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Pepsinogen C in Farm Animals: A Review

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Abstract: Bovine, ovine, caprine, porcine and hen Pepsinogen C (PgC) have been reviewed. Pepsinogens C, together with other gastric proenzymes, are thought to have evolved from a common ancestral aspartyl proteinase. PgC appears to have diverged first followed by prochymosin and pepsinogens A (PgA) and F. PgC is mainly expressed in adult animals. In contrast, in hens and quails, it is synthesized during the embryonic stage before hatching. The purification of PgC requires a combination of weak anion-exchange and strong anion-exchange chromatography. Additionally, size exclusion chromatography may be necessary. PgC in pigs and goats occurs in 2 isoforms, namely PgC-1 and Pg C-2, with approximately 370, 363 and 387 amino acid residues for goat, pig and hen PgC, respectively. The molecular mass porcine pepsinogen C is close to 38-41.4 kDa and 33-36.6 kDa for the zymogen and the active enzyme, respectively. Pepsinogens C in ruminants differ from those of humans and of other animal species in their phosphate content. Unlike PgA, PgC has a higher proteolytic activity against hemoglobin at pH 3 than at pH 2. Also, PgC is less susceptible to the most potent inhibitors of aspartic proteases, such as pepstatin. Investigation of PgC in farm animals is still limited to the gastric mucosal area, despite the fact that PgC C has been reported to be present in blood and in many other tissues. Therefore, studies to further our understanding of the physiological aspects and the clinical relevance of PgC are needed.

Key words: Pepsinogen C, purification, characterization, properties, animals

INTRODUCTION

Pepsinogen C, synonymously referred to as progastricsin, pepsinogen II and parapepsinogen II, is the inactive precursor of pepsin C, amember of the aspartic proteinase family of proteolytic enzymes. Pepsinogen C is less abundant when compared to PgA and is primarily secreted in the gastric mucosa of domestic animals such as cattle (Andren, 1992; Cybulski and Andren, 1990; Eckersall *et al*., 1987; Heishi *et al*., 1995; Kassell and Meitner, 1970, 1971; Kurabayashi *et al*., 1991) pigs (Foltmann *et al*., 1992; Ryle and Porter, 1959; Ryle, 1960, 1966; Ryle and Hamilton, 1966; Samloff and Liebman, 1972) sheep (Fox and Whitaker, 1977; Mostofa *et al*., 1990) goats (Suzuki *et al*., 1999) and hens (Bohak, 1969; Green and Lewellin, 1973; Yasugi and Mizuno, 1981). Under acidic conditions, PgC is activated to the active digestive enzyme pepsin C. Similarly to other digestive enzymes, the main function of pepsin C is to hydrolyze peptide bonds of proteins. Additionally it is now recognized that PgC is involved in other functions that are not directly related to its proteolytic effects on alimentary proteins (e.g digestion of bacteria (Schreiber *et al*., 2006). This study aims to summarize the current knowledge about PgC in farm animals, namely in cattle, sheep, goats, pigs and hens. Although farm animals are targeted, important information may be obtained by comparison with gastric zymogens in other species. The classification, the purification schemes, the genetic evolution and the characterization of the proenzyme will be discussed.

While the less abundant pepsinogen has several synonyms (pepsinogen C, progastricsin, pepsinogen II and parapepsinogen II), for the purpose of this review the term pepsinogen C will be used throughout.

DISTRIBUTION OF PEPSINOGEN C

Distribution in different species: Unlike PgA, PgC has a large extra gastric distribution. Its occurrence is limited to certain species. Species can be categorized into 3 groups: In the first group, PgA and C co-exist, the latter being as the minor component. This group includes cattle (Andren, 1992; Chow and Kassell, 1968; Eckersall *et al*.,

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1987) sheep (Mostafa *et al*., 1990) goats (Suzuki *et al*., 1999) pigs (Foltmann *et al*., 1992; Ryle and Porter, 1959; Ryle, 1960, 1966; Ryle and Hamilton, 1966) and hens (Bohak, 1969; Yasugi and Mizuno, 1981). In the second group, pepsinogen C is the only gastric proenzyme. This is the case in rats (Furihata *et al*., 1980; Lai *et al*., 1988) and guinea pigs (Kageyama *et al*., 1992). Finally in the third group, which is made up by domestic carnivores, PgC is absent and PgA and B are the only proenzymes present (Suchodolski *et al*., 2002; Tress *et al*., 2004).

Localization within the stomach

Ruminants: The cellular localization of PgC within the stomach has been investigated by immunohistochemistry and immunocytochemistry techniques applied to the fundic, pyloric and duodenal areas by using polyclonal antibodies directed towards bovine pepsin C raised in rabbits. According to Heishi *et al*. (1995) in cattle PgC immunoreactive cells are first detected in the fundic and pyloric regions of fetuses of around 52 cm in crown-rump length (about 180 days of gestation). The frequency of PgC-immunoreactive cells and also the intensity of immunoreactive staining increase with the progress of gestation. Immunoreactivity is mostly restricted to the basal portion of the fundic and pyloric glands. After birth, immunoreactivity in the fundic mucosa is found in both the chief cells and also in mucous cells of the gastric pits.

In cattle and lambs, the immunoreactivity of mucous cells mucous cell is mucous neck is even stronger than that of the chief cells (Kitamura *et al*., 2001; Wislinski and Cybulski, 1994). In the pyloric mucosa, PgC immunoreactivitiy is found in the gastric pits and in the basal portion of the pyloric glands, but immunoreactive cells are organized in small groups and show a patchy distribution in the basal area.

The work of Cybulski and Andren (1990) on the influence of age shows that PgC is first found in calves at the age of about 45 days in surface mucous cells in the pit of the fundic gland. In older calves and adults, mucous neck cells also produce PgC. In the pyloric mucosa, traces of PgC immunoreactivity are found in the lower base of pyloric glands even in newborn calves. When the calves grow older, PgC-immunoreactive cells also develop in the pit and later in the neck of the pyloric gland. The number of these cells in this region increases with age. The development of PgC-producing cells appears to be dependant on age rather than the feeding of milk to the calves.

Dogs and cats: There are some early reports about the cellular localization and the antigenic relationship between pepsinogen C of domestic cats and dogs. These studies were based on immunofluorescence, immunoelectrophoresis, electrophoretic analysis, immunodiffusion and immunoabsorption using rabbit antiserum to human and pig PgA and Pg C (Lindgren *et al*., 1998). It was reported that both Pg A and Pg C are present in mucous neck cells and chief cells in the fundic mucosa, in pyloric gland cells in antral mucosa and Brunner's glands in the proximal duodenum. It was further reported that dog and cat pepsinogens are electrophoretically heterogeneous. Despite this, there is more and more evidence that PgC is not expressed in dog and cat stomach, raising questions concerning the antibodies used by Liebmann and Samloff (1978). Their findings might be the result of cross-immunoreactivity of human and pig antibodies against other proteases of the aspartic family, such as PgB, cathepsine D or prochymosine that are widely distributed in the stomach of cats and dogs.

