

Cold-Adapted Proteases as an Emerging Class of Therapeutics

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ABSTRACT

Proteases have been used in medicine for several decades and are an established and well tolerated class of therapeutic agent. These proteases were sourced from mammals or bacteria that exist or have adapted to moderate temperatures (mesophilic organisms); however, proteases derived from organisms from cold environments—cold-adapted or psychrophilic proteases—generally have high specific activity, low substrate affinity, and high catalytic rates at low and moderate temperatures. Made possible by greater flexibility, psychrophilic enzymes interact with and transform the substrate at lower energy costs. Cold-adapted proteases

have been used in a wide range of applications, including industrial functions, textiles, cleaning/hygiene products, molecular biology, environmental bioremediations, consumer food products, cosmetics, and pharmaceutical production. In addition to these applications, they have also shown promise as therapeutic modalities for cosmeceutical applications (by reducing glabellar [frown] lines) and a number of disease conditions, including bacterial infections (by disrupting biofilms to prevent bacterial infection), topical wound management (when used as a debridement agent to remove necrotic tissue and fibrin clots), oral/dental health management (by removing plaque and preventing periodontal disease), and in viral infections (by reducing the infectivity of viruses, such as human rhinovirus 16 and herpes simplex virus). Psychrophilic proteases with greater activity and stability (than the original organism-derived variant) have been developed; this coupled with available manufacturing recombinant production techniques suggests that cold-adapted proteases have a promising future as a distinct therapeutic class with diverse clinical applications.

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INTRODUCTION

Enzymes that cleave peptide bonds in proteins are also known as proteases, proteinases, peptidases, or proteolytic enzymes [1], and function to accelerate the rate of specific biologic reactions by lowering the activation energy of the reaction [2]. Proteases are most often assumed only to be involved in processes relating to digestion, but the fact that over 2% of the human genome encodes protease genes suggests that they play more complex functions than digestion alone [3]. Indeed, proteases have been shown to be involved in the regulation of a number of cellular components from growth factors to receptors, as well as processes including immunity, complement cascades, and blood coagulation [3]. In addition to involvement in homeostatic processes, increased or dysregulated activity of proteases has been implicated in cancer via its link with tumor growth and invasion [4].

Briefly, proteases are initially produced as inactive precursors, or zymogens, and are distributed in specific organs or locations, where they have little catalytic ability until they are activated by proteolytic cleavage [5]. Further posttranslational mechanisms to control the activity of proteases include phosphorylation, cofactor binding, and segregation of enzyme and/or substrate in vesicles or granules. In addition, the effective concentration of active enzyme can also be strictly regulated by protease inhibitors, which can reduce functional efficacy by forming a complex with the protease and effectively

“balance” proteolytic activity [6]. In this short review, the therapeutic uses and future outlook for proteases (notably cold-adapted proteases) will be discussed.

THERAPEUTIC USE OF PROTEASES

Proteases have been used in medicine for several decades and are an established and well tolerated class of therapeutic agent [3]. Early documented use of proteases in the published literature appeared over 100 years ago [7–9]. In general, proteases have been used therapeutically in four areas: the management of gastrointestinal disorders with orally administered agents, as anti-inflammatory agents, as thrombolytic agents for thromboembolic disorders, and as locally administered agents for wound debridement [10]. Since the first approval of a protease drug in 1978 (urokinase, a serine protease indicated for thrombolysis and catheter clearing), a further 11 drugs have been approved for therapeutic use by the US Food and Drug Administration (FDA) [3]. The majority of these are indicated for the treatment of blood disorders and include thrombolytics: alteplase, reteplase, and tenecteplase; and procoagulants: factor IX, factor VIIa, thrombin, and topical thrombin in bandages. The other approved protease therapeutics are indicated for digestion (pancrelipase), muscle spasms, and as cosmeceuticals (cosmetic products with biologically active ingredients intended to have medicinal or drug-like benefits; botulinum toxin A and botulinum toxin B) [3].

