Review Article

The importance of proteolytic enzymes: Revisiting to era

Neelam N. Saraf[1], Surinder Kaur Makhija[2]

[1]Department of Pharmaceutical Analysis, Shri Bhagwan College of Pharmacy, Aurangabad, Maharashtra, India, [2]Department of Biochemistry, S. B. Science College, Aurangabad, Maharashtra, India

Correspondence:
Mrs. Neelam N. Saraf, Department of Pharmaceutical Analysis, Shri Bhagwan College of Pharmacy, Aurangabad, Maharashtra, India.
E-mail: sarafneelam21@gmail.com

How to cite this article:
Saraf NN, Makhija SK. The importance of proteolytic enzymes: Revisiting to era. Innov Pharm Pharmacother 2017;5(4):181-185.

Source of Support: Nil,
Conflict of Interest: None declared.

ABSTRACT

Proteolytic enzymes (also termed peptidases, proteases, and proteinases) are capable of hydrolyzing peptide bonds in proteins. They can be found in all living organisms, from viruses to animals and humans. Proteolytic enzymes have great medical and pharmaceutical importance due to their key role in biological processes and in the lifecycle of many pathogens. These enzymes have amino acid sequences similar to mammalian enzymes, even both insect amylases and serine proteinases differ from mammalian enzymes in substrate specificity and conduct within the sight of protein inhibitors. There is a renewed interest in proteases as targets for developing therapeutic agents against relentlessly spreading fatal diseases such as cancer, malaria, and AIDS. Advances in genetic manipulation of microorganisms by site-directed mutagenesis of the cloned gene open new possibilities for the introduction of predesigned changes, resulting in the production of tailor-made proteases with novel and desirable properties. Proteases are extensively applied enzymes in several sectors of industry and biotechnology, furthermore, numerous research applications require their use, including production of Klenow fragments, peptide synthesis, digestion of unwanted proteins during nucleic acid purification, cell culturing and tissue dissociation, preparation of recombinant antibody fragments for research, diagnostics and therapy, exploration of the structure-function relationships by structural studies, removal of affinity tags from fusion proteins in recombinant protein techniques, peptide sequencing, and proteolytic digestion of proteins in proteomics. The aim of this paper is to review the importance of proteolytic enzyme and molecular biological aspects of proteolytic enzymes and their applications in the life sciences.

Keywords: Biological aspects, life sciences, proteolytic enzymes, therapeutic agents

Introduction

A protease is an enzyme that performs proteolysis; protein catabolism by hydrolysis of peptide bonds. Proteases have evolved multiple times, and different classes of protease can perform the same reaction by completely different catalytic mechanisms. Proteases can be found in animalia, plantae, fungi, bacteria, archaea, and viruses. Proteolytic enzymes catalyze the cleavage of peptide bonds in different proteins. Proteases are degradative enzymes, which catalyze the hydrolysis of proteins. Proteolytic enzymes, also known as proteases, are the enzymes that catalyze the hydrolytic cleavage of particular peptide bonds in their target proteins (TPs). These enzymes have amino acid sequences similar to mammalian enzymes, even both insect amylases and serine proteinases differ from mammalian enzymes in substrate specificity and conduct within the sight of protein inhibitors. Digestive enzymes in insects happen in midgut luminal substance or may be limited to midgut cells. In cells, they might be connected with the glycocalyx or bound to microvillar layers. The discovery in plants of protein inhibitors influencing insect digestive enzymes called attention to the possibility of utilizing these enzymes as targets as a part of the advancement of new insect control techniques.[1] Proteinase inhibitors (PIs) are universal small proteins that are very normal in nature. They are natural, barrier-related proteins frequently present in seeds and affected in certain plant tissues by herbivore or injuring.[2,3] PIs are available in numerous structures in various tissues of animals and plants and, in addition, in microorganisms. In plants, they can be considered as a part of the defensive mechanism showed against phytophagous insects and microorganisms. The defensive capacities of plant PIs depend on the hindrance of proteases present in insect guts or discharged by microorganisms, bringing about a reduction in the accessibility of amino acids vital for the development and improvement.[4]

Proteases are extensively applied enzymes in several sectors of industry and biotechnology, furthermore, numerous research applications require their use, including production of Klenow fragments,

Access this article online

Website: www.innpharmacotherapy.com

e-ISSN: 2321-323X
p-ISSN: 2395-0781

peptide synthesis, digestion of unwanted proteins during nucleic acid purification, cell culturing and tissue dissociation, preparation of recombinant antibody fragments for research, diagnostics and therapy, exploration of the structure-function relationships by structural studies, removal of affinity tags from fusion proteins in recombinant protein techniques, peptide sequencing, and proteolytic digestion of proteins in proteomics.

