CHAPTER 11

CYSTEINE PROTEASES

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1. INTRODUCTION

Cysteine proteases (CPs) are present in all living organisms. More than twenty families of cysteine proteases have been described (Barrett, 1994) many of which (*e.g.* papain, bromelain, ficain , animal cathepsins) are of industrial importance. Recently, cysteine proteases, in particular lysosomal cathepsins, have attracted the interest of the pharmaceutical industry (Leung-Toung *et al.*, 2002). Cathepsins are promising drug targets for many diseases such as osteoporosis, rheumatoid arthritis, arteriosclerosis, cancer, and inflammatory and autoimmune diseases. Caspases, another group of CPs, are important elements of the apoptotic machinery that regulates programmed cell death (Denault and Salvesen, 2002). Comprehensive information on CPs can be found in many excellent books and reviews (Barrett *et al.*, 1998; Bordusa, 2002; Drauz and Waldmann, 2002; Lecaille *et al.*, 2002; McGrath, 1999; Otto and Schirmeister, 1997).

2. STRUCTURE AND FUNCTION

2.1. Classification and Evolution

Cysteine proteases (EC.3.4.22) are proteins of molecular mass about 21-30 kDa. They catalyse the hydrolysis of peptide, amide, ester, thiol ester and thiono ester bonds. The CP family can be subdivided into exopeptidases (*e.g.* cathepsin X, carboxypeptidase B) and endopeptidases (papain, bromelain, ficain, cathepsins). Exopeptidases cleave the peptide bond proximal to the amino or carboxy termini of the substrate, whereas endopeptidases cleave peptide bonds distant from the N- or C-termini. Cysteine proteases are divided into five clans: CA (papain-like enzymes),

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CB (viral chymotrypsin-like CPs), CC (papain-like endopeptidases of RNA viruses), CD (legumain-type caspases) and CE (containing His, Glu/Asp, Gln, Cys residues in the catalytic cleft) (Barrett, 1994, 1998; Rawlings *et al.*, this volume). The majority of CPs that have been characterized are evolutionarily related to papain and share a common fold. They are synthesized as inactive precursors with a N-terminal propeptide and a signal peptide. Some peptidases of family C1 have C-terminal extensions. Activation requires proteolytic cleavage of the N-terminal proregion that also functions as an inhibitor of the enzyme. Most CPs are inhibited by E-64, cystatins and many synthetic inhibitors (Otto and Schirmeister, 1997; Grzonka *et al.*, 2001).

2.2. Papain

Papain (EC 3.4.22.2) is the best known cysteine protease. It was isolated in 1879 from the fruits of *Carica papaya* and was also the first protease for which a crystallographic structure was determined (Drenth *et al.*, 1968; Kamphuis *et al.*, 1984). The crude dried latex of papaya fruit contains a mixture of at least four cysteine proteases (papain, chymopapain, caricain, glycyl endopeptidase) and other enzymes (Baines and Brocklehurst, 1979). Crude papain of the highest quality and activity is found in sunny regions of constant humidity throughout the year. Methods of purification of papain include water extraction with reducing and chelating agents, salt precipitation and solvent extraction. Very pure papain is obtained by affinity chromatography methods. Papain is composed of 212 amino acids with three internal disulphide bridges, resulting in a molecular weight of 23.4 kDa. It is relatively basic protein, with a pI of 8.75. Its three-dimensional structure reveals that the enzyme is composed of two domains of similar size with the active cleft located between them (Fig. 1).

The general mechanism of cysteine protease action has been very well studied, with papain as the model enzyme. The enzymatic activity of papain is exerted by a catalytic dyad formed by Cys^{25} and His^{159} residues, which in the pH interval 3.5-8.0 form an ion-pair (Fig. 2). Asn^{175} is important for orientation of the imidazolium ring of the histidine in the catalytic cleft. The reactive thiol group of the enzyme has to be in the reduced form for catalytic activity. Thus, the cysteine proteases require a rather reducing and acidic environment to be active. The formation of an intermediate, S-acyl enzyme moiety, is a fundamental step in hydrolysis. This intermediate is formed *via* nucleophilic attack of the thiolate group of the cysteine residue on the carbonyl group of the hydrolysed amide (ester) bond with the release of the C-terminal fragment of the cleaved product. In the next step, a water molecule reacts with the intermediate, the N-terminal fragment is released, and the regenerated free CP molecule can begin a new catalytic cycle (Storer and Menard, 1994).

