Spin Echo Double Resonance: a Novel Method for Detecting Decoupling in Fourier Transform Nuclear Magnetic Resonance

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Summary It is shown that the combination of double irradiation and a simple two-pulse spin echo sequence is a powerful and sensitive method for detecting homonuclear decoupling in a complex spectrum.

SEVERAL methods have recently been used to detect coupled resonances in Fourier transform n.m.r. For example, Feeney and Partington[1] continuously irradiated one transition in a coupled spectrum and observed the resulting changes by difference spectroscopy. Pachler and Wessels[2] showed that 'selective population inversion' (SPI) was a better means of achieving the same result.

It is often difficult or impossible, however, selectively to irradiate a single component of a multiplet, *e.g.* in a spectrum of a very large molecule when the spin–spin coupling constant (J) is of the same order as the linewidth. If the SPI method is applied to both A transitions in an AX system, for example, the X resonances are not significantly perturbed (although cross relaxation effects may be observed at X some time after the inversion pulse[3]). One way of getting round this difficulty is to apply enough power completely to decouple X from A during data acquisition, and we have successfully used this method, together with difference spectroscopy, in a wide variety of applications.[4,5] The method is not ideal, however, since the irradiation during acquisition causes a reduction in signal to noise ratio and Bloch Siegert[6] shifts make difference spectroscopy difficult to interpret near the irradiation position (see Figure e). Also in situations where the linewidths are of the same order as J, the difference between, say, a triplet and a doublet produced by irradiation may be only a few percent of the original resonance. This can be a serious problem in macromolecules where the signals are often weak. The method described here overcomes these difficulties.

Carr–Purcell spin echo sequences have recently been applied to Fourier transform n.m.r.[7] We consider here only the simple 90°–τ–180° sequence which refocusses the phase losses due to field inhomogeneities, forming an echo at time 2τ after the 90° pulse, when data acquisition is started. The 90° and 180° pulses applied here were non-selective; thus all coupled spins are equally influenced. This results in the dephasing due to J not being refocussed, but this phase loss follows a precise pattern; for example, a first-order doublet resonance is observed to be inverted at a time $1/J$. Singlets and the central component of a triplet do not change phase but the outer components of a triplet are inverted at time $1/2J$ and so on. This is illustrated in the Figure where the spectrum of the aromatic region of tryptophan is shown under various conditions. In (b) the doublets are inverted but the triplets have approximately regained their correct phase at 2τ 118 ms (J *ca.* 8·5 Hz).

This dependence of the phase on multiplicity is the key to the method used. If selective irradiation is applied during the period between the 90° pulse and the start of data acquisition, the multiplicity and thus the phase of the coupled resonances can be changed. The observed multiplicity is the same as the original since the irradiation is not applied during data acquisition. Figure (c) illustrates how irradiation of the low field doublet causes inversion of the phase of the high-field triplet, since this triplet was a doublet during the irradiation period. The difference spectrum (d) contains a *positive* peak with an area twice that of the original triplet. If a doublet is collapsed to a singlet during the pre-acquisition period the resulting difference spectrum is a *negative* peak at the doublet. The method is therefore also sensitive to the nature of the multiplet.

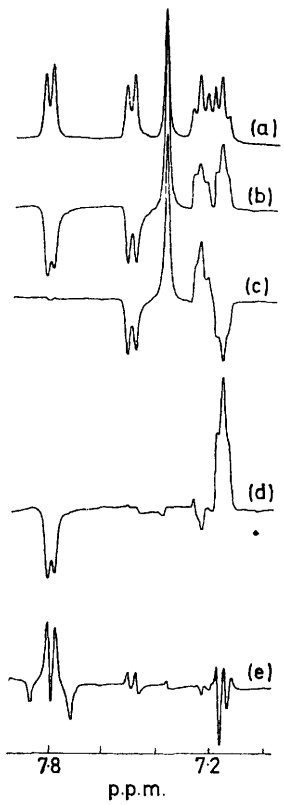

FIGURE. 64 transient 270 MHz spectra of 20mM L-tryptophan plus 1 mM $PrCl_3$ in D_2O. (a) Normal Fourier transform spectrum; (b) spectrum obtained by applying a 90° pulse followed by a delay of 59 ms, a 180° pulse, and then a further 59 ms delay; (c) as in (b) but with selective irradiation applied at the frequency of the low-field doublet during the 118 ms pre-acquisition period; (d) the difference between (b) and (c); (e) the difference between spectrum (a) and a spectrum accumulated while irradiating the low field doublet as described previously.[5]

We have already found this method to be very useful and easy to apply. In proteins, for example, the coupling constants for aromatic groups are all around 8·5 Hz while those for methyl groups are around 6·5 Hz. It is thus easy to set τ to give the most sensitive $1/2J$ value, although this setting is not critical. There is a loss of signal, of course, in the pre-acquisition period due to T_2 decay but the large areas observed in the difference spectra, and the absence of irradiation during data acquisition, more than compensate for this loss.

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