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THE ANALYSIS OF NMR RELAXATION DATA IN TERMS OF MULTIPLE INTERNAL MOTIONS

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A novel formalism for estimating the complex motions of proteins and other flexible macromolecules from NMR relaxation measurements is applied to ^{13}C NMR relaxation data on the Bovine Pancreatic Trypsin Inhibitor (M. W. 6,500). Six experimental parameters measured at two field strengths are accounted for by a minimum of three motions at each carbon group. Low frequency components make small but finite contribution to the relaxation of all resonances, suggesting a general low frequency distortion of the backbone. Rotational diffusion of the protein makes a relatively minor contribution to the relaxation process. For aliphatic groups, rotation of side chains dominates relaxation.

It is well known that NMR relaxation measurements can in principle provide information on the internal dynamics of proteins and other macromolecules. However, the interpretation of relaxation measurements on flexible molecules presents much more serious theoretical difficulties than generally appreciated. It is always possible to derive a correlation time from the measurement of a single relaxation parameter, using any arbitrary mathematical model. It is usually possible to account for two or even three relaxation parameters by any one of several models. The inappropriateness of a specific model, as applied to a specific system, becomes apparent only if the stringent condition is applied that the model account for all, i.e., for a large number of relaxation measurements made on the system.

To evaluate the magnitude of this problem, and to eliminate it if possible, we have collected and analyzed ^{13}C relaxation data (T_1, T_2 and NOE) at frequencies of 45 and 90 MHz on Bovine Pancreatic Trypsin Inhibitor, a small protein (M. W. 6,500). It has been pointed out before that the relaxa-

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tion of proton-bearing ^{13}C nuclei is dominated by dipole-dipole interaction with the nearest proton and therefore permit a relatively simple interpretation in terms of a single relaxation mechanism (1,2). The procedure used for data analysis is based on the formalism developed for the description of relaxation phenomena in systems with multiple degrees of internal motional freedom (3,4). In this formalism, the spectral density function used in the calculation of relaxation parameters is given by

$$J_{F_j}(\omega) = \sum_{\ell_1 \ldots \ell_2} \frac{2\left|\langle F_j \prod_{k=1}^{N} \phi_{\ell k}^{k} \rangle\right|^2 \sum_{k=1}^{N} \lambda_{\ell k}^{k}}{\left\{\sum_{k=1}^{N} \lambda_{\ell k}^{k}\right\}^2 + \omega^2} \qquad (1)$$

where; $J(\omega)$ is the spectral density function at a frequency ω; F_j, a dynamic variable with an autocorrelation function $F_j(t)F_j^*(t + \tau)$; λ_n, ϕ_n are eigenvalues of a transition operator $\Omega\phi_n = \lambda_n\phi_n$ -- i.e. λ_n, ϕ_n are the rate and amplitude parameters of the dynamic variable F_j respectively; $\lambda_n = 1/\tau_n$ where τ_n is the correlation time for the nth individual motion. The formalism thus permits the systematic testing of relaxation equations implying one, two, up to N motions against a given set of experimental data. In the present analysis, an additional simplifying assumption was made, which is not a severe restriction on the generality of the approach -- i.e., that each motion can be represented by an average rate parameter $\bar{\lambda}_k$, rather than a complete set of eigenvalues $(\lambda_1 \ldots \lambda_\ell)_k$. No knowledge of the absolute amplitude ϕ of each motion is required, since the eigenfunctions can be normalized and the condition holds $\sum_i \alpha_i = 1$, where α_i is the average amplitude of a set of motions, $\alpha_i = \left|\langle F_j, \phi_j \rangle\right|^2$ Under these conditions, Eq. (1) can be re-written as

$$J(\omega) = \sum_k \frac{\alpha_k \bar{\lambda}_k}{\omega^2 + \bar{\lambda}_k^2} \qquad (2)$$

Successively setting k = 1, 2, 3...N, all possible combinations of values

α_k and $\bar{\lambda}$ can be found which account for a given set of relaxation data. The computer program required for this analysis will be reported in a more detailed communication. $\bar{\lambda} = 6 \times 10^8$ sec $^{-1}$ describing the rotational diffusion of the molecule was determined by depolarized light scattering (C. C. Wang and R. Pecora, personal communication) and is introduced as an additional known constraint into Eq. (2).

Several important conclusions can be stated from the application of this type of analysis to about 40 identifiable and partially assigned (5,6) ^{13}C resonances in the BPTI spectrum:

(1) For none of the resonances can the set of six (or even three) relaxation parameters (e.g., T_1, T_2, and NOE at 45 and 90 MHz) be simultaneously accounted for by a single motional term.

(2) Two motions, one of which is the rotational diffusion of the protein, suffice to account for relaxation in only two of the resonances, one in the α-CH, the other in the aromatic region of the spectrum. The nature of the second motion, at a frequency of 2×10^8 sec^{-1}, cannot be specified, but it could be the diffusion of the longer axis of the anisotropic molecule (axial ratio ∿3:1). The result in this interpretation is compatible with the notion that the two groups are part of a rigid structure.

