A Disulfide-Linked Collagenous Protein of Nematocyst Capsules

Abstract. *The major protein component present in the capsule and thread of a sea anemone nematocyst consists of monomers of a collagen-like protein linked by disulfide bonds. Purified nematocyst capsules and threads are rich in hydroxyproline, and dissolved by disulfide reducing agents. Electrophoresis of the dissolved component on acrylamide gel results in a single detectable protein band.*

We have found an unusual type of intracellular structural protein composed of monomers of a collagen-like protein linked by disulfide bonds. This protein, together with associated carbohydrates, makes up the major part of the capsule wall and the thread of nematocysts from sea anemones. Nematocysts, highly complex intracellular secretion products diagnostic of the Cnidaria, consist of a capsule containing an eversible thread continuous with the capsule wall at one end and stoppered there by an operculum (Fig. 1). During discharge of the nematocyst, the thread everts and, depending on the type of nematocyst, may either adhere to adjacent surfaces or penetrate the prey, simultaneously injecting a toxin.

Previous reports on the protein composition of these structures appeared contradictory. Based upon the histochemical demonstration of cystine (*1*) and the solubility of some sea anemone nematocysts in alkaline sodium sulfite (*2*) or alkaline sodium thioglycolate (*3*), it was inferred that the capsule might contain a keratin. On the other hand, reports of large amounts of hydroxyproline in nematocyst capsules (*4–6*) indicated that the protein might be a member of the collagen group of proteins. Collagens, however, had not been shown to contain disulfide cross links until recently, when McBride and Harrington (*7*) reported that polypeptide chains of the collagen soluble in neutral salt solution and derived from *Ascaris* cuticle are cross-linked by disulfide bridges.

We used the acontia (*8*) nematocysts of the sea anemone *Aiptasia pallida*. Pure suspensions of undischarged nematocysts, consisting almost entirely of microbasic mastigophores with a few isorhizas, were prepared by a modification and elaboration (*9*) of the method of Yanagita (*10*); acontia threads, when placed in 1.0*M* sodium citrate, expelled many of their nematocysts, undischarged, into the medium. The spent acontia were removed, and the resultant nematocyst suspension was washed three times in fresh citrate solution. Discharged nematocysts were obtained by placing undischarged ones in distilled water for 10 minutes. The discharged nematocysts were washed twice in distilled water to rid them of most of the remaining intracapsular fluids. Some were used immediately after washing; others were dried over $CaCl_2$ for 24 hours before use.

Aiptasia nematocysts, like those of *Hydra littoralis* and *Physalia*, have a high content of hydroxyproline. A purified preparation of discharged nematocysts had 29.9 μg of hydroxyproline per milligram dry weight as measured by a modification (*11*) of the Stegemann method (*12*). The nitrogen content of the preparation, as determined by the micro-Conway adaptation of the Nessler method, was 0.056 mg of nitrogen per milligram of dry weight. If this nitrogen is assumed to be derived solely from protein, then the discharged nematocysts would contain 0.35 mg of protein per milligram dry weight, of which hydroxyproline would compose about 8.5 percent of the total protein. However, paper-chromatographic analysis shows that part of the nitrogen is derived from hexosamines present in the polysaccharides of the discharged nematocysts. Mucopolysaccharide was indicated histochemically in the discharged nematocysts by a positive periodic acid-Schiff stain, and by a faint metachromasia with 1 percent thionine. While the amount of carbohydrates present in these samples has not been determined quantitatively, the amount of polysaccharide associated with invertebrate collagens is considered to be high in comparison to that associated with vertebrate collagens (*13*). Hence, if the contribution of nitrogen by hexosamines were taken into account, then the 8.5 percent value for hydroxyproline content might be higher and similar to that found in vertebrate collagens and in hydra nematocysts (*4*). Despite their high content of hydroxyproline, *Aiptasia* nematocysts were not solubilized by collagenase. Elastase also was ineffective.

