Broadband Proton-decoupled Proton Spectroscopy of Cancer Cell Extract

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Introduction

Even at high fields, high-resolution ^{1}H spectra of metabolic extracts suffer from a large degree of signal overlap due to the limited proton chemical shift dispersion and the broadening effect of *J*-splittings in proton multiplets. It has long been recognised that a broadband proton-decoupled proton spectrum could go a long way to alleviating this crowding, but it is important that any such method retain correct peak integrals for quantitative analysis. A method has recently been developed which allows the acquisition of such a spectrum, resulting in peaks with absorption-mode lineshape and quantitative integrals[1]. In this abstract, we demonstrate the application of the method to a sample of KG1a (AML cancer model) cell extract.

Theory

The anti *z*-COSY spectrum[2] gives reduced diagonal-peak multiplets that contain the same number of peaks as the coupled one-dimensional spectrum (Fig. 1a). These peaks are arranged along the anti-diagonal of the multiplet, at 90° to the main diagonal. Shearing the two-dimensional spectrum by 45° gives a spectrum that is reminiscent of the *J*-spectrum, but with absorption-mode lineshapes (Fig. 1b). A second shear can be applied so that the diagonal-peak multiplets are symmetrically disposed about the ω_2-axis; coupling information is now exclusively displayed along the ω_1-axis, with chemical shift information isolated along the ω_2 axis (Fig. 1c). A projection of the two dimensional spectrum onto the ω_2-axis therefore gives the chemical-shift spectrum, devoid of coupling information.

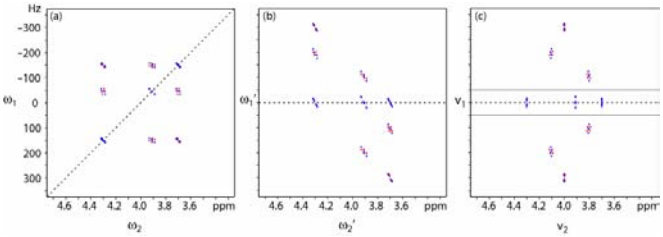

Figure 1 Shear operations on the anti z-COSY spectrum (a). The first shear (b) maps the main diagonal onto the ω_1 axis. The second shear (c) arranges the diagonal-peak multiplets symmetrically about the ω_1 axis.

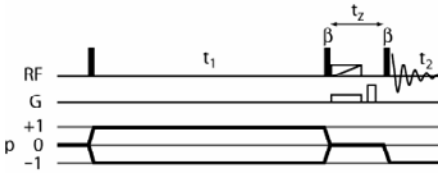

Figure 2 Pulse sequence of the anti *z*-COSY experiment. b is a small flip angle.

Diagonal-peak multiplets must be removed from the spectrum so that they do not contribute to the projection spectrum; this can be achieved in one of two ways: experimentally by co-adding a series of experiments with different timings; or in post-processing by symmetrization or only projecting over a limited range of ω_1 frequencies.

The anti *z*-COSY spectrum is acquired with reduced ω_1 spectral width to maximize the resolution in ω_1 (since ω_1 and ω_2 are subsequently mixed by the shear operations). The resulting spectrum has a diagonal which is folded multiple times, but this wrapping is undone by the first 45° shear.

Material and Methods

The anti *z*-COSY spectrum with reduced ω_1 spectral width of KG1a (AML cancer model) cell extract (in D2O) was recorded, with presaturation of the residual water signal. As sensitivity was at a premium, the flip angle of the mixing pulses was set to 20°; the data were acquired with 80 scans per increment. The spectral widths in ω_1 and ω_2 are 100 Hz and 5000 Hz respectively; the acquisition time in t_2 was 0.8 s, and the number of increments in the indirect dimension was 40, giving a maximum value of t_1 of 0.4 s. The spectrum was processed with a decaying exponential weighting function with 1.3 Hz of line broadening in both dimensions, and was recorded using a cryo probe.

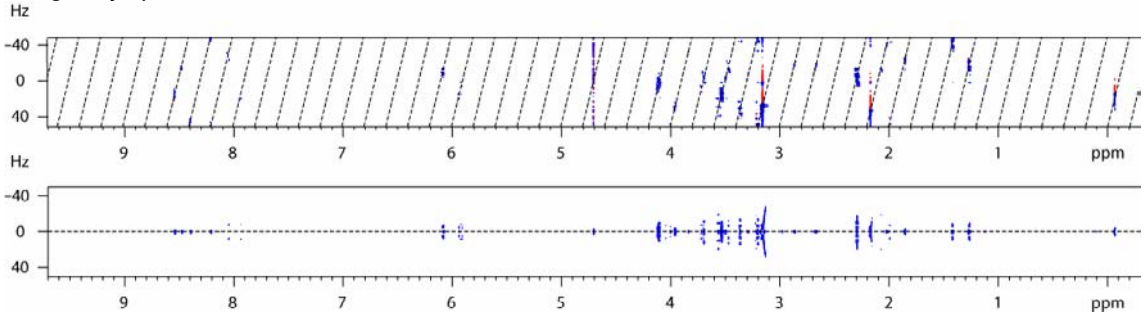

Figure 3 Anti z-COSY spectrum of cell extract. In the upper spectrum, the diagonal (shown as a dashed line) is wrapped multiple times because of the reduced w_1 spectral width. The double sheared spectrum is shown below.

Results

Figure 3 shows the folded anti z-COSY spectrum before (above) and after (below) shearing. The projection, shown in Fig. 4b, results in significant simplification compared to the coupled proton spectrum, shown in Fig. 4a.

Discussion

Simplification of crowded ^{1}H spectra of metabolite extracts should increase the amount of extractable information. The method demonstrated here allows that without overly complex data processing. The primary disadvantages of the method are the low sensitivity, due to the use of small flip-angle pulses, and the introduction of extra peaks to the spectrum due to strong coupling.

References

1. A.J. Pell, R.A.E. Edden and James Keeler, *Magn Reson Chem*, in press. 2. H. Oschkinat, A. Pastore, P. Pfändler and G. Bodenhausen, *J Magn Reson*, 1986, **69**, 559–566.

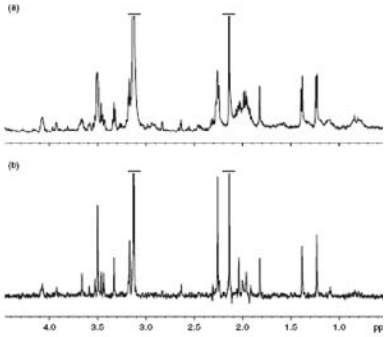

Figure 4 Comparison of the coupled ^{1}H spectrum (a) with the projection (b) of Fig. 3.