IIT Guwahati

Lecture 39

Course BT 631

Protein Structure, Function and Crystallography

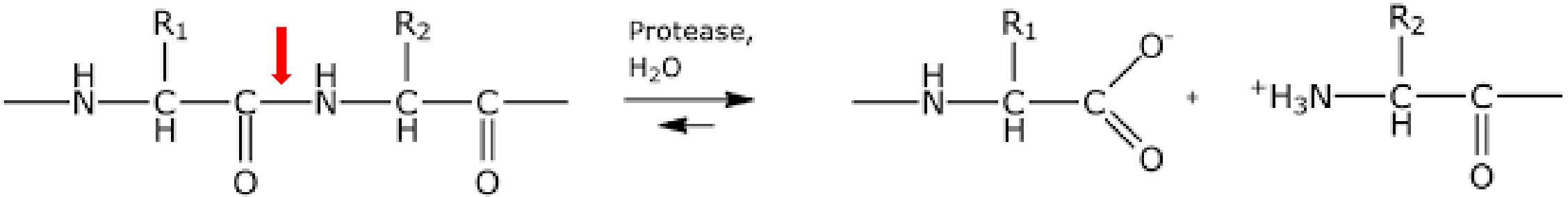
Prof. Arun Goyal

Dept. of Biosciences and Bioengineering

Proteases

- Proteases are protein scissors also known as peptidases/proteinases found in animals, plants, bacteria, archea and viruses.

- They perform proteolysis by catalyzing the hydrolysis of the peptide bonds in the polypeptide chain of proteins liberating free amino acids.



- Proteolysis takes place not only in digestive tract but also in lysosomes, cytoplasm and other parts of the cell. The free amino acids are used up throughout the body.

- Proteases range from small enzymes of molecular mass, 20 kDa (eg. caspases) to sophisticated protein-processing and degradation machines of molecular mass 700 kDa (eg.26S and 30S proteosomes) to 6000 kDa (eg. Metalloproteases like Meprin A).

Types of proteases

There are two types of proteases, based on the cleavage site within the substrate molecule. i) Exo-proteases and ii) Endo-proteases.

i) Exo-proteases: Remove terminal amino acid from the protein chain liberating monomers and dipeptides.

Some examples of Exo-proteases are:

- Aminopeptidases: catalyzes the removal of a single amino acid from amino terminus.
- Dipeptidyl-dipeptidases: catalyzes the removal of dipeptides from amino terminus.
- Carboxypeptidases: catalyzes the removal of single amino acid from carboxy terminus.
- Dipeptidases: hydrolyse dipeptides into two single amino acids.

Types of proteases

ii) Endo-proteases: **Proteases cleaving the peptide bonds in the middle of a protein chain.**

Some examples of endo-proteases are:

Endo-proteases	Cutting sites	Optimal pH
Trypsin	Cleaves at C-terminal of protein after Lys or Arg, except these residues are followed by Pro	8.0
Chymotrypsin	Cleaves proteins at C-terminus after Phe, Tyr or Trp, except these residues are followed by Pro	8.0
Pepsin	Cleaves proteins at N-terminal before Leu, Phe, Trp or Tyr, except there is a Pro before these residues	2.0
Thermolysin	Cleaves at N-terminal before the hydrophobic residues Leu, Phe, Val, Iso, Ala and Met, except when there is a Pro before these residues	5.0 - 8.5
Elastase	Cleaves at C-Terminus after Ala, Val, Ser, Gly, Leu and Ile	9.0

Types of proteases

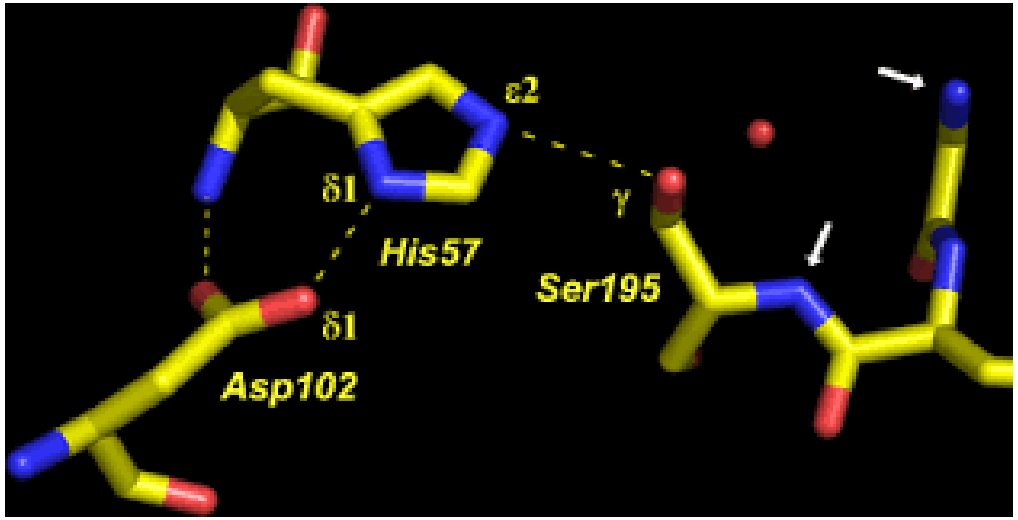
Types of Proteases based on the catalytic activity:

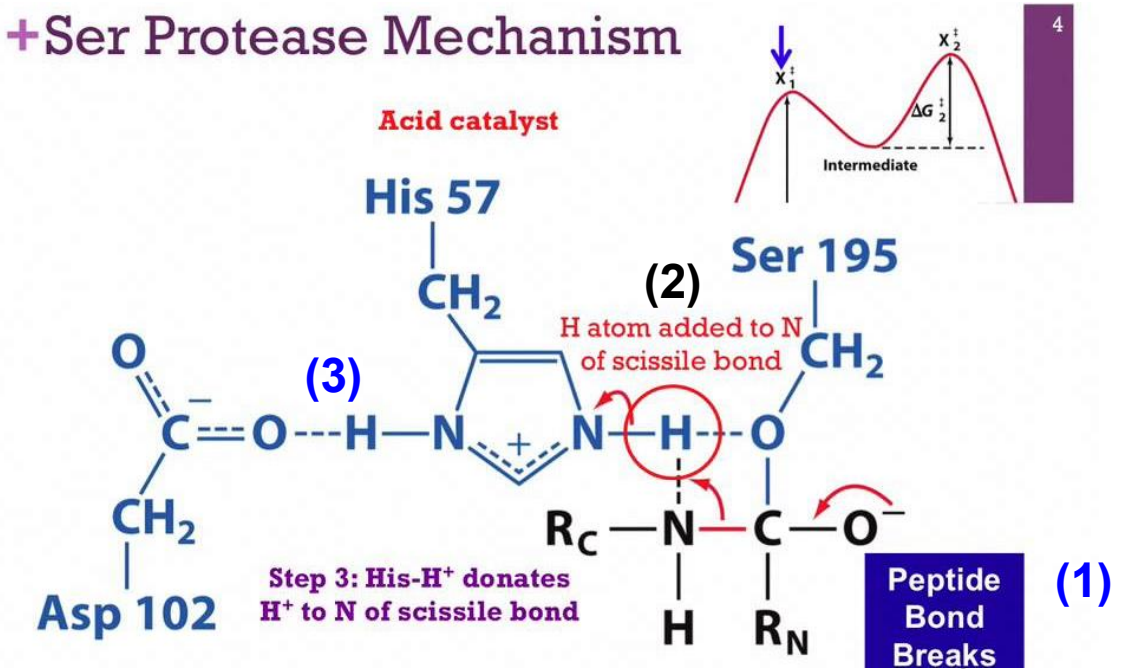
- **Serine proteases:** cleave the peptide bonds in proteins, where Serine serves as the nucleophilic amino acid at the active site, e.g., trypsin, chymotrypsin, subtilisin, elastase.
- **Cysteine (thiol) proteases:** cleave the proteins, in which Cysteine is involved in a nucleophilic attack. e.g., Papain, Cathepsins, Caspases, Actinidain, Bromelain.
- **Threonine proteases:** These enzymes harbour a Threonine residue within the active site. e.g., beta component of archean proteasome, ornithine acetyltransferase.
- **Metalloproteases:** A protease which require a Metal ion for its catalytic action, e.g., thermolysin, meltrin, carboxypeptidase A.
- **Aspartic proteases:** These proteases use an Aspartate residue for catalysis of their peptide substrates, e.g., renin, pepsin, HIV protease.
- **Glutamic acid proteases:** These protease have Glutamic acid and Glutamine at their active site. e.g. scytalido-glutamic peptidase (eqolisin) and aspergilloglutamic peptidase.

