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REVIEW

Serine Proteases of Small Intestine Mucosa – Localization, Functional Properties, and Physiological Role

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Abstract—In this review we present data about small intestine serine proteases, which are a considerable part of the proteolytic apparatus in this major part of the gastrointestinal tract. Serine proteases of intestinal epitheliocytes, their structural–functional features, cellular localization, physiological substrates, and mechanisms of activity regulation are examined. Information about biochemical and functional properties of serine proteases is presented in a common context with morphological and physiological data, this being the basis for understanding the functional processes taking place in upper part of the intestine. Serine proteases play a key role in the physiology of the small intestine and provide the normal functioning of this organ as part of the digestive system in which hydrolysis and suction of food substances occur. They participate in renewal and remodeling of tissues, retractive activity of smooth musculature, hormonal regulation, and defense mechanisms of the intestine.

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Despite significant advances in studies of proteases, in many cases their natural substrates, mechanisms of regulation of activity, and physiological roles remain unknown. Considering the complexity of proteolytic systems taking part in the whole spectrum of life functions in an organism, understanding the function of a particular protease requires as far as possible analyzing it in the context of the functioning of an organ, tissue, or a cell in which the enzyme is found. Therefore, data about the degradome of an organism (tissue, cell), i.e. the full spectrum of proteases and their substrates and inhibitors that function in a given organ (tissue, cell) can be of great help [1].

This review summarizes data on serine proteases of the small intestine mucosa, which represent a significant part of the proteolytic apparatus of this essential section of the digestive track. The small intestine (*intestinum tenue*) is the major organ for digestion, where digestion and absorption of exogenic food substances occurs practically completely. Moreover, the small intestine, acting as an intermedium between the organism and the environment, has protective functions and acts as a barrier against the flow of nutrients. Functional activity of the small intestine is largely determined by biochemical processes involving proteases. Traditionally, the role of intestinal proteases was reduced to degradation of food proteins, but in recent years proteases were found to provide the most precise control of biological processes on the cellular level, which is achieved by highly specific hydrolysis of peptide bonds (proteolytic processing) [2]. Proteases modulate and terminate a variety of cellular reactions controlling essential biological processes such as digestion, proliferation, cell differentiation and migration, tissue morphogenesis and remodulation, DNA replication, neuronal sprouting, angiogenesis, wound healing, immunity, and apoptosis [2]. Deficiency or excess in proteases, as well as untimely exhibition of their activities, underlie the origins of a number of pathologies, including diseases connected with anomalies in small intestine functioning.

In the upper part of the small intestine – the duodenum – the main physiological processes providing constant reparation of intestinal epithelium, secretion of mucus, synthesis of enzymes and inhibitors, and of humoral and other factors occur. An enzymatic digestive conveyor functions in the duodenum that provides complete hydrolysis of the main types of macromolecular compounds composing food particles. The most elegant and well-adjusted mechanism of interaction between pancreatic and intestinal proteases guarantees activation of zymogens, localization, and attaining the necessary concentrations of active enzymes in the respective regions of the digestive apparatus [3]. Regulation of these and

other processes is largely provided by multifunctional serine proteases, which are key enzymes of the small intestine proteolytic systems. The present review systematizes data on small intestine serine proteases in terms of their functional importance.

To more fully represent the physiological environment of the analyzed proteases as well as the conditions of their functioning, we include brief information on small intestine histology and a short characterization of the processes involving various types of cells.

STRUCTURE OF INTESTINAL EPITHELIUM

Figure 1 presents a general histogram of human small intestine mucosa, the localization of serine proteases in different types of epitheliocytes being shown schematically. The surface of the intestinal villi is covered with one layer of cylindrical epithelium with significant cellular polarity. The main part of its mass (about 90%) is comprised by enterocytes with characteristic brush border formed by microvilli of apical plasmatic membrane where parietal digestion takes place. The main function of these cells is absorption of nutrients [3, 4]. Between enterocytes, single goblet cells are located; they secrete mucus covering the surface of the intestinal villi. Near the bottom of villi the epithelial layer forms invaginations called crypts of Lieberkuhn (Fig. 1). Crypts, as well as villi, are layered with epitheliocytes with single goblet cells in between. Deep in the crypts exocrinocytes are located; they contain acidophilic granules and are called Paneth cells (Fig. 1). In crypts as well as in villi epithelium endocrine cells functional activity is found that is connected with hormonal activity of the intestine. In the structure of the epithelium layer there are also lymphocytes, which enter from villi stroma through the basal membrane [5].

Intestinal epithelium has a high rate of cell renewal. In human small intestine every minute from 10 to 60 million epithelium cells are formed. In the same time, the same number of cells dies and is rejected into the intestinal lumen [6]. Renovation of epithelium occurs by multiplication of stem crypt cells. Multiplying stem crypt cells are pushed towards the top of the villus and replace old shedded cells of bordered epithelium. As enterocytes move from the bottom of crypts to the top of a villus, they differentiate and mature. This process is accompanied by changes in metabolism, structure and enzyme spectrum.

