

Chitin in Sea Anemone Shells

Abstract. *Chitin, which is widely distributed among life forms, is well documented in the coelenterate class Hydrozoa and is contained in one member of class Scyphozoa. In class Anthozoa, hard corals synthesize it but soft corals do not. Chitin was identified by infrared spectrophotometry in the trochoid shell of the actinian Stylobates. It constitutes 1.7 percent of the shell by weight, the rest probably being protein. The ability of sea anemones to synthesize chitin is thereby confirmed.*

Chitin is a linear polysaccharide similar in many respects to cellulose (1-3). Jeuniaux (1, 4) concluded that its wide distribution among fungi, plants, protists, and animals is evidence that the capacity to synthesize it evolved early.

Chitin, in the restricted sense, has repeatedly been demonstrated in the perisarc of both calyptoblastic and gymnoblastic hydroids; it occurs in chondrophore and millepore hydrozoans as well (1, 2, 4). Jeuniaux once proposed (1) that this feature distinguishes hydrozoans from other coelenterates but abandoned the idea (4) when chitin was demonstrated, albeit rarely, in members of other classes. Among scyphozoans, only the podocyst of *Aurelia aurita* is known to contain this substance (5).

Chitin is more common among anthozoans, but may be totally absent in some groups of the class; the several species of octocorals that have been analyzed lack it (1, 6). The first record of chitin in a

nonhydrozoan coelenterate was from the reef-forming coral *Pocillopora damicornis* (7). Wainwright (7) proposed that this material, which makes up 0.01 to 0.1 percent of the dry coral skeleton by weight, and which was identified by x-ray diffraction and biochemical tests, forms the organic matrix upon which calcification occurs. Initially Jeuniaux (1) acknowledged only that the physical and chemical properties of the compound were near to those of chitin, but chitin has since been found in other corals (6).

Pelagic anemones of family Minyadidae secrete from their pedal end a "chitinous mass" that keeps them afloat (8); a cuticle envelops the column of many species of actinians; sea anemones of the genus *Adamsia* living on gastropod shells inhabited by hermit crabs may extend the shell's lip by secreting what is variously called a cuticle (9), "chitine" (10), a horny membrane (10, 11), or

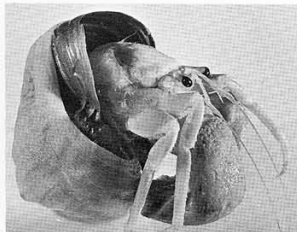


Fig. 1 (left). Shell of *Stylobates aeneus*; greatest width, 60 mm. Fig. 2 (right). Anemone shell occupied by hermit crab *Parapagurus doffeini* and enveloped by actinian *Stylobates aeneus* (apex of shell at lower left, anemone oral disk on opposite side; specimen CASIBP 011029).

solidified mucus (12). None of these materials has been analyzed chemically. Although Muzzarelli (3) reported chitin in *Metridium senile* and Wilfert and Peters (6) obtained a positive chitosan test in preserved specimens of *Urticina* (= *Tealia*) *felina*, neither of these anemones secrete ectodermal products and are therefore unlikely sources of chitin. Even though chitin was suggested to be present in the mesoglea of *Edwardia callimorpha*, it was not found in the only sea anemone examined by Jeuniaux (1).

Dunn *et al.* (13) discussed the actinian *Stylobates aeneus* Dall, which produces a trochoid "chitinous" shell [Fig. 1; figure 3 in (13)] shaped so much like that of a snail that it was initially considered to be one. Thin and parchment-like in texture, it is secreted by the anemone's pedal disk ectoderm, which is glandular [figure 2 in (13)]. More than 100 of these bronze-colored shells, each containing a living hermit crab *Parapagurus doffeini* Balss and covered by the anemone that had produced it (13) (Fig. 2), were trapped in Hawaiian seas (14).

Before chemical analysis of the shell, its apex, which usually contains remnants of a calcareous shell upon which

the anemone first settled (13), was removed. Extraction of chitin (15) followed ultrasonic washing with dilute (about 1 percent) aqueous sodium dodecyl sulfate, several rinses with deionized water, and drying at 100°C, which caused the shells to become brittle. The product, a fluffy, whitish solid, was washed several times with deionized water and collected on filter paper where it dried to form a thin film. The yield was approximately 1.7 percent by weight.

The infrared absorption spectrum obtained from a small portion of the film (Fig. 3C) agreed closely with that from commercial crab chitin (Fig. 3A) and published spectra (3, 16). Especially characteristic are the amide I band around 1640 cm^{-1} and the amide II band near 1540 cm^{-1} , in addition to the numerous intense bands between 1000 and 1150 cm^{-1} that are typical of cellulose. The infrared spectrum of cleaned, dried, and powdered, but otherwise unprocessed shell (Fig. 3B) approximated that of the extracted material but more closely resembled one typical of protein (17). The conventional assay for chitin (18), by first converting it to chitosan through incubation in concentrated KOH, which

did not dissolve the shell, produced the diagnostic violet color upon addition of 0.2 percent iodine solution, and brown with subsequent exposure to 1 percent H_2SO_4 ; 75 percent H_2SO_4 then dissolved it. Thus the shell does contain chitin, in the restricted sense.

Unprocessed shell ignited at 500°C in a muffle furnace yielded 1.2 percent ash. Spectrographic elemental analysis of the ash showed appreciable amounts of Ca, Si, and Mg, small amounts of Al and Cu, and traces of several other metals. The 98+ percent of shell that is not chitin is probably mainly protein, as judged by the infrared spectrum of the untreated shell and the ash content. Insect cuticles generally contain no more than 25 to 50 percent chitin on a dry weight basis, and some arthropods lack detectable chitin altogether (2). *Stylobates* shells exhibit no macroscopic evidence of mineralization. The fact that most shells are easily dented, much like Ping-Pong balls, suggests that the associated metals may be bound to the protein.

Stylobates is the first sea anemone proved capable of synthesizing chitin and the first anthozoan known to do so in appreciable quantities. Our findings support Jeuniaux's (1, 4) assertion that chitin in coelenterates is synthesized by the ectoderm. *Stylobates* is a member of the largest actinian family, Actiniidae, no other member of which is known to secrete a product resembling chitin. The Minydidae belong to the same subtribe (Eudomyaria) as the Actiniidae, but *Adamsia* and most of the cuticle-forming species belong to another subtribe (Acontiarina) (6). Although it is likely that the ability to synthesize chitin is ancient (1), these data suggest that that trait may rarely be entirely lost in a lineage. Rather, it is retained in isolated taxa. Perhaps the responsible biochemical pathways are seldom actually lost, so that chitin production is facultatively possible in most species. The sea anemones appear to be an ideal group to analyze for patterns of secretory ability.

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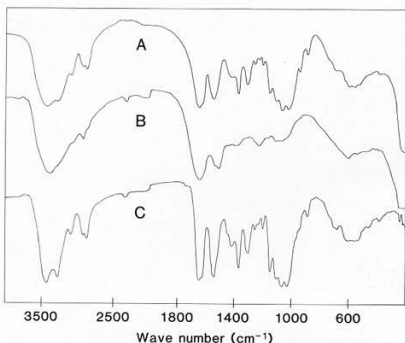


Fig. 3. Infrared absorption spectra (Perkin-Elmer spectrophotometer, model 283). (A) Commercial purified chitin from crab shells (Sigma, No. C-3641) (dispersed in KBr); (B) unprocessed *Stylobates* shell (dispersed in KBr); (C) extracted *Stylobates* shell (un-supported film).

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