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Prediction of methyl-side chain dynamics in proteins

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Abstract

A simple analytical model is presented for the prediction of methyl-side chain dynamics in comparison with S^2 order parameters obtained by NMR relaxation spectroscopy. The model, which is an extension of the local contact model for backbone order parameter prediction, uses a static 3D protein structure as input. It expresses the methyl-group S^2 order parameters as a function of local contacts of the methyl carbon with respect to the neighboring atoms in combination with the number of consecutive mobile dihedral angles between the methyl group and the protein backbone. For six out of seven proteins the prediction results are good when compared with experimentally determined methyl-group S^2 values with an average correlation coefficient $\bar{r} = 0.65 \pm 0.14$. For the unusually rigid cytochrome c_2 no significant correlation between prediction and experiment is found. The presented model provides independent support for the reliability of current side-chain relaxation methods along with their interpretation by the model-free formalism.

Introduction

NMR relaxation of isotopically labeled proteins provides information on amino-acid side chain dynamics with atomic resolution. Because side chains are often intimately involved in protein-ligand and protein-protein interactions, the quantitative characterization of side-chain mobility is important for understanding protein function. Particularly suitable probes of molecular motions are the methyl groups whose dynamics can be assessed via ^{13}C relaxation of the methyl carbon (Nicholson et al., 1992; Wand et al., 1995) or ^{13}C, ^{2}H, and ^{1}H relaxation of partially deuterium substituted methyl groups (Muhandiram et al., 1995). The relaxation data, which probe ns and subns time-scale dynamics, can then be interpreted by the model-free formalism, which, in its simplest form, assigns to each methyl group an order parameter, S^2, and an internal correlation time, τ_e (Lipari and Szabo, 1982). The S^2 parameter, which can take values between 0 and 1, is a measure for the orientational motional restriction of the C-C bond (or C-S bond for methionine) that connects the methyl group with the rest of the side chain. While ^{13}C-derived and ^{2}H order parameters of the same methyl group can show discrepancies (Lee et al., 1999; Ishima et al., 2001), more recently a suite of deuterium relaxation experiments was introduced, which measures at a given magnetic field five different relaxation rates for each methyl group allowing a self-consistent analysis of the relaxation parameters (Millet et al., 2002; Skrynnikov et al., 2002).

The availability of an increasing body of NMR relaxation data allows the testing of hypotheses about the determinants of protein dynamics. For a SH3 domain evolutionarily conserved structural motifs were identified to play an important role for side-chain dynamics (Mittermaier et al., 2003; Mittermaier, 2003). For NMR S^2 order parameters of backbone ^{15}N-^{1}H bonds it was found that they substantially depend on the number and strengths of local atomic contacts between the peptide plane moiety and the rest of the protein (Zhang and Brüschweiler, 2002). An analytical expression was given for the estimation of S^2 values

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from a known 3D structure determined by X-ray crystallography or by NMR. Here, we extend the local contact model for the S^2 prediction of methyl groups and apply it to a number of different proteins for which methyl S^2 order parameters have been measured.

Methods

A measure for the number and strengths of steric contacts between a methyl carbon and its surrounding atoms is given by the contact sum

$$C_i = \sum_k e^{-r_{ij}/r_{eff}}, \quad (1)$$

where r_{ik} is the distance between the methyl carbon atom i and heavy atom k, which includes all heavy atoms that belong to cofactors, prosthetic groups, ligands, and amino acids other than the one that carries the methyl group. r_{eff} is an effective distance, which is the same for all methyl groups throughout the protein. C_i is a measure for the packing density at the methyl site, which differs from a hard surface model and qualitatively conforms with the contact strength between electron densities of Slater orbitals. The S^2 order parameter of the methyl group i is expressed as

