Protease Inhibitors in Porcine Colostrum: Potency Assessment and Initial Characterization

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ABSTRACT : Porcine colostrum and milk were separated into the acid-soluble and casein fractions by acidification followed by centrifuge. The acid-soluble fraction of porcine colostrum was further separated by liquid chromatography and anisotropic membrane filtration. Trypsin and chymotrypsin inhibitory capacity in porcine colostrum, milk and their components was determined by incubating bovine trypsin or chymotrypsin in a medium containing their corresponding substrates with or without addition of various amounts of porcine colostrum, porcine milk or their components. The inhibition of insulin-like growth factor I (IGF-I) and epidermal growth factor (EGF) degradation in pig small intestinal contents by porcine colostrum was measured by incubating iodinated IGF-I or EGF with the intestinal contents with or without addition of porcine colostrum. Degradation of labeled IGF-I or EGF was determined by monitoring the generation of radioactivity soluble in 30% trichloroacetic acid (TCA). The results showed that porcine colostrum had high levels of trypsin and chymotrypsin inhibitory activity and increased the stability of IGF-I and EGF in pig intestinal contents. The inhibitory activity declined rapidly during lactation. It was also found that trypsin and chymotrypsin inhibitory activity and the inhibition on IGF-I and EGF degradation in the acid-soluble fraction were higher than that in the casein fraction. Heat-resistance study indicated that trypsin inhibitors in porcine colostrum survived heat treatments of 100°C water bath for up to 10 min, but exposure to boiling water bath for 30 min significantly decreased the inhibitory activity. Compared with the trypsin inhibitors, the chymotrypsin inhibitors were more heat-sensitive. Separation of the acid-soluble fraction of porcine colostrum by liquid chromatography and anisotropic membrane filtration revealed that the trypsin and chymotrypsin inhibitory capacity was mainly due to a group of small proteins with molecular weight of 10,000-50,000. In conclusion, the present study confirmed the existence of high levels of protease inhibitors in porcine colostrum, and the inhibition of porcine colostrum on degradation of milk-borne growth factors in the pig small intestinal tract was demonstrated for the first time. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 12 : 1822-1829)***

Key Words : Porcine Colostrum, Protease Inhibitors, Growth Factors, GI Tract

INTRODUCTION

Protease inhibitors have been found in the mammary secretions of human (Laskowski and Laskowski, 1951; Lindberg, 1979; Lindberg et al., 1982), rat (Carlsson et al., 1975; Westrom et al., 1976; Telemo et al., 1982; Rao et al., 1990; Rao et al., 1993), cow (Laskowski and Laskowski, 1951; Weber and Nielsen, 1991) and pig (Laskowski et al., 1957; Kress et al., 1971; Carlsson et al., 1980; Westrom et al., 1982; Xu et al., 1996). The amount of trypsin inhibitors in sow colostrum is to a group of colostrum-specific protease inhibitors (Westrom et al., 1982). It has been shown that colostrum-specific protease inhibitors enhance absorption of intact colostrum proteins in neonatal pigs, probably by inhibiting the gastrointestinal proteolysis (Westrom et al., 1985). However, little is known about the physical and chemical characteristics of protease inhibitors in porcine colostrum. In addition, whether porcine colostrum enhances the survival rates of milk-borne growth factors, which have been speculated to play a role in the development of the gastrointestinal (GI) tract in suckling young (Koldovsky, 1996; Xu, 1996), is largely unknown. The objectives of the present study were to investigate the trypsin-chymotrypsin inhibitory activity and heat resistance of various protease inhibitors in porcine colostrum and porcine milk. The protease inhibitors in porcine colostrum were further characterized by anisotropic membrane filtration and liquid chromatography. The inhibition of porcine colostrum on insulin-like growth factor I (IGF-I) and epidermal growth factor (EGF) degradation in the GI lumen was also examined.

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MATERIALS AND METHODS

Collection and preparation of porcine colostrum and milk

Porcine colostrum and milk were collected from four sows by hand milking. Colostrum was collected at the time of parturition and milk was collected at 7-14 days post-parturition. Milk samples collected from the same sow within this period were pooled. Porcine colostrum was collected from the sow without oxytocin injection; while milk was collected after an i.m. injection of 20 IU oxytocin (Sigma, St Louis, MO, USA).

