Role of Collagen and Contractile Elements in Ultimate Tensile Strength of Squid Mantle

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Squid mantle was treated with probes to establish the relative importance of collagen and contractile proteins in tensile strength as a function of sample orientation. Collagenase was used to modify collagen, and sodium chloride and a crude trypsin preparation were used to modify the contractile elements. Treatment of raw mantle with collagenase reduced strength in the longitudinal direction but had no effect in the transverse, while sodium chloride and trypsin reduced strength transversely but had no effect in the longitudinal direction. These results support the hypothesis that collagen is a primary determinant of raw mantle strength in the longitudinal direction and that muscle fibers are relatively more important in the transverse. On the contrary, when samples were heated after treatment with enzymes or sodium chloride, tensile strength was reduced in both orientations. Comparison of the data with a model indicated that collagenous elements other than those of the outer tunic contribute to longitudinal strength.

INTRODUCTION

The texture of squid has been cited as a dominant quality attribute which influences the demand for this mollusc on domestic as well as international markets (Takahashi, 1965; Kalikstein, 1974; Galus, 1975; Otwell and Hamann, 1979a,b; Sheehy and Vik, 1980; Stanley and Hultin, 1982). A better understanding of factors affecting squid texture is important in developing techniques that will produce improved products and increase its utilization as a food. This research was undertaken to determine components of squid mantle that contribute to its tensile properties.

MATERIALS AND METHODS

Materials. Squid (*Loligo pealei* and *Illex illecebrosus*) of 11–18-cm length were obtained from day boats in Gloucester and transported on ice to the laboratory in Amherst, MA. The squid were cleaned (skin, tentacles, viscera removed) and cut open along the dorsal surface, which yielded a flat triangular shaped squid mantle. These were packed in sealed plastic bags and stored at −40 °C until used.

Collagenase (type VII), trypsin (pancreatic, type II), and pepstatin A (an inhibitor of aspartic proteinases like cathepsin D) were obtained from Sigma Chemical Co. Type VII collagenase is a highly purified form of collagenase and was chosen to eliminate nonspecific effects on proteins other than native collagen. The trypsin on the other hand is a crude preparation containing significant chymotryptic activity; it was only necessary to have a preparation that was free of collagenase. *p*-(Chloromercuri)-benzoate (pCMB) and phenylmethanesulfonyl fluoride (PMSF) were from Calbiochem. All other chemicals were of reagent grade.

Methods. *Preparation of Outer Tunic Enriched Mantle.* The squid mantle was scraped with a razor blade to remove the inner tunic and most of the muscle fibers to yield a sheet enriched in outer tunic.

Preparation of Samples Treated with Enzymes and Salt. For raw squid, samples were cut in the shape of a dumbbell of overall length of 4.5 cm; the ends were 0.5 cm in width, and the center was 0.2 cm. This was done to make sure the center portion would be the part that broke during tensile strength measurements (Kuo et al., 1990). Three pieces of the dumbbells were added to 100 mL of solution containing enzyme or NaCl in 125-mL Erlenmeyer flasks and incubated at room temperature (about 25 °C) at 30 rpm in a shaker bath for the indicated times. Room temperature generally varied ±2 °C; comparative samples were always run at the same time so that temperature differences should not be a factor. For samples that were to be tested after heating, half of a cleaned squid mantle (about 15–20 g) was added to 500 mL of the appropriate solution in a 1-L beaker. Shaking speed and temperature were the same as for the dumbbell pieces. After the appropriate period of incubation, the samples were removed and placed in distilled water at a ratio of 1:60 (squid/water) for the indicated time and temperatures. Dumbbell pieces as described above were cut from the heated mantle and evaluated for their tensile properties. The data for samples taken at time 0 were not treated with the modifying solutions.

The collagenase solution was prepared in a 0.2 M Tris buffer (pH 7.4), containing 10 mM $CaCl_2$, with a final concentration of 20 units of enzyme/mL. One unit of collagenase activity is defined as that amount of enzyme which will cause the release of peptides from native collagen equivalent in ninhydrin color to 1.0 μmol of L-leucine in 5 h at pH 7.4 and 37 °C. The trypsin solution was prepared in a 0.1 M phosphate buffer (pH 7.6) with an activity of 555 *N*-benzoyl-L-arginine ethyl ester (BAEE) units/mL. One unit will hydrolyze 1.0 μmol of BAEE/min at pH 7.6 and 25 °C. The concentration of salt solution was 0.6 M NaCl in distilled water.

