

Applications and Future Prospects of Proteases: An Overview

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ABSTRACT

Proteases are enzymes with highly specialized proteolytic functions. Proteases are classified as highly demanded enzymes with numerous plant, animal, and microbial sources. They are present in all living things and are crucial for the development and differentiation of cells. They not only have several physiological functions and roles in living beings but are also of great importance in various industries as well thus providing a lot of economic benefits. This review aims to study and analyze the updated information on various biological aspects of proteases highlighting their sources, types, and mode of action on microbial production of the enzyme and their industrial applications.

INTRODUCTION

The term "proteases" describes a class of enzymes that hydrolyze protein peptide links. They go by the name's proteinases or proteolytic enzymes as well. Within the hydrolase class of enzymes, proteases make up a large group.

They are widely used and have a significant impact on applications in both the physiological and commercial domains.[Raghunath T. et.al. 2010] This is one of the greatest features of proteinases, including the lysosome enzymes cathepsin B and cathepsin D and the mammalian digestive enzymes trypsin, chymotrypsin, and pepsin. Proteolytic enzymes can selectively modify proteins by limiting their cleavage, which is how they can be used to process and transport secretory proteins across membranes, activate zymogenic forms of enzymes, and coagulate blood and fibrin clots. Important biological processes including photogenecity, complement system, apoptotic pathways, invertebrate prophenoloxidase activation cascade, metabolism regulation, enzyme modification, and others employ them as well. Proteolytic enzymes are important as reagents in laboratory, clinical, and industrial operations; their employment in many medical therapies makes studying them valuable as well. About 60% of all enzyme sales globally are made up of proteases, one of the three major classes of industrial enzymes. [Mala B. et.al.1998] Additionally, because protease enzymes cure a variety of illnesses, including pain, lung, heart, eye, digestive tract, and skin ulcer diseases, they are widely utilized in the manufacturing of pharmaceuticals.[Shrivastava et.al.,2019] Protease enzymes are therefore expected to be in greater demand in the future.

CLASSIFICATION OF PROTEASES

Proteases are considered in subgroup 4 of group 3 (hydrolases) as per the International Union of Biochemistry and Molecular Biology (International Union of Biochemistry, 1992). All living things, including viruses, depend on proteases for their physiological function. Enzymes can be categorized according to where they come from microbial (including bacterial, fungal, and viral), plant, animal, and human enzymes.

Based on their site of action on the substrate, Proteases are often divided into two groups: [1] exopeptidase and [2] endopeptidase, depending on whether they work at or away from the termini. Based on their site of action at the N or C terminus, exopeptidases are further classified as aminopeptidases and carboxypeptidases. The peptide bond closest to the substrate's amino or carboxy terminus is broken by exopeptidases. Peptide bonds that are isolated from the termini of the substrate are broken by endopeptidases. Based on the functional group that is present in the active site, endo-peptidases are further classified into four primary classes: metalloproteases, aspartic proteases, cysteine proteases, and serine proteases. [Motyan et.al., 2013]

TABLE 1 - Classification and nomenclature of peptidases. [Rawlings et.al., 2017]

Subclasses	EC code	Catalytic activity
Exopeptidases	3.4.11	Cleave near a terminus of peptides or proteins
Aminopeptidases	3.4.11	Eliminate a single amino acid from the free N-terminus
Dipeptidases	3.4.13	Exopeptidases specific for dipeptides
Dipeptidyl peptidases	3.4.14	Remove a dipeptide from the free N-terminus
Tripeptidyl peptidases	3.4.14	Remove a tripeptide from the free N-terminus
Peptidyl dipeptidases	3.4.15	Release of free C-terminus liberates a dipeptide
Carboxypeptidases	3.4.16	Remove a single amino acid from the C-terminus
Serine proteases	3.4.16	Active sites contain serine
Metalloproteases	3.4.17	Active sites contain metal ions
Cysteine proteases	3.4.18	Active sites contain cysteine
Omega peptidases	3.4.19	Remove terminal residues that are substituted,

		cyclized, or linked by isopeptide bonds
Endopeptidases	3.4.21	Cleave internally in peptides or proteins
Serine proteases	3.4.21	Active sites contain serine
Cysteine proteases	3.4.22	Active sites contain cysteine
Aspartic proteases	3.4.23	Active sites contain aspartate
Metalloproteases	3.4.24	Active sites contain metal ions
Threonine endopeptidases	3.4.25	Active sites contain threonine
Endopeptidases of unknown catalytic mechanism	3.4.99	Acting on peptide bonds

SOURCES OF PROTEASES

Principal Protease Sources: Proteases are produced from microbial, fungal, animal, and plant sources. [Dr. Neethu S. Kumar et al., 2016]

1. Proteases in plants: Among the well-known proteases with plant origins are ficin, papain, keratinases, and bromelin. However, the process of producing plant proteases takes a long time. Pinaceae is derived from the latex of *Carica papaya* fruits, whereas pineapple juice and stem contain bromelain. However, these are difficult and long procedures.

