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DYNAMICS OF OVERHAUSER EFFECTS IN MACROMOLECULES

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Abstract - Estimation of distances between near neighbor protons in macromolecules, using the nuclear Overhauser effect, is often complicated by the phenomenon of spin-diffusion. In order to overcome this difficulty, the time dependence of the Overhauser effect at short times following the selective inversion of one set of spins may be followed. Some experimental results obtained applying this technique to solutions of lysozyme in D_2O are presented, and compared with theory and with data obtained by Poulsen, Hoch and Dobson on this system employing measurements of the nuclear Overhauser effect after a short period of saturation. It appears that internal motions in the protein may bring protons closer together than in the X-ray diffraction derived crystal structure, and produce larger effects than predicted on the basis of a rigid molecule.

INTRODUCTION

The change in intensity of the NMR signal from one set of nuclei when a second set is subjected to saturating irradiation is known as the nuclear Overhauser effect (1). Its magnitude and sign are controlled by the cross-relaxation processes connecting the spin temperatures of the two sets of nuclei and by the relaxation rate of the observed set arising from all processes. In macromolecules, the dominant process is the zero-quantum cross-relaxation, corresponding to exchange of spin-states for two nuclei in the antiparallel arrangement. This has the effect of transferring excitation from the irradiated species to the observed species, partially saturating it, and reducing its signal and intensity. For large macromolecules this mechanism becomes very efficient, and the excitation may be transferred along a chain of nuclei, resulting in a loss of specificity of the effect. This removes the possibility of identifying signals from protons which are close to each other.

It has been suggested that this difficulty may be overcome by observing the time course of the build-up of the Overhauser effect (2,3,4). For a multispin system, the time dependence is given by

$$\dot{\underset{\sim}{z}} = - \underset{\sim\sim}{R}\underset{\sim}{z} \qquad (1)$$

where $\underset{\sim}{z}$ is the vector of deviation from equilibrium of the z-magnetizations of the sets of nuclei, i,j..., and $\underset{\sim\sim}{R}$ is the relaxation matrix with the elements ρ_i, σ_{ij} defined by Noggle (1). The solution to the equations may be found using standard numerical methods (3,4).

Transient Overhauser effects are observed following the inversion at time zero of a selected set of nuclei. The curves of intensity versus time for the non-irradiated sets are sums of exponentials which show maxima, then decay to zero at long times. Nuclei close to the irradiated nucleus generally show steeper initial rises, higher maxima, and maxima at shorter time than remote nuclei.

In this work we have observed the time dependence of specific nuclear Overhauser effects following selective inversion of spins in the protein lysozyme in D_2O solution. Lysozyme is a well studied system, and numerous investigations using ^1H NMR have been reported: McDonald and Phillips (5); Campbell, *et al* (6); Dobson (7). The use of Overhauser effects to probe spatial structure has been reported by Campbell, *et al* (8), Cassels, *et al* (9), Chapman, *et al* (10), and by Poulsen, *et al* (11). In the most recent study by Poulsen, *et al*, effects were observed following 0.25 seconds of saturating irradiation applied to the perturbed nucleus. In this way many revealing inter-residue effects were established for the hydrophobic box region of lysozyme. These effects correlate only roughly with internuclear distance.

In this work we have monitored the time dependence of selected nuclear Overhauser effects

following inversion of a specific spin, and have compared our results with those theoretically expected, and with those obtained using the procedure of Poulsen, et al (11), in order to determine whether more precise characterization of the geometry is practical. An additional possible complication is that breathing motions within the structure will bring nuclei closer together than in the X-ray diffraction structure. Because of the r^{-6} dependence of the relaxation rate, such events would seriously distort the expected relaxation pattern.

EXPERIMENTAL

Lysozyme was obtained from Sigma Chemical Company, and exchanged with D_2O by solution, heating and lyophilization to remove the NH signals. Final solutions were 4% by weight in D_2O.