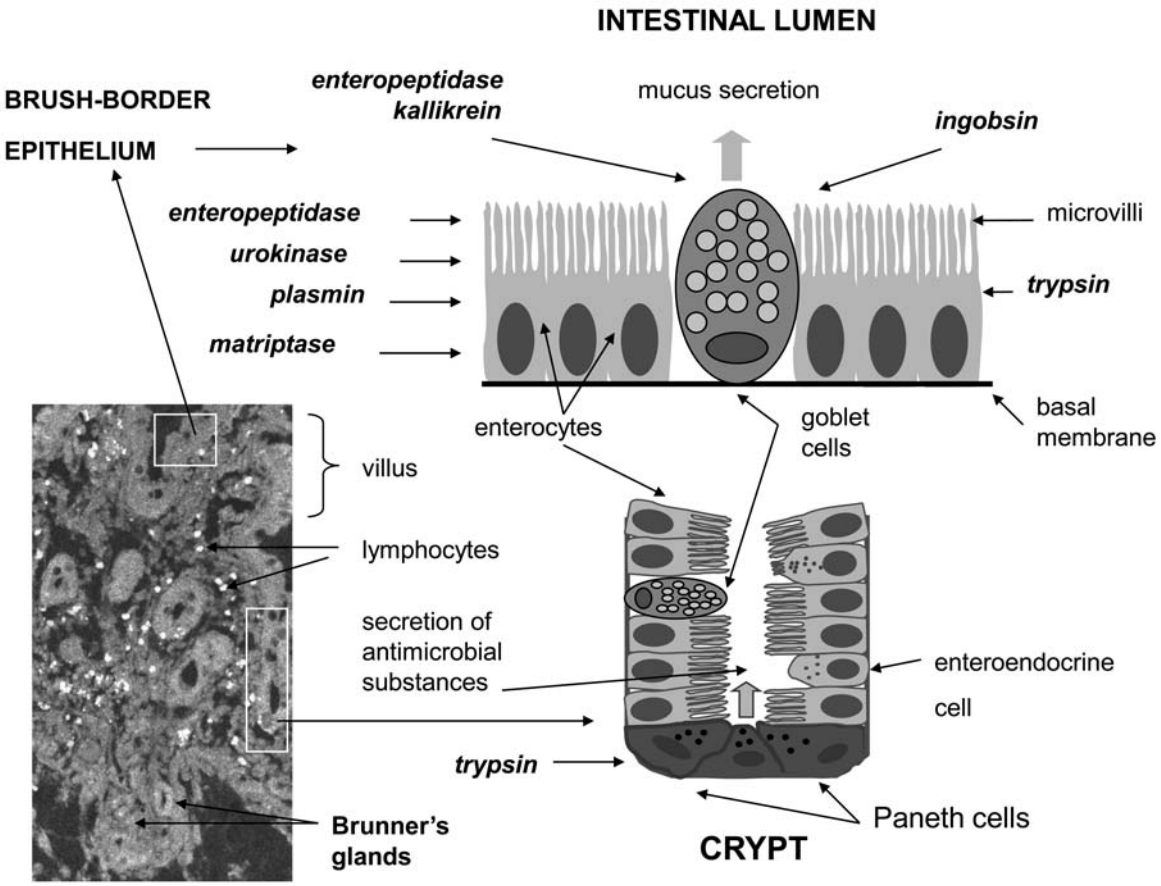

Fig. 1. Localization of serine proteases in epithelial cells of small intestine mucosa (scheme).

In addition to crypts, duodenal mucosa contains duodenal (Brunner's) glands (Fig. 1). They are located in the submucosa, sometimes filling it entirely. Their ducts pierce the muscle plate of mucous membrane and open up between the crypts or on their bottoms [4, 7]. The secretion of these glands contains many biologically active factors involved in various processes occurring in the duodenum, such as recovery and growth of the intestinal epithelium, the formation of protective viscous secretions, nonspecific immunity, proteolysis, and hormonal regulation [7].

MULTIDOMAIN TRANSMEMBRANE SERINE PROTEASES OF ENTEROCYTES: ENTEROPEPTIDASE AND MATRIPTASE (MT-SP1)

Proteases of enterocytes – enteropeptidase and matriptase – belong to the type II multidomain transmembrane serine protease family (TTSP) [8]. This group of enzymes is characterized by the presence of a proteolytic domain, a transmembrane domain, a short cytoplasmic domain, and an extended backbone domain connecting the transmembrane and proteolytic domains. Proteases of this group are synthesized as a single-chain precursor that is activated by enzymatic hydrolysis of a bond formed by an arginine or a lysine carboxyl group located in a characteristic activation area. The active double-chain form of the enzyme remains bound to the membrane, but there is evidence of the existence of a soluble form of some representatives of the TTSP, including the enterocyte proteases enteropeptidase and matriptase.

Substrate specificity of known proteases from the TTSP group is trypsin-like, with a preference for Arg as the P1 residue (Schechter–Berger nomenclature [9]). For some TTSP (including matriptase), autoactivation ability is assumed, whereas enteropeptidase is virtually unable to activate its own zymogen [10, 11].

