Isolation and Partial Characterization of Two Types of Muscle Collagen in Some Cephalopods

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Collagen from muscle of volador (*Illex coindetii*), pota (*Toradopsis eblanae*), and white octopus (*Eledone cirrhosa*) was characterized in terms of anatomical location, sex, and maturity. Collagen content was higher in arms than in mantle in all three species; there were also significant differences in octopus depending on the age of the individual. Concerning sex, the largest differences in the amount of collagen were found in relation to total protein content. In volador and pota, collagen solubility was higher in the mantle than in the arms, and in the case of pota there were also sex-related differences. In octopus males, solubility was higher in the arms. Two types of collagen, I and V, were identified as the principal constituents in all three species and at both anatomical locations (mantle and arms). The electrophoretic mobility of the α2 chain differed in the two types of collagen, but the amino acid compositions of the collagen were similar in the mantle and arms in all three species examined.

Keywords: *Cephalopods; collagen; solubility; types; amino acid composition*

INTRODUCTION

Collagen, one of the major constituents of intramuscular connective tissue, has been shown to exist in different genetic forms. In fish species, collagen has been shown to play an important role in maintaining the structure of muscle in association with swimming movement and meat texture (Yoshinaka et al., 1988; Kuo et al., 1990, 1991; Montero and Borderías, 1989). The collagen content of fish and seafood depends on the species, feeding regime, and state of maturity of the fish. In general, fish muscle contains ∼0.2−2.2% collagen in the case of teleosts and up to 10% in elasmobranchs (Sikorski et al., 1984). Although higher collagen content contributes to the toughness of muscles, no such problems are encountered in fish. Fish connective tissue contributes little, if any, to the eating texture of cooked fish. However, some species of squid may develop a tough, rubbery texture upon heat processing. Mizuta et al. (1994c) proposed the following three characteristics as collagen-related factors determining texture: (1) total collagen content, (2) distribution or morphology of collagen fibers in muscle, and (3) content of a specific collagenous component.

Moreover, cephalopods are fast-growing, reaching sexual maturity in one or two years (Guerra, 1992). Such a short life cycle implies a high rate of protein replacement. The shortness of the time in which females reach sexual maturity can produce considerable changes in the muscle proteins (Mangold, 1987). The thickness of the connective tissue depends on species as well as on age, sexual maturation, and muscle depletion.

Another consideration is the possible influence of specific living patterns. Thus, squid (volador and pota) are very active throughout their lifetimes, whereas octopus are highly sedentary, rousing themselves only to pursue their prey. All of this may considerably influence their composition and suitability for processing. The function of each of the anatomical regions of the live organisms can have a considerable effect on the amount of collagen present and its degree of aggregation. The arms perform a grasping function and therefore have to withstand more strain than the mantle. The squid mantle needs to be more elastic to perform its propulsive function, whereas the octopus is sedentary and moves by means of its tentacles.

The structure of the collagen fibrils must evidently influence the physical properties of the tissue, mainly solubility and texture.

The study of the biochemical characteristics of fish collagen such as amino acid composition, collagen types, and cross-linking is a precursor to further investigation into technical aspects. The object of the present study was to ascertain the biochemical characteristics of cephalopod collagen as these relate to the anatomical location of the collagen (mantle and arms), sex (male and female), and sexual maturity (immature and mature).

MATERIALS AND METHODS

Three cephalopod species were used: (i) volador (*Illex coindetii*; Vérany, 1839), phases IV and V, males averaging 220 mm in length and females averaging 260 mm; (ii) pota (*Todaropsis eblanae*; Ball, 1841), young males averaging 120 mm and mature males averaging 160 mm/young females averaging 180 mm and mature females averaging 270 mm; (iii) white octopus (*Eledone cirrhosa*; Lamarck, 1798) reaching a dorsal mantle length of 160 mm. Females grow larger than males, but the latter are more precocious and can grow to different sizes.

