Type V Collagen in Trout (*Salmo gairdneri*) Muscle and Its Solubility Change during Chilled Storage of Muscle

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Post-mortem changes of types I and V collagens in rainbow trout muscle were examined in relation to the softening of fish muscle during chilled storage. Degradation of helical regions of collagens was not detected. On the other hand, the solubility of type V collagen increased significantly, while that of type I collagen did not change. These facts suggest that degradation of nonhelical regions or intermolecular cross-links occur preferentially in type V collagen.

INTRODUCTION

Texture is an important quality factor of fish products, especially for the sliced raw meat (Sashimi) eaten in Japan and other countries. It has been demonstrated that raw fish meat is softened after only 1 day of chilled storage (Hatae et al., 1985; Toyohara and Shimizu, 1988; Montero and Borderias, 1990; Toyohara et al., 1990; Oka et al., 1990; Ando et al., 1991). Collagen, one of the major constituents of fish intramuscular connective tissues, has been demonstrated to play an important role in fish meat texture (Sato et al., 1986; Hatae et al., 1986). In addition, histological studies showed that pericellular connective tissues are degraded more intensively during chilled storage than interstitial connective tissues (Bremner and Hallett, 1985, 1986; Hallett and Bremner, 1988; Ando et al., 1991). It is now well accepted that genetically and chemically distinct collagens (types) exist within the same individual (Bornstein and Sage, 1980). A tissue-specific localization of distinct collagen types has been demonstrated in skeletal muscle (Duance et al., 1977; Bailey et al., 1979).

Type-specific degradation of collagen may occur during chilled storage of fish muscle, causing tissue-specific degradation of intramuscular connective tissues and consequently softening of the fish muscle. However, post-mortem changes in quantitatively minor collagen types, such as type V, have not been examined in fish muscle nor in avian and mammalian muscles.

Recently, Sato et al. (1988, 1989a) demonstrated the presence of types I and V collagens in the muscle of many fish species. On the other hand, Montero et al. (1990) detected only type I collagen in trout (*Salmo irideus*) muscle.

In this paper, the presence of types I and V collagens in rainbow trout (*Salmo gairdneri*) muscle was determined, and degradation of the type V collagen during chilled storage of rainbow trout muscle was investigated.

MATERIALS AND METHODS

Samples. Live rainbow trout (*S. gairdneri*), weighing 930 g (mean), were collected from a local fish farmer. The white muscle was excised from the dorsal part of trunk and sliced into pieces 10 mm thick. The sliced muscle was divided into three groups. One was used immediately for the chemical and physical analyses, and the others were wrapped in Saran film and stored at 5 °C for 1 and 3 days and analyzed.

Preparation of Collagens. Acid-soluble (ASC), pepsin-solubilized (PSC), and insoluble collage (ISC) fractions were prepared by a method that involved a preliminary extraction with a cold 0.1 N NaOH solution to remove noncollagenous proteins (Sato et al., 1988). The preliminary NaOH extraction did not denature or solubilize the collagen and also excluded the effect of endogenous proteases on collagen during preparation (Sato et al., 1987).

Preparation of types I and V collagens from PSC was performed according to the method of Niyibizi et al. (1984) with a slight modification. The outline of preparation procedures is illustrated in Figure 1. Human type V collagen isolated from pepsin digests of placenta was purchased from Sigma (St. Louis, MO).

Electrophoresis. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) was performed according to the method of Laemmli (1970) using 5% gel. The gel was developed by using Coomassie Brilliant Blue R-250.

Determination of Collagens. Types I and V collagen contents in ASC, PSC, and ISC fractions were determined according to the method described previously (Sato et al., 1989b). A factor of 11.42 was used to convert the amount of hydroxyproline to total collagen. A factor of 1.7 was used to convert the percentage of densitometric area of α1(V) and α3(V) bands on the gel to type V collagen. The value was expressed as the average of three determinations ± standard deviations (SD).

