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Biochemical properties of bone and scale collagens isolated from the subtropical fish black drum (*Pogonia cromis*) and sheepshead seabream (*Archosargus probatocephalus*)

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Abstract

Acid-soluble collagen (ASC) and pepsin-solubilized collagen (PSC) were isolated from the bones and scales of black drum (*Pogonia cromis*) and sheepshead seabream (*Archosargus probatocephalus*) caught in the Gulf of Mexico. ASC and PSC were analyzed for molecular weight by SDS–PAGE, amino acid composition, secondary structure, and denaturation temperature. The molecular masses of the collagen subunits were about 130 kDa for α_1 and 110 kDa for α_2, respectively. The amino acid composition of the PSCs was closer to that of calf skin ASC than to that of cod skin ASC. The melting temperatures of ASC and PSC were >34 °C. Intrinsic viscosity of the PSCs was similar to the intrinsic viscosity of collagen from fish species such as hake, cod, and catfish. Black drum and sheepshead bone and scale collagens were typical type-I collagens and may find applications in the functional food, cosmetic, biomedical, and pharmaceutical industries.

Keywords: Collagen; Denaturation; Fish; Seafood wastes; Bone; Scale; Thermal stability; Viscosity

1. Introduction

Collagen is the most abundant protein in vertebrates and constitutes about 25% of vertebrate total proteins. Collagen is unique in its ability to form insoluble fibres that have high tensile strength and a right-handed triple superhelical rod consisting of three almost identical polypeptide chains.

Collagen has been, traditionally, isolated from the skins of land-based animals, such as cow and pig. Non-denatured collagens from these sources find applications in cosmetics, biomedical, and pharmaceutical industries. Denatured collagen, known as gelatin, finds applications in the food and biomedical industries. Biomedical and pharmaceutical applications of collagen include the treatment of hypertension, urinary incontinence and pain associated with osteoarthritis, use in tissue engineering for implants in humans, inhibition of angiogenic diseases, such as diabetes complications, obesity, and arthritis (Rehn et al., 2001). In recent years, the outbreak of bovine spongiform encephalopathy (BSE) and the foot-and-mouth disease (FMD) crisis have caused restrictions on collagen trade and the need for alternative safe sources of collagen (Helcke, 2000; Trevitt & Singh, 2003).

The southern states of Louisiana, Mississippi and Florida have abundant coastal and marine resources. Seafood processors in LA, MS, and FL generate more than 3,000,000 pounds of fish skins and millions of pounds of bones annually through their processing of black drum (*Pogonias cromis*) and sheepshead (*Archosargus probatocephalus*) fillets for food services. Due to

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compliance with environmental laws and the growing knowledge of the potential of obtaining health-enhancing products from aquatic sources, interest has grown, among seafood processors across the southern states, in obtaining higher value functional foods, fine biochemicals, and pharmaceuticals, such as antimicrobials, antioxidants, enzymes, proteins, nucleic acids, enzyme inhibitors, cosmetics, and pharmaceuticals from fish, shellfish, and algae underutilized components.

Thermal denaturation temperatures of collagen isolated from the skin of black drum (*P. cromis*) and sheepshead seabream (*A. probatocephalus*), measured by melting point using circular dichroism, gave the following values: black drum (ASC), 34.2 °C; sheepshead ASC, 34.0 °C; black drum PSC, 35.8 °C; sheepshead PSC, 34.3 °C (Ogawa et al., 2003). The literature value for the heat stability of calf skin collagen is 36.3 °C. Black drum and sheepshead have large quantities of calcified tissues, such as bones and scales, in addition to skins. The dry weight ratio of bones and scales in the fish body is higher than that of skin.

Like those of other vertebrates, the bones and scales of fish contain a high amount of collagen and are abundantly available as a byproduct of fish processing operations. There are many reports on thermal stability of fish collagen (Kimura, Zhu, Matsui, Shijoh, & Takamizawa, 1988; Nagai & Suzuki, 2000a; Zhu & Kimura, 1991). However, fish species with reported collagen denaturation temperatures higher than 30 °C, such as e.g. skipjack and carp, are very limited. Most fish collagens, known to date, denature at temperatures below 30 °C, indicating that fish collagen is generally less stable than its mammalian counterparts.

