the literature (Dellwo and Wand, 1989; Palmer and Case, 1992), suggest that such anticorrelated crankshaft motions are a common feature of backbone dynamics in polypeptide systems that can be probed by nuclear relaxation measurements and comparisons of N-H and C^{α}-H backbone order parameters.

MD simulations can be used to explore the connections between internal motions and relaxation parameters. For example, the generalized order parameter S^2 (Levy et al., 1981a,b; Lipari et al., 1982; Lipari and Szabo, 1982a,b), providing information on the degree of spatial (both radial and angular) restriction of the internal motion of a bond vector, can be calculated from MD simulations. For two nuclei at a fixed internuclear distance, the internal angular autocorrelation function that governs NMR spin relaxation is given by (Lipari and Szabo, 1982a,b):

$$C_l(t) = \langle P_2[\hat{\mu}(0)\,\hat{\mu}(t)] \rangle \qquad (1)$$

$\hat{\mu}(t)$ is a unit vector in the direction of the vector connecting two nuclei at time t in the molecular reference frame, $P_2(x)$ is the second-order Legendre polynomial, and $\langle\,\rangle$ denotes the time average of the ensemble of conformations sampled during the trajectory. The generalized order parameter, S^2, can also be expressed as:

$$S^2 = \lim_{t \to \infty} C_l(t) \qquad (2)$$

Using the property of correlation functions:

$$\lim_{t \to \infty} \langle A(0)\,B(t) \rangle = \langle A(t) \rangle \langle B(t) \rangle \qquad (3)$$

and the spherical harmonics addition theorem:

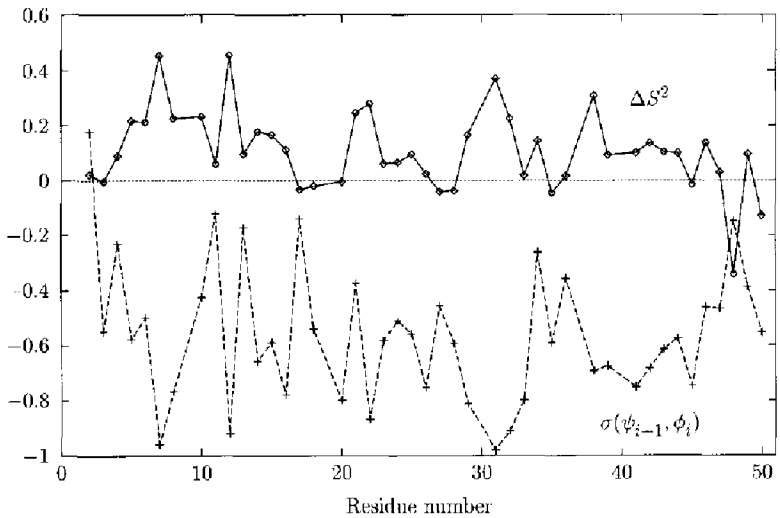

Fig. 1. Comparison of the cross-correlation function for the fluctuations in the dihedral angles $\sigma(\psi_{i-1},\phi_i)$ with the order parameter difference $\Delta S^2 = S^2_{C^{\alpha}\text{-H}} - S^2_{\text{N-H}}$ between C^{α}-H and N-H bond vectors in the polypeptide backbone of hTGFα. The MD trajectory for hTGFα was carried out as follows. Initial atomic velocities were assigned randomly from a Maxwell–Boltzmann distribution at 5 K, followed by slow heating to 298 K. Following a 40 ps MD equilibration trajectory at 298 K, a 150 ps MD trajectory was calculated and stored for further analysis. The simulation employed periodic boundary conditions and was performed in the constant (T,V) ensemble, T = 298 K. The simulations were carried out without restraints using the AMBER force field (Weiner et al., 1984) and the IMPACT modeling program (Kitchen et al., 1990). The starting structure was one of the 16 conformers generated from experimental NMR data (Moy et al., 1993) using the DISMAN structure generation program (Braun and Gō, 1985). It was further energy minimized (without constraints) in vacuum using IMPACT. The protein was then embedded in a 53 Å × 47 Å × 43 Å box containing 3380 water molecules, providing a solvent shell of approximately 8 Å. The overall rotational motion of the protein was removed by superimposing the principal moments of inertia for each coordinate set from the trajectory onto a common reference frame. Order parameters for the C^{α}-H and N-H vectors for each residue were calculated and their difference ΔS^2 was plotted against crankshaft correlation coefficients $\sigma(\psi_{i-1},\phi_i)$. The average values for $S^2_{\text{N-H}}$ and $S^2_{C^{\alpha}\text{-H}}$ were 0.57 and 0.67 units, respectively. The correlation coefficient of the linear regression between ΔS^2 and $\sigma(\psi_{i-1},\phi_i)$ was −0.5.

