The Organisation and Chemistry of the Byssus of some Bivalves of the Waltair Coast, India

BY

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(3 Text figures)

INTRODUCTION

"ADULT BIVALVES and related mussels secrete a complex extra-organismal structure, the 'Byssus' which serves as a mechanism by which these sessile molluscs attach to littoral substrata" (YONGE, 1962). The study on byssus dates back to the late 17th century when HEIDE (1684) made a study of the byssus with its roots in the foot of the animal but he considered it to be a plant. In the early 18th century, RÉAUMUR (1711) gave the first accurate description of the byssus of *Mytilus*. COUPIN (1892) and BOUTAN (1895) investigated the byssus in a wide range of lamellibranchs. X-ray diffraction photographs of the byssus threads of *Mytilus* and *Pinna* were furnished by FAURÉ-FRÉMIET & BOUDONY (1938), and they reported them to be of collagen type. BROWN (1952) claimed that the fibres were collagen hardened and were comparable to those of the insect cuticle. MELNICK (1958) reported the presence of collagen in the phylum Mollusca. PIKKARAINEN *et al.* (1968) reported on the occurrence of collagens in *Mytilus edulis* and *Pinna nobilis*. This was followed by some important contributions on the presence of collagen in the byssus of *M. edulis* (PUJOL *et al.*, 1970), on the analysis of secretions of the byssus (MAHÉO, 1970), on the biophysical characteristics of the byssus threads (BAIRATI & VITELLARO, 1970) and on tanning in byssus threads (RAVINDRANATH & RAMALINGAM, 1972). Description of byssus in *Arca zebra* was furnished by PETER *et al.* (1973), while ENGEL *et al.* (1971), SCHWARTZ *et al.* (1972) and BOWEN *et al.* (1974) made further studies on the attachment discs of the byssus threads. TAMARIN *et al.* (1976) are of the opinion that the formation of a colloidal dispersion in water is necessary for the attachment of active sites. Extensive morphological and histochemical studies on byssus in *Lithodomus lithophaga* were made by BOLOGNONI *et al.* (1975). Recently, ALLEN *et al.* (1976) made observations on the formation and mechanical properties of the byssus threads in *Mytilus edulis*.

MATERIALS AND METHODS

The present work deals with the physical properties as well as the solubility of the byssus threads in 11 species of bivalves, which have been collected in and around Visakhapatnam. They are *Perna viridis, Perna indica, Septifer bilocularis, Modiolus metcalfei, Arca symmetrica, Barbatia obliquata, Isognomon nucleus, Pinctada margaritifera, Pinna vexillum, Beguina variegata* and *Mytilopsis sallei*.

The byssus threads of these bivalves were carefully separated from their foot along with the root region. They were then washed thoroughly with water to remove the adhering sand and other particles. A single, whole byssus thread was separated from the bundle and examined under the microscope.

For the solubility tests, the byssus threads were tested in both cold and boiling water, ammonia, alcohol, concentrated hydrochloric acid, concentrated hot HCl, concentrated sulphuric acid, concentrated nitric acid, hot concentrated acetic acid, cold concentrated acetic acid, aqueous alkaline sodium sulphide, hot concentrated potash, aqueous sodium hypochlorite, aqueous lithium iodide and lead acetate tests. A dilute solution of ferric chloride is used to find the reaction of orthodiphenols.

OBSERVATIONS

In *Arca symmetrica* (Figure 1 A) and *Barbatia obliquata* (Figure 1 B) the byssus threads are yellowish brown in colour and translucent. They are strip-like without a distinct division being made of a root, stem and the threads. The proximal part of the byssus is embedded in the posterior groove of the foot. At the distal part are the attachment discs by which the byssus threads get attached to the substratum. In the case of *Modiolus metcalfei* (Figures 1 C & c), *Septifer bilocularis* (Figures 1 D