Collagen molecules in solution denature close to the upper limit of the physiological temperature or the maximum body temperature of the animal species from which the collagen is extracted (Privalov, 1982). We hypothesized that fish species that inhabit the tropical and subtropical waters of the Gulf of Mexico may contain collagen with denaturation temperatures above 30 °C.

The objective of this research was to investigate the biochemical properties and thermal stability of black drum and sheepshead seabream bone and scale collagens.

2. Materials and methods

2.1. Materials

Adult black drum (*P. cromis*) and sheepshead seabream (*A. probatocephalus*) were caught in the Gulf of Mexico. The fish were filletted and skinned in a seafood processing plant in Kenner, LA. Scales were removed from the skins. Fish vertebral columns were used as bone materials. Fish vertebral column and scales were collected and kept frozen until used. Pepsin, EC 3.4.23.1, was obtained from Fisher Scientific (Fair Lawn, NJ). Calf skin type-I acid-soluble collagen was purchased from ICN Biochemicals Inc. (Irvine, CA). NuPAGE® Tris-acetate gels (3–8%) and staining and destaining kit were products of Invitrogen (Carlsbad, CA). All other chemicals were of reagent grade.

2.2. Methods

2.2.1. Moisture content

Moisture content was determined by using an OHAUS moisture determination balance Model 6010 (Florham, NJ).

2.2.2. Preparation of acid-soluble collagen

All procedures to prepare acid-soluble collagen (ASC) were carried out at ambient temperature (22–23 °C), except for centrifugation at 4 °C. The bones and scales were soaked in 10 volumes of 0.1 M NaOH for 24 h with stirring, using a magnetic stirrer. The alkaline-treated calcified tissues were re-soaked in 20 volumes of 0.1 M NaOH solution with stirring for 24 h. The alkali-insoluble components were filtered using a cheese-cloth and were rinsed with distilled water repeated until a neutral pH was reached. The insoluble components were extracted with 10 volumes of 0.5 M acetic acid for three days. Thus, the solution was centrifuged at 10,000*g* for 20 min at 4 °C. The residue was re-extracted with 10 volumes of 0.5 M acetic acid for three days and the extract was centrifuged as described above. The supernatants of the two extracts were combined and were salted-out by adding NaCl to give a final concentration of 0.9 mol dm^{-3}. The solution was left overnight; the resulting precipitates were collected by centrifuging at 10,000*g* for 20 min. The precipitates were dissolved in 10 volumes of 0.5 M acetic acid. The procedures of salting-out and solubilization were repeated three times. The resulting solution was dialyzed against 0.1 M acetic acid for further experiments.

2.2.3. Preparation of pepsin-solubilized collagen

All procedures to prepare pepsin-solubilized collagen (PSC) were carried out at ambient temperature (22–23 °C), except for the centrifugation steps which were carried at 4 °C. Bone and scales were treated in alkaline solutions as described above and rinsed with distilled water until a neutral pH value was obtained. The insoluble components were solubilized with 10 volumes of 0.5 M acetic acid containing 0.1% (w/v) pepsin for three days. The solution was centrifuged at 10,000*g* for 20 min at 4 °C. The residue was re-extracted with 10 volumes of 0.5 M acetic acid solution containing 0.1% (w/v) pepsin for three days, and the extract was centrifuged as described above. The supernatants of the two extracts were

combined and were salted-out by adding NaCl to give a final concentration of 0.9 mol dm^{-3}. The solution was left overnight; the resulting precipitates were collected by centrifuging at 10,000*g* for 20 min. The precipitates were dissolved in 10 volumes of 0.5 M acetic acid. The procedures for salting-out and solubilization were repeated three times. The resulting solution was dialyzed against 0.1 M acetic acid.

