Backbone Structure of a Helical Integral Membrane Protein in the Absence of NOEs

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Introduction

Conventional solution NMR structure determinations rely on distance restraints from the nuclear overhauser effect (NOE). Due to dynamics within membrane proteins, particularly those examined in detergent micelles, long range NOEs may not be observable. For example, no long range NOEs were observed for the protein Rv1761c from *M. tuberculosis*. To facilitate structure determination of membrane proteins, we provide here, as an example, the backbone structure determination of Rv1761c without NOE restraints.

Experimental

A total of 162 paramagnetic relaxation enhancement (PRE) derived distance restraints were used from MTSL spin labels F30C, S48C and S102C. Residual dipolar couplings (RDC) from two distinct alignments were used. The RDC datasets include 113 dipolar couplings from weak alignment in a compressed 5.5% neutral polyacrylamide gel (PAG) and 105 dipolar couplings from alignment in a compressed 5% negatively charged PAG. 210 dihedral angle restraints were predicted from assigned $^{15}$N, $^{13}$C$_{\alpha}$, $^{13}$C$_{\beta}$ and $^{13}$C' chemical shifts using TALOS. Utilizing a simulated annealing protocol in XPLOR-NIH a family of 10 lowest energy structures were determined to high resolution (Fig. 1). Analysis of the structure family indicates excellent agreement between observed restraints and calculated values for the F30C, S48C and S102C PRE data as well as the two independent alignment RDC data sets (Fig. 2).

Results and Discussion

The backbone structure of Rv1761c, using PRE distance, RDC orientation and dihedral angle restraints has been refined to 1.49Å resolution. The transmembrane helix domain was refined to 0.41Å while the extramembranous helical regions were refine to 0.56Å. With exception of the 21 residue loop region, the structure was well resolved. The results shown here demonstrate that structure determination of helical integral membrane proteins without the use of NOEs is possible and that high resolution backbone structures are achievable.

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