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MECHANICAL PROPERTIES IN RELATION TO CHEMICAL CONSTITUENTS OF POSTMOLT CUTICLE OF THE BLUE CRAB, *CALLINECTES SAPIDUS*

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Abstract—1. Cuticle of the crab, *Callinectes sapidus*, was tested for tensile strength, % elongation, calcium, protein, and chitin at various times postmolt.

2. Tensile strength and % elongation increased rapidly immediately postmolt, decreased rapidly through 10 hr postmolt, and then decreased slowly through 68 hr postmolt.

3. Cuticle protein and chitin content decreased gradually through 48 hr postmolt whereas calcium content increased and began to stabilize during this time period.

4. Selective removal of calcium resulted in an increase of both tensile strength and % elongation whereas removal of protein resulted in a decrease of both physical parameters. Removal of chitin resulted in a small decrease of elongation with no change in tensile strength.

INTRODUCTION

Insect cuticle is a two-phase material composed primarily of chitin plus protein (Andersen, 1973). Sclerotinization or crosslinking of some of this protein appears to be the primary means of "hardening" (Pryor, 1940). Crab cuticle at molt is similar to that of insects in that it is composed primarily of chitin and protein (Travis, 1965). However, crabs differ from insects during the postmolt "hardening" process because, in addition to sclerotinization (Dennell, 1947), there is deposition of calcium and magnesium salts into the protein–chitin matrix (Clarke & Wheller, 1922) and these salts become the major component of the 3-phase "hardened" cuticle.

The purpose of this investigation was to determine the relationship of some mechanical properties of crab cuticle with its postmolt chemical changes as the transition is made from a two-phase to a three-phase material.

MATERIALS AND METHODS

Collection of specimens

Male *Callinectes sapidus* were collected from the Rappahannock River and maintained in crab floats at Urbanna, Virginia. Salinity ranged from 7.5 to 12‰. The animals were allowed to molt and were transferred to a compartmentalized float until they were sacrificed. At various times postmolt, the entire carapace was removed, cleaned of adhering tissue by gently rubbing the cuticle with a soft bristle brush, sealed in labeled plastic bags, and stored at −20°C. At a later time, non-waisted 1 cm wide test strips were cut from the epibranchial-mesobranchial carapace (Rathbun, 1930). The orientation of the strips was diagonal to the anterio-posterior axis of the animal. The orientation should not effect the mechanical measurements as it has been shown that the chitin, and presumably protein, skeleton of prawn shell is laid down with no preferential orientation (Joffee *et al.*, 1975). The test strips were kept wet at all times and tensile properties were measured at room temperature. Thickness was measured using a micrometer.

Tensile strength and elongation were measured using a floor model Instron Tensile Tester at a gage of 12.7 mm and a crosshead speed of 0.508 mm/min.

Extractions from 24 hr *postmolt cuticle*

Calcium was removed from some cuticle strips prior to mechanical testing by treatment with saturated ethylenediamine tetraacetic acid (EDTA) for 4 hr at room temperature. Protein was removed from some cuticle strips prior to mechanical testing by treatment with a solution of Pronase E (Sigma Type XIV) at 116 units per strip in 0.05 M Tris plus 0.04% sodium azide at pH 7.5 for 28 hr at room temperature. Chitin was removed from some cuticle strips prior to mechanical testing by treatment with a solution of chitinase (Sigma C6137) at 22 units per strip in 0.05 M Tris plus 0.04% sodium azide at pH 7.5 for 7 days at room temperature. All extracted strips were rinsed twice with water and tested immediately.

Chemical assays

Total calcium, protein and chitin of all test strips were determined after mechanical testing as was the non-extracted cuticle adjacent to the test strips. Calcium was determined by first ashing preweighed air-dried samples. Residual ash was dissolved in 2 N HCl and tested with a Perkin–Elmer 370A Atomic Absorption Spectrometer using standard addition calibration with commercial standards (Fisher Scientific). Protein was determined by first extracting samples with 2 N HCl for 4 hr at room temperature and then with 2 N NaOH for 4 hr at 100°C. The two solutions were mixed and protein measured using Oyama & Eagle's (1956) modification of the method of Lowry *et*

al. (1951) with bovine serum albumin (Sigma) for standards. The extracted samples were again dried to constant weight and the residual material was a measure of chitin (Stevenson, 1969; Welinder, 1975).

