the absorbance changes of the drug itself to monitor binding and also by using H-D exchange of amino protons in EB, since binding to DNA inhibits this exchange. For the strong binding mode, the simplest model:

$$EB + DNA_{site} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} DNA\ (EB)$$

is consistent with data from both kinds of experiments; k_{-1} is 30 s^{-1} at 20°C 1 M NaCl, pH 7; k_1 is 10^6 M^{-1} s^{-1} where DNA sites are measured in units of 5P/site. The pseudo first order rate of occupying sites exceeds the hydrogen exchange opening rate by factors of 10 or more under typical experimental conditions. Thus intercalative opening does not require H-bond breakage. The change in intercalative opening caused by association of DNA in mononucleosome cores will be described at the meeting.

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CONFORMATIONS AND CONFORMATIONAL DYNAMICS OF PROTEINS IN SOLUTION STUDIED BY NUCLEAR MAGNETIC DOUBLE RESONANCE

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Saturation of single resonances in the proton nuclear magnetic resonance (nmr) spectrum of a protein in solution results in changes in intensities of other resonances (Redfield and Gupta, 1971). These changes come about either through nuclear Overhauser effects which arise because of dipolar coupling between the protons in the molecule, or through cross-saturation effects which arise when the protein fluctuates between different well defined conformations. For lysozyme from hen egg white (mol. wt. ~14,500) the method reveals details of the protein structure and of individual atom fluctuations.

In Fig. 1 *a* a difference spectrum shows intensity changes of methyl group resonances which result from the saturation of the C(5)H resonance of Trp 28 in lysozyme. The resonances showing large effects have been assigned (Dobson, 1977; Poulsen, et al., 1980) and are from groups within 7Å of the Trp 28 C(5)H proton in the crystal structure. These observations show that the solution structure of lysozyme in the region of Trp 28 is close to that of the crystal structure because the magnitude of an Overhauser effect depends on the distance r_{ij} of a nucleus i from the saturated nucleus j.

Many similar Overhauser effects have been measured in the hydrophobic box region of

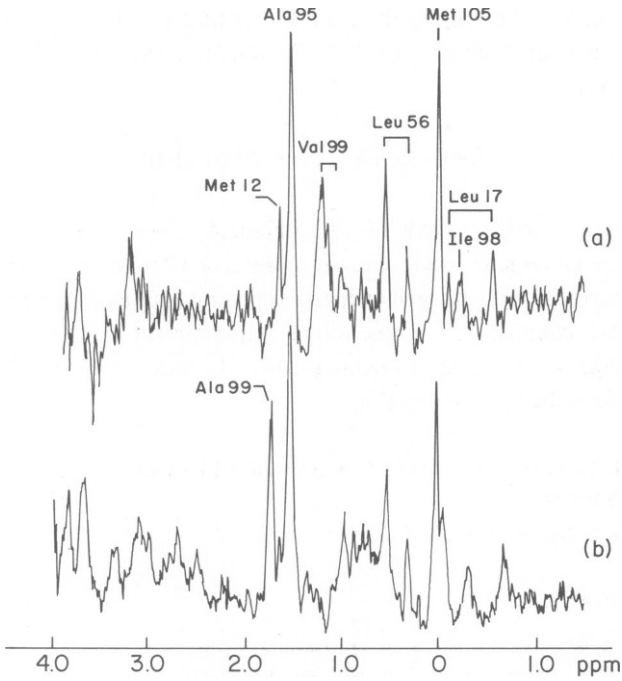

Figure 1 Difference spectra obtained by substracting spectra obtained with a saturation pulse applied at the Trp 28 C(5)H resonant frequency for 1.0 s before acquisition from spectra with the saturation pulse off-resonance as described by Poulsen et al. (1980). (*a*) 7 mM hen lysozyme at 55°C in D_2O, pH 3.6; (*b*) turkey lysozyme under the same conditions. The area of the peaks represent the magnitudes of the nuclear Overhauser effects, which are negative and all <10%.