All three species were trawled on the Galician shelf. When caught, the different species were immediately placed in boxes with ice and freighted to the Instituto del Frío by refrigerated truck. Samples reached the laboratory 36 h after catching. At the laboratory they were separated by sex and stage of sexual development (young specimens, phases II and III; adults,

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10.1021/jf990711k CCC: $19.00
Published on Web 05/13/2000

phases IV and V). They were then gutted, and the mantles were separated from the arms before skinning and chopping. Assays for characterization of the species were carried out on the same day they reached the pilot plant.

Isolation of Connective Tissue. The connective tissue was isolated according to the method of Borderías and Montero (1985), involving removal of noncollagenous material by extensive extraction with 0.14 M NaCl and 0.05 M proteases inhibitors [1 mM phenylmethanesulfonyl fluoride (PMSF), 1 mM *p*-chloromercuribenzoate, and 10 mM ethylenediaminetetraacetic acid (EDTA)] and stirring during 24 h repeated at least three times.

Solubilization of Connective Tissue. To determine the solubility of the isolated connective tissue in acetic acid, 2 g of connective tissue was homogenized with 0.5 M acetic acid in an Ultra-Turrax (ratio 1:25 w/v) for 1 min containing 1 mM PMSF, 1 mM *p*-chloromercuribenzoate, and 10 mM EDTA. The mixture was continuously stirred for 24 h at 2−4 °C and then centrifuged at 35000*g* for 1 h. The resulting supernatant was set aside, and the same operation was repeated with the precipitate. This last supernatant was added to the previous supernatant. The process was carried out three times, and the three supernatants were combined. These supernatants contained the acid-soluble connective tissue fraction, and the precipitate was the insoluble fraction. The results were expressed as percent collagen solubilized in acid, which was calculated by determining the hydroxyproline in the soluble fraction and the insoluble fractions according to the method of Leach (1960). The entire process was carried out at 0−4 °C.

Purification and Fractionation of the Different Collagen Types. The different types of collagen were characterized in the arm and mantle of all three species. In all cases samples were taken from a mixture of skinned, chopped muscle from at least 10 specimens.

The connective tissue was extracted from mantle and arms according to the method of Mizuta et al. (1994b). The muscle was homogenized in an Osterizer homogenizer for 1 min at maximum setting with 0.1 M NaOH containing 1 mM PMSF and 25 mM EDTA in a proportion of 1:20 (w/v). The homogenate was then stirred for 24 h. The purpose of extraction with NaOH and use of enzymatic inhibitors was to remove noncollagenous proteins and protect the collagen from endogenous proteases. After stirring, the homogenate was centrifuged at 15000*g* for 20 min, and the resulting residue was put through three intensive washes with distilled water. The residue was resuspended in 0.5 M acetic acid and digested over 48 h with pepsin (porcine pepsin EC 3.4.23.1; Sigma, St. Louis, MO; crystallized and lyophilized) in a ratio of 1:10 (w/w) enzyme/substrate. Next, the homogenate was centrifuged to produce a supernatant that was the pepsin-solubilized collagen fraction. The entire process was carried out at 0−4 °C. The procedure used to isolate the different types of collagen was a modified version of the methods of Takema and Kimura (1982) and Mizuta et al. (1994b) consisting of controlled saline precipitation of the different types of collagen on the basis of collagen solubilized with pepsin. NaCl was added to this solubilized collagen up to a concentration of 2 M. The resulting precipitate was collected by centrifugation (10000*g*, 20 min) and then solubilized with 0.5 M acetic acid and 0.45 M NaCl over 18 h. The rest of the process is outlined in Figure 1.

For electrophoresis (SDS−PAGE) the collagen types thus obtained were dissolved with 50 mM Tris and 1% SDS, pH 7.4−7.6. Electrophoresis was carried out on a Phast-System horizontal apparatus (Pharmacia LKB Biotechnology AB, Uppsala, Sweden) using 7.5% polyacrylamide gels (Phastgel; Pharmacia LKB Biotechnology). Collagen bands were visualized by staining (Fairbanks et al., 1971).