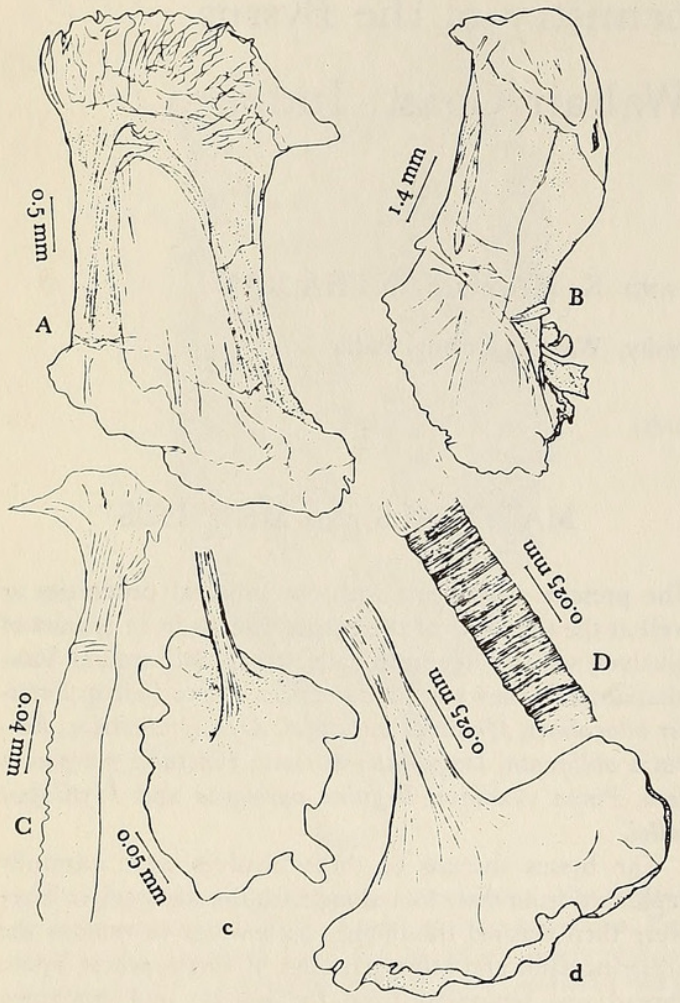

Figure 1

A: Byssus thread of *Arca symmetrica*
B: Byssus thread of *Barbatia obliquata*
C: Corrugations seen on one side of the thread of *Modiolus metcalfei*
c: Attachment disc of *Modiolus metcalfei*
D: Soft flattened part of the thread in *Septifer bilocularis*
d: Attachment disc of *Septifer bilocularis*

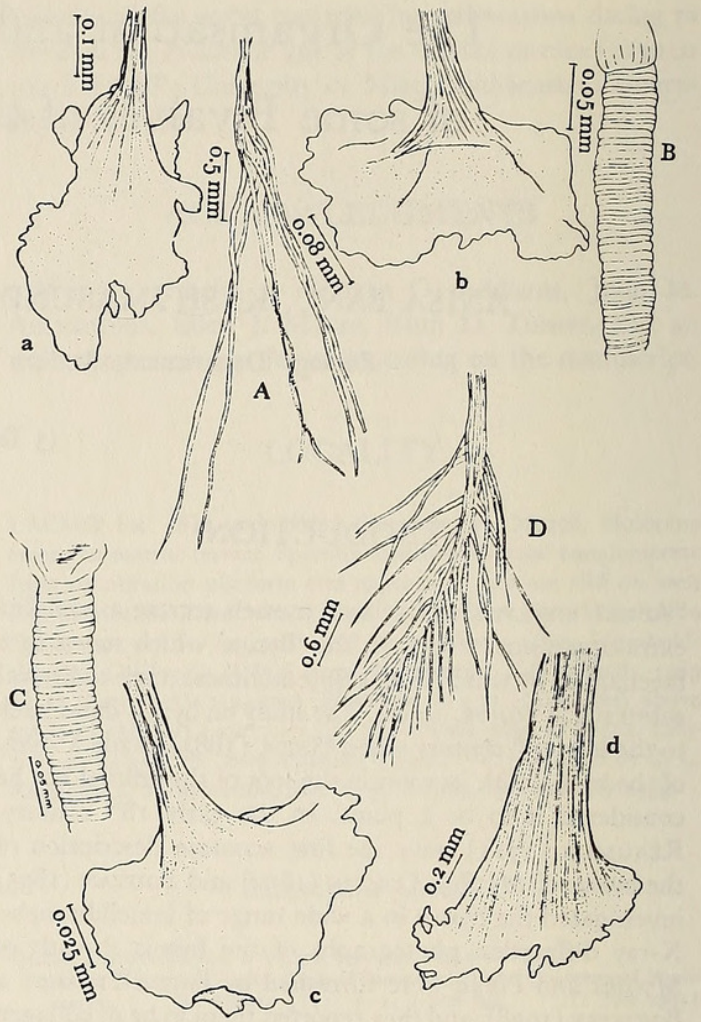

Figure 2

A: Branched proximal part of the byssus thread of *Isognomon nucleus*
a: Attachment disc of *Isognomon nucleus*
B: Soft, flat part of the byssus thread of *Perna viridis*
b: Attachment disc of *Perna viridis*
C: Proximal part of the byssus thread of *Perna indica*
c: Attachment disc of *Perna indica*
D: Branched proximal part of the byssus thread of *Pinctada margaritifera*
d: Attachment disc of *Pinctada margaritifera*

