Analysis of deuterium relaxation-derived methyl axis order parameters and correlation with local structure

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Abstract

Methyl axis ($S^2_{\text{axis}}$) and backbone NH ($S^2_{\text{NH}}$) order parameters derived from eight proteins have been analyzed. Similar distribution profiles for Ala $S^2_{\text{axis}}$ and $S^2_{\text{NH}}$ order parameters were observed. A good correlation between the two $S^2_{\text{axis}}$ values of Val and Leu methyl groups is noted, although differences between order parameters can arise. The relation of $S^2_{\text{axis}}$ or $S^2_{\text{NH}}$ to solvent accessibility and packing density has also been investigated. Correlations are weak, likely reflecting the importance of collective, non-local motions in proteins. The lack of correlation between these simple structural parameters and dynamics emphasizes the importance of motional studies to fully characterize proteins.

Proteins are not rigid. While a great deal can be learned from the static structures obtained using NMR and X-ray crystallography, these models do not provide information on transiently populated conformations which may be biologically relevant (Alber et al., 1983; Varley and Pain, 1991; Zavodsky et al., 1998) nor do they describe the residual entropy of the folded state which is important for protein stability (Karplus et al., 1987). Consequently, a great deal of effort has been devoted to the characterization of protein dynamics using a wide variety of techniques (Brooks et al., 1988; Palmer, 1997).

Over the past several years NMR-based methods have emerged for studying protein dynamics over timescales ranging from ps (Palmer, 1997) to ms (Farro et al., 1994; Tolman et al., 1997). $^{15}$N spin relaxation studies (Kay et al., 1989), for example, have become a routine part of NMR structural analyses and yield information about the motion of backbone as well as sidechain NH bond vectors on the ps–ns timeframe. Typically $^{15}$N relaxation data is interpreted in terms of an order parameter, $S$, describing the amplitude of ps–ns bond vector motions along with an effective correlation time, $\tau_c$, related to the timescale of the motions (Lipari and Szabo, 1982). More recently, methods have been developed for the study of sidechain dynamics in proteins (Muhandiram et al., 1995; LeMaster and Kushlan, 1996; Yang et al., 1998). In one such approach proteins are labeled uniformly with $^{13}$C and fractionally deuterated, allowing measurement of $T_1$ and $T_{1\rho}$ relaxation times of deuterons in CH$_2$D (Muhandiram et al., 1995) and CHD (Yang et al., 1998) groups. This method is particularly attractive because the relaxation of the deuteron is dominated by the well-understood quadrupolar interaction. Dynamics of methyl groups in a number of proteins have now been measured using the $^2$H-based approach. In the present communication we use a database of eight proteins for which structural information, $^{15}$N–$^1$HN $S^2$ values and methyl sidechain dynamics are available (see legend to Figure 1) in order to establish whether there are correlations between
can be described with a single distribution function. Indeed, the two samples have similar distributions. It is noteworthy that NH order parameters ($S^2_{NH}$) are equal to within experimental error. The $S^2_{NH}$ values are similar, the $S^2_{NH}$ values are equal to within experimental error. The $C^1$ axis values were significantly smaller than measured $S^2_{C^1}$ values (Nicholson et al., 1996). Interestingly, the correlation coefficient, $r$ (Zar, 1984), between an Ala $S^2_{ax}$ value and the backbone NH order parameter of the same residue is only 0.34, with $p$, the probability of the two samples derived from uncorrelated populations, equal to 0.022. This low correlation is likely due to the intervening dihedral angle, $\phi$. One would expect the correlation between alanine $S^2_{ax}$ and $S^2_{C^1}$ to be much higher, although sufficient data is not available at present to establish whether this is in fact the case. Statistically significant correlations ($p < 0.05$) were not observed between $S^2_{ax}$ and $S^2_{NH}$ for any of the other residues, suggesting that the degree of bond vector mobility is (weakly) correlated over a separation of one dihedral angle but not, in general, much further. Finally, although the $S^2_{ax}$ distributions for Ile $C^1$ and Leu $C^1$ are similar, the profiles for Ile $C^2$ and Val $C^1$-$C^2$ are statistically distinct suggesting that the presence of the $C^3$ carbon in Ile has the effect of reducing mobility at the $C^2$ position.

Since the two methyl groups of Val and Leu residues belong to a single isopropyl moiety, they must have essentially the same mobility. As noted previously, however, the order parameters of the methyl groups can differ if for example the effective averaging axis for the isopropyl unit makes different angles with the two methyl threefold axes (LeMaster and Kushlan, 1996; Yang et al., 1998). In Figure 2, the $S^2_{ax}$ values for the two methyls of Val (a), Leu (b) and Ile (c) are compared. In the case of Val and Leu the agreement between intra-residue $S^2_{ax}$ values is close, with correlation coefficients of 0.89 and 0.86, respectively. About half the methyl pairs for these residues are equal to within experimental error. The $C^2$ and $C^1$ methyl axis order parameters of Ile correlate less well ($r = 0.44$, $p = 0.016$). This level of correlation is similar to that observed between $S^2_{ax}$ and $S^2_{NH}$ for Ala.

Buck et al. (1995) have found that Asn and Gln NH2 order parameters in lysozyme correlate well with their degree of burial. These residues can be found in both highly solvent exposed as well as buried positions and when buried they often participate in hydrogen bonds or polar interactions. A study which compared the motion of nitroxide spin labels introduced at com-