The colostrum and milk were defatted by centrifugation at 1,000 g for 10 min at 4°C. The casein fraction (CF) was obtained by acidifying defatted colostrum or milk to pH

4.0-4.6 with 2 M acetic acid and centrifugation at 26,000 g for 30 min at 4°C using a Beckman ultracentrifuge. The pellet (casein fraction) was resuspended with distilled water. Both the casein suspension and the supernatant (acid-soluble fraction: SF) were then neutralized with 1.0 M NaOH. Protein concentrations in the preparations of defatted colostrum, defatted milk, and their CF and SF were determined by the Lowry method using bovine serum albumin (BSA) as a standard (Lowry et al., 1951).

Determination of protease inhibitory activity

Trypsin and chymotrypsin activities were determined spectrophotometrically using N-benzoyl-L-arginine-4-nitroanilide (Bz-L-Arg-4-NA) and N-succinyl-L-alanyl-L-alanyl-L-prolyl-L-phenyl-alanine-4-nitroanilide (Suc-Phe-4-NA) as the respective substrate (Greiger and Hans, 1984; Greiger, 1984). Trypsin and chymotrypsin inhibitory activity in porcine colostrum or porcine milk or its components was measured by incubating bovine trypsin (Sigma, St Louis, MO, USA) (10 g in 2 ml incubation medium) or chymotrypsin (Sigma, St Louis, MO, USA) (20 g in 3 ml incubation medium) in the medium containing the corresponding substrate with or without addition of various amounts of defatted porcine colostrum or porcine milk or its components (Carlsson and Karlsson, 1972; Rao et al., 1990, 1993; Xu et al., 1996). The inhibitory activity was calculated as the percentage decrease in the substrate hydrolysis rate, which is directly proportional to absorbance increase during incubation.

Pepsin inhibiting activity was determined by incubating pepsin (Sigma, St Louis, MO, USA) with bovine hemoglobin (Sigma, St Louis, MO, USA) without or with addition of different amounts of the SF of porcine colostrum at 37°C for 15 min. The reaction was terminated by addition of an equal volume of 5% trichloroacetic acid (TCA) followed by centrifugation at 4,000 rpm for 20 min. The soluble protein in the supernatant was quantified by Lowry method (Lowry et al., 1951). The pepsin inhibiting activity was determined by percentage decrease of bovine hemoglobin hydrolysis rate during the incubation.

Inhibition of luminal degradation of IGF-I and EGF by colostrum

Collection and preparation of gastrointestinal fluids : Gastric and intestinal contents were collected from three 45-day-old weaned pigs. The animals were naturally suckled or fed *ad. libitum* prior to euthanasia with an overdose of thiopentone sodium (Pentothal, Abbott Australia Pty Ltd., Sydney, Australia). The GI tract was removed from the abdominal cavity immediately after the animals were euthanatized. The small intestine was separated into three parts of equal length, *i.e.* proximal, mid and distal parts. The intestinal luminal fluids from each segment were collected by holding the segment vertically with gentle shaking. The intestinal contents were suspended in an equal volume of chilled saline and centrifuged at 3,000 rpm for 20 min to remove food particles. All the operations were performed on wet ice. The luminal fluids were then stored in aliquots at -20°C until further use.

Preparation of iodine labeled IGF-I : Recombinant human IGF-I (Sigma, St. Louis, MO, USA) and EGF (Sigma, St. Louis, MO, USA) were labeled with I-125 (Amersham Far East Trading Ltd., Hong Kong) by the Chloramine-T method (Greenwood et al., 1963). Briefly, 10 μl of IGF or EGF (0.5 mg/ml) in an eppendorf was added in sequence with 50 μl phosphated buffer (0.5 M, pH 7.4), 0.5 mCi iodine-125 and 10 μl chloramine-T solution (0.5 mg/ml). After constant mixing for 1 min, 100 μl sodium metabisulphate solution (2 mg/ml) was added followed by 200 μl phosphated buffer (0.05 M, pH 7.4, containing 2.4 M NaCl). All ingredients were dissolved in the above phosphated buffer or otherwise specified. Free iodine was removed by chromatography on a prepacked disposable Sephadex G-25 column (PD-10) which was equilibrated and eluted with phosphated buffer saline (0.05 M, pH 7.4, containing 0.075 M NaCl and 0.1% BSA). The final preparations of the labeled IGF-I and EGF had a specific radioactivity of 80-90 μCi/μg.

Inhibition of luminal degradation of IGF-I and EGF by porcine colostrums : To determine if porcine colostrum is able to protect IGF-I and EGF from hydrolytic degradation in the GI lumen, iodinated IGF-I or EGF was incubated at 37°C for 20 min in a medium (150 μl) containing 20 μl of intestinal fluids of weaned pigs with or without various amounts of porcine colostrum, CF and SFof porcine colostrum, BSA or trypsin-chymotrypsin inhibitor (Sigma, St. Louis, MO, USA). The reaction was terminated by addition of 200 μl of cold 30% TCA followed by 100 μl of 1.5% BSA. TCA soluble and TCA precipitable radioactivity were separated by centrifugation, and total radioactivity and TCA precipitable radioactivity were recorded. Inhibitory activity of porcine colostrum was calculated as a percentage decrease in the generation rate of TCA soluble radioactivity.