Plant proteases

Proteolysis is crucial for plant physiology and improvement. It is responsible for removing abnormal/misfolded proteins, supplying amino acids required to make new proteins, helping the development of zymogens and peptide hormones by restricted cleavage, controlling digestion system, and for programmed cell death of particular cells in plant organs.

Animal proteases

The most commonly known proteases from animal origin are pancreatic trypsin, chymotrypsin, pepsin, and rennins.[5,6] These are derived in pure form in large quantities. However, their production depends on the availability of livestock for slaughter, which in turn is governed by political and agricultural policies.

Trypsin and Chymotrypsin

Trypsin and chymotrypsin, like most proteolytic enzymes, are synthesized as inactive zymogen precursors (trypsinogen and chymotrypsinogen) to prevent unwanted destruction of cellular proteins and to regulate when and where enzyme activity occurs. The inactive zymogens are secreted into the duodenum, where they travel the small and large intestines before excretion. Zymogens also enter the bloodstream, where they can be detected in serum before excretion in urine. Zymogens are converted to the mature, active enzyme by proteolysis to split off a pro-peptide, either in a subcellular compartment or in an extracellular space where they are required for digestion [Figure 1].

Trypsin and chymotrypsin are structurally very similar, although they recognize different substrates. Trypsin acts on lysine and arginine residues, while chymotrypsin acts on large hydrophobic residues such as tryptophan, tyrosine, and phenylalanine, both with extraordinary catalytic efficiency. Both enzymes have a catalytic triad of serine, histidine, and aspartate within the S1 binding pocket; although the hydrophobic nature of this pocket varies between the two, as do other structural interactions beyond the S1 pocket.

The human pancreas secretes three isoforms of trypsinogen: Cationic (trypsinogen 1), anionic (trypsinogen 2), and mesotrypsinogen (trypsinogen 3). Cationic and anionic trypsins are the major isoforms responsible for digestive protein degradation, occurring in a ratio of 2:1, while mesotrypsinogen accounts for <5% of pancreatic secretions. Mesotrypsin is a specialized protease known for its resistance to trypsin inhibitors. It is thought to play a special role in the degradation of trypsin inhibitors, possibly to aid in the digestion of inhibitor-rich foods such as soybeans and lima beans. An alternatively spliced mesotrypsinogen in which the signal peptide is replaced with a different exon 1 is expressed in the human brain; the function of this brain trypsinogen is unknown. There are two isoforms of pancreatic chymotrypsin, A and B, which are known to cleave proteins selectively at specific peptide bonds formed by the hydrophobic residues tryptophan, phenylalanine, and tyrosine [Figure 2].

The inability to examine trypsin in midgut homogenates shows a low sensitivity of their assay procedure as opposed to for the presence of a trypsinogen[7] sequenced, what appeared to be a precursor of midgut trypsin in *Aedes aegypti*. Its sequence is similar to that of most trypsins, despite the fact that it showed significant differences from the vertebrate trypsin precursors in the region of the activation peptide. Comparable results were found with a putative trypsinogen from *Drosophila melanogaster* (Meigen)[8] and from *Simulium vittatum* (Zetterstedt) (Diptera: Simuliidae).[9] These differences suggest that the processing of precursors of insect trypsins might be distinctive from that of vertebrates. There is confirmation in *Tineola bisselliella* (Hummel),[10] furthermore, *Bombyx mori*[11,12] that dissolvable trypsin is derived from membrane-bound structures. *Erinnyis ello*[13,14] and in *Musca domestica* (Linnaeus)[15-18] trypsin is synthesized in midgut cells in an active structure, however, is connected with membranes of little vesicles. In any case, some properties of insect chymotrypsin contrast to those of vertebrate chymotrypsin, for example, their instability at acid pH[10,19] and their strong inhibition by soybean trypsin inhibitor [Figure 3].[20-22]