Enteropeptidase as a key enzyme in the activation cascade of digestive enzymes. Enteropeptidase is localized at the apical membrane of mature enterocytes (within the brush border) and in goblet cells [12] (Fig. 1). The presence of the enzyme is more pronounced at the villi apex, where mature enterocytes are localized. Enteropeptidase content in the area of the crypts is minimal. The enzyme is also present in the dense part of the intestinal secretions, where it appears after rejection of enterocytes [13].

Enteropeptidase is a highly specific protease of the small intestine that activates trypsinogen. Trypsin, in turn, is an activator for zymogens of pancreatic digestive enzymes such as chymotrypsin, elastase, kallikrein, carboxypeptidase, and some others [14]. Trypsin as a component of the destructive proteases participates in cavitary digestion.

Studies of enteropeptidase substrate properties using synthetic peptide and protein substrates demonstrated the necessity of four acidic residues at substrate positions P5-P2 for efficient enzyme hydrolysis. The physiological substrate of enteropeptidase, trypsinogen, has the typical conservative sequence (Asp12-Asp-Asp-Asp-Lys16) in the activation peptide [15] except in human trypsinogen [16].

According to X-ray analysis, residues Asp P2 and P4 of trypsinogen form ionic bonds with the unique primary exosite of the enteropeptidase catalytic subunit (Lys886-Arg-Arg-Lys889) [17]. It is assumed that there are two additional secondary substrate-binding sites in the light and heavy chains, each specific to the natural substrate – trypsinogen [18, 19]. The influence of the heavy chain of enteropeptidase during the interaction of the enzyme with low molecular weight substrates is insignificant, but it becomes crucial in recognition of macromolecular substrates and inhibitors [20].

Bovine proenteropeptidase can be activated *in vitro* with trypsin and duodenase (see below) [21, 22]. The active form of enteropeptidase from different species of animals and humans is a double-chain glycoprotein with high carbohydrate content (35-57%), with the heavy (82-140 kDa) and light (35-62 kDa) chains linked by disulfide bonds. The light chain contains catalytically active residues of a serine triad – Ser987, His841, and Asp892 [23]. The heavy chain has complex mosaic structure that includes a number of domains that enable interaction with the membrane, the polarity of enzyme localization in the cell (apical membrane), specific substrate recognition, and some other functions [10, 21].

Lack of enteropeptidase activity in the intestinal mucosa is associated with serious disorders of the digestive process as it impedes digestion of proteins, which leads to severe pathological conditions [24, 25].

Matriptase is a key enzyme in the process of epithelium renewal. Epithelial cells of the apical region of the villi of the small intestine express an arginine-specific protease – matriptase or MT-SP1 (membrane-type serine protease 1) [26, 27]. Matriptase is localized in the basal-lateral region of enterocytes where intercellular contacts and epithelial cells adhere with the basal membrane [28] (Fig. 1).

Matriptase is a membrane-bound glycoprotein with molecular mass of 80-90 kDa. The mechanism of matriptase activation involves the formation of an activation complex in which intermolecular activation of neighboring zymogen molecules occurs [29]. The complex is formed by noncatalytic domains of the enzyme and a physiological matriptase inhibitor (HAI-1), which facilitate the induction of the zymogen conformation required for the activation process [30]. Mature matriptase is a double-chain heterodimer with characteristic features of TTSP proteases [27]. The active form of matriptase can be released into the intercellular space; this is made possible by the processing of the *N*-terminal region of the enzyme molecule (hydrolysis of the Gly149–Ser150 bond) [28].

Localization of matriptase on the basolateral membrane allows the enzyme to interact with factors involved in intercellular and cell–substrate interactions. The biological role of matriptase is associated with epithelium

renewal [28]. As mentioned above, intestinal epithelial cells are a highly mobile population of cells. Migration of epithelial cells from crypts, where the division of epithelium stem cells occurs, to the apex of the villi is accompanied by differentiation, maturation, functioning, and termination of the life cycle of epithelial cells, and it involves the controlled destruction of cells adhesion sites and their subsequent recovery. The control mechanism of intestinal epithelium renewal by the regulation of enterocytes adhesion with basal membrane involving matriptase and other proteases is shown on Fig. 2.

Matriptase (MT-SP1) activates the precursor of urokinase (uPA), which is involved in regulation of cell–substrate interactions through activation of proteases in the basal membrane of the intestinal epithelium [31]. In addition, matriptase activates hepatocyte growth factor (HGF), which is a signaling protein for epithelial cell dissociation [32].

The secretory form of matriptase is able to cleave extracellular matrix proteins – fibronectin and laminin – which disrupts the binding of these proteins with their receptors (integrins) on the surface of epithelial cells resulting in a weakening of cell adhesion and facilitation of their migration to the apex of the villi and subsequent release in the luminal surface of the intestine [28].

Thus, matriptase plays a key role in migration of intestinal epithelia by direct (hydrolysis of cellular matrix proteins) and indirect (activation of uPA and HGF) participation in the weakening of cell adhesion to the basal membrane.