2.2.4. Circular dichroism

The collagen sample, fully dialyzed against 0.1 M acetic acid solution using a dialysis tube with molecular weight cut off of 12,000–14,000, was diluted to a concentration of 0.3 g dm^{-3} with 0.1 M acetic acid solution. Circular dichroism (CD) measurements were carried out using a AVIV CD spectrometer 62 DS (Lakewood, NJ) calibrated with re-crystallized D-10-camphorsulfonic acid, $[\theta]_{290.5} = 7800$ (deg cm^2 $dmol^{-1}$). The CD spectrum of the sample was taken using a 0.1 cm path-length quartz cell. A mean amino acid residue weight of 91 g mol^{-1} for collagen (Harrington & Von Hippel, 1961) was assumed to determine its molar ellipticity [θ]. The melting curve of collagen was determined by monitoring [θ] at the wavelength of a positive extreme at 220 nm. The $[\theta]_{220}$ was recorded while heating the sample at the rate of 0.5 °C min^{-1} (15–45 °C). To convert the data into plots of the fractional change against temperature, the following equation was used: $F = (Y_{obsd} - Y_U)/(Y_N - Y_U)$, where F is the fraction, Y_{obsd} is the observed molar ellipticity value at 220 nm, and Y_N and Y_U are the values of $[\theta]_{220}$ for native-folded (at 15 °C) and completely-unfolded (at 45 °C) forms, respectively. Transition temperature, $T_m - CV$, was determined as a temperature indicative of a midpoint, namely $F = 0.5$, between the native and unfolded forms.

2.2.5. Viscosity

Collagen samples (0.04 to 0.4 g/dm^3) were prepared in the same manner as the CD sample. A Cannon-Fenske type Kinematic Viscometer tube (Fisher Scientific) with the efflux time for water of about 135 s at 20 °C was employed for the viscosity measurement. Eight ml of the sample solution were incubated for 30 min at 20 °C, and then the efflux time of the solution was measured in the tube. Specific viscosity (η_{sp}) was calculated by the equation $(t - t_0)/t_0$, assuming that the densities of the solution and solvent were the same (t = efflux time of the collagen solution and t_0 = efflux time of the solvent). The reduced viscosity (η_{sp}/c), where c is the protein concentration (g/dl), was plotted against the concentration c. The intrinsic viscosity, [η] dl/g, was given as the intercept of the curve of the equation $\eta_{sp}/c = [\eta] + kc$, where k dl^2/g^2 is the slope.

The thermal denaturation of the collagen was determined from viscosity changes using the same viscometer tube. Eight ml of the collagen solution (0.3 g/dl^3) were incubated for 30 min at a given temperature from 15 to 45 °C, and then its viscosity was determined by measuring the efflux time at the same temperature. The thermal denaturation curve of the collagen solution was determined by plotting the reduced viscosity η_{sp}/c against temperature. The thermal denaturation temperature, $T_d - V$, was expressed as a mid-point temperature between the extrapolated line for native collagen and that for fully denatured collagen on the η_{sp}/c vs. temperature plot.

2.2.6. Sodium dodecyl sulfate–gel electrophoresis (SDS–PAGE)

Electrophoresis was carried out using NuPAGE® Tris-acetate gel (3–8%). Dialyzed samples were heated at 100 °C for 5 min in NuPAGE® LDS sample buffer (Invitrogen) including NuPAGE reducing agent (Invitrogen). The electrophoretic separation was carried out according to the manufacturer's procedure. The gel was stained using the NOVEX® colloidal blue staining kit (Invitrogen) for 8 h with shaking. After staining, the staining solution was decanted and replaced with 400 ml de-ionized water overnight with shaking. Band intensities of the gel were analyzed using Scion Image Version Beta 4.0.2 (Scion Corp., Frederic, MD).

2.2.7. Protein determination

The protein concentration was determined by a Perkin a Elmer Series II Nitrogen Analyzer 2410 (Shelton, CT). The nitrogen–protein conversion factor for collagen is 5.4 (Benedict & Ellis, 1987).