Some other proteases such as pepsin and renin. Pepsin is an acidic protease found in the stomach of the vertebrates. Pepsin is one of the central protein degrading or proteolytic enzymes in the digestive system. Rennin is an enzyme secreted by the kidney that separates protein and produces an ascent in blood pressure. Rennet is a pepsin-like protease (rennin, chymosin; EC 3.4.23.4) that is produced as an inactive precursor, prorennin, in the stomach of all nursing mammals.

Microbial Protease

The ability of the plant and animal proteases to meet current world demand has led to an increased interest in microbial proteases. Microorganisms represent an excellent source of enzyme owing to their broad biochemical diversity and their susceptibility to genetic manipulation. Microbial proteases account for approximately 40% of the total worldwide enzymes sales.[23]

Bacteria

Most commercial proteases, mainly neutral and alkaline, are produced by organisms belonging to the genus bacillus. Bacterial neutral proteases are active in a narrow pH range (pH 5–8) and have relatively low thermotolerance. The bacterial neutral proteases are characterized by their high affinity for hydrophobic amino acid pairs. Their low thermotolerance is advantages for controlling their reactivity during the production of food hydrolysates with a low degree of hydrolysis.

Fungi

Fungi elaborate a wider variety of enzymes than do bacteria. For example, *Aspergillus oryzae* produces acid, neutral, and alkaline proteases. The fungal proteases are active over a wide pH range (pH 4–11) and exhibit broad substrate specificity.

Viruses

Viral proteases have gained importance due to their functional involvement in the processing of proteins of viruses that cause certain fatal diseases such as AIDS and cancer. Serine, aspartic, and cysteine peptidases are found in various viruses.[24]

Currently, proteases are classified on the basis of three major criteria:

1. Type of reaction catalyzed
2. Chemical nature of the catalytic site
3. Evolutionary relationship with reference of structure.

Proteases are grossly subdivided into two major groups, i.e., exopeptidases and endopeptidases depending on their site of action. Exopeptidases cleave the peptide bond proximal to the amino or carboxyl termini of the substrate, whereas endopeptidases cleave peptide bonds distant from the termini of the substrate. Based on the functional group present at the active site, proteases are future classified into four prominent groups, i.e., serine proteases, aspartic proteases, cysteine proteases, and metalloproteases. There are a few miscellaneous proteases which do not precisely fit into the standard classification, for example, ATP-dependent proteases which require ATP for activity.[25]

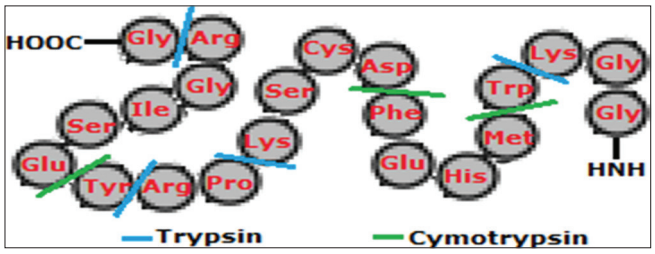

Figure 1: Trypsin and chymotrypsin cleavage the protein at different site

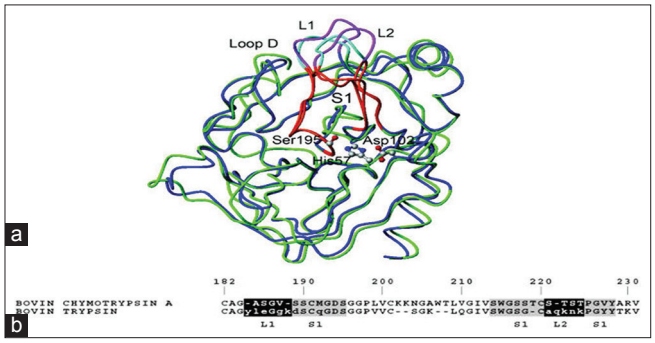

Figure 2: Superposition of trypsin and chymotrypsin. (a) The two enzymes have very similar tertiary structure. Trypsin is shown in green ribbon and chymotrypsin in blue. Active site residues of trypsin are shown in ball and stick. Loops of trypsin are shown in magenta; loops of chymotrypsin are shown in pale green. S1 binding pocket is shown in red[1]

