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Capturing intrinsic nanomechanics of allostery

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ABSTRACT The Hsp70 chaperone exploits allosteric communication between its substrate binding domain and its nucleotide binding domain to regulate the loading and release of misfolded polypeptides in an ATP-hydrolysis-dependent manner. In this issue of Biophysical Journal, Singh, Rief, and Zolda ´k report an exquisitely detailed study of the nanomechanical aspects of the allosteric mechanism in DnaK, an Escherichia coli heat shock protein 70 chaperone.

Most proteins are fragile and, during their lifetime, break structurally and functionally when subjected to harsh changes in the cellular milieu (e.g., thermal shock) or other stresses during organismal aging (1). Chaperones come to the rescue by binding to the damaged protein at its vulnerable stretches (such as those exposed hydrophobic residues), preventing protein aggregation (2). The heat shock protein (Hsp) 70 family provides chaperone services to many proteins, helping them to recover after misfolding or to get transported across membranes (2). DnaK is an Escherichia coli member of the Hsp70 family. DnaK (638 amino acids, 70 kDa) is composed of an N-terminal nucleotide-binding domain (NBD) that is connected through a flexible linker to a C-terminal substrate-binding domain (SBD).

The allosteric coupling between DnaK's NBD and SBD is at the heart of its chaperone function, and over the years, this topic has been addressed in many studies and significant progress has been made to characterize the allosteric behavior of DnaK, mostly through structural, computer modeling, and mutagenesis approaches (3–5). Single-molecule force spectroscopy (SMFS) (6), by accurately measuring changes in molecular length when the molecule is subjected to applied forces and by registering characteristic patterns of force peaks when the tensed molecule unravels either completely or partially, seems to be uniquely qualified for very challenging measurements aimed at the direct capturing of complex submolecular rearrangements accompanying allosteric interdomain communication in chaperones such as DnaK. Rief, Zolda ´k, and their colleagues have been exploiting SMFS implemented on ultra-high-resolution, high-stability, dual-beam optical traps (laser tweezers) to examine DnaK in a series of impressive papers that characterized in detail the nanomechanical properties of separate NBDs and SBDs of DnaK (7,8) as well as the effect of nucleotides on NBD's substructures and unfolding pathways(9). In the paper published in this issue of Biophysical Journal,

Singh, Rief, and Zolda ´k capitalize on these previous successes and, more broadly, also on the effort of the biophysics community studying Hsp70 allostery and present yet another fascinating story about the internal mechanics of the DnaK chaperone as affected by nucleotide binding, hydrolysis, and interactions with a model substrate, using, in that study, an almost complete two-domain DnaK construct (10). Before being able to directly capture these allosteric interactions at a single-DnaK molecule level, the authors had to overcome obstacles in establishing stable molecular bridges between optically trapped beads due to the difficult-to-control flexibility of the disordered C-terminal tail of DnaK. After truncating this unruly fragment, SMFS measurements became feasible, but a new problem surfaced: DnaK revealed its chaperone appetite for one of its own fragments, including two hydrophobic leucine residues (positions 542 and 543) that became exposed during SMFS stretching and were locking themselves in the still-folded substrate binding cavity, creating undesired autoinhibitory effects. After truncating this problematic fragment and two additional mutations, the authors engineered a smaller, more stable SBD within the final DnaK construct named DnaK*ye, which was originally developed and examined for their full allosteric potential by the Gierasch group (4). This construct proved to be amenable to SMFS measurements in the absence and presence of ATP, ADP, and a model NRLLLTG (NR) peptide substrate. Due to the stochastic nature of forced domain

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unfolding, the mechanically stronger NBD may occasionally unfold before
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the SBD does. However, the identification of the two is relatively straightforward in SMFS measurement as the polypeptide chain folded within the NBD is roughly twice as long as in the SBD. In the apo and ADP-bound form, the mechanical unfolding force spectrogram of DnaK*ye was essentially a combination of force peaks displayed by isolated NBDs and SBDs, strongly suggesting that, under these conditions, the domains are separated and behave independently. However, under ATP-bound conditions, SMFS captured a new behavior for the SBD, which became mechanically weaker and partially unfolded, strongly suggesting that the SBD underwent a conformational transition to an open form, with the alpha helical lid detached from the beta-sandwich. The NBD remained mechanically unchanged when bound to ATP. A mutation in the interdomain linker that is known to abolish the allosteric communication in DnaK, as well as the mutation that inhibits ATP-ase activity of the NBD separately, and totally eliminated the effect of ATP (within the NBD) on the mechanics of SBD*ye, strongly suggesting that the weakening of the SBD is a direct manifestation of the allosteric action of the NBD on the SBD. Interestingly, and most amazingly, under ATP conditions, in a small fraction of measurements (ca. 10%), SMFS captured behavior of the SBD that was indistinguishable from that observed under ADP conditions. These results, supported by some additional control measurements involving DnaK mutants, strongly suggested that the transitions of the SBD from a mechanically weak, partly unfolded open structure to a mechanically strong and well folded (closed) structure revealed the response of the SBD to ATP hydrolysis within the NBD. Next, the authors observed the effect of the model NR substrate on the allosteric coupling between the NBD and the SBD, exploiting its known propensity to stimulate the ATP-ase activity of the NBD up to threefold. As the NBD needed to remain active (and thus folded) during these measurements, the force applied to the DnaK*ye construct had to be low, and the detection of SBD*ye status needed to rely on minute changes in the length of the trapped protein when SBD*ye transitioned between open (ATP in the NBD), closed (ADP in the NBD), and open (new ATP in the NBD) states. The effect of ADP release and (re)entry of a new ATP molecule into the NBD on the state of SBD*ye could also be directly observed in real time as force-relaxation steps accompanying SBD opening. Interesting tension fluctuations in the SBD were observed under ATP binding in the presence of NR peptide, suggestive of partial unfolding/refolding of SBD segments, which, based on previous research, could be traced to detachment/refolding of specific beta strands within the SBD beta core, in response to the allosteric interactions between docked NBDs and SBDs. According to their previous paper, these specific two beta strands form a mechanical hinge that is moved in an allosteric fashion upon ATP binding and helps to drive lid opening over the substrate-binding cleft (8). The level of mechanistic detail involved in the allosteric communication in DnaK captured in this paper is astonishing. The paper highlights the power of SMFS, and laser tweezers specifically, for their unique capabilities in unraveling complex chemo-mechanical interactions in individual protein nanomachines fueled by ATP for their allosteric operations. We look forward to this story being continued and hope that capturing directly DnaK allostery during DnaK chaperone activity is an attainable target for the Rief-Zolda ´k team.

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DECLARATION OF INTERESTS

The author declares no competing interests.

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