Initial characterization of protease inhibitors in porcine colostrum

The protease inhibitors in porcine colostrum were further characterized through the following two methods.

Gel filtration : Separation of the SF of porcine colostrum was performed on a 1×50 cm column of Sephadex G-100 (Pharmacia, Sweden). The column was equilibrated and eluted with 0.05 M Tris-HCl buffer (pH 7.4) at room temperature. A constant flow of 8 ml/h was maintained using a Tris pump (Isco). The absorbance at 280 nm in a flow-cell was recorded with Isco UA-6 Absorbance Detector. Protein elution fractions were concentrated by

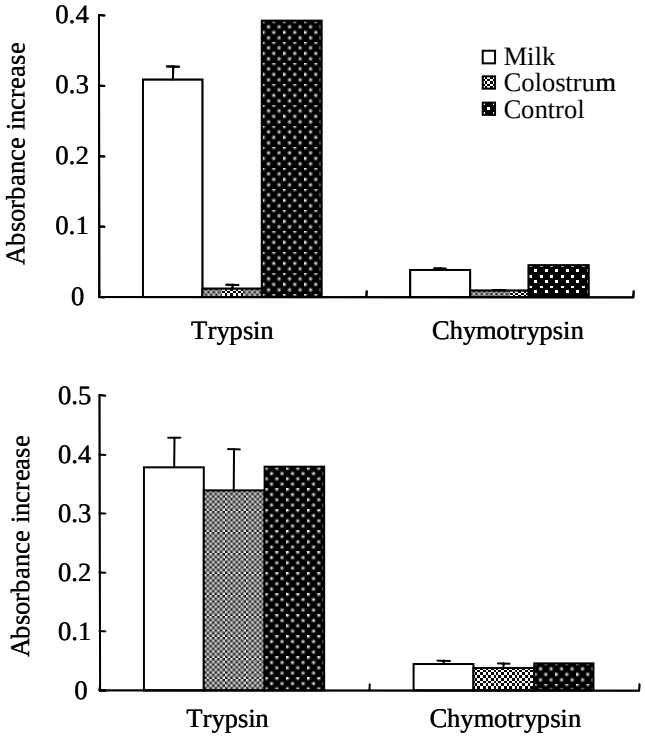

Figure 1. Inhibitory activity of the SF (upper panel) and CF (lower panel) of porcine colostrum and milk. Trypsin (5 g/ml) or chymotrypsin (6.7 g/ml) was incubated in a medium containing the corresponding substrate without (control) or with the presence of 200 g protein equivalent of the SF or the CF of either colostrum or milk (milk and colostrum samples: mean±SEM, n=4; control: mean of duplicate assays).

lyophilization or centricon centrifugal concentrator. The trypsin and chymotrypsin inhibitory activity in the protein elution fractions was examined by the methods described above.

Centricon concentrators : Concentration and separation of the SF and CF was achieved by ultrafiltering the sample solution through an anisotropic membrane. Three different concentrators were used in this study, namely Centricon 10, Centricon 50 and Centricon 100 [Amicon Polymers (HK) Ltd.] with molecular weight cut-offs of 10,000, 50,000 and 100,000 daltons, respectively. Samples were added to the concentrator's sample reservoir, followed by centrifugation forcing solvents and low molecular weight solutes through the membrane and into the filtrate cup. Molecules with molecular weights greater than the membrane cut-off were retained in the sample reservoir. The filtrate in the filtrate cup was collected and the trypsin inhibitory activity was determined as described above.

Data analysis

Statistical analysis was carried out using a computer package: Minitab (Minitab Inc., USA, 1994). The difference between the mean values of colostrum and milk or between