Amino acids in isolated collagen were determined from a hydrolysate with 6 M HCl (110 °C, 24 h) using a Biochron 20 automatic analyzer (Pharmacia LKB). Amino acids were determined by derivatization with ninhydrin and measurement of absorbance at 570 nm except for proline and hydroxyproline, for which absorbance at 440 nm was measured.

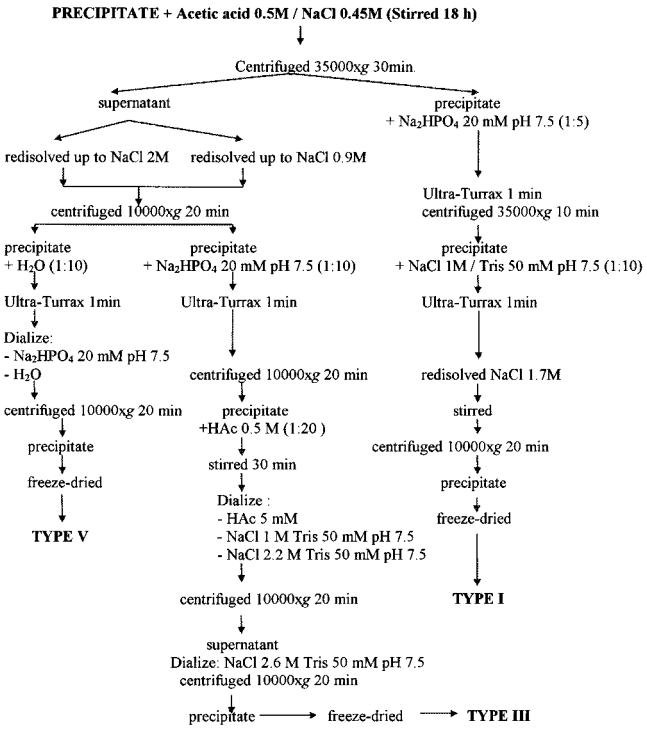

Figure 1. Outline of preparation for types I, III, and V collagen fractions.

Table 1. Muscle Collagen Content (Percent)

	mantle		arms	
species[a]	male	female	male	female
volador, immature	1.1[a]	0.9[b]	1.5[c]	1.7[d]
pota, immature	1.4[a]	1.4[a]	1.8[bc]	1.9[cd]
pota, mature	1.6[ab]	1.8[bc]	2.0[cd]	2.1[d]
octopus, immature	1.4[a]	1.3[a]	2.0[c]	2.0[c]
octopus, mature	1.2[a]	1.7[b]	2.1[cd]	2.3[d]

[a] Immature (phases II−III) and mature (phases IV−V). Diferent letters in the same species indicate significant differences ($p \leq 0.05$).

The amino acid content was expressed by the number of residues per 1000 residues.

Two-way analysis of variance (ANOVA) was carried out for the different samples. The computer program used was Statgraphics (STATC Inc., Rockville, MD). The difference of means between pairs was resolved by means of confidence intervals using a least significant difference range test. The level of significance was set for $p \leq 0.05$.

RESULTS AND DISCUSSION

Table 1 shows the percentage of collagen in the muscle from either anatomical region of the three species. In all three species the collagen content was higher in the arms than in the mantle. By sex, collagen content in volador was significantly higher in the mantle of males and higher in the arms of females. In terms of maturity, unlike the volador, there were no appreciable differences ($p \leq 0.05$) between collagen contents of male and female potas in either anatomical region. In pota the only significant differences ($p \leq 0.05$) were in the mantles of young and adult females. In female octopus, the percentage of collagen was higher ($p \leq 0.05$) in adults than in young specimens. In adult octopus the collagen content was significantly ($p \leq 0.05$) higher in females than in males. These differences may be partly due to the different biological development of the octopus. First, males are smaller and younger than

