カツオ・マグロ類の普通,血合両筋肉の化学的研究

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著者名	加納,哲 鈴木,敏博 前山,薫 武輪,俊彦 渡部,終五 橋本,周久
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Comparative Studies on Ordinary and Dark Muscles of Tuna Fish

Satoshi Kanoh,*1,4 Toshihiro Suzuki,*2 Kaoru Maeyama,*3
Toshihiko Takewa,*1 Shugo Watabe,*1 and Kanehisa Hashimoto*1

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The ordinary and dark muscles of albacore, yellowfin and skipjack tunas were examined for protein composition, along with the contents of myoglobin (Mb), and ATP and related compounds. The stroma protein and Mb contents were around 5 and 10–40 times, respectively, higher in the dark than in the ordinary muscle in the three fishes. The K value, a parameter of freshness, of the dark muscle was 3–14 times higher than that of the ordinary muscle in every analyzed specimen.

The proportions of collagen to elastin in connective tissues were fairly constant regardless of the species and the part of the muscle, 88–98: 2–12.

Tuna fishes contain a large quantity of deep-seated dark muscle in addition to the superficial dark muscle which can be observed in all fishes. Tuna fishes are distinct from other scombrid fishes since the ordinary muscle of the former is clearly red-colored and the adult body size is commonly larger. By these reasons, tuna fishes have been regarded as suitable specimens for comparative studies on the ordinary and dark muscles. Braekkan[1] described that the contents of various vitamins were much higher in the dark than ordinary muscle. There have so far been published many data on the muscle pigment (myoglobin= Mb) from tuna fishes.[2–7] Rather few data are available, however, on their muscle proteins, except for some studies on myofibrillar and myosin ATPases of the tuna.[8–12]

These situations led us to examine for the muscle protein composition, and the contents of Mb, ATP and related substances in both muscles from three tuna fishes. In addition, their connective tissues were assayed for the collagen to elastin ratio.

Materials and Methods

Materials

Fresh specimens of the albacore *Thunnus alalunga* (1.7 kg in average body weight), yellowfin tuna *Neothunnus albacora* (3.2 kg) and skipjack *Katsuwonus pelamis* (2.0 kg) were obtained and kept frozen at −20°C until use not longer than several months. Frozen yellowfin tuna blocks for sashimi were also purchased and used as occasion demanded.

Frozen fishes were partially thawed in a cold room at 2–4°C. After removing the head and caudal fin, each specimen was dissected into three roughly equal body parts at a right angle to the backbone and designated 1st to 3rd part from the head. The ordinary and deep-seated dark muscles on the dorsal side were carefully excised from each part and used for the following analyses. In the present study, the deep-seated dark muscle was used throughout and designated simply "dark muscle", and the superficial one was used only when necessary.

Protein Composition

The ordinary and dark muscle proteins of each tuna fish were separated into sarcoplasmic, myofibrillar, alkali-soluble and stroma fractions by the procedure reported previously,[13] and assayed for nitrogen content by the micro-Kjeldahl method. Some muscle specimens were packed into a polyethylene bag, heated at 60°C for 1 h, and examined for protein composition. The protein fractions separated as above were analyzed by SDS-gel

*1 Laboratory of Marine Biochemistry, Faculty of Agriculture, The University of Tokyo, Bunkyo, Tokyo 113, Japan (加納 哲, 武輪俊彦, 渡部終五, 橋本周久: 東京大学農学部).

*2 Shizuoka Prefectural Technology Center, Makigaya, Shizuoka 421–12, Japan (鈴木敏博: 静岡県工業技術センター).

*3 Faculty of Marine Science and Technology, Tokai University, Shimizu 424, Japan (前山 薫: 東海大学海洋学部).

*4 Present address: Faculty of Fisheries, Mie University, Tsu 514, Japan (現所属 三重大学水産学部).

Table 1. Protein composition of ordinary and dark muscles of three tuna fishes before and after heating

(mgN/g muscle)