The use of topical proteases as a tool for selective tissue destruction (i.e., ablation of diseased tissue to expedite improvement or cure) is an attractive one. Epidermally confined dermatologic disorders, i.e., those for

which the primary disease is confined to the epidermis (e.g., verruca or actinic keratosis), are known to be cured using superficial destructive techniques to remove diseased skin, allowing the regeneration of healthy tissue from adjacent/accessory structures [2]. Precise tissue destruction is also a desirable property and is possible with topically administered proteases. For example, the ideal method to destroy an epidermal neoplasm would involve selective elimination of malignant tissue without causing damage to healthy tissue or deeper structures. Therefore, exploiting the unique ability of proteases to cause selective epidermal separation is an attractive approach to achieve such desired precision. However, such a level of precision is currently not achievable using conventional methods of therapeutic tissue destruction, such as cryosurgery (with liquid nitrogen), electrosurgery, laser surgery, chemosurgery, and cold-steel surgery, which can produce tissue damage (to varying degrees) that extend unnecessarily beyond the epidermis, which can result in delayed healing, scar formation, and alterations to pigmentation [2]. As a consequence, there has been great interest in using the selective properties of enzymes and, thus, proteases have been examined for effectiveness in a number of such topical applications, including animal models of acne vulgaris, wound healing, epidermal ablation, and debridement of necrotic ulcers.

Trypsin demonstrated antiaging properties and a comedolytic effect (i.e., opening up of clogged pores and lysis of comedones [hard plugs of keratin and sebum within hair follicles]) in a murine model of acne [11]. The principle physiological change that leads to acne vulgaris is the process of a sebaceous follicle transforming to a comedone via hypercornification and hyper-keratinization of the

infundibulum (i.e., the funnel in which the hair follicle grows). A murine model was used to quantify the effects of daily topical trypsin over 5 days' treatment and resulted in improved skin plasticity, increased cell layers in the dermis and epidermis, as well as increased skin elasticity when compared with control treatment. In addition, when compared with controls, trypsin induced follicular-epidermal differentiation and also prevented extensive programmed cell death and apoptosis [11].

Collagenase ointment has also shown benefits in wound healing by achieving selective debridement in porcine models [12]. Partial or full-thickness wounds in Yorkshire pigs were contaminated with *Staphylococcus aureus* and *Pseudomonas aeruginosa*, then treated with *Clostridium* collagenase ointment (Santyl®; Healthpoint Ltd., Fort Worth, Texas, USA), or controls of white petrolatum or moist dressing and untreated wounds. Following treatment over 8 days, collagenase ointment achieved complete re-epithelialization in 85% of animals with partial-thickness wounds compared with only 10% using petrolatum and 0% using moist dressing and untreated wounds. Furthermore, significantly less inflammation and less neutrophil infiltration was observed by histology in the animals treated with collagenase, and re-epithelialization was enhanced, compared with petrolatum [12].

The potential of topically applied proteases for epidermal ablation has also been demonstrated through the in vitro and in vivo use of subtilisin, trypsin, and dispase in murine and human skin samples. These proteases target keratin, desmosomes, and collagen IV, respectively. Following application, they all demonstrated subcorneal separation, intraepidermal acantholysis, and subepidermal dissociation [2]. Furthermore, topical

application of a 2.5% (w/v) solution of bovine trypsin to two seborrheic keratosis for 15 min on the trunk of a human participant destroyed the lesions after 1 month, and after 3 months there was no evidence of scarring, pigment changes, or residual seborrhoea keratosis [2].

The use of streptokinase-streptodornase or crystalline trypsin (Trypure®; Novo Nordisk, Bagsvaerd, Denmark) impregnated in wound dressings was examined in patients with necrotic varicose or arteriosclerotic leg ulcers. Treatment with either protease resulted in a significant reduction in pus and debris associated with the ulcers, as well as a significant increase in tissue granulation ($P < 0.01$ in both groups). Compared with trypsin, the streptokinase-streptodornase formulation was associated with less pain ($P < 0.01$) [13].

