

Protein Degradation

76

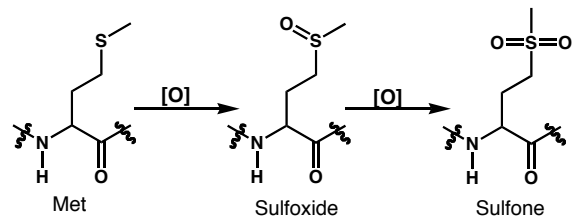
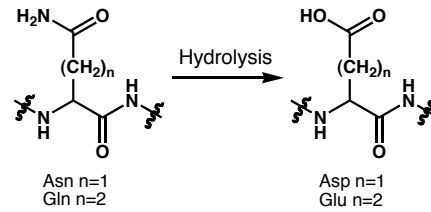
Protein Degradation

- Proteins are not immortal nor are they immutable.
- Over time, proteins can be subjected to gradual covalent modification as a result of non-enzymatic and enzyme-mediated chemical reactions.
- Cellular proteins are in a state of flux, they are being synthesized, and they are being broken down. The relative rates of these processes determines the amount of protein present.
- Degradation provides a means of eliminating proteins that have become damaged, denatured or misfolded as well as abnormal proteins (errors in biosynthesis).
- Degradation is also used to regulate the levels of various proteins .
- The various protein degradation pathways are highly regulated, and are compartmentalized within organelles (lysosomes) and macromolecular structures (proteasomes).

77

Chemical Aging

- Proteins age and are susceptible to chemical change and modification (wear and tear).
 - Oxidation of sulfur atoms of Cys and Met residues.
 - Deamidation of side chains of Asn and Gln residues.
 - Racemization of C_α and epimerization of side chain chiral centers (Thr and Ile).
 - ◆ Asn and Asp residues particularly susceptible to racemization.
 - ◆ Most proteins are degraded before racemization becomes an issue.
 - Disruption of disulfide bonds.
 - Hydrolysis of peptide bonds (especially at Asp residues).
- Enzymes are present that repair some of this damage/modifications (especially Cys and Met oxidation).
 - Maintain intracellular glutathione in reduced form.

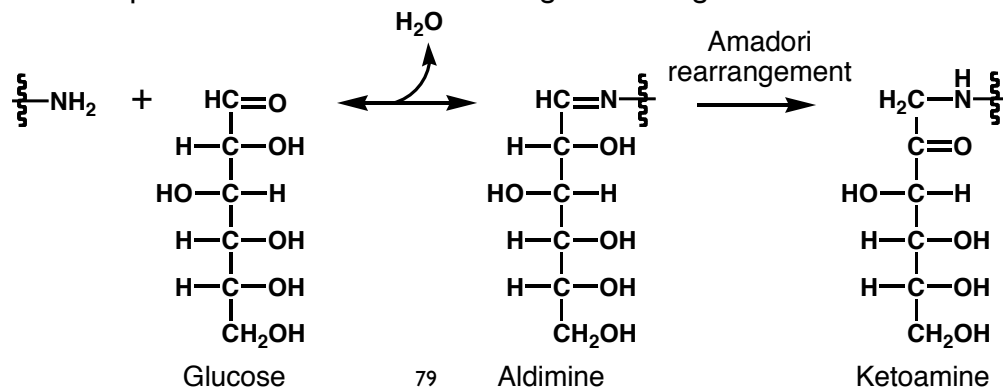


Peptide bonds adjacent to Asp, Ser and Thr are particularly susceptible to hydrolysis.

78

Chemical Aging

- Proteins are covalently modified by many chemicals present in the environment (many of which are man-made).
- Non-enzymatic reaction between primary amino groups and reducing sugars (such as glucose) results in formation of a Schiff base.
 - Schiff base then rearranges to form a stable ketoamine.
 - Dehydration of ketoamines results in yellow-brown fluorescent products and cross-links between protein molecules.
 - Particular problem when concentration of glucose is high.



