Lecture 31
5/3/04

Outline
1. brief overview of proteases
2. describe the machines (cryoEM, x-ray crystallography) compare and contrast proteosome vs. proteases
3. interacting proteins-unfold, translocate proteins to be degraded; this process requires ATP
4. targeting proteins for degradation
   -eukaryotes- ubiquitin which is a post-translational modification by a protein
   - bacteria - tmRNA tag

1. Proteases:

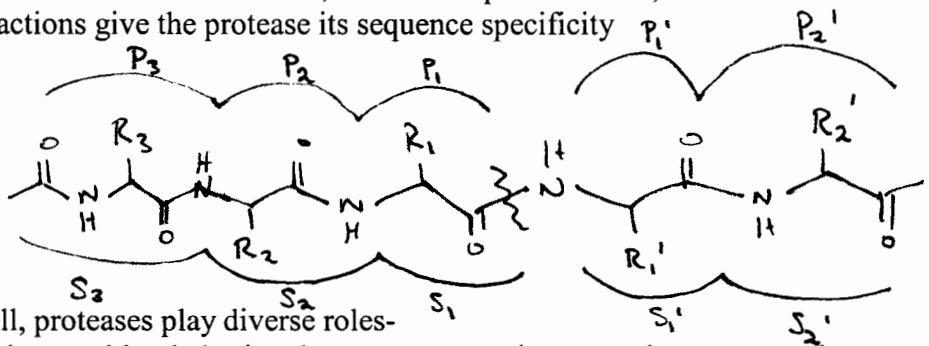
Nomenclature
P= amino acid side chain in protein (substrate) that is being degraded, S= binding pocket in protease for the P side chains

From left of bond to be cleaved, residues are called P1, P2, P3 (complementary to S1,S2, S3 etc)

To right of bond to be cleaved P1', P2' etc complements S1`, S2'

These intereactions give the protease its sequence specificity

Cartoon



Inside the cell, proteases play diverse roles-
Some examples are: blood clotting, hormone processing, complement cascade, generate amino acids for homeostasis

What would happen if you tried to overproduce a protease? All hell would break loose inside the cell due to non-specific degradation! So how can we overexpress them to make these proteins?

To control proteolysis- most proteases are made as precursor proteins (proproteins or zymogens) this form is inactive or has very low activity

To get to the active form, need a protease or can have slow auto-proteolysis, it depends upon the system.

2nd method in eukaryotes, almost every protease has a small protein inhibitor. protease and protease inhibitor form a complex and therefore, one must destroy the inhibitor to make the protease active

Proteases are well understood
We can make femtoM inhibitors based on what we know about the mechanisms of all four classes of proteases (ex HIV protease inhibitors)
What kind of proteases are there?
There exist 4 classes of proteases

a. Serine (threonine) proteases
Catalytic triad= S,D,H
Covalent catalysis using S as the active site nucleophile
Ex: chymotrypsin, serine proteases are also involved in the blood clotting cascade
Also, a subset of this class that use OH nucleophiles in covalent catalysis is use threonine instead of serine (yeast and human proteosome use T) and the N-terminal amino group (also T) as a general base (instead of His)

b. cysteine proteases
C and H
Covalent catalysis
Involved in "apoptosis", caspases

c. aspartate proteases
non covalent catalysis
activate H_20, general base and protonate leaving group, general acid catalysis
"captopril"- is a potent inhibitor of an enzyme involved in regulation of blood pressure and is consequently used clinically to regulate High blood pressure

d. metalloproteases
non covalent catalysis
Zn^{2+} most common, the metals act as Lewis acids and can also activate water for nucleophilic attack
Also Ca^{2+}, Fe^{2+}, Cu^{2+}, Mn^{2+}
Many drug targets

Chymotrypsin

1) conversion to active form
self-proteolysis or other proteases

N-------Phe15-Ile16------His57------Asp102----Asp194-Ser195--------C

The catalytic triad is H57, D102, S195
proteases have P1, S1 complementarity, the S1 site of chymotrypsin likes a hydrophobic or aromatic residues and this binding site in part accounts for the specificity of the protease. The S1 site of trypsin or enzymes involved in blood coagulation likes basic amino acids such as K or R.

As noted above most proteases are made with additional amino acids attached to their N-terminus (pro-proteins). To activate a protein such as chymotrypsin or trypsin,

cleavage of the pro-protein occurs between residues 15 and 16 (F at P1) and gives rise to an N-terminus with a NH_3^+. This NH_3^+ forms a salt bridge with D194 (note it is adjacent to the active site S195)-opens up the S1 binding site so that P1 can bind. The protein in this form is now an active protease

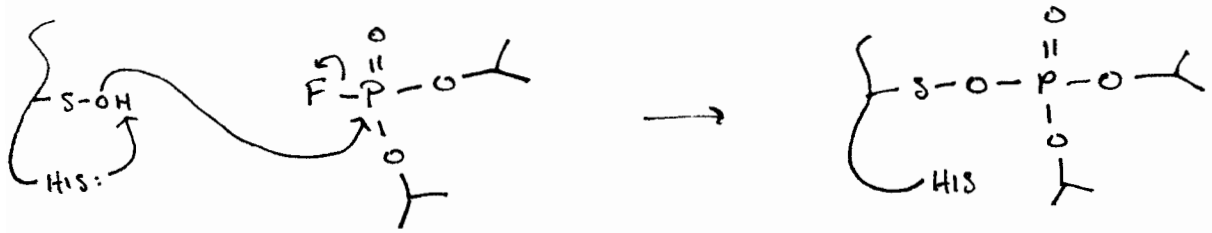
NH_3^+-------F15 NH_3^+Ile16--------D194-S195---------C

2) look at mechanism, role of S,D,H
covalent catalysis
remember we previously discussed this mechanism as Steitz used chymotrypsin as a model mechanism for peptide bond formation on the ribosome
See p 12 handout 3b

This mechanism applies to the proteosome, but replace S with a T, H with the N-terminal amino group of the T. Threonine is at the N-terminus of the proteosome subunit and is actually generated by self process which will be discussed in the next lecture. Note also that one stabilizes the tetrahedral intermediate or transition state of the reaction, by unique hydrogen bonding interaction which occur only at this step in the reaction.

3) Inhibitors (of serine proteases)
Lactocystin- specifically inactivates human and yeast proteosome. As you have seen in ps 6, this inhibitor has been very useful in understanding the mechanism of antigen presentation.
General inhibitor for serine proteases is isopropyl-fluorophosphate. The P-F bond is cleaved and the active site serine or threonine becomes acylated. The covalent modified protein in this form is only very slowly hydrolyzed and thus the enzymes become inactivated.

DRAW



ISOPROPYL-FLUOROPHOSPHATE

Model of tertrahedral intermediate

2. **Proteosomes:** found from bacteria to man

Machine that has been called "chamber of doom", but is necessary for life

Composed of 2 to 4 seven membered rings stacked on top of each other

In yeast and in humans the subunits are isozymes-structurally homologous (~21kDa) and each of the 7 constituents of the ring can have a different sequence. In the bacterial proteosome, all of the subunits of the 7 membered ring are identical.

Structures from cryoEM and crystallography
See page 12 handout 4a

One type alpha/beta/beta/alpha
Only beta rings are active, even though alpha and beta are structurally homologous

How do we get unfolded protein through the small hole in the proteosome and translocate??