Table 2. Collagen (Percent) from Muscle Protein Content

	mantle		arms	
species[a]	male	female	male	female
volador, immature	10.6[a]	9.7[a]	16.2[b]	16.7[b]
pota, mature	13.3[a]	18.0[b]	19.0[bc]	20.7[ce]
pota, mature	18.0[b]	23.5[d]	19.1[bc]	21.1[e]
octopus, immature	11.0[a]	20.0[b]	16.7[ce]	19.0[bf]
octopus, mature	14.5[d]	15.7[cd]	17.6[ef]	19.0[bf]

[a] Immature (phases II−III) and mature (phases IV−V). Different letters in the same species indicate significant differences ($p \leq 0.05$).

Table 3. Solubility in 0.5 M Acetic Acid of Muscle Collagen (Percent)

	mantle		arms	
species[a]	male	female	male	female
volador, immature	84.05[a]	87.00[a]	78.30[b]	78.29[b]
pota, immature	77.94[a]	84.26[b]	74.48[c]	72.28[ce]
pota, mature	81.07[d]	80.83[d]	71.45[e]	70.31[e]
octopus, immature	84.00[a]	87.63[b]	79.65[c]	86.11[ab]
octopus, mature	80.27[c]	79.17[c]	72.30[d]	78.09[c]

[a] Immature (phases II−III) and mature (phases IV−V). Different letters in the same species indicate significant differences ($p \leq 0.05$).

females when they reach maturity, and, second, when females reach maturitt, feeding and growth may be altered. Growth can be irregular, static, or even negative due largely to mobilization of muscle proteins (Mangold, 1987).

The fact that there was more collagen in arms than in mantles could be explained by the function that either part performs in the living organism. Whereas the arms perform a grasping function and have to stand more strain than the mantle, the latter needs to be more elastic to fulfill its propelling function. Some studies suggest a relationship between the way a fish moves and the collagen content of the muscle and show that the greater the elasticity of the body and the more it participates in propulsive movement, the higher the collagen content in the muscle (Sato et al., 1986; Montero and Borderías, 1990).

Sato et al. (1986) studied the amount of muscle collagen in 24 different fish species. They arrived at a figure between 0.3 and 2.2%, representing between 1.6 and 12.4% of crude protein. There have been few studies on this aspect of cephalopods, but there is one by Sadowska and Sikorski (1987) that gives figures of 0.97% collagen in the muscle of *Illex argentinus* and 0.32% in the muscle of *Loligo patagonica*.

It is helpful to know the ratio of collagen to crude protein in the muscle, given that there are various physiological states in which mobilization of some proteins can occur, along with variations in the proximate composition of the muscle (Sikorski et al., 1984). As Table 2 shows, in both young and adult volador the difference was in the anatomical region, the arm protein fraction containing a significantly larger amount of collagen than the mantle fraction. In pota, the ratio of collagen to crude protein as relating to sexual maturity differed significantly ($p \leq 0.05$) in the mantle; in adult specimens collagen represented a higher percentage of the protein fraction than it did in immature specimens. By sex, the ratio of collagen to total protein was higher ($p \leq 0.05$) in female than in male mantles and arms irrespective of maturity, although in immature females the difference was not significant ($p \leq 0.05$). In terms of anatomical region, in young pota specimens the ratio of collagen to total protein was higher in arms than in mantle; in adults, however, there was either no difference ($p \leq 0.05$) (males) or the reverse was true (females). In octopus mantle the collagen content was higher ($p \leq 0.05$) in adult than in immature males, whereas in females the reverse was true (Table 2). Of the different lots of adult octopus, collagen content was higher ($p \leq 0.05$) in the arms, with no sex-related differentiation. In immature male specimens collagen content was higher ($p \leq 0.05$) in arms than in mantle, whereas in immature female specimens there was no significant difference between the contents of arms and mantle. As noted earlier, growth of the female octopus is irregular owing to mobilization of the muscle proteins (Mangold, 1987).