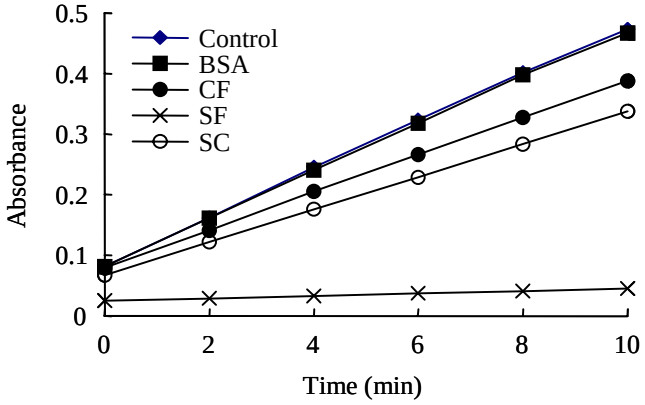

Figure 2. Typical trypsin activity kinetics when bovine trypsin (5 g/ml) was incubated with Bz-L-Arg-4-NA without (control) or with the presence of 200 g protein equivalent of BSA, skim colostrum (SC), porcine casein (CF) or the SF of porcine colostrums at 25°C for 10 min. The results are expressed as absorbance at the wavelength of 405 nm.

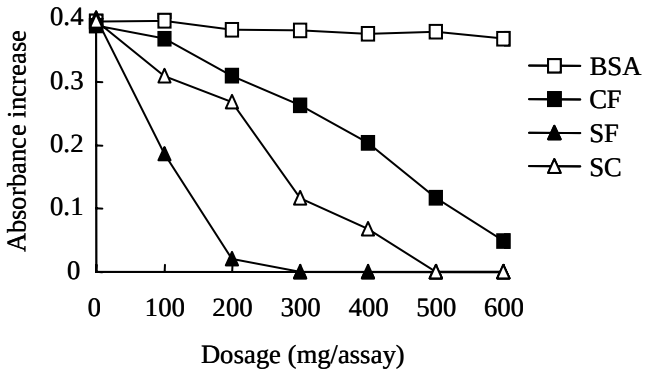

Figure 3. Inhibition of trypsin activity by various amounts (protein equivalent, g/assay) of BSA, SC, CF or the SF of porcine colostrum. Bovine trypsin (5 g/ml) was incubated with these samples in a medium containing the corresponding substrate at 25°C for 10 min (means of duplicate assays).

the SF and CF of porcine colostrum was tested by Student t-test (Freund and Wilson, 1993).

RESULTS

Protease inhibitory activity in porcine colostrum and milk

The trypsin and chymotrypsin inhibitory activity of porcine colostrum and milk was shown in Figure 1. The results indicated that the inhibitory potency in porcine colostrum was significantly higher than that in milk ($p < 0.01$). The inhibitory potency of the SF of porcine colostrum was nearly 5-fold higher than that of milk. The trypsin and chymotrypsin inhibitory activity in the CF of porcine milk was too low to be detected in the present assay system, which contained 5 g/ml bovine trypsin or 6.7 g/ml chymotrypsin.

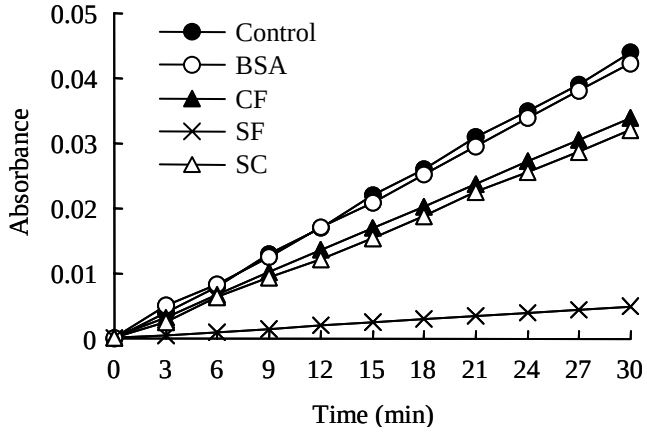

Figure 4. Typical chymotrypsin kinetics when bovine chymotrypsin (6.7 g/ml) was incubated with Suc-Phe-4-NA without (control) or with the presence of 200 g protein equivalent of BSA, SC, CF or the SF of porcine colostrums for 30 min at 25°C. The results are expressed as absorbance at the wavelength of 405 nm.

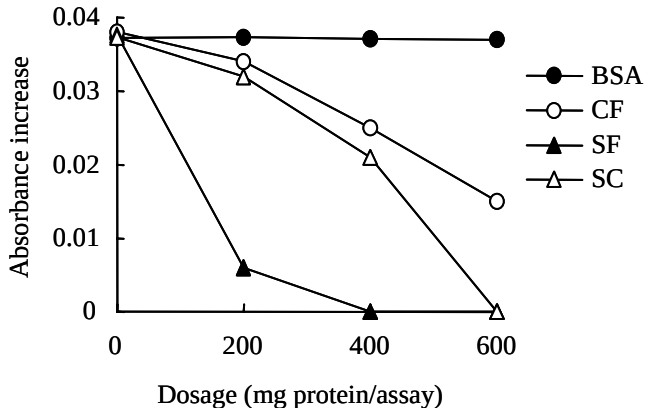

Figure 5. Inhibition of chymotrypsin activity by various amounts (protein equivalent, g/assay) of BSA, SC, CF or the SF of porcine colostrum. Bovine chymotrypsin (6.7 g/ml) was incubated with these samples in a medium containing the corresponding substrate at 25°C for 30 min (means of duplicate assays).

