Effect of Subdomain Interactions on Methyl Group Dynamics of Villin Headpiece Protein

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Introduction

Villin headpiece (HP67) is an F-actin binding protein and is a very popular system for protein folding studies. It consists of an N-terminal (residues 9-41) and C-terminal (residues 42-76) subdomains, the latter of which is folded in isolation. The X-ray structures of the isolated C-terminal subdomain (HP36) and its counterpart in HP67 are very similar for the hydrophobic core residues. However, fine rearrangements of the free energy landscape are expected to occur because of the interactions between the two subdomains. We detect and characterize these changes by comparing the μs-ms time scale dynamics of the methyl-bearing side chains in isolated HP36 and in HP67.

Experimental

Using NHMFL 400 MHz NMR spectrometer equipped with a home-build static probe, we conducted deuteron line shape measurements on a native headpiece and a mutant lacking the salt bridge. The measurements were conducted in 298-233K temperature range.

Results and Discussion

We probed three hydrophobic side chains at the interface of the two subdomains (L42, V50, and L75) as well as at two residues far from the interface (L61 and L69). Solid-state deuteron NMR techniques are combined with computational modeling for the detailed characterization of motional modes in terms of their kinetic and thermodynamic parameters. The effect of inter-domain interactions on side chain dynamics is seen for all residues but L75. Thus, changes in dynamics because of subdomain interactions are not confined to the site of perturbation. One of the main results is a two- to threefold increase in the value of the activation energies for the rotameric mode of motions in HP67 compared with HP36, indicating that rotameric jumps are kinetically restricted due to subdomain contact. Detailed analysis of configurational entropies and heat capacities complement the kinetic view of the degree of the disorder in the folded state. The results are accepted to “Protein Science”, with the issue coming out in February 2014.

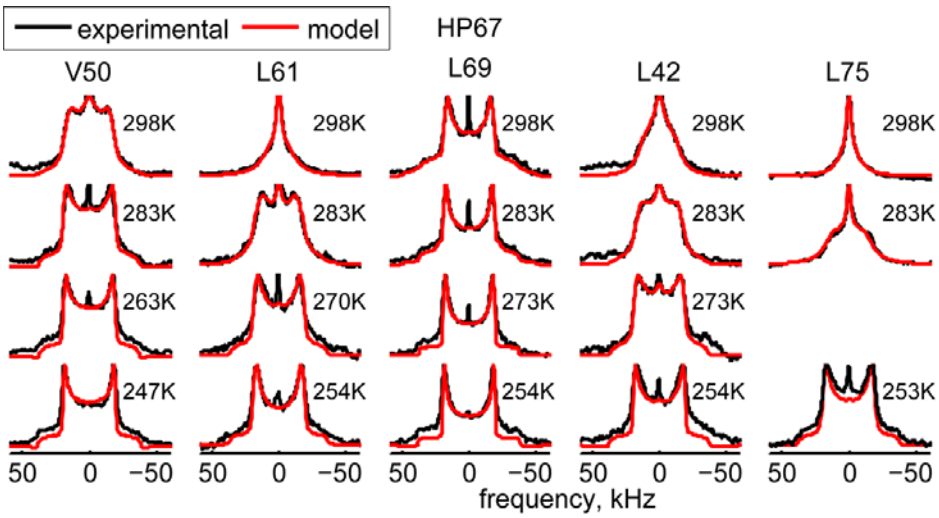

Figure 1. Experimental and simulated spectra of HP67 at all temperatures.

Conclusion

Our study allowed us to obtain a detailed kinetic and thermodynamic view of the changes in the local landscape due to the subdomain interactions in HP67 compared with isolated HP36.

Acknowledgements

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