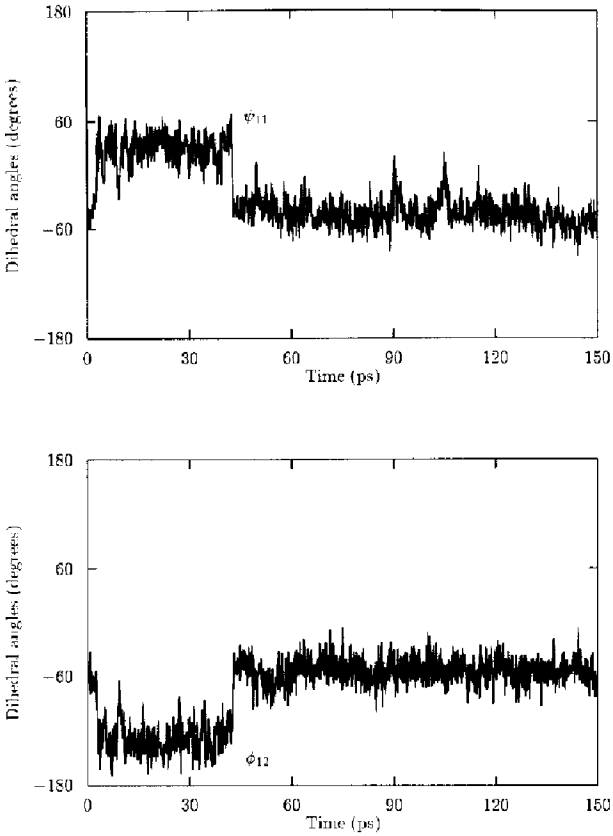

Fig. 2. Plots of the time evolution of the backbone dihedral angles ψ_{11} and ϕ_{12}. The amide bond linking residues Ser[11] and His[12] exhibits two crankshaft flips, the first at approximately t = 3 ps and the second at t = 42 ps. The crankshaft librations correspond to the small-amplitude high-frequency oscillations of the dihedral angles about local minima, e.g. dihedral angle ψ_{11} oscillates about +55° between 3 ps and 42 ps and about −50° between 43 ps and 150 ps.

$$P_2(\mu_1\mu_2) = \frac{4\pi}{5}\sum_{m=-2}^{2} Y_{2m}(\Omega_1)Y^*_{2m}(\Omega_2) \qquad (4)$$

yields an expression for the order parameter (Levy et al., 1981b; Lipari and Szabo, 1982a):

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

where Y_{2m} (Ω) are second-order spherical harmonic expressions and $\Omega(t)$ denotes the polar angles Θ and Φ of the bond vector in the molecular frame at time t.

MD calculations were carried out for hTGFα in water. Details of the MD protocol are summarized in the legend to Fig. 1. For each amino acid residue, order parameters S^2 were calculated from the 150 ps trajectory (following a 40 ps equilibration trajectory) using Eq. 5 for all backbone N-H (except for N-terminal valine and proline residues) and C^α-H (except for glycine residues) bond vectors.

The MD trajectory exhibits two kinds of crankshaft motions involving anticorrelated variations in backbone dihedral angles ψ_{i-1} and ϕ_i. The first is a fast (~1 ps) time scale motion corresponding to small-amplitude librational rigid-body rocking of backbone peptide groups. A second, slower time scale (> 100 ps) motion involves flipping of the peptide group from one local minimum to another, with correlated changes in ψ_{i-1} and ϕ_i. The two kinds of crankshaft motions, referred to here as 'crankshaft librations' and 'crankshaft flips', are shown in Fig. 2. The peptide groups preceding residues Asp[7], His[12], Thr[20], Arg[22], Gln[26], Ala[31] and Cys[32] exhibit crankshaft flips during the 150 ps trajectory.