Trypsin and chymotrypsin inhibitory activity in porcine colostrum

Trypsin enzyme kinetics was shown in Figure 2. Addition of BSA into the incubation medium had no effect on trypsin activity, while addition of defatted colostrum (skim colostrum) markedly reduced the hydrolysis rate. The results also indicated that the SF had greater inhibition potency than the CF of porcine colostrum. The findings were confirmed by dose-response studies (Figure 3), which showed that the SF of porcine colostrum was over three times more potent than the CF in inhibiting trypsin activity. Addition of 300 g protein equivalent of the SF of porcine colostrum or 500 g protein equivalent of skim colostrum into the assay medium containing 5 g bovine trypsin completely blocked the enzyme action (Figure 3).

Chymotrypsin inhibitory activity was also demonstrated in porcine colostrum. The enzyme kinetics studies showed that porcine colostrum exhibited potent inhibitory activity against chymotrypsin (Figure 4), and the inhibitory activity in the SF was much higher than that in the CF, which was further supported by dose-response studies (Figure 5).

The pepsin inhibitory activity was not found in porcine colostrum.

Table 1. Inhibition of IGF-I hydrolysis in the mid intestinal fluids of 45-day-old weaned pigs by colostrum, SF and CF of colostrum, BSA and trypsin-chymotrypsin inhibitor

Protein	Dosage (g)[1]	IGF-I degradation[2]	Inhibitory activity[3]
No inhibitor		69.6	0
Colostrum	120	70.9	0
	240	51.8	25.6
	600	18.8	73.0
	1,200	0	100
Acid soluble fraction	80	56.9	18.3
	160	34.3	50.7
	400	13.4	80.8
	800	0	100
Casein fraction	40	72.0	0
	80	69.6	0
	200	43.6	37.8
	400	37.8	45.7
BSA	150	70.0	0
	300	67.2	4.0
	450	68.1	2.2
Trypsin-chymotrypsin inhibitor	1	55.5	20.3
	2	52.3	24.9
	3	53.6	23.1

[1] Protein equivalent. [2] IGF-I degradation rates were expressed as the percentage of TCA soluble radioactivity after incubation of iodine labeled IGF-I with the pooled mid intestinal fluids of weaned pigs with or without addition of various amounts of colostrum or its fractions. The values were means of duplicate assays. [3] Inhibitory activity was expressed as difference of IGF-I degradation of the "no inhibitor" group and the inhibitor group divided by the IGF-I degradation of the "no inhibitor" group.

Inhibition of IGF-I and EGF degradation in luminal fluids by colostrum

Both SF and CF of porcine colostrum exhibited a potent inhibitory effect on IGF-I degradation in the intestinal fluids in weaned pigs, and the inhibitory activity was dose-dependent (Table 1). It was also found that the SF possessed higher inhibitory activity than the CF. IGF-I degradation in the assay system was completely inhibited after incubation with the SF at the dose of 0.8 mg protein equivalent or porcine colostrum at the dose of 1.2 mg protein equivalent. However, bovine serum albumin had no significant effect on the IGF-I degradation.

EGF degradation was also reduced after incubation with porcine colostrum. Both the SF and CF of porcine colostrum showed inhibitory activity on EGF degradation in a dose-dependent manner (Figure 6). Addition of 300 g protein equivalent of the SF or CF of porcine colostrum to

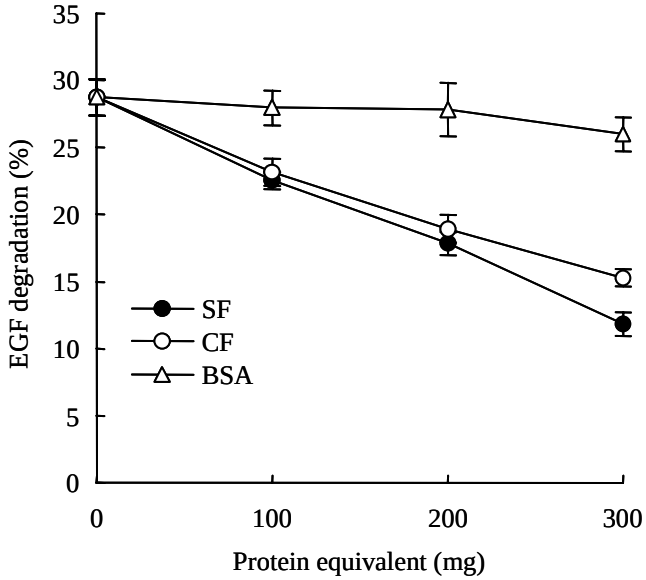

Figure 6. Inhibition of the CF, SF of porcine colostrum and BSA on EGF degradation in the proximal luminal fluids of weaned pigs.