(3) A minimum of three motions is required to account for the relaxation data on all other resonances. Thus, except for the preceding two, none of the observed resonances can be said to represent groups rigidly held in the protein structure. This indicates that greater caution is necessary in the use of the common assumption that the relaxation of C_α resonances reflects only the motion of the rigid protein backbone (7-9). With three motions and six measured parameters, an exact solution of the six simultaneous relaxation equations is possible. In our procedure, the best fit was obtained separately for the three relaxation measurements at each frequency, showing that a set of α_k, $\bar{\lambda}_k$ which provided a solution for one set also

TABLE I BPTI--TYPICAL MOTIONAL FREQUENCIES (λ) AND AMPLITUDES (α) FROM ^{13}C RELAXATION DATA

CHEMICAL SHIFT(PPM)† ASSIGNED RESIDUE(S)	FIELD STRENGTH (MHz)	T_1 (SEC)	T_2 (SEC)	NOE	MOTION 1 λ_1(Hz)	MOTION 1 α_1(%)	MOTION 2 λ_2(Hz)	MOTION 2 α_2(%)	MOTION 3 λ_3(Hz)	MOTION 3 α_3(%)
7.55	45	.256	.100	2.77	6E8	2%	1E7	1%	2E10	97%
ILE^{18} OR ILE^{19} CH_3	90	.370	.135	2.46	6E8	3%	1E7	1%	3E10	96%
13.129	45	.322	.068	1.73	6E8	5%	1E7	1%	7E10	94%
MET^{52} SCH_3	90	.350	.057	1.74	6E8	10%	1E7	1%	8E10	89%
16.708	45	.276	.085	2.61	6E8	1%	1E7	1%	2E10	98%
ALA^{16,27,58} CH_3	90	.242	.083	2.19	6E8	5%	1E7	<1%	2E10	94%
33.286	45	.111	.026	1.67	6E8	25%	3E7	10%	2E10	65%
ASP β CH_2	90	.122	.033	1.55	6E8	48%	3E7	7%	1E10	45%
39.355	45	.303	.082	2.13	6E8	7%	1E7	1%	3E10	92%
LYS ϵ CH_2	90	.264	.050	2.20	6E8	14%	1E7	3%	2E10	83%
51.738	45	.117	.062	1.45	6E8	33%	2E8	35%	9E11	32%
ALA^{58} α-CH	90	.220	.062	1.18	6E8	23%	2E8	61%	9E11	15%
53.546	45	.106	.044	1.27	6E8	1%	2E8	74%	3E10	24%
UNASSIGNED α-CH	90	.207	.036	1.20	6E8	63%	1E7	6%	2E10	31%
116.866	45	.121	.026	1.16	6E8	4%	9E7	87%	9E10	9%
TYR^{3,5} C	90	.322	.035	1.15	6E8	13%	1E8	87%	3E10	<1%

†RELAXATION DATA AT 17°C, pD 5--CHEMICAL SHIFTS ARE REFERENCED TO EXTERNAL TMS.

constituted a solution of the other. A representative sample of the data and calculated frequencies and amplitudes is seen in Table I. Two noteworthy points are immediately apparent: (1) A low frequency motion (λ_2 = 1-2 x 10^7 sec^{-1}) makes a small but consistent contribution to the relaxation of virtually all resonances, and (2) a very high frequency component (λ_3 = 10^{10}-10^{11} sec^{-1}) makes a contribution to the relaxation of several C_α resonances. This component is easily understood in the case of C_α of Ala 58, since the residue is near the C-terminal of the protein and could wobble freely. It speaks against the dynamic stability of the salt bridge between Arg 1 and Ala 58 postulated on the basis of chemical shift measurements(6). No such easy explanation can be advanced for its appearance amoung other C_α's, but it is possible that it reflects the small rearrangements predicted by molecular dynamics calculations (10,11) in this frequency range.

The nature of the low frequency component is not defined by this type of analysis. If one assumes that it represents a low amplitude wobble (4) the average angle of the wobble can be estimated to be <45°. Other models

can be proposed and cannot be distinguished from a wobble on the basis of relaxation data alone. The near ubiquity of the component suggests that it represents a general relatively slow warp of the entire backbone, which would be reflected in the motion of both the backbone and all side chain carbons. The frequency of this component suggests that it may originate from diffusional collisions between protein molecules.

The rapid component of the motion of methyl groups largely represents methyl group rotation. However, the fractional contribution of this motion (92-98%) exceeds the theoretical maximum (88.8%) predicted by the appropriate rigid rotor model (12). This and the observation that alternative values of λ_3 in the same frequency range can account for the relaxation data suggests that methyl group rotation may not be the only significant motion at this frequency. Discrimination may be possible if the analysis is extended to include four or more degrees of motional freedom.

The type of analysis carried out here allows a rigorous determination of (a) the minimum number of motions required to account for a set of relaxation data and (b) the frequency of each motion to within a factor of 2-3. It appears to sacrifice information on the absolute amplitude of each motion. However, the sacrifice is more apparent than real. Whenever two or more specific models account for the data equally well, no real information on amplitudes can be derived from relaxation data. Since this is frequently the case (4), it can be said that the information on the nature and amplitudes of individual motions -- as contrasted to their frequencies -- which NMR can provide is very limited. Accurate knowledge of the frequencies, however, permits comparisons of mobilities in different parts of the protein structure and thus a limited but significant understanding of its dynamics.

Viewed in the light of the known structure of BPTI, the data in Table I show that, even in this relatively small and rigid protein, rotational diffusion of the molecule as a whole makes only a minor contribution to relaxation. Rotation of side chains dominates the relaxation process.

The rates of these rotations are nearly equal for all aliphatic groups observed, but differ somewhat for the aromatics, which appear more sensitive to the rotational diffusion of the protein. This is understandable from an examination of a molecular model of BPTI. All of the aliphatic groups examined are on the surface of the structure, whereas the aromatics are not. Two additional motions -- one of low, one of high frequency -- are present in the backbone. While relaxation measurements by themselves cannot shed light on their nature, interpretation may be possible on the basis of molecular dynamics calculations.

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