Measurement of Muscle Firmness. The firmness of muscle was determined by testing its resistance to penetration by a Rheometer RUD-J (Fudoh Kogyo Ltd.). The probe (5 mm in diameter) penetrated parallel to the muscle fiber at a speed of 1 mm/s. The firmness value (g) was expressed as the average of 6–10 measurements ± SD.

Statistical Analyses. The significance of the differences between the means of muscle firmness, collagen content, and collagen solubility were determined by t-test.

RESULTS AND DISCUSSION

Collagen Types in Rainbow Trout Muscle. The electrophoretic patterns of types I and V collagen fractions (Figure 2a,b) were typical for fish type I (Kimura et al., 1987) and type V collagens (Sato et al., 1988, 1989a). Three distinct bands with mobility corresponding to α1(V), α3-(V), and α2(V) chains of human type V collagen (Figure 2c) were observed in the type V collagen fraction.

From the precipitation properties in sodium chloride and ammonium sulfate solutions and from the electrophoretic patterns, it can be concluded that type V collagen existed in rainbow trout muscle with type I collagen. Montero et al. (1990) did not find type V collagen because they used 2.4 M NaCl in Tris-HCl buffer, pH 7.4, to precipitate the collagen. Under these conditions, we found type V collagen to be soluble (see Figure 1). Type V collagen was present at 35 mg % of dry muscle (Table I).

Post-Mortem Changes in Muscle Firmness. The firmness of rainbow trout muscle decreased significantly ($P < 0.01$) after 1 day of storage at 5 °C (Table I). Similar results have been reported (Hatae et al., 1985; Toyohara and Shimizu, 1988; Montero and Borderias, 1990; Toy-

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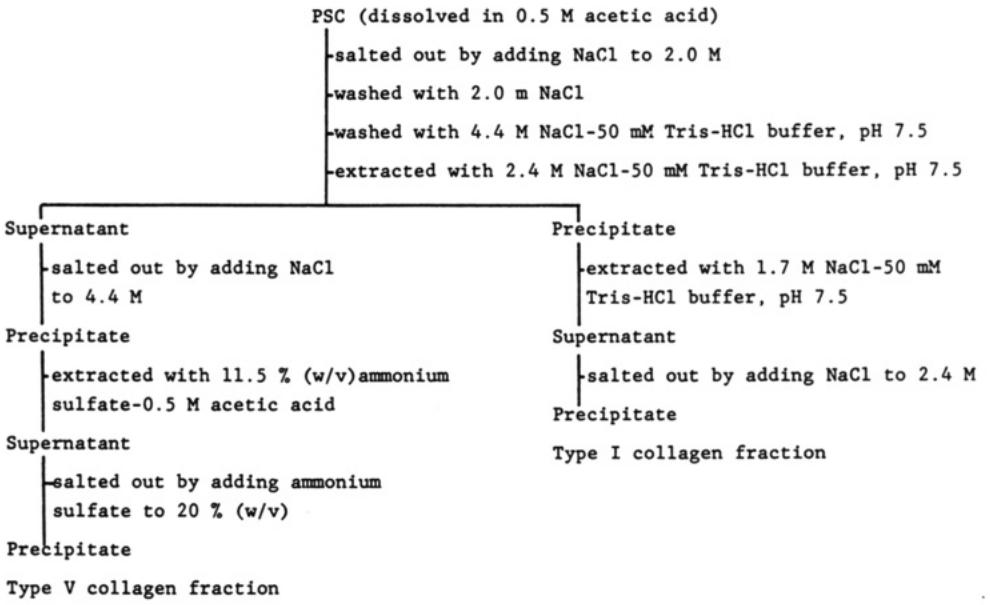

Figure 1. Outline of preparation for types I and V collagen fractions.