Pigs: In contrast to dogs and cats, the investigation of the cellular localization of PgC was carried out by using a specific antibody. The study has demonstrated the presence of four peptic cells in the pig. Furthermore, a similar cellular localization to man has been confirmed in pigs, with Pg A being localized in mucous neck and chief cells in the fundic mucosa and Pg C in mucous neck and chief cells in fundic mucosa and in pyloric glands in the antral region (Liebman and Samloff, 1979).

Pepsinogen C in the vascular space: Like other gastric proenzymes, PgC is found in the vascular space in measurable quantities, both in healthy and diseased subjects. This has been demonstrated in humans by immunoassays of serum (Biemond *et al*., 1993; Hengels and Strohmeyer, 1989; Samloff, 1982) and by chromatography of serum of cattle with ostertagiasis (Eckersall *et al*., 1987). No published information about the presence of PgC in the blood of other species is available. While the exact mechanism, whereby a small amount of pepsinogen enters the vascular space is not well understood, the ratio of PgA to PgC in serum reflects the morphological and functional status of the gastric mucosa and more specifically, of the chief cells, which are the main source of pepsinogens C in the gastric mucosa. Also, the ratio of PgA to PgC has been used as a biomarker for gastric disorders in humans (Borch *et al*., 1989).

Tissue distribution: In humans PgC is also found in other tissues, including the lung, ovarian tissue, seminal fluid, pancreatic islet cells, spleen, striated muscle cells, semen, vagina, cystic breast fluid, amniotic fluid, urine, breast tumors, breast milk, intestine, prostate and cerebral fluid

(Reese *et al*., 1986; Reid *et al*., 1984; Samloff and Liebman, 1972,a, b; Sanchez *et al*., 1992; Seijffers *et al*., 1965).

High expression levels of PgC have been reported in the esophagus of the frog (Inokuchi *et al*., 1991) and the ovarian tissue of the trout (Bobe and Goetz, 2001). These last two findings suggest that PgC still retains some characteristics of an ancestral housekeeping tissue proteinase. However, in domestic animals, the investigation on the extra gastric localization of PgC remains to be clarified.

CLASSIFICATION

Pepsin A and pepsin C differ in their enzymatic and physicochemical characteristics. They elute separately during column chromatography and both have a different electrophoretic mobility (Foltmann, 1985). They also differ in molecular mass, amino acid composition and the pH optimum at which they exhibit maximum proteolytic activity (Foltmann, 1985). Based on above characteristics, three classification schemes have been suggested.

Classification according to the proteolytic activity of the enzymes: The letter A has been attributed to enzymes with high proteolytic activity at pH 2 and letters B and C to enzymes with a lower proteolytic activity at pH 2. Also, numbers have been used to distinguish major and minor pepsinogens. Consequently, PgA, the major form, is also known as pepsinogen I and PgC is also known as pepsinogen (Bergmann, 1942; Hartley, 1960).

Classification according to the electrophoretic mobility: The different forms of pepsinogens show different motilities on SDS PAGE: the letters A and C are followed by the arabic numbers 1, 2 or 3 for the designation of isoforms (Foltmann, 1981). For example, PgC-1 and PgC-2 in goats are both zymogens of PgC (Suzuki *et al*., 1999).

Classification according to the catalytic mechanism: Another classification system is summarized in Fig. 1 and has been suggested by the Enzyme Commission (EC) of the International Union of Biochemistry (Commission of Editors, 1965). Based on its catalytic mechanism, pepsin belongs to the class-3 hydrolases, subclass-4, which is made up by peptides hydrolases (i.e., peptidases). Peptide hydrolases (EC 3.4) act on peptide bonds and constitute a large family of enzymes that are divided into endopeptidases (EC 3.4 21-99) and exopeptidases (EC 3.4.11-19), depending on the location at which they break the peptide chain.

According to Rawling and Barret (1994) the endopeptidases are subdivided into four families: The serine-, cysteine-, aspartic-and metallo endopeptidases. Serine peptidases have a serine residue in the active center, aspartic peptidases have two aspartic acid residues in the catalytic center, cysteine peptidases have a cysteine residue and metallo-proteinases use a metal ion in the catalytic mechanism.. The aspartic endopeptidases are composed of pepsin A, pepsin B, pepsin C, chymosin, retropepsin of HIV1 and retropepsin of HIV2 (Bergmann, 1942; Rawling and Barret, 1994).

ONTOGENESIS OF PEPSINOGEN C

Not all gastric zymogens are synthesized in the gastric mucosa during the same life stage.

In cattle, PgC is co- localized with prochymosin and PgA in the same secretory granules in the fundic, pyloric

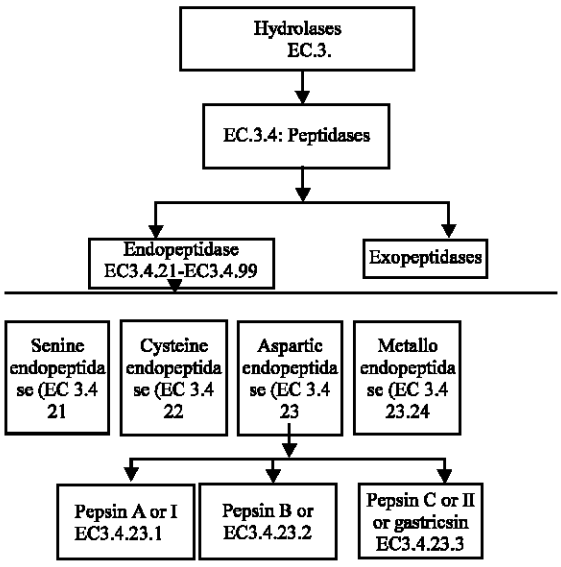

Fig. 1: Systematic classification of gastric enzymes based on their catalytic mechanism

Table 1: Chromatographic steps for the purification of pepsinogen C in farm animals

Species	Step 1	Step 2	Step 3	Step 4	Step 5	References
Bovine	DEAE cellulose	Gel filtration	Mono Q			Eckersall *et al*., 1987
Bovine	DEAE cellulose	Gel filtration	DEAE cellulose			Chow and Kassell, 1968
Pig	DEAE cellulose	DEAE cellulose	Gel filtration	DEAE cellulose	Gel filtration G-100	Ryle and Hamilton, 1966
Pig	DEAE Cellulose	DEAE sepharose	Mono Q			Foltmann *et al*., 1992
Goat	DEAE Sephacel	Gel filtration	Mono Q			Suzuki *et al*., 1999
Hen	DEAE cellulose	Gel filtration	Gel filtration			Bohak, 1969