FIBRINOLYTIC FACTORS – PLASMINOGEN ACTIVATORS AND PLASMIN – PROMOTE EPITHELIOCYTE MIGRATION IN INTESTINAL MUCOSA

In small intestine mucosa serine proteases are synthesized that belong to the fibrinolysis system – plasminogen activators of urokinase and tissue type (uPA or urokinase and tPA, respectively) and plasminogen [33]. The rate of synthesis of these factors and the concentration of active

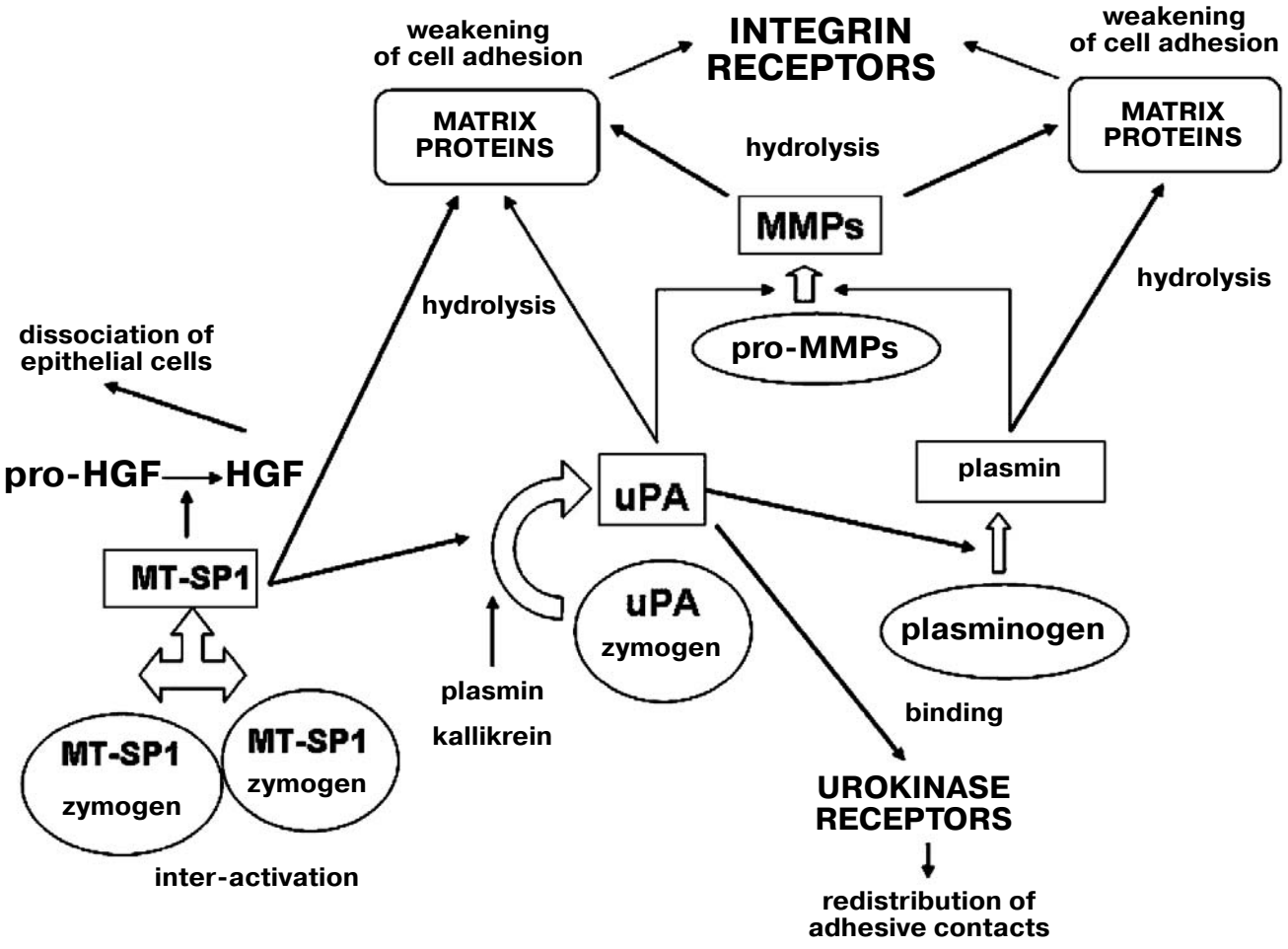

Fig. 2. Control mechanism of intestinal epithelium renewal involving epithelial proteases. MMPs, matrix metalloproteases; uPA, urokinase; HGF, hepatocyte growth factor; MT-SP1, matriptase.

enzymes in the small intestine is significantly higher than in other segments of the intestine. The physiological role of tissue fibrinolytic proteases is traditionally connected with pathologies of the respective organs, in this case the intestines. The rate of fibrinolysis in intestinal mucosa increases in inflammation and hemorrhagic lesions of the small intestine caused by such diseases as duodenitis, Crohn's disease, peptic ulcer, ulcerative colitis, etc. [34-36]. Proteolytic events in acute the stage of inflammation are a highly coordinated process that initiates mechanisms of remodeling and regeneration of damaged tissues [37]. Intensive extracellular proteolysis plays an important role in mucosa functioning in the absence of pathologies as well. This fact is primarily connected with promoting renewal of intestinal epithelium. The plasminogen activator–plasmin system, besides its participation in fibrinolysis, is directly involved in regulation of functions of a wide spectrum of extracellular matrix components [38] and is an important factor promoting epithelium renewal.