Spectra were obtained at 600 MHz, using the spectrometer at the NMR Facility for Biomedical Studies, Pittsburgh, in the correlation mode. Isolated peaks in the spectrum were inverted by application of a soft-pulse exactly on resonance; after a variable delay, the spectrum was scanned. In order to follow the Overhauser effect, difference spectra were formed by subtracting the spectrum from a control spectrum obtained without initial inversion. Peak heights were measured and the Overhauser effect is expressed in units of intensity such that a single unperturbed proton peak has unit intensity. In plotting intensities, the time was taken as the total time until observation, i.e. the sum of the delay time and the sweep interval until observation.

RESULTS AND DISCUSSION

The aromatic region of the 600 MHz proton spectrum of lysozyme is shown in Fig. 1, together with identification of a number of the peaks according to Poulsen, et al (11). From the width of individual proton signals (12) it is judged that the rotational correlation time in our solutions is about 20 - 25 ns, and this agrees with the correlation time found from α-^{13}C relaxation rates by Oldfield, et al (13), who give 20 ns as the value. For this rotational rate, $\omega\tau_c$ is found to be 75, well beyound the extreme narrowing limit and into the high-field region favoring spin diffusion.

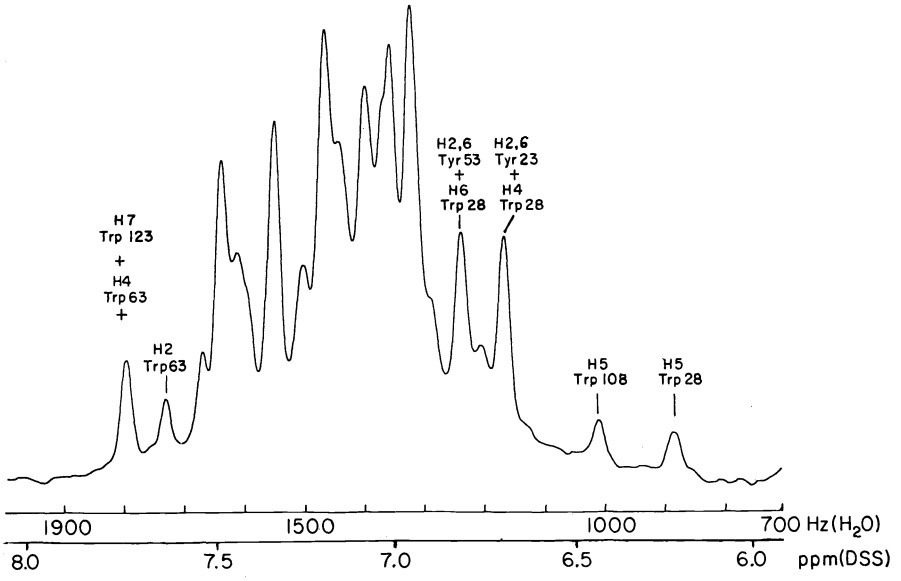

Fig. 1. Aromatic region of the 600 MHz proton spectrum of lysozyme in D_2O solution. Amide proton signals have been removed by exchange.

In order to calculate the expected behavior of the proton spin system after inversion of selected spins, the coordinates C, O, N atoms in residues immediately surrounding TRP 28 and TRP 108 were extracted from the crystal coordinates in the Brookhaven Data File (14) and displayed using the PROPHET system (Fig. 2). In a typical calculation a table of all interproton distances was compiled for these residues, and the set of 16 protons closest to the perturbed protons were selected for computation of the effects. The protons selected