Preparation of Samples Treated with Protease Inhibitors. Squid was soaked in a mixture of protease inhibitors (1 mM pCMB, 1 mM PMSF, 1 mM EDTA, and 1 μg of pepstatin A per milliliter of 0.1 M phosphate buffer, pH 8.0) for 1 h in a shaker at room temperature and then incubated in the water bath at 60 °C for different time periods (2–30 min). Both the treated and control samples were then cut in the longitudinal or transverse direction.

Analytical Methods. All analyses were performed in triplicate on each squid, and at least three squid mantles were used. Data were tested for significance by means of Duncan's multiple range test for the incubation studies and Student's *t*-test at the 95% confidence level for other parameters.

Ultimate Tensile Strength Measurement. The samples (raw or cooked squid) were cut longitudinally or transversely or at

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Table I. Effect of Specimen Orientation on Ultimate Tensile Strength[a] of Squid

treatment	longitudinal	transverse	27° [b]	45°
Loligo				
raw	31 ● 8	13 ± 5	21 ± 4	16 ± 3
60 °C, 2 min	6 ± 2	10 ± 3	6 ± 1	5 ± 1
100 °C, 2 min	10 ● 4	22 ± 7	7 ± 1	11 ± 2
Illex				
raw	45 ± 8	14 ± 3	17 ± 3	8 ± 1
60 °C, 2 min	11 ± 4	15 ± 3	7 ± 1	4 ± 1
100 °C, 2 min	17 ● 5	11 ± 3	7 ± 1	6 ± 1

[a] Ultimate tensile strength in N/cm² ● SD. [b] Angles are given with respect to the long axis of the squid mantle.

specific angles to the long axis. Preparation procedures and measurement have been described (Kuo et al., 1990). Dumbbell-shaped pieces of squid mantle were stretched on an Instron universal testing machine, and the resistance to stretch was measured. Ultimate tensile strength is the force produced at breakage. Strain at failure measured the length of stretch before breaking.

Soluble Hydroxyproline and Protein Measurement. Soluble hydroxyproline (HOP) was assayed directly from the medium containing the samples that had been treated with enzymes or salt. HOP was determined by the colorimetric method described by Woessner (1961). For soluble protein content, 4 mL of biuret reagent was added to 1 mL of the medium containing the samples that had been treated with enzymes or salt. The protein content was determined according to the biuret method described by Cooper (1977).

RESULTS

Ultimate Tensile Strength of Raw and Heated Mantle. Mantles of the two species of squid were tested for their ultimate tensile strength at four different orientations, both in unheated samples and in samples that were placed in water for 2 min at either 60 or 100 °C (Table I). The samples were cut parallel to the long axis of the mantle, perpendicular to it, or at angles of either 27° or 45° from the long axis. The angle of 27° was chosen because it is the angle between the longitudinal axis and the direction of the collagen fibers in the outer tunic of *Loligo*. Collagen fibers in *Loligo* are at an angle of 27° on both sides of the perpendicular. In both species the highest ultimate tensile strength was found in the longitudinal direction when the sample was not heated. The values were considerably less in the other directions. The ultimate longitudinal tensile strength of raw *Illex* was greater than that of raw *Loligo*, but little difference was observed between species when ultimate tensile strength was measured in the transverse direction or at an angle of 27°.

On heating, there was a large decrease of the ultimate tensile strength in the longitudinal direction while there was no change in this parameter in the transverse direction, except in the case of *Loligo* mantle heated for 2 min in water at 100 °C, where the ultimate tensile strength increased. The changes observed at an orientation of 27° from the long axis were similar in nature to those in the longitudinal direction. A decrease on heating was also observed at an orientation of 45° for *Loligo*. This decrease was not significant with *Illex* since the ultimate tensile strength of this mantle at 45° was initially very low.

