COMPARISON OF GRADIENT-ENHANCED HSQC VERSUS TROSY PULSE SEQUENCES FOR SOLUTION NMR CHARACTERIZATION OF HELICAL INTEGRAL MEMBRANE PROTEINS

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Introduction

The study of large biomacromolecules by solution NMR is complicated by the overlap of resonances within conventionally acquired spectra. Even small helical integral membrane proteins (IMPs) are difficult to study by solution NMR since they must be studied in either detergent micelles or lipid bicelles, either of which may add more than 50kDa to the weight of the protein. The large weight of the protein-membrane mimetic complex leads to fast relaxation times due to the long rotational correlation time τ_c. The spectral consequences are line broadening, reductions in spectral sensitivity and even the disappearance of resonances. Deuteration of helical IMPs can reduce transverse relaxation rates and increase spectral sensitivity. However, deuteration significantly reduces the amount of protein that can be produced. Transverse relaxation optimized spectroscopy (TROSY) can significantly extend the size of proteins that may be studied by solution NMR (1). Here we examine the effect of TROSY pulse sequences on the heteronuclear single quantum coherence (HSQC) spectra of helical IMPs.

Experimental

Two-dimensional HSQC spectra correlating amide protons and nitrogens were collected on a number of helical IMPs originating from *Mycobacterium tuberculosis*. Spectra for one such protein, kdpC (Rv1031) shown here in Fig. 1 were acquired as either HSQC or TROSY spectra. Datasets designated as HSQC were acquired utilizing a gradient-enhanced HSQC pulse sequence (2). TROSY spectra were acquired with a spin-state selective gradient-enhanced TROSY pulse sequence (3).

Results and Discussion

The results shown here in Fig. 1 for kdpC (Rv1031) illustrate that for helical IMPs there is relatively little difference between HSQC (blue) and TROSY (red) spectra. The differences in linewidths between the two spectra differ by less than 1 Hz in both the ^{1}H and ^{15}N dimensions. The exact reasoning for the lack of improvement with TROSY implementation is unclear at this point, yet it may be due to intrinsic dynamics present in membrane proteins that are not typically present in soluble proteins. In addition, TROSY spectra suffer from reduced sensitivity compared to HSQC spectra. However, while the improvement in spectra seen with the implementation of TROSY is limited, it is promising that high quality, high resolution spectra of helical IMPs may be obtained with both HSQC and TROSY pulse sequences.

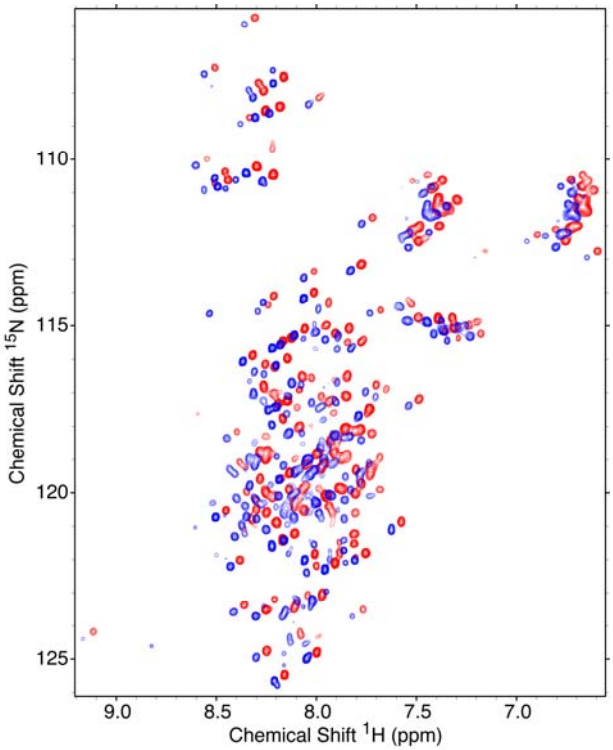

Fig. 1 – ^{1}H/^{15}N HSQC (blue) and TROSY (red) spectra of kdpC (Rv1031) in 20mM Sodium Acetate, pH 4, 50°C illustrate less than 1Hz difference between the two spectra.

References

[1] K. Pervushin, *et al*., Proceedings of the National Academy of Sciences, **94**, 12366-12371 (1997).
[2] L. Kay, *et al*., Journal of the American Chemical Society, **114**, 10663-10665 (1992).
[3] J. Weigelt, Journal of the American Chemical Society, **120**, 10778-10779 (1998).