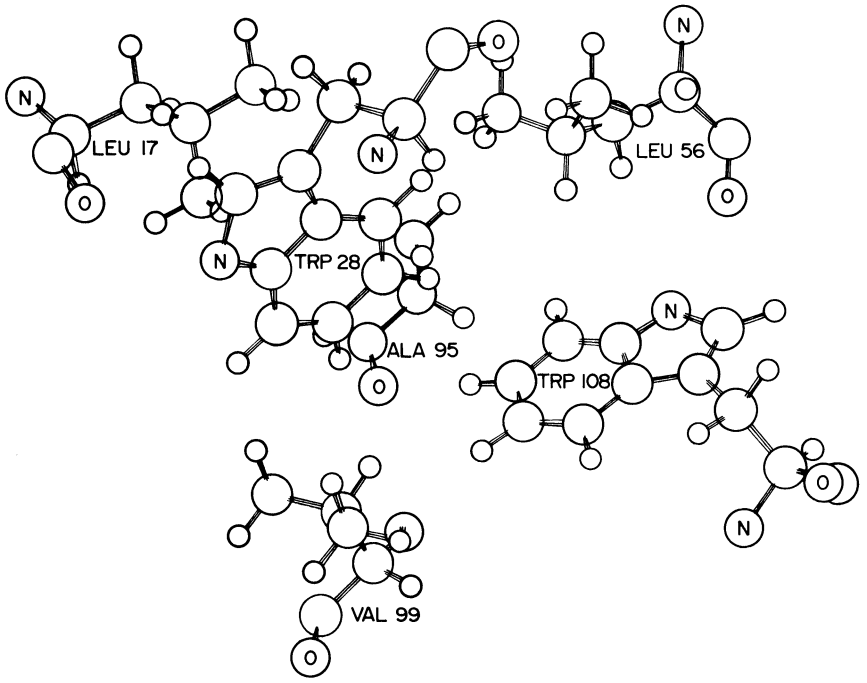

Fig. 2. View of amino acid residues in the vicinity of TRP 28 and TRP 108 of lysozyme, as they occur in the structure obtained by X-ray diffraction.

were: H4, H5 and H6 of TRP 108, a 2δ protons of leu 56, 3γ protons of val 99, β val 99, one β ala 95, H4, H5, H6 and H7 of TRP 28, and the α and one β proton of TRP 28. The calculated nuclear Overhauser effects as a function of time following inversion of H5 TRP 28, H5 TRP 108 and H4 TRP 28 were obtained using the computer program ZONLY (4). Similar calculations were performed for other sets of nuclei with various initially inverted nuclei.

The theoretical curves for H4 TRP 28 and H5 TRP 108 are shown in Fig. 3, following initial inversion of H5 TRP 28. Also shown in this figure are the experimental data points, labeled to correspond with the curves. The points for H4 TRP 28 agree approximately with the predicted curves. In other cases observed in this work, where the inverted and observed protons are on the same ring, and thus held rigidly at a constant distance from each other, it was generally found that the experimental data points and theoretical curve agreed satisfactorily at short times. A difficulty with the present calculation is that the restricted set of protons chosen limits the spin-diffusion to within its "box", so that the effects at long time, (>200 ms) which would be dissipated into the rest of the molecule, are too large.

In cases where the irradiated and observed protons are on separate residues, the agreement is not as good. In some cases the observed effect is greater than predicted. An example of this is also shown in Fig. 3. The observed effects on H5 TRP 108 are well above the predicted curve. Examination of the structure of lysozyme suggests, however, that a closer approach of TRP 28 and TRP 108 would be readily accomplished without strains; since the magnitude of the Overhauser effect is influenced in first order by r_{ij}^{-6}, such a deformation would greatly increase the observed effect without changing $\langle r_{ij} \rangle$. One would also expect to see a larger effect in the reverse direction, and Fig. 4 shows the theoretical curves and data points for the effect on H4 TRP 28 following inversion of H5 TRP 108. The effect here is also larger than predicted on the basis of rigid structure, consistent with the suggested motion.

In all cases the rate of recovery of the initially inverted spin agrees well with that calculated, although the agreement might be improved by the use of a slightly longer correlation time.

Freely rotating methyl groups in the protein are known to act as sinks for the excitation, draining excitation rapidly away from protons close by them (Kalk and Berendsen, (15)). Thus the valine 99 protons would be expected to diminish the effects on H7 TRP 28, by draining excitation from it rapidly. In fact such effects are less than predicted, both in this work and that of Poulsen, et al. Calculations on several model spin systems confirm this effect.

