Examining the Adverse Effect of Detergent Upon the Soluble Domain of KdpC

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Introduction

Structural characterizations of integral membrane proteins by (IMPs) solution NMR have long focused on small proteins or peptides with very small extramembranous (EM) domains or loops. Future applications of solution NMR to membrane proteins must tackle increasingly larger membrane proteins with significant EM domains. Unfortunately, the need for a membrane mimetic environment to solublize the transmembrane (TM) domains may result in unwanted interactions with large EM domains. Here we begin to examine the effect of detergent micelles upon the soluble EM domain of KdpC, a 21kDa integral membrane protein that is responsible for intersubunit communication in the potassium transporting Kdp protein complex.

Results and Discussion

While detergent micelle environments may be appropriate membrane mimetics for TM domains of IMPs, as supported by recent structures for IMP TM domains, the micellar environment may be detrimental to large EM domains. $^{1}H/^{15}N$-HSQC spectra of the KdpC-EM domain in the absence of detergent micelles indicate that the EM is an autonomously folded soluble protein with primarily α-helical architecture (Fig. 1 *A*). Upon addition of dodecylphosphocholine (DPC) detergent micelles the KdpC-EM domain undergoes a dramatic conformational change as indicated by the significant shifting of resonances in HSQC spectra as shown in the glycine fingerprint region of HSQC spectra in Fig. 1 *B*. Interestingly, inclusion of the TM domain of KdpC changes the interaction between DPC and the KdpC-EM domain (Fig. 1 *C*).

Conclusions

The KdpC EM domain likely interacts with other subunits of the Kdp complex through three highly conserved regions in the EM domain. These highly conserved regions are predominantly hydrophobic, and we suggest that it is these regions that interact with DPC as indicated in Fig. 1 *B*. Additionally, results indicate that the nature of the interaction between KdpC-EM and DPC changes upon inclusion of the KdpC-TM domain. We suggest that the TM domain tethers the EM into close proximity with DPC micelles thereby changing the interaction surface by 'forcing' interactions between DPC and portions of KdpC-EM proximal to the TM domain in addition to the presence of preferential interactions of DPC molecules with conserved hydrophobic regions on KdpC-EM. Through the results shown here, we emphasize that great care must be taken when examining membrane proteins with large extramembranous domains by solution NMR. Clearly detergent interactions with EM domains can be significant, particularly for proteins purported to have significant protein-protein interaction sites.

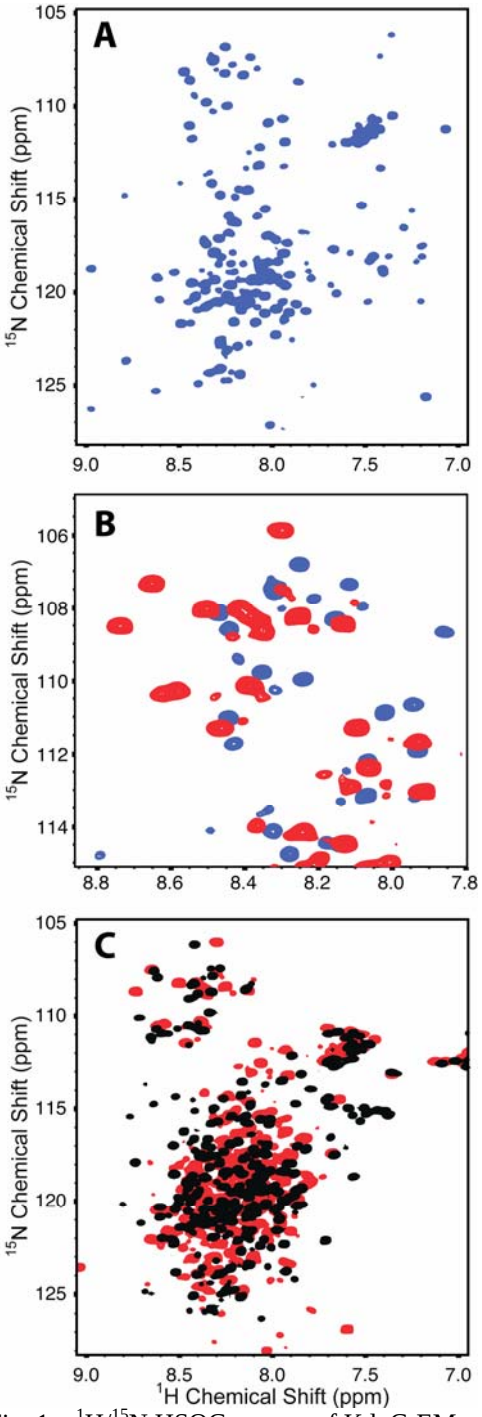

Fig. 1 – $^{1}H/^{15}N$ HSQC spectra of KdpC-EM without DPC (*blue*), KdpC-EM with DPC (*red*) and KdpC Full Length (TM+EM) with DPC (*black*) indicate different modes of interaction between DPC and KdpC-EM based upon the presence or absence of the TM domain.

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