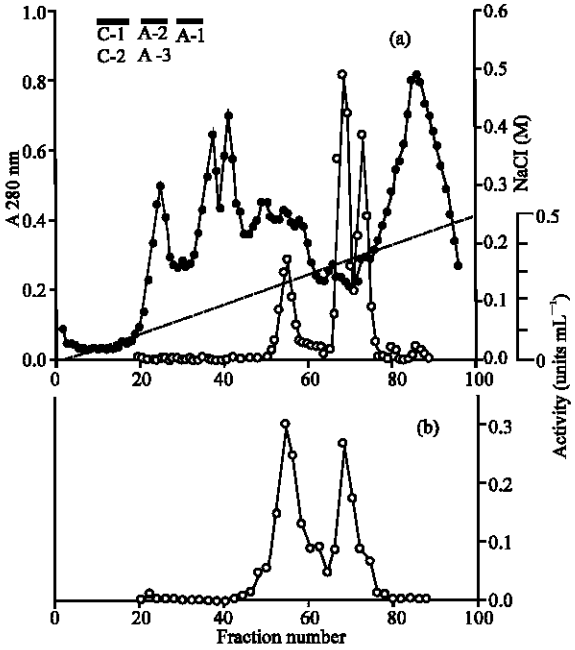

Fig. 2: Chromatography of goat PgA and PgC crude homogenate supernatant of goat abomasal mucosa was loaded onto a DEAE-sephacel column (1.5±30cm).The column was washed with 500 mL 0.01M sodium phosphate, pH 7.0 and proteins were eluted by the same buffer containing 1M NaCl at a flow rate of 1mL per minute. Proteolytic activity was carried out by hemoglobin -digestion method at pH 2(Fig. A) and 3.5 (Fig. B). As shown in the chromatogram PgC elutes before PgA under these conditions. A clear separation between PgA and Pgc was obtained. (Reprinted from Suzuki *et al.* (1999) with the permission from Elsevier)

and duodenal areas. However, PgC is not observed in the parietal cells (Andren, 1992; Andren *et al.*, 1982; Kurbayashi *et al.*, 1991; Yamada *et al.*, 1988).

In this species, the ontogeny of PgC, prochymosin and PgA exhibits an interesting pattern, as they start to be produced at different ages and show different patterns of development in the cells of the abomasal mucosa. The number of cells producing prochymosin is closely correlated with milk-feeding, while the development of PgC is related to the age of the calf. The most consistent factor during the development of cells of the abomasum is the number of cells producing PgA (Cybulski and Andren, 1990).

In pigs, prochymosin is known to be expressed during the fetal age, while PgA and PgC secretion predominates in adult animals (Sangild *et al.*, 1992).

In hens and quails, expression of PgC begins in embryos before hatching (Yasugi and Mizuno, 1981; Yasugi *et al.*, 1979). In these species, prochymosin is expressed at high levels in the proventriculus of embryos, with a peak synthesis at day 15. Subsequently, prochymosin is replaced by PgA and progastricsin prior to hatching (Sakamoto *et al.*, 1998).

PURIFICATION

Purification protocol: The general purification protocol for PgC is shown in Table 1. In the last two decades, PgC has generally been purified from gastric mucosa by combining weak anion-exchange chromatography (e.g., using a DEAE column) and strong anion-exchange chromatography (e.g., using a Mono-Q column). In some cases an additional size exclusion chromatography step is required (Eckersall *et al.*, 1987; Foltmann *et al.*, 1992; Ryle and Hamilton, 1966; Suzuki *et al.*, 1999).

The elution order: During the purification protocol, PgA and C closely co-elute. The order of elution from an anion-exchange column is generally dependant on electrophoretic mobility with components with the lowest electrophoretic mobility being eluted first. This is the case in humans, cattle and goats (Fig. 2), where PgC is eluted before PgA (Eckersall *et al.*, 1987; Foltmann and Jensen, 1982; Foltmann *et al.*, 1992; Suzuki *et al.*, 1999). However, the order of elution may also depend on the buffer composition used. In one purification protocol of porcine PgC (Foltmann *et al.*, 1992) PgC elutes after PgA on a weak anion-exchange chromatography (DEAE Sepharose) by using the following buffers: buffer A, 2 mM sodium phosphate, pH 6.9 and buffer B, 2 mM sodium phosphate, pH 6.9 containing 1 M NaCl, as shown in Fig. 3.

Ratio of pepsinogenA to pepsinogen C: Both types of PgA and C have been shown to be present in appreciable amounts in mammalian stomachs. PgC has been shown to account for less than 16% of total pepsinogen in the stomach of cattle (Martin *et al.*, 1982) less than 12% in the stomach of pigs (Foltmann *et al.*, 1992) and 40% in the stomach of goats (Suzuki *et al.*, 1999).

CHARACTERIZATION

Phosphorylation: Phosphorylation is a post-translational modification, which may play a role in protein transport and localization, protein function, interaction within proteins and cell signaling (Blom *et al.*, 1999). According to the sequence analysis, bovine PgA has 24 available sites for phosphorylation, namely 20 serine sites, 2 threonine sites and 2 tyrosine sites (Blom *et al.*, 1999)

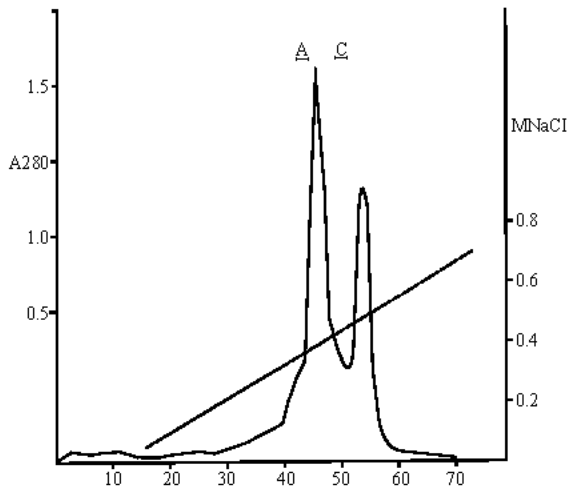

Fig. 3: Chromatography of PgA and PgC. Fractions with bovine hemoglobin activity, from DEAE cellulose chromatography column, were pooled and loaded onto a DEAE-sepharose column (6L6B; 30×1.6cm).The column was washed with 500 mL 0.02M sodium phosphate, pH 6.9 and proteins were eluted by the same buffer containing 1M NaCl at a flow rate of 30 mL h^{-1}. As shown in the chromatogram PgC elutes before PgA under these conditions. A clear separation between PgA and Pgc was obtained. (Reprinted from Foltmann *et al.* (1992) with permission, from Elsevier)

Bovine, ovine and caprine pepsinogen I and pepsinogen II (the latter is synonymous with PgC) have been isolated and characterized (Eckersall *et al.*, 1987; Mostfa *et al.*, 1990). Based on the phosphate content, pepsinogen I differs from pepsinogen II by a higher content of phosphate per mol pepsinogen (Eckersall *et al.*, 1987; Mostofa *et al.*, 1990). Pure bovine PgC has never been obtained, because it is always contaminated with serum albumin (Eckersall *et al.*, 1987; Mostofa *et al.*, 1990). To date, most studies reported the purification of the active enzyme pepsin C only (Kassell and Meitner, 1970, 1971; Martin *et al.*, 1982). Unlike in ruminants, PgC in humans and pigs does not contain any phosphate (Foltmann *et al.*, 1992; Suzuki *et al.*, 1999).

