The Binding of Cdc42 with Its Intrinsically Disordered Binding Partner, WASp GBD

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Introduction

Protein-protein interactions play a key role in a great variety of biological processes. It has been suggested that intrinsically disordered proteins (IDPs), due to their lack of a stable structure under native conditions, are able to associate with a wide variety of protein targets with high specificity but low affinity [1]. Recently, a dock-and-coalesce model [2] was put forth to describe the binding kinetics of IDPs. Here, we investigate the binding of the intrinsically disordered WASp GTP Binding Domain (GBD) to Cdc42 to validate this model. Previous structural study of this protein complex showed that these proteins bind through oppositely charged surfaces and calculations based on the TransComp method suggests that this charge-charge interaction is responsible for the docking step of the interaction. We are using relaxation dispersion NMR to characterize the Cdc42:WASp GBD interaction and to validate the dock-and-coalesce model. Further experimental verification of this model is sought by designing mutants altering the charge-charge interactions stabilizing the complex.

Experimental

Both Cdc42 and WASP GBD were expressed in E.coli, and purified by His-tag and GST-tag, respectively. The interaction between Cdc42 and WASP GBD was evaluated by ^{15}N relaxation dispersion NMR.

Results and Discussion

Resonances in the 2D ^{1}H-^{15}N chemical shift correlation spectrum of ^{15}N-labeled wildtype WASp GBD (in the presence of excess Cdc42) were identified according to Abdul-Manan *et al*. [3]. ^{15}N backbone relaxation measurements were conducted on a sample of 1.2 mM ^{15}N-labeled wildtype WASp GBD with 1.0 mM unlabeled Cdc42, revealing three residues that exhibit comparatively large dispersions (Fig. 1).

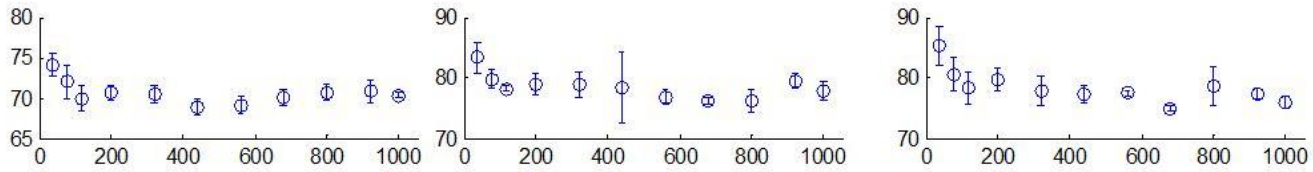

Figure 1: ^{15}N CPMG data for residues Ser232 (left), Ser242 (middle), Leu270(right)

Conclusions

Here, we utilized ^{15}N relaxation dispersion to study the mechanism whereby WASp GBD binds to Cdc42. The results of this work are anticipated to help us better understand the kinetics of coupled folding and binding processes in IDPs.

Acknowledgements

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References

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