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REVIEW ARTICLE

# Proteolytic Enzymes Delivery Systems: A Review

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## ABSTRACT

Until fairly recently, proteases were considered primarily to be protein-degrading enzymes. However, this view has dramatically changed and proteases are now seen as extremely important signalling molecules that are involved in numerous vital processes. Protease signalling pathways are strictly regulated, and the dysregulation of protease activity can lead to pathologies such as cardiovascular and inflammatory diseases, cancer, osteoporosis and neurological disorders. Several small-molecule drugs targeting proteases are already on the market and many more are in development. The status of human protease research and prospects for future protease-targeted drugs are reviewed here, with reference to some key examples where protease drugs have succeeded or failed.

## KEYWORDS

Proteases, Digestive Enzymes, FCC (Food Chemical Codex), Enzyme Activity, Synergistic effect, JECFA (Joint (FAO/WHO) Expert Committee on Food Additives), GRAS (Generally Recognized As Safe)

## INTRODUCTION

Protease refers to a group of enzymes whose catalytic function is to hydrolyze (breakdown) peptide bonds of proteins. They are also called proteolytic enzymes or proteinases. Proteases differ in their ability to hydrolyze various peptide bonds. Each type of protease has a specific kind of peptide bonds it breaks. Examples of proteases include: fungal protease, pepsin, trypsin, chymotrypsin, papain, bromelain, and subtilisin.

Proteolytic enzymes are very important in digestion as they breakdown the protein foods to liberate the amino acids needed by the body. Additionally, proteolytic enzymes have been used for a long time in various forms of therapy. Their use in medicine is gaining more and more attention as several clinical studies are indicating their benefits in oncology, inflammatory conditions, blood rheology control, and immune regulation. Contrary to old beliefs several studies have shown that orally ingested enzymes can bypass the conditions of the GI tract and be absorbed into the blood stream while still maintaining their enzymatic activity. Commercially, proteases are produced in highly controlled aseptic conditions for food supplementation and systemic enzyme therapy. The organisms most often used are *Aspergillus niger* and *oryzae*.[1]

Any enzyme that breaks down protein into its building blocks, amino acids, is called a protease, which is a general term. The digestive tract produces a number of these enzymes, but the three main proteases are pepsin, trypsin and chymotrypsin. Special cells in your stomach produce an inactive enzyme, pepsinogen, which changes into pepsin when it contacts the acid environment in your stomach. Pepsin breaks

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certain chemical bonds in proteins, producing smaller molecules called peptides and beginning protein digestion. Your pancreas makes trypsin and chymotrypsin, enzymes that are released into your small intestine through the pancreatic duct. When partially digested food moves from your stomach into your intestine, trypsin and chymotrypsin complete protein digestion, producing simple amino acids that are absorbed into your circulation.

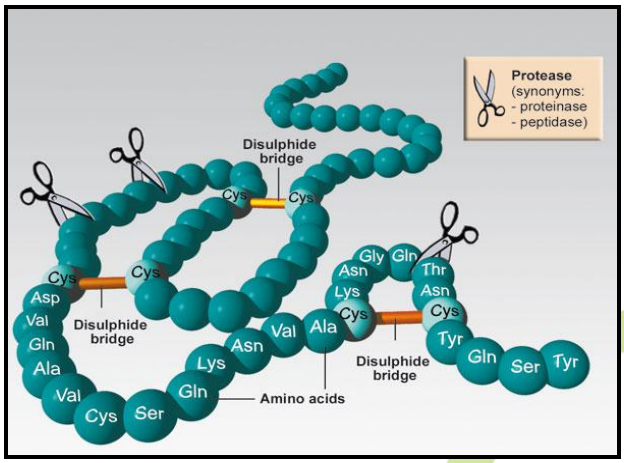


Figure 1: Schematic Representation of Site of Action of Protease Enzymes

(Courtesy: SternEnzym – The Enzyme Designer GmbH& Co. Germany)

