

EFFECT OF SAMPLE PREPARATION AND PARAMAGNETIC RELAXATION ON Rv0008c – AN INTEGRAL MEMBRANE PROTEIN FROM *MYCOBACTERIUM TUBERCULOSIS*

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Introduction

Sample preparation methodology is a crucial factor for obtaining high quality membrane protein NMR spectra, several approaches such as reconstitution and detergent exchange are widely used to prepare for membrane protein NMR samples. We present here a comparison of these two methods and also show how paramagnetic Mn^{2+} ions affect Rv0008c, an integral membrane protein from *Mycobacterium tuberculosis*.

Experimental

Rv0008c was purified using reconstitution and detergent exchange sample preparation methods. In the reconstitution method, eluted protein was dialyzed against water to completely remove emipgen, the protein was then lyophilized and reconstituted in a desired detergent. In the detergent exchange method, protein was bound to a Ni^{2+} column and was washed and eluted in the desired detergent. The effect of paramagnetic Mn^{2+} ions on Rv0008c was evaluated through comparison of TROSY spectra of proteins with and without Mn^{2+} . All spectra were recorded on a Varian 720 MHz spectrometer.

Results and Discussion

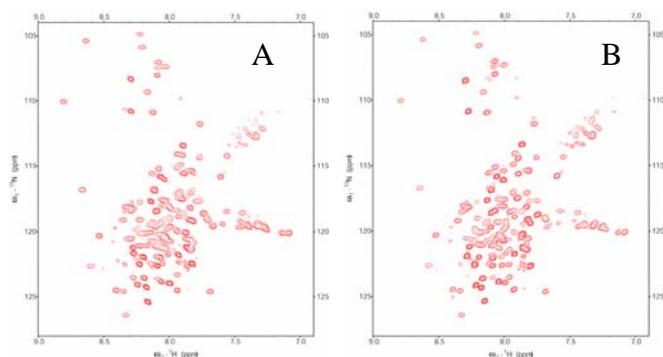


Figure 1. $^{15}N/^1H$ -TROSY spectra of Rv0008c protein obtained by (A) reconstitute and (B) detergent exchange sample preparation methods

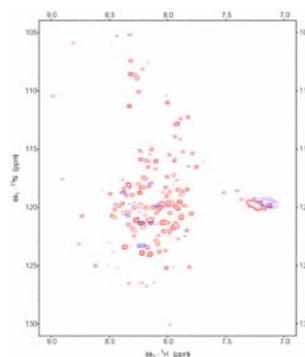


Figure 2. Superimposition of the $^{15}N/^1H$ -TROSY spectra of Rv0008c in the presence (blue) and in the absence of Mn^{2+} ion (red)

TROSY spectra obtained for Rv0008c via reconstitution and detergent exchange both showed the same well-folded homogenous samples (Figure 1A, B). The dispersion and number of observed peaks in two spectra are consistent indicating that these two sample preparation methods are capable of producing identical high quality samples.

In addition, in the presence of paramagnetic Mn^{2+} ions, most resonances were totally broadened out, leaving about 20 peaks with reasonable intensity in the spectra (Figure 2). This result indicates that Mn^{2+} bound to protein/detergent complex so that not only solvent-exposed but also some transmembrane residues were quenched through paramagnetic relaxation.

Acknowledgements

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