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MOLECULAR DYNAMICS STUDIES OF NMR RELAXATION IN PROTEINS

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There is a growing awareness that the visualization of proteins as large rigid molecules, each of which can be described by a single static structure, is inadequate. Instead, it is now realized that proteins undergo structural fluctuations which span a range of times from subpicoseconds to milliseconds or longer. In this report we are concerned with the relation between protein fluctuations occurring on the picosecond to nanosecond time scale and nuclear magnetic resonance (NMR) relaxation experiments.

NMR relaxation parameters provide an important probe for molecular motion. The spin lattice (T_1) and spin-spin (T_2) relaxation times and NOE factor η depend on the thermal motions of the system under study. Carbon 13 NMR is particularly well suited for probing the dynamics because the relaxation is predominantly determined by the dipolar interactions between the ^{13}C nuclei and their directly bonded protons. ^{13}C NMR has been used extensively to study the dynamics of small molecules (1) and of proteins (2). For proteins, which have many internal degrees of freedom, the interpretation of NMR experiments in terms of molecular motion is not unambiguous. We report here the use of molecular dynamics simulation techniques for the analysis of relaxation due to structural fluctuations within protein; the relaxation of the backbone atoms and of side chains protruding into the solvent are considered.

NMR relaxation experiments probe angular correlation functions of the relaxing nucleus. In a ^{13}C experiment the dynamical quantities are the spherical polar coordinates with respect to a laboratory frame of the C-H internuclear vectors. The time correlation functions that determine the NMR relaxation can be evaluated from the phase space trajectories that are obtained by solving the equations of motion for the atoms comprising the protein.

To study protein structural fluctuations Karplus and McCammon (3, 4) calculated a 100-ps molecular dynamics simulation of the pancreatic trypsin inhibitor protein (PTI). The effect of the high frequency (picosecond) fluctuations determined in this kind of simulation is to average out the protein tumbling contribution to the spectral density and increase the observed NMR T_1. Of particular interest is the contribution of fast motional averaging to the NMR relaxation of backbone carbons in proteins. Since the motion of these atoms is expected to be extremely hindered, it has often been assumed that the relaxation of these carbons is accounted for entirely by the protein molecular tumbling. From an analysis of the 100-ps PTI molecular dynamics simulation we find that fast relaxation processes increase the backbone C^{α} carbon T_1's by ~20% over the rigidly tumbling values. For example, at 45 MHz the T_1 of a backbone carbon is predicted to be 76 ms if PTI were tumbling rigidly in solution whereas

TABLE I
SIDE CHAIN NMR PARAMETERS*

Side chain carbon	Exact result			Lattice jump model		
	T_1	T_2	NOE	T_1	T_2	NOE
C_0‡	58	48	1.70	58	48	1.70
C_1	179	163	2.34	162	147	2.31
C_2	312	308	2.90	252	247	2.85
C_3	493	486	2.90	358	355	2.92
C_4	682	672	2.90	520	518	2.97

*Spectrometer frequency 68 MHz, protein tumbling time 1 ns, NMR relaxation times in milliseconds.
‡Carbon C_0 assumed to tumble rigidly with the protein.

from the trajectory analysis the T_1's are calculated to be close to 100 ms. The T_1's predicted from the trajectories are close to the measured values for backbone C^α carbons of PTI (5). We also find that the predicted T_1's for the glycine residues in PTI are not greater than the predicted values for the C^α carbons of bulkier residues. However, the calculated T_1's of the C^α carbons of a few of the residues are found to be significantly greater than 100 ms. For example, the fast motional averaging is predicted to increase the T_1 for the C^α carbon of lysine 15 in PTI by almost four times relative to the rigid body value. We note that differences in the T_1's of this magnitude as predicted from molecular dynamics trajectories could be used as an aid in assigning resonances in protein spectra.

For many of the ^{13}C nuclei in a protein larger time fluctuations than those probed in a 100-ps simulation are important; in general, motions up to nanoseconds (the PTI tumbling time is estimated to be 1.6 ns) can make significant contributions. Stochastic molecular dynamics methods have been used to study the complete spectrum of relaxation effects in a model side chain protruding into the solvent. The diffusive Langevin equation of motion was solved to simulate the equilibrium and dynamic properties of an alkane chain in aqueous solution for times up to 100 ns (6). The chain has an overall tumbling time corresponding to that of a protein and, in addition, has four internal rotational degrees of freedom with barriers of ~2.8kcal/mol. The internal dynamics of the side chain were found to separate into two very different time scales. The shorter, on the order of tenths of picoseconds, corresponds to torsional fluctuations within a potential well (corresponding to that for the backbone α carbons), and the longer, on the order of two hundred picoseconds, corresponds to transitions between minima. Levy et al.[1] have compared the NMR relaxation times calculated exactly from the stochastic trajectory with a lattice jump model for the relaxation; in the latter, each carbon-carbon bond is allowed to make uncorrelated jumps between trans, gauche +, and gauche - states with rate constants determined from the dynamics. There is a large gradient in the relaxation times along the chain (see Table I). The T_1 values range from 58 ms for a carbon moving rigidly with the protein (τ = 1 ns) to >500 ms for the carbon furthest from the protein backbone. There is general agreement between the NMR relaxation times calculated from the exact dynamics and the lattice jump model (Table I). The NMR times calculated from the jump model are within 10% of the exact results for the carbon reorienting about one side chain rotational axis and within 30% of the exact values for carbons furthest out along the chain. While the lattice jump model provides a satisfactory description of the NMR relaxation of the free side chain, an alternative treatment is required for side chains in the

[1]R. M. Levy, M. Karplus, and P. G. Wolynes. Manuscript submitted for publication.

protein interior where both rapid oscillations and longer time processes are expected to contribute to the relaxation. As the time scale separation between the internal rotations and tumbling increases, the independent lattice jump model provides a poorer description of the NMR relaxation and improved models are required (7).

In summary, the present work provides a theoretical foundation for the continuing effort to obtain information about the internal dynamics of proteins from NMR measurements.

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PROTON NUCLEAR MAGNETIC RESONANCE AND FLUORESCENCE SPECTROSCOPIC STUDIES OF SEGMENTAL MOBILITY IN AEQUORIN AND A GREEN FLUORESCENT PROTEIN FROM *AEQUOREA FORSKALEA*

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Aequorin is a protein of low molecular weight (20,000) isolated from the jellyfish *Aequorea forskalea* which emits blue light upon the binding of Ca^{2+} ions (for review, see reference 1). This bioluminescence requires neither exogenous oxygen nor any other cofactors. The light emission occurs from an excited state of a chromophore (an imidazolopyrazinone) which is tightly and noncovalently bound to the protein. Apparently the binding of Ca^{2+} by the protein induces changes in the protein conformation which allow oxygen, already bound or otherwise held by the protein, to react with and therein oxidize the chromophore. The resulting "discharged" protein remains intact, with the Ca^{2+} and the chromophore still bound, but is incapable of further luminescence. The fluorescence spectrum of this discharged protein and the bioluminescence spectrum of the original "charged" aequorin are identical. A green