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H. M. Kalisz
Microbial Proteinases

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The Amidases from a Brevibacterium Strain:
Study and Applications

Enzyme Studies

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Bland Symington Montenecourt

Bland Symington Montenecourt, Professor of Biology, Lehigh University, died of cancer Saturday, December 19, 1987.

Bland received her B.A. in biology, magna cum laude, from Rosemont College in 1964. While attending Rosemont, she achieved national ranking in tennis and played against the great women tennis stars of the 1960's. From Rosemont College she went to Rutgers University, receiving a Ph.D. in Microbiology in 1968 working under the guidance of Joel O. Lampen on the regulation of enzyme synthesis in yeast. After a two year post-doctoral appointment at Rutgers Medical School, Bland took time off from her active career to raise a family — two sons and a daughter.

In 1976, she joined the Department of Biochemistry and Microbiology, Cook College, Rutgers University. There, as an Assistant Research Professor, in collaboration with Douglas E. Eveleigh, she established a world-renowned program in the isolation of hyper-cellulolytic strains of *Trichoderma reesei* for use in the fermentative conversion of biomass into industrial chemicals.

Bland came to Lehigh as Associate Professor in the Department of Biology in the Fall of 1981. She was promoted to Professor in 1985. During her tenure at Lehigh she continued her interest in biomass conversion, investigating the genetics of *Trichoderma reesei* and *Thermomonospora sp.* cellulase production and, more recently, expanding her program to include the study of thermophilic clostridia, the microbial fermentation of cheese whey, and the microbial desulfurization of coal.

Bland was a prolific researcher. In the past five years she authored or co-authored 52 publications. She was an international leader in the development of microbial strains for the fermentative conversion of biomass into industrial chemicals. She was a consultant for several national and international companies. She served on the editorial boards of four biotechnology journals including *Applied Microbiology and Biotechnology*, *Trends in Biotechnology*, *Journal of Biotechnology*, and *Applied and Environmental Microbiology* and not to forget *Advances in Biochemical Engineering/ Biotechnology*.

In recognition of her many accomplishments she was awarded the Annual Research Award of the Division of Microbial Chemistry and Technology of the American Chemical Society, the Novo Award for Excellence in Research, and the Eleanor and Joseph F. Libsch Research Award from Lehigh University.

Through her dedicated and prominent involvement in professional activities, she served as a role model for students. She encouraged their active collaboration with visiting scientists and solicited their involvement in proposal writing and grant management.

Bland integrated her professional career with numerous outside interests, serving a an active member of the church and on the Bethlehem Town Environmental Commission. She had a love for animals and was a farmer/scientist who had experience with and understood the use of steam exploded wood as a ruminant feed for her cows, goats and sheep. She was a nurturing parent and took particular pride in her three children — Dorsey, Ned and Marc. Their support and that of her husband, Gene, sustained her through her illness and allowed her to work under increasingly difficult circumstances.

We will remember Bland for her great activity and forthright manner. Most of all we will miss her as a good friend and caring, valued colleague. May she rest in peace.

Janice A. Phillips
Associate Professor
and Arthur Humphrey, Doug Eveleigh and Kathy Gottlund

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Microbial Proteinases

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Up to 10 years ago proteinases were regarded as degradative enzymes which could only catalyse the total hydrolysis of proteins. However, recent advances in assay techniques, such as the use of more selective substrates, have demonstrated that proteolytic enzymes carry out highly specific and selective modifications of proteins by limited hydrolysis. Furthermore, proteinases play an important role as reagents in laboratory and clinical analyses and in industrial processes. Thus, considerable emphasis is placed on research on the proteinases. The discovery of new, highly specific proteinases and improved enzyme technology, such as immobilisation and novel purification methods, should make the microbial enzymes even more attractive in biotechnology. Development and modification of existing industrial systems is likely to be the main factor in increasing the industrial application of the proteinases. In genetic biotechnology proteinases have mainly a detrimental effect in protein purification and the elimination of these enzymes may be an important factor in potential progress in this field. This review describes some of the specific functions and properties of the microbial proteinases and discusses some of the most important commercial applications of these enzymes.