Enzymes promote the body's own regeneration processes. Certain proteolytic enzymes possess pronounced anti-inflammatory and anti-edematous properties. They promote the breakdown of toxic metabolites and inflammatory products and thus contribute substantially to the detoxification of the human body. Simultaneously additional fibrinolytic activity of the enzymes and "vessel-sealing" effect of rutin accelerate the blood flow with dissolution of fresh blood clots (microthrombin) and this allows normalization of the microcirculation. Therefore, the inflammatory products that mediate pain are eliminated more quickly, the improved blood supply enhances local oxygenation and tissue tension decreases in parallel with the reduction in edema. All of this provides some analgesic effect. In sharp contrast to the mechanisms of action of conventional non-steroidal and steroidal anti-inflammatory drugs, systemic enzyme therapy thus does not block the natural healing processes of inflammation. It is also characteristic for some enzymes, that they activate or stimulate macrophages and natural killer cells. These cells have well-recognized essential role in the body's own immunological defense. They are part of a larger system, the reticulo-endothelial system. Therefore, enzymes also affect the whole body's immune system as a "biological response modifier". Macrophages stimulated by the enzymes secrete tumor necrosis factor (TNF) and other cytokines. For these two reasons enzymes are predestined to become a future of chemotherapeutic agent, they do not lead to the destruction of all cells with high mitotic activity, but only of those that are actually malignant.[2]

**Activity Measurement for Digestive Enzymes (Proteolytic)**

Enzyme strength is measured in terms of activity. Enzymes may be present, but unless they are functional, they will not do any good. While most food, supplement, and drug comparisons use weight (such as milligrams), the most important measurement with enzymes is the activity and potency of the enzyme. A product label should list enzyme strength in standard activity units rather than by weight. To measure activity of digestive enzymes, tests or assays determine the quantity of digestion that occurs under specific conditions. This activity depends on concentration, quantity, pH, temperature, and substrate. When you review the labeling on a digestive enzyme package, activity mentioned as per Food Chemical Codex (FCC) units. This labeling certifies that the enzymes went through thorough testing for activity and potency. The American food industry accepts these units as set forth by the National Academy of Sciences. Some companies promoting enzymes list measurements based on dosage, weights such as milligrams (mg), or a other things. Weight, dosage, and any other units do not give any information on enzyme activity – 220 mg per capsule does not tell anything about enzyme activity. You may have 220 mg of nothing, or 10% activity or 90% activity.

FCC labeling is the only national standard for the evaluation of activity and potency of enzymes in the United States. The higher the activity number, the quicker the food is digested. A lower number will still be digesting food, but it will take longer. Since enzymes do not get used up in the process, we do not 'run out' of enzymes before all the food is digested, but the stomach and intestines are absorbing food, completely broken down or not, at the same time.

Since we are 'on the clock,' with possible unbroken-down peptides (or other food components) being absorbed, we want the food to be digested by the enzymes before it gets absorbed in a partially broken-down state. FCC labeling example: If Product # 1 has 15,000 HUT of protease and Product # 2 has 45,000 HUT of protease. Product #2 can break down three times more protein than product # 1 in a given period of time. This is how to compare digestive enzyme activity and formulations.[3]

Table 1: Some available Domestic and International Multienzyme preparations

| Brand Name | Contents | Dosage Form |
|---|---|---|
| Enractin* | Trypsin, Bromelain, Rutoside trihydrate | E.C. Tablet |
| Enzomac Plus* | Trypsin, Bromelain, Rutoside trihydrate | E.C. Tablet |
| Phlogam Tabs* | Trypsin, Bromelain, Rutoside trihydrate | E.C. Tablet |
| DigestZyme# | Amylase, Lipase, Protese, Cellulase, Invertase, Diastase, Lactase, Bifidobacterium longum, Lactobacillus Acidophyllus | Capsule |
| Creon# | Pancreatin, Amylase, Lipase, Protese | Capsule |
| VitalzymXe# | Serrapeptase, Papain, Bromelain, Amylase, Lipase, Rutin, Amla | Capsule |
| Wobenzyme® N# | Papain, Trypsin, Chemotrypsin, Bromelain, Rutoside trihydrate, Pancreatin | Capsule |
| Celiac Aid ™ # | Strong Acid Protease(Aspergillus Oryzae higher), Protease (Aspergillus Oryzae), (Bacillus Subtilis), (Carica Papaya), Amylase I (Aspergillus Oryzae), Amylase II (Bacillus Subtilis), Glucomylase, Cellulase | Capsule |
| Vitabase#Systemic Enzyme Blend | Bromelain, Papain, Trypsin, Chemotrypsin, Pancreatin | E.C. Tablet |
| DigeSEB® Super# | Protease(I,II,III), Bromelain, Papain, Peptizyme SP | Capsule |
| DIGEST ASSURE# | Bromelain, Papain, Amylase, Lipase, Lactase, Cellulase | Capsule |