Residue His[12], which exhibits two 'crankshaft flips' during the trajectory (Fig. 2), was chosen to illustrate these large-amplitude correlated structural changes. Two structures were selected from the trajectory immediately before (t = 42 ps) and after (t = 43 ps) a crankshaft flip. Pictures of the polypeptide backbone including the peptide bond which spans (ψ_{11}, ϕ_{12}) are shown in Fig. 3. As a result of the crankshaft flip, the orientation of the peptide plane formed by the bond between Ser[11] and His[12] at t = 43 ps is rotated by about 100° relative to the corresponding conformation immediately prior to the flip at t = 42 ps. Note, however, that the orientations of the C^α-H bond vectors for these two conformations are almost identical (Fig. 3). Atomic coordinate changes observed for crankshaft librations are similar, but of smaller magnitude. Interestingly, the crankshaft librations and flips represent similar motions of the polypeptide backbone that occur on different time and amplitude scales. These crankshaft motions are conservative, in the sense that the localized rocking of the amide plane is not propagated up or down the polypeptide chain.

The crankshaft motions (librations and flips) of the peptide group are characterized by anticorrelated fluctuations of ψ_{i-1} with ϕ_i. For each conformation, $\Delta\psi_{i-1}$ and $\Delta\phi_i$ correspond to fluctuations of these dihedral angles from their average values calculated over the entire MD simulation. One measure of the crankshaft motion is obtained by calculating the normalized equal time correlation functions (McCammon et al., 1976) for the joint fluctuations in ψ_{i-1} and ϕ_i:

$$\sigma(\psi_{i-1},\phi_i) = \frac{\langle \Delta\psi_{i-1}\Delta\phi_i\rangle}{\langle(\Delta\psi_{i-1})^2\rangle^{1/2}\langle(\Delta\phi_i)^2\rangle^{1/2}} \qquad (6)$$

Anticorrelated fluctuations of the ψ_{i-1} and ϕ_i dihedral angles are a common feature of all hTGFα residues (Fig. 1); the average value of the normalized correlation function is −0.6. The magnitude of $\sigma(\psi_{i-1},\phi_i)$ appears to be independent of residue size and polarity, and is not correlated with the hydrogen-bonded secondary structure of hTGFα (Moy et al., 1993). However, the magnitude of the cross-correlation function is related to the difference between the N-H and C^α-H order parameters $\Delta S^2 = S^2_{C^\alpha\text{-}H} -$