1 Introduction

Since their introduction in the 1960's as detergent additives, the commercial usage of proteinases has progressed rapidly. As the most important industrial enzymes, proteinases account for nearly 60% of total enzymes sales [1] (Table 1), with two-thirds of the proteinases produced commercially being of microbial origin. The most important microbial proteinases employed commercially are the alkaline proteinase of *Bacillus licheniformis* used in detergent manufacture; *Mucor* proteinase used in cheese manufacture, and *Aspergillus oryzae* proteinases used for dough modification and soy sauce production. These enzymes account for the bulk of proteinase production world-wide. However, other proteinases, both microbial and non-microbial, are also employed in numerous industrial processes. This is especially true in the food industry where fungal proteinases are of particular importance.

Table 1. Total worldwide enzyme sales

Enzyme		Total Sales	(%)
Proteinases	Alkaline (Detergents)	25	
	Rennins	10	*59*
	Trypsin	3	
	Others	21	
Carbohydrases	Amylases	18	
	Isomerase	6	
	Pectinases	3	*28*
	Cellulases + Lactases	1	
Lipases			*3*
Others	Analytical		
	Pharmaceutical		*10*
	Developmental		

2 Detection of Proteinases

Most proteinases are normally detected qualitatively by using proteins as substrates. Proteins such as haemoglobin, casein, azocoll, and Remazoll Brilliant Blue have been used extensively. Proteolytic activity is usually detected by either;

1) Following the decrease of the initial substrate, or
2) Measuring the increase of defined peptide products. In many cases proteolytic activity is followed by measuring the release of peptides from dye-, fluorescence-, radioactively-, or enzyme-labelled proteins. This simplifies the measurement of peptides and increases the sensitivity of the assay.

Activities of proteinases, such as the blood clotting enzymes, kallikreins and many other mammalian proteinases, which catalyse the hydrolysis of specific peptide bonds are usually measured using the natural substrates of the enzymes.

Detection of highly specific proteolytic enzymes has advanced considerably with the introduction of synthetic peptide substrates for the assay of enzyme activity.

Suitable peptide portions, located on the amino-terminal side of the bond to be cleaved, are used as the specific recognition sequences. Hydrolysis of the selected peptide releases a chromogenic (e.g. 4-nitroaniline) or fluorigenic (e.g. 7-amino-4-methyl coumarin) compound, which can subsequently be measured spectrophotometrically or fluorimetrically, respectively.

In addition to detection of proteinases by specific substrates they can be identified and characterized by affinity labelling methods. Compounds such as DFP, TLCK, TPCK, peptide chloromethyl ketones and peptidyl diazomethyl ketones have been used to distinguish between individual proteinases present in biological samples.

Details of the above-mentioned methodological approaches are described elsewhere [2-6].

3 Proteinase Functions

3.1 General Characteristics

Proteinases are a highly complex group of enzymes which vary enormously in their physico-chemical and catalytic properties. The proteolytic enzymes, which are produced intra- and extracellularly, play an important role in the metabolic and regulatory processes of animal and plant cells, as well as in those of prokaryotic and eukaryotic microorganisms.

Extracellular proteinases are involved mainly in the hydrolysis of large polypeptide substrates, such as proteins, into smaller molecular entities which can subsequently be absorbed by the cell [7,8].

Intracellular enzymes play a key role in the regulation of metabolic processes [9,10]. They also play a vital role in protein turnover [10,11], maintaining a balance between protein synthesis and degradation [10,11]. Proteinases are also involved in the control of many other physiological functions, such as digestion [12,13], maturation of hormones [14-16], viral assembly [17-19], immune response [3,20-22], inflammation [23-25], fertilisation [23,26], blood coagulation [3,23,27], fibrinolysis [3,23,28], control of blood pressure [23,29-31], sporulation [15,32,33], germination [13,34,35] and pathogenesis [36,37]. Proteinases have also been implicated in the regulation of gene expression [35,38], DNA repair [39,40], and DNA synthesis [41].

3.2 Protein Turnover

Protein turnover is a continual process in all living cells [10,42,43]. The process is similar in all organisms, with different rates of turnover for individual proteins and subcellular fractions [35,44]. Protein turnover eliminates abnormal proteins [45], such as mutant human haemoglobins [9], and is essential for adaptation of cells to new environmental conditions, especially in response to starvation for nutrients [10,15,35]. Degradation of a protein appears to be initiated by exposure of a unique cleavage site at the C-terminal end of the protein [46].

