COVARIANCE DOSY NMR

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Introduction

Determining the composition of a chemical mixture, preferably without destroying the sample, is both challenging and essential to analytical chemistry in general and the emerging field of metabolomics in particular. Diffusion ordered spectroscopy (DOSY) is one of the most powerful methods available for achieving this goal. [1] In the DOSY experiment, the translational diffusion rate of mixture components are correlated with their NMR chemical shifts, allowing spectral “demixing” of the sample. Recently, covariance NMR processing has emerged as an alternative means for achieving spectral demixing of aqueous samples through singular value decomposition of homonuclear 2D NMR data. [2] The same pulse sequences which are amenable to covariance processing, such as 2D COSY and 2D TOCSY, are also well suited for use in pseudo-3D DOSY experiments, where it may be possible to combine the demixing properties of these two approaches to produce a unified protocol with improved performance.

Experimental

A mixture of 100 mM Valine, 100 mM Glutamate, 100 mM Lysine in 99.9% D$_2$O was prepared in a 5mm Wilmad NMR tube. Spectra were collected on the Varian 600 MHz spectrometer using pulse sequences developed by the authors by combining the Varian supplied DOSY (bppled) and 2D TOCSY (tntocsy) pulse sequences. Spectra were collected for eight diffusion gradient strengths spaced evenly from 2% to 95% of the maximum z-gradient power. Spectra were averaged over four TOCSY mixing times (45ms, 62ms, 76ms, 97ms) in order to achieve greater uniformity in crosspeak intensity.

Spectra for each mixing time were added and processed using the covariance method to produce eight 2D TOCSY data sets corresponding to the eight diffusion gradient strengths. Peaks were picked in nmrDraw and the peak heights recorded. Analysis of the diffusion dimension proceeded in two stages. First, non-linear least squares fitting of the intensity decays and estimated uncertainties was performed for a single exponential decay model, yielding a decay constant proportional to the diffusion coefficient of the molecule corresponding to each peak. Peaks with identical diffusion coefficient, within the fit error, were clustered to identify mixture components. Second, the peak intensity over the diffusion series was numerically integrated for each peak and the resulting diffusion weighted intensity entered into a row vector of dimension equal to the number of peaks in the spectrum. A covariance matrix was constructed from this data and diagonalized, yielding eigenvectors hypothesized to reveal the single component NMR spectra.

Results and Discussion

One of the most severe limitations the ability of both the DOSY and covariance processing methods to achieve spectral demixing is peak overlap in the NMR dimensions. Overcoming this problem was the original motivation for extending the DOSY method into a second NMR dimension in the DOSY-TOCSY or related experiments. For mixtures where overlap persists even in the higher dimension spectra, it is still not possible to achieve clean demixing. Likewise, peak overlap confounds the singular value decomposition method used to demix spectra with the covariance processing method. Although computer simulations suggested that the combined application of the two methods should yield resolution of modestly overlapped systems, this was not found to be the case experimentally.

Acknowledgements

This work was funded by the NIH (R.B.).

References