Urokinase or plasminogen activator of urokinase type (uPA). Urokinase is a multidomain extracellular protein synthesized in various types of cells including enterocytes of the small intestine [38]. Urokinase binds to the cell surface through specific interaction with membrane-bound proteins. The enzyme is localized in the epithelial cells of the upper part of villi, where the adhesion of cells to the basal membrane is the least durable, and enterocytes are released into the intestinal lumen [38]. The maximal activity of urokinase correlates with the index of proliferation and renewal of enterocytes, which, according to Gibson et al. [38], clearly indicates the role of this enzyme in epithelium renewal.

Urokinase is synthesized as a single-chain precursor with a molecular mass of 54 kDa. The mature double-chain form of urokinase is formed by proteolytic activation under the action of plasmin. Urokinase can also be activated by kallikrein, blood clotting factor XIIa, or cathepsin B [39]. The structure of urokinase contains three domains. The *N*-terminal domain is homologous to epidermal growth factor and interacts with urokinase receptor u-PAR/CD87. The kringle domain contains sites of interaction with the specific protein inhibitor PAI-1, heparin, and another receptor different from u-PAR/CD87. Finally, the proteinase domain has the characteristic catalytic triad of the active site of serine proteases [40]. Each of the protein domains has rigid structure supported by disulfide bonds. The light chain of urokinase contains the *N*-terminal and kringle domains, and the heavy chain contains the catalytic domain. The enzyme chains are connected by a single disulfide bond. Plasmin can sequentially cleave the *N*-terminal and kringle domains with the formation of low molecular weight active forms of urokinase on the cell surface. Single-chain urokinase in complex with receptor is efficiently activated by plasmin, but it is resistant to further proteolysis [39].

Urokinase plays a significant role in processes associated with cell–substrate adhesion, in particular, it promotes cell migration that accompanies the renewal of intestinal epithelium. Urokinase cleaves some proteins of the extracellular matrix (fibronectin) and activates matrix metalloproteases that hydrolyze matrix proteins [41] (Fig. 2). In addition, urokinase activates plasminogen on the cell surface, which, in turn, is an activator of metalloproteases and can directly cleave extracellular matrix proteins [42, 43]. Matrix proteins are specifically recognized by integrin receptors, which are associated with intracellular signaling systems that regulate the cytoskeleton state, adhesion, and chemotaxis [44, 45]. Urokinase affects the functional state of the cell by modifying the surrounding matrix. In addition, urokinase, either directly or through plasmin formation, activates growth factors or releases them from the matrix [40].

In addition to providing extracellular proteolysis, urokinase affects the adhesion and migration of cells independently of proteolytic activity. Urokinase binds to membrane receptors as a ligand, which activates intracellular signaling processes that lead to cytoskeletal reorganization and redistribution of adhesive contacts.

Tissue plasminogen activator (tPA), also presenting in the intestinal mucosa [32], is structurally and functionally similar to urokinase [42]. There are some differences in structural determinants located in the noncatalytic sites of these proteins responsible for the binding of these enzymes with various components of cell surface and cell environment. The *N*-terminal region of tPA molecules contains two additional domains – a finger domain and a second kringle domain responsible for specific binding with fibrin [42]. In contrast to urokinase, no specific receptor has been found for tPA. It is believed that tPA is primarily involved in fibrinolysis, while urokinase plays a role in the mechanisms of regulation of cell migration. In some cases, functional interchangeability is possible for both types of plasminogen activators.

Plasmin is found in rat small intestine tissues in the form of an inactive precursor (plasminogen) and as a mature enzyme [33]. Plasminogen is secreted as a single-chain glycoprotein (92 kDa). It is structurally characterized by the presence of five kringle domains containing the so-called lysine-binding sites [46]. The kringle domains provide binding of plasminogen with fibrin and cell membranes, which is necessary both for its activation and localization of plasmin action. Plasminogen is activated by cleavage of the Arg561–Val562 bond, forming the mature enzyme consisting of two polypeptide chains connected by a disulfide bond [39, 46]. The catalytic triad of the active site of serine proteases is in the light B-chain of plasmin and includes residues His602, Asp645, and Ser740. Plasmin hydrolyzes peptide bonds containing lysine residue in P1-position [47]. The specificity of plasmin on natural substrates is ensured by the kringle domains of the heavy A-chain containing binding sites

with fibrin and the physiological inhibitor antiplasmin [47].

The plasmin precursor is activated by urokinase and tPA. Active plasmin cleaves fibrin (fibrinogen), coagulation factors (V/Va, VIII/VIIIa), some growth factors, and extracellular matrix components and can activate zymogens of matrix metalloproteases [46, 48]. In turn, matrix metalloproteases are involved in the degradation of extracellular matrix components resistant to other proteases, which promotes cell migration and tissue remodeling. Thus, plasmin plays a dominant role in extracellular proteolysis [39, 41].

