Decoupling Proton Magnetic Resonance Signals Located Under the H_2O Peak

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Summary A series of successful decoupling experiments on $C^{\alpha}H$ protons under the H_2O peak in aqueous solutions of oligopeptides is described.

IN order to use the information available in an n.m.r. spectrum ($J_{NH-C^{\alpha}H}$ and $d\delta/dT$) to obtain the three-dimensional structure of an oligopeptide, it is necessary to assign each backbone proton peak to a particular amino-acid in the molecule. This is generally done by establishing the spin-decoupling relationships from the NH to $C^{\alpha}H$ peaks and from the $C^{\alpha}H$ to $C^{\beta}H$ peaks, and then using the chemical shifts and splitting patterns of the $C^{\beta}H$ protons to assign the decoupling-related series of peaks to each specific amino-acid in the molecule. Most of the ^1H n.m.r. studies of biologically interesting oligopeptides have been carried out on materials dissolved in organic solvents since previous attempts to decouple the $C^{\alpha}H$ protons from either the NH or $C^{\beta}H$ protons were unsuccessful because the $C^{\alpha}H$ peak is masked by a large peak of H_2O.

The main problem in a decoupling experiment in any water solution is the strong H_2O signal entering the n.m.r. spectrometer each time when the second radio frequency (R.F.) field H_2 irradiates a line close to the resonance of the water protons. The strong signal from H_2O will usually saturate one or more amplifier stages in the lock or signal channel (or in both channels) before it is removed by filter action of the selective circuits in the amplifiers. A saturated amplifier stage will prevent the proper functioning of the appropriate channel, and we either lose the internal lock or obtain distorted spectra or both. The most probable

amplifier stages to be overloaded are the last audio-frequency (A.F.) stages and the A.F. synchronous detectors. A successful experiment can be performed if we are able to prevent the saturation of any stage in the spectrometer by properly adjusting the amplification along the path of the signals. Practically, this means that the R.F. and A.F. amplification must be decreased, and the direct current (D.C.) amplification must be increased in both channels in such a way that the overall signal-to-noise ratio is not substantially affected.

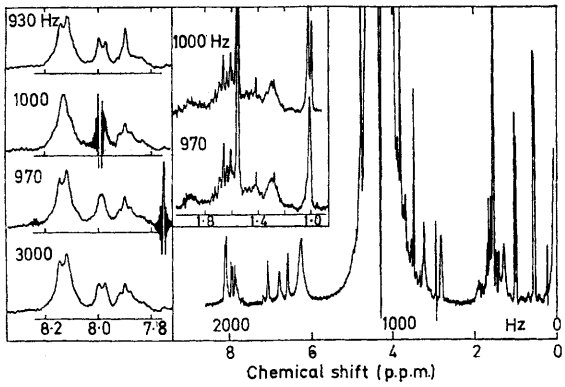

FIGURE. *The 250 MHz ^1H n.m.r. spectrum of the acetate salt of H-Gly-Val-Arg-Gly-Pro-Ala-NH_2 in H_2O at pH 5·3 and 30 °C. The inset shows the spectra observed when irradiating at the frequency shown by each trace.*

In the course of this work we noted that, under certain circumstances, the success of this decoupling experiment is much more difficult to achieve than under others. If the $C^{\alpha}H$ proton is located under the H_2O peak but not at the H_2O resonance frequency, then the H_2O signal is of much larger magnitude than if the $C^{\alpha}H$ being irradiated is either not located under the H_2O peak or is located at the resonance frequency of H_2O. The explanation of this behaviour is as follows. If H_2 is applied at the exact H_2O resonance frequency, then the H_2O transition is saturated, and practically no H_2O signal enters the spectrometer. As we move away from the resonance frequency (toward either higher or lower frequencies) the H_2O signal increases and reaches a maximum on either side of the H_2O resonance frequency; the decoupling experiment is most difficult to perform in the region of these two maxima. As we move even further away from the resonance frequency (toward the tails of the peak where the H_2O transition is not excited), the H_2O signal decreases. The location of the maxima depends on the magnitude of H_2, being close to the resonance frequency of H_2O at low H_2 and moving away from the resonance frequency (toward either higher or lower frequencies) as H_2 increases.[1]

Using oxidized glutathione (**1**) in water, we studied two

γ-Glu–Cys–Gly
|
γ-Glu–Cys–Gly
(**1**)

situations in which the $C^{\alpha}H$ proton can be decoupled from the corresponding NH proton with relative ease: (1) when the $C^{\alpha}H$ peak is almost out from under the H_2O peak (4·36 p.p.m.), as in the case of the $C^{\alpha}H_2$ peak (3·58 p.p.m. or 895 Hz) of glycine which is coupled to an NH peak at 8·12 p.p.m. and (2) when the $C^{\alpha}H$ peak is at the same frequency as the H_2O peak, as in the case of the $C^{\alpha}H$ peak (4·38 p.p.m. or 1095 Hz) of cystine which is coupled to an NH peak at 8·16 p.p.m.

When the $C^{\alpha}H$ peak occurs at intermediate frequencies, which are neither exactly at the resonance frequency of the H_2O peak nor sufficiently removed from the resonance frequency that the H_2O peak has no amplitude, the decoupling experiment is more difficult to perform. Nevertheless, it is still possible to carry out the experiment successfully, (Figure), in which irradiation at 3·72 p.p.m., 930 Hz ($C^{\alpha}H_2$) decouples the glycine NH triplet at 7·90 p.p.m.; irradiation at 3·88 p.p.m., 970 Hz ($C^{\alpha}H$) decouples the three-proton doublet at 1·01 p.p.m. ($C^{\beta}H_3$ of Ala) and the doublet at 7·99 p.p.m. (NH of Ala); irradiation at 4·00 p.p.m., 1000 Hz ($C^{\alpha}H$) decouples peaks at 1·50 p.p.m. ($C^{\beta}H_2$ of Arg) and the doublet at 8·13 p.p.m. (NH of Arg).

It is no longer necessary to use an organic solvent to obtain structural information on oligopeptides. It is now possible to establish the NH–$C^{\alpha}H$–$C^{\beta}H$ coupling relations and carry out the concomitant peak assignments in the biologically interesting solvent, water.

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[1] A. Abragam, "The Principles of Nuclear Magnetism," Oxford Univ. Press, 1961, p. 47.