Structure and mechanism of proteases

Serine proteases

- The catalytic triad of the serine proteases consists of Serine-Histidine-Aspartic acid.
- Serine acts as a nucleophile which cleaves the substrate peptide bond by nucleophilic attack. (1)
- Histidine acts as a general base where its imidazole ring positions the serine side-chain and polarizes the hydroxyl group by abstracting a proton. (2)
- Aspartate residue plays a role in correcting the orientation of histidine, making it a better proton acceptor through electrostatic effects. (3)

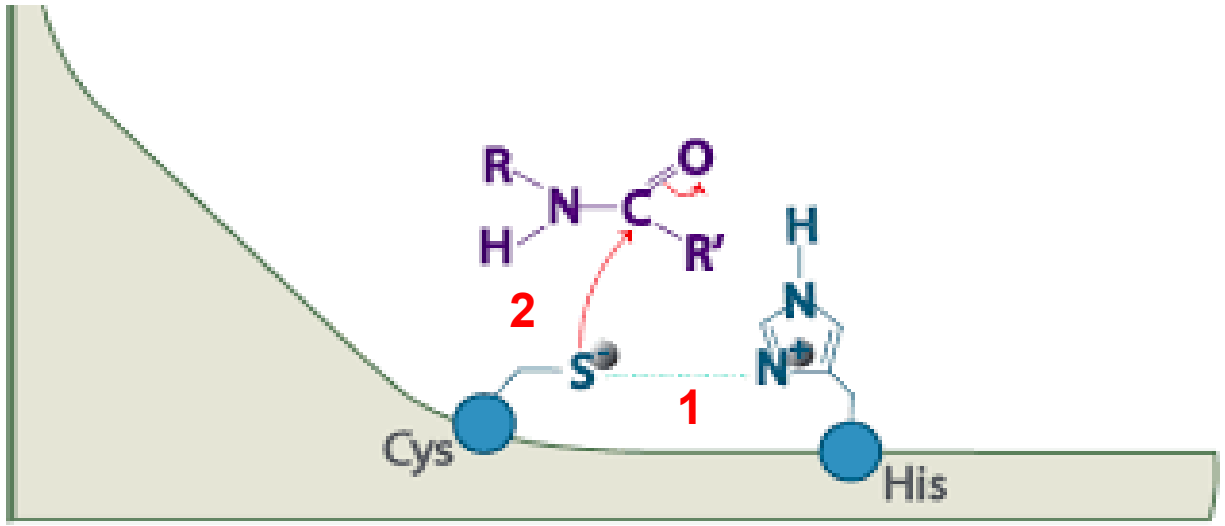




Structure and mechanism of proteases

Cysteine proteases

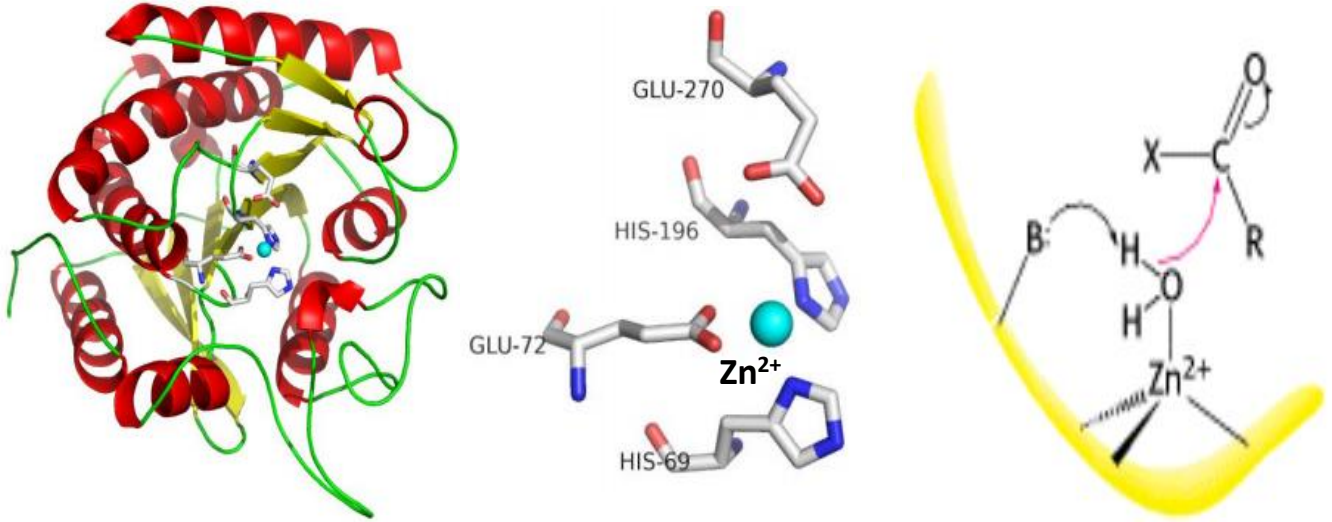
- The catalytic dyad in cysteine consists of Cysteine and Histidine.
- The imidazole ring of histidine deprotonates the cysteine's thiol group at the enzyme's active site. (1)
- Deprotonated cysteine catalyzes the hydrolysis of substrate's peptide bond releasing a fragment of the substrate (2)



Structure and mechanism of proteases

Metalloproteases

- **Metalloproteases involves a Metal ion to break peptide bonds.**
  Most metalloproteases use Zinc as their metal, but a few use Cobalt.

