Structural and functional studies of a 1 MDa chaperonin in action by combined NMR and EM approaches

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The study of the assembly, structural and functional properties of biomolecular nanomachines remains a considerable practical challenge. The sheer size of these protein assemblies, the complexity of the structural rearrangements involved present an array of logistical problems. Even if X-ray crystallography and cryo-EM methods can provide static pictures of the system, kinetic data are necessary for a full, atomic resolution understanding of the mode of action. NMR spectroscopy offers a unique ability to monitor structural and dynamic changes in real-time and at atomic resolution. However, the NMR studies of large proteins and complexes has been a real challenge for a long time. Recent developments in specific isotope labeling of methyl groups in a perdeuterated protein has significantly extended the frontier of liquid state [1].

In this communication, I will present that a combination of methyl specific labeling and optimized NMR spectroscopy integrated with EM can be used to probe different functional states and the refolding cycle of a 1 MDa active chaperonin. To decipher this mechanism, we reconstituted the functional assembly specifically labeled on methionine methyl groups. Thereby the methionine residues have been use as probes of the chaperonin structure allowing the identification of NMR spectra corresponding to the intermediate states and the active species of the functional cycle. NMR allowed us to investigate in an atomic- and time-resolved manner the structural rearrangement corresponding to the different states during the functional cycle of a large biological machinery. We have characterized the interaction between the chaperonin and an unfolded substrate protein. We have observed that the unfolded proteins are stabilized by the chaperonin, allowing us to identify the holdase activity of the chaperonin. Using the combination of different structural biology approaches, it has been possible to follow the refolding of the unfolded protein by the chaperonin and the effects of the unfolded protein on the functional cycle of the chaperonin in action.

References

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