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Large-scale conformational changes of GroEL chaperonin induced by nonnative protein target

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Introduction

Esherichia coli oligomeric protein GroEL is known to assist protein folding [1] and binds various nonnative polypeptides preventing their nonspecific aggregation [2] both *in vivo* and *in vitro*. GroEL quaternary structure consists of 14 identical subunits arranged into two stacked seven-membered rings with a molecular mass nearly 800kDa. Each subunit consists of apical, intermediate and equatorial domains which are involved in stabilizing of inter-subunit and inter-ring contacts as well as in binding of nonnative protein targets, ADP, ATP, and co-chaperonin GroES [3]. Here we demonstrate large-scale conformational changes of GroEL particle induced by the binding with nonnative proteins.

Materials and methods

GroEL was purified after super-expression of the corresponding gene in *E. coli* cells. To remove some tryptophan-containing polypeptide impurities which are usually tightly associated with GroEL during its purification, Butyl Toyopearl and Reactive RED 120 chromatography was used.

The complex of GroEL with nonnative protein targets (pepsin and lysozyme in the presence of 20mM DTT) was controlled using size-exclusion chromatography on Superose 6. The samples were prepared in 50mM Tris-HCl buffer, pH7.5 containing 100mM KCl and 10mM Mg-acetate. The GroEL concentration was 5mg/ml. The SAXS patterns were measured on BL-15A small-angle installation using 2.35m length camera and CCD detector.

Results

The small-angle X-ray scattering data within the scattering vector values from $0.01A^{-1}$ to $0.15A^{-1}$ were collected for GroEL in the absence and in the presence of nonnative protein targets. Figure 1 represents the SAXS patterns for free GroEL and in the presence of nonnative pepsin with stoichiometry 1:1. Inset represents the difference between these SAXS patterns within the experimentally measured scattering vector values (S). One can see that the SAXS pattern for GroEL-pepsin complex is lower then for free GroEL within S values between 0.03 and 0.095. This interval of GroEL SAXS pattern is sensitive to the change of the orientation of apical and intermediate domains as well as the inter-ring distance. The modeling of the GroEL solution structure in the complex with nonnative protein target shows that one ring is unchangeable while in the other ring the apical domains change their orientation of +10° and intermediate domains of -10°. Besides, the interring distance decreases of 4Å. The same result was obtained with nonnative lysozyme. Thus, the GroEL solution structure in the complex with nonnative protein targets with stoichiometry 1:1 is more closed then the free GroEL solution structure at least with respect to one ring (the orifice of one ring is decreased of two-fold).

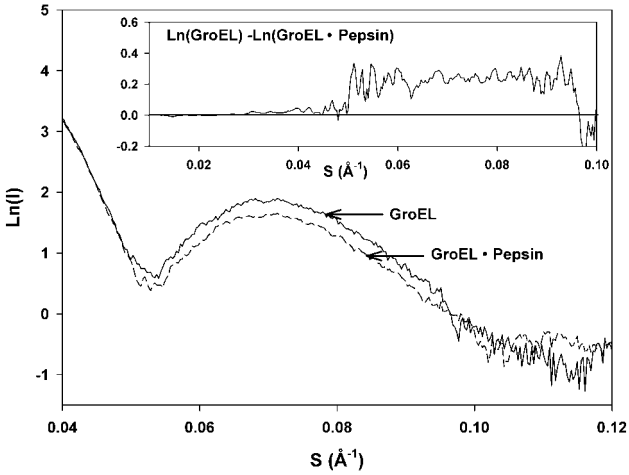

Figure 1.

References

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