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Comparison of physical–chemical properties of type I collagen from different species

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Abstract

Type I collagen is an important biopolymer and has been widely used in biomaterials due to its excellent biocompatibility and biodegradable properties. However, only a few studies have been reported on its comparison in different species. The amino acid composition, SDS-PAGE, UV–Vis spectrum, thermal transition temperatures, extractable uronic acid/protein ratio and enzymatic sensitivity of type I collagen from bird feet (BF), bovine skin (BS), frog skin (FS), porcine skin (PS) and shark skin (SS) were evaluated. The amino acid composition of type I collagens were different from different species, BF collagen contained higher glutamic acid (Glu) and aspartic acid (Asp), SS collagen contained lower aspartic acid and hydroxyproline (Hyp). Similar SDS-PAGE profiles were found from different animal's collagen, all samples were composed of two α1-chain and one α2-chain. All UV–Vis spectrums exhibited a typical absorption peak at 218 nm. The UV absorption spectrum of BF collagen ranged from 190 to 340 nm, FS collagen ranged from 190 to 270 nm; the other species collagen ranged from 190 to 240 nm. Thermal transition temperatures of type I collagen from different animals decreased in the order of BF > BS > PS > FS > SS. PS collagen had higher extractable uronic acid/protein ratio and the lowest enzymatic sensitivity. Summarizing these results, the BF collagen had higher hyproxyproline (Hyp) + proline (Pro) value and exhibited higher thermal stability; the PS collagen contained larger amount of glycosaminoglycan and resulted in a high enzymes resistance. However, the BF and PS collagen should be used as a suitable material in biomaterial utilitys because of its better biostability.
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Keywords: Physical–chemical properties; Species; Collagen

1. Introduction

Type I collagen are major component of mammalian connective tissue, accounting for approximately 30% of all protein in the human body (Lee, Singla, & Lee, 2001). This collagen can be fabricated into various forms such as a gel, sponge, fiber, and film to serve as a scaffold for tissue engineering with unique biocompatibility and biodegradable properties (Lee et al., 2001; Pachence, 1996). Type I collagen has been extensively isolated from various animals, including bird feet (Liu, Lin, & Chen, 2001; Lin & Liu, 2006), bovine skin (Huang & Nimni, 1993; Yoshimura, Hozan, Chona, & Shirai, 1996), equine tendon (Angele et al., 2004), frog skin (Li, Liu, Gao, & Chen, 2004; Purna & Babu, 2001; Sathya, Kumar, Purna, & Babu, 2002), fish scale (Nomura, Sakai, Ishii, & Shirai, 1996), jellyfish (Nagai et al., 2000), porcine skin (Nomura, Toki, Ishii, & Shirai, 2000; Wu et al., 1999), rat tail tendon (Robinson, 1997), sea urchin (Robinson, 1997), and shark skin (Nomura, Yamano, Hayakawa, Ishii, & Shirai, 1997). Collagens from domestic animals are usually utilized in industry and they are chemically modified using proteases, a cross-linking agent or physical methods to

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vary and control their function for multiple uses (Angele et al., 2004; Lee et al., 2001; Pachence, 1996). Mammalian skin is as excellent source of type I collagen for biomedical use (Angele et al., 2004; Nomura et al., 2000), but bovine collagen has some potential risks of infectious diseases (Angele et al., 2004; Lee et al., 2001; Pachence, 1996) such as bovine spongiform encephalopathy (BSE) or transmissible spongiform encephalopathy (TSE). The infectious agent that causes BSE disease was found to be a "prion" (a protenacious particle resistant to acids, alkalie and enzymes) and thus concerns have raisen over the use of bovine products in medical devices (Pachence, 1996; Purna & Babu, 2001). Currently, the roles of collagen in disease transmission have not been established. But, in order to reduce the risk to manufacture biomaterials, an alternative safe collagen must be considered. Inherent chemical or physical differences in properties of collagen varies depending on the species of collagen (Angele et al., 2004; Nomura et al., 2000). Many studies indicated the bird feet (Liu, Lin, & Chen, 2001; Lin & Liu, 2006), frog skin (Li et al., 2004; Purna & Babu, 2001; Sathya et al., 2002), sea urchin (Robinson, 1997) and shark skin (Nomura et al., 1997) collagen have a molecular structure different than domestic animals. Their amino acid composition, peptide constitution, glycosaminoglycan content and thermal behavior are significantly different from land animals. However, there are limited numbers of systematic comparative study on these species-related properties. In this study, the main objective was to compare the structural relationship of collagen and to evaluate the function of different animal collagens by comparative biochemistry approaches, to provide more information that can be used to design collagen-based biomaterials with desirable properties.

