Nucleotide-Induced Conformational Changes of Tetradecameric GroEL Mapped by Hydrogen/Deuterium Exchange Monitored by Ultrahigh Resolution 14.5 T FT-ICR Mass Spectrometry

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Results and Discussion

The detailed characterization of large protein assemblies in solution remains challenging to impossible. Nonetheless, these large complexes are common and often of exceptional importance. Here we employ hydrogen/deuterium exchange mass spectrometry (HDX-MS) to access *E. coli* chaperonin GroEL conformation in solution. The ~800 kDa tetradecameric GroEL plays an essential role in the proper folding of many proteins via an ATP-driven cycle of conformational changes. Previous studies of the structural dynamics of GroEL upon ATP binding have been inconsistent, showing either minimal or major allosteric changes. Our results, which are based on the native, non-mutated, protein under physiological conditions in solution, demonstrate substantial changes in conformation and/or flexibility upon ATP binding. We capture the pivotal step in its functional cycle by use of a non-hydrolyzable ATP analog, ATPγS, to mimic the ATP-bound GroEL state. Comparison of HDX-MS results for apo GroEL and GroEL-ATPγS enables the characterization of the nucleotide-regulated conformational changes throughout the entire protein with high sequence resolution. GroEL is by far the largest protein assembly yet mapped by HDX-MS, and the results achieved here establish the groundwork for further HDX-MS characterization of such large complexes.

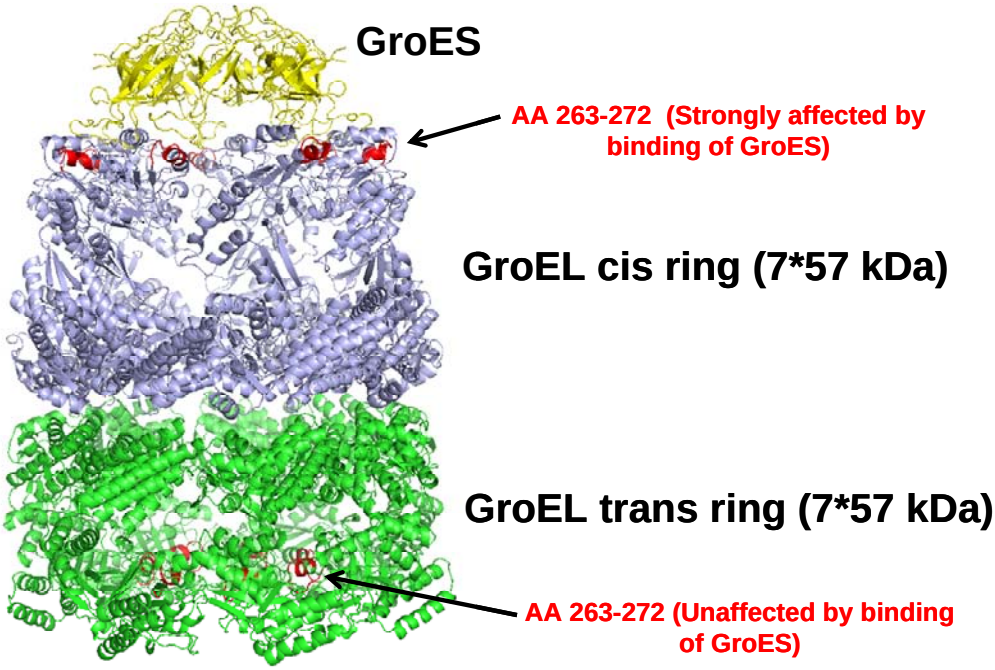

Figure 1. Structural models of the GroEL:GroES chaperone complex, showing changes in solvent accessibility for GroEL subunits on binding of the GroES "cap". Changes are seen in only one of the two GroEL heptamers.

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References

[1] Zhang, Q.; et al., Scientific Rpts, **3**, 1247 (2013).