We conclude that the observation of the time dependence of the transient Overhauser effects in these systems in conjunction with the structure determined by X-rays has potential for giving information which is quantitatively useful in confirming sets of assignments of

signals from protons which are substituted on rigid frameworks, such as tryptophan or tyrosine rings; deviations from predictions may provide indications of deformations of the structure from that in the crystal as determined by X-ray diffraction; finally diminished effects could possibly indicate the proximity of methyl groups acting as excitation sinks.

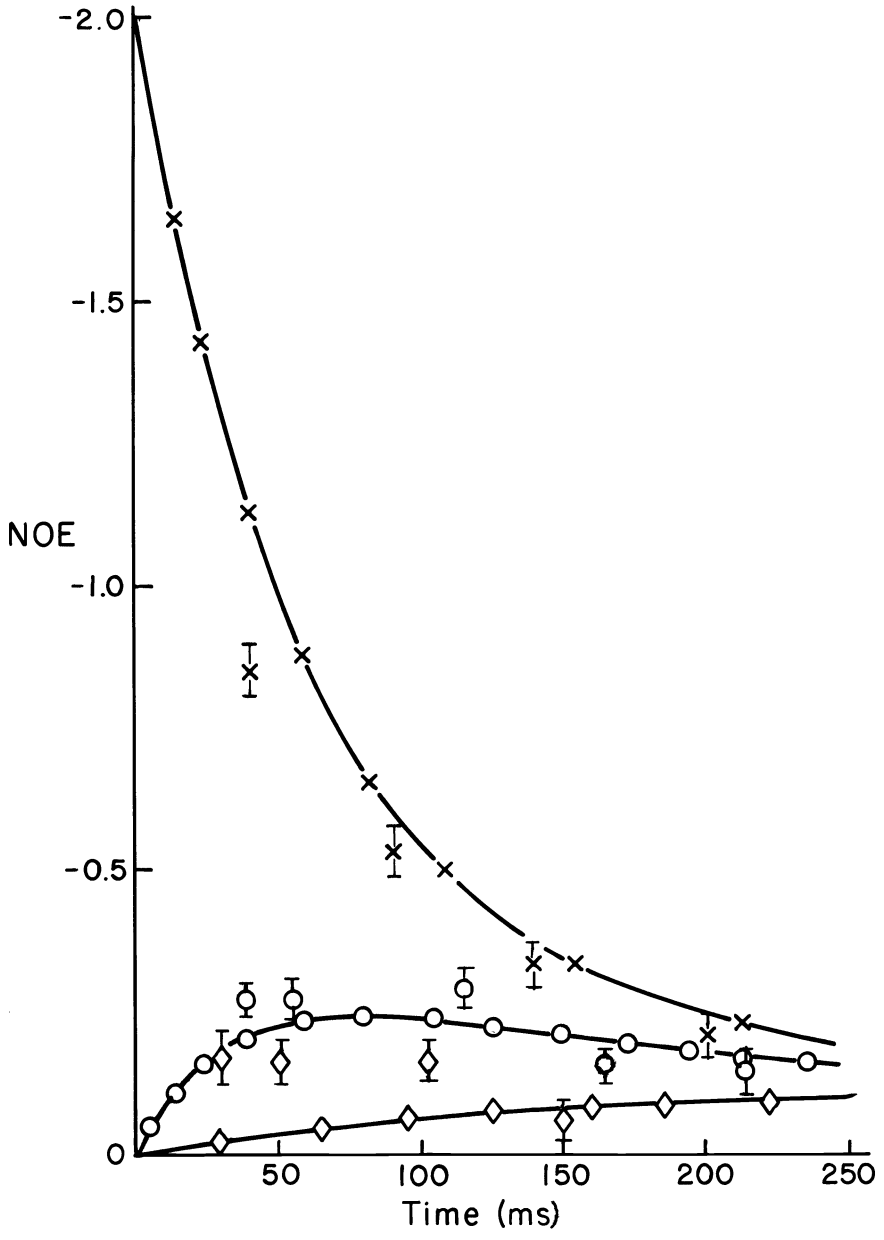

Fig. 3. Calculated and observed Overhauser effects on selected protons following inversion of H5 TRP 28. The predicted effect and the observed effect at different times are labeled as follows: X = H5 TRP 28 (the inverted spin); O = H4 TRP 28 ; ◊ = H5 TRP 108 .