2. Material and methods

2.1. Preparation of type I collagen from different animal species

Collagen can be prepared from bird feet (BF) according to the method of Lin and Liu (2006). Frozen broiler feet were obtained from a local packing poultry plant and thawed overnight at 4 °C. The toenails of bird feet were excised, then the feet were cut into 2 cm pieces, ground by a meat grinder and frozen at −20 °C. All the preparations were performed at 4 °C. Removal of fat and pigments were accommodated by stirring in 20% ethanol for 24 h, centrifugation at 10,000*g* for 20 min and discarding the supernatant. A 5% (w/w) pepsin (EC 3.4.23.1)/sample weight and a 10 fold volume of 0.5 M acetic acid was later added to the precipitate. Crystalline NaCl was slowly added to a final concentration of 0.9 M and centrifuged at 20,000*g* at 4 °C for 40 min. The resultant precipitate was dialyzed against 0.05 M acetic acid, and then the dry material was obtained by lyophilization.

Bovine skin (BS) from 3–5 year old cattle was obtained from a local slaughterhouse. The preparation was according to the modified method of Huang and Nimni (1993). One hundred gram of skin strips were defatted twice with 1 L of chloroform/methanol solution (50/50, v/v) at room temperature for 24 h, washed with distilled water and homogenized in 0.05 M Tris–HCl buffer containing 0.15 M NaCl and stirred 24 h at 4 °C. After 10,000*g* centrifugation at 4 °C, a supernatant was added containing crystalline NaCl to a concentration of 1.8 M, and centrifuged at 20,000*g* for 1 h. The precipitates were dialyzed against 0.05 M acetic acid at 4 °C and lyophilized.

Frog skin (FS, edible bullfrog) was obtained from a local market, the skin was soaked in 0.5 M acetic acid for 2 h and the epidermis was dissected, then the extraction procedures were the same as that used for bird feet collagen (Lin & Liu, 2006), except a lower (4 °C) pepsin digestion temperature was used to avoid excessive hydrolysis.

Porcine skin (PS) from 6 month old large white pigs (Landrace × Yorkshire) was obtained from a local slaughterhouse. The preparation was according to the modified method of Wu et al. (1999). Subcutaneous fat was removed from the fresh porcine skin which was then cut into 3–5 × 15–20 mm strips, and soaked in acetone for 24 h. Porcine skin was then soaked in 1 N lactic acid at room temperature for 24 h to cause swelling of the collagen. This was then homogenized, and 5% pepsin/sample weight (w/w) was added and sequentially stirred at 4 °C. The collagen solutions were filtered with sterilized gauze. The filtrate were sequentially adjusted to pH 10 with 1 N NaOH and maintained for 24 h to inhibit pepsin activity. The pH was adjusted to 7.0 with 0.5 M acetic acid. Finally, the solution was centrifuged at 20,000*g* for 40 min at 4 °C, and the precipitate was lyophilized.

Shark skin (SS, *Sphyrna lewini)* was purchased from a local fishing outlet, type I collagen prepared form shark skin was by the modified method of Sivakumar and Chandrakasan (1998). The skin was soaked in 0.5 M acetic acid for 2 h and the epidermis was dissected. It was then washed with cold distilled water, cut and homogenized in 6 M urea containing 0.5 M sodium acetate (pH 6.8) in order to remove proteoglycan and non-collagenous proteins. After centrifugation at 20,000*g* for 40 min at 4 °C, the precipitate were sequentially extracted with 0.05 M Tris–HCl containing 1.0 M NaCl, 0.5 M acetic acid and a pepsin solution (10 mg/mL in 0.5 M acetic acid). The precipitates were redissolved in 0.5 M acetic acid and thoroughly dialyzed against 0.05M acetic acid and lyophilized.