2.2.8. Amino acid composition

Samples were hydrolyzed under vacuum with 6 M HCl at 110 °C for 24 h under argon atmosphere in the presence of phenol. Amino acid analysis was performed using a Hewlett Packard AminoQuant II system (Palo Alto, CA).

3. Results and discussion

3.1. Chemical properties

Fig. 1 shows SDS–PAGE patterns of the isolated collagens. Bone ASC and PSC had at least two different α chains (α_1 and α_2) and their cross-linked chains (Fig. 1(a)). In both species, the molecular mass of bone ASC subunit was about 130 kDa for α_1 and 110 kDa for α_2. The existence of at least two different subunits shows that a major collagen from the fish bones is a type-I collagen. There were no significant differences in subunit molecular mass between bone and scale collagens (Fig. 1(b)). In addition, the electrophoretic patterns and migration of scale and bone collagens were similar to the electrophoretic patterns of collagens isolated from the

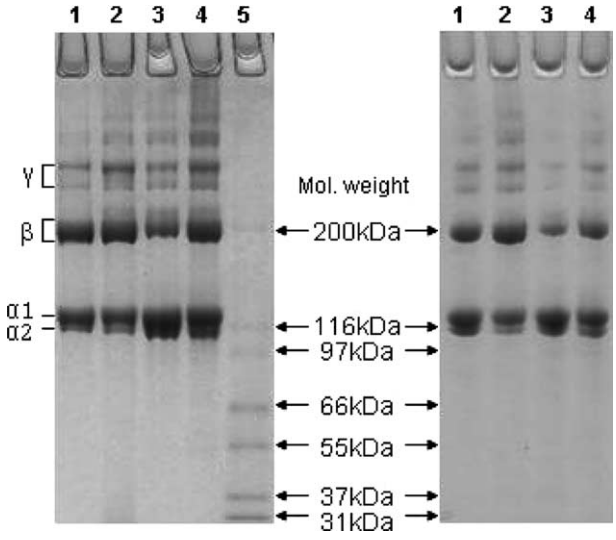

Fig. 1. SDS–PAGE patterns of (a) bone and (b) scale collagens. 1. Black drum ASC; 2. sheepshead ASC; 3. black drum PSC; 4. sheepshead PSC; 5. Molecular weight marker.

skin of the same fish species (Ogawa et al., 2003). Kimura, Miyauchi, and Uchida (1991) reported that soluble collagen from scale and bone of lathyritic carp consisted of two molecular forms, $(\alpha_1)_2\alpha_2$ as a main component and $\alpha_1\alpha_2\alpha_3$ as a minor one. Therefore, the calcified tissues of black drum and sheepshead might contain $\alpha_1\alpha_2\alpha_3$ as a minor component as well. Table 1 shows the band intensity ratio of cross-linked chain (dimer form, β or trimer form, and γ form) to total non-cross-linked monomer chains ($\alpha_1 + \alpha_2$) on the SDS–PAGE gel. In both species, acid-soluble collagen contained higher population of cross-linked components than its pepsin-solubilized counterpart, showing that the intra- and/or inter-molecular cross-linking of collagens, that is, β and γ components, was richer in ASC than in PSC. ASC from black drum scale had a lower β cross-linking rate (0.66) than sheepshead ASCs. ASC and PSC from sheepshead bones and scales had higher cross-linked rates than ASC and PSC from Blackdrum bones and scales.

The amino acid compositions of the pepsin-solubilized collagens (PSCs) are shown in Table 2. Black drum and sheepshead collagens had similar amino acid profiles. The collagens were high in proline (Pro), glycine (Gly), and alanine (Ala), which are characteristic of all collagens. High levels of hydroxylysine (Hyl) and hydroxyproline (Hyp), as observed in collagens from animal species, were measured in the fish collagens. The distribution patterns of amino acid composition were closer, especially for Pro and Hyp, to calf collagen rather than to cod collagen (Herbage, Bouillet, & Bernengo, 1977; Yamaguchi, Lavéty, & Love, 1976). The same amino acid distribution patterns were observed in the collagens isolated from the skin of black drum and sheepshead (Ogawa et al., 2003). The similarity in the electrophoretic migration and amino acid composition suggested that the chemical compositions of type I collagen were highly conserved among the tissues.