RESULTS AND DISCUSSION

The cuticle tensile strength was found to increase rapidly immediately after molt from 5.9 to 15 N/mm^2 and then decrease to a value of 5.6 N/mm^2 by 24 hr postmolt and remained fairly constant through 68 hr postmolt (Fig. 1). Very similar results have been reported for *Locusta* adult femur cuticle (Hepburn & Joffe, 1974a). The percent elongation of crab cuticle was also found to increase postmolt starting at a value of 20.5% at molt and increasing to 28.9% by 15 min postmolt (Fig. 1). The elongation then steadily declined until at least 48 hr postmolt.

The chemical changes in blue crab cuticle postmolt are shown in Fig. 2. At molt, the dry cuticle is composed of 57% protein and 36% chitin with no measurable calcium. A few hours postmolt the percentage of cuticle as protein and chitin decreased and calcium salts became the major constituent. During this postmolt "hardening" of crab cuticle, there is a continued synthesis and deposition of protein, chitin and calcium into the cuticle for at least 30 days, along with continuous increase in cuticle thickness (unpublished). The increase in tensile strength immediately postmolt correlates with a rapid increase in exocuticle sclerotin immediately postmolt before the endocuticle begins to contribute to cuticle thickness (Vigh & Dendinger, 1982).

Insect cuticle "hardening" is primarily by sclerotinization of cuticular proteins (Anderson, 1973), and a correlation has been found between deposition of formamide-insoluble protein and the tensile breaking force of *Locusta* cuticle (Hepburn & Joffe, 1974b; Hepburn & Roberts, 1975). Crab cuticle development and "hardening" is more complex than in insects. In both, the epicuticle, along with the chitin–protein matrix of the exocuticle, are formed before molt. *Callinectes* cuticle at molt is 57% protein of which 28% of this is sclerotin (Vigh & Dendinger, 1982). After molt, the chitin–protein matrix of the endocuticle is deposited with concomitante mineralization and, at the same time, mineralization of the exocuticle occurs (Travis, 1965). By 68 hr postmolt, total protein has decreased to 12.8% of the cuticle and only 27% of this protein is sclerotin (Vigh & Dendinger, 1982).

The rapid decrease of tensile strength and elongation between 1 and 10 hr postmolt probably reflects mineralization prior to 10 hr postmolt at a level

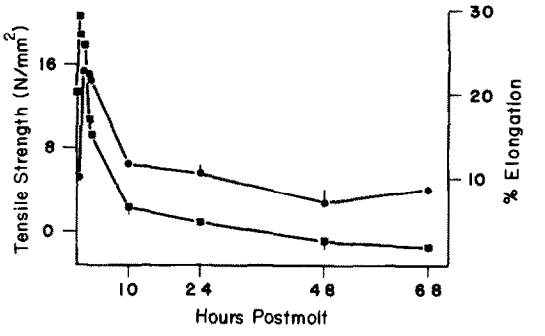

Fig. 1. Tensile strength and elongation of *Callinectes sapidus* carapace at various times postmolt. ● = tensile strength, ■ = % elongation.

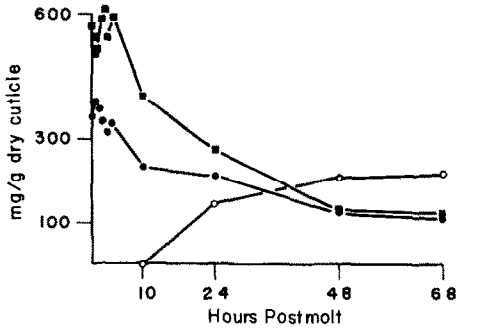

Fig. 2. Calcium, protein, and chitin content of *Callinectes sapidus* carapace at various times postmolt. ○ = calcium, ■ = protein, ● = chitin.

below the detection limits of the methods used here (see below). Calcium carbonate crystals in the cuticle of *Camarus pulex* have been detected 5 min postmolt (Reid, 1943) and in *Carcinus* sp. at 4 hr postmolt (Drach, 1937).

Calcium, protein and chitin were each selectively removed from 24 hr postmolt cuticle test strips in order to determine to what extent each component contributed to tensile strength. The amount of each component removed is shown in Table 1.