In the face of increasing antibiotic resistance among bacteria, development of therapeutics has broadened to compounds that target virulence factors rather than viability. Antivirulence strategies would be less likely to result in the emergence of mutations leading to resistance, due to the reduced impact on the level of selective pressure on the bacterial population [14]. A virulence factor recognized as a tremendous burden on our healthcare system is the formation of bacteria into biofilm. Biofilms, complex structures notoriously difficult to disassemble, protect the colonizing bacteria from the host's immune system and from antibiotic therapy. Much research effort to develop new prophylactic and therapeutic agents has focused on the inhibition of biofilm formation or dispersal of established biofilms, as this would release the bacteria into their planktonic phenotype, which is much more sensitive to antibiotics and to the immune defenses [14].

Efforts to discover effective antibiofilm therapeutic alternatives to antibiotics have been plentiful, and much of that effort has focused on enzyme-based treatments. For example, proteinase K and trypsin were shown to be effective in disrupting biofilm formed by certain staphylococcal strains [15]. The overexpression of bacterial extracellular proteases inhibited biofilm formation [16], and esperase HPF (subtilisin) is effective against multispecies biofilms [17].

PSYCHROPHILIC OR COLD-ADAPTED PROTEASES

The proteases so far approved by the US FDA are sourced from a range of mammals or bacteria that exist or have adapted to moderate temperatures—i.e., mesophilic organisms. In the pursuit of more effective and more flexible proteases, the therapeutic potential of molecules derived from organisms from cold environments has been examined. Those organisms from the three domains of life (bacteria, archaea, eucarya) that thrive in cold environments (i.e., psychrophiles) have developed enzymes that generally have high specific activity, low substrate affinity, and high catalytic rates at low and moderate temperatures [18–20]. In general, when compared with mesophilic variants, the property of greater flexibility in psychrophilic enzymes allows the protease to interact with and transform the substrate at lower energy costs. The comparative ease of interaction is possible because the catalytic site of the psychrophilic protease can accommodate the substrate more easily [20]. However, this increased flexibility is often accompanied by a trade-off in stability [21]. Therefore, in contrast to mammalian analogs, psychrophilic proteases

are more sensitive to inactivation by heat, low pH, and autolysis [18, 19, 21–25].

Comparisons between psychrophilic and mesophilic trypsin suggested that there are a number of structural features that are unique to the cold-adapted trypsin that give greater efficiency, but also reduced stability. Their greater efficiency and catalytic ability arise because of deletions from the surrounding loop regions of the structure. This increased flexibility is generally most pronounced around the site of catalytic activity and enables the protease to move and facilitate reactions at low temperatures, and in a low energy environment [26]. The increased catalytic activity is thought to result from optimization of the electrostatic forces (hydrogen bonds, van der Waals interactions, and ion pairs) at the active site [27]; for cold-adapted serine proteases, this is thought to result from the lower electrostatic potential of the S1 binding pocket caused by the lack of hydrogen bonds adjacent to the catalytic triad [25].

Catalytic activity or enzyme efficiency is often expressed as k_{cat}/K_M (i.e., the specificity constant), where k_{cat} represents the catalytic

production of a product under ideal conditions (i.e., turnover number) and K_M represents the Michaelis constant or the affinity of the protease for the substrate. Generally, the high catalytic rates observed in cold-adapted proteases are the result of modifications in enthalpy favoring higher turnover numbers. However, when looking at proteases that have adapted through strong K_M improvement, such as trypsin (that does not only increase k_{cat} but also increases its catalytic efficiency by lowering its K_M), the distinction between these mesophilic and psychrophilic proteases become more pronounced. An example of this is seen by a 17 times greater catalytic efficiency with trypsin from Atlantic cod, compared with trypsin from bovine sources (Fig. 1) [22]. Detailed examination of the temperature performance of cod and bovine trypsin demonstrated that the cod-derived protease displayed a twofold increase in k_{cat} and a more than eightfold improvement (reduction) in K_M . Practically, the main implication of a lower K_M is that a lesser amount of enzyme is required to gain a high catalytic efficiency. Furthermore, in a study comparing Atlantic cod trypsin with