Table 2
Amino acid compositions of PSCs

	Black drum		Sheepshead	
	Bone	Scale	Bone	Scale
Hyp	84.6 ± 1.0	87.9 ± 4.8	88.5 ± 5.7	86.4 ± 4.8
Asx	44.4 ± 1.1	41.6 ± 2.7	43.1 ± 1.8	41.5 ± 2.5
Thr	26.6 ± 0.5	25.3 ± 2.0	26.6 ± 2.3	24.8 ± 1.4
Ser	35.5 ± 1.3	37.0 ± 2.8	34.5 ± 3.5	38.2 ± 2.4
Glx	68.7 ± 1.0	63.9 ± 4.1	65.8 ± 3.7	63.5 ± 4.3
Pro	107 ± 2.3	111 ± 9.7	106 ± 1.3	113 ± 3.1
Gly	338 ± 10.5	345 ± 25.9	342 ± 29.2	347 ± 20.2
Ala	130 ± 1.6	124 ± 7.8	129 ± 9.2	124 ± 6.8
Cys	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Val	18.0 ± 0.3	18.6 ± 1.4	17.9 ± 1.3	16.2 ± 3.5
Met	12.4 ± 0.9	11.6 ± 0.8	13.2 ± 0.5	12.2 ± 0.8
Ile	6.3 ± 0.1	6.7 ± 0.6	6.7 ± 0.6	7.0 ± 0.5
Leu	20.0 ± 0.3	18.7 ± 1.2	18.5 ± 1.3	17.7 ± 1.0
Tyr	1.8 ± 0.0	1.7 ± 0.1	1.9 ± 0.1	1.8 ± 0.2
Phe	12.8 ± 0.1	12.4 ± 1.0	12.9 ± 0.9	12.9 ± 0.9
Hyl	8.5 ± 0.5	7.4 ± 0.9	9.1 ± 0.7	8.1 ± 0.5
Lys	26.2 ± 1.7	30.2 ± 2.5	27.7 ± 2.8	29.7 ± 1.8
His	6.3 ± 0.5	5.9 ± 0.3	4.8 ± 0.5	5.4 ± 0.3
Arg	53.6 ± 1.0	51.5 ± 2.8	52.2 ± 3.9	51.0 ± 2.9
Total	1000	1000	1000	1000

The mean ± standard deviation of three determinations for the same sample preparations; Asx = Asp + Asn; Glx = Gln + Glu.

3.2. Secondary structure and collagen thermal behavior

CD spectra of collagen are shown in Fig. 2. Native collagen from bones gave a characteristic CD spectrum with a positive extreme at 220 nm and a negative peak that appeared at 197–199 nm (Fig. 2(a)). Slight devia-

Table 1
Band intensity ratios[a] of cross-linked chain to total monomer chains in collagen

	Bone				Scale			
	Black drum ASC	Sheepshead ASC	Black drum PSC	Sheepshead PSC	Black drum ASC	Sheepshead ASC	Black drum PSC	Sheepshead PSC
$\beta/(\alpha1 + \alpha2)$	1.15 ± 0.11	1.36 ± 0.06	0.49 ± 0.04	0.80 ± 0.05	0.66 ± 0.07	1.29 ± 0.05	0.39 ± 0.12	0.61 ± 0.08
$\gamma/(\alpha1 + \alpha2)$	0.43 ± 0.04	0.65 ± 0.03	0.21 ± 0.03	0.44 ± 0.07	0.32 ± 0.12	0.57 ± 0.18	0.14 ± 0.08	0.27 ± 0.11

[a] The results of two independent collagen isolations.

