Lecture 30
4/30/04

Review Session: Tuesday may 4, 7-8:30
EXAM: may 5th, 7:30-9:30

PS 6 due Monday

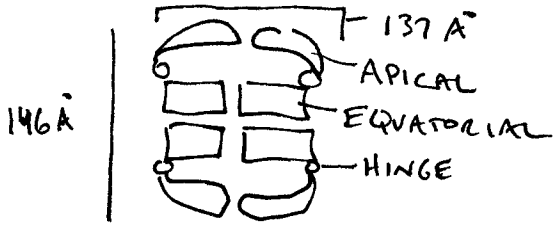
See handout 4b for more info and references on disulfide oxidases and isomerases

GroEL/GroES

1) Structure

GroEL is compsed of 2 stacked 7 membered rings. Each monomer is 57 kDa and is composed of an equatorial (ATP binding) domain, a hinge domain, and an apical domain.

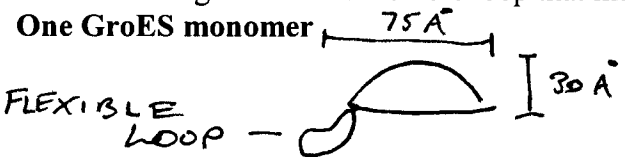
Cross section showing 4 monomers (137 angstroms by 146 angstroms)



In the absence of nucleotides, the two halves are completely symmetrical

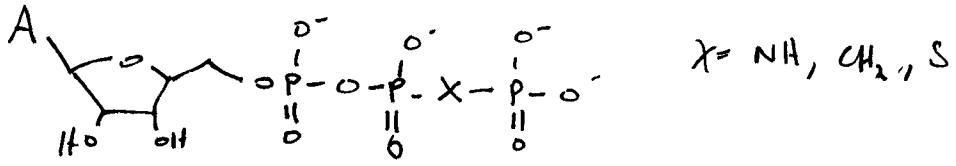
GroES - lid, each monomer is 10 kDa (75 angstroms by 30 angstroms), also a 7 membered ring. There is a flexible loop that interacts with GroEL

One GroES monomer



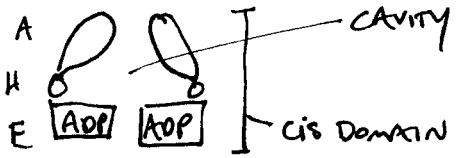
The active form of the GroEl/GroES complex is bullet shaped, with GroES capping one end of GroEL- no longer symmetric

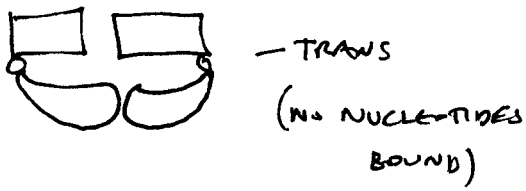
Efforts to obtain informative structures crystallographically and by cryoEM have been difficult because of lack of small molecules (such as the antibiotics used to study the ribosome) to freeze out specfic conformations of a given machine.
Have tried non-hydrolizable ATP analogues
Ex. **Non-hydrolysable ATP** to freeze in ATP bound state, x= NH, CH_2, or S



Or, make mutants of GroEL – Mg^{2+} is a cofactor that interacts with ATP and protein and is often liganded to D,N or Es of the protein or the phosphates of ATP.
Use site-directed mutagenesis to mutate one of the amino acids D to A, can inhibit ATPase activity and potentially freeze out the ATP bound state.

Structure of the ADP bound form
ATP bound form with D-> mutant
"**cis**" form, cavity can hold proteins from 15-60 kDa





"**trans**" form, no nucleotides bound
Check out 1aon.pdb

Conformational changes on nucleotide binding
Open vs. Closed States

Trans domain- exposed hydrophobic patch on interior
Cis domain- capped with groES, hydrophobic region is buried and the protein has moved to the interior of the enclosed cavity

Functional Model (see p. 10 handout 4a)

1) chamber formed (15-60 kDa unfolded protein can fit into the chamber)
2) folding process is protein dependent and more than one cycle may be necessary to fold a multidomain protein
3) function of ATP
    a. positive cooperative allosteric effector
    one ATP binds to one equatorial position, causes rapid binding of 6 additional ATPs. Thus all 7 subunits have an ATP bound which undergoes hydrolysis before release of the protein from the cavity
    b. negative effector
    once ATP is bound in one ring (cis), prevents any ATP binding to the other ring (trans)

See page 10 of handout 4a (bottom, figure 1) for a cartoon diagram
The GroEL with no GroES bound is at very low concentrations inside the cell
Cycles though with one ring active, then the other ring active

Question: still unanswered
What is the physical basis for more rapid folding w/in a chamber vs. in solution?

Method to determine substrate specificity (detail in T & D 11)

1) 2-D gel electreophoresis (separate proteins based on charge and MW)
2) pulse-chase labeling experiments allows radiolabeling a protein with ^{35}S-Met and then monitoring the fate of the label relative to the GroEL/GroES cavity
3) stop rxn with EDTA, presumeably removes Mg. It is interesting that the chamber remains in tact with the protein that is undergoing search of its folding space; then one uses immunoprecipitation to precipitate both the GroEL/GroES and the encapsulated proteins. The proteins in the immunoprecipitate are all run out on 2D gels and each protein can then be identified
4) MALDI-TOF mass spec to ID proteins

From the Hartl experiments, they estimated that 10% of bacterial proteins interact with GroEL/GroES during the folding process *in vivo*

CRYPTS!! – "The Proteosome"

Overview: why is the proteosome important?

1) protein misfolding from the ribosome or under stress results in aggregation- may lead to disease
2) regulation
    a. protein degradation could destroy a regulatory protein
    b. protein "A" is inactive due to its association with an inhibitor; when a signal is sent to make protein "A" active (ex. phosphorylation), the inhibitor protein is targeted for degradation by proteosome
3) degradation plays a key role in generating immuno-competent peptides (8-15 amino acids) which are displaced in MHC complexes on the surface of T cells and B cells. This presentation triggers the immune response
4) homeostasis- under starvation conditions, proteoylsis provides amino acids

Outline

1. brief overview of proteases (mechanism, proenzymes, small inhibitors of proteases as tools) The serine proteases are excellent models for the proteosome
2. describe the proteosome machines: structure by cryoEM, x-ray crystallography; mechanism of assembly of subunits into their active form; the mechanism of peptide bond formation and comparison with serine proteases
3. interacting proteins- required for the protease to function. These proteins unfold the protein to be degrades and then translocate the unfolded protein into the proteosome. ATP is required for both of these processes. the coordination of these processes with proteolysis is a major focus of interest
4. targeting proteins for degradation
    -eukaryotes- ubiquitin
    - bacteria - tmRNA tag