79

Chemical Aging

- Chemical modification can significantly impact protein structure and function.
- Modifications that compromise structure, stability or function usually result in quick degradation of the affected protein.
- Alterations that do not trigger the degradation of a protein tend to accumulate throughout the lifetime of the protein.
- Most proteins turn over at rates that are determined by enzymatic processes.
- The only type of chemical modification that appears to be the primary determinant of the rate of degradation is the oxidation of bound metal centers, which results in the destruction of coordinating side chains.

80

Factors that Determine the Rate of Degradation

- Originally thought that the rate of degradation of a protein was related to:
 - susceptibility to thermal denaturation
 - absence of stabilizing ligands
 - intrinsic rate of deamidation of Gln and Asn residues
 - susceptibility of Cys, His and Met residues to oxidation
 - glycosylation or phosphorylation
 - presence of free α -amino group
 - net negative charge
 - flexibility of unfolded state.
- These make sense if susceptibility to enzyme proteolysis were the limiting factor.
- Too limiting... it assumes the same degradation mechanism is used in all cases, but this is likely not the case.

81

Factors that Determine the Rate of Degradation

- The variety of degradation mechanisms and rates suggest more specific signals are involved.
- Rapidly degraded proteins contain one or more 16-20 residue segments that are rich in Pro, Glu, Ser and Thr residues (PEST regions).
- The N-terminal amino acid can determine the rate of degradation in the ubiquitin-dependent system.
- Folded conformation is important in minimizing rate of degradation. Abnormalities such as chemical modifications, mutations, chain terminations or incorporation of amino acid analogs usually result in rapid degradation through enzymatic proteolysis.

N-terminal residue	Half-life
Stabilizing	
Met, Gly, Ala, Ser, Thr Val	>20 h
Destabilizing	
Ile, Gln	~30 min
Tyr, Glu	~10 min
Pro	~7 min
Leu, Phe, Asp, Lys	~3 min
Arg	~2 min

82

Proteases Involved in Protein Turnover

- A variety of proteases of varying specificities appear to be involved in the degradation of certain proteins.
- Many intracellular proteases such as the Ca²⁺-dependent calpains appear to be involved with the regulatory cleavage of specific enzymes and not protein degradation.
- The most interesting protein degradation is that which occurs within the cell, whereas most of the well-characterized proteases normally function extracellularly.
- Intracellular proteases tend to be larger than comparable extracellular proteases, with additional domains that function primarily in regulatory capacities.
- While hydrolysis of peptide bonds does not require energy input, the process of protein degradation within the cell usually does. The energy (in the form of ATP) is required for initiation of proteolytic degradation.

83

Proteases Involved in Protein Turnover

- Several different ATP-dependent proteases have been discovered in a variety of organisms.
- Some of the isolated ATP-dependent proteases are thiol proteases, and are activated by binding of ATP.
- The other group of ATP-dependent proteases are serine proteases that require ATP hydrolysis for proteolytic activity. They also require bound Mg^{2+} ions. ATPase activity is stimulated by the presence of protein/peptide substrates for degradation.
- Only two proteases, *La* and *Ti* (out of >20 known proteases) in *E. coli* are known to require ATP for enzyme activity.

84

Proteases Involved in Protein Turnover

- Only two proteases, *La* and *Ti* (out of >20 known proteases) in *E. coli* are known to require ATP for enzyme activity.
- *La* is a tetrameric serine protease, assembled from four identical polypeptide chains (87KD each).
 - Production of *La* is stimulated by environmental stress such as heat shock. It appears to degrade abnormal and denatured proteins that can result under stressful conditions (appears to degrade short lived regulatory proteins as well).
 - *La* requires ATP hydrolysis for proteolytic activity. ATPase activity is stimulated by the presence of protein substrates.
 - ATP appears to play a regulatory role and does not contribute directly to the proteolytic mechanism.
 - Bound ATP is hydrolyzed following proteolysis of a peptide bond in the substrate. (rate limiting step appears to be release of ADP)
 - As a homotetramer, the complex incorporates 4 active sites. All four appear to be under allosteric control.

