Towards Solid State NMR Structure-Activity Relationships for Large Membrane Proteins

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Introduction

Solid state NMR allows for the characterization of structure-activity relationships for membrane proteins in liquid crystalline lipid bilayers, in a nearly native environment. An important factor in the success of this technique is the production of full length, functional and highly pure protein samples. The structural characterization of large membrane proteins will require the use of amino acid specific isotope labeling schemes due to crowding in the 2D separated local field spectra observed for these samples.[1] Here we present the initial analysis of three membrane proteins from *Mycobacterium tuberculosis*.

Experimental

Proteins were expressed and purified as described,[2] with slight modifications. Expression vectors with either six or ten histidine purification tag were utilized. Expression and purification yields were determined using 12% SDS-PAGE and UV absorption spectrophotometry. Prediction of transmembrane alpha helices was performed using the program TMHMM.

Results and Discussion

Table 1 details the purification yields for the three proteins under study. Initial purification of the SugA/B proteins yielded high levels of impurities and low affinity for the nickel resin. To resolve this issue a 10-histidine affinity tag was employed and observed an increase in affinity of the protein as judged by increased elution and a marked decrease in impurities. The smaller protein, PgsA1, did not require the extra histidine residues for a highly pure yield.

Table 1: Purification data for three membrane proteins. Dashes indicate data was not available.

Protein	Molecular Weight (kDa)	# Transmembrane Alpha Helices	6x His Yield (mg)	10x His Yield (mg)
PgsA1	23.3	3	34	-
SugA	33.0	5	3	15
SugB	29.1	6	6	-

Sequence analysis of PgsA1 is shown in Fig. 1. Based on the hydrophobicity analysis the three proteins SugA, SugB and PgsA1 have five, six and three transmembrane alpha helices each. All hydrophobic residue types are observed in both the extramembranous and the transmembrane domains. However, a specific label such as phenylalanine would probe primarily helix #1, while isoleucine specific labeling would probe helices #2 and #3. A glutamate or an aspartate specific label could be used to observe only the extramembranous region of the protein. Interestingly all three helices each contain a cysteine residue and helix #1 contains a lysine residue, uncommon occurrences in alpha helical transmembrane domains. Similar strategies are devised for the SugA and SugB proteins based on a similar analysis.

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  1 - MSKLPFLSRA AFARITTPIA RGLLRVGLTP DVVTILGTTA SVAGALTLFP MGKLFAGACV
 61 - VWFFVLFDML DGAMARERGG GTRFGAVLDA TCDRISDGAV FCGLLWWIAF HMRDRPLVIA
121 - TLICLVTSQV ISYIKARAEA SGLRGDGGFI ERPERLIIVL TGAGVSDFPF VPWPPALSVG
181 - MWLLAVASVI TCVQRLHTVW TSPGAIDRMA IPGKGDR
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Figure 1: Sequence information for PgsA1. Predicted transmembrane alpha helices are highlighted in red.

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References

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