A necessary condition for the effective epithelial barrier is strong cell–cell and cell–substrate adhesion. The intestinal epithelium is a mobile population of cells, migrating from crypt area towards the apex of intestinal villi. The proteolytic cascade system including matriptase (localized on the basal membrane of enterocytes) and fibrinolytic system factors (urokinase and plasmin) is a necessary component of the control system of matrix component exchange of small intestine mucosa. It promotes controlled destruction and subsequent restoration of migrating cells adhesion sites, which provides intensive renewal of intestinal epithelium (in Fig. 2).

INGOBSIN – TRYPSIN-LIKE SERINE PROTEASE OF GOBLET CELLS

Goblet cells are exocrine cells of the epithelial layer of the small intestine; their main role is associated with continuous secretion of mucus. Ingobsin is a poorly studied protease localized in goblet cells of rats, pigs, and humans [49] (Fig. 1). The most intense synthesis of ingobsin occurs in duodenal goblet cells, and the enzyme is predominantly localized in the lower part of the villi and crypts [50]. Ingobsin secretion increases under the influence of vasoactive intestinal peptide (VIP) and acetylcholine [51].

Substrate specificity of ingobsin on synthetic substrate is expressed by the following series of preferences of P1-position residues hydrolysis: Lys > Arg >> Tyr. Ingobsin shows endoprotease activity on protein substrates. Based on the results of enzyme inhibitor analysis, ingobsin is a serine protease [50]. The molecular weight of ingobsin is 33 kDa, and the pH optimum (7.4-8.0) corresponds to the physiological pH in the duodenum. Ingobsin is secreted into the intestine lumen, and its activity was found in duodenal juice.

Ingobsin cleaves epidermal growth factor (EGF) at very low enzyme concentrations. EGF consists of 53 amino acid residues; its physiological functions include a protective role for the intestinal epithelium caused by the inhibition of gastric acid secretion [52]. EGF, cleaved by ingobsin, loses mitogenic properties but retains protective functions. Ingobsin can degrade the endogenous glycoprotein haptocorrin, which binds vitamin B12 and may have an effect on B12 transport in the duodenum [50].

INTESTINAL KALLIKREIN

Kallikrein-like proteins are expressed in the small intestine of rats [53] and humans [54, 55]. Immunocytochemical methods demonstrated kallikrein localization in goblet cells of the intestinal epithelium and their secretions [56]. A significant amount of kallikrein is also available in submucosal macrophages [57] and mast cells [55].

Kallikreins are arginine-specific serine proteases involved in hormone processing by releasing kinins from kininogens. There are two main types of kallikreins: kallikrein of blood plasma and tissue kallikrein, the latter synthesized in tissues of certain organs such as pancreas, salivary gland, intestine, and kidney [58, 59]. Tissue kallikreins are trypsin-like proteases that are biochemically characterized as acidic glycoproteins (p*I* 3.5-4.5) with molecular mass of 24 to 40 kDa. These proteases are encoded by a family of genes, and their number varies in different mammalian species [59]. Kallikrein isolated from the small intestine of rats and humans (32 kDa) is biochemically similar to pancreatic kallikrein [58].

Factors that determine secretion and activation of intestinal kallikrein remain unknown. It is known that the inactive precursor of tissue kallikrein can be activated *in vitro* by trypsin, plasmin, or plasmatic kallikrein [60]. Tissue kallikrein activity is regulated by kallistatin – the main inhibitor of tissue kallikrein and some of the endogenous tissue and plasma serpins [57]. In the intestine kallistatin is found in epithelial cells and macrophages [58].

The role of the intestinal kallikrein has been studied insufficiently. It is assumed that intestinal kallikrein may be involved in processing of mucoprotein peptides from secretion of goblet cells [56]. Participation of the enzyme in the transport of chloride ions in intestinal epithelium is also a possible function [61]. Intestine inflammation is accompanied by increased kallikrein secretion and release of the enzyme in the extracellular space [57].

Kininogen is the natural substrate of intestinal kallikrein; it was not detected in the intestine, however, and the role of kininogen as the activator for intestinal kallikrein is highly probable. In favor of this is the presence of kinins on the surface of epithelial cells; in addition, kininase 2 (angiotensin-converting enzyme) is localized in the area of glycocalyx of enterocytes [62]. Thus, small intestinal mucosa contains all three components of the kallikrein–kinin system (kallikrein, kininase 2, kinins). Kallikrein, independently of kinins, can affect intestinal motility, secretion, and metabolism of connective tissue directly. Kallikrein and kinins are suggested to be involved in the regulation of absorption of electrolytes and nutrients [56].

INTESTINAL TRYPSIN

The main source of trypsin is the pancreas, and trypsin is localized in enterocytes of small intestine of human and mouse [63] as well as in Paneth cells of humans [64, 65]. Thus, trypsin is a properly intestinal enzyme. According to the authors of [63], trypsin extracted from the tissue of small intestine is in an active form; enteropeptidase is probably the activator of intestinal trypsin. Autoactivation of trypsin is also possible [66].