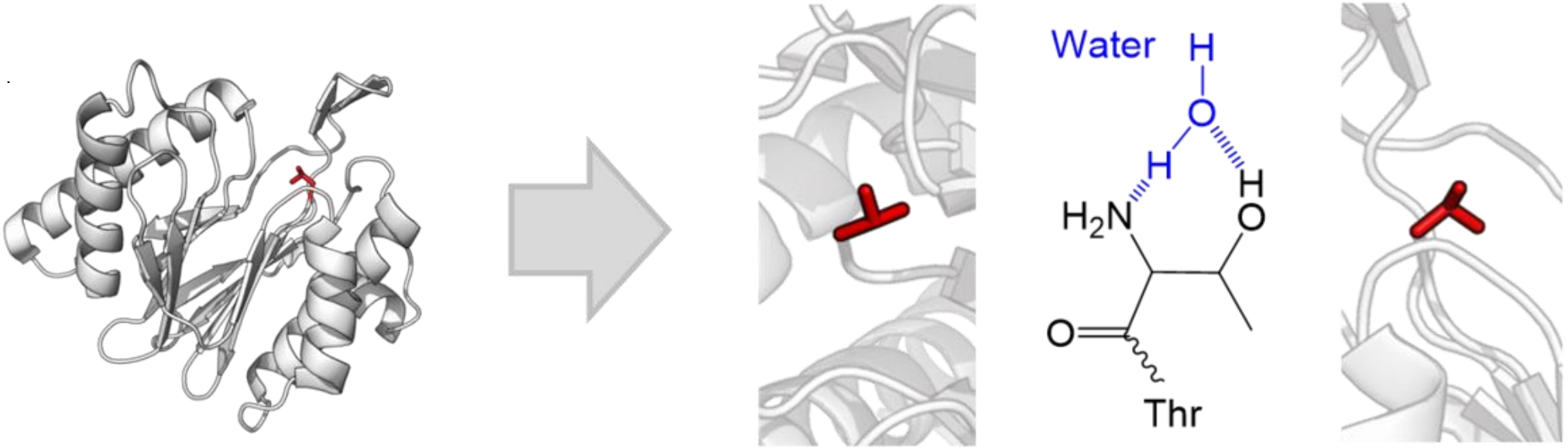


- **The metal ion is coordinated by three amino acid residues with a labile water at the fourth position.**
- **The metal ion activates the water molecule to act as a nucleophile to cleave the peptide bond in the substrate.**

Structure and mechanism of proteases

Threonine proteases

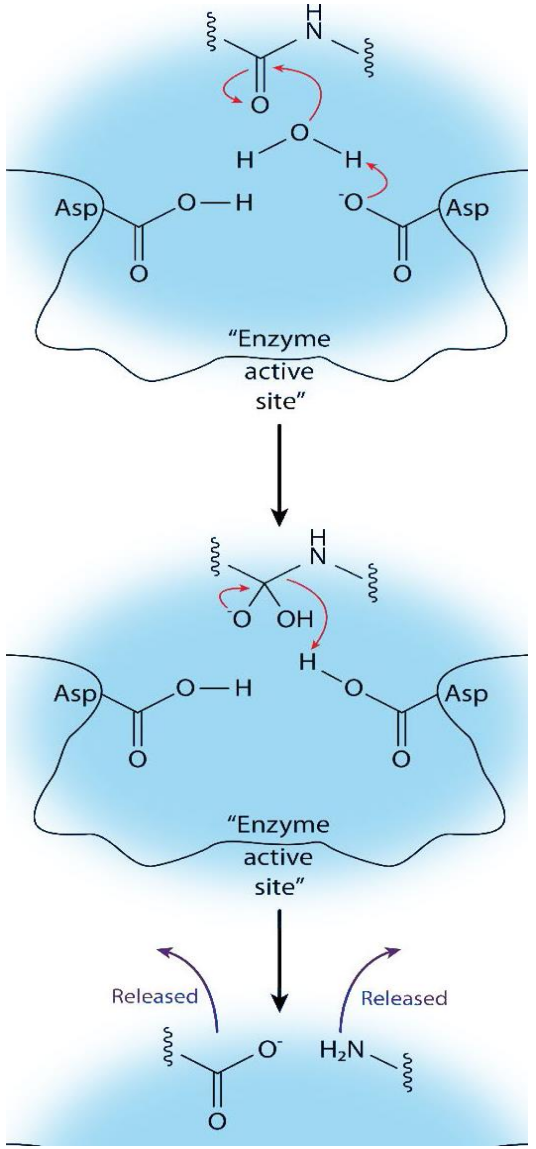
- These proteases have threonine in their active site.
- Threonine proteases use their own amino and hydroxyl group for cleaving the substrate.
- Amino group of threonine deprotonates and activates the hydroxyl group with the help of water leading to the cleavage of peptide bond of the substrate.