Table I. Post-Mortem Changes in Muscle Firmness and Collagens during Storage of Rainbow Trout Muscle at 5 °C[a]

storage time, days	mean hardness,[b] g	collagen content, mg % of dry muscle		solubility of collagens,[c] %					
				type I			type V		
		type I	type V	ASC	PSC	ISC	ASC	PSC	ISC
0	277 ± 34 (6)a	1318 ± 108a	35 ± 6a	64.7 ± 4.0a	22.3 ± 0.6a	13.3 ± 3.0a	19.3 ± 16.8a	38.0 ± 10.8a	43.3 ± 7.6a
1	202 ± 51 (10)b	1451 ± 57a	33 ± 8a	67.0 ± 5.0a	23.0 ± 5.1a	9.0 ± 1.0a	39.3 ± 14.3a	39.0 ± 15.5a	21.7 ± 2.5b
3	165 ± 24 (9)b	1301 ± 97a	51 ± 6a	72.0 ± 3.6a	21.0 ± 3.0a	7.0 ± 1.0a	36.6 ± 4.0a	52.6 ± 5.0b	10.7 ± 1.5c

[a] Different letters in the same column indicate significant differences ($P < 0.01$). [b] Numbers of measurements are given in parentheses. [c] ASC, acid-soluble collagen; PSC, pepsin-solubilized collagen; ISC, insoluble collagen.

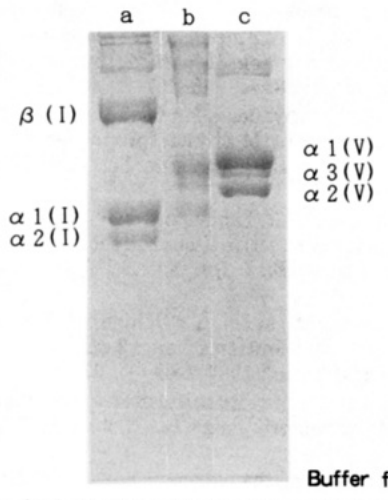

Figure 2. SDS–PAGE patterns of types I (a) and V collagen (b) fractions from rainbow trout muscle compared with that of human type V collagen (c).

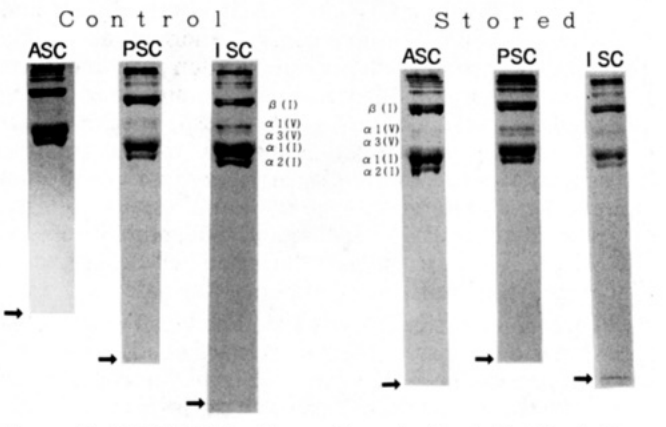

Figure 3. SDS–PAGE patterns of pepsin-digested acid-soluble (ASC), pepsin-solubilized (PSC), and insoluble collagen (ISC) fractions from rainbow trout muscle immediately after death (control) and after 3 days of storage (stored). Arrows show buffer front.

ohara et al., 1990; Oka et al., 1990; Ando et al., 1991). This type of test has been demonstrated to be highly correlated with sensory tests (Hatae et al., 1985; Oka et al., 1990).

When the firmness of sliced raw muscle was evaluated orally and manually, the perception of firmness of all samples decreased after 1 day of storage.

Post-Mortem Changes in Collagens. No significant decrease in collagen content occurred during 3 days (Table I). In addition, no fragment with lower molecular weight than α chain was detected in the collagen fractions, which were digested with pepsin to remove nonhelical regions, by SDS–PAGE after 3 days of storage (see Figure 3). Cleavage of the triple-helical region by collagenase did not occur during 3 days of storage.