Breakdown of polypeptides of no value for the cell provides a pool of amino acids as precursors for the resynthesis of proteins. Under conditions of starvation, proteins serving newly required functions in the cell can be synthesized with little net change in protein content [47,48].

Evidence for the involvement of intracellular proteinases in protein turnover is available [15,30,49]. ATP-dependent proteinases of *Escherichia coli* and resting yeast cells are responsible for the hydrolysis of the abnormal proteins [50,51]. In *E. coli*, the energy required for the degradation of these proteins is due to proteinase La, the *lon* gene product [52,53], which hydrolyses proteins and ATP in a coupled process [54]. The rates of protein degradation in vivo depend on the cellular content of the ATP-dependent proteinase, with enhanced proteolysis being deleterious to the *E. coli* cell [55]. Proteinase La, a 450,000 Daltons tetramer composed of identical subunits [53], catalyses an initial rate-limiting step in abnormal protein degradation in vivo [54]. Energy-dependent proteolytic enzymes other than proteinase La also exist in *E. coli* [56,57]. The energy-dependent degradation of proteins is a multi-step process involving activation of the proteinase by the binding of ATP; cleavage of the peptide bond; and hydrolysis of the ATP to ADP which stops protein cleavage [58]. Protein degradation appears to require two molecules of ATP per peptide bond cleaved [59]. Hydrolysis of ATP, however, is required only for the degradation of proteins; cleavage of peptides of less than 10,000 Daltons requires only binding of the nucleotide to the enzyme [58]. In eukaryotes, the highly selective non-lysosomal turnover of intracellular proteins is also effected by ATP-requiring mechanisms [51], involving a ubiquitin-dependent pathway [51,60]. The degradation process requires ATP for the binding of ubiquitin to the substrate protein [61]. Degradation of the ubiquitin-protein conjugate also requires ATP and may involve ATP-dependent proteinases [62].

3.3 Sporulation

Conditions of starvation induce the formation of bacterial spores [35,63], yeast ascospores [64], and the fruiting of slime moulds [13] and fungi [65]. These processes involve intensive protein turnover [35]. Proteolytic processes play a key role in the transition from vegetative growth to spore formation in yeasts and bacteria [15,33,66].

During the aggregation of unicellular slime moulds into multicellular units and subsequent differentiation, sweeping changes in enzyme patterns occur, with a large amount of protein breakdown for the synthesis of new proteins and carbohydrates [67]. Large increases in proteinase activity are also observed during fruiting body formation in the cultivated mushroom *Agaricus bisporus* [65] and the related "bracket fungus" *Schizophyllum commune* [68]; the proteinases in the latter being involved in enzyme inactivation reactions.

In bacteria, at least two intracellular serine proteinases are activated by a post-translational event at the end of vegetative growth and during the early stages of sporulation [69]. A membrane bound proteinase, which shows unique properties and is produced earlier than the cytoplasmic enzymes during sporulation [70,71], controls the proteinase system in the sporulating cells of *Bacillus subtilis* by regulating the level of a proteinaceous inhibitor which specifically inactivates the cellular proteinases [72]. Production of extracellular proteinases also coincides with sporulation [73], the increase in activity resulting from de novo synthesis. Experiments with proteinase

inhibitors have shown a need for both extracellular [32] and intracellular [74] proteinases in sporulation. However, *B. subtilis* mutants deficient in the two major extracellular proteinases sporulate normally, indicating that these sporulation-associated enzymes are not essential for spore development [75, 76]. Proteinase inhibitors have also been used to show the need for proteinases in sporulation of yeast cells. Protein breakdown during yeast sporulation is energy dependent. In *Aspergillus* species a correlation between proteinase secretion and sporulation has been reported. The region of proteinase formation is at a constant distance from the hyphal tip, and has a constant spatial relationship with the membrane site where the first conidiation septum is formed [77].

3.4 Germination

During bacterial spore germination, proteinases make amino acids available for the synthesis of new enzymes required for the process and provide a nitrogen source for nucleotide biosynthesis [35]. About 20% of the protein in dormant spores of *Bacillus* and *Clostridium* species is degraded in the first minutes of germination [78]. Proteolysis is necessary for protein synthesis by the germinating and outgrowing spore, since the dormant spore lacks most free amino acids and is incapable of de novo synthesis of a number of amino acids due to an absence of the necessary enzymes [79]. Degradation is initiated by endoproteinase activity of at least two serine enzymes [34]. Both proteinases have unique specificity for storage proteins, which are a group of small, acid-soluble spore proteins synthesised only during sporulation under transcriptional control [80, 81] and do not affect other spore proteins, and are present only in the developing forespore. They are absent from vegetative and young sporulating cells, and from the surrounding mother cell. Activity of both enzymes is rapidly lost on germination [34].