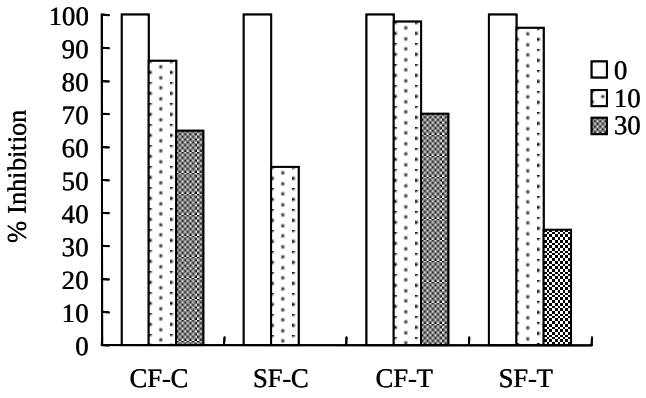

Figure 7. Effects of heat treatment (100°C up to 30 min) on trypsin (T) and chymotrypsin (C) inhibitory activity of CF and the SF of porcine colostrum. Inhibition is expressed as percentage (means of duplicate assays) of control, and the control (100%) is the inhibition obtained by unheated colostrum component (SF or CF).

the incubation medium containing 20 l of the proximal intestinal fluids of weaned pigs reduced the degradation rate of EGF by 59% and 47% respectively, while addition of the same amount of BSA to the incubation medium had no significant effect. Soybean isolated trypsin-chymotrypsin inhibitors also showed a potent protection of EGF from the *in vitro* hydrolysis, and addition of 2 g of the inhibitor reduced the degradation rate by 72%.

Effect of heat treatment on the inhibiting activity of porcine colostrum

The components inhibiting trypsin and chymotrypsin activity in the CF and SF of porcine colostrum could

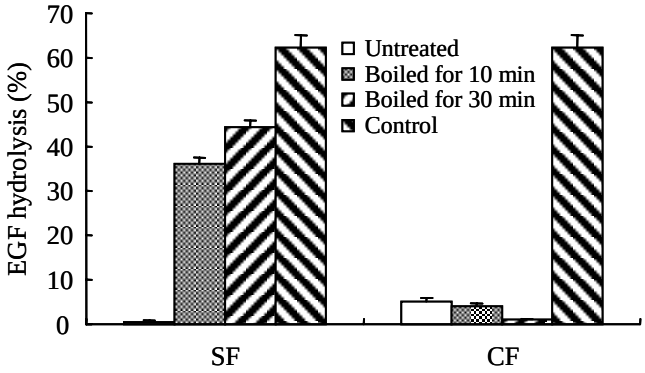

Figure 8. Effects of heat treatment of SF or CF of porcine colostrum on their ability to protect EGF degradation in the intestinal lumen. Iodinated EGF was incubated with 20 l mid intestinal fluids of weaned pigs without (Control) or with addition of SF (1,100 g protein) or CF (500 g protein) which were boiled for 0, 10 or 30 min). The degradation rates (mean±SEM) of EGF were calculated from the generation of TCA-soluble radioactivity.

survive heat treatment to varying extents (Figure 7). The trypsin inhibitory activity in porcine colostrum remained unchanged after heat treatment in boiling water bath for 10 min. However, exposure of the CF or SF of porcine colostrum to boiling water bath for 30 min reduced their trypsin inhibitory activity significantly and the SF was more heat-labile than the CF. Interestingly, it was found that chymotrypsin inhibitory activity was much more heat-sensitive than trypsin inhibitory activity in the SF of porcine colostrum. Heat treatment in boiling water bath for 30 min completely destroyed the chymotrypsin inhibitory activity in the SF of porcine colostrum. When the SF was boiled for 10 or 30 min, the inhibitory activity on the EGF degradation in the intestinal fluids decreased significantly. However, the same heat treatment had no effects on the inhibitory activity of the CF (Figure 8).