85

Proteases Involved in Protein Turnover

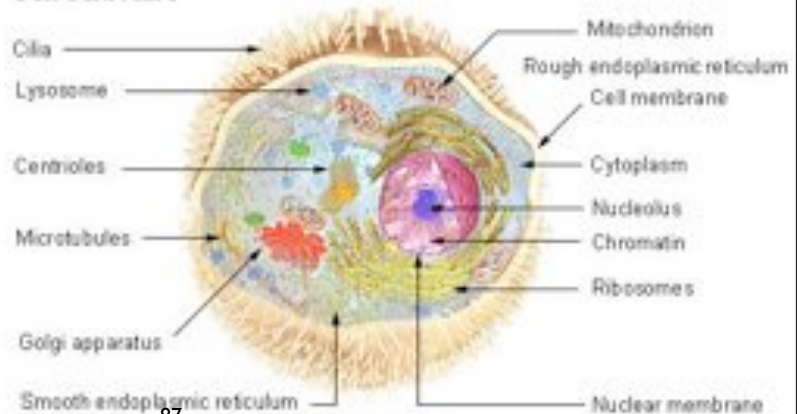
- Only two proteases, *La* and *Ti* (out of >20 known proteases) in *E. coli* are known to require ATP for enzyme activity.
- *Ti* is a complex of two polypeptide chains. One chain provides proteolytic site the other the ATPase site. (the isolated proteolytic chain demonstrates ATP independent proteolytic activity in the absence of the ATPase chain.
 - ATP hydrolysis by the ATPase chain activates the proteolytic chain.
 - Like *La*, *Ti* is a heat-shock protein that is produced to degrade damaged proteins.

86

Lysosomes

- Lysosomes are intracellular compartments involved with protein degradation. They contain a variety of proteases that require acidic conditions for enzyme activity (collectively known as cathepsins).
- Some intracellular protein degradation takes place in the lysosomes.
- Cytoplasmic proteins are believed to enter lysosomes when a small volume of cytoplasm is sequestered by cell membrane, which then fuses with the lysosome. (believed to be mainly in response to starvation conditions)
- Alternatively, intracellular proteins may be introduced into the lysosome when invaginations in the lysosome membrane occurs. (believed to be the normal pathway for the degradation of long-lived proteins)

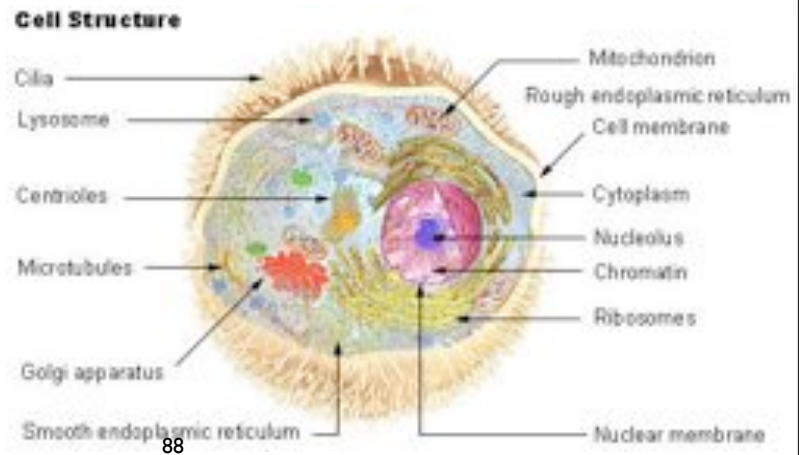
Cell Structure



87

Lysosomes

- It has been proposed that a third pathway exists that is activated under specific conditions. This pathway involves the selective uptake of proteins and peptides based on a specific peptide motif.
- Lysosomes are involved with the uptake and digestion of exogenous proteins, which are taken up from the extracellular medium through endocytosis.