The active site residues Cys^{25} and His^{159} are positioned on opposite sides of the cleft. A number of structures of papain complexes with ligands and inhibitors have been elucidated by X-ray crystallography. Following the notation of Schechter and Berger (1967), the substrate pocket of papain binds at least seven amino acid

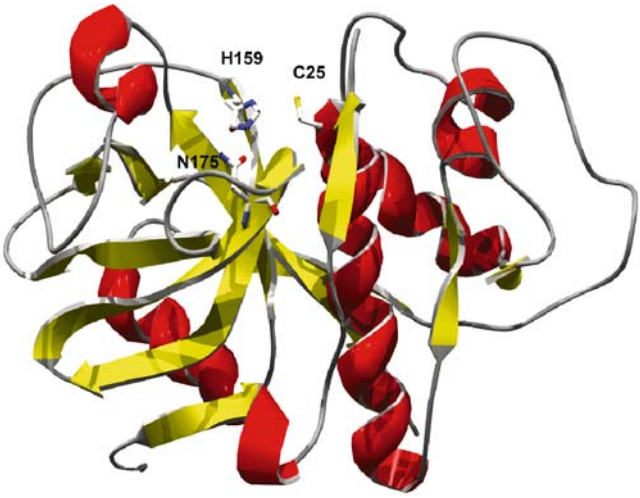

Figure 1. Ribbon representation of the three-dimensional structure of papain (Kamphuis *et al.*, 1984)

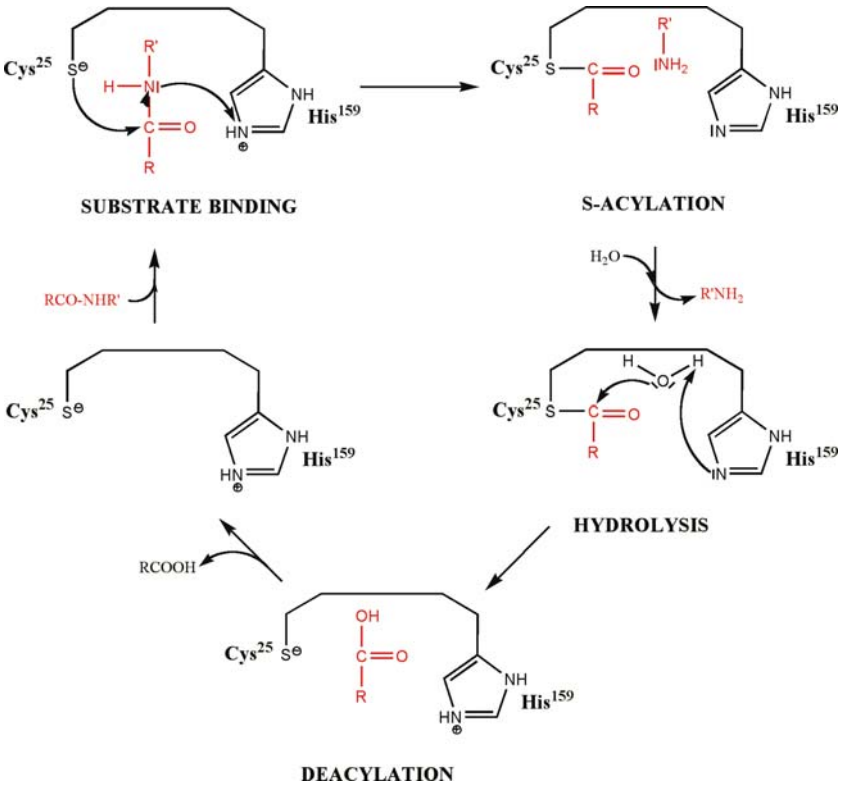

Figure 2. Enzymatic mechanism of protein hydrolysis by cysteine proteases

residues in appropriate S_n and S_n' subsites (Fig. 3). On the basis of kinetic and structural data Turk *et al.* (1998) proposed that only five subsites are important for substrate binding. According to their proposal, the S_2, S_1 and S_1' subsites are important for both backbone and side-chain binding, whereas the S_3 and S_2' pockets are crucial only for amino acid side-chain binding. A preference for those substrates containing a bulky hydrophobic chain (Phe, Leu, Ile *etc.*) in P_2 position was found; the amino acid residue in position P_1 of the substrate influences substrate binding to the enzyme to a lesser degree. There is some preference for basic amino acids (Arg, Lys) in this position but Val is not accepted. The S_3 binding site of the enzyme is less constrained; it can accommodate different amino acids side chains. Generally, papain possesses fairly broad specificity and can cleave various peptide bonds. The optimal activity of papain occurs at pH 5.8–7.0 and at temperature 50–57° C when casein is used as the substrate. Papain is stable and active for several months when stored at 4° C. Decreased activity during storage is due to oxidation of the active site thiol group. This oxidation can be partially reversed by thiol reagents (cysteine, mercaptoethanol, dimercaptopropanol *etc.*).