Characterization of the protease inhibitors in porcine colostrum

Separation by Sephadex G-100 column : On a Sephadex G-100 column, the proteins in the SF of porcine colostrum were eluted in two peaks (Figure 9) with a Ve/Vt ratio of 0.36 and 0.87, respectively. The two peaks contributed 80% of the total trypsin-chymotrypsin inhibitory activity in the SF of porcine colostrum, and the inhibitory activity in the second peak was over 9 times more potent than that in the first peak.

Characterization by centricon concentrators : The trypsin and chymotrypsin inhibitory activities in the filtrates of three different centricon concentrators were shown in Table 2. The results showed that the protease inhibitory activity was found in the filtrate of Centricon 50 but not in the filtrate of Centricon 10.

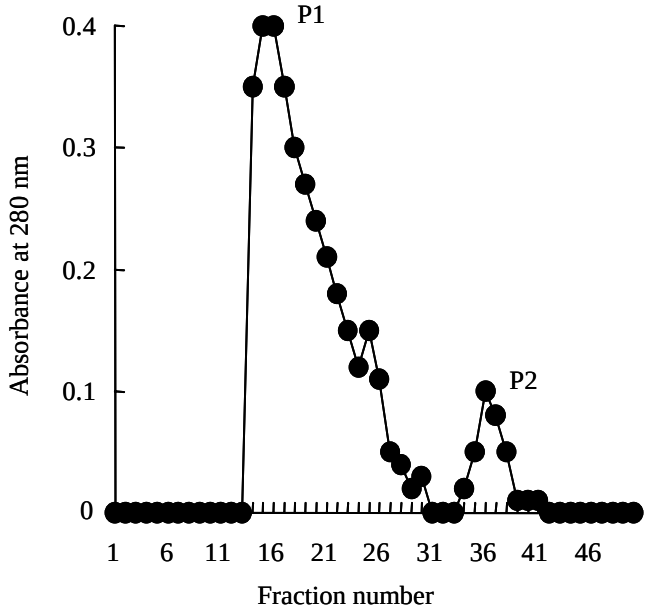

Figure 9. Protein profile of the SF of porcine colostrum eluted on a Sephadex G100 column as described in the "Materials and Methods". Protein contents were monitored by reading absorbance at 280 nm. The void volume and column volume were initially determined by eluting blue dextran and sodium chloride on the column, respectively.

DISCUSSION

The present study demonstrated that porcine colostrum contained high levels of trypsin-chymotrypsin inhibitory activity and one ml of porcine colostrum could inactivate 1 mg of bovine trypsin. However, the inhibitory activity decreased markedly during lactation (Figure 1). The trypsin inhibitory activity has been previously reported in porcine colostrum (Laskowski et al., 1957; Kress et al., 1971; Westrom et al., 1982; Xu et al., 1996), and the inhibitory activity is highest at the time of parturition and declines rapidly with the progress of lactation (Laskowski et al., 1957; Westrom et al., 1982). Laskowski et al. (1957) reported that the trypsin inhibitor concentration was highest on the first day after birth and fell gradually almost to zero by the fifth day. Westrom et al. (1982) also found that the total trypsin inhibitory capacity (TIC) and the content of the individual protease inhibitors, both the specific colostrum protease inhibitors and the serum-type inhibitors, decreased significantly during the first day of lactation, and the specific colostrum protease inhibitors disappeared from the milk after 5-7 days.

The present study indicated that the inhibitory activity was present in both the CF and SF of porcine colostrum, but the inhibitory capacity in the SF was much more potent than that in the CF (Figure 2, 3, 4, and 5). In addition, their heat resistance was also different (Figure 7), suggesting that the inhibitory mechanism of the CF may be different from that of the SF of porcine colostrum.

Table 2. Percentage of the inhibitory activity against trypsin and chymotrypsin in the centricon filtrates of the acid-soluble and casein fractions of porcine colostrum*

	Centricon 10		Centricon 50		Centricon 100	
	SF	CF	SF	CF	SF	CF
Trypsin	0	0	90	48	100	67
Chymotrypsin	0	0	95	71	100	87

*The data (means of duplicate assays) are expressed as percentages of protease inhibitory activity of the original colostrum components, the SF and CF.