Mechanism of Action

Purification of proteases to homogeneity is a prerequisite for studying their mechanism of action. Vast numbers of purification procedures for proteases, involving affinity chromatography, ion-exchange chromatography, and gel filtration techniques have been well documented. Preparative polyacrylamide gel electrophoresis has been used for the purification of proteases from *Conidiobolus coronatus*. Purification of staphylocoagulase to homogeneity was carried out from culture filtrates of *Staphylococcus aureus* by affinity chromatography with a bovine prothrombin-sepharose hydrolases have been isolated and purified from *Escherichia coli* by DEAE-cellulose chromatography.[26]

The catalytic site of protease is flanked on one or both sides by specificity subsites, each able to accommodate this side chain of a single amino acid residue from the substrate. These sites are numbered from the catalytic site S1 through Sn toward the N terminus of the structure and S19 through Sn9 toward the C terminus. The residues which they accommodate from the substrate are numbered PI through Pn and P19 through Pn9, respectively [Figure 4].[27]

Genetic engineering, a rapidly developing field of biotechnology relates to the utilization of transgenic plants as bioreactors to getting proteins of commercial or medical importance (enzymes, hormones, antibodies, blood plasma proteins, etc.).[28] Plant expression systems utilized for obtaining recombinant proteins have certain advantages over their microbial or animal counterparts. The low net cost of the

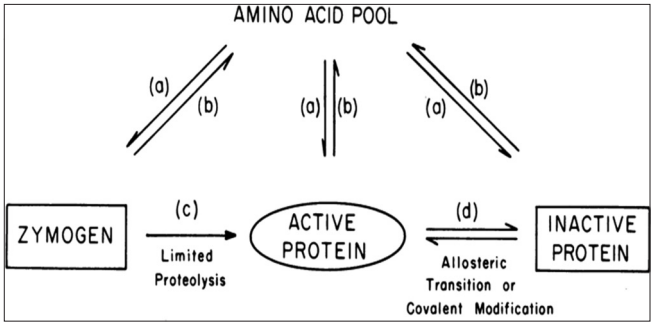

Figure 3: Schematic representation of major control mechanisms. (a) Transcription and translation regulate the rate of formation of the various proteins from the amino acid pool. (b) Other controls regulate the rate of degradation of the various proteins to their constituent amino acids. (c) The activity of the protease that catalyzes zymogen activation may be, in turn, regulated by a series of consecutive reactions of limited proteolysis. (d) Reversible conformational changes are responsive to effector concentrations or to the activities of specific group transferases and hydrolases

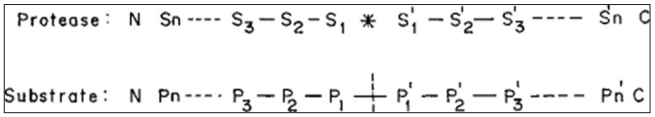

Figure 4: Active sites of proteases. The catalytic site of proteases is indicated by p and the scissile bond is indicated by; S1 through Sn and S19 through Sn9 are the specificity subsites on the enzyme, while P1 through Pn and P19 through Pn9 are the residues on the substrate accommodated by the subsites on the enzyme

final product is the major advantage.[28,29] Among the disadvantages in the low TP yield, this was seen in a number of cases. One of the reasons for low yield is the quick cleavage of foreign proteins by proteolytic enzymes.[30] In this connection, broad utilization of natural inhibitors of proteinases may turn into a possibly encouraging approach to increase the yield of recombinant proteins in plant expression systems.[31,32] The utilization of strategies of genetic engineering for acquiring plant forms that would be resistant to insect pests is a noteworthy field of contemporary biotechnology.[33-35] Representatives of the first generation of such plants harbor the genes of delta endotoxins (cry proteins) of the Gram-positive soil bacterium *Bacillus thuringiensis* (Berliner) (*Bt* toxins).[36,37] The cultivation area of such plants added up to 22 million hectares in 2004.[38]