Primary structure: PgC consists of a single polypeptide chain of 370, 363 and 387 amino acid residues in goats (Suzuki *et al.*, 1999) pigs (Foltmann *et al.*, 1992) and hens (Bohak, 1982), respectively. The number of amino acid residues that constitute the prosegment range from 35 to 49 residues and are 43 amino acid residues in both, pigs (Foltmann *et al.*, 1992) and humans (Foltmann and Jensen, 1992). The prosegment moieties at the N-terminus of the zymogen are highly basic, whereas the pepsin moieties contain a large number of Asp and Glu residues that are highly negatively charged (Foltmann *et al.*, 1992). The prosegments of all gastric zymogens show high homology of their amino acid sequences. More significantly, the residues Pro-5p, Leu-6p, Lys-10p, Arg-13p, Gly-20

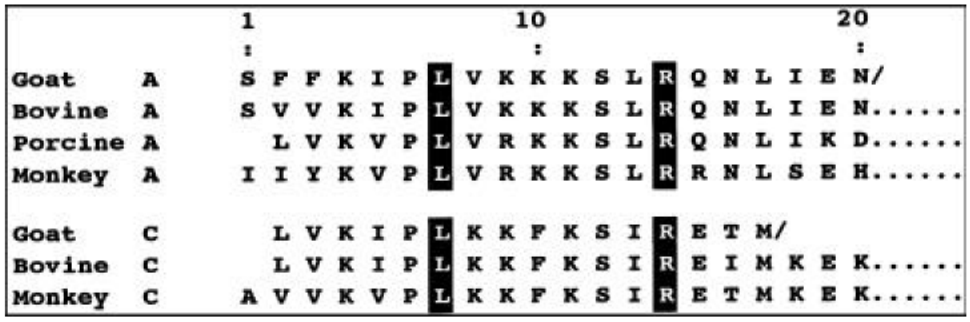

Fig. 4: Goat Pepsinogen C and pepsin C sequence analysis. The conserved residues between known pepsinogens are shown in filled boxes. (Reprinted from Suzuki *et al.* (1977) with permission from Elsevier)

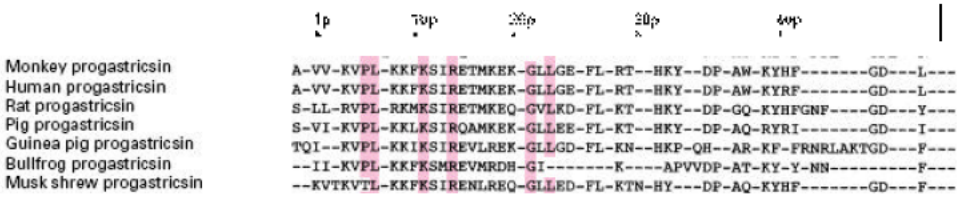

Fig. 5: Alignment of the amino acid sequences of the prosegments of Pepsinogen C from different species (Reprinted from Richter *et al.* (1998) with permission from R.Y. Yada)

and Leu-22-p are conserved in gastric proteinases whose sequences are known (Foltmann *et al*., 1981).

Although the primary structure of zymogens of many species are known, the three dimensional structures of farm animal PgC has not yet been described.

Molecular mass: Pig, goat and human PgC occurs in 2 isoforms each, with a molecular mass of approximately from 38 to 41.4 kDa in pigs (Foltmann *et al*., 1992; Ryle and Hamilton, 1966) 38 kDa in humans (Foltmann and Jensen, 1992) and 39 kDa in goats (Suzuki *et al*., 1999) Pepsin C has a molecular mass of approximately 33-36.6 kDa in pigs (Chiang *et al*., 1967; Foltmann *et al*., 1992; Ryle and Hamilton, 1966; Tang *et al*., 1973) and 32 kDa in humans (Foltmann and Jensen, 1982).

Amino acid sequencing: Both goat and pig PgCs have been partially sequenced. These sequences are shown in Fig. 4 and 5. In pigs, the sequence of the first amino acid residues shows 67% homology with PgC from humans, monkeys and rats. In porcine PgC, the highly conserved Lys-36p (porcine PgA numbering) is changed to an Arg (Foltmann *et al*., 1992).

PROPERTIES

Activation: Pepsinogen C is secreted into the gastric lumen and at acidic conditions is activated through the release of an NH2 terminal segment. The activation pathway occurs in two steps via one intermediate; this has been demonstrated for pig PgC (Christensen *et al*., 1977; Dykes and Kay, 1976; Kageyama and Takahashi, 1983, 1985). During the sequential pathway, the prosegment is released in a stepwise manner by proteolytic cleavage of one or more peptide bonds within the prosegment, followed by cleavage of the peptide bond that is connecting the prosegment to the active enzyme. Finally, the initial cleavage site in the sequential pathway occurs at a peptide bond located at the C-terminal end of either the first or the second helix in the prosegment. These findings suggest that the amino acid sequence of the internal proteolytic processing site is more critical to the activation reaction than is the sequence at the cleavage site between the prosegment and the active enzyme (Foltmann, 1981).

Digestive activity against hemoglobin: During purification, the proteolytic activity of pepsin C can be measured by the conventional hemoglobin digestion method at pH 2 (Anson and Mirsky, 1932) Like pepsin A, pepsin C exhibits high proteolytic activity at an acidic pH. In several mammalian species it is known, that pepsin C is maximally active against hemoglobin at a pH of approximately 3, a slightly higher pH optimum than that observed for pepsin A (Suzuki *et al*., 1999).

The proteolytic specificity of pepsin C has not been studied as extensively as that of pepsin A, but comparison of human pepsin A and pepsin C showed, that although the latter has generally similar substrate specificity, pepsin C prefers Tyr at the P1 position and, thus, preferentially cleaves Tyr-X bonds (Tang, 1970, 2004). This difference in proteolytic activity towards hemoglobin at different pHs is an interesting approach for identification of PgC, especially in species that synthesze both, PgA and C.

Pepsin C inhibitors: Since the work of Rich and Sun (1980), Valler *et al*. (1985) some naturally occurring inhibitors of aspartic proteases are known. The most potent inhibitor of aspartic gastric enzymes is pepstatin.