Role of Outer Tunic. Ultimate tensile force and failure strain were measured in the raw mantle of *Loligo* and *Illex* in the longitudinal and transverse directions before and after removal of the inner tunic and a large portion of the muscle fibers by careful scraping with a razor blade (Table II). Initially, all samples had the same cross-sectional area, and the amount of tissue removed was

Table II. Tensile Properties of Squid Mantle before and after Removal of the Inner Tunic and Some Muscle Fibers[a]

	Loligo		*Illex*	
	unscraped	scraped[b]	unscraped	scraped[c]
	Longitudinal Direction			
ultimate tensile force, N	1.3 ± 0.2[a]	1.6 ± 0.3[a]	2.2 ± 0.8[b]	2.1 ± 0.6[b]
failure strain, cm	0.26 ± 0.04[c]	0.26 ± 0.04[c]	0.19 ± 0.03[d]	0.18 ± 0.02[d]
no. of samples	6	6	9	9
	Transverse Direction			
ultimate tensile force, N	0.5 ± 0.1[a]	0.3 ± 0.1[b]	0.5 ± 0.1[a]	0.3 ± 0.1[b]
failure strain, cm	0.26 ± 0.03[c]	0.19 ± 0.03[d]	0.23 ± 0.03[c]	0.17 ± 0.02[d]
no. of samples	9	9	9	9

[a] The thickness and thus the cross-sectional area of all samples before scraping were the same. Values with different superscripts in either the longitudinal or transverse direction are significantly different ($P < 0.05$). [b] 72% off for longitudinal direction; 73% for transverse direction. [c] 73% off for longitudinal direction; 70% for transverse direction.

relatively uniform in all samples (70–73%). Greater amounts of muscle fibers were not removed for fear of damaging the outer tunic. The tensile measurement is presented as the total force rather than based on the cross-sectional area since all cross-sectional areas were initially equal. The samples were thus enriched in the outer tunic compared to the muscle fibers, and the inner tunic was completely removed. Neither the ultimate tensile force nor the failure strain in the mantle of either species of squid was affected by the removal of the inner tunic and the muscle fibers when these parameters were measured in the longitudinal direction. The process of scraping did, however, decrease both the ultimate tensile force and failure strain when they were measured in the transverse direction, i.e., perpendicular to the longitudinal axis.

Use of Probes To Determine Role of Collagen and Contractile Proteins. In the next series of experiments we examined the use of probes on the tensile properties of the squid mantle. These "probes" were agents that were expected to have an effect selectively on either collagen or the contractile proteins in the muscle cells (Hultin, 1985). A commercial collagenase was used in an attempt to modify collagen components, while NaCl was used for its known ability to solubilize contractile proteins at high ionic strength. Trypsin was used because it is an enzyme with very limited activity against native collagen molecules but would be expected to be more active against the proteins of the contractile apparatus. Strips of mantle were incubated in solutions of these probes for the indicated periods of time. Then the ultimate tensile strength was measured in the sample treated with the probe as well as a sample that was treated the same way except the probe was absent. Measurements were made on both raw mantle and mantle that had been heated after treatment by salt or enzyme by placing in water at 100 °C for 2 min. Results are given only for *Loligo* mantle, but results with *Illex* mantle were similar in all respects.

Data obtained when type VII collagenase (Sigma) was the probe are given in Figure 1a for the longitudinal orientation of mantle strips and in Figure 1b for the parameters in the transverse direction. In the longitudinal direction, collagenase produced a significant ($P < 0.05$) lowering of the ultimate tensile strength vs the control after incubation times of 6 and 8 h at 25 °C. Conversely, when raw *Loligo* mantle was examined in the transverse direction, there was no significant reduction of the ultimate tensile strength over the time period examined. After the mantle samples cut in the longitudinal direction were heated, the ultimate tensile strengths of both the control