& d), *Perna viridis* (Figures 2 B & b), *Perna indica* (Figures 2 C & c) and *Pinna vexillum* (Figures 3 C & c), the byssus threads when just formed are yellowish brown, translucent and soft in appearance. But in all these forms the mature byssus threads are tough and dark brown in colour. In all these cases the threads are divisible into a root region which is embedded in the byssus gland at the region of the posterior groove of the foot, and a stem region that is continuous with the root region and is hard to the touch. To this stem are attached thousands of byssus threads. Each byssus thread again shows a posterior region which is flat and soft and which occupies about $\frac{1}{4}$ of the length of the byssus thread. These threads are attached to the stem by a ring of material at the proximal region. The surface of this proximal part is corrugated completely in *Perna viridis, Perna indica* and *Septifer bilocularis*. In *S. bilocularis* the corrugations are more numerous compared to those of the former 2 species.

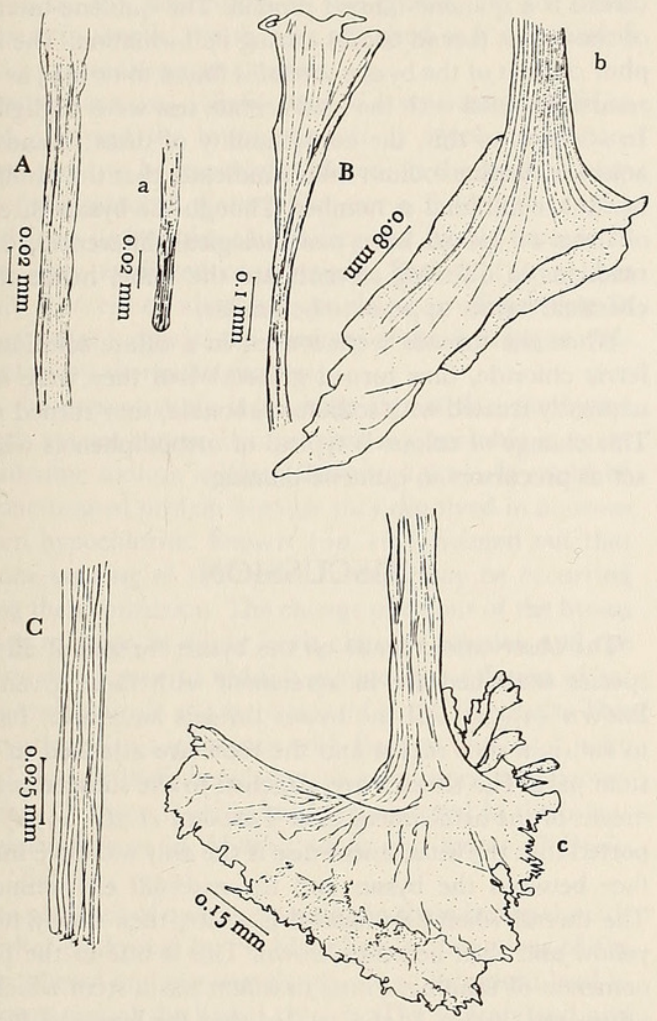

Figure 3

A: Proximal region of the byssus thread of *Beguina variegata*
a: Rounded tip of *Beguina variegata*
B: Flattened part of the byssus thread of *Mytilopsis sallei*
b: Attachment disc of *Mytilopsis sallei*
C: Proximal part of the byssus thread of *Pinna vexillum*
c: Attachment disc of *Pinna vexillum*

In *Modiolus metcalfei* these corrugations are seen only on one side, and the other side is a straight line. In *Mytilopsis sallei* (Figures 3 B & b) the posterior region of the byssus thread is flat without any corrugations of any sort. It is quite hard when compared to the byssus of *Perna viridis* and *Perna indica*. But the ring part by which this is attached to the stem region of the byssus thread is quite conspicuous. In *Beguina variegata* (Figures 3 A & a) there is no distinct division into a posterior soft and a distal hard cylindrical portion. There is also no distinct, separate attachment disc by which these threads get attached to the substratum. The entire thread is cylindrical and hard, with a distal rounded tip. It is attached to the substratum with this rounded tip. Continuous with this proximal, soft, flat portion is a rounded cylindrical hard portion of the byssus thread which occupies the remaining $\frac{3}{4}$ of the length of the byssus thread. This situation is observed in *Perna viridis, Perna indica, Modiolus metcalfei, Mytilopsis sallei* and *Septifer bilocularis*.

In *Pinna vexillum* the byssus thread is remarkable in that the stem region is bifurcated in the proximal portion, each branch having a separate root region by which it is embedded in the byssus gland. To the stem part are attached the byssus threads. In this species there is no soft, flat, corrugated proximal region, the entire thread being straight, cylindrical and hard.