Antibody molecules are produced by the immune system against foreign substances and are classified into the immunoglobulin superfamily of the proteins [Figure 5a]. They consist of four polypeptide chains, two identical heavy chains (H) and two identical light chains (L) which are connected by disulfide bridges. Both the H and the L chains contain variable (VH and VL) and constant (CH1, CH2, CH3, and CL) regions, respectively. The VH and VL chains, containing hypervariable regions, are responsible for the antigen-antibody interactions and determine the antigen specificity.[39] Fragments of the monoclonal antibodies are widely used in diagnostics, therapeutics, and in biopharmaceutical research having beneficial properties compared to the whole immunoglobulin molecules due to their smaller size and lower immunogenicity.[40] Fragments of whole immunoglobulin molecules can be produced using recombinant DNA technology or can be generated by enzymatic digestion. Here, we discuss the proteolytic antibody fragmentation method. In general, the papain, pepsin, and ficin proteases are used for the specific digestion of IgG molecules. Digestion of an antibody by the cysteine protease papain results in three fragments due to the cleavage of peptide bonds in the hinge region between CH1 and CH2 domains: One Fc (crystallizable) and two identical Fab (antigen binding) fragments are released [Figure 5b]. While both released Fab fragments carry one antigen-binding site, the Fc fragment does not have antigen-binding ability. The aspartic acid protease pepsin cleaves the peptide bonds of the antibody near the disulfide bonds connecting the H chains [Figure 5c]. This digestion results in the release of the peptides of the Fc region and one F(ab')2 fragment containing both antigen-binding sites. The cysteine protease ficin can release both F(ab')2 or Fab fragments [Figure 5d], depending on the cysteine concentration [Figure 6].[40]

Another so many properties use in proteomic application: [41]

Summary

Proteases are a unique class of enzymes since they are of immense physiological as well as commercial importance. They possess both degradative and synthetic properties. Since proteases are physiologically necessary, they occur ubiquitously in animals, plants, and microbes. Microbial proteases have been extensively used in the food, dairy, and detergent industries since ancient times. There is a renewed interest in proteases as targets for developing therapeutic agents against relentlessly spreading fatal diseases such as cancer,

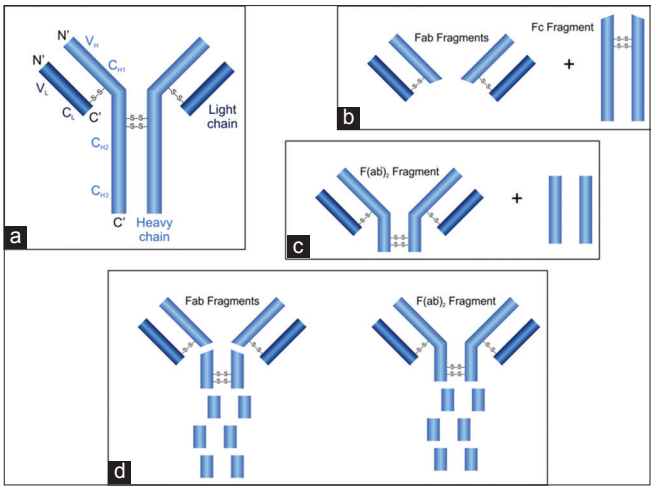

Figure 5: Structure of IgG antibody molecules (a) and fragments released after proteolytic digestion using papain (b), pepsin (c), or ficin (d)