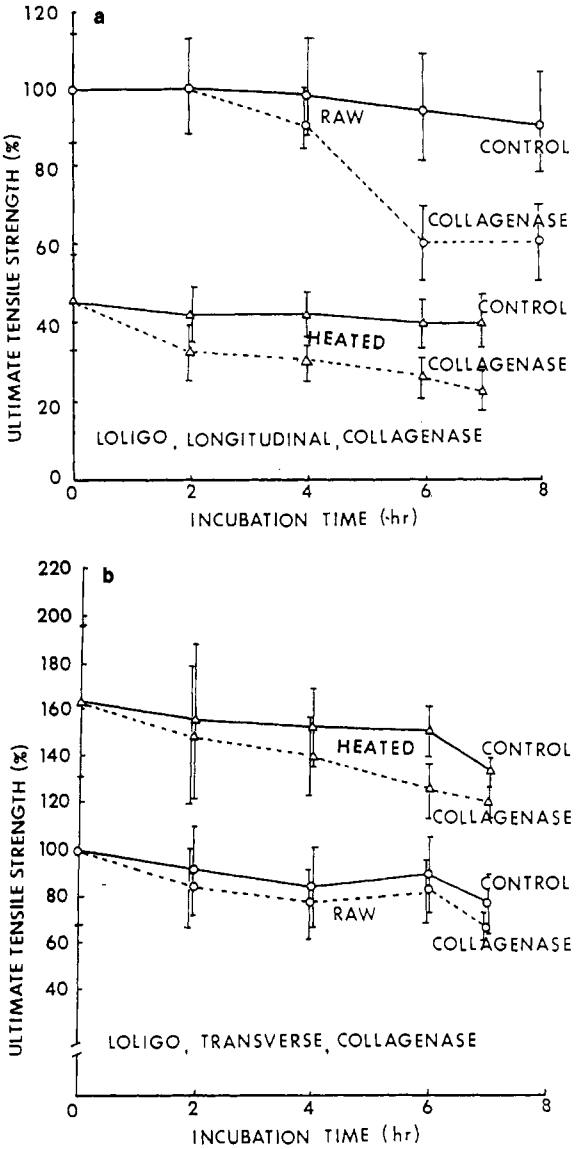

Figure 1. Effect of collagenase on ultimate tensile strength (±SD) of raw and heated *L. pealei* oriented in the longitudinal (a) and transverse (b) directions. Heating conditions were 100 °C for 2 min. The 100% value in the longitudinal direction was 33 N/cm² (a), and that in the transverse was 17 N/cm² (b).

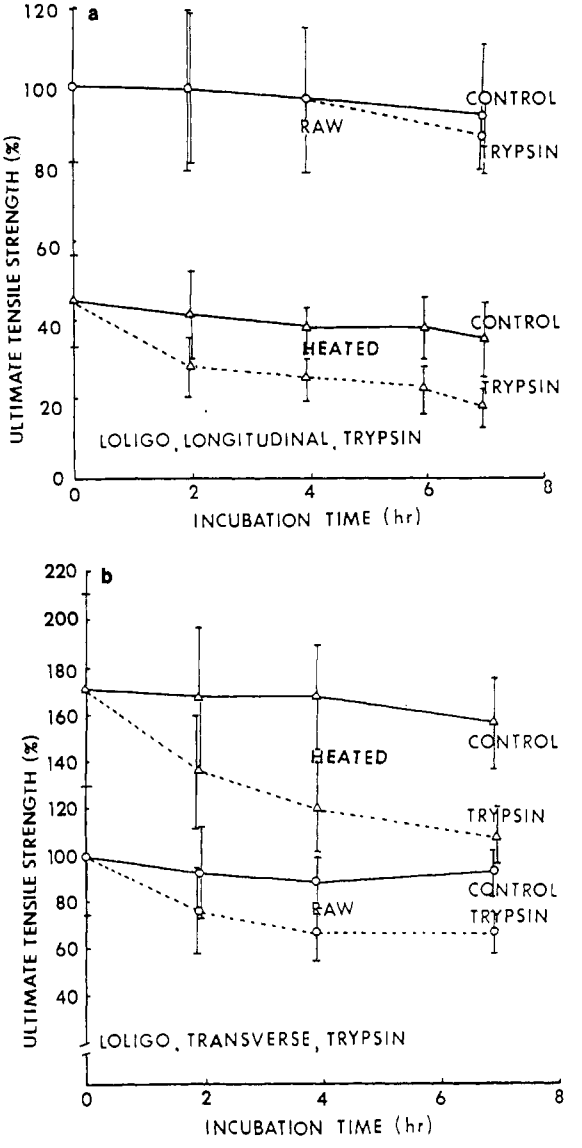

Figure 2. Effect of trypsin on ultimate tensile strength (±SD) of raw and heated *L. pealei* oriented in the longitudinal (a) and transverse (b) directions. Heating conditions were 100 °C for 2 min. The 100% value in the longitudinal direction was 29 N/cm² (a), and that in the transverse was 16 N/cm² (b).