2.2. Amino acid composition

Collagen samples were hydrolyzed in 6 N HCl at 110 °C for 24 h in a dry heater (HACH COD reactor, USA) and their amino acid composition were determined by an amino acid analyzer (Beckman 6300, USA). The 4-hydroxyproline (4-Hyp) contents were determined by the method of Reddy and Enwemeka (1996). Two-hundred μL of 1% collagen suspension (in 0.5 M acetic acid) were plus added 300 μL of 3.3 N NaOH which were then sequentially hydrolyzed by autoclaving at 121 °C for 40 min. Then 4500 μL of chloramine solution were added for oxidation, plus 5000 μL of Ehrlich's reagent for chromophore formation, and the results were recorded at 550 nm with a spectrophotometer (Hitachi U-2001, Japan).

2.3. SDS-PAGE analysis

SDS-PAGE of different species of collagen was performed by the method of Laemmli (1970), using 7.5% separating gels and 5% stacking gels. The gels were stained with Coomassie Blue R250 (Sigma, B7920) and destained in methanol: acetic acid (2:1). The separated protein bands were identified by comparison to a standard molecular mixture marker (Sigma, SDS-6H) which included myosin (205 kDa), β-galcactosidase (116 kDa), phosphorylase b (97.4 kDa), bovine serum albumin (66 kDa), ovalbumin (45 kDa), and carbonic anhydrase (29 kDa).

2.4. Determination of extractable uronic acid

One hundred mg of collagen powder were stirred in 20 mL 0.5 M of guanidine at 4 °C for 24 h. The suspension was centrifuged at 20,000*g* for 20 min. The supernatant was analyzed for its protein and uronic acid contents. The uronic acid (μg/mL)/protein content (mg/mL) was used as an indicator of glycosaminoglycan. Protein and uronic acid concentration were determined by the biuret method (Itzhaki & Gill, 1964) and the sulfuric acid-cabazole method (Bitter & Muir, 1964), respectively.

2.5. UV–Vis spectrums

The UV–Vis adsorption spectrums of different species of collagen were determined utilizing a spectrophotometer (Hitachi U-2001, Japan) which was maintained at 37 °C by a temperature controller and this instrument recorded the increase in wavelength intervals over time (10 nm/min) with the aid of a spectrophotometer program.

2.6. Thermal behavior

The denaturation temperature of different species of type I collagen were measured by differential scanning calorimetry (Seiko DSC131, France). Type I collagens were dissolved in 200 fold 0.5 M acetic acid and injected (approximately 300 μL) into an aluminum cell and subjected to DSC. The heat rate was 5 °C/min and the temperature was increased from 5 to 150 °C. Three temperatures were measured: the onset temperature (T_o), the maximal peak 1 (T_{p1}) and the maximal peak 2 (T_{p2}) as characteristics of the denaturation process of different species of type I collagen.

2.7. Enzymatic sensitivity

Collagen degradation in bacterial collagenase (EC 3.4.24.3; 7 FALGPA units/mg solid), pepsin (EC 3.4.23.1; 463 units/mg solid) and trypsin (EC 3.4.21.4; 1200 BAEE units/mg solid) solution were measured to estimate the enzymatic sensitivity of different collagen species. Five mg samples of different species of pre-soluble collagen in 500μL of 0.5 M acetic acid were shaken (100 rpm) in an incubator at 37 °C with 2000 μL of collagenase solution (0.5 mg/mL dissolved by 0.01M Tris–HCl, pH 7.2, containing 50 mM $CaCl_2$), pepsin solution (1.0 mg/mL dissolved by 0.5 M acetic acid, pH 3.2) and trypsin solution (1.0 mg/mL dissolved by a sodium phosphate buffer solution, pH 7.2), respectively, for 8, 16, 24, 48, 72 and 96 h. For isolation, the degraded products was precipitated by 10 mL of 5% trichloroacteic acid (TCA) solution and filtered through a Whatman No. 2 (Toyo) filter paper. The 4-Hyp content of the filtrate were evaluated by the procedure of Reddy and Enwemeka (1996). The results were calculated according to following equation:

$$\text{Enzymatic sensitivity } (\%) = [H_2/H_1] \times 100,$$

where H_1 is initial 4-Hyp amounts of collagen sample and H_2 is the 4-Hyp amounts of enzyme degraded collagen. Higher amount of release 4-Hyp value indicate more collagen molecules degraded with the enzyme solution.

2.8. Statistical analysis

All data from this experiment were analyzed by a GLM program and Duncans new multiple range tests contained in the SAS system (2000).

3. Results and discussion

3.1. Amino acid composition

The amino acid composition directly influenced the collagen's physical–chemical properties (e.g., solubility in NaCl solution, cross-linking ability and thermal stability). The analysis of amino acid content in collagens indicated that the molecular structure of species

is different and therefore results in a different structure (Table 1). Glycine was the most abundant amino acid in all collagen species and the amount was approximately 40%. BF collagen had the lowest amount of glycine (382.1/1000 residues) in this study. Although all collagen species contained trace amounts of tyrosine, which was an indicator of telopeptide remained. These values were in an acceptable range (1.1–5.9/1000 residues) of that reported in the literature (Angele et al., 2004; Li et al., 2004; Nomura et al., 1997). The BF collagen proportionally contained higher glutamic acid and aspartic acid. SS collagen revealed the lowest proline and hydroxyproline values of all collagen species evaluated. In previous studies, difference in collagen denaturation temperatures have been correlated with proline (Pro) and hydroxyproline (Hyp) content which are believed to play a substantial role in the stabilization of the triple helix due to the non-covalent bonding of their pyrrolidine ring (Jose & Harrington, 1964). Therefore, the higher value of Pro + Hyp contributed to a higher thermal stability and is discussed later.

3.2. SDS-PAGE

The SDS-PAGE and the profiles of all species collagen contained two main α-chains such as α-1 and α-2 (Fig. 1). Therefore, our results also indicated that type I collagen is a major component in collagen extracted from these species. SDS-PAGE profile of FS collagen exhibited higher concentration of low molecular weight protein fragments. The literature also indicates that frog collagen was more easily degraded by pepsin (Li et al., 2004) or had higher protease sensitivity than mammalian collagens (Purna & Babu, 2001). In this study, it was also found that 1% pepsin in 0.5 M acetic acid incubated for 24 h is optimal to produce telopeptide-poor collagen for all species except for FS collagen due to excess hydrolysis.

Table 1
Amino acid composition of type I collagen from different animal species[a]

Composition	Residues/1000 residues				
	BF	BS	FS	PS	SS
Aspartic acid	57.1	40.3	34.3	34.6	29.3
Threonine	16.0	10.9	14.9	12.0	12.5
Serine	6.2	2.4	2.1	2.0	1.4
Glutamic acid	46.5	18.4	12.1	9.9	13.4
Proloine	42.9	49.8	49.6	52.0	44.1
Glycine	382.1	411.8	389.0	396.8	402.7
Alanine	127.8	146.6	162.3	153.5	155.0
1/2 Cysteine	1.7	2.4	4.1	3.1	4.3
Valine	14.6	17.1	15.9	20.6	18.3
Methionine	8.0	12.1	11.9	10.2	20.1
Isoleucine	23.9	26.8	32.2	27.8	43.8
Leucine	37.3	37.1	40.3	42.8	34.3
Tyrosine	1.1	2.7	5.9	4.2	2.3
Phenylalanine	12.2	11.7	14.2	13.4	11.8
Histine	9.1	10.1	11.1	12.8	12.1
Lysine	49.3	55.2	54.1	63.6	51.1
Arginine	23.8	26.1	25.2	26.9	26.5
4-Hydroxyproline[b]	136.6	129.1	117.5	125.4	97.8

[a] BF, bird feet; BS, bovine skin; FS, frog skin; PS, pig skin; SS, shark skin.
[b] The 4-hydroxyproline (4-Hyp) contents was measured by Reddy and Enwemeka (1996) method and expressed as μg 4-Hyp per mg dry mass.