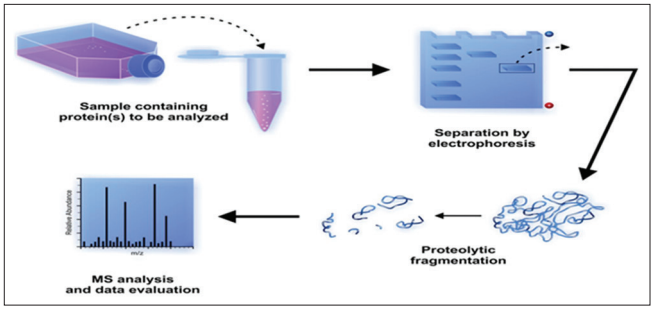

Figure 6: Proteomic analysis using mass spectrometry after separation and in-gel digestion of proteins of interest

malaria, and AIDS. Their degradative properties make them useful for general protein digestion in tissue dissociation, cell isolation, and cell culturing. The specificity and the predictability of cleavages by proteases enables their use for more specific tasks such as antibody fragment production, the removal of affinity tags from recombinant proteins, and specific protein digestion in the proteomics field mainly for protein sequencing.

References

1. Ryan CA. Proteinase inhibitors in plants: Genes for improving defenses against insects and pathogens. Ann Rev Phytopathol 1990;28:425-49.
2. Koiwa H, Bressan RA, Hasegawa PM. Regulation of protease inhibitors and plant defense. Trends Plant Sci 1997;2:379-84.
3. Browse J, Howe GA. New weapons and a rapid response against insect attack. Plant Physiol 2008;146:832-8.
4. De Leo F, Volpicella M, Licciulli F, Liuni S, Gallerani R, Ceci LR. PLANT-PIs: A database for plant protease inhibitors and their genes. Nucleic Acids Res 2002;1:347-8.
5. Boyer PD. The Enzymes. 3rd ed. New York, N.Y: Academic Press, Inc.; 1971.
6. Hoffman T. Food related enzymes. Adv Chem Ser 1974;136:146-85.
7. Barillas-Mury C, Graf R, Hagedorn HH, Wells MA. cDNA and deduced amino acid sequence of a blood meal-induced trypsin from the mosquito, *Aedes aegypti*. Insect Biochem 1991;21:825-31.
8. Davis CA, Riddell DC, Higgins MJ, Holden JJ, White BN. A gene family in drosophila melanogaster coding for trypsin-like enzymes. Nucleic Acids Res

1985;13:6605-19.