*Domestic Product, # International Product, E.C.-Enteric Coated

Table 2: Selection of Some Publically Available Food-Use Enzyme Safety and Toxicity Testing[4]

| Enzyme | Publically Available Data |
|---|---|
| Fungal & Bacterial amylase | FDA GRAS Notification 22, FDA GRAS Notification 24<br>FDA GRAS Notification 79, FDA GRAS Notification 126<br>21 C.F.R. 184.1148<br>JECFA Evaluations-FAS 22-JECFA 31/5<br>JECFA Evaluations -TRS 759-JECFA 31/17<br>JECFA Evaluations -FAS 22-JECFA 31/11 (1987)<br>JECFA Evaluations -TRS 789-JECFA 35/15<br>JECFA Evaluations -FAS 28-JECFA 37/67<br>JECFA Evaluations -TRS 806-JECFA 37/10<br>Australia New Zealand Application A467 |
| Fungal & Bacterial protease | FDA GRAS Notification 89, FDA GRAS Notification 90,<br>FDA GRAS Notification 333, 21 C.F.R. 184.1150<br>JECFA Evaluations -TRS 789-JECFA 31/15<br>JECFA Evaluations -FAS 22-JECFA 31/8<br>JECFA Evaluations -TRS 759-JECFA 31/17<br>JECFA Evaluations -NMRS 50/TRS 488-JECFA 15/12<br>JECFA Evaluations -FAS 1/NMRS 50A—JECFA 15/9<br>Australia New Zealand Application A1057 |
| Bromelain | 21 C.F.R. 1024<br>JECFA Evaluations -NMRS 50/TRS 488-JECFA 15/11 |
| Papain | 21 C.F.R. 184.1585<br>JECFA Evaluations -NMRS 50/TRS 488-JECFA 15/11 |
| Pepsin | 21 C.F.R. 184.1595<br>JECFA Evaluations -NMRS 50/TRS 488-JECFA 15/11 |
| Cellulase | FDA GRAS Notification 292<br>JECFA Evaluations -FAS 30-JECFA 39/15<br>JECFA Evaluations -TRS 828-JECFA 39/10<br>Australia New Zealand Application A1011 |
| Fungal lipase | FDA GRAS Notification 81, FDA GRAS Notification 111,<br>FDA GRAS Notification 216<br>JECFA Evaluations -NMRS 54/TRS 557-JECFA 18/20<br>Australia New Zealand Application A1036<br>Australia New Zealand Application A569<br>Australia New Zealand Application A519<br>Australia New Zealand Application A517<br>Australia New Zealand Application A516<br>Australia New Zealand Application A402<br>Australia New Zealand Application A435 |

JECFA- Joint (FAO/WHO) Expert Committee on Food Additives, GRAS- Generally Recognized As Safe, C.F.R.-Code of Federal Regulation , FAS-Food Additive Series, TRS-Technical Report Series, NMRS-Nutrition Meetings Reports Series, FDA-Food & Drug Administration,

Table 3: Proteolytic Activity Plant & Animal Origin with its Units as Per FCC[5]

