APPLICATION OF SITE DIRECTED NITROXIDE SPIN LABELS AND PARAMAGNETIC ION BINDING TAGS TO SOLUTION NMR PARAMAGNETIC RELAXATION ENHANCEMENT

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Introduction

Determining the three-dimensional structure of proteins via solution nuclear magnetic resonance (NMR) spectroscopy typically relies on the use of nuclear overhauser effect (NOE) data to obtain distance constraints. Although short to medium range NOEs can be collected on helical integral membrane proteins (IMPs), long range NOEs have proven elusive (1). Paramagnetic relaxation enhancement (PRE) offers the chance recover the distance constraints necessary for structure determination. Nitroxide spin labels and paramagnetic ions induce fast relaxation in neighboring nuclei through a distance dependent interaction. The distance between the paramagnetic group, either a nitroxide spin label or paramagnetic ion, can be calculated from the comparison of relative relaxation rates in the presence and absence of the paramagnetic group.

Experimental

Two approaches have been taken towards introducing paramagnetic groups into Rv1761c at unique sites. The first approach, the three residue amino terminal Cu²⁺(Ni²⁺)-binding peptide (ATCUN) motif (2) has been cloned into the helical IMP Rv1761c from Mycobacterium tuberculosis. Heteronuclear single quantum coherence (HSQC) spectra in the presence and absence of Cu²⁺ ions have been obtained and are being evaluated to obtain relaxation rates. The second approach utilizes site directed spin labeling. To this end unique cysteines have been introduced via site directed mutagenesis to create the mutants S10C, F30C and S48C. The nitroxide spin label methanethiosulfonate (MTSL) has been successfully coupled to each of these three unique cysteine mutations.

Results and Discussion

The ATCUN motif has been shown to be very promising as indicated by the data in Fig. 1 illustrating Rv1761c in the presence (green) and absence (red) of Cu(SO₄). The copper ions have been successfully bound and through the PRE effect have completely wiped out a number of resonances (indicated by asterisks in Fig. 1). There are a number of resonances with significantly enhanced relaxation rates, that while not completely absent from the spectrum with Cu²⁺ do exhibit significantly reduced intensities. It is likely that these resonances are located from 15 to 25Å away from the ATCUN motif.

The MTSL spin label has been successfully introduced into Rv1761c at S10C, F30C and S48C as indicated in Fig. 2 by electron paramagnetic resonance (EPR) experiments performed here at the NHMFL. Both the F30C and S48C indicated motion of the spin label on the order of 1ns while the S10C spin label data indicates motions on a 20ns timescale. These results are expected since both F30C and S48C are located outside the transmembrane (TM) region of the protein while S10C is located within the TM region. Experiments are underway to obtain PRE data with MTSL and evaluate enhanced relaxation rates.

References