Intramolecular Nuclear Overhauser Effects in Proton Magnetic Resonance Spectra of Proteins

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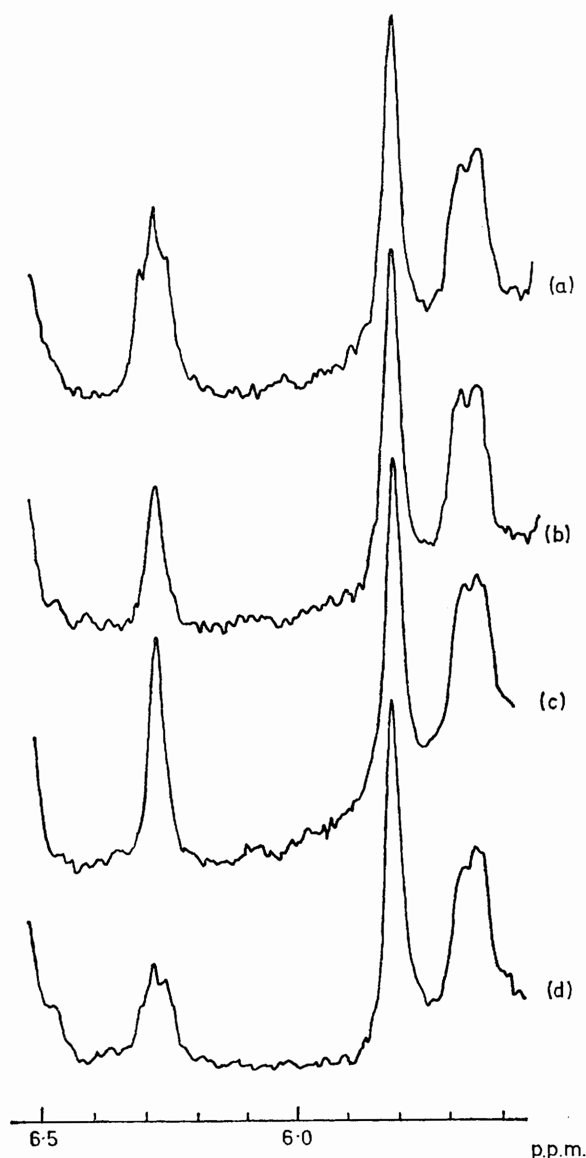
Summary The observed negative nuclear Overhauser enhancements in the ^{1}H n.m.r. spectra of lysozyme are used to obtain information about molecular motion which is compared to similar information obtained from measurements of spin-lattice relaxation times.

The nuclear Overhauser effect, which arises from selective saturation of transitions in a spin system coupled through dipolar relaxation mechanisms has been used extensively in the study of small molecules by ^{1}H n.m.r.[1,2] For a homonuclear double-resonance experiment, the signal can be enhanced by as much as 50%. However, Balaram *et al.*[3] observed, on irradiating some resonances of a large molecule, a reduction in the signal from a small molecule in rapid exchange between free solution and the large molecule. The signal from protein bound ^{19}F has also been observed to disappear on irradiating specific ^{1}H resonances[4] *i.e.* the "enhancement" approaches −100% in this system.

In the course of double-resonance experiments on the spectrum of hen-eggwhite lysozyme[5,6] we have observed that the irradiation of certain aromatic protons causes a substantial decrease in the area of the resonances of other aromatic protons. In this communication we report an analysis of this effect for one of several coupled systems which we have studied in the lysozyme spectrum.

Figure (a) shows a small part of the spectrum of lysozyme. The resonance at 6·28 p.p.m. is a triplet (clearly observed using resolution enhancement techniques[7]) and has an area corresponding to one proton. It is shifted by ring current effects outside the main envelope of aromatic proton resonances and is thus easy to observe. The 6·28 p.p.m. triplet can be shown to be coupled to a doublet at 6·76 p.p.m. and a triplet at 6·82 p.p.m.[6] The triplet at 6·82 p.p.m. is also coupled to a doublet at 7·76 p.p.m. These decoupling experiments confirm that these resonances are from the benzenoid ring of a tryptophan residue. Since the two resonances coupled to the observed peak at 6·28 p.p.m. are close together, irradiation at 6·8 p.p.m. can be used to saturate these two resonances and to decouple totally the triplet resonance [Figure (b)]. The area of the triplet



(a) A small part of the 270 MHz Fourier transform spectrum of 10 mM hen lysozyme in D_2O, pH = 4·0 at 68 °C, (b) effect of 'continuous' irradiation at 6·8 p.p.m., (c) resonance at 6·28 p.p.m. decoupled, without Overhauser effect, (d) with Overhauser effect, without decoupling.

resonance is reduced by 45 ± 10% in this experiment, whilst other nearby resonances are unaffected. Irradiation at positions other than 6·8 p.p.m. causes no decoupling or area change at 6·28 p.p.m.

