Dynamics of the N-terminal domain of Human MDM2 protein bound to a p53 peptide

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Introduction

The oncoprotein MDM2 is of special interest as a potential therapeutic target due to its critical role in regulating p53 activity. The structure of the N-terminal domain of MDM2 is known, [1] as is the structure of the domain bound to a p53 derived peptide. [2] These structures and other data indicate conformational rearrangements of both MDM2 and p53 upon complex formation, but little is known about their conformational dynamics. Several small molecule inhibitors of the MDM2-p53 interaction have recently been reported, enhancing the need to more fully understand the influence of ligand binding on MDM2 structure and dynamics, and through them, the protein's biological activity.

Experimental

^{15}N spin relaxation data was collected at 298K on the 800 MHz Bruker Avance 2 spectrometer at NHMFL (Tallahassee, FL), equipped with a triple resonance cryogenic probe.

Results and Discussion

Preliminary characterization of the backbone dynamics of MDM2 in the presence of p53 has been performed using ^{15}N NMR spin relaxation. ^{15}N T_1, ^{15}N T_2, and $\{^1H\}$-^{15}N NOE parameters were collected at 800 MHz using pulse programs designed in house to make optimal use of the 800 MHz spectrometer and the cryogenic probe at NHMFL.

The spin relaxation data were quantitatively interpreted in terms of motional order parameters (S^2), which reveal the amplitude of ps-ns timescale dynamics along the protein backbone. Trends in the order parameters (Fig. 1) are consistent with the secondary structure of MDM2. Notably, the order parameters of the loops at residues 40-48 and 70-73 are low, as are those of the N- and C-terminal tails.

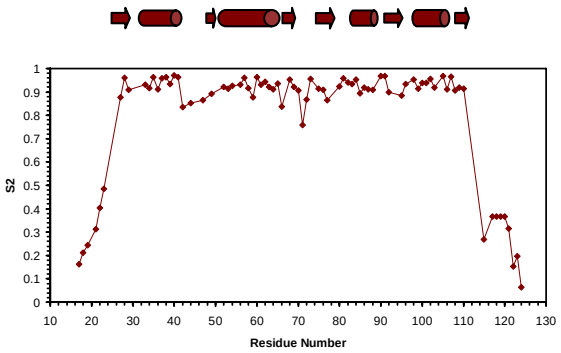

Figure 1: MDM2-p53 backbone order parameters (S^2) derived from modelfree fitting of 800 MHz spin relaxation data. The secondary structure of MDM2 is displayed at the top of the figure for reference.

Conclusions

NMR spin relaxation data collected on the MDM2(17-125)-p53(17-29) complex indicate several sites which are highly mobile on the ps-ns timescale in the presence of the ligand. Measurement of the apo-state dynamics will indicate how the dynamics profiles relate to specific aspects of ligand binding, which is essential for this protein's function.

Acknowledgements

SAS is the recipient of an NIH-NRSA post doctoral fellowship. This project was funded by NSF grant 0621482 to RB.

References

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[2] Kussie, P.H., et al. Science, **274**, 948-953 (1996).