$$S_i^2 = \tanh(aC_i/n_i^b) - c, \quad (2)$$

where prefactor a, exponent b and offset c are empirical parameters and the hyperbolic tangent function is defined as usual as $\tanh x = (e^x - e^{-x})/(e^x + e^{-x})$. n_i is the number of consecutive mobile dihedral angles between the methyl carbon and the backbone C^α atom of the same amino acid, i.e., $n_i = 1$ for the alanine $C^\beta H_3$ methyl group, $n_i = 2$ for the two valine methyl groups $C^{\gamma 1}H_3$ and $C^{\gamma 2}H_3$ and the threonine methyl group $C^{\gamma 2}H_3$, $n_i = 3$ for the two leucine methyl groups $C^{\delta 1}H_3$ and $C^{\delta 2}H_3$, $n_i = 2{,}3$ for the $C^{\gamma 2}H_3$ and $C^\delta H_3$ methyl groups of isoleucine, and $n_i = 4$ for the methionine $C^\epsilon H_3$. The n_i-dependence takes into account that methyl group order parameters tend to decrease with increasing separation from the backbone (LeMaster and Kushlan, 1996; Mittermaier et al., 1999), most likely due to the cumulative motion about sequential dihedral angles. Not all side chains follow this trend, however, as is discussed below.

Equation 2 predicts a low order parameter for a methyl group that has a high n_i value with only few or loose contacts to other heavy atoms located within an effective distance r_{eff}. Except for the $1/n_i^b$ term, Equation 2 is analogous to the expression for the prediction of backbone S^2 order parameters (Zhang and Brüschweiler, 2002). Equation 2 was implemented in the Python programming language and applied to PDB files that were preprocessed using MMTK (Hinsen, 2000). Parameters a, b, c, r_{eff} were optimized using the procedure described in the following section.

Results

Quantitative experimental methyl group order parameters are publicly available for an increasing number of proteins. In this study seven proteins were used, which are compiled in Table 1 together with the corresponding PDB codes. For HIV-protease the methyl-group S^2 order parameters had been determined from ^{13}C-^1H relaxation data, for all other proteins the S^2 values had been determined for partially deuterated methyl groups. For the B1 domain of protein L, which forms a trimer in the crystal structure, the prediction using Equation 2 was applied to chain A. For the dimeric HIV-1 protease the S^2 prediction was applied to both chains and the results were then averaged. For M-FABP and cytochrome c_2 the prediction was applied using both a X-ray and a NMR structure.

For an initial choice of the parameters a, b, c, and r_{eff}, Equation 2 was applied to the atomic coordinates extracted from the PDB files. For each protein the computed S^2 values were compared with the experimental S^2 values in terms of both $\chi^2 = \sum_i (S^2_{i,calc} - S^2_{i,exp})^2$ and the Pearson correlation coefficient $r = cov(S^2_{i,calc}, S^2_{i,exp})/(var(S^2_{i,calc}) \cdot var(S^2_{i,exp}))^{1/2}$. The four parameters a, b, c, and r_{eff} were varied on a grid and the sum of the χ^2 values, $\Sigma(\chi^2)$, and the sum of the correlation coefficients, $\Sigma(r)$, of the five best fitting proteins (ubiquitin, L B1 domain, A-LBP, flavodoxin, and HIV-protease) was monitored (Figure 1). Both sums show a single although relatively flat extremum between $r_{eff} = 3.0$ and 7.0 Å. For $r_{eff} = 3.4$ Å optimized values for the other fitting parameters were obtained as $a = 0.26$, $b = 2.2$, and $c = 0.125$.

Table 1 summarizes the results for all proteins analyzed in this study. It gives the correlation coefficients and χ^2 values between predicted and experimental order parameters using the above values for a, b, c, and r_{eff}. The correlation coefficients vary between 0.814 for ubiquitin and 0.048 for cytochrome c_2. Cytochrome c_2 with its unusually rigid behavior (Flynn et al., 2001) is the only protein for which no correlation is found neither for the crystal structure ($r_{X-ray} = 0.070$) nor for the NMR structure ($r_{NMR} = 0.048$). When cytochrome c_2 is excluded, the average cor-

Table 1. Comparison between experimental and predicted methyl group order parameters using Equation 1 with a = 0.26, b = 2.2, c = 0.125, r_{eff} = 3.4 Å