High potent and heat labile inhibitory activity in the SF indicated the possible presence of specific protease inhibitors. So far, both colostrum-specific and serum-type protease inhibitors have been found in porcine colostrum (Westrom et al., 1982). The colostrum-specific inhibitor was first reported in porcine colostrum by Laskowski et al. (1957). Later on, it was found that sow colostrum trypsin inhibitors (SCTI) were a group of small proteins, which had a higher relative affinity for trypsin than the porcine serum inhibitors (Laskowski et al., 1957; Carlsson et al., 1974; Ohlsson et al., 1982; Ohlsson, 1987). By using ion exchange chromatography, Kress et al. (1971) isolated four homogeneous fractions, which were active against trypsin, α-chymotrypsin and chymotrypsin B, but inactive against carboxypeptidase B and the milk clotting activity of pepsin. Westrom et al. (1979, 1982) reported that the high protease inhibitory capacity of porcine colostrum was particularly due to colostrum-specific protease inhibitors. The marked decrease of TIC in porcine milk was due mainly to the disappearance of colostrum-specific protease inhibitors from porcine milk. Similar developmental pattern of colostrum-specific protease inhibitory capacities was observed by Jensen and Pedersen (1979). However, the studies of Westrom et al. (1979, 1982) also indicated that about 10-20% of trypsin inhibitory activity in porcine colostrum was derived from the inhibitors with molecular weight of about 70,000 daltons. These inhibitors resembled, with respect to physiochemical characteristics and immunological identities, those found in sow serum. The levels of these inhibitors in porcine colostrum are related to their molecular weights, suggesting that the inhibitors with larger molecular weights may probably originate from sow serum rather than produced by mammary gland. These protease inhibitors are supposed to be transferred from serum to colostrum via both a transcellular and paracellular route (Westrom et al., 1982). Like sow colostrum protease inhibitors, all the serum-type inhibitors, namely α_2-macroglobulin f and s, inter-α-trypsin inhibitor, α_2-antitrypsin and α_1-protease inhibitor, also decrease significantly during lactation (Westrom et al., 1982). Since colostrum specific protease inhibitors disappear from

mature milk, these serum-type inhibitors are thus responsible for the remaining low trypsin-inhibitory capacity observed in milk.

It remains unclear as to the exact mechanism by which porcine casein fraction inhibits trypsin and chymotrypsin activity. Presumably, casein may non-specifically bind to the proteases and block the active site. Alternatively, casein could act as preferential substrate since it is flexible in structure and very susceptible to the proteases (Lehninger, 1982; Xian et al., 1985). In addition, some protease inhibitors in porcine colostrum may be precipitated or wrapped in the casein pellets during acid precipitation (Fox, 1992), and these inhibitors may also contribute to the protease inhibitory activity detected in the CF.

By using centricon centrifugal concentrators, the present study suggested that the protease inhibitors in porcine colostrum were due mainly to a group of small proteins with molecular weights of about 10,000-50,000, whereas the protease inhibitors with molecular weights larger than 50,000 contributed little (Table 2). The findings were supported by liquid chromatography (Figure 9), which showed that the protease inhibitory activity in porcine colostrums was eluted mainly in the fraction corresponding to small proteins. The results generally agreed with the previous reports (Carlsson et al., 1974; Westrom et al., 1979), which suggested that the trypsin inhibitory activity in porcine colostrum may be mainly due to a group of small peptides.

The physiological function of the protease inhibitors in colostrum and milk may be exerted both in the mammary gland of the lactating female and in her nursing spring. Dietary protease inhibitors have been implicated in the facilitation of preclosure macromolecular absorption in pig, rat and rabbit (Carlsson et al., 1980; Telemo et al., 1987; Udall et al., 1984). So far, a number of growth factors have been found in milk of various species and their physiological functions in the development of neonatal animals have been proposed (Odle et al., 1996; Koldovsky, 1996; Xu, 1996; K. Kita et al., 2001). Conceivably, milk-derived protease inhibitors may help protect growth factors from the luminal digestion, and subsequently allow them to survive the GI tract and to play a role locally or systematically in the neonatal animal. The present study demonstrated that porcine colostrum enhanced IGF-I and EGF survival rate in the luminal fluids of the GI tract in neonatal pigs (Table 1, Figure 6 and Figure 8). The results confirmed and extended the previous findings that porcine colostrum had an ability to protect gastrin from hydrolysis in pig small intestinal fluids (Xu et al., 1996). The enhanced stability of peptide growth factors or hormone in the GI lumen by colostrum was also reported in rodents (Rao et al., 1990, 1993; Xian et al., 1995).

Taken together, high levels of protease inhibitory capacities were detected in porcine colostrum and the inhibitory capacity decreased markedly with the progress of lactation. The protease inhibitors in porcine colostrum existed mainly in the SF and they were heat-labile small proteins. The protease inhibitors in porcine colostrum enhanced the stability of EGF and IGF-I in the GI lumen of weaned pigs.

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