and the collagenase-treated samples decreased (Figure 1a). In the case of the heated mantle cut longitudinally, there was a further significant decrease at time periods of 4 h and after in ultimate tensile strength in the sample treated with collagenase compared to the control ($P < 0.05$). When the *Loligo* mantles cut in the transverse direction were heated in 100 °C water for 2 min, there was an increase in the ultimate tensile strength (Figure 1b). Although collagenase had no effect on the ultimate tensile strength in the transverse direction of the raw mantle, an effect appeared after heating in samples incubated for 6 h or more ($P < 0.05$).

Results obtained with trypsin were different from those observed with collagenase (Figure 2). Trypsin had no effect on the ultimate tensile strength of raw *Loligo* mantle cut in the longitudinal direction but did significantly decrease the ultimate tensile strength of raw mantle cut in the transverse direction ($P < 0.05$). Incubation with trypsin significantly affected the ultimate tensile strength of squid mantle cut in either direction and heated in water at 100 °C for 2 min.

Results obtained by incubating mantle in the presence of 0.6 M NaCl were qualitatively similar to those obtained with trypsin (Figure 3). Treatment with NaCl did not affect raw *Loligo* mantle cut in the longitudinal direction but did affect it after heating and significantly affected both raw and cooked mantle cut in the transverse direction ($P < 0.05$). *Illex* mantle behaved in a similar way in all respects to *Loligo*.

During each treatment both soluble protein and soluble hydroxyproline were determined as a function of time (Table III). This was done to determine if the probes, i.e., collagenase, trypsin, and NaCl, caused changes consistent with their predicted function. Adding probes such as enzymes to intact tissue is no guarantee that the probes can interact with the target proteins. The data of Table III show that the probes brought about the predicted changes. Collagenase had little effect on the change in soluble protein but increased soluble hydroxyproline much more than the other treatments. On the other hand, both trypsin and NaCl caused large increases in the soluble protein from the mantles of both *Loligo* and *Illex*. Trypsin and NaCl also increased soluble hydroxyproline but to a lesser extent than did collagenase. It is clear that collagenase was solubilizing more collagen than the other agents

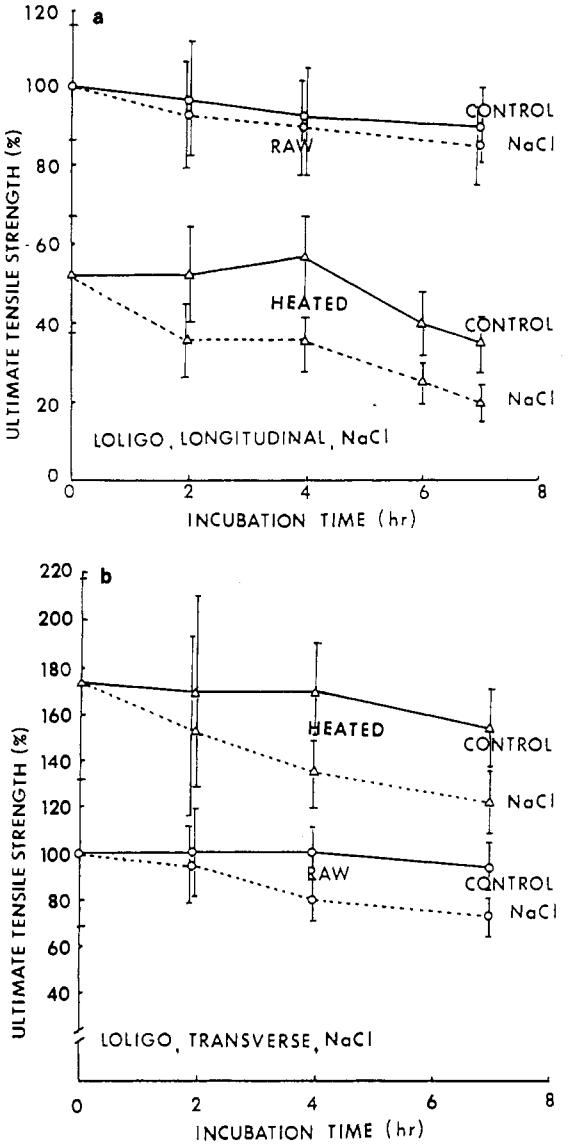

Figure 3. Effect of NaCl on ultimate tensile strength (±SD) of raw and heated *L. pealei* oriented in the longitudinal (a) and transverse (b) directions. Heating conditions were 100 °C for 2 min. The 100% value in the longitudinal direction was 31 N/cm^2 (a), and that in the transverse was 15 N/cm^2 (b).

while a greater increase in total soluble protein occurred on treatment with trypsin and NaCl.