Species	Muscle type	Part of body	Non-protein N	Protein N			
				Sarco-plasmic	Myo-fibrillar	Alkali-soluble	Stroma
Albacore	Raw ordinary	1st	6.7	15.3 (46.8)*	16.0 (49.2)	0.4 (1.2)	1.5 (2.9)
		2nd	6.2	15.5 (46.0)	17.0 (50.4)	0.3 (0.9)	0.9 (2.7)
		3rd	8.1	19.0 (51.4)	17.0 (45.9)	0.1 (0.3)	0.9 (2.4)
	Raw dark	1st	3.9	11.0 (40.1)	11.2 (40.9)	1.7 (6.9)	3.5 (12.8)
		2nd	3.5	11.6 (42.1)	10.3 (37.2)	1.6 (5.9)	4.1 (14.9)
	Heated ordinary	2nd	7.5	1.5 (4.9)	0.7 (2.3)	28.5 (92.3)	0.1 (0.3)
	Heated dark	2nd	4.0	2.5 (8.2)	1.5 (4.9)	25.2 (82.4)	1.4 (4.6)
Yellowfin tuna	Raw ordinary	1st	6.7	13.2 (42.2)	17.4 (55.6)	0.2 (0.6)	0.5 (1.5)
		2nd	6.9	13.9 (42.6)	18.2 (55.9)	0.1 (0.2)	0.4 (1.3)
		3rd	6.6	13.2 (40.9)	18.0 (56.0)	0.6 (1.8)	0.4 (1.3)
	Raw dark	1st	3.5	10.5 (44.3)	8.8 (37.2)	1.4 (12.8)	3.0 (6.1)
		2nd	3.5	12.2 (45.4)	11.3 (42.1)	2.7 (10.1)	0.7 (2.4)
		3rd	3.4	11.1 (41.9)	11.7 (44.1)	2.2 (8.3)	1.5 (5.8)
	Heated ordinary	2nd	7.5	1.6 (4.5)	1.0 (2.8)	32.9 (92.5)	0.1 (0.2)
	Heated dark	2nd	4.0	2.2 (7.4)	1.3 (4.2)	26.0 (86.5)	0.6 (2.0)
Skipjack	Raw ordinary	1st	8.4	15.5 (45.7)	17.3 (51.0)	0.3 (0.9)	0.8 (2.4)
		2nd	8.2	15.0 (44.2)	18.1 (53.4)	0.2 (0.6)	0.6 (1.8)
		3rd	8.0	15.3 (45.4)	17.9 (53.1)	0.1 (0.3)	0.4 (1.2)
	Raw dark	1st	4.4	11.9 (37.8)	12.8 (41.6)	5.2 (16.5)	1.6 (5.1)
		2nd	4.4	11.9 (37.0)	15.8 (49.1)	3.0 (9.3)	1.5 (4.7)
		3rd	4.2	12.1 (36.8)	11.9 (36.2)	4.8 (14.5)	4.1 (12.5)
	Heated ordinary	2nd	8.8	1.4 (4.5)	1.5 (4.8)	28.3 (90.1)	0.2 (0.6)
	Heated dark	2nd	5.2	1.6 (5.1)	1.0 (3.2)	27.7 (88.2)	1.1 (3.5)

* Numbers in parenthesis represent percentage distribution.

electrophoresis[14] in 10% polyacrylamide gels containing 0.1% SDS.

Determination of Hemoprotein

Muscle specimen was extracted with 4 volumes of water according to the method reported previously.[15,16] To each extract was added a small amount of KCN and $NaNO_2$, in order to change Mb and hemoglobin (Hb) into the cyanmet form and the absorbance of the resulting solution was measured at 540 nm. Subsequently, the sum of both cyanmet-hemoproteins was calculated on the assumption that the molecular weight and molecular extinction coefficient of Mb (or average subunit of Hb) were 16,400 and 11,300,[17] respectively. A portion of the cyanmet-hemoprotein solution was applied to high-speed gel filtration on a TSK-GEL G3000SW column (7.5×600 mm) with a Toyo Soda HLC-803D high performance liquid chromatograph equilibrated with 0.2 M phosphate buffer (pH 7.0) containing 0.01% each of KCN and $NaNO_2$. The proportions of both hemoproteins were determined by monitoring at 420 nm.

Determination of ATP and Related Compounds

ATP and related compounds were extracted with 10% perchloric acid from each muscle specimen according to the method of Ehira *et al.*[18] After neutralization with a KOH solution, the extract was centrifuged and the resulting supernatant was subjected to the same high performance liquid chromatography (HPLC) as mentioned above. A TSK-GEL ODS-120A column (4.0×300 mm) and a TSK-GEL DEAE-2SW column (4.0×250 mm) were connected to each other and used. HPLC was run with a linear gradient using 0.05 M phosphate buffer (pH 6.5 or 6.0) containing 5% methanol or 0.5% acetonitrile, and 0.3 M or 0.5 M phosphate buffer (pH 3.0) containing 20% acetonitrile as shown in Table 3. The contents of ATP and related compounds were determined by absorbance at 260 nm.

Determination of Collagen and Elastin

Connective tissues were obtained from the ordinary and dark muscles, and also from the boundary portion between both muscles. Each material was homogenized with an enough volume of 0.02 M sodium phosphate buffer (pH 7.4) containing 0.2 M NaCl in a Waring-type blender equipped with a buffle. The connective tissue was collected as flocks by filtering the homogenate through a 1.5-mm mesh wire screen. The connective tissue fibers thus obtained were fractionated into collagen and elastin by the method of Davis and Mackle.[19] One portion of each protein fraction was assayed for dry matter weight. Another portion of each fraction was hydrolyzed in 6 N HCl in a sealed evaculated tube, at 110°C for 24 h, and analyzed for amino acids with a Hitachi 835 amino acid analyzer. Desmosine and isodesmosine were purchased from Wako Pure Chemical Co., Osaka.