2.3. Bromelain

The name 'bromelain' was originally given to the mixture of proteases found in the juice of the stem and fruit of pineapple (*Ananas comosus)*. Even now, bromelain is still used as the collective name for enzymes found in various members of the Bromeliaceae family. The major endopeptidase present in extracts of plant stem is termed 'stem bromelain', whereas the major enzyme fraction found in the juice of the pineapple fruit is named 'fruit bromelain'. Some other minor cysteine endopeptidases (ananain, comosain) are also found in the pineapple stem.

Stem bromelain (EC 3.4.22.32) belongs to the papain family. It is a glycosylated single-chain protein of molecular weight 24.5 kDa. It contains 212 amino acid residues, including seven cysteines, one of which is involved in catalysis. The other six are associated in pairs forming three disulphide bridges. The crystal structure of stem bromelain has not yet been reported. Stem bromelain can be purified

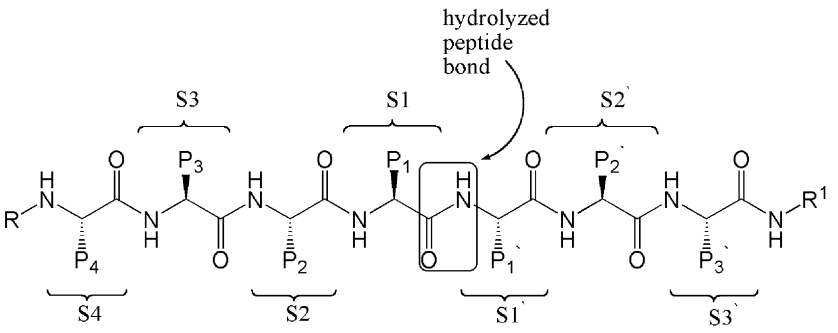

Figure 3. Interaction of papain with substrate

from dried pineapple stem powder by cation-exchange or affinity chromatography methods (Rowan *et al.*, 1990). Pure stem bromelain is stable when stored at −20° C. The pH optimum for bromelain activity is 6–8.5 for most of its substrates, and the temperature optimum range of this enzyme is 50 to 60° C. Cysteine is commonly used as an activating compound for bromelain, other thiols being less effective. Stem bromelain has high proteolytic activity for protein substrates, with a preference for polar amino acids in the P_1 and P_1' positions. It has strong preference for Z-Arg-Arg-NHMec among small molecule substrates. It is scarcely inhibited by chicken cystatin and very slowly inactivated by E-64.

Fruit bromelain (EC 3.4.22.33), the major endopeptidase present in the juice of the pineapple fruit, is immunologically distinct from stem bromelain. Fruit bromelain is a single-chain glycosylated protein of molecular weight 25 kDa. It has much higher proteolytic activity compared to stem bromelain and a broader specificity for peptide bonds.

2.4. Ficain (ficin)

Ficain (EC 3.4.22.3; synonym: ficin) is the name for the cysteine protease isolated from dried latex of *Ficus glabrata*. It is also present in other species of *Ficus, e.g. F. carica, F. elastica.* Ficain can be purified by gel filtration followed by covalent chromatography (Paul *et al.*, 1976). The optimum pH range is from 5 to 8, whereas the temperature optimum is from 45 to 55° C. Ficain requires cysteine or other reducing agents for activation. The enzyme has broad specificity with the acceptance of hydrophobic amino acid residues (Phe, Leu, Val) in the S_2 pocket. Ficain like papain is inhibited by chicken cystatin.