Trypsin, single-chain monomer with a molecular mass of 23-26 kDa, cleaves protein substrates by hydrolyzing peptide bonds formed by the carboxyl group of Arg and Lys. Intestinal trypsin is supposed to participate in the hydrolysis of food proteins and extracellular matrix proteins and activation of zymogens, including matrix metalloproteases [63]. In addition, trypsin can fulfill another signal role. Trypsin stimulates secretion of arachidonic acid and prostaglandins by enterocytes of the small intestine by proteolytic activation of receptors such as PAR-2 [67]. It is believed that PAR-2 receptors, located on endocrine cells of the intestinal mucosa epithelium, are involved in regulation of intestinal hormone release under the influence of trypsin [68].

Relatively recently the trypsin role as an activator of the antimicrobial peptide human alpha-defensin HD-5 was established; this peptide together with other defensins is expressed in Paneth cells [65]. Paneth cells are localized at the bottom of intestinal crypts (glands of Lieberkuhn); their secretory granules contain a variety of antimicrobial agents (Fig. 1). In addition to different types of defensins, these cells synthesize lysozyme, phospholipase 2, IgA, and several other factors involved in defense reactions. Paneth cells, while being stationary cells of the intestinal epithelium, are the first echelon of the antibacterial protection of intestinal mucosa [65]. The presence of trypsin isoforms in Paneth cells was demonstrated in [65].

The case of trypsin can demonstrate multifunctionality of serine proteases of the intestine. Trypsin, a classical digestion protease of the pancreas, is present in different types of epithelial cells of the intestinal mucosa where its functional role is expanded and includes signaling and protective functions.

DUODENASE – MULTIFUNCTIONAL PROTEASE OF BRUNNER'S (DUODENAL) GLANDS AND MAST CELLS

Brunner's (duodenal) glands are located in the submucosa of the duodenum (Fig. 1). Brunner's gland secretions contain glycoproteins, forming a viscous protective layer, bicarbonates, providing alkaline reaction of the secretion, and several other compounds with different functional properties, such as lysozyme, epidermal growth factor, trefoil peptides, IgA, IgM, pepsinogens, and inhibitors of serine proteases [7].

Epithelial cells and secretory ducts of bovine Brunner's gland were demonstrated to contain a previously unknown serine protease – duodenase [69]. Duodenase is also synthesized in mast cells of the intestinal mucosa [70].

The enzyme molecule (26.5 kDa) consists of a single polypeptide chain with a small (3%) sugar content. Duodenase is a very alkaline protein (pI > 9.5). The enzyme has dual trypsin- and chymotrypsin-like specificity, thus showing selectivity to their substrates. X-Ray analysis and molecular modeling of duodenase revealed distinctive architectural features of the substrate-binding area of duodenase that enables recognition and binding of both charged and hydrophobic substrates by the enzyme [71, 72]. The effectiveness of polypeptide substrate recognition by duodenase largely depends on the substrate conformational features [73].

The physiological role of duodenase is presumably associated with processing of proenteropeptidase – the precursor of the key enzyme of the activation cascade of digestive proteases [22]. The ability of duodenase to transform *in vitro* recombinant zymogen of enteropeptidase into active double-chain form was proven [22]. The secretion of Brunner's gland, containing duodenase, enters the inter-villi space where the interaction of duodenase with enteropeptidase zymogen, which is located on the apical membrane of enterocytes (see above), is likely. The activator of duodenase itself remains unknown. Duodenase zymogen has a short activation dipeptide (GlyLys) and can be activated intracellularly during *N*-terminal processing [72]. Alpha 1-antitrypsin is probable a physiological inhibitor of duodenase [74].

Duodenase activates peritoneal mast cells *in vitro*, which may indicate proinflammatory activity of duodenase [75]. The ability of the enzyme to initiate mitosis of fibroblasts was also shown. The effect of duodenase on cells in one study, the authors believe, is implemented by the proteolytic activation of receptors such as PAR [70]. These data indicate a possible role of duodenase in inflammatory reactions and remodeling of tissues.

This review presents information on the biochemical and functional properties of the intestinal serine proteases together with morphological and physiological data that underlie the understanding of functional processes in the upper intestine. The role of serine proteases in the physiology of the small intestine is quite significant, covering almost all the important control mechanisms of life and functional activity of this segment of the digestive system in health and disease (table). As activators of enzyme zymogens, serine proteases trigger activation cascades leading to increased proteolytic presence in a particular area of the intestine that is required to carry out a specific physiological process. Thus, enteropeptidase

Serine proteases of epithelial cells of the small intestines –localization, substrates, physiological role