Results and Discussion

Protein Composition

The protein compositions of the ordinary and dark muscles from the three fishes are shown in Table 1 and Fig. 1. Their protein compositions roughly resembled each other: Sarcoplasmic and myofibrillar protein fractions were 41–51 and 46–56% respectively in the ordinary muscle, and 37–45 and 36–49% respectively in the dark muscle. Proportions of both protein fractions were somewhat higher in the ordinary than the dark muscle. It is worth to mention that stroma protein fraction was around 5 times higher in the dark than the ordinary muscle, through the three fishes. When compared in detail, they exhibited some species-specificity in protein composition. For example, the proportion of stroma protein fraction in albacore dark muscle was particularly higher (13–15%) than those of the two other fishes or the teleosts so far reported (around 3%).[13,20] The ratio in albacore dark muscle was rather similar to those of shark ordinary muscle (7–12%)[21,22] and of rabbit fast muscle (17–18%).[23] There were found some differences depending on body part: The proportion of stroma protein fraction from the 3rd part of skipjack dark muscle was 12.5%, which was clearly higher than those of the other parts (around 5%).

The 2nd part muscle of each fish was heated at 60°C for 1 h and determined for protein composition. The results obtained are given in Table 1 and Fig. 1, along with those of the corresponding raw muscle. The sarcoplasmic and myofibrillar protein fractions markedly decreased by the heat treatment through the three fishes, and alkali-soluble protein fraction sharply increased instead. The increment of this fraction was about 100 times with the ordinary muscle, whereas around 10 times with the dark muscle.

Each protein fraction from both muscles of albacore 2nd part gave rise to SDS-gel electropherograms in Fig. 2. A thick band, presumably

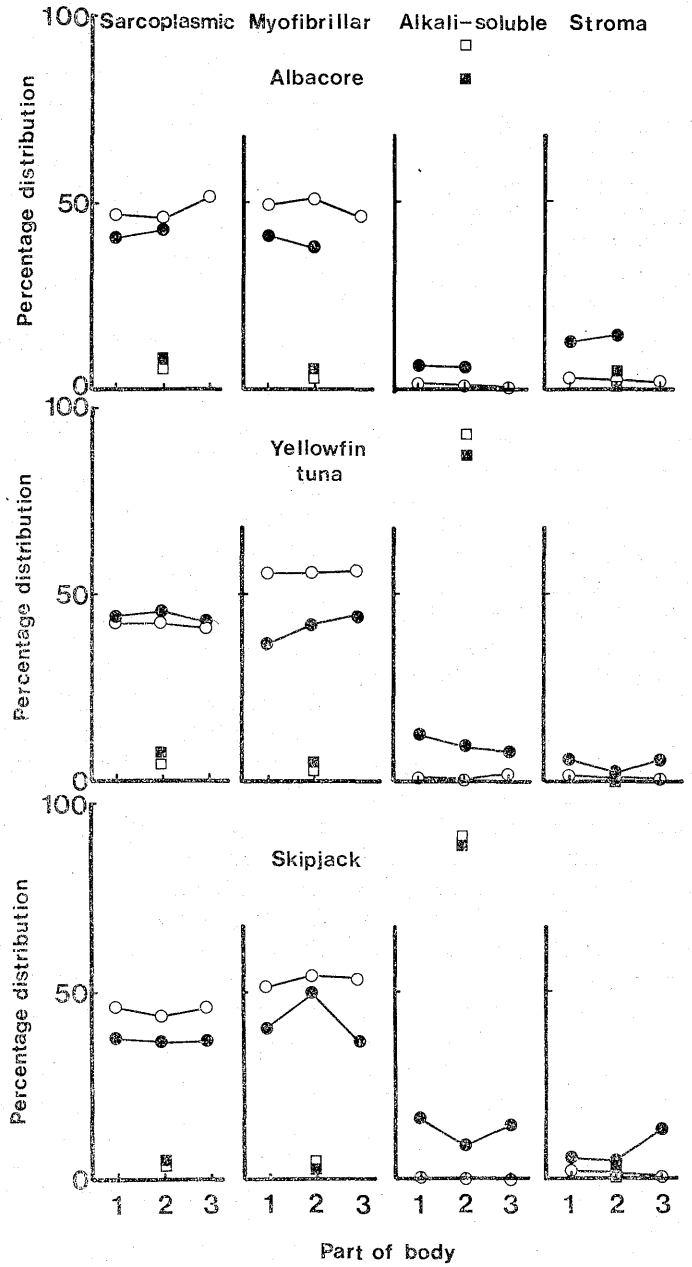

Fig. 1. Protein composition of ordinary and dark muscles of three tuna fishes before and after heating. Symbols are: raw ordinary (○—○), raw dark (●—●), heated ordinary (□), and heated dark muscles (■).