3.3. Extractable uronic acid/protein ratio

Purified collagen usually contains traces of non-collagenous proteins and glycosaminoglycan, which are easily soluble in guanidine hydrochloride (Gu-HCl) solution (Angele et al., 2004; Yoshimura et al., 1996). The extractable protein and uronic acid contents were determined by the biuret method and the sulfuric acid-cabazole method, respectively, and used as indicator of non-collagenous protein and glycosaminoglycan. In present study, BF and FS collagen had significant ($p<0.05$) higher extractable protein. However, extractable protein is regarded as an indicator of non-collagenous protein contaminants in collagen production. The histological composition of bird feet (BF) and frog skin (FS) collagen were more complicated than other animal skin collagen (e.g., porcine skin and bovine skin). The frog skin is covered with a firm epidermis that is difficult to remove, and bird feet contained abundant amount of fat and minerals (Liu et al., 2001). Moreover, uronic acid/protein ratio of PS collagen were significantly ($p<0.05$) higher than other animal's collagen (BF, BS, FS and SS collagen) and revealed that the glycosaminoglycan content of PS collagen was higher than in other animal collagen. Glycosaminoglycans are negatively

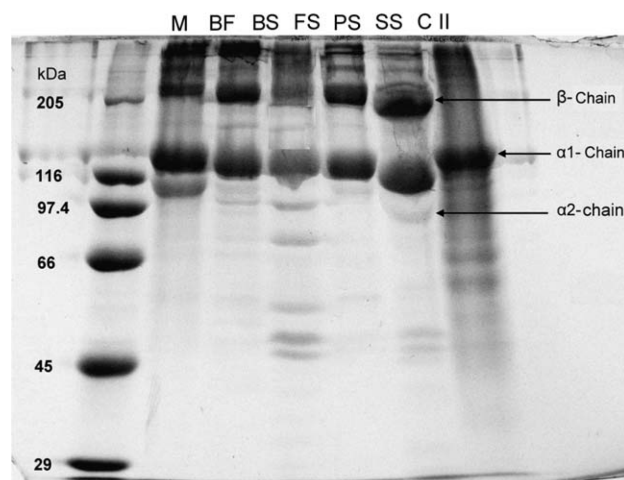

Fig. 1. Electrophretogram (SDS-PAGE) of type I collagen from different animal species (M: Sigma high molecular marker. BF, bird feet; BS, bovine skin; FS, frog skin; PS, pig skin; SS, shark skin; C II, sigma chicken type II collagen).

charged heteropolysaccharides that serve a function in the formation of the matrix to hold together the collagen fiber of connective tissue (Angele et al., 2004; Yoshimura et al., 1996). Therefore, the PS collagen is not only associated with a high degree of glycosaminoglycan but this also affects other physico-chemical properties.

3.4. UV–Vis spectrum

The near UV absorption spectrum of type I collagen can be used to measure its tyrosine content and the integrity of the non-helical telopeptides (Jose & Harrington, 1964; Na, 1988). All species of collagen exhibited a bell shape-like spectrum and displayed a major absorption peak at 218 nm in this study (Fig. 2). The spectrum of BF collagen (190–340 nm) and FS collagen (190–270 nm) were wider than other animal (BS, PS and SS collagen) collagen (190–240 nm). Tyrosine and phenylalanine are sensitive chromophores and absorb UV light at 283 and 251 nm (Doyle & Bello, 1968; Jose & Harrington, 1964). This property is used to express the integrity of the non-helical telopeptides and other protein contaminants when collagen is extracted and purified. The spectra of BF collagen and FS collagen shifted to the far end of the UV spectrum (270–340 nm) and were closely related to their higher proteinous contaminants which were not removed by the same procedures. This hypothesis is also demonstrated by the amount of extractable protein as presented in Table 2.