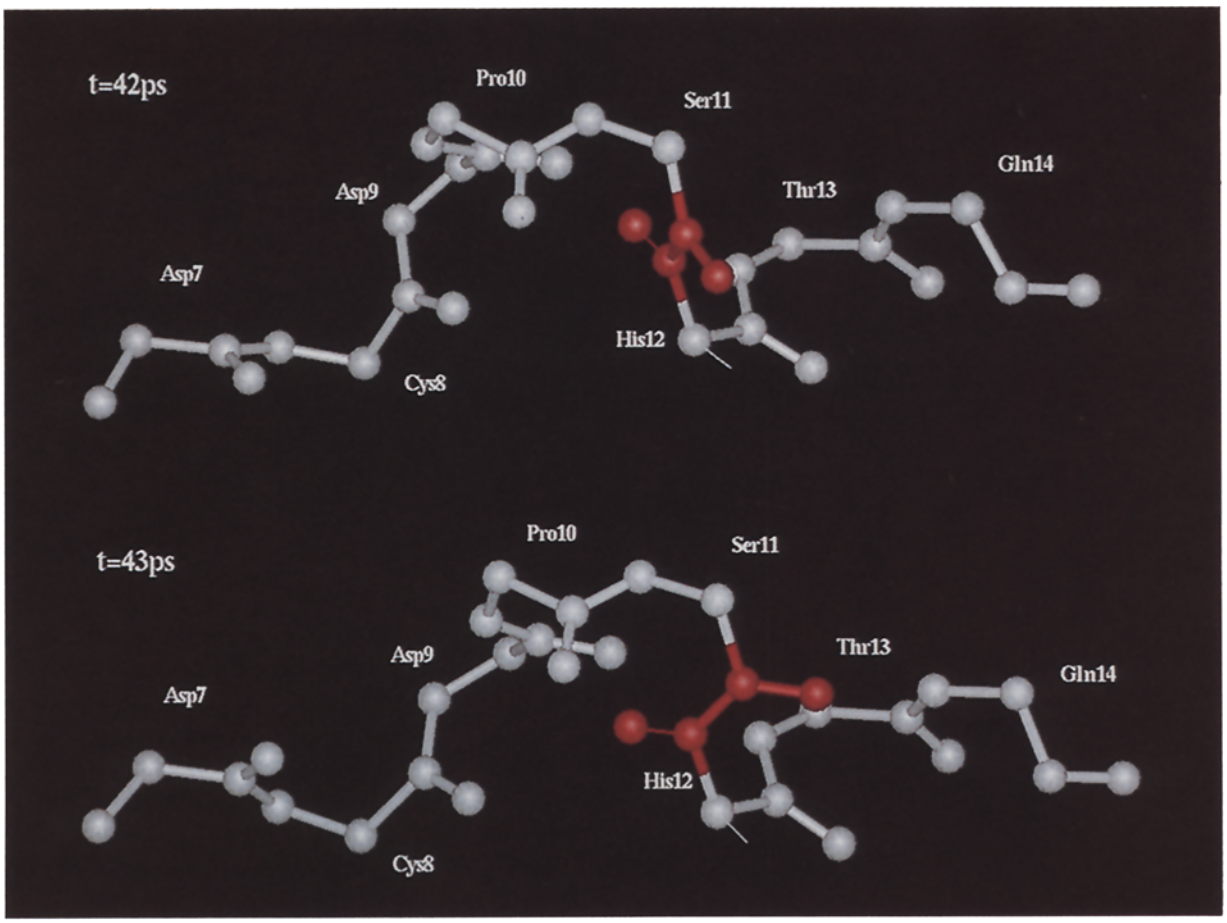

Fig. 3. A portion of the polypeptide backbone of hTGFα, including the peptide bond (shown in red) linking residues Ser^{11} and His^{12}. The figure shows two structures selected from the trajectory immediately before (top, t = 42 ps) and after (bottom, t = 43 ps) the 'crankshaft flip' of the amide plane.

$S^2_{N\text{-}H}$, with the most negative cross-correlations between the fluctuations in the ψ_{i-1} and ϕ_i dihedral angles observed for those residues where the differences between the motional averaging of the C^α-H bond vector and the corresponding N-H bond vector were the greatest. The correlation coefficient for a linear fit between ΔS^2 and $\sigma(\psi_{i-1},\phi_i)$ from the data in Fig. 1 was calculated to be −0.5.

The average values of order parameters S^2 obtained for the N-H and C^α-H bond vectors in the trajectory are 0.57 and 0.67 units, respectively. However, the average N-H bond vector order parameter determined experimentally for hTGFα is significantly higher, $S^2 = 0.78$ at pH 6.5 and a temperature of 303 K (Li and Montelione, 1995), while the C^α-H bond vector order parameters have not yet been determined experimentally. The discrepancy between calculated and observed N-H bond order parameters is due primarily to the effects of the longer time scale internal motions in the MD trajectories. Similar observations have been made by other workers, who often omit residues for which no clear plateaus are observed in the calculated autocorrelation functions when comparing predicted and measured motional order parameters (Kördel and Teleman, 1992).

The crankshaft flips are infrequent on the time scale of the hTGFα trajectory and therefore it is difficult to estimate the rate of these motions with statistical accuracy. A very simple analysis suggests that the time scale for these motions is on the order of a nanosecond.* A careful study of the possible effects of infrequent large-amplitude flips of the peptide bond on NMR observables (e.g. HNOEs and T_1 and T_2 relaxation times) is needed in order to make a direct comparison with experiment. Such an analysis is underway.

In the present study, the contribution of these low-frequency motions to the order parameters was approximately removed from the trajectory analysis by calculating the order parameters (Eq. 5) over 10 ps segments of

*A very simple estimate of the rate of crankshaft flips may be obtained by treating all amide groups as equivalent. In that case, one simply counts the total number of crankshaft flips and divides by the sampling period. If the amide groups are treated as equivalent, the sampling period is the product of the simulation length (150 ps) and the number of amide groups (50). The total number of crankshaft flips observed during the 150 ps simulation of hTGFα was seven. Therefore, the simplest estimate of the period for this motion is ((150 ps) × 50) / 7 = 1.07 ns.