Mb, appeared in the case of sarcoplasmic protein fraction irrespective of muscle type. In the case of myofibrillar protein fraction from either muscle, myosin heavy chain and actin were clearly detected. These electrophoretic patterns resembled those of sardine and mackerel.[18] As shown in the lower part of Fig. 2, dense protein bands newly appeared when the alkali-soluble protein fraction from the heat-treated muscle was electrophoresed. Those bands may have originated from myofibrillar proteins.

Hemoprotein

Hemoprotein contents in both muscles of albacore, yellowfin tuna, and skipjack were determined by HPLC. As shown in Table 2, Mb

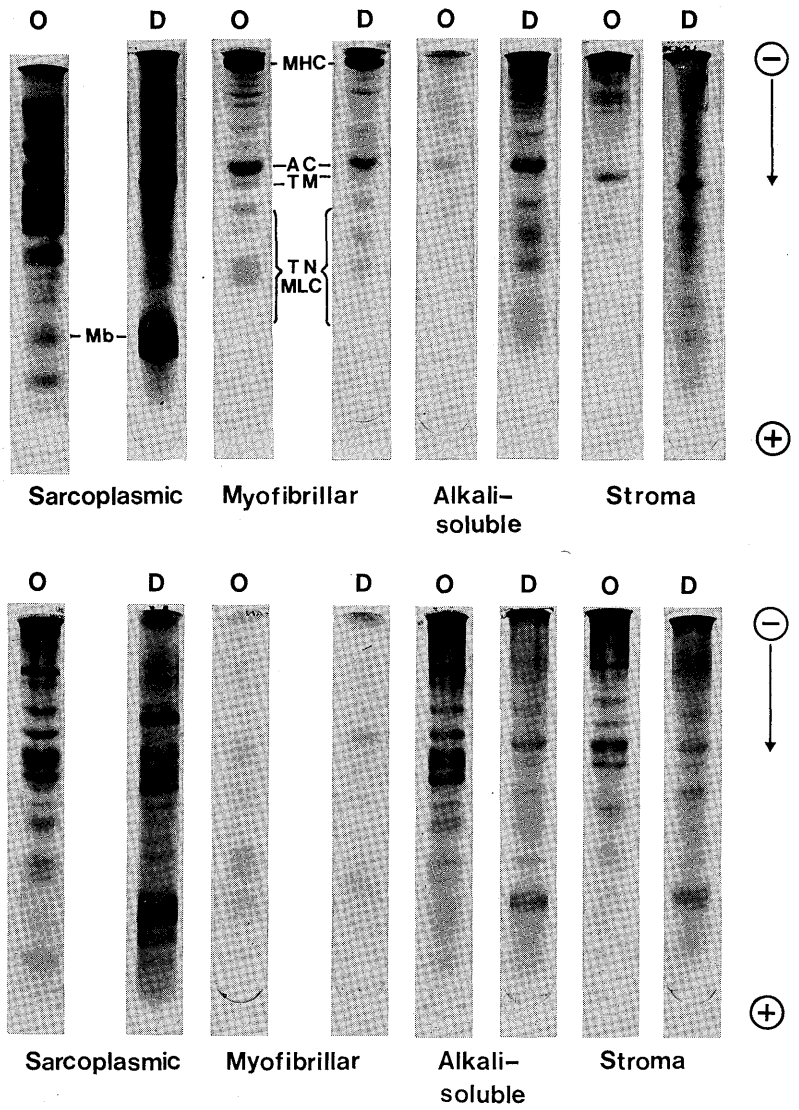

Fig. 2. SDS-gel electrophoretic patterns of four protein fractions of raw (upper) and heated (lower) albacore 2nd-part muscle (10% gel). The amount applied to a column was 30 μl sarcoplasmic, 150 μl myofibrillar, 120 μl alkali-soluble and 50 μl stroma protein fraction from ordinary and dark muscles. In heated ordinary and dark muscles, in the above order, 80 μl, 150 μl, 40 μl, and 100 μl, respectively. Heated sarcoplasmic and myofibrillar fractions were concentrated before application.

Abbreviations: O=ordinary muscle; D=dark muscle; Mb=myoglobin; MHC=myosin heavy chain; AC=actin; TM=tropomyosin; TN=troponin; MLC=myosin light chain.

content in each muscle did not differ so widely among body parts, nor among fish species. The dark muscle of albacore, yellowfin tuna, and skipjack contained 24–46, 24–44 and 8–12 times as much Mb as the ordinary muscle, respectively, in a rough agreement with the data in literature.[7,15]

A small amount of Hb was detected in the superficial dark muscle of yellowfin tuna.