9. Ramos A, Mahowald A, Jacobs-Lorena M. Gut-specific genes from the black fly simulium vittatum encoding trypsin-like and carboxypeptidase-like proteins. Insect Mol Biol 1993;1:149-63.
10. Ward CW. Resolution of proteases in the keratinolytic larvae of the webbing clothes moth. Aust J Biol Sci 1975;28:1-23.
11. Eguchi M, Iwamoto A. Alkaline proteases in the midgut tissue and digestive fluid of the silkworm, *Bombyx mori*. Insect Biochem 1976;6:491-6.
12. Eguchi M, Iwamoto A, Yamauchi K. Interrelation of proteases from the midgut lumen, epithelia and peritrophic membrane of the silkworm, *Bombyx mori*. Comp Biochem Physiol 1982;72A:359-63.
13. Santos CD, Terra WR. Plasma membrane-associated amylase and trypsin: intracellular distribution of digestive enzymes in the midgut of the cassava hornworm, *Erinnyis ello*. Insect Biochem 1984;14:587-95.
14. Santos CD, Ribeiro AF, Terra WR. Differential centrifugation, calcium precipitation and ultrasonic disruption of midgut cells of *Erinnyis ello* caterpillars. Purification of cell microvilli and inferences concerning secretory mechanisms. Can J Zool 1986;64:490-500.
15. Espinoza-Fuentes FP, Ribeiro AF, Terra WR. Microvillar and secreted digestive enzymes from *Musca domestica* larvae. Subcellular fractionation of midgut cells with electron microscopy monitoring. Insect Biochem 1987;17:819-27.
16. Terra WR, Espinoza-Fuentes FP, Ribeiro AF, Ferreira C. The larval midgut of the housefly (*Musca domestica*): Ultrastructure, fluid fluxes and ion secretion in relation to the organization of digestion. J Insect Physiol 1988;34:463-72.
17. Lemos FJ, Terra WR. Properties and intracellular distribution of a cathepsin D-like proteinase active at the acid region of *Musca domestica* midgut. Insect Biochem 1991a;21:457-65.
18. Lemos FJ, Terra WR. Digestion of bacteria and the role of midgut lysozyme in some insect larvae. Comp Biochem Physiol 1991b;100B:265-8.
19. Jany KD, Gerhard P. Purification and some physical properties of a chymotrypsin-like protease of the larva of the hornet, *Vespa orientalis*. Eur J Biochem 1974;42:419-28.
20. Jany KD, Haug H, Pfleiderer G, Ishay J. Enzymatic and chemical properties of an endopeptidase from the larva of the hornet vespa crabro. Biochemistry 1978;17:4675-82.
21. Baker JE. Resolution and partial characterization of the digestive proteinases from larvae of the black carpet beetle. In: Current Topics in Insect Endocrinology and Nutrition. New York: Plenum Press; 1981.
22. Sakal E, Applebaum SW, Birk Y. Purification and characterization of *Locusta migratoria* chymotrypsin. Int J Pept Protein Res 1988;32:590-8.
23. Godfrey T, West S. Industrial Enzymology. 2nd ed. New York, N.Y: Macmillan Publishers Inc.; 1996. p. 3.
24. Rawlings ND, Barrett AJ. Evolutionary families of peptidases. Biochem J 1993;290:205-18.
25. Menon AS, Goldberg AL. Protein substrates activate the ATP-dependent protease La by promoting nucleotide binding and release of bound ADP. J Biol Chem 1987;262:14929-34.
26. Perlmann GE, Lorand L. Proteolytic enzymes. Methods Enzymol 1970;19:732-5.
27. Rao MB, Tanksale AM, Ghatge MS, Deshpande VV. Molecular and biotechnological aspects of microbial proteases. Microbiol Mol Biol Rev 1998;62:597-635.
28. Daniell H, Lee SB, Panchal T, Wiebe PO. Expression of the native cholera toxin B subunit gene and assembly as functional oligomers in transgenic tobacco chloroplasts. J Mol Biol 2001;311:1001-9.
29. Goldstein DA, Thomas JA. Biopharmaceuticals derived from genetically modified plants. QJM 2004;97:705-16.
30. Doran PM. Foreign protein degradation and instability in plants and plant tissue cultures. Trends Biotechnol 2006;24:426-32.
31. Michaud D. Development and reproduction of ladybeetles (Coleoptera Coccinellidae) on the citrus aphids *Aphis spiraecola* patch and *Toxoptera citricida* (Kirkaldy) (Homoptera: Aphididae). Biol Control 2000;18:287-97.
32. Outchkourov NS, Peters J, de Jong J, Rademakers W, Jongsma MA. The promoter-terminator of chrysanthemum rbcS1 directs very high expression levels in plants. Planta 2003;216:1003-12.
33. Schuler TH, Poppy GM, Kerry BR, Denholm I. Insectresistant transgenic plants. Trends Biotechnol 1998;16:168-75.
34. Christou P, Capell T, Kohli A, Gatehouse JA, Gatehouse AM. Recent developments and future prospects in insect pest control in transgenic crops. Trends Plant Sci 2006;11:302-8.
35. Sharma HC, Sharma KK, Crouch JH. Genetic transformation of crops for insect resistance: Potential and limitations. Crit Rev Plant Sci 2004;23:47-72.
36. de Maagd RA, Bosch D, Stiekema W. Toxin-mediated insect resistance in plants. Trends Plant Sci 1999;4:9-13.
37. Pigott CR, Ellar DJ. Role of receptors in *Bacillus thuringiensis* crystal toxin activity. Microbiol Mol Biol Rev 2007;71:255-81.
38. Ferry N, Edwards MG, Gatehouse JA, Gatehouse AM. Plant–insect interactions: Molecular approaches to insect resistance. Curr Opin Biotechnol 2006;15:55-161.
39. Devlin TM. Textbook of Biochemistry with Clinical Correlations. 5th ed. New York, NY, USA: Wiley & Sons; 2002.
40. Rader C. Overview on concepts and applications of fab antibody fragments. Curr Protoc Protein Sci 2009;Chapter 6:Unit 6.9.
41. Mótyán JA, Tóth F, Tőzsér J. Research applications of proteolytic enzymes in molecular biology. Biomolecules 2013;3:923-42.