The data in Table I show that lowering of tensile strength occurs when squid mantle is placed in water at a temperature of 60 °C for 2 min. It has been hypothesized that fish contain alkaline proteases which are activated at a temperature of 60 °C (Iwata et al., 1973; Lin and Lanier, 1980). In this work, squid mantle was incubated for 1 h at room temperature in the presence of a variety of protease inhibitors that should inhibit the major classes of proteolytic enzymes, i.e., 1 mM pCMB, 1 mM PMSF, 2 mM EDTA, and 1 μg per milliliter of pepstatin A. The samples were then held for times up to 20 min at 60 °C at pH 8.0. No effect was observed on the ultimate tensile strength in the longitudinal direction of the squid mantle thus treated. Thus, no evidence in support of an active alkaline protease was obtained.

DISCUSSION

Texture is the predominant quality factor in squid, while flavor is generally more important than texture in finfish (Hultin, 1985). This work focused on the measurement of the ultimate tensile strength in squid mantle subjected to various processes with the aim of determining the factors responsible for this mechanical property. Ultimate tensile strength was chosen because it is a property that can be related to the structural characteristics of the tissue.

The mantle tissue of squid is highly structured, and this structure has been discussed (Otwell and Hamann, 1979a,b; Kuo et al., 1990). Basically, it consists of circular muscle fibers in the mantle that are oriented perpendicular to the long axis of the squid body. In addition, there are radial muscle fibers that connect the inner and outer tunics which exist to modify the thickness of the mantle. In general, the muscle fibers do not run parallel to the long axis of the squid mantle. Collagen fibers also have a specific orientation with the major fibers in the outer tunic running at approximately a 27° angle to the longitudinal axis in *Loligo* (Ward and Wainwright, 1972) and a 23° angle for *Illex* (Kuo, 1989). It would be expected that resistance to tensile forces of these collagen fibers would be greatest when the force was applied in the direction longitudinal to the long axis of the squid. The collagen fibers of the inner tunic are smaller than those of the outer. In addition, there are collagen fibers interspersed among the muscle cells (Gosline and Shadwick, 1983a,b). Some intramuscular fibers (IMF-1) run between the inner and outer tunics in the longitudinal direction. Comparable intramuscular fibers (IMF-2) connecting the tunics are found in a plane perpendicular to the long axis. The IMF-1 fibers would contribute to the force of resistance parallel to the longitudinal axis, while those of the IMF-2 would contribute to those perpendicular to this axis.

When sections of raw squid mantle were cut at various angles from the longitudinal direction, the force required to cause the mantle to break was highest in a direction parallel to the long axis of the mantle. This would suggest that collagen fibers were very important contributors to this ultimate tensile strength since the contractile elements were oriented primarily in a direction transverse to this long axis. In addition, the number and size of the collagen fibers in the outer tunic raise the clear possibility that the outer tunic is a particularly important contributor to the tensile properties of raw squid mantle. To test this proposition, the inner tunic and some 70% of the muscle fibers were cut away from the mantle, leaving the outer tunic with approximately 30% of the muscle fibers. When this was done, there was no significant effect on the ultimate tensile strength in the longitudinal direction, indicating that in fact the collagen fibers of the outer tunic did play a major role in the tensile properties of the squid mantle parallel to its long axis.

On the other hand, removing the inner tunic and the attached 70% of the muscle fibers did cause a significant decrease in the tensile force required to break the mantle when this force was applied transverse to the long axis. This may be interpreted as indicating that the muscle fibers contribute more to the tensile properties of the squid mantle in the transverse direction than do the collagen fibers of the outer tunic. Thus, it would appear that the tensile properties of raw squid mantle in the longitudinal direction are contributed primarily by collagen fibrils of the outer tunic, while muscle fibers play a significantly greater role in the transverse direction. Since the muscle fibers comprise some 98% of the thickness of the mantle (Otwell and Hamann, 1979a), the ultimate tensile strength of the collagen fibers in the outer tunic must be large.