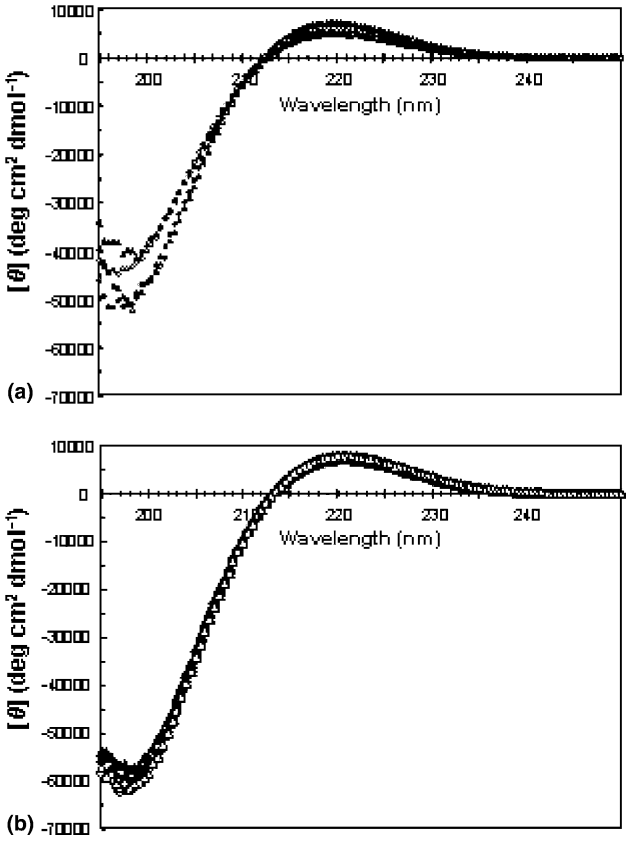

Fig. 2. CD spectra of (a) bone and (b) scale collagens. The spectra were taken at 15 °C. Filled diamond, black drum ASC; unfilled diamond, black drum PSC; filled triangle, sheepshead ASC; unfilled triangle, PSC, sheepshead PSC.

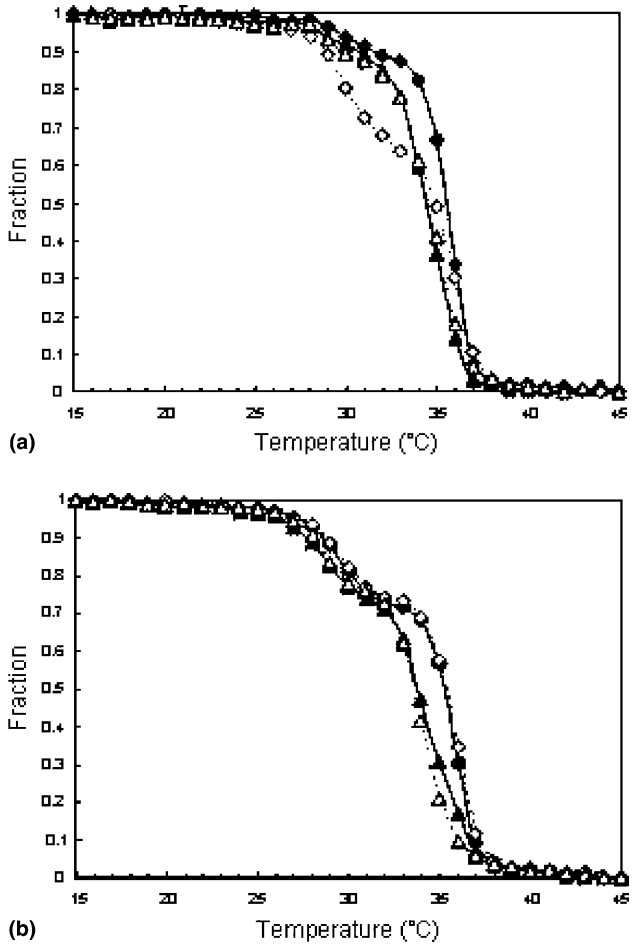

Fig. 3. Melting curves of collagens from (a) bones and (b) scales. The fraction change of $[\theta]_{220}$ was plotted against temperature. Filled diamond with solid line, black drum ASC; unfilled diamond with broken line, black drum PSC; filled triangle with solid line, sheepshead ASC; unfilled triangle with broken line, PSC, sheepshead PSC.