Pepstatin is isolated from *Streptomyces* species, which contains two residues of a novel amino acid called statine. There is a consensus that pepstatin strongly inhibits pepsin A but not pepsin C (Rich and Sun, 1980) Pepsin C and chymosin are less susceptible to pepstatin and a 100-and 10-fold molar excess is required for inhibition (Roberts and Taylor, 2003).

In the same way, *Ascaris lumbricoides*, a parasitic nematode that survives in acidic conditions of the stomach is also known to secrete a 149-residue proteinase inhibitor (Kageyama, 1998) that shows high affinity for pepsin A but not for pepsin C (Ng *et al*., 2000).

Although the low affinity of pepstatin for pepsin C and chymosin is still being investigated, pepstatin may be a useful tool to discriminate PgA and PgC during purification (Suzuki *et al*., 1999; Tress *et al*., 2004) Pepstatin may also be a useful tool to inhibit the activation of pepsin A in immunoassays for the measurement of PgA (Banga *et al*., 2003a).

Bactericidal role of the prosegment: Pepsinogens are secreted into the gastric lumen and autocatalytically activated to pepsins under acidic conditions, releasing the N-terminal prosequences (Hartley, 1960). So far, the released prosequences of pepsinogens do not have any known significant function of their own, even though the prosequences of pepsinogens have the role of inactivating the proteolytic activity of pepsins by blocking access of substrates to the catalytic site.

Minn and co-workers (Minn *et al*., 1998) reported a novel function of the prosequences of pepsinogens by demonstrating a strong antimicrobial activity of the prosegment against various microorganisms in the bullfrog, namely Gram+ and Gram- bacteria and some fungi (Minn *et al*., 1998). The authors speculated that such activity may also be found in other species.

Antibacterial role of pepsin C: *Helicobacter pylori* is unique among bacteria, in that it can persist in the mucus of the antral mucosa causing gastritis, gastric and duodenal ulcers, lymphoma and adenocarcinoma (Suerbaum and Michetti, 2002). Since the mucus has a rapid turnover and the ecological niche of the bacterium is the narrow juxtamucosal mucus layer, high bacterial motility is essential for the initial colonization of the mucus, as well as for the maintenance of persistent infection (Ottemann and Lowenthal, 2002). Schreiber *et al.* (2006) studied the bacterial effect of pepsin C and recently reported that pepsin C inactivates *H. pylori in vivo* within several minutes at a pH range between 2 and 5 (Schreiber *et al.*, 2006). The anti-bacterial effects of pepsin C against *Helicobacter pylori* were observed in two phases: A rapid immobilization, leaving the bacteria viable and destruction of the bacteria after exposure to a higher pepsin concentration for at least 15 min. The efficiency of pepsin C in terms of immobilization of bacteria is higher than that of pepsin A or chymosin (Schreiber *et al.*, 2006).

In mammals it is believed that the PgC found in the seminal fluid is activated in the vagina and may serve to degrade other seminal proteins to decrease the immunogenic load of the vagina and to prevent immunoinfertility (Szecsi and Lilja, 1993).

PEPSINOGEN C GENES

A complete gene structure has been described for pepsinogens C in humans (Hayano *et al.*, 1988; Pals *et al.*, 1989; Takahashi, 1992) rats, pigs (Ichihara *et al.*, 1985) and hens (Hayashi *et al.*, 1988). The gene for PgC consists of nine exons separated by eight introns. The positions of the introns, which interrupt the coding sequence for the zymogen, are conserved among pepsinogens and are similar to those for other mammalian aspartic proteinases, including cathepsin D and E and renin, suggesting the evolution of all pepsinogens from a common ancestral gene (Hayano *et al.*, 1988).

Mutations affecting phosphorylation, glycosylation and amino acid sequence have occasionally been found. There is no evidence for multiple genes that code for pepsinogens (Pals *et al.*, 1989; Takahashi, 1992).

PHYLOGENETIC DEVELOPMENT OF PEPSINOGEN C

The evolutionary relationships of proenzymes have been investigated by Tang *et al.* (1978) who showed the evidence of gene duplication in the evolution of gastric aspartic proteases. Later, Kageyama (2000) 1investigated the molecular evolution in monkeys and highlighted the evolutionary relationships of pepsinogens in other species. Based on their findings, pepsinogens may be classified into 4 groups, namely, PgA, C, F and prochymosin. The four different types of pepsinogen are thought to have evolved from a common ancestral aspartyl proteinase and then have diverged. Pepsinogen C seems to have diverged at an earlier time than the others and was followed by prochymosin, then PgA and F. The separation of the latter three forms from PgC likely occurred in tetrapod species. Also, since the occurrence of progastricsin in trout has been established (Bobe and Goetz, 2001) there might be at least two types of pepsinogen in ancestral fish (Kageyama, 2000).

Pepsinogen A isozymogens in humans and rabbits (Kageyama *et al.*, 1990) and bovine prochymosin isozymogens are very closely related, suggesting that gene duplication events may have occurred independently two or more times in the genes for these pepsinogens.

The phylogeny of pepsinogens is considered to be useful for estimating the phylogeny of vertebrates. PgC might be the most suitable molecular marker for this purpose, since multiplication of its gene has not been identified (Kageyama, 2000).

CLINICAL APPLICATIONS OF PEPSINOGENS IN DOMESTIC ANIMALS

Cattle: Blood PgC concentrations have been used for four decades as a diagnostic tool for ostertagiosis in cattle (Jennings *et al.*, 1966). In the first grazing season, high pepsin A activities in cattle correlate with the occurrence of parasitic gastroenteritis. Different cut-off values (measured in mU Tyrosine/L of serum) have been reported for the diagnosis of clinical ostertagiosis in young calves. Also, a problem in interpretation of these differences is that no standardized method for measurement has been agreed upon and comparisons between different laboratories are lacking. An elevated blood pepsinogen proteolytic activity can also be found in healthy cows (Berghen *et al.*, 1988; Chiejina, 1977, 1978; Mckellar, 1984, 1985). This implies that the measurememt of PgA proteolytic activity is not suitable for the diagnosis of ostertagiasis unless it is used in conjunction with other clinical and parasitological tests.

Pigs: In pigs, gastric ulceration is a worldwide problem and minimally-invasive diagnostic tests for gastric lesions would be of great clinical value as early diagnosis may allow early intervention and lead to improved longevity of affected pigs.

Although mean PgA concentrations were found to be elevated in herds of pigs with ulcers compared to a non-ulcer group, there is no clear correlation between serum PgA concentrations and gastric ulcers, since

some pigs with a normal gastric mucosa also have high PgA concentrations (Banga *et al*., 2003b).

Taking in account the results of other investigations in cattle and pigs, serum concentrations of PgA are not useful for the diagnosis of gastric parasites and gastric ulcers in individual animals.