lysozyme and there is a strong correlation between the effects produced and interproton distances derived from x-ray data (Poulsen et al., 1980). In Fig. 2 a double logarithmic plot shows that all of the x-ray distances are within ±0.8 Å of a line drawn with a slope of -6. This slope would be expected for the short saturation pulse used in this experiment because in the simplest case Overhauser effects then depend simply on r_{ij}^{-6}. This correlation is virtually at the level of the accuracy of the x-ray and nmr data and has been used without further analysis to examine conformational differences and changes. In Fig. 1 *b*, for example, a difference spectrum for lysozyme from turkey egg white is shown. Although the spectrum is similar to that of hen lysozyme, the resonances assigned to Val 99 are absent and a new resonance of an alanine residue is now present. A structural difference is therefore apparent, and in this case it is very simply explained because in the sequence of turkey lysozyme residue 99 is alanine. This is the only sequence difference from hen lysozyme in this region of the protein and the new resonance can be assigned to Ala 99. The magnitude of the Overhauser effect enables this methyl group to be placed 3.5 ± 0.8 Å from the Trp 28 C(5)H proton. The close similarity between the other resonances in the two spectra shows that no other structural differences exist near to Trp 28 although minor differences between the proteins involving other residues have been detected.

Even in the short-pulse experiment (Fig. 2), deviations from a simple r^{-6} dependence are observed. At longer distances larger effects than expected arise because the finite length of the saturation pulse permits significant diffusion of magnetisation through near protons to more distant ones. Overhauser effects have been studied as a function of the length of the saturation pulse. Calculations show that the experimental data are broadly consistent with the

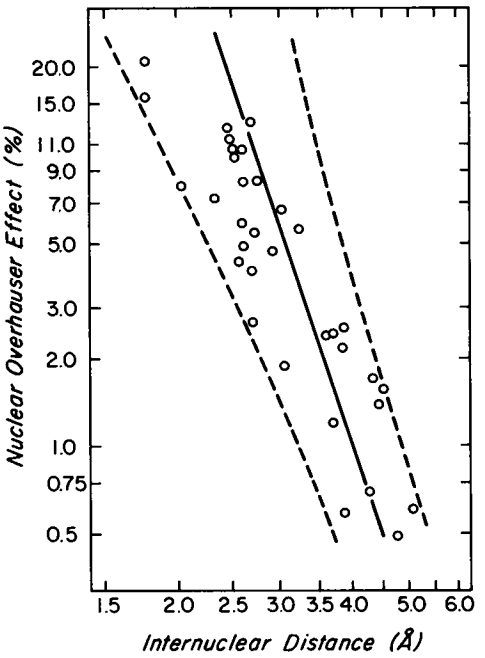

Figure 2 Plot of the magnitude of nuclear Overhauser effects obtained during a 0.25-s saturation pulse, against interproton distances calculated from x-ray crystallographic coordinates. The saturated and observed resonances are all from residues in the hydrophobic box region of hen lysozyme. Allowance for rapid methyl group and phenylalanine and tyrosine rotations has been made. The full line has a slope of -6, and the dashed lines are ±0.8 Å from this. The data are from Poulsen et al. (1980).

crystal structure, and with expected correlation times for molecular tumbling and rotation of methyl groups.

To study dynamic aspects of the protein we are examining the significance in a more detailed analysis of the double resonance experiments of various types of internal motion, and particularly the influence on Overhauser effects of an averaging of interproton distances over various interconverting conformations. The relevant motions include both subpicosecond fluctuations about the average structure and slower fluctuations between different conformational states such as those already detected in lysozyme (Dobson, 1977). Very slow fluctuations can result in separate resonances for a nucleus in two or more conformations, and intensity changes in a double resonance experiment can then arise from cross-saturation as well as from Overhauser effects. The rotation of a tyrosine residue in cytochrome *c* was studied in this way (Campbell et al., 1976) and for lysozyme the method is being used to study the rates of conformational changes which follow oligosaccharide binding.

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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