ATP-Induced Shape Change in a Model Protein Complexed in Chaperonins

The role of molecular chaperones in mediating and controlling intracellular, as well as *in vitro* protein folding, has broad implications for biotechnology. There is now considerable insight into the possible mechanisms whereby chaperone proteins recognize, stabilize, and release unfolded polypeptide chains in a manner whereby they are able to productively refold. However, there are a number of important, fundamental gaps in the understanding of chaperone action, which remain to be resolved. Among the growing list of chaperone families are the chaperonins GroEL (EL) and GroES (ES), which have been intensively studied. Knowledge of how misfolded protein substrates physically interact with EL should provide vital clues necessary to unravel the process by which EL mediates their proper folding. The ultimate goal is to determine the mechanism by which EL transforms its substrate proteins and then releases them in a form able to refold to their native conformation.

One of the key issues in establishing a molecular mechanism for EL is to describe in structural terms the conformations of polypeptide substrates when bound to various chaperonin complexes. For example, does a nonnative polypeptide substrate unfold further upon binding to EL? On the other hand, when a chain is released from EL in the presence of the co-chaperonin ES, does it adopt a more folded, or less folded conformation? These questions are difficult to resolve with naturally occurring proteins since they refold so readily when released from chaperonin complexes. One approach to address these issues, however, is to utilize a family of mutationally altered protein substrates that are unable to adopt their native conformation. The nonnative subtilisin variant, PJ9 (*p*), which is unable to refold when released, is one such system that is readily available [1].

Single-ring EL mutants have been shown to assist in the refolding of nonnative polypeptide chains [2, 3] and they form unusually stable complexes with ES upon the addition of nucleotides. Capitalizing on these properties, a single-ring EL variant (sEL) was used to trap *p* within EL by ES upon the addition of the nucleotides adenosine di- or tri-phosphate (ADP or ATP). Small angle neutron scattering (SANS) experiments were then performed to examine the structure of sEL/ES/*p* complexes and investigate changes in *p* conformation in the presence of both nucleotides.

p was 86 % deuterated (d*p*) so that it contrasted sufficiently with the chaperonin, allowing the contrast variation technique to be used to separate the scattering from the two components bound in the complex. The sEL mutant assured that d*p* and ES were each bound in a 1:1 stoichiometry with the sEL, providing an advantage over previous SANS experiments [4] which included mixed stoichiometries of EL and ES.

Unlike ATP, ADP does not cause dissociation of d*p* from EL. If d*p* is no longer covalently bonded to EL, would its location in the EL/ES complex change? To answer this question, two contrast variation series of measurements, one with ADP and the other with ATP present, were performed on the sEL/ES/d*p* complex [5]. Measurements were made in five D_2O buffers in each case, as shown in Fig. 1. The extensive $I(Q)$ data sets in Fig. 1 allow the separation of the scattering intensities from each of the components in the complex and also the cross-term intensity. Modeling is then used to try to reproduce these measured intensities.

Using this method, the location and approximate shape of d*p* in the sEL/ES/d*p* + ADP complex was determined as modeled in Fig. 2. The significant result is that the d*p* component has an asymmetric shape: part of the polypeptide must extend beyond the cavity inside the sEL ring up into the space surrounded by ES. The best fit to the data from both complexes (*i.e.*, with ADP or ATP) was

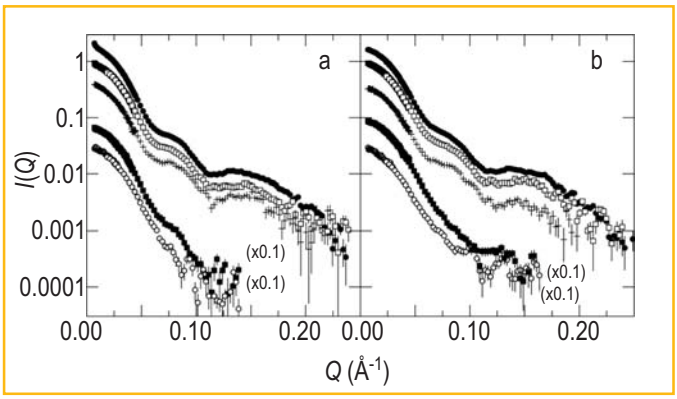

Fig. 1. Contrast variation data from sEL/ES/d*p* complexes a) + ADP and b) + ATP in 100 % (●), 85 % (□), 70 % (+), 20 % (O) and 0 % (■) D_2O solutions. The data in 20 % and 0 % D_2O solution are shifted by the factor 0.1, as indicated, for clarity.