The decoupling and Overhauser effects can be separated using the gated decoupling methods described for coupling between ^{1}H and ^{13}C nuclei.[8,9] The only difference in a homonuclear experiment is that the decoupling power, switched on during data accumulation, is applied using a time-shared method.[10] Figure (c) shows the spectrum decoupled but without an Overhauser effect. The area of the 6·28 p.p.m. resonance is the same in Figures (a) and (c) within experimental error (±10%). A spectrum showing the Overhauser effect, without decoupling is shown in Figure (d). The area of the 6·28 p.p.m. resonance in Figure (d) is 55 ± 10% less than that in Figure (a).

Using the formula relating the enhancement and the molecular correlation time, τ_c, presented by Balaram *et al.*[2] an enhancement of $-50 \pm 10\%$ at 270 MHz corresponds to a value of $1·5 \pm 0·3 \times 10^{-9}$ s for τ_c. This value is a lower limit since relaxation from sources other than the irradiated transitions are neglected in this treatment.

We have also used a 180°–τ–90° pulse sequence[11] to measure the spin-lattice relaxation times (T_1) of the lysozyme protons at two frequencies. At 270 MHz, under the above conditions, $T_1 = 1·2 \pm 0·1$ s and at 90 MHz, $T_1 = 0·26 \pm 0·05$ s, for the proton at 6·28 p.p.m. This T_1 ratio[12] gives a value for τ_c of $1·1 \pm 0·4 \times 10^{-9}$ s. The agreement between this value and that calculated from the Overhauser enhancement implies that the relaxation of the proton at 6·28 p.p.m. is totally dominated by the two protons which resonate around 6·8 p.p.m. If the hydrodynamic radius of lysozyme is assumed to be 14 Å and if the viscosity at 68 °C is 0·5 centipoise, the value for τ_c obtained using the Stokes equation is $1·2 \times 10^{-9}$ s, which agrees very well with the above experimental values. Assuming the usual temperature dependence of viscosity and correlation times the value of τ_c at 25 °C is expected to be about $2·8 \times 10^{-9}$ s from these results.

We have also decoupled the resonances of the methyl groups of lysozyme but no Overhauser effects have been detected. This is expected since the relaxation of each proton in a methyl group is dominated by its two nearest neighbours.

The observation of the large negative enhancement parameters may be particularly valuable when macromolecules larger than lysozyme are studied. In such systems the spin-coupling is unlikely to be observable and the Overhauser effects would then have to be used to assist assignment. As we have shown previously, resonances affected by applied irradiation can be readily identified using difference spectroscopy.[5,13]

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[1] J. H. Noggle and R. E. Schirmer, 'The Nuclear Overhauser Effect,' Academic Press, New York, 1971.
[2] I. D. Campbell and R. Freeman, *J. Magnetic Resonance*, 1973, **11**, 143.
[3] P. Balaram, A. A. Bothner-By, and J. Dadok, *J. Amer. Chem. Soc.*, 1972, **94**, 4015.
[4] B. D. Sykes, H. I. Weingarten, and M. J. Schlesinger, *Proc. Nat. Acad. Sci. U.S.A.*, 1974, **71**, 469.
[5] I. D. Campbell, C. M. Dobson, R. J. P. Williams, and A. V. Xavier, *Ann. N.Y. Acad. Sci.*, 1973, **222**, 163.
[6] I. D. Campbell, C. M. Dobson, and R. J. P. Williams, *Proc. Roy. Soc.*, in the press.
[7] I. D. Campbell, C. M. Dobson, and R. J. P. Williams, *J. Magnetic Resonance*, 1973, **11**, 172.
[8] R. Freeman and H. D. W. Hill, *J. Magnetic Resonance*, 1971, **5**, 278.
[9] R. Freeman, H. D. W. Hill, and R. Kaptein, *J. Magnetic Resonance*, 1972, **7**, 327.
[10] J. P. Jesson, P. Meakin, and G. Kneissel, *J. Amer. Chem. Soc.*, 1973, **95**, 618.
[11] R. L. Vold, J. S. Waugh, M. P. Klein, and D. E. Phelps, *J. Chem. Phys.*, 1968, **48**, 3831.
[12] H. B. Coates, K. A. McLauchlan, I. D. Campbell, and C. E. McColl, *Biochem. Biophys. Acta*, 1973, **310**, 1.
[13] C. D. Barry, C. M. Dobson, L. O. Ford, D. A. Sweigart, and R. J. P. Williams in 'Nuclear Magnetic Resonance Shift Reagents,' ed. R. E. Sievers, Academic Press, New York, 1973, 173.