PROBING THE INTERACTIONS OF MICELLAR ENVIRONMENTS WITH THE EXTRAMEMBRANOUS DOMAINS OF INTEGRAL MEMBRANE PROTEINS

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Introduction

Solution NMR spectroscopy can provide high resolution structural and dynamics information for integral membrane proteins (IMPs) provided that the IMPs are solubilized in a suitable membrane mimetic. IMPs are typically comprised of hydrophobic transmembrane (TM) domains that span the cell membrane, and domains outside the membrane (extramembranous domains) that perform a variety of functions including enzymatic processes. A desirable membrane mimetic would solubilize the IMP solely through interaction with the TM domain. Detergent micelles are currently the only available membrane mimetics suitable for studying IMPs via solution NMR. While detergent micelles are a good mimetic of lipid bilayers, unintended interactions of detergent molecules with the extramembranous (EM) domains of IMPs can cause problems. These interactions may result in small, localized changes within the EM, or may cause larger changes including structural rearrangements and unfolding. To avoid these undesirable interactions, we are approaching IMP structural characterization through a 'Divide-and-Conquer' approach in which TM domains are studied in dodecyl phosphocholine (DPC) detergent micelles and EM domains are studied separately in aqueous solution without detergent.

Results and Discussion

To probe the interactions of detergent micelles with an IMP EM domain, the protein KdpC was chosen (1). KdpC, the C subunit of the potassium transporting ATPase Kdp, contains a single TM helix and a 19 kDa EM domain ($KdpC^{EM}$). $^{1}H/^{15}N$-HSQC spectra of $KdpC^{EM}$ without detergent exhibit high quality spectra indicative of a well-folded α-helical protein. Upon addition of 150mM DPC, resonances in $^{1}H/^{15}N$-HSQC spectra of $KdpC^{EM}$ collapse to a width of approximately 1 ppm in the ^{1}H dimension. The shifting of resonances examined by titration with DPC reveal that spectral changes are complete upon reaching the critical micelle concentration of DPC (1.1mM). $^{1}H/^{15}N$-HSQC spectra of $KdpC^{EM}$ with DPC overlay well with $^{1}H/^{15}N$-HSQC spectra of $KdpC^{FL}$ as shown in Fig. 1. The results indicate that even low concentrations of detergent may have significant detrimental effects upon the EM domains of some IMPs. As a result, the 'Divide-and-Conquer' method of structural characterization may be uniquely applicable to membrane protein systems exhibiting undesirable EM domain-detergent interactions.

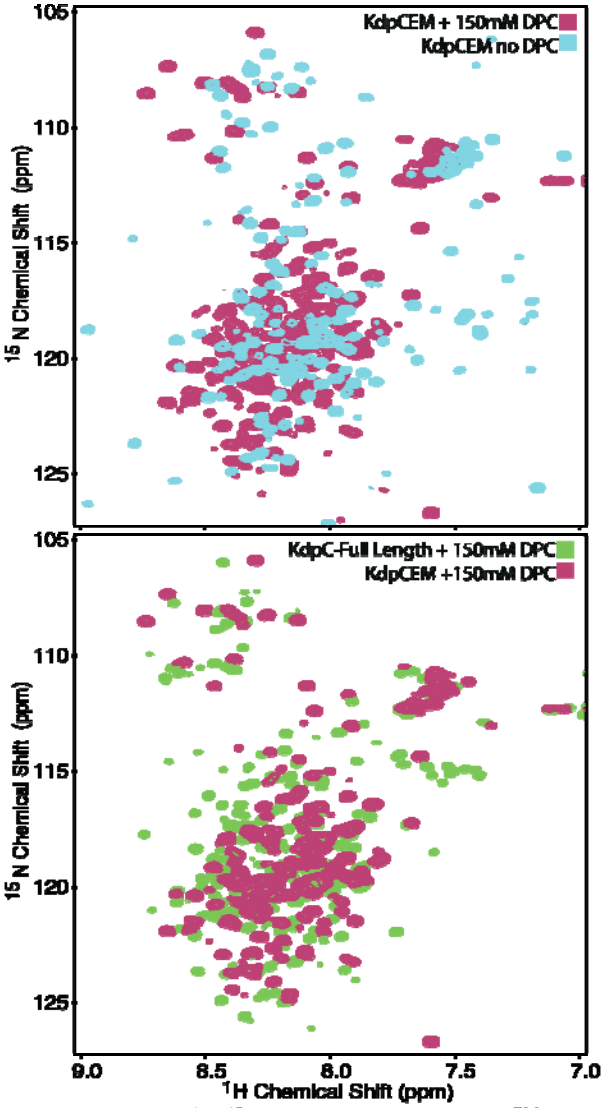

Fig. 1 – Overlaid $^{1}H/^{15}N$ HSQC specta of $KdpC^{EM}$ with no DPC (cyan), $KdpC^{EM}$ with 150mM DPC (magenta) and $KdpC^{FL}$ with 150mM DPC (green)obtained at 720 MHz.

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References

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