The solubility of type I collagen did not change significantly after 1 day of storage, but that of type V collagen increased significantly ($P < 0.01$). After 3 days of storage, the insoluble fraction of type I collagen decreased to approximately half and that of type V collagen decreased to one-fourth. Collagens in tissues are stabilized by covalent cross-links which are formed mainly in nonhelical regions and can be solubilized by cleaving the cross-links or nonhelical regions [see review by Miller (1984)]. In addition, Sato et al. (1987) demonstrated the presence of proteases in fish muscle, which degraded nonhelical regions of collagen and solubilized collagen in vitro. These facts suggest that nonhelical regions may be

degraded preferentially in type V collagen rather than in type I collagen during short chilled storage of trout muscle. There is, however, a possibility that cleavage of cross-links occurred preferentially in type V collagen.

It has been demonstrated by histochemical studies that bovine and chicken type V collagen is distributed mainly in the pericellular connective tissues of muscle (Duance et al., 1977; Bailey et al., 1979). Recently, Sato et al., (1989b) demonstrated that the ratio of type V collagen of type I collagen was higher in the pericellular connective tissue fraction than in the interstitial connective tissue fraction of fish. In addition, it has been demonstrated by transmission electron micrograph that the diameter of the collagen fiber in fish endomysium, which encloses muscle fiber, is thinner than in myocommata, which separate fish muscle into myomers (Hallett and Bremner, 1988). It has been demonstrated that type V collagen forms thinner fibrils than type I collagen in vitro (Adachi and Hayashi, 1986). Together with these studies, it could be suggested that type V collagen may be one of the major constituents of fish pericellular connective tissues such as endomysium and perimysium, which encloses muscle bundle, while type V collagen content in whole muscle was much lower than type I collagen. Then cleavage of nonhelical regions and/or cross-links of type V collagen may weaken pericellular connective tissues and consequently cause disintegration of muscle fibers and muscle softening.

It has been demonstrated in scanning and transmission electron micrographs that fish endomysium, perimysium, and the fine network which connects myomers to myocommata were degraded and thin collagen fibrils in these tissues disappeared during chilled storage. On the other hand, myocommata and thick collagen fibers were still evident (Bremner and Hallett, 1985, 1986; Hallett and Bremner, 1988). More recently, Ando et al. (1991) demonstrated that gradual disintegration of endomysium is mainly responsible for post-mortem muscle softening of rainbow trout. The post-mortem tissue-specific breakdown of fish pericellular connective tissues and the disappearance of thin collagen fibers in these tissues support the preferential degradation of type V collagen demonstrated by the present study. The solubility of type I collagen seemed to increase during chilled storage, while nonsignificant statistical difference was detected.

There is a possibility that type I collagen in specified tissues is degraded, causing muscle softening. However, there is no evidence that can explain why degradation of quantitatively major type I collagen causes specific breakdown of fish pericellular connective tissues.

The increase of type V collagen solubility after 1 day of storage was manifested by a reduction of firmness value. In contrast, biochemical change observed in myofibrillar proteins could not be linked to softening of fish muscle (Hatae et al., 1985; Toyohara and Shimizu, 1988; Azan et al., 1989). In addition, no evident breakdown of muscle fiber has been observed during chilled storage (Bremner and Hallett, 1985, 1986; Hallett and Bremner, 1988). Then degradation of type V collagen may play an important role in fish meat softening during 1 day of chilled storage.

The change of collagen solubility in fish muscle (Montero and Borderias, 1990) during chilled storage was examined in relation to proteolytic activity on collagen. In this study, total collagen solubility increased slightly but significantly after 1 day of storage. The present study is the first to demonstrate that solubility of type V collagen increases preferentially after 1 day of chilled storage of rainbow trout. This might be caused by enzymes with high specificity to type V collagen.

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