2.5. Cathepsins

Lysosomal cathepsins are an important group of enzymes that are responsible for a number of physiological processes including cellular protein degradation (Brömme and Kaleta, 2002). All cathepsins have mature domains of 214–260 amino acids. The structure of cathepsins shows an L-domain containing the active cysteine residue and a conserved α-helix and R-domain with the histidine residue and four to six β-strands. With the exception of cathepsin S, human cathepsins have acidic pH optima characteristic of the lysosomal compartment, and they are rapidly inactivated at neutral pH. Cathepsins have different specificities which are related to their specific functions in different tissues (Lecaille *et al.*, 2002).

3. INDUSTRIAL APPLICATIONS OF CYSTEINE PROTEASES

Proteases, which firmly maintain first place in the world enzyme market, play an important role in biotechnology. The cysteine proteases of plants and animal cathepsins are of considerable commercial importance due to their strong proteolytic activity against a broad range of protein substrates. Most industrial applications of these enzymes are described in excellent books and review articles published

Table 1. Major industrial applications of cysteine proteases

Application	Enzymes used	Reason (uses)
Biological detergent	papain, bromelain	protein stain removing
Baking industry	bromelain, papain	lowering the protein level of flour in biscuit manufacturing, dough relaxation, preventing dough shrinkback, better bread volume, crumbliness and browning uniformity
Brewing industry	bromelain, papain	removing cloudines during storage of beers, spliting proteins in the malt
Dairy industry	bromelain, papain	whey hydrolyzates, sweetener, cheese rippening
Photographic industry	ficin	dissolving gelatin of the scraped film allowing to recovery of silver present
Food industry	bromelain, papain, cathepsins	tenderizer for meat, make high-level nutriments, make soluble protein products and breakfast, cereal and beverage, gelatin stabilization, health food, dry fermented food rippening
Waste removing (effluent)	bromelain, papain	lowering viscosity of water extract (stick water), protein and peptides production
Chitooligosaccharides production	crude bromelain, crude papain	chitosan depolymerization to use in pharmacy, animal food, medicine
Sea food	bromelain, papain	surimi production, protein hydrolyzates
Cosmetic industry	bromelain, papain	peeling effect, tooth whitening, can help to dispel taches ad pimples, clean face
Parmaceutic industry and medicine	bromelain, papain	kill the lymphatic leukemia cells, probacteria, parasite and bacillus tuberculars, helping diminish inflammation, normalize the functioning of the gallbladder, alleviating pain and promote digestion, soft lens cleaning
Textile	bromelain, papain	used for processing wool, boiling off cocoons and refining silks
Leather industry	papain	depilatory for tanning the leathers
Forage (animal‘s food)	bromelain, papain	to increase availability and inversion of proteins decreasing the cost of forages and exploiting sources of protein
Chemical industry (organic sythesis)	bromelain, papain	synthesis of aspartam, antitumor compounds, bioactive peptides

in recent years (Adler-Nissen, 1986; Vilhelmsson, 1997; Godfrey and West, 1996; Uhlig, 1998; Rao *et al.*, 1998; Leisola *et al.*, 2001; Shahidi and Kamil, 2001; Sentandreu *et al.*, 2002; Clemente, 2000; Aehle, 2004; Liu *et al.*, 2004). In Table 1 some major industrial applications are presented.

3.1. Beer and Alcohol Production

Light and clear beers are preferred by consumers. Different ingredients used during beer manufacture incorporate proteins which form insoluble complexes that appear

as a permanent haze. When the beer is chilled the insolubility increases and a more intense haze, known as chill-haze, is produced. Treatment with a proteolytic enzyme (usually crude papain or bromelain) results in a beer that remains clear and bright when chilled. Enzyme serum is also excellent as a wort clarifier (Esnault, 1995; Jones, 2005). Currently papain is not so widely used because of the trend for additive free beers prevailing in some European countries.

3.2. Baking Industry

Proteases are used in the baking industry because dough may be prepared more quickly if the gluten it contains has been partially hydrolysed. When high-gluten varieties of wheat are used the gluten must be extensively degraded for making biscuits or preventing shrinkage of commercial pie pastry. Bromelain has been widely used in the baking industry because of its rapid rate of reaction, broad pH and temperature optima and its lack of amylase or pentosanase side activities. Protease treatment improves dough relaxing and bread volume, prevents dough shrink back, and allows faster bakery throughput (Tanabe *et al.*, 1996).