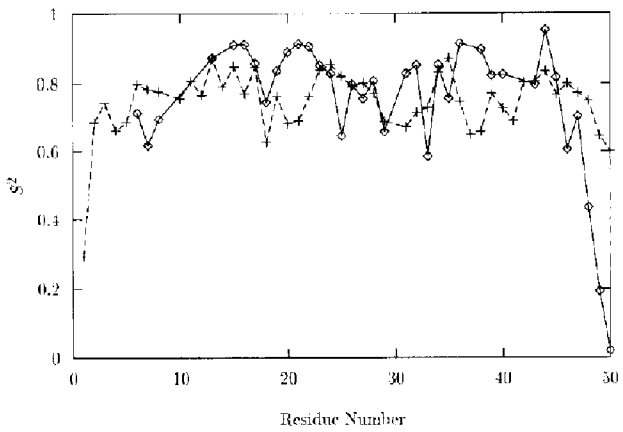

Fig. 4. Comparison of the experimentally obtained (solid line) order parameters with those calculated from the MD simulation (dashed line) averaged over 15 segments of 10 ps each of the trajectory.

the trajectory and then averaging the values over 15 such segments. By this procedure, motions of the N-H and C^{α}-H vectors with time constants longer than about 10 ps are filtered out. The resulting average C^{α}-H and N-H order parameters are 0.85 and 0.75 units, respectively. The short-time block averaged N-H order parameters are in reasonably good agreement with the average experimentally measured N-H order parameter (Li and Montelione, 1995); the average S^2 is 0.78 units at pH 6.5 and a temperature of 303 K. A comparison by residue of the experimental and calculated N-H order parameters is shown in Fig. 4. Discrepancies between order parameters extracted from experiments and simulations may originate from the simulations, the analysis of the experiments, or both. This is a complex issue, which will be the focus of future papers.

A scatter plot of $\Delta S^2 = S^2_{C^{\alpha}\text{-H}} - S^2_{\text{N-H}}$ against the dihedral angle cross-correlation function $\sigma(\psi_{i-1},\phi_i)$ obtained after block averaging during 10 ps of the order parameter and dihedral angle cross-correlation function data is shown in Fig. 5. The mean value of the C^{α}-H order parameters is still 0.1 units greater than that of the N-H order parameters, which is consistent with the difference obtained without block averaging. Furthermore, the correlation coefficient between the block average values for ΔS^2_{avg} and $\sigma_{avg}(\psi_{i-1},\phi_i)$ increases to –0.7 (Fig. 5). While the observed difference between the average N-H and C^{α}-H order parameters could be accounted for by an adjustment of the NH (C^{α}-H) bond length used to calculate S^2, this would not account for the good correlation observed in Fig. 5 between ΔS^2_{avg} and $\sigma_{avg}(\psi_{i-1},\phi_i)$. The attenuation of the effects of the low-frequency crankshaft flips leads to an even better correlation between the difference in the C^{α}-H and N-H order parameters ΔS^2 and the high-frequency crankshaft librations. This is because the highest frequency crankshaft librations are localized to the four atoms (N, H^N, C', O) that define the peptide plane, and the pure libration corresponds to perfect anticorrelated motion between ψ_{i-1} and ϕ_i, whereas the flips involve more extensive rearrangements of additional atoms, including some backbone C^{α}-H bond vectors.

Crankshaft flips of the polypeptide backbone observed in an MD trajectory of human Il-1β have been proposed to correlate with ^{15}N relaxation data, indicating unusually long internal correlation times τ_e of 400 ps to 5 ns (Chandrasekhar et al., 1992). While several backbone N-H bonds of hTGFα exhibit complex relaxation behavior, characterized by long (>400 ps) τ_e values (Li and Montelione, 1995), the ^{15}N relaxation parameters for five of the seven residues exhibiting crankshaft flips in this 150 ps trajectory are fit to τ_e values less than 250 ps. Of the other two sites, one (i.e., the N-H of residue Arg^{22}) was fit with a τ_e value of 1.27 ns and the other (i.e., His^{12}) could not be analyzed because of significant solvent saturation transfer effects (Li and Montelione, 1995). Accordingly, there is no clear correlation between the observed crankshaft flips and long values of τ_e in these studies of hTGFα.