The presence of disulfides as components of the nematocyst capsule and thread was demonstrated through experiments in which the nematocyst was dissolved when placed in disulfide reducing agents such as dithiothreitol, sodium thioglycolate, cysteine, and mercaptoethanol at alkaline *p*H. Upon addition of these reagents to undischarged nematocysts, nearly all discharged with the thread everting. In some cases, however, nematocyst threads were propelled through the opercular opening uneverted and remained in this condition. After discharge, the first sign of nematocyst dissolution was a series of marked bucklings occurring along the capsule wall. Soon the wall was no longer visible; only a fine membrane delineating the original capsule form persisted.

In all cases, the higher the *p*H, the faster the reducing agent dissolved the nematocyst capsules (*14*). Each reagent showed different degrees of effectiveness in dissolving the capsules; dithiothreitol, which can maintain proteins in a permanently reduced state (*15*), was very effective (*14*), acting at low concentrations and at only a slightly alkaline *p*H.

The dissolution of the everted thread, in contrast to that of the capsule, re-

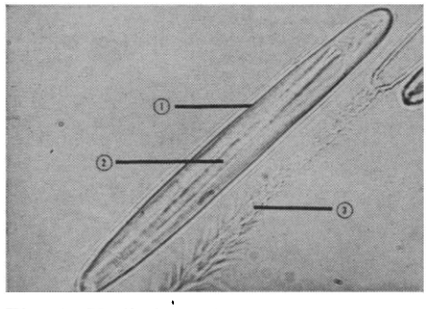

Fig. 1. Undischarged nematocyst capsule (1) containing the uneverted thread (2) attached at the lower end. A discharged, everted thread (3) can be seen to contain numerous barbs which constitute the armature (× 3000).

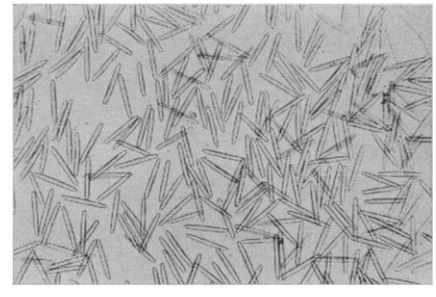

Fig. 2. Purified suspension of *Aiptasia* nematocysts consisting almost entirely of the large microbasic mastigophores and a few smaller isorhizas (× 320).

quired a relatively higher *p*H and longer periods of exposure to the reducing agents (*14*). Regardless of the reagent used, it was not possible to dissolve the uneverted threads nor the barbs of the everted ones, even at a *p*H as high as 11. These observations show that the disulfides of the thread are not available for reduction until the thread everts. Also, although these observations cannot account for the insolubility of the barbs, they do suggest a means for purifying the barbs for future analyses.

Disulfide reducing agents are not known to dissolve other proteins containing hydroxyproline. Accordingly, we obtained negative results with collagen of bovine tendon and with mesoglea from *Aequorea aequorea*. Hydroxyproline is present in significant amounts in mesoglea (3.6 percent) of scyphomedusae (*16*) and in the mesoglea (2 to 3 percent) of the hydromedusae used in these experiments. On the other hand, we find nematocysts from hydra and *Physalia*, both rich in hydroxyproline (*4–6*), are dissolved in alkaline thioglycolate.

The nematocysts were treated with performic acid, a reagent which converts disulfides to sulfonates. After the acid was added to them, nematocyst capsules buckled in a manner similar to that observed when they were treated with reducing agents. In performic acid, however, dissolution did not follow. The nematocysts treated with performate stained with alcian blue; this staining procedure is used to detect proteins containing disulfides, such as keratins (*17*). Such staining of the capsule wall and thread did not occur unless the treatment with performate preceded the application of the dye. Furthermore, neither buckling nor dissolution occurred in sodium citrate, formic acid, alkaline sodium glycolate, methionine, or ethanol.