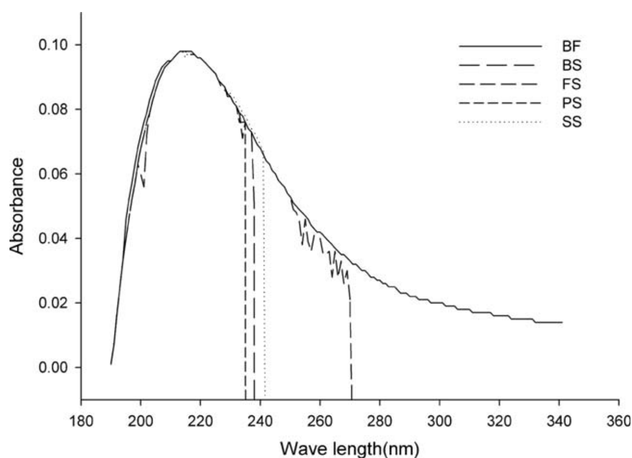

Fig. 2. UV–Vis absorption spectrums of type I collagen form different species.

3.5. Thermal behavior

In order to further examine the relationship between amino acid content and thermal stability, differential scanning calorimetry (DSC) detection was used to study the thermal behavior of the different collagen species. The higher transition temperature indicates that the collagen had higher stability in a high temperature environment. Thermal stability also influences on the durability of the collagen-based biomaterials (Rault, Frei, Herbage, Abdul-Malak, & Huc, 1996). DSC measurements were made to study the thermal behaviors of different species of collagen (Table 3). The thermal transition temperature of type I collagen from different animals displayed a wide range (T_o: 44.70–59.58 °C, T_{p1}: 68.76–88.41 °C, T_{p2}: 69.01–88.77 °C, respectively) and decreased in the order of BF > BS > PS > FS > SS. These results indicated that the BF collagen had the highest value of thermal stability. On the contrary, the aquatic animal (FS and SS) collagen showed a lower transition temperature. Previous studies revealed a correlation between the environmental temperature and amino acid composition at which in exist particular spe-

Table 3
Thermal transition temperatures of type I collagen from different animal species[a]

Species	Thermal transition temperature (°C)[b]		
	T_o	T_{p1}	T_{p2}
BF	59.58	88.41	88.77
BS	56.93	82.11	82.71
FS	52.53	73.93	74.50
PS	48.30	79.31	79.59
SS	44.70	68.76	69.01

[a] BF, bird feet; BS, bovine skin; FS, frog skin; PS, pig skin; SS, shark skin.
[b] Three temperatures were measured: the onset temperature (T_o), the maximal peak 1 (T_{p1}) and the maximal peak 2 (T_{p2}) as characteristics of the denaturation process of different species type I collagen.

Table 2
Extractable protein and uronic acid of type I collagen from different animal species[a]

	Uronic acid (μg/mL)[b]	Protein (mg/mL)	Uronic acid/protein
BF	32.68 ± 3.67^z	6.59 ± 0.68^z	4.96 ± 0.07^y
BS	9.88 ± 1.44^w	2.30 ± 0.05^x	4.29 ± 0.58^x
FS	8.65 ± 0.95^w	3.96 ± 0.06^y	2.18 ± 0.24^w
PS	23.87 ± 1.78^y	1.97 ± 0.13^w	12.11 ± 0.86^z
SS	17.09 ± 1.52^x	2.82 ± 0.01^x	5.92 ± 0.65^y

^{w,x,y,z} Means within the same column without the same superscript are significantly different ($p < 0.05$).
[a] BF, bird feet; BS, bovine skin; FS, frog skin; PS, pig skin; SS, shark skin.
[b] Mean ± standard deviation ($n = 6$ replicates/treatment).

cies. It has been widely known that aquatic collagen has significantly lower denaturation temperature (Li et al., 2004; Nagai et al., 2000; Nomura et al., 2000; Yoshimura et al., 1996). Frog and shark which live in a low temperature, and evolutionary reduced the quantities of Pro + Hyp content of collagen and exhibited a lower thermal transition temperature. According to the distinctive thermal properties of aquatic animal's (FS and SS) collagen, this study also confirms the structural relationship of collagen to the animal's habitat and function of its respective collagen.