Proteinases are also involved in macroconidial germination and hyphal fusion. Macroconidial germination in *Microsporum gypseum*, and an alkaline proteinase found in the germination supernatant, can be inhibited by a specific serine proteinase inhibitor. Addition of the proteinase increases spore germination [82]. Following hyphal fusion between *Podospora anserina* strains which are heterogenic for their incompatibility alleles, an incompatibility reaction occurs which is correlated with the release of high proteolytic activity [83]. During germination of *Dictyostelium discoideum* spores [84] and *Polysphondylium pallidum* microcysts [85], excreted acid proteinases are thought to be involved in the breakage of cell wall polypeptide linkages.

3.5 Enzyme Modification

3.5.1 Activation

Many inactive precursors of zymogens are converted to active forms by proteinases which cleave one or more peptide bonds. The proteinases involved in such reactions are usually highly specific. Zymogen activation represents an important part in physiological regulation, being a rate-controlling step in many processes such as the generation of protein hormones [15, 16], activation of enzymes [12], assembly of fibrils [86] and viruses [17, 18], blood coagulation [23, 27] and fertilisation of ova by sperm [87].

Only a few examples of enzyme activation in lower eukaryotes have been observed. In yeast, proteinase contained in vesicles activates a zymogen of chitin synthase by limited proteolysis in vitro [88]. The active chitin synthase is involved in primary septum formation in budding yeast cells [89]. Activation of chitin synthase has also been observed in *Phycomyces, Candida albicans, Mucor rouxii, Aspergillus nidulans* and related species [89,90]. In vitro activation of the *Mucor rouxii* cAMP phosphodiesterase has also been demonstrated [91]. The vacuolar enzymes of *Saccharomyces cerevisiae* are also activated [15,92]. Proteolytic processing of prohormones occurs at sites containing paired basic residues [93]. The processing of α-mating factor in *Saccharomyces cerevisiae* appears to involve two novel proteinases; a serine proteinase in the α-cell soluble fraction [14]; and a calcium-dependent cysteine proteinase in the α-cell membrane fraction [94].

3.5.2 Inactivation

Inactivation is defined as an irreversible loss of in vivo catalytic activity in the physiologically significant reaction of an enzyme [47]. It is distinct from protein turnover, where proteins are degraded to their constituent amino acids. In vivo enzyme inactivation has frequently been observed in microorganisms in response to physiological and developmental changes [47]. Several enzymes are irreversibly inactivated after a metabolic shift. Enzymes involved in gluconeogenesis, such as phosphoenolpyruvate carboxykinase [95], fructose-1,6-bisphosphatase [96] and cytoplasmic malate dehydrogenase [97] become inactivated when glucose is added to *Saccharomyces cerevisiae* cells growing on acetate and the mechanism of inactivation involves proteolysis.

Proteinase B from yeast has been shown to inactivate several enzymes in vitro, such as tryptophan synthetase, chitin synthase, fructose-1,6-biphosphatase and glutamate-oxaloacetate transaminase, while yeast proteinase A inactivates threonine dehydratase and tryptophan synthetase [98]. However, studies with mutants lacking these two proteinases have shown that neither enzyme is solely responsible for inactivation [15,33]. In the inactivation of fructose bisphosphatase, proteinases are involved in the second of a two stage inactivation process which starts with a reversible phosphorylation [99]. The enzyme appears to be proteolysed by extravacuolar proteinases [100]. The proteolytic degradation of a protein has been shown to depend upon the conformational stability of the molecule [101]. Highly specific degradation of proteins may be achieved in a two-step process involving the covalent modification of the proteins as a marking mechanism for proteolysis [43].