Structure and mechanism of proteases

Aspartic proteases

- Aspartic proteases have two highly conserved aspartate residues at the active site.
- One of them act as an catalytic acid and the other one as catalytic base.
- The acidic aspartate deprotonates water, (1).
- Deprotonated water cleaves the substrate's peptide bond by nucleophilic attack (2).



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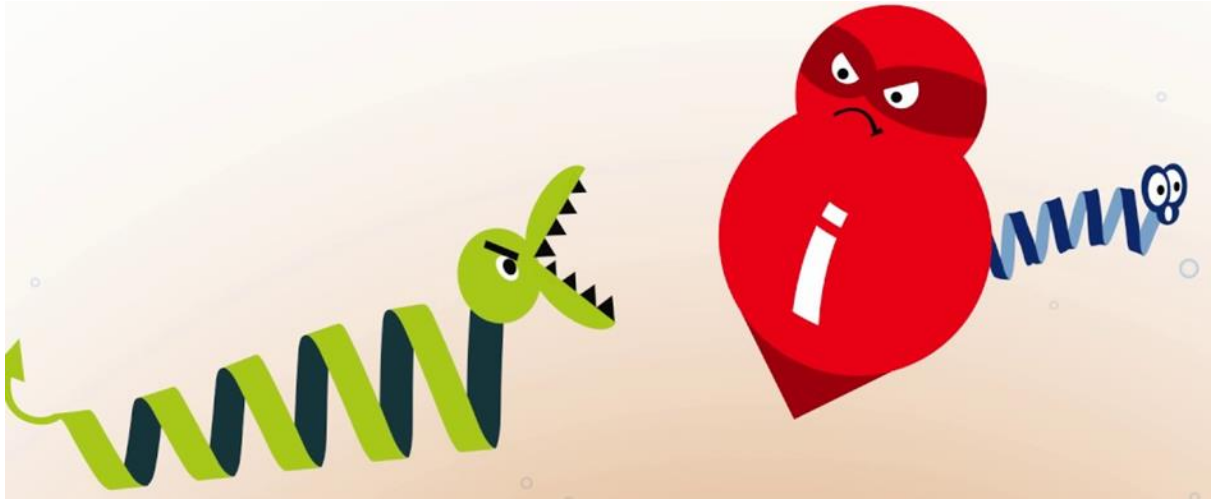
Protease inhibitors

Protease inhibitors are agents that can prevent a protease from splitting a protein into peptides.



Some common examples of protease inhibitors are:

- **Captopril:** an inhibitor of the metalloprotease angiotensin-converting enzyme (ACE).
- **Crixivan:** an inhibitor of the HIV protease, is used in the treatment of AIDS.
- **Pepstatin:** an inhibitor of aspartyl proteases.
- **Serpins:** an inhibitor of serine and cysteine proteases.

Function of Proteases

Papain: This enzyme can hydrolyze primary connective tissues and therefore can be used as meat tenderizer. Papain also cleave the Fc portion of immunoglobulins from the Fab portion. It can also dissociate cells in the first step of cell culture preparations.

Cathepsin: Mammalian cathepsins have a role in the immune system and bone resorption.

Caspases: plays a major role in apoptosis.

Ficain: Used for differentiating many blood group antigens.

Bromelain: Used as meat tenderizer and also for treatment of breast cancer by systemic enzyme therapy.

Pepsin: One of the most important digestive enzymes and used as dietary supplement.

Rennin: Widely used in the production of milk products like curd and cheese.

Trypsin: Used for resuspension of adherent cells in tissue culture lab, digestion of proteins into peptides for mass spectrometry analysis, used as baking enzyme to improve the workability of dough, used in the manufacture of sauces, improve the texture of fish products, meat tenderization and for stabilization of cold beer. Besides it is also used to produce hypoallergic food by breaking specific allergenic proteins.