3.3. Food Processing

Hydrolysis of animal or vegetable food proteins is carried out for different purposes: to improve nutritional characteristics, to retard deterioration, the modification of different functional properties (solubility, foaming, coagulation, and emulsifying capacities), the prevention of undesired interactions, to change flavours and odours, and the removal of toxic or inhibitory factors, among others. Enzymatic hydrolysis is strongly preferred over chemical methods because it yields hydrolysates containing well-defined peptide mixtures and avoids the destruction of L-amino acids and the formation of toxic substances. Cysteine proteases, especially papain and bromelain, are widely used to prepare protein hydrolysates having excellent taste properties because of the absence of bitterness. Seafood (Vilhelmsson, 1997; Aspmo *et al.*, 2005), eggs (Lee and Chen, 2002) and vegetable (soya, wheat, rice, sunflower, sesame and maize - Wu *et al.*, 1998; Bandyopadhyay and Ghosh, 2002) protein hydrolysates not only provide excellent enhanced flavour in a wide range of foods but also improve protein assimilation (Adler-Nissen, 1986; Clemente, 2000).

Caseins and whey are some of the important protein substrates available in nature. Whey proteins generate a significant increase in foam formation and stable foam structure that can be reduced by proteolysis (Lieske and Konrad, 1996). Hydrolysis of milk proteins reduce the allergenic properties of dairy products. Milk protein hydrolysates are also used in health and fortifying sports drinks, in infant and low-digestible enteral nutrition and dietetic food.

Proteinases are widely applied in the formulation of marinades and tenderising recipes. Softness and tenderness have been identified as the most important factors affecting consumer satisfaction and the perception of taste. Tenderisation can be effected by breaking the cross-links between the fibrous protein of meat (collagen

and elastin) or by breaking meat into shreds. The traditional enzymes for this are papain, bromelain or ficin (Godfrey and West, 1996) which are sprayed or dusted onto meat. However, native meat enzymes – cathepsins and calpains – play a special role in tenderising meat by controlled ageing (Sentandreu *et al.*, 2002; Thomas *et al.*, 2004). Meat from older animals remains tough but can be tenderised by injecting inactive papain into the jugular vein of the live animal shortly before slaughtering. Upon slaughter, the resultant reducing conditions cause the accumulation of free thiols in the muscle, activating the papain and hence tenderising the meat. This is a very effective process as only 2–5 ppm of inactive enzyme need to be injected. Recently, however, it has been found that this destroys the animal's heart, liver and kidneys which cannot be sold. Papain activity is difficult to control and persists into the cooking process. Papain and bromelain as well as endogenous cysteine proteases are used for accelerated ripening of dry fermented sausages (Diaz *et al.*, 1996) and dry-cured ham (Scannell *et al.*, 2004). The activity of endogenous muscle cysteine proteases (mainly cathepsins) activated during cooking caused myosin degradation and subsequent loss of texture. In surimi production, too much cysteine protease activity is also undesirable (An *et al.*, 1996), therefore proteinase inhibitors (Gracia-Carreńo, 1996) are applied to prevent gel weakening (Kang and Lanier, 2000; Rawdkuen *et al.*, 2004). Other applications include: producing dehydrated beans, baby food, food that can be easily digested by the patients, soft sweets, food deodorization (Schmidl *et al.*, 1994; Clemente, 2000).

3.4. Animal Feed

The addition of papain to some mixed forages can greatly increase the availability of protein, decreasing the cost of the forage and exploiting sources of protein (Wong *et al.*, 1996). An important application of proteases in the pet food industry is to produce a digest which liquefies the raw material and creates an acceptable flavour. This is then coated onto or mixed into dry pet food to improve its palatability.

3.5. By-product Utilization

Recently, chitosan-related materials have received a considerable amount of attention because they are useful in the food (Muzzarelli, 1996) and agriculture (Koga, 1999) industries and have various biological activities of interest (Ravi Kumar *et al.*, 2004). Chitosan is a deacylated derivative of chitin which is an abundant natural polysaccharide found in the exoskeleton of creatures such as crustaceans and insects, and in fungi. Chitinous material is obtained from the marine products' industry as a solid waste product. Chitosan depolymerisation enhances its water solubility and reduces solution viscosity as well as suppressing gel formation during storage. Therefore the depolymerisation of chitosan could facilitate the application of chitosan-related materials in a variety of fields. Commercial crude papain, bromelain and ficin are widely used for chitosan depolymerisation (Li *et al.*, 2005; Chang *et al.*, 2005). However, the hydrolysis of chitin and chitosan by means of

stem bromelain was the result of chitinase and chitosanase activities present in the crude enzyme and not bromelain itself (Hung *et al.*, 2002).