The collagen content in this study (expressed as a percentage of muscle protein) was higher than that reported by Sadowska and Sikorski (1987), who found 2−11% collagen in skinned mantle and 2−16% collagen in skinned arms of *I. argentinus*. Sikorski and Kolodziejska (1986) studied the collagen in the arms and mantle of *I. argentinus* and in the mantle of *L. patagonica* and reported figures of 16, 11, and 3%, respectively. In a sampling of 300 *L. argentinus* and 500 *L. patagonica* individuals, Sadowska and Sikorski (1987) found a high percentage of collagen in mantle protein, exceeding 10%. Kariya et al. (1986) reported 5.9 and 5.0% collagen in protein of arms and mantle, respectively, of *Octopus vulgaris*.

According to Dyer and Dingle (1961), 3% of the proteins in teleosts and 10% of the proteins in elasmobranchii are stromal. Kimura et al. (1979) found that 3% of the muscle proteins in carp were collagenous proteins. In a study of various crustacean species (shrimp, prawn, lobster, king prawn, and crab) Mizuta et al. (1994a) found that collagen represented 0.3−3.5% of the proteins and 0.04−0.58% of the muscle weight. None of the references consulted make any mention of the stage of sexual development of specimens. It has been shown that cephalopods contain more collagen than most marine animals, including elasmobranchs. The collagen content of the species studied here was somewhere between that of marine animals and land animals, with collagen making up one-third of the protein in the latter.

In volador, collagen solubility in 0.5 M acetic acid was significantly higher ($p \leq 0.05$) in mantle than in arms, irrespective of sex (Table 3). In pota mantles, collagen solubility differed ($p \leq 0.05$) in immature and adult specimens; in male specimens solubility was higher in adults, and in female specimens it was higher in immature mantles; in the arms of male specimens, collagen solubility was higher ($p \leq 0.05$) in immature than in adult specimens (Table 3). By sex, collagen solubility in the mantle was higher for young females than for young males. Finally, collagen solubility in 0.5 M acetic acid was higher ($p \leq 0.05$) in mantle than in arms, irrespective of maturity or sex. The figures for solubility of octopus collagen in 0.5 M acetic acid were consistently higher ($p \leq 0.05$) in the sexually immature lots than in the adults. In immature specimens solubility was significantly higher ($p \leq 0.05$) in the females (both mantle and arms); in adult specimens solubility of collagen in the arms was significantly higher ($p \leq 0.05$) in females. Solubilities of mantle and arm collagen differed significantly ($p \leq 0.05$) only in male specimens; mantle collagen was the more soluble (Table 3).

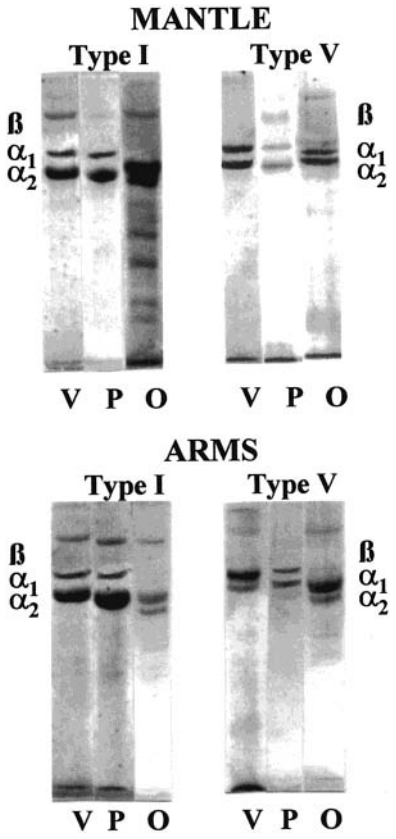

Figure 2. Electrophoresis profile of types I and V collagen fractions from mantle of volador, pota, and octopus.