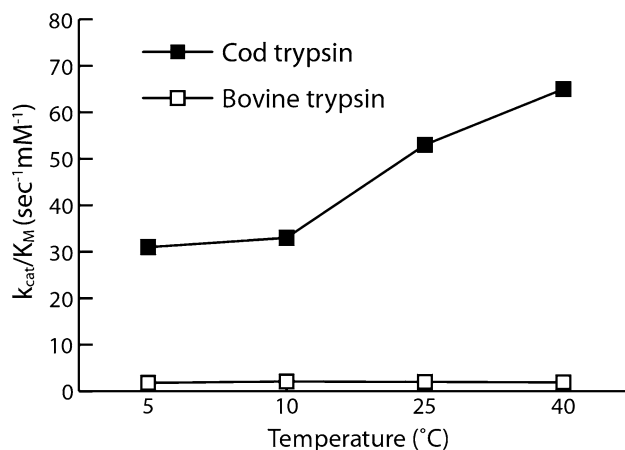


Fig. 1 Example of the large impact of temperature on the specificity constant k_{cat}/K_M on a cold-adapted protease, here determined for Atlantic cod trypsin and its mesophilic homolog, bovine trypsin, using the synthetic substrate,

benzoyl-L-arginine *p*-nitroanilide. The same pattern also applies to other substrates. k_{cat} turnover number, K_M Michaelis constant. Adapted with permission from Asgeirsson et al. [22]

bovine trypsin [28], the cod trypsin cleaved proteins more effectively across a range of temperatures. For example, at temperatures up to 25°C, cod trypsin more effectively cleaved intercellular adhesion molecule 1, myoglobin, lactoferrin, and lysozyme when compared with bovine trypsin. At lower temperatures (4°C), this difference in effect was even more pronounced. Overall, it appears that for cold-adapted proteases, the enzyme activity curve as a function of temperature is shifted toward low temperature (compared with their mesophile counterparts). Therefore, either due to improved k_{cat} or K_{M} , the catalytic activity ($k_{\text{cat}}/K_{\text{M}}$) values are higher for psychrophilic proteases than their mesophilic counterpart over a temperature range from 0°C to at least 30°C. In fact, many cold-adapted enzymes have temperature optima in the range of, or even closer to, the temperature range in which mesophilic enzymes operate naturally, than mesophilic enzymes themselves [18, 22]. However, the greater efficacy is accompanied by a reduced thermal stability, evident in the fast denaturation at moderate temperatures [18, 27]. Variations in the flexibility and rigidity of the psychrophilic protein may explain the greater adaptability and efficacy at lower temperatures, and also the reduced stability. Structural changes, such as fewer hydrogen bonds, fewer salt bridges, and poorer van der Waals packing interactions in the core, are evident in psychrophilic proteases [25]. However, this is not a widespread rule; while some psychrophilic proteases have lower stability than mesophilic analogs, some have decreased stability only at the sites of substrate binding and catalysis [10, 29].

Clearly, if a psychrophilic protease were to be the most effective in a mesophilic environment, there is the obvious requirement to enhance its fundamental stability and functionality. Before

applying the thermal stability traits of a mesophilic protease to a psychrophilic analog, an understanding of the relationship between stability, static and dynamic flexibility or plasticity, and catalytic efficiency of cold-adapted proteases is required. Site-directed mutagenesis and directed evolution are among the methods expected to produce proteases that exhibit the stability of a mesophilic product while retaining the efficiency of a psychrophilic molecule [21, 30–33].