To determine the number and relative sizes of the subunits released from the nematocyst by reduction with disulfide, electrophoresis of the dissolution products on acrylamide gel was analyzed. Samples of dried, discharged nematocysts (0.5 mg) were dissolved either in 0.1 ml of 0.25*M* thioglycolate, *p*H 9.6, or in 6.5 $\times$ $10^{-3}M$ dithiothreitol, *p*H 9.6. The samples were applied to tubes containing 7.5 percent gel in a tris(hydroxymethyl) aminomethane-glycine buffer, *p*H 8.5, and the current adjusted to 5 ma per tube of gel. Gels were stained in amido-schwarz or Coomassie blue. Those stained with amido-schwarz were destained electrophoretically in 7.5 percent acetic acid. As little as 0.1 μg of protein (Armour serum albumin) could be detected with these dyes.

Electrophoresis of dissolved nematocysts repeatedly resulted in one sharp detectable band. Since the migration of this band was approximately 80 percent of the distance traveled by the buffer front, as compared with 48 percent for serum albumin, the protein may be of low molecular weight, or of high negative charge, or both.

Our results resolve the apparently contradictory findings that nematocysts show some chemical characteristics of both collagens and keratins. Furthermore, they help explain such properties of nematocysts as their resistance to gelatinization by autoclaving (*5*), a property usually associated with collagens rich in hydroxyproline. Perhaps the disulfide bridges help stabilize the protein of the nematocyst so that gelatinization does not occur. The resistance of nematocysts to digestion by collagenase might be similarly explained. The differential solubilization of the capsule and everted thread (*14*) may reflect variations either in the aggregation of the nematocyst protein or in the association of that protein with various polysaccharides.

As pointed out by McBride and Harrington (*7*), some of the previously described collagens (or collagen-like proteins) may also have cystine cross-linkages. Piez and Gross (*18*) report the presence of cystine in some collagens isolated from sponges, coelenterates, and echinoderms. Hence, it might be profitable to reexamine other collagens.

RICHARD BLANQUET*
Laboratory for Quantitative Biology, University of Miami, Coral Gables, Florida, and *Duke University Marine Laboratory, Beaufort, North Carolina*
HOWARD M. LENHOFF
Laboratory for Quantitative Biology and *Department of Biology, University of Miami, Coral Gables, Florida*

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14. The times for the capsule to dissolve at the respective stated *p*H values: (i) in 0.0065*M* dithiothreitol at *p*H 9.4, 8.5, and 7.5 were 1, 3, and 9 minutes; (ii) in 0.25*M* sodium thioglycolate at *p*H 9.6, 8.9, 8.1, 7.1 and 6.2 were 1, 3, 29, 90, and ∞ minutes; (iii) in cysteine at *p*H 10.1, 9.1, and 8.6 were 1, 3, and ∞ minutes; and (iv) in 14.2*M* mercaptoethanol at *p*H 8.4 were 5 minutes. The infinity symbol designates that no visible dissolution took place during 2 hours of observation. The everted threads, although observed under all the above conditions, dissolved only in the dithiothreitol at *p*H 9.4 and 8.5 in 3 and 30 minutes, respectively, and in sodium thioglycolate at *p*H 9.6 in 5 minutes.
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* Present address: Laboratory for Quantitative Biology, University of Miami, Coral Gables, Florida 33124.

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Pholidostrophiid Brachiopods: Origin of the Nacreous Luster

Abstract. *The "nacreous" luster characteristic of the pholidostrophiid group of fossil brachiopods results from a shell structure that produces superimposed sets of natural optical-diffraction gratings made of calcite. The wall structure is crossed lamellar, parallel to the shell surface; thus flakiness and development of reflecting surfaces are facilitated.*

The iridescent pearly luster characteristic of many molluscan shells is commonly found in the shell layer that is composed of horizontal lamellae consisting of many tabular aragonite crystals, and which is referred to as the nacre or nacreous layer. Shell layers that are composed of similar lamellae but mineralogically made up of calcite crystals are referred to as calcitostracum (*1*). The luster of calcitostracum is seldom as well developed as it is in most nacreous layers; thus it is species whose shells are wholly or partly aragonitic that are of commercial interest as sources of pearls (cultured and natural) and mother-of-pearl.

The luster associated with this nacre results from two combined optical phenomena (*2*): (i) thin-film interference