In general terms, collagen solubility in acetic acid was higher in the mantle for all three species and tended to be higher in immature than in adult specimens. This could indicate that there is more cross-linking of collagen in arms and in adult specimens due to the conversion of reducible bonds into more complex structures which are not acid-soluble. These structures increase with age and also during frozen storage, and they are responsible for reducing the solubility of the collagen in acid (Miller, 1984).

There are no data for collagen solubility in 0.5 M acetic acid in cephalopods, but there are references to solubility in other kinds of solvent in both cephalopod and fish species other than the ones considered here, such as *I. argentinus* and *L. patagonica* (Sadowska and Sikorski, 1987), *Sepia officinalis* (Babu et al.., 1980), hake and cod (Yamaguchi et al., 1976; Sikorski et al., 1984; Montero et al., 1990), and trout (Montero et al., 1990; Sato et al., 1991); in those cases that considered the degree of maturity or sexual development, solubility was found to be lower in mature specimens, as the number of acid- and heat-stable bonds increases with age (Montero and Borderías, 1990).

The electrophoretic profiles of the types of collagen isolated in each species and anatomical region are shown in Figure 2. In the three species only types I and V were identified in both anatomical regions. The two isolated collagen types precipitated a saline concentration characteristic of types I and V, bearing out the findings of numerous authors, coinciding with quantitatively major and minor collagens, respectively (Trelstad et al., 1972; Chung and Miller, 1974; Epstein, 1974; Burgeson et al., 1976; Miller, 1984; Mizuta et al., 1994b). Similarities and differences have been found between the types of collagen from one to the other species. In this sense, the major collagen of cyclotome skin collagen greatly resembles vertebrate type I collagen; meanwhile, body one resembles invertebrate major collagens (Kelly et al., 1988; Sato et al., 1989; Kimura and Matsui, 1990; Matsui et al., 1990), one of the main properties of which is that only a negligible amount of the collagen can be extracted by acid or salt solutions from tissues, whereas type I collagen of superior vertebrates can be solubilized by this extraction, so pepsin digestion has been used to solibilize the cyclotome body collagen as it is formed with the invertebrate quantitatively major collagen (Sato, 1993).

In the isolation of collagen types, a higher proportion of type I was isolated than of type V, in a ratio ranging from 8:1 to 10:1 (type I/type V). Mizuta et al. (1994b) found a comparable ratio in the mantle of *T. pacificus*: 9:1 (type I/type V). In the electrophoretic profile of these two types of collagen there were two clearly differentiated protein bands in proximate positions; this suggests that the molecules were heterotrimers, that is, comprising at least two different types of α chain. Although the proportion of the α2 chain band was greater than that of the α1 chain band, the presence of β chains rules out any speculation as to the structure of the collagen molecule. However, most of the studies in different species of cephalopods coincide with a heterotrimer structure of the form (α1)2α2. According to the effect observed by Mizuta et al. (1996, 1997) the alkali and pepsin treatment could produce more labile α1 chain and increase the intensity of α2 chain in the electrophoretic pattern. Mizuta et al. (1996, 1997) observed that the SDS−PAGE pattern of the quantitatively major (SQ-I) and minor (SQ-II) collagens of squid mantle (*T. pacificus*) changed after alkali (0.1 M NaOH) and digestion with pepsin, decreasing the staining intensity of the upper bands of the chains with a concomitant increase of the staining intensity of the lower chains. The α1 chains are quite susceptible to digestion on both types of collagen; meanwhile, the α2 chain of the quantitatively major collagen (SQ-I) component seemed to be more stable to pepsin digestion than the chain α2-(SQ-II) of the quantitatively minor collagen. The bands of all collagen types electrophoretically isolated in each species and anatomical region were unaffected by the presence of β-mercaptoethanol, indicating that there were no disulfide bridges characteristic of type III collagen. This ruled out any contamination by type III collagen in the other isolated types.

