INDUCTION OF MEMBRANE PROTEIN TUBULAR STRUCTURES IN *ESHRISHA COLI*

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Introduction

Helical membrane tubules are a particularly desirable specimen for the determination of high resolution structures using X-ray crystallography, cryoelectron microscopy and NMR spectroscoppy. We previously reported about 20% of the tuberculosis membrane proteins were found in tubular structures when overexpressed in *E. coli*. Here we show that these proteins are at high concentration and purity. Furthermore, some membrane proteins can be induced into these lipid structures when fused with maltose binding protein.

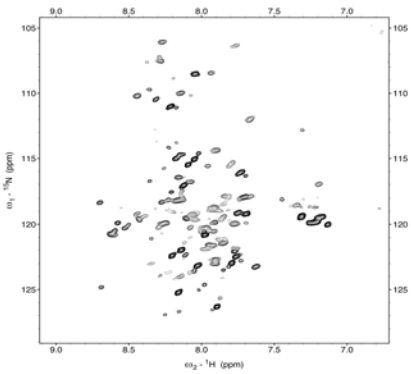

Fig. 1: HSQC spectrum of the *Mtb* protein, Rv2433c, obtained at 600 MHz from a 5% LPPG solubilized sample of proliferated tubule membranes at 50 ^{0}C.

Experimental

E. coli cells harboring membrane proteins from tuberculosis were grown in LB media at 37 ^{0}C. Protein was induced with IPTG for 3 hours. For isotope labeling, harvested cells from 4 liters of LB media were washed once in M9 media and then inoculated into 1 liter of M9 media. Tubules were collected in the soluble fraction using a sucrose density gradient and ultracentrifugation. A band of tubules was extracted from the 53% band on the gradient by means of a Pasteur pipet and dialyzed against 50mM Tris pH 7.5. Eight membrane protein constructs that were previously expressed as inclusion bodies were fused with maltose binding protein and induced at 30 ^{0}C for 6 hours. To remove proteins weakly associated with membrane, a denaturing solution containing 5 M urea, 50 mM Na_2CO_3, 25 mM EDTA, 25 mM DTT, pH 10 was used to wash the membrane.

Results and Discussion

The recovery rate of protein in tubules prepared using sucrose unltracentrifigation is <50%, but usually quite well purified from the native membranes. A solution NMR spectra of a detergent solubilized sample of tubules of Rv2433 from *M. tuberculosis* is shown in Fig. 1. While the spectrum is not ideal it is important to realize that this sample still contains the native *E. coli* lipids. The spectrum is virtually superimposable on the spectrum of a highly purified sample as previously published (Page et al., 2006). When fused with maltose binding protein, all eight constructs produced significant amounts of tubular structures in *E. coli*, ranging from 30% to 70% of the total protein expressed. Figure 2 shows the abundant population of tubes observed within the *E. coli* cell.

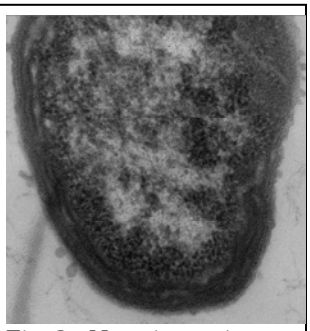

Fig. 2: Negative-stain electron microscopic cross section of CorA-MBP expressed in E. coli.

Conclusions

Our work indicated that membrane proteins can be overexpressed in tubular structures at high purity. Membrane protein in tubular form can be induced by fusion with maltose binding protein and expressed at low temperature. Not only can such tubular protein arrays lead more readily to 2D arrays for electron microscopy, but we anticipate that the high concentration of protein in a bilayer environment will facilitate solid state NMR sample preparation and protein purification for all of the technologies.

References

Page RC, Moore JD, Nguyen HB, Sharma M, Chase R, Gao FP, Mobley CK, Sanders CR, Ma L, Sonnichsen FD, Lee S, Howell SC, Opella SJ, Cross TA. J. Struct. Funct. Genomics. 2006 Mar; 7(1):51-64.

Acknowledgement
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