Protein[a]	PDB entry[b]	Resolution (Å)	Number of S^2 values	std[c]	r[d]	χ^{2}[e]	$(\chi^2/N)^{1/2}$[f]
Ubiquitin	1UBQ	1.8	44	0.249	0.814	1.311	0.173
L B1 domain	1HZ6	1.7	27	0.191	0.780	0.492	0.128
A-LBP	1LIB	1.6	37	0.235	0.681	1.365	0.192
Flavodoxin	1FLV	2.0	86	0.230	0.664	2.885	0.183
HIV protease	1EBK	2.1	52	0.223	0.459	2.349	0.212
M-FABP	1G5W	NMR	51	0.260	0.467	3.144	0.248
	1HMT	1.4			0.452	3.248	0.252
Cytochrome c_2	1C2R	2.5	50	0.208	0.070	3.401	0.261
	1C2N	NMR			0.048	4.328	0.294

[a]Methyl-deuterium relaxation used are the ones published for ubiquitin (Lee et al., 1999), B1 domain of protein L (Millet et al., 2003), adipocyte lipid-binding protein (A-LBP) (Constantine et al., 1998), flavodoxin (Liu et al., 2001), HIV protease (Ishima et al., 2001), muscle fatty acid-binding protein (M-FABP) (Constantine et al., 1998), and cytochrome c_2 (Flynn et al., 2001).
[b]References of PDB entries: Vijay-Kumar et al. (1987) (1UBQ), O'Neill et al. (2001) (1HZ6), Xu et al. (1993) (1LIB), Rao et al. (1992) (1FLV), Mahalingam et al. (1999) (1EBK), Lucke et al. (2001) (1G5W), Young et al. (1994) (1HMT), Benning et al. (1991) (1C2R), Cordier et al. (1998) (1C2N).
[c]Standard deviation of experimental S^2 values.
[d]Pearson's correlation coefficient between predicted and experimental S^2 values.
[e]$\chi^2 = \sum_i (S^2_{i,calc} - S^2_{i,exp})^2$.
[f]Rescaled χ^2 where N is S^2 the number of values.

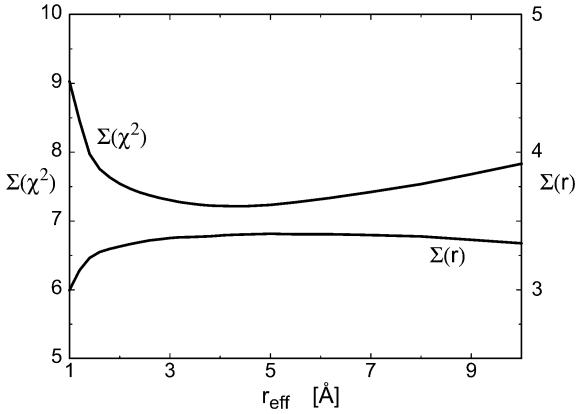

Figure 1. Optimization of Eq. (2) as a function of r_{eff} by varying parameters a, b, c. $\Sigma(\chi^2)$ is the sum of $\chi^2 = \sum_i (S^2_{i,calc} - S^2_{i,exp})^2$ terms and $\Sigma(r) = \sum_i r_i$ is the sum of the correlation coefficients between experimental and predicted methyl-group S^2 values for the five proteins ubiquitin, L B1 domain, A-LBP, flavodoxin, and HIV-protease.

relation coefficient for the remaining six proteins is $\bar{r} = 0.65 \pm 0.14$.