The electrophoretic profiles from type I collagen showed no apparent differences between mantle and arms in any of the species studied, which suggests that type I collagen is the same in mantle and arms of all three species and that they do not differ structurally. However, octopus did differ from volador and pota in the electrophoretic mobility of the type I collagen bands of mantle and arms. The mobility of the α1 of type I octopus collagen was higher in both mantle and arms, resulting in greater proximity among the α chains.

The electropherogram of type V collagen was similar in all three species. This electrophoretic profile consisted of two protein bands, which were equally mobile in volador and pota. In the case of octopus, the pattern was like that of type I collagen; the mobility of the α1(V) chain was greater in octopus than in the other two species in both mantle and arms. The electrophoretic profile of this collagen type was the same for mantle and arms in all three species.

Table 4. Amino Acid Composition (Number of Residues/1000 Amino Acids) of the Types of Collagen Fractions[a]

	Vm-I	Va-I	Pm-I	Pa-I	Om-I	Oa-I	Vm-V	Va-V	Pm-V	Pa-V	Om-V	Oa-V
threonine	21	22	28	24	24	28	22	25	24	25	25	27
valine	19	22	25	23	25	27	30	31	31	31	33	31
methionine	11	11	12	11	11	13	11	11	11	12	12	12
isoleucine	16	16	15	17	19	20	35	34	33	26	31	34
leucine	30	27	29	29	31	30	51	50	41	44	43	41
phenylalanine	13	20	11	10	11	11	14	13	13	14	14	12
lysine	17	16	16	17	15	15	32	32	24	25	30	27
histidine	8	14	8	8	14	16	9	8	9	9	12	14
arginine	64	59	60	59	57	60	51	50	53	52	50	49
aspartic	61	65	75	67	61	59	71	71	72	74	70	73
glutamic	94	94	99	98	96	88	102	104	101	104	89	101
serine	19	24	21	24	28	28	40	39	43	43	41	45
gyicine	333	322	330	341	329	322	303	304	307	309	304	292
alanine	101	94	91	92	93	95	66	67	74	67	65	63
tyrosine	3	3	3	3	3	3	4	4	4	3	4	4
proline	94	92	97	97	88	81	82	82	77	71	77	85
hydroxyproline	97	98	79	80	96	103	77	75	82	91	91	90

[a] V, volador; P, pota; O, octopus; m, mantle; a, arms; I, type I; V, type V.

Kimura et al. (1981) identified a majority collagen type in skin of octopus (*Octopus vulgaris*) with a structure $[\alpha1(I)]_2\alpha2(I)$ and composition closely resembling that of type I bovine collagen. Takema and Kimura (1982) found that the majority of collagen in arms of octopus (*O. vulgaris*) was type I and was identical to the type I collagen in the same species. Kimura and Karasawa (1985) showed that, unlike what was previously thought, the majority of collagen in the cartilage of *T. pacificus* was type I and not type III and that they had virtually the same amino acid composition as type I collagen of the same species. Type I collagen at different locations would therefore seem to be the same.

Shadwick (1985) posed the existence of at least two different collagen types in skin, tunic, and mantle of squid (*Loligo vulgaris*) and cuttlefish (*S. officinalis*). A number of subsequent studies reported the presence of types I and V collagen in the mantle of *T. pacificus*, which were isolated by saline precipitation (Mizuta et al., 1994b,c, 1995, 1996). These collagen types possessed a heterotrimer structure of the form $[\alpha1(I)]_2\alpha2(I)$ and $[\alpha1(V)]_2\alpha2(V)$; the amino acid composition of the type I collagen was similar to that of the type I cartilage identified by Kimura and Karasawa (1985), whereas the composition of the type V collagen was similar to that of type V in carp muscle as determined by Sato et al. (1988) (Mizuta et al., 1994b,c, 1995, 1996).