| Proteolytic Activity Source | Application & Principle | Unit |
|---|---|---|
| Plant | This procedure is used to determine the proteolytic activity of papain, ficin, and bromelain. The assay is based on a 60 min. proteolytic hydrolysis of a casein substrate at pH 6.0 and $40^0$C. Unhydrolyzed substrate is precipitated with trichloroacetic acid and removed by filtration; solubilized casein is then measured spectrophotometrically. | FCC Papain Unit (PU) |
| Bacterial | This procedure is used to determine protease activity, expressed as PC units, of preparations derived from *Bacillus subtilis* var. and *Bacillus licheniformis* var. The assay is based on a 30 min. proteolytic hydrolysis of casein at $40^0$C and pH 7.0. Unhydrolyzed casein is removed by filtration, and the solubilized casein is determined spectrophoto metrically | bacterial protease unit (PC) |
| Fungal | This procedure is used to determine the proteolytic activity, expressed as hemoglobin units on the tyrosine basis (HUT), of preparations derived from *Aspergillus oryzae* var. and *Aspergillus niger* var., and it may be used to determine the activity of other proteases at pH 4.7. The test is based on the 30 min. enzymatic hydrolysis of a hemoglobin substrate at pH 4.7 and$40^0$C.Unhydrolyzed substrate is precipitated with trichloroacetic acid and removed by filtration. The quantity of solubilized hemoglobin in the filtrate is determined spectrophotometrically. | Hemoglobin Units on the Tyrosine basis (HUT) |
| Fungal | This procedure is used to determine proteolytic activity, expressed in spectrophotometric acid protease units (SAP), of preparations derived from *Aspergillus niger* var. and *Aspergillus oryzae* var. The test is based on a 30 min. enzymatic hydrolysis of a Hammarsten Casein Substrate at pH 3.0 and at $37^0$C. Unhydrolyzed substrate is precipitated with trichloroacetic acid and removed by filtration. The quantity of solubilized casein in the filtrate is determined spectrophotometrically | Spectrophoto metric Acid protease (SAP) |

FCC- Food Chemical Codex

**Definitions[5]**

- Papain Unit - One papain unit (PU) is defined in this assay as that quantity of enzyme
  that liberates the equivalent of 1 µg of tyrosine per hour under the conditions of the assay.
- PC Unit - Calculation One bacterial protease unit (PC) is defined as that quantity of enzyme that produces the equivalent of 1.5 µg/mL of L-tyrosine per min under the conditions of the assay.
- HUT Unit - Calculation One HUT unit of proteolytic (protease) activity is defined as that amount of enzyme that produces, in 1

min under the specified conditions, a hydrolysate whose absorbance at 275 nm is the same as that of a solution containing 1.10 µg/mL of tyrosine in 0.006 *N* hydrochloric acid.

- SAP Unit - Calculation One spectrophotometric acid protease unit is that activity that will liberate 1 µmol of tyrosine per min under the conditions specified.

Following are some Review articles on proteolytic enzymes

Jon Barron, Chatsworth, claimed, method for treating a subject with a proteolytic formulation which consist of Fungal protease, Papain, Bromelain, Fungal pancreatin, Nattokinase, Amylase, Lipase, Rutin, Ginger and a combination of calcium, magnesium, ionic trace minerals and potassium which is useful in reduces the release or migration, reduces scarring, facilitates digestion, removes impurities from circulatory or respiratory system, reduces allergic reaction, reduces inflammation, enhance immune system, treats an acid reflux or gastro esophageal disease, reduces dental plaque and arterial plaque etc.[6]

Coenen TM, Aughton P. et al, An amino peptidase enzyme preparation obtained from Aspergillus niger was subjected to a series of toxicological tests to document the safety for use as a processing aid for food. The enzyme preparation was examined for sub-acute and sub chronic oral toxicity, and mutagenic potential. No evidence of oral toxicity or mutagenicity was found. Administration of the amino peptidase enzyme preparation at doses of 500, 1000 and 2000 mg/kg body weight/day for 90 days did not induce noticeable signs of toxicity. The no-observed-adverse-effect level (NOAEL) of the enzyme preparation in the sub chronic toxicity study was 2000 mg/kg body weight/day (equivalent to 1152 PHEA units/kg body weight/day). It can be concluded that no safety concerns were identified in the studies conducted with this amino peptidase enzyme preparation derived from Aspergillus niger and produced under controlled fermentation conditions.[7]

William Wong, N.D., et al, No known toxicity has been demonstrated at any level of enzyme dosing in animal studies or in humans. Animals survived outrageously large quantities of enzymes without damage so it has been impossible to find a lethal dose (no LD50). In 'Enzymes – The Fountain of Life,' Lopez relates how researchers fed guinea pigs and rats a daily enzyme dose for six months which would have corresponded to around 250 tablets per day for a 60 kilogram (134 pound) person. No ill effects. In another, clinical study was performed on Rats and Horses. Rats were fed enzymes of 2,500 tablets equivalent to a human dose for 6 months and Horses fed with 250 tablets daily for 6 months. Result of these test found with no significant changes and no harmful effects as well as no toxicity has ever been found. Since enzymes are natural agents, they can be included in the category of the biological response modifiers (BRM). This means that they do not alter the physiology of our body; instead, they just stimulate our bodies to act according to its own inner wisdom. Enzymes will work only if they are needed to do so.