tions in ellipticity were observed among the four collagen species measured, suggesting that there was a minor discrepancy in structure. On the other hand, scale collagens showed similar secondary structures, as seen in Fig. 2(b), denoting a positive extreme at 220 nm and a negative peak at 198 nm. These spectral characteristics in bone and scale collagens are typical of the collagen triple-helix structure (Engel, 1987). Fig. 3 provides the melting curves of the collagen triple-helix. All collagens showed apparently bi-phase thermal transition, as shown by collagens from fish skins (Ogawa et al., 2003) and animal skins (Brown, Farrell, & Wildermuth, 2000; Sato et al., 2000). The existence of bi-phase thermal transitions suggests that those collagens possessed at least two inner domains with diverse stabilities or two different collagen molecules with diverse stabilities. In the transition curve, the contribution of the first transition was less than that of the second transition. The first transitions for bone collagens began at about 28 °C and the first transition for scale collagen occurred at 26 °C (Fig. 3(a) and (b), respectively). In both bone and scale, the second transition started at 33 °C for black drum and 32 °C for sheepshead. The transition was completed at around 38 °C for every collagen species. Thermal behaviour of sardine scale collagen using optical rotation was investigated by Nomura, Sakai, Ishii, and Shirai (1996). The structure of the collagen altered in the temperature range 23–30 °C with a T_m of 27.3 °C; this T_m value was 6–8 °C lower than the T_m of black drum and sheepshead scale collagens.

3.3. Viscosity and its thermal behaviours

One of the physicochemical characteristics of collagen is its high viscosity. The results of the intrinsic viscosity of bone PSCs are given in Table 3. The intrinsic viscosity of black drum collagen was 13.7 dl/g, which was similar to the intrinsic viscosity values reported for cod skin ASC (12.8 dl/g), hake skin ASC (13.7 dl/g), and marine cat-fish muscle (12.7 dl/g) (Ciarlo et al., 1997; Gordon Young & Lorimer, 1960; Rose & Mandal, 1996). On the other hand, sheepshead PSC had a higher viscosity value of 19.1 dl/g. The high viscosity can be accounted for by the high proportion of β- and γ-chains,

Table 3
Intrinsic viscosities of collagens at 20 °C

	[η] (dl/g)	Slope k (dl^2/g^2)
Black drum bone PSC	13.7	216.4
Sheepshead bone PSC	19.1	309.6
Cod skin ASC	12.8[a]	135.8[a]
Hake skin ASC	13.7[b]	ND
Marine cat-fish	12.7[c]	ND

ND, not determined.
[a] Gordon Young and Lorimer (1960).
[b] Ciarlo, Paredi, and Fraga (1997).
[c] Rose and Mandal (1996).

resulting in a higher average molecular weight (Table 1). Thus, the viscosity of bone PSCs was similar to the viscosity of collagens from other parts, such as skin, of the fish. Changes in the viscosity upon heating are shown in Fig. 4. The viscosity started declining at 30 °C. It decreased completely at 40 °C for black drum and 39 °C for sheepshead and remained low above 40 °C. The denaturation temperatures $T_d - V$ were 35.5 and 34.8 °C