A residue by residue comparison of experimental and back-calculated methyl-group order parameters using Equation 2 is shown in Figure 2 for ubiquitin, the B1 domain of protein L, flavodoxin, and M-FABP. For ubiquitin, auto-relaxation of the multispin terms H_zC_z, $H_zC_zD_z$, $H_zC_zD_y$ (Muhandiram et al., 1995) were measured at 600 MHz and 750 MHz B_0-field strengths, converted into ^{2}H T_1 and $T_{1,\rho}$ relaxation times, and interpreted using the model-free approach (Lipari and Szabo, 1982) in terms of an internal correlation time τ_e and a S^2 order parameter (LS-2 model) (Lee et al., 1999). The prediction performs well for most methyl groups in ubiquitin (Figure 2a). The data for both methyl groups of L8 with low order parameters of 0.27 and 0.21 are remarkably well reproduced as are the disparate order parameters of I44 with $S^2_\gamma = 0.71$ and $S^2_\delta = 0.31$. Good agreement is also found for most methyl groups located in the C-terminal region. For some residues the prediction does not perform well, such as in the case of L50 for which the prediction yields S^2 values of 0.49 and 0.45, whereas the experimental values are 0.89 and 0.86, respectively. In this case, the division by $n_i^b = 3^{2.2} = 11.2$ in Equation 2 is insufficiently compensated by the contact term C_i leading to an underestimation of S^2.

For the B1 domain of protein L the quality of agreement between experiment (Millet et al., 2003) and prediction is striking (Figure 2b). Of the 30 methyl

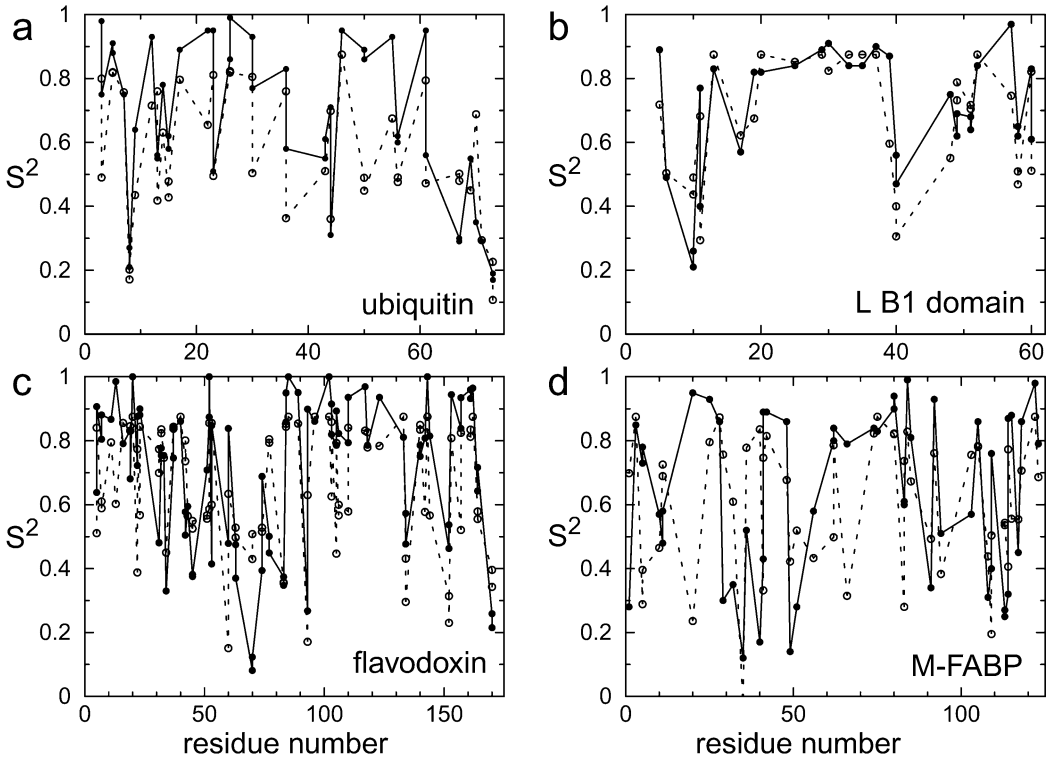

Figure 2. Experimental (closed circles connected by solid line) and predicted (open circles connected by dashed line) of methyl-group S^2 order parameters using Equation 2 of (a) ubiquitin, (b) L B1 domain, (c) flavodoxin, (d) M-FABP (using the NMR structure (Lucke et al., 2001)).