Crankshaft flip can lead to significantly reduced values of both the N-H and C^{α}-H order parameters if these motions occur on a time scale that is significantly faster than that of the molecular tumbling. On the other hand, if the crankshaft flips occur on a time scale that is significantly slower than that of the molecular tumbling, they will not be detected in T_1 or HNOE experiments, and will not contribute to the order parameter. The third case, where the time scale of the crankshaft flips is commensurate with the molecular tumbling, requires further analysis to determine the possible effects that uncertainties in measured relaxation times have on the values of S^2 and τ_e

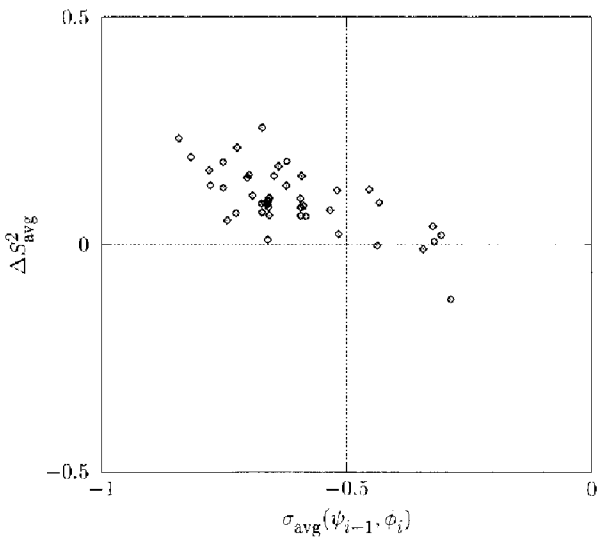

Fig. 5. Scatter plot of the cross correlation in the dihedral angle fluctuations $\sigma(\psi_{i-1},\phi_i)$ against the order parameter difference $\Delta S^2 = S^2_{C^{\alpha}\text{-H}} - S^2_{\text{N-H}}$. As in Fig. 4, the dihedral angle correlation functions and order parameters were calculated over 10 ps segments of the trajectory and then averaged over 15 such segments. The correlation coefficient of the linear regression between ΔS^2 and $\sigma(\psi_{i-1},\phi_i)$ is –0.7.

extracted from the conventional model-free analysis. In model calculations, we observe that when the relaxation time for the internal motions τ_e approaches the rotational correlation time τ_M, small changes in values of the experimental relaxation times (T_1, T_2, NOE) can lead to large variations in order parameters. For example, considering a protein like hTGF-α with a rotational correlation time of about 4 ns, if the relaxation time for the internal motions τ_e is on the order of 2 ns, then the relative error in S^2 is five times greater than the relative error in the spin-lattice relaxation rate when S^2 is on the order of 0.8. Thus, for this case, if there is a 5% uncertainty in $1/T_1$ then the uncertainty in S^2 is ±0.24. While the relatively large experimental values of the backbone N-H order parameters for hTGF-α appear to rule out crankshaft flips on a time scale of ~100 ps, much longer trajectories are needed to obtain statistically reliable estimates of the time scale for these motions in the MD simulations. Protein backbone C^α-H order parameters predicted from nanosecond MD simulations have recently been reported (Balasubramanian et al., 1994; Smith et al., 1995). Smith et al. (1995) compared longitudinal and transverse relaxation times for the backbone ^{15}N and $^{13}C^\alpha$ nuclei of BPTI, calculated from a 1 ns MD trajectory, and concluded that the disagreement between simulation and experiment appears to be the result of incorrect or missing long time scale (>200 ps) relaxation processes for certain residues. Furthermore, these authors suggested that the problem of elucidating the correct behavior of the internal motions on the same time scales as the overall rotation is complicated by the breakdown of the model-free approach in this regime. A more detailed analysis of the effects of infrequent dynamical events on apparent order parameters extracted from NMR observables is underway in our group.

In summary, the hTGFα MD trajectories demonstrate that amino acid residues with N-H order parameters that are smaller than their C^α-H order parameters exhibit 'crankshaft librational' motions of their peptide backbone on the picosecond time scale. These 'crankshaft' motions involve rocking of the rigid peptide bond due to anticorrelated changes in the backbone dihedral angles ψ_{i-1} and ϕ_i. In addition to these high-frequency librational motions, infrequent 'crankshaft flips' from one local minimum to another are also observed for seven out of 50 amino acid residues. It is not yet certain how these crankshaft flip motions are manifested in nuclear relaxation data. Overall, the present analysis of the hTGFα MD simulations suggests that comparisons of N-H and C^α-H order parameters determined from nuclear relaxation data provide a useful approach for identifying and characterizing motions of the polypeptide backbone on different time scales.

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