Many enzymes of vegetative cells are selectively inactivated during or prior to sporulation. Hydrolysis of anabolic enzymes not needed during the stationary phase may make available peptides and amino acids for other purposes, such as spore synthesis or formation of new enzymes necessary for adaptation to the changing growth environment [102,103]. Most examples of inactivation have been found in bacilli and yeasts. They include NADP-dependent glutamate dehydrogenase, isocitrate lyase, as well as the gluconeogenic enzymes, malate dehydrogenase and fructose-bisphosphatase, whose activities decrease from the onset of yeast sporulation [103], and aspartate transcarbamylase [104], glutamine phosphoribosylpyrophosphate amidotransferase [105], and threonine dehydratase [106], which are inactivated in starving

B. subtilis cells prior to sporulation [48]. Inactivation of aspartate transcarbamylase either consists of, or is immediately followed by, selective proteolysis, in an energy-requiring process [107]. Inactivation of glutamine phosphoribosylpyrophosphate amidotransferase requires oxygen and is followed by proteolysis [108]. Several enzymes, including the glutamine synthetase of *Klebsiella aerogenes* and *Escherichia coli* become proteolysed following covalent modification by mixed function oxidation [43, 109]. The glucose dehydrogenase of *Bacillus megaterium* is cleaved into fragments by proteinase K, resulting in a loss of activity [110]. In *Neurospora crassa* tryptophan synthetase is inactivated by a proteinase during transition from exponential to stationary phase [111]. In *Schizophyllum commune* iso-enyzmes of phosphoglucomutase are selectively inactivated during fruiting by a proteinase which is only detected during inactivation of the enzyme [68].

3.5.3 Modification

A number of enzymes are modified so that their physiological reactions become altered but not lost. Leucyl-tRNA synthetase from *Escherichia coli* is modified from an enzyme catalysing synthesis of leucyl tRNA to one which catalyses leucine-dependent pyrophosphate-ATP exchange, by a proteinase which splits a polypeptide of about 3000 molecular weight from the native synthetase [112]. The fructose bisphosphate aldolase of *Bacillus cereus* [113] and the RNA-polymerase of *B. thuringiensis* and *B. subtilis* [114] become modified by proteinases, probably during transition from vegetative growth to sporulation.

Enzymes modified by proteinases in yeasts include 3-phosphoglyceric acid mutase, hexokinase, aldehyde dehydrogenase, phosphofructokinase and cytochrome b2 [98]. These modifications could, however, be in vitro artifacts, and may play no physiological role in vivo.

3.6 Protein Maturation and Secretion

Many proteins are synthesised as precursors and are subsequently processed by limited proteolysis to the mature (authentic) products. Four protein maturation events involving proteolysis have been recognised [33]. These are:

a) Removal of N-terminal formylmethionine or methionine from nascent polypeptide chains;
b) Removal of peptide extensions or "signal peptides" from proteins passing through cell membranes;
c) Cleavage of the translation product of polycistronic mRNA coding for several distinct polypeptide chains;
d) Conversion of inactive pro-proteins into biologically active products, i.e. enzyme activation.

Proteins translocated across or integrated into cellular membranes are synthesised as precursors containing a short amino terminal extension of 15–30 amino acid residues, called "signal peptide" [115]. At least three structurally and functionally distinct regions have been defined; a positively charged basic amino-terminal region containing 2–8 amino acids (n-region), followed by a 7–15 residue long uncharged, mainly hydrophobic region (h-region), and a 5–6 residue long polar

C-terminal region (c-region) [116]. The amino acid before the cleavage site has a short side chain [117]. Prokaryotic proteins have a considerably higher incidence of acidic than basic residues around the cleavage site [118]. The signal peptides are essential for the translocation of proteins across cell membranes [117]. They are recognised by the signal recognition particle (SRP), a cytoplasmic ribonucleoprotein, which binds to ribosomes and may cause arrest of elongation of the initiated polypeptide chain at a discrete site. The SRP-ribosome complex binds to the SRP receptor, or "docking protein", an integral membrane protein of the endoplasmic reticulum [119]. The SRP is released on binding, the ribosome resumes chain elongation and the growing polypeptide chain passes through the membrane [120]. The signal peptides are cleaved off during or shortly after membrane passage to yield the mature product [117, 121, 122].

3.7 Extracellular Proteinases

Extracellular proteinase activity has been observed in numerous prokaryotic and eukaryotic microorganisms [7, 13, 123, 124]. Extracellular proteinases are involved mainly in the degradation of exogenous proteins to peptides and amino acids before cellular uptake. They usually have wide substrate-specificities and can degrade most non-structural proteins, such as albumin, casein, insulin or haemoglobin. Extracellular proteinase activity has also been observed during sporulation [73] and spore germination [85]. Synthesis of the *B. subtilis* serine proteinase appears to be regulated either by a catabolite repressor or an inducer produced only at the end of logarithmic growth [125].