Plant cysteine proteases are also used to improve the recovery of protein from slaughterhouse waste (Gómez-Juárez *et al.*, 1999) and soy processing (Moure *et al.*, 2005). The recovered proteins are subsequently used in both the feed and food industries owing to their good nutritional value and excellent functional properties (Silva *et al.*, 2002). Nowadays papain and alkaline bacterial proteases are also employed for solubilizing fish wastes (Gildberg *et al.*, 2002; Guerard *et al.*, 2002) and to lower the viscosity of expressed fish fluids (stick water) in fodder manufacture, as well as to extract carotenoproteins from brown shrimps (Chakrabarti, 2002). Cysteine proteases are also used in skeletal muscle wasting (bone cleaning) and meat recovery processes. To recover this material, bones are mashed and incubated at 60° C with neutral or alkaline proteases for up to 4 hours. The meat slurry produced is used in canned meat and soups and protein-free bones are used as a source of gelatin.

Photographic films and plates essentially consist of an emulsion on a firm support of cellulose acetate, or polyester, or glass. The emulsion is composed of a suspension of minute silver halide crystals in gelatin. Spent films which have lost their usefulness could be utilized as a source of valuable chemicals recovered by means of the proteolytic action of papain (*i.e.* recovery of silver). Papain and bromelain are also applied to biodegrade polymers (Dupret *et al.*, 2000; Howard, 2002; Chiellini *et al.*, 2003).

3.6. Leather Industry

The bating of leather is a technique which takes place before tanning, and is employed to provide hides and skins with the requisite malleability and softness. Bating materials, which contain proteases, serve this purpose by breaking down the proteinaceous material of skins and hides. However, the proteolytic action should only be allowed to continue to a specific level to avoid destruction of the basic structure of the leather. In addition, papain also acts as a dehairing agent. A conventional dehairing process with sodium sulphide and lime is a major source of the pollution associated with the tanning industry. Several enzymatic (including protease and amylase activities) and non-enzymatic dehairing methods have evolved during the last century. Papain together with soluble silicates (water glass) can be used as a depilatory for tanning leathers, making the products smooth and shiny and eliminating the formation of chrome bearing leather waste (Saravanabhavan *et al.*, 2005).

3.7. Textile Industry

Papain can be used for processing wool, boiling off cocoons and refining silks (Freddi *et al.*, 2003). As a result, the products will not shrink and will be quite soft. Natural silk and the engulfing gums produced by silk worms are both proteinaceous

in nature. Since papain can dissolve sericin but is unable to affect silk fibre protein it can be used for the refinement of the mixture of bombycine and vinegar fibre. In the past, papain has been widely used to 'shrink-proof' wool. A successful method involved the partial hydrolysis of the scale tips. This method also gave wool a silky lustre and added to its value. The method was abandoned a few years ago for economic reasons.

3.8. Cosmetic Industry

Enzyme baths containing bacteria and/or enzymes are popular as treatments for giving a smooth skin. Papain can help dispel blotches and pimples, clean the face and promote blood circulation making the skin healthier and tender. Papain and bromelain are used in face-care products to provide gentle peeling effects.

3.9. Organic Chemistry

Papain is used in the synthesis of amino acids (Rai and Taneja, 1998), biologically active peptides (Gill *et al.*, 1996), anticancer drugs (Du, 2003) and polyaspartate (Soeda *et al.*, 2003).

4. USE OF CYSTEINE PROTEASES IN PHARMACY AND MEDICINE

Due to their availability, proteases isolated from plants have a special place in these areas. A wide range of therapeutic benefits are claimed for bromelain, introduced as a therapeutic compound since 1957. Bromelain's principle activities include: the reversible inhibition of platelet aggregation (Morita *et al.*, 1979), fibrinolytic activity (Maurer *et al.*, 2000), anti-inflammatory action (Inoue *et al.*, 1994), the modulation of cytokines and immunity (Desser *et al.*, 1994; Munzig *et al.*, 1995), skin debridement of burns (Rosenberg *et al.*, 2004), anti-tumour activity (Batkin *et al.*, 1988), enhanced absorption of other drugs (Tinozzi and Venegoni, 1978), mucolytic properties (Hunter *et al.*, 1957), a digestion aid (Knill-Jones *et al.*, 1970), enhanced wound healing (Tassman *et al.*, 1965) and cardiovascular and circulatory improvement (Taussig and Nieper, 1979). In addition to the cysteine protease, bromelain preparations also contains other biologically active compounds such as peroxidase, acid phosphatase, several protease inhibitors and organically bound calcium. It was found that isolation of the proteolytic fraction of bromelain leads to loss of the many beneficial effects observed *in vivo* for crude extracts (Taussig and Nieper, 1979). Results obtained from pharmaceutical and preclinical studies recommend bromelain as an orally given drug for complementary tumour therapy. The anti-metastatic activity of bromelain and its ability to inhibit metastasis-associated platelet aggregation as well as the growth and invasiveness of tumour cells is especially promising. The anti-invasive effect was found to be independent