3.6. Enzymatic sensitivity

In order to enhance the durability of the collagen-based biomaterials, the cross-linking procedure becomes necessary to improve their biological stability (Angele et al., 2004; Rault et al., 1996). Chemical modification with chemical agents (e.g., glutaraldehyde, formaldehyde and isocyanate) confers remarkably high strength and durability, but also induces potential cytotoxity or poor biocompatibility (Rault et al., 1996). Selection of a higher enzymatic resistance collagen should be an alternative way to reduce the quantity of the cross-linking agent. Collagen degraded products with collagenase, pepsin and trypsin solution were measured to estimate the enzymatic sensitivity of different collagen species.

The release of 4-Hyp amount of all animal collagen species and with enzyme solutions were constantly increased as incubation time increased (Fig. 3). All collagen samples revealed only partial degradation (up to 50%) by collagenase. FS and SS collagen had higher amounts of released 4-Hyp at 48 h (Fig. 2a). But the BF collagen was more easily degraded by collagenese at 72 h and 96 h. The BS and PS collagen seem more resistance to collagenase treatment compared with BF, FF and SS collagens. Collagenase has the unique ability to cleave all three α-chains of type I collagen at a single site, producing fragments about three quarter and one quarter the size of the original molecule. Once the initial cleavage of collagen takes place, the two fragments of collagen are no longer stable at body temperature, the triple helix is lost and the polypeptide chains can be further degraded by other proteases (Shingleton, Hodges, Brick, & Cawston, 1996). However, the PS collagen was more stable in collagenase solution compared to other collagen species. This result could be related to its higher glycosaminoglycan content. Angele et al. (2004) studied the difference between equine and bovine-collagen-based matrix and pointed out that the collagenase cleavage sites could be blocked because of a higher glycosaminoglycan content in equine collagen compared to bovine collagen. In the present study, we also detected the collagen cleavage sites of PS collagen were effectively masked or blocked by high amounts of glycosaminoglycan.