Similar results were observed when the strain at failure was compared in the scraped and unscraped mantle samples. Scraping had no effect on failure strain in either

Table III. Net Increase of Soluble Protein and Hydroxyproline (HOP) of Squid Incubated with Collagenase, Salt, or Trypsin at Room Temperature

	soluble protein, mg/g ww, after incubation for				soluble HOP, μg/g ww, after incubation for			
	0 h	2 h	4 h	7 h	0 h	2 h	4 h	7 h
Illex								
collagenase	0	0.1 ± 0.01	0.1 ± 0.01	0.3 ± 0.01	0	60 ± 3	80 ± 6	183 ± 9
trypsin	0	10 ± 0.5	18 ± 0.9	28 ± 1.4	0	22 ± 1	33 ± 1	58 ± 2
NaCl	0	20 ± 1.0	35 ± 1.7	39 ± 1.9	0	27 ± 1	50 ± 2	55 ± 2
Loligo								
collagenase	0	0.1 ± 0.01	0.1 ± 0.01	0.5 ± 0.01	0	55 ± 2	81 ± 6	210 ± 10
trypsin	0	5 ± 0.2	10 ± 0.5	15 ± 0.9	0	18 ± 1	30 ± 1	57 ± 2
NaCl	0	25 ± 1.2	38 ± 1.9	39 ± 1.9	0	30 ± 1	57 ± 2	55 ± 2

species in the longitudinal direction but reduced it significantly in the transverse direction. This indicates that strain in the longitudinal direction is also primarily a function of the collagen fibers of the outer tunic but that in the transverse direction other factors play a more important role. These factors probably include the muscle fibers themselves.

The effect of sample orientation relative to the long axis on ultimate tensile strength was very different when the mantles were heated. There was a dramatic decrease in the ultimate tensile strength in the longitudinal direction at either 60 or 100 °C, while there was no effect or an increase in the transverse direction. This indicates that there was a major effect of heating on the collagen fibers of the outer tunic as previously observed (Otwell and Hamann, 1979b). It seems likely that this involved the denaturation or melting of the collagen molecules. The effects of high temperatures on the tensile properties of the squid mantle in the transverse direction are most likely complex, with a decrease in the contribution of collagen fibers being offset by an increased resistance to force generated in the contractile apparatus of the muscle fibers. Loss of water and increase in protein density could contribute to this effect. While collagen fibers apparently play a major role in the tensile properties of raw squid mantle, the contractile proteins become relatively more important after heating.

With squid mantle cut at angles of 27° and 45° to the long axis, results were obtained that are difficult to analyze due to the complexity of the structures involved. In general, mantle strips cut at an angle of 45° have relatively low tensile strengths whether raw or heated. If ultimate tensile strength is an indicator of tenderness, it would suggest that this is a good angle from which to cut strips for consumption. Further work is needed in this area.

To further test the proposition that the collagen fibers in the outer tunic were an important contributor to tensile properties in the longitudinal direction and that the contractile proteins of the muscle fibers contributed rather more in the transverse direction, a series of probes were used. These probes were chosen since they should have had a preferential effect on either collagen fibers or the contractile proteins of the muscle fibers. In general, the probes behaved in a way that was consistent with the suggestion that collagen contributed to tensile strength in the longitudinal direction and that the contractile proteins had relatively more importance in the transverse direction. Only the collagenase probe, which should act on collagen, had an effect on raw mantle strength in the longitudinal direction, while trypsin and NaCl, whose effects should be directed to the contractile proteins, modified raw mantle strength only in the transverse direction. Analysis of the samples for soluble protein and soluble hydroxyproline indicated that the probes were functioning in the predicted manner.

