Conformations and Exchange Dynamics of FlgM, an Intrinsically Disordered Protein, in Dilute and Crowded Conditions

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Introduction

The majority of biochemical and biophysical studies are performed using dilute solutions of macromolecules, however, the high concentration (up to 400 g/L) of biomacromolecules in cells can affect protein dynamics and structure. FlgM, a regulator of the ordered synthesis of flagellar proteins, is an intrinsically disordered protein with transient helices in the C-terminal region that becomes structured upon binding its sigma factor [1]. Structure formation can also be induced by high concentrations of glucose and bovine serum albumin [2]. The conformations and exchange dynamics of FlgM in dilute and crowded conditions, were investigated with backbone ^{15}N NMR relaxation and CPMG relaxation dispersion experiments.

Experimental

Uniformly ^{15}N isotopically labelled *S. typhimurium* FlgM was expressed in *E. coli* and purified by size exclusion and anion-exchange chromatography. For crowding experiments, 100 g/L dextran-20, 150 g/L BSA, or 100 g/L hen egg white lysozyme was incorporated into the sample. Backbone ^{15}N NMR relaxation experiments and relaxation dispersion experiments were carried out on the 600 MHz 52 mm and 800 MHz 52 mm magnets in the NMR facility. The 700 MHz 52 mm magnet in the Florida State U. Chemistry building was also used.

Results and Discussion

In crowded environments, ^{15}N backbone relaxation measurements suggest slowed tumbling of FlgM. Moreover, crowded environments facilitate conformational exchange occurring on the μs-ms timescale, as probed by CPMG relaxation dispersion experiments performed at 16.5 and 18.8 T (Figs. 1 and 2).

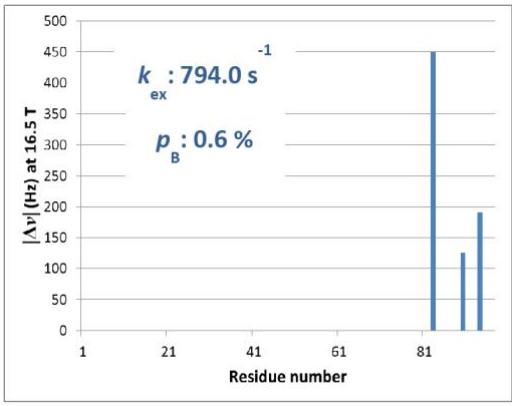

Figure 1: Fitting of ^{15}N CPMG data for residues 83, 90, and 94 of FlgM in 100 g/L dextran-20 to a global k_{ex} and minor population p_B.

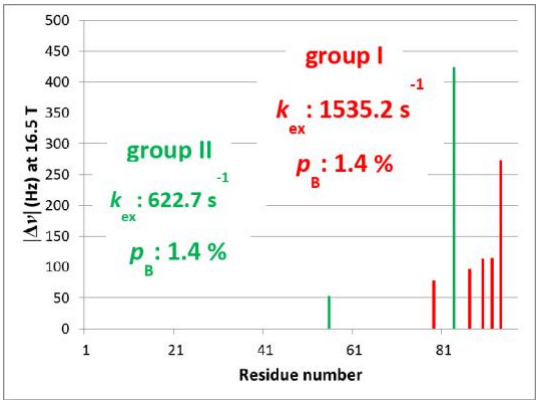

Figure 2: Fitting of ^{15}N CPMG data for group I residues (55 and 83) and group II residues (79, 87, 90, 92, and 94) of FlgM in 100 g/L dextran-20 to a global k_{ex} and minor population p_B values.

Conclusions

Here, we observed μs-ms timescale conformational exchange occurs in FlgM in crowded environments. Previous small angle neutron scattering results obtained in our lab show that FlgM adopts distinct extended and compact conformations in crowded environments (manuscript submitted) and here we show that exchange occurs between these distinct conformations.

Acknowledgements

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References

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[2] Dedmon, M.M., *et al*., Proc. Natl. Acad. Sci. USA, **99**, 12681-12684 (2002).