In Situ Study of *Mycobacterium tuberculosis* Membrane Proteins

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Introduction

Recently membrane-mimetic environments for native structure, dynamics, and function of membrane proteins have attracted attention. The newly developed "in-cell" NMR spectroscopy has shown the importance of studying proteins in living cells [1]. The MASssNMR data in the E. coli membranes show that analyzing a small recombinant transmembrane peptide [2] and full length transmembrane protein [3] is possible. Even some differences in the conformation of amino acid side chains were observed [1].

In cells the membranes normally constitute only a small fraction of the cell volume, and membrane proteins are typically expressed at low levels compared to soluble proteins. We developed a method that can increase the ratio of *Mycobacterium tuberculosis* membrane protein in E. coli membranes and improve sensitivity and resolution of the spectra by MASssNMR spectroscopy.

Experimental

Recombinant transmembrane proteins were expressed at low temperature with specific isotope amino acid labels. The membrane fraction was isolated by ultra-centrifugation. The sample was packed into a 3.2 mm MAS rotor. Solid-state MAS NMR spectra were acquired on a Bruker Avance 600.1 MHz NMR spectrometer using an NHMFL 3.2 mm Low-E triple-resonance biosolids MAS probe.

Results and Discussion

Shown in Figure 1 are spectra of the isotopically labeled proteins with no significant labeling of the other proteins in the E. coli membrane.

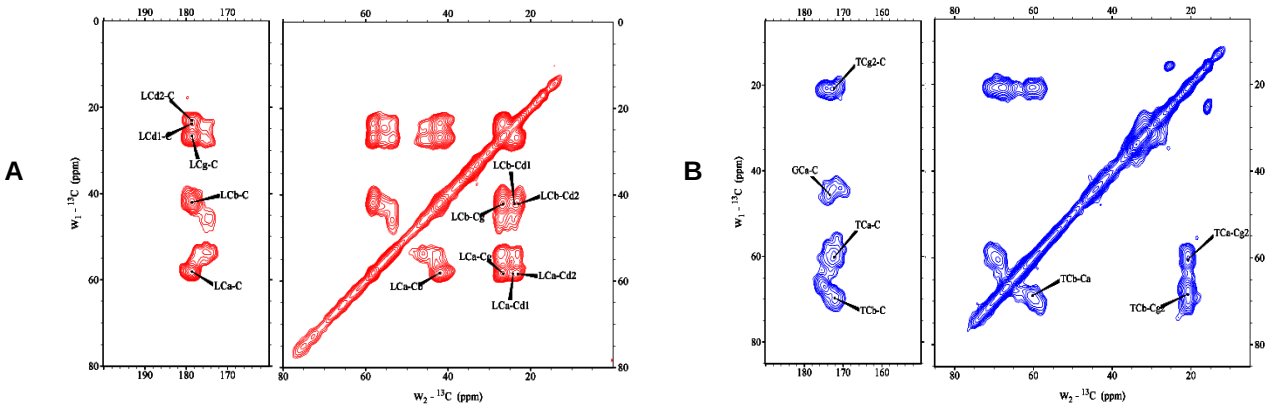

Figure 1. ^{13}C-^{13}C correlation spectra using 50 ms mixing time at 243K in *E. coli* membranes at pH 8.0. (A) Leucine [^{15}N, ^{13}C] labeled CrgA. (B) Threonine [^{15}N, ^{13}C] labeled LspA.

Conclusions

The result indicated that this method will make possible the study the structures of membrane proteins in their natural environments and as they are inserted by the native machinery without the tedious process of protein purification and reconstruction.

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References

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