# A Nuclear Magnetic Resonance Study of Hapten-Antibody Interaction

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SUMMARY

In the presence of purified specific antigalactosyl antibody, the hapten *p*-nitrophenyl-β-D-galactoside showed changes in its nuclear magnetic resonance (NMR) spectrum for those protons attached to the aromatic ring. These changes were interpreted as line broadening and thus indicated the most energetic site of attachment of the hapten to the antibody.

The initial observation by Jardetzky (1) of changes in the NMR spectrum of diphosphopyridine nucleotide upon binding to yeast alcohol dehydrogenase has now been extended to other binding phenomena (2–4). This note further indicates (see 4) that NMR can serve as an adjunct in the study of hapten-antibody interaction. The system studied was the binding of *p*-nitrophenyl-β-D-galactoside to antigalactosyl antibody.

The hapten, as its tetraacetate, was synthesized by the method of Latham *et al.* (5) and deacetylated with ammonia in anhydrous methanol. Antigalactosyl antibody was purified from rabbit antisera by the method of Mage *et al.* (6). A purified rabbit γ-globulin fraction (7, 8) contained antibody directed against bovine serum albumin. NMR spectra were obtained on a Varian A-60 spectrometer whose output was coupled to a Varian C-1024 time average computer (CAT). The sweep of the CAT was triggered by a reference capillary in the sample tube and the width of the spectrum corresponds to 400 cps. Each component was exchanged 3 times in 99.9% $D_2O$ prior to mixing. All spectra were obtained in deuterated 0.01 M sodium phosphate, 0.15 M NaCl, pH 7.4 buffer, (pD not recorded). Temperature control (4°) was maintained by the Varian V-6057 variable temperature probe.

Figure 1A is an NMR spectrum of the hapten ($3.2 \times 10^{-3}$ M) alone. Peak assignments agree well with those recorded for the glycosidic structure (9). Figure 1B is the spectrum of the hapten ($3.2 \times 10^{-3}$ M) in the presence of the purified γ-globulin fraction ($1.25 \times 10^{-4}$ M). Figure 1C is the spectrum of the hapten and antigalactosyl antibody at concentrations identical to Fig. 1B. One notes that in the presence of specific antibody there is a loss of height intensity of the aromatic protons of the hapten without significant changes for those protons attached to the hexopyranoside ring. The anomeric proton is unfortunately buried in the spinning $H_2O$ side bands. This change is consistent with, although not definitive evidence for, selective line broadening as a result of changes in relaxation rates due to restricted mobility of those protons most firmly bound. Confirmatory data are provided by binding experiments to be reported elsewhere, where ~65% of the binding energy of the hapten to the antibody is contributed by the nitrophenyl group. Stoichiometrically less than 5% of

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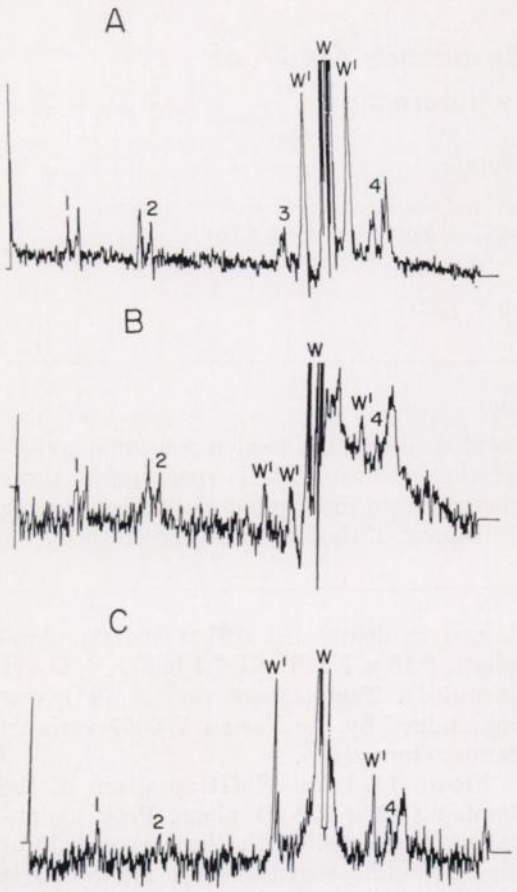


FIG. 1. *NMR spectra of hapten under varying experimental conditions*

See text. Following are the peak assignments: *1* and *2*, protons of nitrophenyl ring; *3*, anomeric proton; *W*, residual $H_2O$ peak; $W^1$, spinning side bands of $H_2O$; *4*, protons other than anomeric proton attached to carbon atoms of hexopyranoside ring structure; two separate groups of peaks are represented, and these are not amenable to simple first-order interpretation.

the hapten is in the bound form, pointing out that changes in line width can occur even when most of the ligand is in the free form. A kinetic analysis of this state has been given by Fischer (10) and requires rapid exchange between the free and bound forms. As a consequence, therefore, the binding site must be readily accessible to solvent and hapten.

What has been interpreted as a preferential stabilization of the aglycon moiety of the hapten, requires a more stringent confirmation by measurement of relaxation rates for the different peaks in the presence and absence of specific antibody. This and other experiments are to be undertaken. The effect, however, appears to be a specific binding effect since it is not observed in the presence of pooled γ-globulin.

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