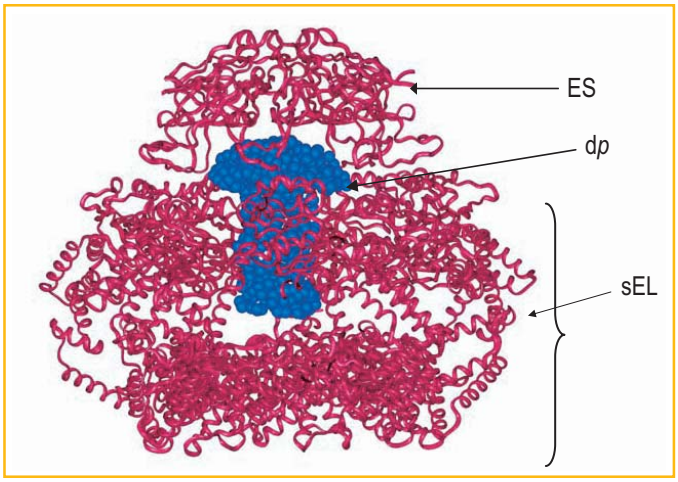

Fig. 2. Side view of a model for the sEL/ES/d*p* + ADP complex constructed from SANS contrast variation and crystallography data. The sEL/ES complex is represented by the red ribbon structure and the d*p* is represented by the blue spheres.

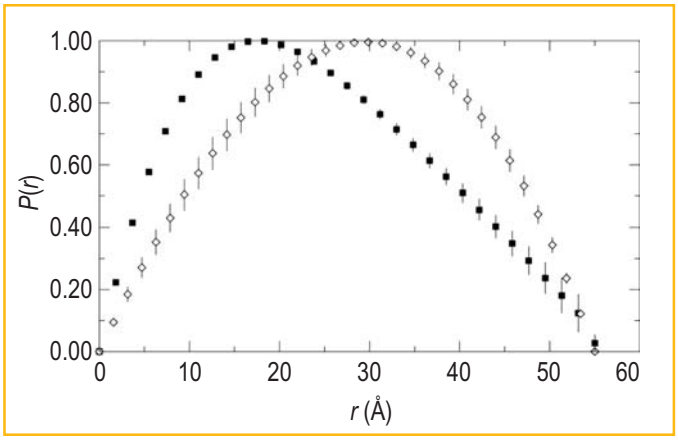

Fig. 3. Normalized distance distribution functions for d*p* bound to sEL/ES complexes + ADP (■) and + ATP (◇).

achieved using one ring of the EL/ES solution structure of Ref. 6 for the sEL/ES component of the complex, one ring of the x-ray structure for EL of Ref. 7 for the free sEL, and the x-ray structure for free ES of Ref. 8 in a ratio of 75:21:4 by mass.

The significant difference in the sEL/ES/d*p* complex formed from ATP is that the shape of the bound d*p* molecule changes from an asymmetric shape such as that shown in Fig. 2 to a more symmetric shape. Figure 3 shows the distance distribution functions obtained by Fourier inversion of the $I(Q)$ for bound d*p* in both the sEL/ES/d*p* + ADP and sEL/ES/d*p* + ATP complexes. The most probable distance increases from approximately 22 Å to 30 Å, with a similar increase in the radius of gyration, R_g, from 19.0 ± 0.5 Å to 21.0 ± 0.5 Å.

The shape of d*p* in the presence of ATP is clearly more symmetric, as indicated by the greater symmetry of the distance distribution function. This suggests that d*p* is transformed into a more expanded form in the ATP complex. However, the symmetry of this curve cannot be used to unambiguously assign a shape to d*p* in the ATP complex. Because the sEL/ES/d*p* complex dissociates in solution, which contains about 21 % of sEL/d*p* complex, it is possible that the distance distribution curve represents a composite of data from d*p* bound to sEL alone and to the sEL/ES complex formed from ATP. For the same reason, it is unclear from the data whether d*p* is still located partially in the sEL cavity. However, what is clear is that there has been a transformation in the shape of d*p* associated with the complex formed from ATP. The d*p* shape change was significant enough to be detected as a change in the shape of the scattering from this component, in spite of the fact that dissociation is likely to be present in the sample. A conformational change of d*p* either was not supported by the complex formed from ADP or was insufficient to generate a lasting change in shape in that case, and d*p* instead relaxed back to a form close to its original conformation. This important observation reflects the relative ability of ATP to promote refolding of protein substrates relative to ADP.

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