Some associated problem obtaining an, effective formula of enzymes, as some governmental authorities do not permit the use of a mixture of active substances. It is essential to have a mixture otherwise the therapeutic effect desired will not be produced. In the literal sense, any food has a mixture of “drugs” [or chemicals] such as vitamins, minerals, trace elements, amino acids, etc. And, we combine these more complexly when we combine foods. There are some monoenzymatic preparations, but their therapeutic effect is very limited. Many studies have been done regarding the effect of an enzyme used alone and also used in a mixture. There is a clear synergistic effect when combined with more than one enzyme. For example, bromelain has an effect factor of 1, but if it combine with papain which also has an effect factor of 1 the resulting effect will not be their sum of 1+1 = 2, but rather 1+1 = 3, or perhaps even 1+1 = 4. This is due to their natural ability to act synergistically. Generally monoenzymatic preparation is not so much

 

available. Eg. pancreatin itself is composed of at least 12 enzymes.

Caution must be taken in certain specific cases like hemophilic patients also the patients who have a risk of hemorrhage. The reason is that enzymes liquefy blood and make coagulation slower. In case of pregnancy, we must ponder the risk. But all the studies in animals have demonstrated no teratogenic effect by enzyme mixtures.[8]

Michael T. Murray, N.D et al, Proteolytic enzymes or proteases refer to the various enzymes that digest or break down into smaller units protein. These enzymes include the pancreatic protease trypsin, bromelain, papain, fungal proteases, and Serratia peptidase. Preparations of proteolytic enzymes have been shown to be useful in the situations like Cancer, Digestion support, Inflammation, sports injuries and trauma, Sinusitis, asthma, bronchitis, and chronic obstructive pulmonary disease etc. Orally administered most of the proteolytic enzymes absorption studies have confirmed that they are absorbed intact. In fact, they appear to be actively transported across the gut wall .Protease, Lipase and Amylase are most of the enzymes that are used for systemic route. Proteases and lipase's have systemic functions that means they perform jobs all over the body in most every system. Only amylase, the carbohydrate lysing (cleaving or eating) enzyme acts almost solely in digestion.[9]

Bodhankar S.L., et al, Mazzone A, et al, Kee W., H. Tan S, L., Lee V. Salmon Y.M. et al, Proteolytic enzymes are the first line of defense against inflammation. Inflammation is a reaction by the immune system to an irritation. For eg.injury occurred in right knee. The immune system sensing the irritation the knee is undergoing creates a protein chain called a Circulating Immune Complex (CIC) for short, tagged specifically for that right knee. This CIC floats down to the right knee and causes inflammation signs like pain, redness and swelling. But, inflammation is self-perpetuating, itself creating an irritation that the body makes CIC's to in response.[10,11,12]

L Bucci, J Stiles et al, "Sports Injuries and Proteolytic Enzymes", the authors summarized 14 studies on proteolytic enzymes. Over 1500 subjects were studied. The type of proteolytic enzymes used in the studies varied. Trypsin/ chymotrypsin tablets were used in six studies, bromelain in four, papain in two, streptokinase/streptodornase in one, and an unspecified mix of proteolytic enzymes in another. "Favorable results were obtained in every study, with all reporting significant improvements in reduction of pain, swelling, edema, recovery time, period of disability, time of return to normal activities and leg-raise stiffness (for low back pain). The amount of time needed to resolve injuries was halved in most subjects with supplements."[13]

Ernst E., Matrai A, Klin Wschr. et al, The blood is not only the river of life; it is also the river through which the cells and organs dispose of their garbage and dead material. Enzymes improve circulation by eating the excess fibrin that causes blood to sometimes get as thick as ketchup or yogurt creating the perfect environment for the formation of clots. All of this material is supposed to be cleaned off by the liver on "first pass" or the first time it goes through but given the sluggish and near toxic or toxic states of everyone's liver these days that seldom happens. Breaking dead material down small enough that it can immediately pass into the bowel. Cleanse the FC receptors on the white blood cells improving their function and availability to fight off infection. And here we come to the only warning we have to give concerning the use of systemic enzyme – don't use the product if you are a hemophiliac or are on prescription blood thinners like Coumadin, heparin and Plavix. The enzymes cause the drugs to work better so there is the possibility of thinning the blood too much.[14]

