X-ray crystal structure of peptide-bound GroEL

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Molecular chaperon GroEL assists protein folding *in vivo* in an ATP-dependent mechanism. GroEL is a homotetradecameric molecule, arranged radially in a double-ring structure [1]. The two rings stacked back to back share a seven-fold rotational symmetry with a central channel that is split at the inter-ring interface into two functional compartments. Each subunit consists of three domains, the apical domain, the intermediate domain, and the equatorial domain. GroEL also requires a cochaperon, GroES, for function. GroES is a homoheptemer, with seven subunits distributed rotationally in a dome-like arrangement [2]. In the GroEL/GroES structure, GroES caps one end of GroEL and connects one of the central compartments to a continuous sealed chamber, doubling the volume of the central cavity. In the substrate-binding competent GroEL, the surface of the inner wall of the central channel is hydrophobic; this surface becomes hydrophilic in the GroEL/GroES complex [3]. The first step in GroE-assisted protein folding is the binding of partially folded or misfolded substrate protein to GroEL. Small-angle neutron scattering and cryo-electron microscopy show that the substrate protein binds to the opening of the central cavity of GroEL [4, 5] where the apical domains are located. Structure-based mutagenesis studies [6] further implicate that the apical domain is the involved in substrate binding and that the interactions are largely hydrophobic. The intriguing aspect of this GroEL/substrate interaction is that GroEL can bind to a wide spectrum of substrate proteins in their nonnative forms [7]. So far, both the stereochemical details of GroEL/substrate interactions and the mechanism for the substrate promiscuity are not clear.

To circumvent the seven-fold averaging effect and to minimize the multiple conformations of the bound substrate in the binding sites, we chose to use the apical domain of GroEL to select for the strong-binding peptides (SBP) from a peptide library displayed on phage M13, and study the interactions by x-ray crystallography [8]. The affinity of strong peptides selected by the bio-panning technique to the apical domain was measured by fluorescent anisotropy, and one of the peptides has a K_d of 2 μM. This peptide was then cocrystallized with both the apical domain and the tetradecameric GroEL, and the crystals diffracted to 2.1 Å and 3.0 Å respectively. The peptide binds to a groove formed by helix H and helix I of the apical domain, in a manner similar to that of the GroES mobile loop (see Figure 1). Crystal structure of the apical domain in apo form was also determined at 2.0 Å, revealing that the peptide binding site exists in different conformations. Our structural analysis, combined with other results, suggests that various modes of molecular plasticity are responsible for the tight promiscuous binding of nonnative substrates, and provides a mechanism for the substrates' release into the shielded cis assembly.

Figure 1: Top view of SBP/GroEL complex. Current partial refinement gives R_{work} ~ 0.245 and R_{free} ~ 0.318. Except for the SBP binding sites, no substantial structural arrangements occur in GroEL when compared to the unliganded GroEL. SBPs are coloured in yellow, and GroEL is in red. Helices H and I of one subunit are labelled. For clarity, only one ring of GroEL is shown. This figure was generated in Setor.

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