The fact that type III collagen was not found and type V was found suggests that type V probably performs a function similar to that of type III in mammalian muscle, where it forms copolymers with type I and controls the diameter of the collagen fibers (Adachi and Hayashi, 1986; Keene et al., 1987; Birk et al., 1990).

The amino acid compositions of both types I and V isolated in volador, pota, and octopus were similar in mantle and arms (Table 4). In other words, the composition of any one type of collagen did not differ appreciably according to species or anatomical region, which suggests that these are collagens of the same type. The composition of type I collagen in skin of pota (*T. eblanae*) as determined by various authors (Kimura and Matsuura, 1974; Mizuta et al., 1994b,c, 1995, 1996) is similar to that of type I isolated in cartilage and muscle of the same species (Kimura and Karasawa, 1985; Mizuta et al., 1994b,c, 1995, 1996), in skin of octopus (*O. vulgaris*) (Kimura et al., 1981b), in arms of cuttlefish (*S. officinalis*) (Babu et al., 1980), and in muscle of the three experimental species. The composition of type V collagen isolated in muscle of pota (*T. eblanae*) (Mizuta et al., 1994b,c, 1995, 1996) was very similar at both anatomical locations and resembled that of type V isolated in mantle and arm muscle of volador, pota, and octopus. This suggests that the collagen types may not differ according to species or anatomical location.

One of the characteristics of both types of collagen shared by all types of collagen isolated in living beings is the glycine content, which represents between 30 and 34% of total collagen molecule residues.

The differences in type I and V collagen compositions in the three experimental species lies in the fact that type V contains higher proportions of isoleucine, leucine, lysine, and serine and smaller proportions of alanine and hydroxyproline.

A comparison of the proportion of hydroxyproline in fish and cephalopod muscle shows that in volador, pota, and octopus this is 7.7−10.3%, whereas in fish species such as carp, eel, mackerel, salmon, hake, and trout the amount of hydroxyproline ranges from 7 to 8.4% (Sato et al., 1988; Kimura et al., 1988; Montero et al., 1990). It should be noted that cephalopod and fish muscle collagen contain less hydroxyproline than collagen of food animals and other vertebrates (McClain et al., 1970; Sikorski et al., 1984).

Values of aspartic acid, glutamic acid, and lysine in type I collagen of the experimental species were also higher than in fish but were consistent with the values reported by other authors for collagen of different cephalopod species (Kimura and Matsusura, 1974; Kimura et al., 1981, 1988; Takema and Kimura, 1982; Kimura and Karasawa, 1985; Sato et al., 1988; Mizuta et al., 1994b).

Fish, mollusc, and crustacean collagens differ from mammalian collagens in that they contain more essential amino acids and less hydroxyproline. The proportions of aspartic acid and lysine were higher in type V collagen of the three experimental species than in type V collagen of carp and pota (*T. pacificus*) reported in the literature (Sato et al., 1988; Mizuta et al., 1994b,c).

The amino acid composition of type V collagen from the experimental species was similar to that of type V from vertebrates and differed from type I in that it contained less alanine and more leucine. These differences were also reported by Sato et al. (1988) and Mizuta et al. (1994c).

Of particular interest are the high hydroxyproline content found in both types of collagen, which has an

important function in stabilization of the triple helix, and the fact that lysine content was normal with respect to other cephalopod and fish species. It is worth bearing in mind that lysine is critical for the formation of acid-stable intermolecular bonds. Triple-helix stability in type I collagen is therefore presumably greater because of hydrogen bridges, whereas type V collagen contains more lysine residues and may therefore be susceptible to cross-linking.

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Received for review June 28, 1999. Revised manuscript received March 7, 2000. Accepted March 16, 2000. This research was financed by the UE under Project TS3*-CT92-0109 and by the Comisión Interministerial Científica y Técnica under Project ALI93-1146-CO2-01-CE.

JF990711K