Humans: Alterations in serum concentrations of PgA and PgC in serum have been described in human patients with stomach diseases, such as gastric ulcers, gastritis and gastric cancer. Despite typical findings, i.e., hyperpepsinogenemia in duodenal ulcer disease, or hypopepsinogenemia in atrophic gastritis or stomach cancer and *Helicobacter* infections, there is a considerable overlap in serum concentrations between patients with different gastric diseases, limiting the clinical value of routine measurements of serum concentrations of pepsinogens. However, a ratio of PgA to PgC concentration of less than 3 is considered a useful indicator for chronic atrophic gastritis (Borch *et al*., 1989).

It has also been reported that the measurement of serum PgC, when combined with that of serum PgA or gastrin concentration, may be used as a tool for the evaluation of patients with pernicious anemia due to atrophic gastritis (Lindgren *et al*., 1998).

Based on these promising reports about the diagnostic utility of the ratio of serum PgA/PgC concentrations in humans, the development of an immunoassay for the measurement of PgC and the evaluation of the diagnostic utility of the ratio of the serum concentrations of Pg A and Pg C for the diagnosis of gastric diseases in pigs appears warranted.

CONCLUSION

The understanding of PgC in farm animals is incomplete. Purification protocols allowing high recovery, involving only few steps and clear separation of PgC from other zymogens need to be evaluated. Also, study of the importance of extragastric localization of PgC is needed. Lastly, the evaluation of the clinical utility of the measurement of serum concentrations of PgA and PgC for the diagnosis of gastric diseases in pigs is warranted.

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REFERENCES

Andrén, A., 1992. Production of prochymosin, pepsinogen and progastricsin and their cellular and intracellular localization in bovine abomasal mucosa. Scand. J. Clin. Lab. Invest., 52: 59-64.

Andrén, A., L. Bjorck and O. Claesson, 1982. Immunohistochemical studies on the development of prochymosin- and pepsinogen-containing cells in bovine abomasal mucosa. J. Physiol., 327: 247-254.

Anson, M.L. and A.E. Mirsky, 1932. The estimation of pepsin with hemoglobin. J. Gen. Physiol., 16: 59-63.

Banga-Mboko H., J. Sulon, B. Closset, B. Remy, I. Youssao I.N.M. De Sousa, B. El Amiri, P.T. Sangild, D. Maes, J.F. Beckers, 2003a. An improved Radioimmunoassay for measurement of pepsinogen in porcine blood samples. Vet. J., 165: 288-295.

Banga-Mboko, H., H.H. Tamboura, D. Maes, H. Traore, I. Youssao, P.T. Sangild, B. El Amiri, B, Bayala, B. Remy B. and J.F. Beckers , 2003b. Survey of gastric lesions and blood pepsinogen levels in pigs in Burkina Faso. Vet. Res. Commun., 27: 595-602.

Berghen, P., P. Dorny, J. Vercruysse and H. Hilderson, 1988. The use of the serum pepsinogen assay in the epidemiological study of *Ostertagia ostertagi* (Dutch). Vlaams Diergeneeskundig Tijdschrift, 57: 157-173.

Bergmann, M.A., 1942. Classification of proteolytic enzymes. Adv. Enzymol., 2: 49-68.

Biemond, I., J. Kreuning, J.B.M. Janssen and C.B. Lamers, 1993. Diagnostic value of serum pepsinogen C in patients with raised serum concentrations pepsinogen A. Gut., 34: 1315-1318.

Blom, N., S. Gammeltoff and S. Brunak S., 1999. Sequence and structure based prediction of eukaryot protein phosphorylation sites. J. Mol. Biol., 294: 1351-1362.

Bobe, J. and F.W. Goetz , 2001. An ovarian progastricsin is present in the trout coelomic fluid after ovulation. Biol. Reprod., 64: 1048-1055.

Bohak, Z., 1969. Purification and characterization of chicken pepsinogen and chicken pepsin. J. Biol. Chem., 244: 4638-4648.

Borch, K., C.K. Axelsson, H. Halgreen, M.D. Nielsen, T. Ledin, P.B. Szesci, 1989: The ratio of pepsinogen A to pepsinogen C: A sensitive test for atrophic gastritis. Scand. J. Gastroenterol., 24: 870-876.

Chiang, L., L. Sanchez-Chiang, J.N. Mills and J. Tang, 1967. Purification and properties of porcine gastricsin. J. Biol. Chem., 242: 3098-3102.

Chiejina, S.N., 1977. Plasma pepsinogen levels in relation to ostertagiasis in adult cattle. Vet. Rec., 100: 120.

Chiejina, S.N., 1978. Field observations on the blood pepsinogen levels in clinically normal cows and calves and in diarrhoeic adult cattle. Vet. Rec., pp: 103.

Chow, R.B. and B. Kassell, 1968. Bovine pepsinogen and pepsin.I Isolation purification and some properties of the pepsinogens. J. Biol. Chem., 243: 1718-1724.

Christensen, K.A., V.B. Pedersen and B. Foltmann, 1977. Identification of an enzymatically active intermediate in the activation of porcine pepsinogen. FEBS. Lett., 76: 214-218.

Commission of Editors of Biochemical Journals, 1965. Enzyme nomenclature. Report on the recommendations 1964 of the International Union of Biochemistry on nomenclature and classification of enzymes. Science, 150: 719-721.

Cybulski, W. and A. Andren, 1990. Immunohistochemical studies on the development of cells containing progastricsin (minor pepsinogen) in comparison to prochymosin and pepsinogen in bovine abomasal mucosa. Anatomical Rec., 227: 458-463.

Dykes, C.W. and J. Kay, 1976. Conversion of pepsinogen into pepsin is not a one-step process. Biochem. J., 153: 141-144.

Eckersall, P.D., J. Macaskill, Q.A. Mckellar and K.L. Bryce, 1987. Multiple forms of bovine pepsinogen. Isolation and identification in serum from calves with ostertagiasis. Res. Vet. Sci., 43: 279-283.

Foltmann B., 1981. Gastric proteinases-Structures, function, evolution and mechanism of action. Essays Biochem., 17: 52-84.

Foltmann, B., 1985. Purification, structure and activation of pepsinogens. Prog. Clin. Biol. Res., 173: 1-13.

Foltmann, B. and A. L. Jensen, 1982. Human progastricsin: analysis of intermediates during activation into gastricsin and determination of the amino acid sequence of the propart. Eur. J. Biochem., 128: 63-70.

Foltmann, B., H.B. Drohse, P.K. Nielsen and M.N. James, 1992. Separation of porcine pepsinogen A and progastricsin: Sequencing of the first 73 amino acid residues in progastricsin. Biochem. Biophys. Acta, 1121: 75-82.

Fox P.F. and J.R. Whitaker, 1977. Isolation and Characterization of Sheep Pepsin. Biochem. J., 161: 389-398.

Furihata C., D. Saito, H. Fujiki, Y. Kanai, T. Matsushima and T. Sugimura, 1980. Purification and characterization of pepsinogens and unique pepsin from rat stomach. Eur. J. Biochem., 105: 43-50.