As expected, whenever the probes affected the ultimate tensile strength in the raw mantle tissue, they also brought about a decrease in the ultimate tensile strength when the samples were heated at 100 °C for 2 min. In addition, however, treatment with salt or the enzyme preparations caused a significant decrease in ultimate tensile strength in heated samples compared to samples heated but not treated with the probes in the orientation that they did not affect in the raw state. Thus, collagenase decreased ultimate tensile strength in the transverse direction of heated mantle relative to the control while trypsin and NaCl did the same for the heated mantle in the longitudinal direction, again in comparison to the untreated, but heated, control. It is clear that there was an interaction between these probes and high-temperature treatment and that the interplay of factors such as collagen fibers vs contractile proteins of the muscle fibers has more complex interactions in the heated tissue than they do in the unheated.

There is also the possibility that the probes have some effect on the molecules that are not their primary targets, such effects not showing up in the unheated mantle but being brought out when the further stress of high temperature is applied. Sikorski and Kolodziejska (1986) added squid mantle to a curing solution containing 1.5% NaCl and found that this decreased the increase in hardness that was ordinarily caused by soaking the sample in a 2% acetic acid cure. Soaking the mantle in a 5% NaCl solution for 15–17 h slightly decreased the hardness, while treatment with either NaCl and phosphate or only phosphate significantly decreased the sensory toughness and cooking loss (Kolodziejska et al., 1985). Hardness of mantle decreased continuously during cooking in boiling 2% NaCl up to at least 60 min (Kolodziejska et al., 1987).

Results with the probes indicate that the factors in squid mantle contributing to the tensile properties of raw squid are complex and most likely involve several structural elements. Nevertheless, it may be useful to devise a simple model to reflect the experimental results. Toward this end, we propose a model based solely on the collagen fibers of the outer tunic. Vectorial analysis was used to assess the force of resistance at the different angles by the relative contribution that the collagen fibers would make in that particular direction on the basis of their orientation to the long axis. An angle of 27° from the long axis was assumed for *Loligo* (Shadwick and Gosline, 1984) and 23° for *Illex* (Kuo, 1989). Under these assumptions, predicted values of the relative ultimate tensile strengths compared to that in the longitudinal direction were calculated for squid mantle oriented perpendicular to the longitudinal axis (transverse) and at an angle of 45°. These were then compared to the actual measured values of the raw squid mantle for the two species of squid (Table IV).

As expected, the measured values were different from those predicted by our simple model that was based solely on the collagen fibers in the outer tunic. This model ignored the potential effect of the circumferential muscle fibers which would be expected to make their greatest contribution in the transverse direction. However, it is interesting and important to note that the predicted values

Table IV. Ratios of Predicted and Measured Values of Ultimate Tensile Strengths of Raw Squid Mantle

collagen fiber angle, deg	ratio of ultimate tensile strengths		
	longitudinal	transverse	45°
27 (*Loligo*)			
pred	1	0.51	0.71
measd	1.00 ■ 0.10	0.42 ± 0.08	0.52 ■ 0.07
23 (*Illex*)			
pred	1	0.42	0.71
measd	1.00 ± 0.15	0.31 ± 0.05	0.18 ± 0.02

in the transverse direction turned out to be overestimates of the actual measured values. This is the opposite of what would be expected if the muscle fibers were making an important contribution to the texture of the raw mantle. Comparison to the predicted values indicates that there must be other elements in the longitudinal direction which contribute to tensile properties. From what is known about the structure and location of collagen fibers in squid mantle, this would indicate that other longitudinal elements such as the IMF-1 fibers contribute to the tensile properties of raw squid mantle. In addition, it seems that these longitudinal IMF-1 fibers make more of a relative impact on the tensile properties than do the IMF-2 fibers which are oriented in a plane perpendicular to the longitudinal axis of the squid mantle.

The results obtained with the mantle oriented at an angle of 45° to the long axis are also of interest since there is a clear difference between the two species (Table IV). With *Loligo*, there is an increase in the measured ultimate tensile strength at 45° compared to that obtained in the transverse direction. This is expected, and the magnitude of the increase is not greatly different from that predicted, i.e., an increase of 0.1 (0.42 to 0.52) in the ratio compared to the expected 0.2 (0.51 to 0.71). With *Illex*, however, the situation is quite different, and the measured value of mantle cut with an angle of orientation at 45° is less than it is in the transverse direction and gives the lowest ultimate tensile strength of all the samples evaluated. The reason for this is not known. Thus, unique differences between the predicted and measured values of *Loligo* and *Illex* at a 45° angle might prove of particular interest if it can be related to structural or chemical differences between the species.

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