Removal of 96% of the calcium caused an increase in tensile strength by about 47% (Table 2). This result is consistant with the developmental sequence in that as calcium crystals are deposited in the protein–chitin matrix, it becomes more rigid and brittle. Generally, rigid materials have a lower percent elongation than

Table 1. Calcium, protein and chitin content of cuticle test strips from the carapace of 24 hr postmolt *Callinectes sapidus* before and after treatment

	Treatment									
	None	EDTA			Pronase			Chitinase		
		Pre	Post	% Change	Pre	Post	% Change	Pre	Post	% Change
Calcium (mg/g)	177.4	151.9	6.2	−95.9	138.9	146.4	+5	148.9	155.4	+4
Protein (mg/g)	185.5	193.7	186.1	−4	190.4	119.2	−37	181.5	172.6	−4.8
Chitin (mg/g)	271.6	233.5	147.5	+6	257.6	244.7	−5	243.1	176.5	−27.4

do vasoelastic materials (Wainwright *et al.*, 1976). Cuticle from the crab *Scylla serrata* has been shown to fail in an entirely brittle manner with a uniform fracture edge and no delamination of the lamellae (Hepburn *et al.*, 1975). Cuticle from the prawn *Penaeus mondon* was broken with brittle failure of the exocuticle and delamination of the endocuticle (Joffe *et al.*, 1975). Calcification is more extensive in the exocuticle than in the endocuticle. Apparently the hard, rigid crystals propagate the fracture plane through the softer protein–chitin matrix.

Removal of 27% of the chitin by chitinase resulted in a 47% reduction of tensile strength (Table 2). This is consistant with crab cuticle development in that chitin was 36% of the cuticle dry weight at molt, decreased to 21.5% by 24 hr postmolt and then to 11.4% by 10 days postmolt (Vigh & Dendinger, 1982). When crab shell has all calcium and protein removed, the breaking stress was decreased by 34% or, pure chitin contributed 66% of the tensile strength (Hepburn *et al.*, 1975). The small amount of chitin removed in our work resulted in a much greater decrease of tensile strength than expected. This could be due to one or both of the following: (1) our reduced chitin test strips still contained the normal amount of calcium crystals and these could have increased fracture propagation which would have resulted in artificially low tensile strength; (2) the small amount of chitin removed could have been involved in chitin–protein bonding which may contribute more strength than the chitin itself.

Removal of 37% of the cuticle protein resulted in only an 11% reduction of tensile strength. This was somewhat less than expected if the protein by itself, or the protein portion of the protein–chitin complex, contributes significantly to the tensile strength. Selective removal of proteins from larval cuticle of the beetle *Agenius zebra* indicate that sclerotin or sclerotin-chitin bonds are the most important factors contributing to tensile strength (Hepburn & Levy, 1975).

Although the pronase used in this work is fairly non-specific, it is possible that either the protein removed was not, by itself, important to tensile strength, or that the enzyme did not remove protein which was covalented to chitin.

A postmolt tensile strength decrease has also been reported in *Locusta* (Hepburn & Joffe, 1974a). In insects and crabs the pre-exuvial exocuticle becomes sclerotinized and then the less-sclerotinized endocuticle is synthesized postmolt. Therefore, the cuticle thickens faster than the rate of sclerotinization, resulting in decreasing tensile strength (Hepburn & Joffe, 1974a,b). This may also be true for Crustacea but calcification also appears to play a role in this decreased tensile strength. Calcium removal without any change in protein or chitin content resulted in tensile strength increase. Calcified Crustacea cuticle generally has a lower tensile strength than does insect cuticle when tested by the same investigator. *Carcinus* is 32 N/mm^2 vs 69 N/mm^2 for *Pachynoda* (Wainwright *et al.*, 1976). Prawn was 17 to 28 N/mm^2 (Joffe *et al.*, 1975) and crab was 30 N/mm^2 (Hepburn *et al.*, 1975) vs a range of 78 to 257 N/mm^2 for *Locusta* (Hepburn & Joffe, 1974a) and 52 to 506 N/mm^2 for various other insects (Hepburn & Roberts, 1975).

This greater postmolt decrease in tensile strength with calcification of Crustacean cuticle is indicated by the fact that in one postmolt study of an insect, the tensile strength increased immediately after molt by a factor of 2.8 and then declined to the value at molt (Hepburn & Joffe, 1974a). In this study, crab cuticle tensile strength also increased immediately postmolt by a factor of 2.7 but then declined to a value only half that at molt.

Table 2. Tensile strength and elongation of cuticle test strips from the carapace of 24 hr postmolt *Callinectes sapidus* with removal of calcium, protein, or chitin

Material removed	Tensile strength (Newtons/mm^2)	Elongation (%)
None	9.65 ± 1.33 (8)	7.53 ± 1.15 (8)
Calcium	14.23 ± 0.80 (12)	13.43 ± 1.07 (12)
Protein	5.10 ± 0.48 (14)	3.50 ± 0.19 (14)
Chitin	8.63 ± 0.88 (14)	5.10 ± 0.27 (14)

Values are means ± SEM of (*n*) samples.

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