Green, M L. and J.M. Lewellin, 1973. The purification and properties of a single chicken pepsinogen fraction and the pepsin derived from it. Biochem. J., 133: 105-115.

Hartley, B.S., 1960. Proteolytic enzymes. Ann. Rev. Biochem., 29: 45-72.

Hayano, T., K. Sogawa, Y. Ichihara, Y. Fujii-Kuriyama and K. Takahashi, 1988. Primary structure of human pepsinogen C gene. J. Biol. Chem., 263: 1382-1385.

Hayashi, K., S. Yasugi and T. Mizuno, 1988. Isolation and structural analysis of embryonic chicken pepsinogen gene: Avian homologue of prochymosin gene. Biochem. Biophys. Res. Commun., 152: 776-782.

Heishi, Y., J. Yamada, N. Kitamura, T. Yamashita, A. and A. Andren, 1995. An immunohistochemical study on the development of progastricsin-immunoreactive cells in the bovine abomasal mucosa. Eur. J. Histochem., 39: 39-46.

Hengels, K.J. and G. Strohmeyer,1989. Pepsinogens A and C: purification from human gastric mucosa and determination in serum by optimized radioimmunoassays. Z. Gastroenterol., 27: 406-411.

Ichihara, Y., K. Sogawa and K. Takahashi, 1985. Isolation of human, swine and rat prepepsinogens and calf preprochymosin and determination of the primary structures of Their NH_2-terminal signal sequences. J. Biochem., (Tokyo), 98: 483-492.

Inokuchi, T., K.I. Kobayashi and S. Horiuchi, 1991. Acid proteinases of the fore-gut in metamorphosing tadpoles of Rana catesbeiana. Comp. Biochem. Physiol. B., 99: 653-662.

Jennings, F.W., J. Armour, D.D. Lanson and R. Roberts, 1966. Experimental *Ostertagia ostertagi* infections in calves: studies with abomasal cannulas. Am. J. Vet. Res., 27: 1249-1257.

Kageyama, T. and K. Takahashi, 1983. Occurrence of two different pathways in the activation of porcine pepsinogen to pepsin. J. Biochem., 93: 743-754.

Kageyama, T., 1998. Molecular cloning, expression and characterization of an *Ascaris* inhibitor for pepsin and cathepsin. E. Eur. J. Biochem., 253: 804-809.

Kageyama, T., K. Tanabe, O. Koiwai, 1990. Structure and development of rabbit pepsinogens. J. Biol. Chem., 265: 17031-17038.

Kageyama, T., M. Ichinose, S. Tsukada, K. Miki, K. Kurokawa, O. Koiwai, M. Tanji, E. Yakabe, S.P.B Athanda and K. Takahashi, 1992. Gastric procathepsin E and progastricsin from guinea pig: purification, molecular cloning of cDNAs and characterization of enzymatic properties, with special reference to procathepsin. E. J. Biol. Chem., 267: 16450-16459.

Kageyama, T., 2000. New World monkey pepsinogens A and C and prochymosins. Purification, characterization of enzymatic properties, cDNA cloning and molecular evolution. J. Biochem., (Tokyo), 127: 761.

Kassell, B. and P.A. Meitner, 1970. Bovin Pepsinogens and Pepsins. In: Perlmann G.E. and L. Lorand (Eds.), Methods in Enzymology XIX, Academic Press, New York and London, pp: 337-347.

Kitamura, N., A. Tanimoto, E. Hondo, A. Andren, D.F. Cottrell, M. Sasaki and J. Yamada, 2001. Immunohistochemical study of the ontogeny of prochymosin and pepsinogen-producing cells in the abomasum of sheep. Anat Histol. Embryol., 30: 231-5.

Kurabayashi, Y., J. Yamada, A. Andrea, N. Kitamura and T. Yamashita, T. 1991. Cellular and subcellular localization of progastricsin in calf fundic mucosa: colocalization with pepsinogen and prochymosin. *Acta Anat* (Basel), 140: 75-84.

Lai, K.H., J.B. Wyckoff and I.M. Samloff, 1988. Aspartic proteinases in gastric mucosa of the rat: Absence of pepsinogen I, genetic polymorphism of pepsinogen II and presence of slow moving proteinase, 95: 295-301.

Liebman, W.M. and I. M. Samloff, 1978. Immunochemical characterization and cellular localization of pepsinogens in cat and dog. J. Histochem. Cytochem., 26: 1115-1120.

Liebman, W.M. and I.M. Samloff, 1979. Cellular localization of hog pepsinogens. (letter to the Ed.), J. Histochem. Cytochem., 27: 1112-1113.

Lindgren, A., G.K. Lindstedt and A.F. Kilander, 1998. Advantages of serum pepsinogen A combined with gastrin po pepsinogen C as first-line analytes in the evaluation of suspected cobalamin deficiency: A study in patients previously not suspected to gastrointestinal surgery. J. Int. Med., 244: 341-349.

Martin, P., P. Trieu-Cuot, J.C. Collin and B. Ribadeau Dumas, 1982. Purification and characterization of bovine gastricsin. Eur. J. Biochem., 122: 31-39.

Mckellar, Q.A., 1984-1985. The role of the pepsinogen test in the diagnosis of ostertagiasis. B. Cattle Vet. Assoc. Proc., 1: 11-14.

Meitner, P.A. and B. Kassell, 1971. Bovine pepsinogens and pepsins. A series of zymogens and enzymes that differ in organic phosphate content. Biochem. J., 121: 249-256.

Minn, I., H.S. Kim and S.C. Kim, 1998. Antimicrobial peptides derived from pepsinogens in the stomach of the bulfrog (Rana catesbesiana). Biochem. Biophys. Acta Mol. Basis Dis., 1407: 31-39.

Mostofa, M., Q.A. Mckellar and P.D. Eckersall, 1990. Comparison of pepsinogen forms in cattle, sheep and goats. Res. Vet. Sci., 48: 33-37.

Ng, K.K., J.F. Petersen, M.M. Cherney, C. Garen, J.J. Zalatoris and C. Rao-Naik, 2000. Structural basis for the inhibition of porcine pepsin by Ascaris pepsin inhibitor-3. Nat. Struct. Biol., 7: 653-657.

Ottemann, K.M. and A.C. Lowenthal, 2002. Helicobacter pylori uses motility for initial colonization and to attain robust infection. Infect. Immun., 70: 1984-1990.

Pals, G., T. Azuma, T.K. Mohandas, G.I. Bell, J. Bacon, I.M. Samloff, D.A. Walz, P.J. Barr and R.T. Taggart, 1989. Human pepsinogen C (progastricsin) polymorphism: Evidence for a single locus located at 6p21.1-pter. Genomics, 4: 137-148.

Rawling, N.D. and A. Barret, 1994. Families of aspartic peptidases and those of unknown mechanism. Meth. Enzymol., 248: 105-120.