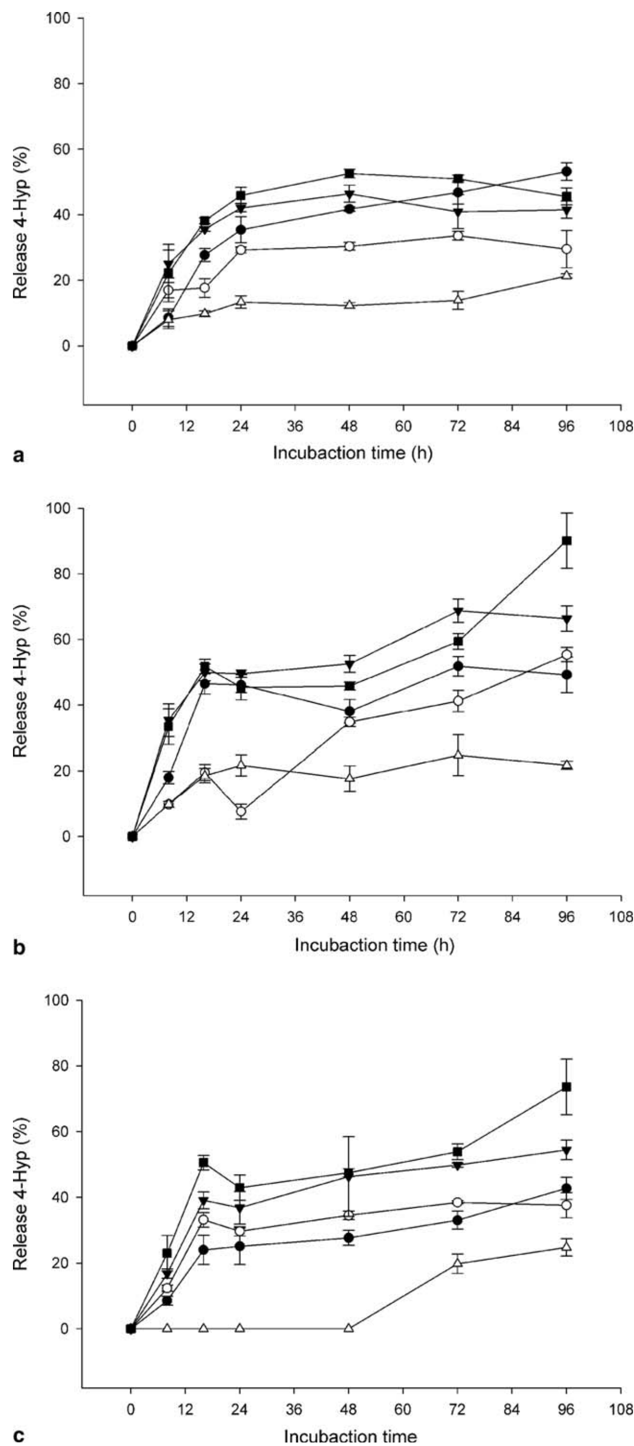

Fig. 3. Enzymatic sensitivity of type I collagen from different animal species (closed circle, BF; open circle, BS; closed triangle, FS; open triangle, PS; closed square, SS) against collagenase (a), pepsin (b), trypsin (c) solution.

To understand the differences in enzymatic sensitivity among the five species of collagen, pepsin and tryspin sensitivities were measured. Trypsin is a non-specific collagenlytic enzyme, but Weadock, Miller, Keuffel, and Dunn (1996) demonstrated that the application of

collagen degradation in trypsin is a useful means of measuring the extent of denaturation. However, these bonds are resistant to cleavage within intact rod-like collagen molecule and these bonds become susceptible to cleavage only after denaturation occurs (Weadock et al., 1996). The degradation effect of pepsin is the same as trypsin, but an confined to acidic condition and also is useful as a meaning index of the degree of denaturation of collagen and collagen-based biomaterials. In the present study, all species of collagen revealed partial degradation by trypsin (up to 90%) and pepsin (up to 73%) which indicates that the land animal collagens (BF, BS and PS collagen) were more stable and significantly resisted proteolysis, therefore, it can be presumed that theses collagens are more lose in the native state than aquatic animal collagen. These results also agreed with those of previous studies (Li et al., 2004; Purna & Babu, 2001; Robinson, 1997; Yoshimura et al., 1996). The aquatic animal collagens are not only easily degraded in the steps used in the preparation procedures, but also more sensitive to non-specific enzymatic hydrolysis (Li et al., 2004; Nomura et al., 1996; Purna & Babu, 2001). This phenomenon may be correlated with thermal stability. The higher thermal stability of purified collagen may also indicate that the preservation is an important element of these structures. On the other hand, previous studies (Li et al., 2004; Nomura et al., 1996; Purna & Babu, 2001) also indicated that aquatic animal collagen were not stable at experimental temperature (37 °C) and sensitive to non-specific enzymatic hydrolysis. However, the PS collagen revealed the lowest enzyme sensitivity among all collagen species. These results were probably caused by to its relatively large amount of glycosaminoglycan content and sterically hindering access to active sites.

4. Conclusion

The amino acid composition directly influenced the thermal stability of different collagen species, the aquatic animal collagen contained lower Pro + Hyp values and displayed a lower thermal stability. The BF collagen had higher Pro + Hyp values and exhibited higher stability. Similar SDS-PAGE profiles were found for different animal collagens, and all samples were composed of two α1-chains and one α2-chain. Moreover, the SDS-PAGE profile of FS collagen exhibited higher amounts of low molecular protein fragment than other species of collagen. All species of collagen had a typical absorption peak at 218 nm, the UV–Vis spectrum of BF collagen and FS collagen shifted to far end of the UV–Vis due to their higher proteinous contaminants. PS collagen contained a relatively large amount of glycosaminoglycan and this could block the cleavage sites for the enzymes. For biostability purposes, the high thermal stability of BF collagen and high enzymatic resistance of PS collagen could be utilized as a suitable material for preparing stable, biocompatible collagen-based biomaterials.

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