of the proteolytic activity. (For a more comprehensive review of applications and activities of this complex of cysteine proteases see Kelly, 1996).

Another enzyme widely used in medical and para-medical practice is papain. This enzyme is used for wound debridement, the removal of necrotic tissue (Mekkes *et al.*, 1997), the external treatment of hard tissues, wart and scar tissue removal, acne treatment, depilation, skin cleansing treatments and as a component of toothpaste. Papain is used in the preparation of tyrosine derivatives which are used for the treatment of Parkinsonism, and for the preparation of tetanus vaccines and immunoglobulin samples for intravenous injections (Brocklehurst *et al.*, 1981). Chymopapain is applied in the chemonucleolysis of damaged human intervertebral spinal discs (Watts *et al.*, 1975).

Although the toxicity of the above mentioned enzymes is rather low, exposure to the dust or aerosols of their solutions is harmful. Such exposure may induce asthma, rhinitis and allergy (Baur and Fruhmann, 1979; Flindt, 1978; Novey *et al.*, 1979). Papain is used in laboratory practice for artificial induction of emphysema (Martorana *et al.*, 1982) and osteoarthritis (Kopp *et al.*, 1983) in experimental animals. Anaphylaxis is one of the complications caused by chymopapain used in chemonucleolysis (Watts *et al.*, 1975; Ford, 1977; DiMaio, 1976). Others are subarachnoid haemorrhage (Buchman *et al.*, 1985), nerve injury (Mackinnon *et al.*, 1984) and intervertebral disk-space infections (Deeb *et al.*, 1985).

Cysteine proteases have also been recognized as critical enzymes in degenerative and autoimmune states. Lysosomal cysteine proteases of the papain family are involved in different pathological states. Deficiency of enzymatic activity of this group of enzymes was found to occur in two diseases: pycnodysostosis, a skeletal bone dysplasia caused by cathepsin K deficiency, and Pappilon-Lefevre syndrome, a periodontopathia caused by cathepsin C defficiency (Lecaille *et al.*, 2002). However, the major role of papain-like cysteine proteases in pathological states is not related to their deficiency but the overexpression of such enzymes or their activity outside their normal site of action. An understanding of the physiopathological functions of cysteine proteases will permit the design of new selective therapeutic agents.

Tumour cell invasion and metastasis are associated with the proteolytic activities of various types of proteases, including lysosomal proteases. Elevated expression of certain cathepsins and diminished levels of their inhibitors have been observed in several human cancers, including breast, gastric, glioma and prostate cancers, and especially in cases of aggressive cells (Lecaille *et al.*, 2002; Otto and Schirmeister, 1997).

Cathepsins of the papain family seem to play a critical role in rheumatoid arthritis (Taubert *et al.*, 2002) and atherosclerosis (Lecaille *et al.*, 2002; Otto and Schirmeister, 1997).

Cysteine proteases of the papain family play an important role in microbial (viral, bacterial) and parasitic infections (Tong, 2002; Han *et al.*, 2005). They are virulence factors and/or participate in tissue penetration, feeding, replication and immune evasion. The lack of redundancy of the cysteine proteases in these

organisms compared to their mammalian hosts makes them attractive targets for the development of new medically useful compounds.

Intense development of enzyme applications for food and animal feeds, the detergent and textile industries as well as in medicine mean that the current list of cysteine protease applications is incomplete. However, variability in the properties of plant enzymes which depend on weather conditions amongst others may well result in their dispacement by microbial enzymes. Genetic engineering techniques will be applicable not only to source valued enzymes in easy-to-grow micro-organisms but also to modify and tailor enzyme properties to consumer requirements.

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