ATP and Related Compounds

ATP and its related compounds in both muscles of the three fishes were determined by HPLC, and the results are summarized in Table 3. The contents of these compounds did not differ significantly depending on body part. The total amount of ATP and related compounds was somewhat higher in the ordinary (12–16 μmol/g)

Table 2. Distribution of hemoproteins in ordinary and dark muscles of three tuna fishes

(mg/g)

	Muscle type	Hemoprotein	Part of body			
			1	2	3	Av.
Albacore	Ordinary	Mb	0.6	0.4	0.6	0.5
	Dark	Mb	14.8	18.2	14.6	16.0
Yellowfin tuna	Ordinary	Mb	0.5	0.7	0.7	0.6
	Dark (superficial)	Hb	0.7	0.8	0.2	0.6
		Mb	21.4	17.5	9.9	16.3
	Dark	Mb	22.3	23.6	24.2	23.4
Skipjack	Ordinary	Mb	2.2	2.1	1.6	2.0
	Dark (superficial)	Mb	16.8	17.8	—	17.3
	Dark	Mb	16.5	17.2	19.0	17.6

Table 3. ATP and its related compounds in ordinary and dark muscles of three tuna fishes

(μmol/g)

Species	Muscle type	Part of body	ATP	ADP	AMP	IMP	HxR	Hx	Total	K value*
Albacore	Ordinary	1	0.06	0.13	0.68	10.62	1.25	—	12.74	9.7
		2	0.04	0.14	0.77	12.19	—	—	13.14	5.0
		3	0.05	0.18	0.31	12.70	0.93	—	13.70	6.2
	Dark	1	0.17	0.11	0.37	4.05	5.55	0.92	11.17	57.9
		2	0.08	0.10	0.30	2.73	5.63	1.24	10.08	67.9
		3	0.07	0.07	0.14	2.81	3.72	0.53	7.34	57.9
Yellowfin tuna	Ordinary	1	1.46	0.44	0.25	11.49	1.02	0.29	14.95	8.8
		2	0.52	0.33	0.29	11.77	0.92	0.13	13.96	7.5
		3	1.77	0.51	0.52	11.84	1.13	0 48	16.20	9.6
	Dark	1	1.99	0.37	—	4.07	3.91	0.43	10.77	40.3
		2	0.23	0.51	—	5.10	3.88	0.50	10.22	42.9
		3	1.72	0.59	—	4.66	3.73	0.75	11.45	39.1
Skipjack	Ordinary	1	1.27	0.31	—	7.05	5.99	0.47	14.69	41.3
		2	1.86	0.35	—	8.40	3.99	0.25	14.85	28.6
		3	0.86	0.23	—	7.62	3.20	0.21	11.62	29.3
	Dark	1	0.96	0.22	0.12	0.37	7.25	0.69	9.61	82.5
		2	1.00	0.30	0.26	1.03	7.62	0.68	10.89	76.2
		3	0.76	0.22	0.27	0.63	7.16	0.64	9.68	80.6

Column, ODS-120A+DEAE-2SW; flow rate, 0.6 ml/min.

Eluant for albacore,

A: 5% MeOH/0.05 M phosphate buffer (pH 6.5)

B: 20% CH_3CN/0.5 M phosphate buffer (pH 3.0)

5 min 45 min 5 min

A——→A——→B——→A

Eluant for yellowfin tuna and skipjack,

A: 0.5% CH_3CN/0.05 M phosphate buffer (pH 6.0)

B: 20% CH_3CN/0.3 M phosphate buffer (pH 3.0)

5 min 30 min 28 min 2 min

A——→A——→B——→B——→A

* K value (%) $= \dfrac{\text{Inosine (HxR)} + \text{hypoxanthine (Hx)}}{\text{ATP} + \text{ADP} + \text{AMP} + \text{IMP} + \text{HxR} + \text{Hx}}$

than dark muscle (7–11 μmol/g).

In the ordinary muscle of albacore, IMP showed the highest content (11–13 μmol/g) among ATP and related compounds. In this muscle, ATP content was extremely low (0.05 μmol/g), and hypoxanthine was hardly detected. On the other hand, inosine content was highest (4–6 μmol/g) and hypoxanthine was found to be about 1 μmol/g in the dark muscle of albacore. The IMP content (3–4 μmol/g) was much lower than that in the

Table 4. Collagen and elastin contents of the connective tissue of ordinary and dark muscles and the boundary portion of three fishes

(%)