Ubiquitination

- In eukaryotes, proteins destined for degradation are tagged by the addition of many molecules of ubiquitin.
- Ubiquitination is carried out via a multi-enzyme pathway (three enzymes/enzyme complexes E₁, E₂ and E₃).
 1. Ubiquitin-activating enzyme (E₁) is a 105-kD dimer.
 2. Initially, the α -carboxyl group of the C-terminal Gly residue of ubiquitin is activated by E₁ with formation of an adenylated ubiquitin intermediate (using ATP).
 3. The activated α -carboxyl group of ubiquitin is then transferred to the thiol side chain of a cys residue on the ubiquitin-carrier protein E₂ (family of at least 7 different proteins).
 4. E₂ transfers the ubiquitin to the α - and ϵ -amino groups of target proteins.



Ubiquitination

- E2 transfers ubiquitin to free primary amino groups on proteins that have been selected/bound by ubiquitinating-protein ligase (E₃).
- Like E₂, there are a variety of E₃ proteins (each is ~180-kD).
- Once one ubiquitin molecule has been added, additional ubiquitin molecules are added to the attached ubiquitin molecule(s) via their ϵ -amino groups, resulting in a ubiquitin chain.
- E₃ plays central role in targeting proteins for degradation. Selects proteins based on nature of N-terminal amino acid:
 - Protein must have free N-terminus.
 - Proteins with N-terminal Met, Ser, Ala, Thr, Val, Gly or Cys are resistant to ubiquitin-mediated degradation.
 - Those with N-terminal Arg, Lys, His, Tyr, Phe, Trp, Leu, Asn, Gln, Asp or Glu are targeted and have half lives of 2-30 minutes.
 - Arg is added at N-terminus of proteins with N-terminal Asp or Glu residues prior to degradation. 90



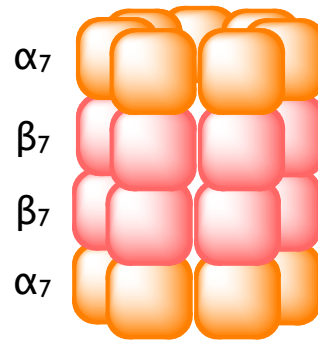
Ubiquitination

- Most proteins susceptible to degradation through N-terminal degradation are normally secreted proteins, not normally found intracellularly.
- PEST sequence also targets proteins for ubiquitin-mediated degradation.
- Unfolded, partially-unfolded and damaged proteins are also targets for ubiquitination.



Proteasome

- Special case of ATP-dependent protease.
- Proteasomes are large oligomeric structures, with a large central cavity which is site of proteolysis.
- Eukaryotic cells contain two forms of proteasomes: 20S proteasome and 26S proteasome.
 - 20S proteasome is a 700-kD barrel-shaped structure assembled from two different types of polypeptide chain (α and β).
 - Assemble into four stacked heptameric rings: $\alpha_7\beta_7\beta_7\alpha_7$ organization.
 - Proteolytic sites are located within cavity that is 15nm long and 11nm in diameter.
 - Access to cavity mediated by outer α_7 rings (also believed to unfold target proteins).
 - subunits possess proteolytic site (N-terminal Thr residues functions much like Ser in serine proteases.)
 - Targets broken down into oligopeptides ranging from 7-9 residues in length.



Note: ubiquitin not observed in prokaryotes.

- Eukaryotic cells contain two forms of proteasomes: 20S proteasome and 26S proteasome.
 - Full 26S proteasome is on the order of 2.5 megadaltons, and is 45 nm in length.
 - 26S proteasome incorporates a 20S proteasome plus two additional structures known as 19S regulators (a.k.a. 19S caps or PA700).
 - Unlike archaeal 20S proteasomes, each α_7 ring is assembled from 7 different α subunits, and each β_7 ring composed of 7 different β subunits.
 - Only three of the 7 β subunits in each ring have protease active sites.
 - Many of the 19S regulator subunits have ATPase activity, and play a role in substrate specificity.
 - The 19S regulator units play a role in recognizing and selecting ubiquitinated proteins for degradation.
 - The 19S regulator units also carry out unfolding and transport of ubiquitinated proteins into the proteolytic central cavity.