Using random mutagenesis, saturation mutagenesis, and in vitro recombination/DNA shuffling, Miyazaki and colleagues [31] generated mutant libraries of the psychrophilic protease, subtilisin S41. Of the resulting proteases, one variant (3-2G7) had an optimal operating temperature increased by 10°C, without compromising activity at low temperatures, and exhibited threefold greater catalytic efficiency. Subsequent generations of this protease have also been developed and have demonstrated even greater levels of activity and stability [32]. One of the authors postulated that a protease with increased activity at low temperature and stability at higher temperatures can exist physically, but it had not been found naturally due to the course of evolution [31].

While it has been shown that it is possible to modify psychrophilic proteases to be more stable at higher temperatures, the opposite is also true: existing mesophilic proteases can be engineered to achieve improved function at low temperatures. For example, based on subtilisin BPN', an alkaline serine protease, sequential in vitro mutagenesis was employed to produce a cold-adapted mutant. Using three mutations in the structure of subtilisin, two that enhanced activity and one that reduced activity, a cold-adapted variant was produced that had a 100% increase in activity compared with the

wild type. The increase in activity was primarily attributed to increased affinity of the mutant variant for the substrate [33].

That the cold-adapted proteases exhibit reduced stability at moderate temperatures need not be considered a disadvantage; in fact, it could prove to be an important property for exploitation if considered for therapeutic use, in particular, topical administration. Therefore, while psychrophilic proteases have high activity and efficiency when applied locally at ambient temperatures, because they are optimized for activity at lower temperatures, they will lose activity and be degraded once equilibrium with the warmer *in vivo* environment is reached; thereby limiting the duration of activity and ensuring that the protease only exhibits a controlled localized effect. Another factor favoring cold-adapted proteases with regard to safety in therapeutic use is that the high catalytic efficiency requires exposure to a smaller amount of enzyme. This is particularly true for proteases with a low K_M , such as cod trypsin. Furthermore, the inherent greater flexibility of cold-adapted proteases has been reported to be particularly useful in conditions, such as low water conditions (e.g., targeting lipid membrane proteins, lipid layer of mucus), wherein the activity of mesophilic and thermophilic enzymes is severely impaired by the high level of structural rigidity [34].

In the event that an extended half-life or greater exposure may be required, proteases can be administered in their inactive zymogen form (to be subsequently activated *in vivo*). Furthermore, greater tolerability may be achieved by engineering the protease to have reduced antigenicity and immunogenicity [35].

While psychrophilic proteases have been obtained from biological sources, such as Atlantic cod (*Gadus morhua*) or Antarctic krill (*Euphausia superba*), the large-scale production

of suitable quantities of homogenous cold-adapted proteases could be obtained using recombinant technologies. A wide variety of fish enzymes and proteases has already been identified, cloned, and expressed in microorganisms [36]. In the production of other proteases for therapeutic purposes, non-human sources or production hosts are preferred so that the potential for contamination can be avoided. Recombinant technologies are thus widely employed to produce approved mammalian (recombinant) therapeutic proteins, such as blood clotting factors (from recombinant Chinese hamster ovary or baby hamster kidney cells), thrombolytics (from *Escherichia coli*), or botulinum toxin (*Clostridium botulinum*) [3]. Therefore, it would appear logical to explore the possibility of producing cold-adapted proteases through recombinant technology. There have been several, more or less successful, attempts to do this in the laboratory. However, large-scale production of recombinant cold-adapted enzymes is associated with several complicating factors, such as the short half-life and autolytic activity of cold-adapted enzymes, which makes production difficult under more standardized industrial conditions and temperatures.

THE USE OF COLD-ADAPTED PROTEASES AS THERAPEUTICS

To date, cold-adapted proteases have been used in a wide range of applications, including industrial functions, textiles, cleaning/hygiene products (detergents), molecular biology, environmental bioremediations (reducing contamination), consumer food products (dairy manufacturing and preparation), cosmetics, and pharmaceuticals (as biocatalysis

in organic synthesis of drugs and/or intermediates in their generation) [1, 10, 29].