Many pathogenic microorganisms secrete proteinases, some of which are involved in the infection process [13, 126, 127]. The virulence of a few pathogenic fungi and bacteria, such as *Vibrio cholerae* [128], is correlated with extracellular proteinase activity of the organisms. The proteinases of viruses have also been implicated in infectious diseases [129]. Several species release specific proteinases which can hydrolyse structural and connective tissue proteins resistant to attack by most proteinases. These include the collagenases of *Clostridium histolyticum*, *Pseudomonas aeruginosa* [13] and *Lagenidium giganteum* [130]; the elastases of *B. subtilis*, *Ps. aeruginosa* and *Flavobacterium*; and the keratinases of *Streptomyces fradiae* [13]. Some species, such as the dermatophytes *Microsporum* and *Trichophyton* produce all three types of proteinases. A number of bacteria, including *Neisseria gonorrhoeae* and *N. meningitidis*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, *S. sanguis* and *S. mitio*, responsible for various human infections release IgA1 proteinases, which specifically cleave the immunoglobulin IgA1 which normally provides for antibody defence of mucosal surfaces [36]. All of the bacterial IgA1 proteinases tested are metalloproteinases [131], except for the *Bacteroides melaninogenicus* enzyme, which is a cysteine proteinase dependent on divalent cations [132]. Several pathogenic *Candida* species secrete acid proteinases which can degrade IgA and which exhibit keratinolytic or collagenolytic activity [133].

Many pathogenic species, including the apple pathogenic fungus *Monilinia fructigena*, utilise host proteins for nutrition [134]. Some species release several proteinases, each with different metabolic functions. One such is the parasitic flagellate

Trypanosoma cruzi, which secretes three proteinases which are believed to be involved in nutrition, parasitism and escape from the host's immune response [135].

4 Localisation and Control of Proteinases

4.1 Localisation of Intracellular Proteinases

Many organelle-specific proteinases have been observed [136]. Proteinase activities have been found in nuclear chromatin [137] and ribonucleoprotein particles, in mitochondria [138, 139], chloroplasts, and ribosomes. Signal peptidases, catalysing the removal of signal peptides from secretory proteins have been observed in rough microsomes. Membrane-bound acid proteinases have been found in rough and smooth microsomes of *Aspergillus oryzae* [140]. A number of enzymes have been found in *Escherichia coli* cell membranes [141]. In eukaryotic microorganisms proteinases are contained in membrane-surrounded compartments similar to the mammalian lysosomes [98, 142], such as the vacuoles of *Saccharomyces cerevisiae*, *Neurospora crassa*, *Candida albicans* and *Microsporum gypseum*. Proteinase activity has also been found in large vacuoles of starved *Euglena gracilis* cells and in digestive vacuoles of various flagellates [143]. In prokaryotes, proteinases are present in the cytosol and are connected with the membrane fraction [141].

4.2 Control of Proteinase Activity

4.2.1 Intracellular Proteinases

4.2.1.1 General Mechanisms

A number of mechanisms operate to control proteolysis. These include modulation of substrate proteins by covalent interconversion, change in hydrophobicity and interaction with various molecules, which change their susceptibility to proteolysis [51, 136]. Proteinase activity is also controlled by nutritional conditions and catabolite repression. The proteinase activity increases in both microbial and mammalian tissues under conditions of nutrient starvation [9]. Nutrients such as glucose repress proteolytic activity in yeasts, bacilli, *Escherichia coli* and other microorganisms [98, 136].

Control of proteinase activity can also be effected by limited proteolysis, as in the activation of zymogens and processing of proteins [15, 92, 144], and by specific localisation of the enzymes. Low molecular weight effectors, such as ATP [9, 51], divalent cations, especially calcium [145], and charged tRNA [146] are able to modify proteinase activity. In the case of charged tRNA, guanosine tetraphosphate and other guanosine nucleotides are thought to serve as allosteric effectors of the proteolytic system.

4.2.1.2 Control by Inhibitors

An extensive number of proteinase inhibitors of microbial origin has been observed [147]. Many of the inhibitors are active against a large number of proteinases