Tissue	Albacore		Yellowfin tuna		Skipjack	
	Collagen	Elastin	Collagen	Elastin	Collagen	Elastin
Ordinary	98.0	2.0	97.4	2.6	90.8	9.2
Dark	97.4	2.6	94.1	5.9	87.6	12.4
Boundary portion	96.4	3.6	91.9	8.1	91.5	8.5

ordinary muscle. Consequently, K value, a parameter of freshness,* of albacore ordinary muscle was calculated to be 5–10%, which was around 1/10 the K value of the dark muscle (60–70%). Roughly comparable data were obtained with yellowfin tuna and skipjack. K value of the dark muscle of yellowfin tuna was 40% and considerably higher than that of the ordinary muscle (8–10%), as was the case with albacore. In the case of skipjack, on the other hand, even the ordinary muscle exhibited a K value of 30–40%, whereas the dark muscle showed a value of 80%. In this connection, ATP contents of the ordinary and dark muscles from yellowfin tuna and skipjack were around 1 μmol/g, which was clearly higher than those of both albacore muscles (0.1–0.2 μmol/g). Saito *et al.*[24] found that the decomposition rate of ATP in rainbow trout or mackerel dark muscle was higher than that of their ordinary muscle. Though ATP decomposition pattern no doubt depends on the freshness of sample specimen, the results obtained here suggested that the pattern differed from one another even among these tuna fishes.

Collagen and Elastin

Collagen and elastin contents in the connective tissues of the tuna fishes are shown in Table 4. Collagen accounted for 88–98% of those connective tissues. Its content was somewhat higher in the ordinary than in the dark muscle, irrespective of the fish species. However, the elastin content of either muscle was highest in skipjack among the three fishes.

Fish,[25] as well as mammalian,[26] collagens are featured by high contents of some amino acids: Glycine residues account for about one third the whole residues, and the contents of imino acids such as proline and hydroxyproline are also high. Furthermore this protein contains hydroxylysine rather specifically. As shown in Table 5, amino acid compositions of the collagens from three tuna fishes resembled each other inrespective of muscle type. The tuna fish collagens differ from human aorta[19] and sheep jugular vein[19] collagens by lower hydroxyproline and higher proline contents, and resemble carp muscle collagen.[25]

Mammalian elastins are featured by high contents of glycine and proline, the former accounting for about 1/3 the total amino acid residues.[19,26–28] These two amino acids, along with alanine and valine, account for 70–80% of the total residues. In addition, mammalian elastins specifically contain two cross-linking amino acids, desmosine and isodesmosine.

Amino acid compositions of the tuna fish elastins are shown in Table 6. The total of glycine, proline, alanine and valine contents was only 28–44%, clearly differing from those of the arterial wall of the trout *Salmo gairdneri* and Atlantic salmon *Salmo salar* (71–72%).[29] It was noteworthy that tuna fish elastins showed high cysteine contents, though they were hydrolyzed without performic acid oxidation. Trace to small amounts of desmosine and isodesmosine were detected in all our elastins.

It was also noted that albacore elastins contained some hydroxylysine, whereas yellowfin tuna or skipjack elastin did not at all.

Incidentally, the collagen from the boundary portion of each fish showed an amino acid composition similar to those of both muscle collagens. However, this was not the case with elastin. For example, the boundary portion elastin of albacore clearly differed from both muscle elastins in lower glycine and proline, and higher glutamic acid and leucine contents. In particular, hydroxyproline was not detected at all in the boundary portion elastin, whereas both muscle elastins contained it at a fairly high level.

As described above, the dark muscle of albacore, yellowfin tuna and skipjack contained Mb 10–40 times, and stroma protein 5 times higher than their ordinary muscle. The latter data is con-

* Refer to the footnote in Table 3.

Table 5. Amino acid composition of collagens from ordinary and dark muscles and the boundary portion of three tuna fishes

(No. of residues/1000 residues)