Cosmeceuticals and Dermatology

The use of proteases for cosmeceuticals is of great interest and potential. One example of such a protease is the FDA-approved botulinum toxin A indicated for muscle spasms. Botulinum toxin A disrupts neurotransmission by inhibiting acetylcholine release and inactivates soluble N-ethylmaleimide-sensitive factor-attachment protein 25 (SNAP-25). Although indicated for the treatment of muscle spasms, botulinum toxins are probably best known for their utility in reducing glabellar (frown) lines [3].

The removal of necrotic tissue and fibrin clots is considered a critical phase in wound care and, as such, proteases are considered to have the potential to be important in the removal of barriers to tissue regeneration and tissue healing [37]. Investigations have shown that proteolytic enzymes from Antarctic krill (acidic endopeptidases [trypsin and chymotrypsin-like enzymes] and exopeptidases [carboxypeptidase A and B]) were shown to be superior to saline control in facilitating recovery in a standard porcine model of wound management [38]. Electrokeratome and trichloroacetic acid were used to create necrotic ulcers in a model of wound recovery in domestic pigs, which were then treated twice daily with dressings impregnated with various concentrations of krill or saline control for 7 days. The krill proteases (at a concentration of ≥ 3.0 casein units/mL) were found to be an effective debridement tool that, when compared with the control treatment, significantly reduced the degree of necrotic tissue ($P < 0.05$), improved tissue granulation, and enhanced wound healing [38]. In addition,

the krill proteases achieved wound cleaning 3–4 days earlier when compared with control treatment.

Periodontal Disease

Tooth decay or dental cavities are caused by the build-up of bacterial plaque and can lead to oral disease. Formed through a number of steps whereby “pioneer” bacteria adhere to dental pellicle (the protein film on the surface of tooth enamel) and subsequent bacteria adhere to the pioneer colonizers, a matrix is formed of salivary components and bacteria. If not adequately managed by mechanical removal (e.g., by brushing or flossing), the toxic bacterial products from accumulated plaque can lead to gingivitis and periodontal disease, the most common oral disorder in industrialized populations [39, 40]. Preventive measures to reduce periodontal disease and the requirement for dental treatments would have obvious benefits.

Krill-derived proteases have shown potential in the management of periodontal disease [41]. In vitro examination indicated that krill enzymes were able to inhibit the binding of oral bacteria to saliva-covered surfaces and detach bacterial plaque; accumulated plaque was effectively removed from dentures without having an effect on the normal (beneficial) microbial flora of the oral environment [39]. Furthermore, when used in a chewing gum formulation, krill proteases were shown to reduce gingivitis. In addition to regular dental care, krill proteases (0.06 or 6.0 U) were delivered via a chewing gum (for 10 min, four times a day for 10 days) to healthy volunteers. Compared with placebo gum, the krill protease-containing gum disrupted bacterial adherence to dental pellicle and a significant reduction (54%, $P < 0.05$) in gingival bleeding was seen

with a therapeutic dose of 0.06 U krill enzymes compared with placebo chewing gum [41]. The gum containing proteolytic enzymes was found to be well tolerated as none of the subjects reported any adverse reactions or events during the entire trial period.

Viral Infections

Acute nasopharyngitis, or the common cold, caused by any one of a large number of antigenically distinct viruses and as one of the most common infectious syndromes in humans, is associated with significant health burden, both in terms of financial and quality of life outcomes [42, 43]. Pathogens of the enterovirus family (human rhinoviruses and Coxsackie A virus serotypes) are the principal causative agent in viral infections and can result in symptoms such as sore throat, sneezing and rhinorrhea, and secondary bacterial infections, as well as more severe symptoms by exacerbating asthma, chronic obstructive pulmonary disease, and cystic fibrosis [42, 43].