groups, only 8 show S^2 differences between prediction and experiment larger than 0.15. These are L10δ1/2, T39γ2, L40δ1/2, T48γ2, T57γ2, and L58δ2. Four of these methyl groups (L10δ1/2, T39γ2, T48γ2) belong to the six methyl groups whose relaxation data could not be interpreted using the simplest version of the model-free approach (LS-2 model) and instead required a model that, in addition to S^2 and τ_e, involves the fitting of an individual tumbling correlation time for each methyl group (LS-3 model). These residues undergo in addition to picosecond motions slower nanosecond time-scale dynamics (Millet et al., 2003). The L B1 domain is the only protein used in this study whose methyl order parameters were determined using the recent deuterium-only relaxation methodology yielding five different deuterium relaxation times for each methyl group (Millet et al., 2002).

Oxidized flavodoxin (170 amino acids) together with a noncovalently bound flavin mononucleotide cofactor (FMN) is the largest protein of this study. Its methyl-group relaxation data were reported using the same NMR methods as for ubiquitin (Liu et al., 2001). Experimental S^2 order parameters that were reported to be larger than 1 were set to 1.0 in Figure 2c. The overall correlation coefficient between experiment and the prediction using Equation 2 is 0.664. If only the 42 methyl groups belonging to the C-terminal half of the protein are considered starting at L83, the correlation coefficient is 0.83, which is slightly higher than the correlation found for ubiquitin. Conversely, the correlation coefficient for the 44 methyl groups belonging to the N-terminal half (I5 to V77) is lowered to 0.52. For a total of 24 methyl groups the difference between experiment and prediction is larger than 0.2 and for 11 methyl groups the S^2 difference is larger than 0.3. The largest discrepancies are exhibited by T13γ2 ($S^2_{\text{exp}} = 0.985$ and $S^2_{calc} = 0.603$) and L70δ2 ($S^2_{\text{exp}} = 0.123$ and $S^2_{calc} = 0.508$), which are located in a bend and a helix, respectively.

Experimental methyl-group order parameters of the apo forms of the human muscle fatty acid-binding protein (M-FABP) and human adipocyte lipid-binding protein (A-LBP) were reported (Constantine et al., 1998) using similar relaxation experiments used for ubiquitin and flavodoxin. For the M-FABP S^2 prediction, the 3D NMR structure (Lucke et al., 2001) of

human M-FAB and the 1.4 Å resolution crystal structure of human M-FAB complexed with stearate was used (Young et al., 1994). The correlations between experimental and predicted order parameters is around 0.46 for both types of structures. The largest discrepancy between prediction and experiment is found for the methionine methyl group M20ε ($S^2_{calc} = 0.20$ vs. $S^2_{\exp} = 0.95$). For the other methionine methyl group, M35ε, the prediction result is clearly better ($S^2_{calc} = 0.0$ vs. $S^2_{\exp} = 0.12$). This behavior stems from differences of the contact sums C_i calculated for the sulfur atoms: for M20εC_i is three times larger than for M35ε, which may cause the observed differential mobilities of the directly attached methyl groups.

For human A-LBP $r = 0.681$ is obtained, which is above average, despite the fact that the crystal structure of murine A-LBP (1LIB (Xu et al., 1993)) was used, which contains 11 mutations with respect to human A-LBP (Constantine et al., 1998). For HIV protease the model reproduces the trends observed in the experimental data (Ishima et al., 2001) reasonably well with the exception of the V82 methyl groups for which the experimental S^2 values are 0.26 and 0.21 whereas the model predicts considerably less mobility with S^2 values of 0.82 and 0.80, respectively. This discrepancy may be due to the fact that in the crystal structure the protease is bound to a notably larger inhibitor (∼800 Da) than in the NMR relaxation study (∼600 Da).

