

Structure of Protein Complex Obtained by Electron Microscopy

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The structure of Ca^{2+} -ATPase was obtained from frozen-hydrated specimen using cryo-microscopy and image processing. Comparison of projection structure from multilamellar crystals and helical tubes. Multilamellar crystals comprises molecules in the calcium-bound state, whereas helical tubes are composed of the calcium-free state; thus, the difference in the projection map may be due to conformational changes induced by calcium binding to Ca^{2+} -ATPase.

HtrA (also called DegP or protease Do), which has a high molecular mass of 500 kDa, is periplasmic heat shock protein whose proteolytic activity is essential for survival of *E. coli* at high temperature. To determine the structural organization of HtrA, we have used electron microscopy and chemical cross-linking analysis. The averaged image of HtrA with end-on orientation revealed a six-membered, ring-shaped structure with a central cavity, and its side-on view showed a two-layered structure. Thus, HtrA behaves as a dodecamer consisting of two stacks of hexameric ring.

The heat shock protein ClpB (HSP 100 family) is a protein-activated ATPase and has also recently been suggested to function as a chaperone in reactivation of aggregated proteins. In addition, the *clpB* gene has been shown to contain two translational initiation sites and therefore encode two polypeptides with different size, the 93- and 79-kDa ClpB. We have determined the molecular architecture of ClpB complex using electron microscopy and image processing. Herein we also discuss the substrate binding site based on comparison of the structure of ClpB93, ClpB79 and His6-ClpB93.