Reese, J.H., J.E. McNeal, E.A. Redwine, I.M. Samloff and T.A Stamey, 1986. Differential distribution of pepsinogen II betyween the zones of the human prostate and the seminal vesicle. J. Urol., 136: 1148-1152.

Reid, W.A., L. Vongsorasak, J. Svasti, M.J. Valler and J. Kay, 1984. Identification of the acid proteinase in human seminal fluid as a gastricsin originating in the prostate. Cell Tissue Res., 236: 597-600.

Rich, D.H. and E.T. Sun, 1980. Mechanism of inhibition of pepsin by pepstatin: Effect of inhibitor structure on dissociation constant and time-dependent inhibition. Biochem. Pharmacol., pp: 2205-2212.

Richter, C., T. Tanaka and R. Y. Yada, 1998. Mechanism of activation of the gastric aspartic proteinases: Pepsinogen, progastricsin and prochymosin. Biochem. J., 335: 481-490.

Roberts, N.B. and W.H. Taylor, 2003. Comparative pepstatin inhibitor studies on individual human pepsins and pepsinogens 1, 3 and 5 (gastricsin) and pig pepsin A.J. Enzyme Inhibition Med. Chem., 18: 209-217.

Ryle, A.P. and R.R. Porter, 1959. Parapepsins: Two proteolytic enzymes associated with porcine pepsin. Biochem. J., 73: 75-86.

Ryle, A.P., 1960. Parapepsinogen II: The zymogen of parapepsin II. Biochemistry, 75: 145-150.

Ryle, A.P., 1966. Minor proteases in the stomach of the pig. Biochem. J., 98: 485

Ryle, A.P. and P.M. Hamilton, 1966. Pepsinogen C and pepsin C. Further purification and amino acid composition. Biochem. J., 101: 176-183.

Sakamoto, N., H. Saiga and S. Yasugi, 1998. Analysis of temporal expression pattern and cis-regulatory sequences of chicken pepsinogen A and C. Biochem. Biophys. Res. Commun., 250: 420-424.

Samloff, I. M., 1982. Pepsinogens I and II: Purification from gastric mucosa and radioimmunoassay in serum. Gastroenterology, 82; 26-33.

Samloff, I.M. and W.M. Liebman, 1972. Purification and immunochemical characterization of group II pepsinogens in human seminal fluid. Clin. Exp. Immunol., 11: 405-414.

Samloff, I.M. and W.M. Liebman, 1972..Immunochemical heterogenety of commercial hog pepsinogens. Immunochemistry, 9: 603.

Sánchez, L.M., J.P. Freije, A.M. Merino, F. Vizoso, B. Foltmann and C. López-Otin, 1992. Isolation and characterization of a pepsin C zymogen produced by human breast tissues. J. Biol. Chem., 267: 24725-24731.

Sangild, P.T., P.D. Crawell and L. Hilsted, 1992. Ontogeny of gastric function in the pig: Acid secretion and the synthesis and secretion of gastrin. Biology of the Neonate, 62: 363-72.

Schreiber, S., R. Bucker, C. Groll, M. Azevedo-Vethacke, S. Scheid, P. Schreiber, S. Gatermann, C. Josenhans and S. Suerbaum, 2006. Gastric antibacterial efficiency is different for pepsin A and C. Arch. Microbiol., 184: 335-340.

Seijffers, M. J., H.L. Segal and L.L. Miller, 1965. Separation of pepsinogen II and pepsinogen III from human urine. Am. J. Physiol., 206: I 1106-1110.

Suchodolski, J.S., J.M. Steiner, C.G. Ruaux, A. Boari, D.A. Williams, 2002. Purification and partial characterization of canine pepsinogen A and B. Am Vet. J., 63: 1585-1590.

Suerbaum, S. and P. Michetti, 2002. Helicobacter pylori infection. N. Engl. J. Med., 347: 1175-1186.

Suzuki, M., Y. Narita, S. Oda, A. Moriyama, O. Takenaka and T. Kageyama, 1999. Purification and characterization of goat pepsinogens and pepsins. Comp. Biochem. Physiol., B 122: 453-460.

Szecsi, P.B. and H. Lilja, 1993. Gastricsin-mediated proteolytic degradation of human seminal fluid proteins at pH levels found in the human vagina. J. Androl., 14: 351-358.

Takahashi, K., 1992. Gene structures of pepsinogens A and C. Scand. J. Clin. Lab. Invest., 52: 97-110.

Takahashi, K. and T. Kageyama, 1985. Multiplicity and intermediates of the activation mechanism of zymogens of gastric aspartic proteinases. In: Aspartic Proteinases and their inhibitors, Kostka V. (Ed.), de Gruyter, Berlin, pp: 265-282.

Tang, J., P. Sepulveda, J. Marciniszyn, K.C. Chen, W.Y. Huang and N. Tao, 1973. Amino-acid sequence of porcine pepsin. Proc. Natl. Acad. Sci. (USA)., 70: 3437-3439.

Tang, J., M.N. James, I.N. Hsu, J.A. Jenkins and T.L. Blundell, 1978. Structural evidence for gene duplication in the evolution of the acid proteases. Nature, 271: 618-621.

Tang, J., 1970. Gastricsin and pepsin. Methods Enzymol., 19: 406-421.

Tang, J., 2004. Gastricsin. In Handbook of Proteolytic Enzymes, (2nd Edn.), (Barrett,A.J., Rawlings, N.D. and Woessner, J.F. Eds.), Elsevier, London, pp: 38-43.

Tress, U., J.M. Steiner, C.G. Ruaux, J. S. Suchodolski and D.A. Williams, 2004. Purification and partial characterization of feline pepsinogen. Am. J. Vet. Res., 65: 1195-1199.

Valler, M.J., J. Kay, T. Aoyagi and B.M. Dunn, 1985. The interaction of aspartic proteinases with naturally-occurring inhibitors from actinomycetes and *Ascaris lumbricoides*. J. Enzyme Inhib., 1: 77-82.

Wislinski, M. and W. Cybulski, 1994. Prochymosin and pepsinogen potential activity and electrophoretic study of proteolytic fractions in the gastric and duodenal mucosal extracts from the first four day old lambs. Arch. Vet. Pol., 34: 75-89.

Yamada, J., A. Andren, N. Kitamura and T. Yamashita, 1988. Electron immunocytochemical co-localization of prochymosin and pepsinogen in chief cells, mucosa neck cells and transitional mucous neck/chief cells of the calf fundic glands. *Acta. Anat* (Basel), 3: 246-252.

Yasugi, S. and T. Mizuno, 1981. Development changes in acid proteases of avian proventriculus. J. Exp. Zool., 216: 331-335.

Yasugi, S., T. Mizuno and H. Esumi, 1979. Changes in molecular species of pepsinogens in the development of the chick. Experientia, 35: 814-815.