Discussion

Side-chain relaxation parameters have often been compared with chemical and structural features, such as amino-acid type, solvent-accessible surface area of side-chain atoms, and local packing. While for the polar asparagine and glutamine side chains of hen-egg white lysozyme a correlation between the solvent accessibility and NH_2 order parameters was found (Buck et al., 1995), an analysis of methyl order parameters of eight proteins, with the exception of methionine, did not show correlations with either methyl solvent accessibility or packing density (Mittermaier et al., 1999). In a subsequent study, an average correlation coefficient of -0.25 was found between normalized order parameters and solvent accessibility (Mittermaier et al., 2003). The near lack of correlation between methyl solvent accessibility and methyl S^2 order parameters was also noted for other proteins (Constantine et al., 1998; Flynn et al., 2001). For flavodoxin, which is one of the largest proteins studied so far, a systematic decrease of methyl S^2 order parameters with increasing distance to the protein surface was observed (Liu et al., 2001).

The contact sum C_i of Equation 1 is positively correlated with the methyl order parameters for the nine different types of methyl groups in the first six proteins of Table 1 covering the range between $r = 0.66$ for Ileγ1 and Valγ2 and $r = 0.17$ for Thrγ2 with an average $\bar{r} = 0.43 \pm 0.17$. If only the first four proteins of Table 1 are included $\bar{r} = 0.48 \pm 0.13$. The low correlation for Thrγ2 reflects the influence of other mechanisms than packing, such as hydrogen bonding involving the ThrO$^{\gamma}$H group. By contrast, if only the n_i-dependence is considered in Equation 2 by setting $C_i = 1$, the average correlation between experiment and prediction for the proteins of Table 1 is $\bar{r} = 0.38 \pm 0.13$. These results indicate that the local contact sums C_i and the dihedral angle numbers n_i are both required for a meaningful analysis of the methyl order parameters (see Equation 2).

The agreement between prediction and experiment is generally determined by the interplay of three principal factors: (i) the quality of the analytical model, (ii) the quality of the experimental data, and (iii) the quality of the 3D protein structure. It is interesting to note that in Table 1 the best agreement is found with crystal structures that have a resolution below 2 Å. The 1.4 Å structure of M-FABP (Young et al., 1994) is the complex with stearate and might slightly differ from the apo state used in the relaxation study. For cytochrome c_2, which escapes a parametrization based on Equation 2, the available crystal structure has only 2.5 Å resolution.

The simple model of Equation 2 has limitations that become apparent, for example when examining the methionines M20ε and M35ε of M-FABP mentioned above. Due to the large $n_i = 4$ value, the predictions are generally low, which works well for M35ε, but not for M20ε where the stabilization of the sulfur atom by local contacts with the environment causes an effective reduction of n_i for the attached methyl group to a value $n_i \approx 1$ explaining the high experimental order parameter $S^2 = 0.95$. Another limitation of Equation 2 is the neglect of collective reorientational dynamics of bond vectors (Prompers and Brüschweiler, 2002). The importance of such effects could explain instances of increased mobility in densely packed environments (Finerty et al., 2002).

Conclusion

In summary, the extended version of the local contact model presented here provides a semi-quantitative prediction of side-chain methyl group dynamics from average protein structures. The best results are obtained for proteins for which a representative high-resolution structure is available. Equation 2 solely includes local contacts together with the number of mobile dihedral angles separating the methyl group from the backbone. Although the contact term with its steric character does not directly take into account the charge distribution in the protein, the latter influences the secondary and tertiary structure and thereby indirectly affects also packing and local contacts.

Considering the independence of NMR side-chain relaxation measurements from the protein structure determination process using X-ray crystallogrophy or NMR, the existence of the simple relationship expressed in Equation 2 independently supports the significance and reliability of the current side-chain measurement methodology along with the interpretation in terms of model-free dynamics parameters. As side-chain dynamics data are becoming available for an increasing number of proteins, it should be possible to further improve this relationship, for example by separate parametrizations of the different amino acid types. Such relationships will be useful for the cross-validation of local features of protein structures and they will contribute to the quantitative understanding of the subtle interplay between protein structure and side-chain mobility.

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