Protease	Localization	Physiological substrate	Proteolysis-mediated processes	Role of protease	Reference
Enteropeptidase	apical membrane of mature enterocytes	trypsinogen	trypsin activation of zymogens of digestive enzymes	triggering of activation cascade of digestive enzymes	[12] [14] [15]
Matriptase	basolateral membrane of epithelial cells of upper region of villi	zymogen of urokinase, precursor of hepatocyte growth factor, extracellular matrix proteins	activation of proteases on basal membrane of epithelial cells by urokinase, weakening of cell adhesion of epithelial cells, exfoliation of "exhausted" epithelial cells	control of intestinal epithelium renewal	[26-28] [31] [32]
Urokinase (uPA)	surface of epithelial cells (upper part of villi) in complex with membrane-bound proteins	plasminogen, zymogens of matrix metalloproteases, fibronectin	cleavage of extracellular matrix proteins by metalloproteases and plasmin	activation of proteolysis on cell surface, modification of matrix environment of cells, uPA-receptor-mediated regulation of intracellular processes (cytoskeletal rearrangement, redistribution of adhesive contacts), regulation of cell migration	[33] [38] [40-43] [46] [48]
Plasmin		prourokinase, zymogens of matrix metalloproteases, extracellular matrix proteins			
Ingobsin	goblet cells of crypts and lower part of intestinal villi	epidermal growth factor (presumably), haptocorrin	loss of mitogenic properties of growth factors, degradation of haptocorrin	presumably involved in transport of vitamin B12 in duodenum	[49] [50] [52]
Kallikrein	goblet cells, their secretion, macrophages, mast cells	low molecular weight kininogen (presumably); predecessors of mucoprotein of goblet cell secretion, growth factors, and peptide hormones, bradykinin receptor BR2	formation of kinins, peptide hormones and growth factors, maturation of goblet cell secretion, release of histamine from mast cells, synthesis and release of prostaglandins, TNF, interleukins	participation in development of inflammation, tissue morphogenesis, and repair; presumably signaling role; absorption (of electrolytes, nutrients); effects on intestinal motor activity, secretion, metabolism of connective tissue	[55] [56] [58] [61] [63]
Intestinal trypsin	enterocytes of intestinal villi, Paneth cells	PAR-2 of enterocytes and enteroendocrine cells; zymogens of enzymes (digestive hydrolases and matrix metalloproteases), alpha-defensin (propeptide), extracellular matrix proteins, food particle proteins	secretion of eicosanoids by enterocytes; release of intestinal hormones; activation of zymogens of proteases and other hydrolases; hydrolysis of food substances, extracellular matrix; activation of alpha-defensin	signaling role, participation in digestion, modification of matrix surroundings of cells; antimicrobial protection	[63] [66-68]
Duodenase	secretory epithelial cells of Brunner's gland, mast cells	zymogen of enteropeptidase (presumably); PAR-type receptors of mast cells, fibroblasts	enteropeptidase activation, degranulation of mast cells, mitogenic effect (fibroblasts)	participation in activation cascade of digestive proteases; proinflammatory role; morphogenesis and tissue repair	[70] [73] [74]

activates trypsinogen, setting up a chain reaction of zymogen activations of digestive hydrolases in the intestinal lumen, which leads to "explosion" of enzyme activity required for the exhaustive hydrolysis of various macromolecular compounds forming food particles. Constant renewal of the intestinal epithelium is controlled by serine proteases located in the area of epithelial cell contact with basal membrane (matriptase, urokinase, plasmin). These enzymes activate cascading processes that lead to a weakening of cell adhesion and migration of epithelial cells towards the apical region of the intestinal villi and the subsequent detachment of cells that have completed their life cycle into the lumen of the intestine. The above-mentioned proteases play a special role in the remodeling and regeneration of damaged tissues in pathological conditions of the intestine.

Understanding of the physiological role of proteases is inseparable from the identification of target proteins on which proteolytic attack of the enzymes is directed. Traditionally viewed as destructive enzymes, proteases are important signaling agents that regulate many cellular processes. The wide range of physiological substrates of serine proteases in the intestine should be emphasized (table). It is interesting to note that some enzymes, zymogen activators, which are at the top of proteolytic cascade, despite their rather limited substrate specificity, characteristic of regulatory proteases, can directly hydrolyze proteins that are substrates of proteases – the end products of the activation cascade. For example, matriptase and urokinase, which trigger the activation cascade of matrix metalloproteases, can hydrolyze substrates of these enzymes – extracellular matrix proteins. At the same time, trypsin, a classic destructive protease of digestion with a large proteolytic potential, promotes the activation of proenzymes and propeptide by limited proteolysis.

A special role in the signaling pathways mediated by proteases is assigned to PAR-like receptors, the activation of which involves proteolytic action on the corresponding domain of the receptor leading to a change in its conformation and launch of a chain of intracellular events that results in a particular cellular response [76]. To date, PAR expression was proven in intestine in mucosal cells (epithelial cells, including enterocytes), myocytes, myofibroblasts, enteral neurons, mast cells, and endothelial cells of blood vessels. Activation of PAR in the intestine regulates processes such as ion transport, secretion of electrolytes and mucins by epithelial cells, peristalsis, inflammation, and changes in the epithelial barrier permeability [77, 78].

Summarizing the given information on serine proteases synthesized in the small intestine, we can distinguish their main physiologically relevant substrates: 1) zymogens of proteases and other enzymes; 2) precursors of peptide hormones, growth factors; 3) extracellular matrix proteins, and 4) PAR-type receptors.

Therefore, serine proteases play a key role in the physiology of the small intestine, and they ensure the normal functioning of this organ as a part of digestive system in which the hydrolysis and absorption of nutrients occur. They are involved in renovation and remodeling of tissues, contractile activity of smooth muscles, hormonal regulation, and protection mechanisms of the intestine.

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