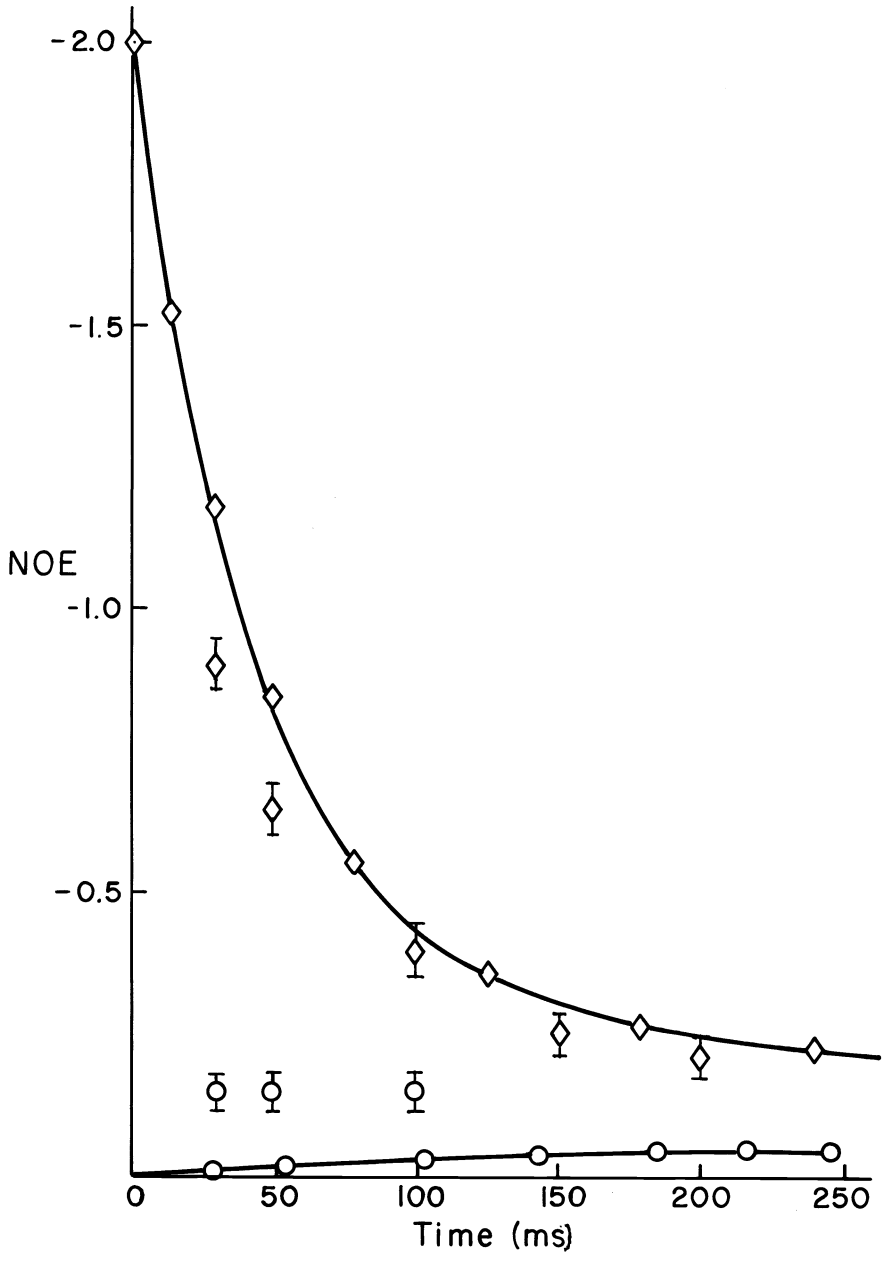

Fig. 4. Calculated and observed Overhauser effect following inversion of H5 TRP 108. The calculated curves and observed effects are labeled as follows: ◊ = H5 TRP 108 ; o = H4 TRP 28.

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