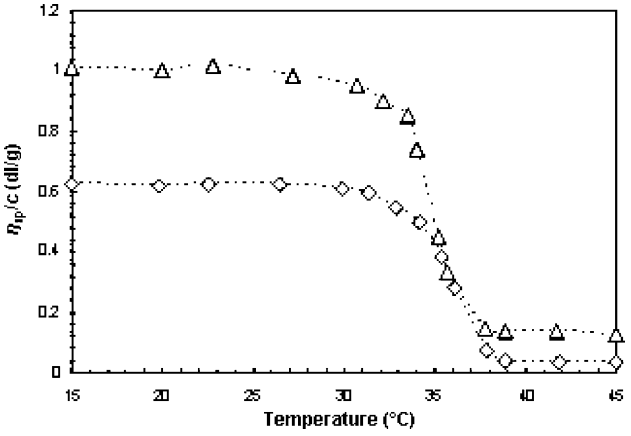

Fig. 4. Thermal denaturation curves of bone PSCs measured by viscosity. Each value was the mean of three determinations. Unfilled diamond with broken line, black drum. Unfilled triangle with broken line, sheepshead.

for black drum and sheepshead, respectively. Those values were much higher than those of bone collagens from other fish species, such as skipjack tuna (29.7 °C) and yellow seabream (29.5 °C) (Nagai & Suzuki, 2000b). The high heat resistance will be favourable for practical applications.

3.4. Correlation between chemical and stability characteristics

In both species of black drum and sheepshead, collagens from the calcified tissues bones and scales had amino acid compositions analogous to those from the skins (Ogawa et al., 2003). The imino acid (Pro + Hyp) content of collagen is closely related to thermostability (Privalov, 1982). Imino acid contents of black drum and sheepshead collagens were relatively high and closer to mammal calf rather than to cod, which inhabit cool-temperature to sub-arctic waters (Table 4). The sub-tropical fish collagens acquired high thermostability ($T_m - CD$, 33.7–35.4 °C) which is comparable to calf skin collagen, owing to the high imino acid content. The $T_m - CD$ value was similar to denaturation temperature $T_d - V$ value (34.8–35.7 °C). This is reasonable because the unfolding of collagen helical structure causes loss of viscosity. So, the two kinds of denaturation temperatures could be compared to each other at the same protein concentration and in the same solvent. Denaturation temperatures of collagen from over 30 marine source collagen (including 25 teleostei sources) were determined by viscosity measurement under the same conditions, that is, in 0.1 M acetic acid as solvent and at a protein concentration of 0.3 g/dm^3 (Kimura et al., 1988; Nagai & Suzuki, 2000b; Zhu & Kimura, 1991). Black drum bone PSC had the highest $T_d - V$ (35.7 °C), followed by sheepshead bone PSC (34.8 °C) for all collagens of marine origin. Judging from the CD melting curve results, the scale collagens are anticipated to have high $T_d - V$ values as well. Thus, the collagens from bones and scales of black drum and sheepshead were

Table 4
Imino acid contents and thermal transition temperatures of collagens

Source of collagen	Imino acids (Pro + Hyp) residues per 1000 residues	$T_m - CD$/°C	$T_d - V$/°C	Physiological temperature[a]/°C
Black drum bone PSC	191	34.9	35.7	15–35
Sheepshead bone PSC	195	34.5	34.8	10–35
Black drum scale PSC	199	35.3	ND	15–35
Sheepshead scale PSC	199	33.6	ND	10–35
Cod (*Gadus morhua*) skin ASC	130	13.0[b]	ND	−0.5–10
Calf skin ASC	215	ND	36.3[c]	37[d]

ND, not determined.
[a] Marine and Coastal Species Information System (1996), Fish and Wildlife Information Exchange, Conservation Management Institute, Virginia Polytechnic Institute and State University. (http://fwie.fw.vt.edu/WWW/macsis/fish.htm).
[b] Burge and Hynes (1959).
[c] Sikorski, Scott, and Buisson (1984).
[d] Body temperature.

found to be quite heat-stable. It is possible to suggest that other fish species in the Gulf coast may also have collagens with high denaturation temperatures, because collagen's thermostability is correlated with the physiological temperature of the fish (Privalov, 1982; Rigby, 1968). The high heat resistances of bone and scale collagens suggest the possibility of using them as substitutes for the land-based animal collagens, about which there are some concerns from consumers and manufacturers.

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