	Albacore			Yellowfin tuna			Skipjack			Carp[25] ordinary muscle	Human[19] aorta	Sheep[19] jugular vein
	Ordinary muscle	Dark muscle	Boundary portion	Ordinary muscle	Dark muscle	Boundary portion	Ordinary muscle	Dark muscle	Boundary portion			
Asp	45.1	43.9	50.9	35.5	33.3	36.9	43.8	44.2	42.3	50.2	47	47.3
Hyp	69.2	75.8	65.7	76.2	80.0	76.0	74.1	75.8	75.8	73.4	118	91.9
Thr	31.2	30.9	35.0	30.8	29.6	31.8	31.8	32.9	32.2	26.4	21	25.1
Ser	38.3	37.7	40.9	37.8	36.2	39.3	33.5	35.5	34.1	33.3	37	31.4
Glu	73.3	72.4	81.5	80.3	75.6	82.3	73.5	74.4	72.5	87.4	76	63.2
Pro	119.6	110.4	99.3	111.5	114.5	104.0	113.0	101.4	107.7	108	95	89.0
Gly	311.6	319.8	295.8	315.2	328.8	307.0	313.5	319.5	323.8	309	347	328.9
Ala	119.6	122.5	117.8	121.6	124.2	118.5	121.4	122.7	123.9	109	77	123.6
Val	22.4	20.7	25.4	21.9	19.4	21.8	21.1	21.1	19.5	24.5	20	46.3
Cys/2	(4.8)	(4.4)	(6.0)	(2.5)	(2.3)	(3.8)	(4.7)	(4.4)	(4.6)	0	3	3.9
Met	16.3	16.5	18.1	15.3	15.1	16.1	17.8	17.4	17.5	14.5	8	4.7
Ile	13.0	12.0	16.5	12.1	10.1	13.3	13.3	12.4	11.7	16.9	17	15.7
Leu	25.7	24.6	31.7	26.7	23.3	28.5	27.5	26.8	25.8	31.3	27	35.2
Tyr	5.5	4.8	8.2	5.7	4.3	6.7	5.6	5.1	4.7	5.6	6	8.7
Phe	14.7	14.1	12.2	15.5	14.9	16.2	15.4	15.3	15.0	14.5	13	17.5
Hyl	7.9	7.6	6.6	8.7	8.8	6.7	7.2	10.2	9.4	5.4	15	3.8
Lys	25.5	24.8	31.1	27.6	24.2	28.8	24.7	21.1	22.0	33.4	24	22.0
His	5.6	5.2	7.7	5.3	4.4	5.7	6.1	5.7	6.0	6.2	7	4.3
Arg	50.6	51.8	49.6	49.8	50.9	56.7	52.0	54.1	51.6	51.0	40	37.3
isoDes*	0	0	0	0	0	0	0	0	0	—	—	—
Des*	0	0	0	0	0	0	0	0	0	—	—	—

* isoDes=isodesmosine; Des=desmosine.

Table 6. Amino acid composition of elastins from ordinary and dark muscles and the boundary portion of three tuna fishes

(No. of residues/1000 residues)

	Albacore			Yellowfin tuna			Skipjack			Human[19] aorta	Sheep[19] jugular vein	Sheep[27] vascular tissue
	Ordinary muscle	Dark muscle	Boundary portion	Ordinary muscle	Dark muscle	Boundary portion	Ordinary muscle	Dark muscle	Boundary portion			
Asp	68.6	63.0	79.7	64.4	66.0	52.3	91.3	90.2	91.5	3.8	15.2	1.8
Hyp	25.5	45.9	0	7.9	6.0	8.5	0	0	0	6.9	22.4	0
Thr	46.7	42.2	51.5	52.0	51.0	46.7	53.8	54.2	54.7	11.1	16.6	9.2
Ser	52.4	52.3	57.7	50.3	53.0	45.4	51.7	56.5	56.7	8.2	17.8	9.0
Glu	103.9	98.0	117.6	115.0	134.6	109.4	141.0	128.7	128.3	16.4	25.8	20.4
Pro	78.2	88.2	60.2	70.3	65.1	78.3	40.3	52.4	54.7	113.3	103.0	105.3
Gly	181.3	215.7	143.7	156.6	128.5	185.1	87.1	104.5	102.9	303.1	304.5	241.1
Ala	96.8	98.8	92.7	88.9	91.7	104.2	89.8	91.7	89.1	227.6	214.7	288.7
Val	52.1	42.1	59.7	59.9	55.9	60.7	59.9	59.8	58.8	140.2	118.3	111.7
Cys/2	(17.3)	(13.3)	(17.8)	(27.4)	(17.6)	(17.8)	(12.4)	(14.5)	(16.3)	0	0	
Met	22.1	19.0	22.5	21.9	22.3	18.6	29.5	25.6	25.7	Trace	1.7	—
Ile	31.9	25.3	37.3	38.6	38.2	31.8	48.4	44.0	43.3	23.8	23.2	17.5
Leu	61.7	50.3	78.6	74.7	82.9	74.2	90.0	87.2	85.5	57.5	58.9	57.5
Tyr	25.7	19.8	32.1	33.6	31.7	37.0	31.1	30.0	30.2	21.1	13.9	13.4
Phe	23.4	20.8	29.2	27.7	29.9	26.8	32.7	34.3	33.4	22.9	29.7	34.5
Hyl	3.3	4.6	0	0	0	0	0	0	0	0	0	
Lys	46.3	40.2	55.8	49.9	62.2	46.4	67.5	57.6	57.8	3.8	7.9	30.7
His	17.8	14.0	17.8	15.2	14.8	12.6	22.0	19.0	20.1	Trace	3.0	13.6
Arg	43.9	46.6	45.8	44.9	48.0	42.5	51.6	49.8	50.3	5.9	11.1	6.8
isoDes*1	0.5	Trace	0.4	0.6	0.5	1.0	Trace	Trace	0.3	3.4*2	4.4*3	29.3*2
Des*1	0.4	Trace	Trace	0.3	0.3	0.6	Trace	0.2	0.2	5.6*2	6.2*3	7.2*2

*1 isoDes=isodesmosine; Des=desmosine.