Kunze R., Ransberger K., et al, Enzymes are adaptogenic seeking to restore a steady state to the body. When the immune system is running low we become susceptible to infectious disease, when it's cranked up too high then the system creates antibodies that attack its own tissues as are seen in the auto immune diseases

 

of MS, Rheumatoid Arthritis, and Lupus. Here therapeutic dosing of oral administered systemic enzymes will tone down immune function and eat away at the antibodies the immune system is making to attack its bodies own tissue. When the immune system is run down too low the enzymes increase immune response, producing more Natural Killer cells, and improving the efficiency of the white blood cells, all leading to improved immunity.[15]

Steffen C, Menzel J. Z Rheumatol, et al, 4 types of immune complexes according to the Heidelberger curve was prepared as an in vitro model for immune complexes in rheumatoid arthritis and collagen diseases. Immune complexes of high and moderate antigen excess and immune complexes of high and moderate antibody excess were incubated with increasing concentrations of a mixture of enzymes or papain or pancreatin. The concentration of immune complexes was determined by a solid phase C1q radioimmunoassay before and after incubation. Up to 90% of the complexes of antigen excess were disintegrated even by low doses of enzymes (5-10 mg %); total cleavage appeared at 80 mg% enzyme concentration. Complexes of the antibody excess zone were gradually disintegrated by enzyme concentrations gradually increasing from 5 to 80 mg%, where total cleavage appeared. Single enzymes showed less cleavage activity than enzyme mixtures. Although enzymes were administered to supernatants of the Heidelberger precipitation containing soluble immune complexes as well as enzyme inhibitors, enzymatic activity was not impaired and immune complexes were disintegrated. The results of these investigations are discussed in regard to treatment with enzymes.[16]

Heinrich Wrba MD and Otto Pecher MD et al, Systemic enzymes are perfectly safe and free of dangerous side effects. They have no LD-50, or toxic dose. Best of all systemic enzymes can tell the difference between the good CIC's and the bad ones because hydrolytic enzymes are lock and key mechanisms and their will only fit over the bad CIC's. So instead of preventing the creation of all CIC's, systemic enzymes just finish/eat the bad ones and in so doing lower inflammation everywhere and with that pain are lowered also.[17]

A Cichoke et al, proteolytic enzyme use will: I) speed up the inflammatory process and bring it to a conclusion; II) help clean up the waste products in the area; III) decrease pain and swelling; IV) dissolve any small blood clots floating nearby; V) improve the supply of nutrients to the tissue, improving circulation; VI) aid in easing blood flow."[18]

Normal cellular metabolism and exposure to environmental toxins and pollutants generate toxic compounds called free radicals, which can provoke cellular injury and promote disease. According to the Linus Pauling Institute at Oregon State University, Vitamin C effectively reduces the formation of such free radicals even when it is present in small amounts. Rutin is a potent antioxidant in its own right, and when consumed with Vitamin C, it improves the latter's absorption and prevents Vitamin C itself from becoming oxidized, thus prolonging and improving its function.[19]

M.A. Puertollano, et al, Capillaries are the microscopic blood vessels that transport blood, oxygen and nutrients into the tiniest recesses of your body. Sometimes only a few cells in diameter, these living channels are extremely susceptible to oxidative insults. While Vitamin C helps provide a healthy collagen matrix that supports your capillaries, rutin and other bioflavonoids lend integrity to their walls and regulate their permeability, or leakiness. The concerted actions of Vitamin C and rutin prevent capillary rupture, bruising and hemorrhage.[20]

## CONCLUSION

The major areas of interest for protease-targeted therapies are likely to remain the cardiovascular, inflammatory and infectious diseases areas, but discovery efforts will probably increase for cancer and neurodegenerative disorders. A more detailed look at the proteases currently considered as potential targets shows that endogenous proteases are often linked to

chronic diseases, and are therefore attractive to pharmaceutical companies. However, there has recently been a revived interest in protease-targeted drugs for infectious diseases, although only a few (AIDS, Hepatitis C) were considered seriously until recently.

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