2. Animal proteases: Animal-derived proteases include chymotrypsin, pancreatic trypsin, pepsin, and rennin. The breakdown of dietary proteins is carried out by trypsin, one of the primary digestive enzymes found in the intestinal tract. The costly enzyme chymotrypsin, which is made from animal pancreatic extract, is only utilized in analytical and diagnostic processes. Laundry detergents have included pepsin, an acidic protease mostly present in the stomachs of all vertebrates. Another important protease is rennin, which is mostly found in animal stomachs as the inactive precursor rennet, which is subsequently transformed into active rennin by the action of pepsin.

3. Microbial Proteases: Due to its rapid growth and ease of manipulation for the creation of novel recombinant enzymes with desired characteristics, the microbial community is generally chosen over other communities for the large-scale synthesis of proteases. A significant portion of the global demand for commercial proteases is met by microbial proteases.

4. Proteases from fungi: The main sources of fungal proteases are numerous *Aspergillus* species, including *A. fumigatus*, *A. flavus*, and *A. candidus*, as well as species like *Cephalosporium* and *Chrysosporium*. Additionally, it can be applied to change the proteins in meals.

5. Bacterial Proteases: Due to their high catalytic activity and production capacity, bacterial proteases are more commercially important in the food, leather, and washing sectors. The primary sources of bacterial proteases are *Alteromonas* sp., *Brevibacterium linens*, *Lactobacterium helveticus*, etc.

PHYSIOLOGICAL FUNCTIONS OF PROTEASES

Many physiological and pathological processes, including protein catabolism, blood coagulation, cell migration and growth, tissue organization, morphogenesis during development, inflammation, tumor growth and metastasis, activation of zymogens, the release of hormones and pharmacologically active peptides from precursor proteins, and secretory protein transport across membranes, are influenced by proteases.

Large polypeptides are hydrolyzed by proteases into smaller peptides and amino acids, which makes it easier for the cell to absorb them. For germination, the dormant spores are devoid of the necessary amino acids. It is thought that during *Dictyostelium discoideum* spore germination, extracellular acid proteases break down cell wall polypeptide connections. A range of regulatory proteins, including those that govern the SOS response to DNA damage, the heat shock response, and the bacteriophage life cycle, are broken down by proteases. [Gottesman et.al.,2010]

INDUSTRIAL APPLICATION OF PROTEASE

The breakdown of protein peptides into amino acids is the primary purpose of protease in industry. The global industrial market for proteolytic enzymes is estimated to be approximately 60%. Application areas for them include food processing, medicines, leather, silk, baking, soy processing, meat tendering, and the brewing industries. At 60% of the whole enzyme industry, proteases are a highly sought-after class of enzymes across a wide range of businesses. [Gaur et.al.,2010]

TABLE 2- Industrial Uses of Proteases [Anwar et.al.,1997, Kim et.al.,2013]

Industry	Enzymes	Application
Leather	Trypsin, Alkaline protease	Bating of leathers dehairing
Food processing	Several proteases	Alteration of a protein-rich substance, such as soy protein
Dairy	Calf rennet and other ficin, trypsin, chymotrypsin	Coagulation of milk protein, production of enzyme-modified cheese, Chey processing

Detergent	Alkaline protease	Extensive use in laundrydetergents for proteinstain removal
Beverages	Papain	Removal of turbidity
Pharmaceutical	Trypsin	Treatment of certain types of hemia, Production of human insulin
Meat	Papain, Ginger protease, alkaline elastase, and thermophilic alkaline protease	Meat tenderization

FUTURE SCOPE

Proteases are a large class of very important industrial enzymes that are engaged in many different physiological and cellular processes. Proteases are found in all living things, including microorganisms, plants, and animals, because they are physiologically required. However, due to their quick growth, small cultivation area requirements, and easy accessibility to genetic modification, microorganisms are a goldmine of proteases and the preferred source of enzymes. Since ancient times, the food, dairy, and detergent industries have made substantial use of microbial proteases. Proteases are attracting fresh attention as potential targets for the development of therapeutic drugs to combat the deadly diseases that are spreading unchecked, such AIDS, malaria, and cancer.

Proteases are useful in basic research in addition to their industrial and medical uses. Their selective cleavage of peptide bonds is employed in protein sequencing, peptide synthesis, and the deciphering of structure-function relationships. The field of protease research is broad. Proteases have uses in molecular biology and genetic engineering. Proteases are necessary to dissolve cell wall membranes in order to examine subcellular compartments such as the nucleus, golgi bodies, mitochondria, lysosomes, and endoplasmic reticulum, among others.

CONCLUSION

The primary focus of this review is on the general characteristics of proteases, with a concentration on the challenges and potential applications of proteases in industry. The food, detergent, leather, and pharmaceutical sectors all heavily rely on the broad specificity of proteases' hydrolytic action. It is also utilized in the structural clarification of proteins, while the synthesis of proteins is facilitated by their synthetic capabilities. It has become clear that a study of the proteinases of plant origin can provide an insight into the role of proteinases in general that are desirable for their various applications, even though the importance of microbial proteinases in industry and medicine has been and will continue to be a significant influence on the choice of organisms and enzymes investigated. Since current technical advancements are developing proteases with unique characteristics and substrate specificities, the potential commercial applications of proteases are expanding rapidly. The development of proteases is

positively positioned by advances in biotechnology, which will also support their continued use to create a sustainable environment that enhances human life quality.

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