*2 Expressed in lysine equivalents.

*3 Expressed in leucine equivalents.

sistent with the tougher texture of the dark muscle.

On the other hand, the dark muscle of each fish analyzed contained inosine as the major ATP-related compound, in contrast to IMP which the ordinary muscle contained as the major component.

Collagens from both muscles of each fish exhibited similar amino acid profiles. This was not true for elastins. Elastins from the boundary portion situated between both muscles of albacore, and to a less degree, yellowfin tuna, showed an amino acid profile which clearly differed from those of both muscle counterparts.

Further studies are now in progress on the changes in toughness of muscles, as caused by heating.

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References

1) O.R. Braekkan: *Fisk. Dir. Skr. Tekn. Undersok.*, **3**, 1–42 (1959).
2) F. Matsuura and K. Hashimoto: *Bull. Japan. Soc. Sci. Fish.*, **22**, 408–412 (1956).
3) S. Konosu, K. Hashimoto, and F. Matsuura: *Bull. Japan. Soc. Sci. Fish.*, **24**, 563–566 (1958).
4) F. Matsuura and K. Hashimoto: *Bull. Japan. Soc. Sci. Fish.*, **24**, 809–815 (1959).
5) F. Matsuura and K. Hashimoto: *Bull. Japan. Soc. Sci. Fish.*, **27**, 162–165 (1961).
6) F. Matsuura, K. Hashimoto, S. Kikawada, and K. Yamaguchi: *Bull. Japan. Soc. Sci. Fish.*, **28**, 210–216 (1962).
7) N. Suzuki, K. Hashimoto, and F. Matsuura: *Bull. Japan. Soc. Sci. Fish.*, **39**, 35–41 (1973).
8) I. A. Johnston, N. Frearson, and G. Goldspink: *Experientia*, **28**, 713–714 (1972).
9) I. A. Johnston and B. Tota: *Comp. Biochem. Physiol.*, **49B**, 367–373 (1974).
10) E. G. Richards, C.-S. Chung, D. B. Menzel, and H. S. Olcott: *Biochemistry*, **6**, 528–540 (1967).
11) C.-S. Chung, E. G. Richards, and H. S. Olcott: *Biochemistry*, **6**, 3154–3161 (1967).
12) T. Murozuka and K. Arai: *Bull. Japan. Soc. Sci. Fish.*, **42**, 65–70 (1976).
13) K. Hashimoto, S. Watabe, M. Kono, and K. Shiro: *Bull. Japan. Soc. Sci. Fish.*, **45**, 1435–1441 (1979).
14) K. Weber and M. Osborn: *J. Biol. Chem.*, **244**, 4406–4412 (1969).
15) F. Matsuura and K. Hashimoto: *Bull. Japan. Soc. Sci. Fish.*, **20**, 308–312 (1954).
16) K. Hashimoto, K. Yamaguchi, N. Takeda, and K. Ogawa: *Bull. Japan. Soc. Sci. Fish.*, **45**, 1331–1333 (1979).
17) D. L. Drabkin: *Am. J. Med. Sci.*, **209**, 268–270 (1945).
18) S. Ehira, H. Uchiyama, F. Uda, and H. Matsumiya: *Bull. Japan. Soc. Sci. Fish.*, **36**, 491–496 (1970).
19) P. F. Davis and Z. M. Mackle: *Anal. Biochem.*, **115**, 11–17 (1981).
20) Y. Shimizu and W. Simidu: *Bull. Japan. Soc. Sci. Fish.*, **26**, 806–809 (1960).
21) K. Bailey: *Biochem. J.*, **33**, 255–263 (1939).
22) S. Watabe, Y. Ochiai, S. Kanoh, and K. Hashimoto: *Bull. Japan. Soc. Sci. Fish.*, **49**, 265–268 (1983).
23) W. Hasselbach and G. Schneider: *Biochem. Z.*, **321**, 462–475 (1951).
24) T. Saito, K. Arai, and T. Yajima: *Bull. Japan. Soc. Sci. Fish.*, **25**, 573–575 (1959).
25) M. Kubota and S. Kimura: *Hikaku Kagaku*, **21**, 80–85 (1975).
26) S. Seifter and P. M. Gallop: in "The Proteins", 2nd Ed. (ed. by H. Neurath), Vol. 4, Academic Press, New York, 1966, pp. 153–304.
27) P. F. Davis, P. A. Ryan, J. N. Manning, W. E. Stehbens, and S. J. M. Skinner: *Angiology*, **35**, 38–44 (1984).
28) H. Toyoda and K. Uehara: *Hikaku Kagaku*, **21**, 109–127 (1975).
29) A. Serafini-Fracassini, J. M. Field, M. Spina, S. Garbisa, and R. J. Stuart: *J. Ultrastr. Res.*, **65**, 1–12 (1978).