Rhinovirus, the most common cause of colds and acute respiratory tract illness [34], gains entry into host cells of the nose and throat by interacting with the human intercellular adhesion molecule 1 (or CD54) [15]. This suggests that proteases that target these molecules, such as those from cod trypsin [28], may have therapeutic potential in the management of viral infections. Indeed, *in vitro* studies have shown that exposing viruses to trypsins results in a reduction in infectivity/activation [44, 45]. Furthermore, data from postmarket studies suggest that the use of ColdZyme® (Enzymatica AB, Lund, Sweden) mouth spray, an oral solution containing glycerol and a cold-adapted cod trypsin, can reduce the incidence of the common cold [46]. Marketed for use as a

moisturizer and to improve oral hygiene, users of ColdZyme noted a reduced occurrence of cold symptoms. The ColdZyme mouth spray creates a thin film in the mouth and throat cavity that acts as an active surface barrier with proteolytic activity. Furthermore, the cold-adapted trypsin used in ColdZyme mouth spray has shown high efficiency in reducing the infectivity of human rhinovirus 16 [46] and herpes simplex virus 1 *in vitro* [47].

A summary of the proteases can be found in Table 1 [2, 3, 11–13, 38, 39, 41, 46, 47].

CONCLUSION AND FUTURE SCENARIOS

Proteases, a recognized, effective, and well tolerated drug class that have been used therapeutically over a number of years, illustrate great potential in the management of a number of disease conditions. This recognition is a stimulus for the investigation of promising proteolytic enzyme variants, including cold-adapted proteases (and other enzymes), for further therapeutic applications. Cold-adapted proteases, therefore, have a promising future as a distinct therapeutic class with diverse clinical applications. This is illustrated by their ability to catalyze biological processes more effectively than mesophilic analogs at lower temperatures, demonstrate good safety profiles, have efficacy in topical applications with a relatively localized effect, and be readily manufactured through recombinant production processes.

As our understanding of their structure and function has broadened, proteases with greater efficacy and stability have been produced while retaining high specificity constants, which provides a tantalizing insight into how they might be employed as therapeutics in the

Table 1 A summary of proteases for therapeutic use [2, 3, 11–13, 38, 39, 41, 46, 47]

FDA approved	Use	Protease description
Cardiovascular/hematology	Thrombolytics	Serine proteases urokinase; alteplase, reteplase, and tenecteplase
	Procoagulants	Factor IX, factor VIIa, thrombin, and topical thrombin in bandages
Dermatology	Wound healing	Collagenase
Digestion	Digestion aid	Pancrelipase
Neuromuscular	Muscle spasms	Botulinum toxin A and botulinum toxin B
Other	Protease action/effect	Protease
Dermatology	Anti-aging properties and comedolytic effect	Trypsin
	Epidermal ablation	Subtilisin, trypsin, and dispase
	Wound healing	Streptokinase-streptodomas
	Wound debridement and management	Acidic endopeptidases (trypsin and chymotrypsin-like enzymes) and exopeptidases (carboxypeptidase A and B) ^a
Periodontal disease	Reducing plaque and gingivitis	Acidic endopeptidases (trypsin and chymotrypsin-like enzymes) and exopeptidases (carboxypeptidase A and B) ^a
Infection	Prevent contracting a cold (rhinovirus)	Cod trypsin
	Biofilm disruption/antibacterial	Proteinase K and trypsin

FDA US Food and Drug Administration

^a From Arctic krill

future. Applications in which proteases may hold promise in the future include the prevention of infection and disease, enhancing the management of peripheral artery disease and thrombosis, dermatology, and wound care.

It is imperative that we continue investigating ways in which potent candidates such as cold-adapted proteases can offer competent alternatives to traditional pharmaceutical therapy, in particular when systemically active agents, such as antibiotics, are used to treat local bacterial or viral infections. Therefore, the authors strongly propose the consideration of cold-adapted

proteases as an emerging class of therapeutics for the treatment of infectious diseases.

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Conflict of interest. Both authors are employees of Enzymatica AB, as stated in their affiliations.

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