In *Isognomon nucleus* (Figure 2 A & a) and *Pinctada margaritifera* (Figure 2 D & d) the proximal region of the thread is soft, flat, yellowish green in colour and not corrugated, but is branched. This forms $\frac{1}{3}$ of the length of the thread. The remaining $\frac{2}{3}$ of the thread is hard, flat, and green in colour.

At the distal end of the byssus thread is the attachment disc which is flower-like except in *Pinctada margaritifera, Isognomon nucleus, Barbatia obliquata* and *Arca symmetrica*. The attachment proper is soft and translucent in appearance and light yellowish in colour.

Byssus threads in *Barbatia obliquata* and *Arca symmetrica* appear to occupy the entire length of the groove. They are soft in both forms. In *Pinna vexillum* they are hard and cylindrical. In *Beguina variegata* each byssus thread is smooth and all the threads joined together give a very soft, smooth and bushy appearance. In *Pinctada margaritifera* and *Isognomon nucleus* the threads are dark green in colour except for the root part which is of a lighter shade and transparent. Unlike the byssus root of other species, the root in these 2 species is branched.

In order to determine the nature of the byssus threads, the following solubility tests were conducted. In all the species no part of the byssus is soluble in cold water. Even in boiling water they are not soluble except that they undergo writhing contractions. Maximum shortening is observed in the byssus root region, but the contractions in the thread region are not much pronounced. None of them are soluble either in ammonia or in alcohol. In concentrated HCl the byssus threads dissolved slowly, the byssus itself turning to a wine-red colour. In concentrated hot HCl the reaction is hastened. However, in *Pinna vexillum,* the stem region of the byssus thread dissolves at a moderate rate. Swelling or dissolution in dilute acids or alkalis is stated to indicate the presence of electrovalent

linkages. The dissolution of byssus threads in concentrated sulfuric acid is quite rapid except in *Mytilopsis sallei, Isognomon nucleus* and *Pinctada margaritifera,* where it is slow. In *Arca symmetrica* and *Barbatia obliquata* rapid dissolution is observed. In nitric acid all the threads dissolve slowly, yielding a yellow solution, but in *Arca symmetrica* and *Barbatia obliquata* the process is very slow. They do not dissolve in aqueous alkaline sodium sulfide, which generally is supposed to dissolve all types of keratin material. Hot concentrated potash dissolves the byssus material except in *Mytilopsis sallei* and *Barbatia obliquata.* All the byssus threads are slightly soluble in hot concentrated acetic acid, but complete dissolution does not occur. In cold concentrated acetic acid they dissolve very slowly.

Aqueous sodium hypochlorite, which dissolves quinone tanned proteins (Brown, 1952), has been found to dissolve all parts of the byssus threads of all the species examined. This suggests the presence of aromatic tanning. The dissolution process progressively increases on application of heat. The lead acetate test for sulphur has little effect on the byssus threads, indicating that sulphur content is low. Agents, such as aqueous lithium iodide, cause swelling of the root region of the byssus threads. The fact that mature threads dissolve at a slower rate than the younger threads indicates that they are older and harder and hence slow to dissolve.

Orthodiphenols react with even a dilute solution of ferric chloride to produce a green colour turning to red on addition of sodium bicarbonate. A dilute solution of ferric chloride was used for soaking the byssus threads. The change of colour to green in the stem and the threads in the case of *Perna viridis, Perna indica, Modiolus metcalfei, Isognomon nucleus, Pinna vexillum* and *Pinctada margaritifera* is very pronounced. In *Mytilopsis sallei* no change could be observed in mature byssus threads, but the younger threads turned green. This may be due to the fact that in the mature threads, being dark brown in colour, the change in colour was masked. The change of colour in the byssus threads of *Septifer bilocularis* and *Beguina variegata* is faint. In *Barbatia obliquata* and *Arca symmetrica* the root part turns green. On soaking them in sodium carbonate, the whole part turns red in all the species.

From these solubility tests it could be inferred that the byssus threads are not keratin, because none of them is soluble in aqueous alkaline sodium sulfide, which specifically dissolves keratin by breaking the electrovalent and disulfide bonds. But they dissolved completely in aqueous sodium hypochlorite, which dissolves quinone-tanned proteins. Hence it could be concluded that the byssus thread is a quinone-tanned protein. The quinone-tanning of the byssus thread occurs during its formation. The sulphur content of the byssus thread is found to be low, as the results obtained with the lead acetate test were negligible. In addition to this, the non-solubility of these threads in aqueous alkaline sodium sulfide indicates that the disulfide bonds are minimal in number. Though the byssus threads of these 11 species have morphological differences, their reactions in differing solvents are the same, hence their chemical nature appears to be similar.

When the threads were soaked in a dilute solution of ferric chloride, they turned green. When they were subsequently treated with sodium carbonate, they turned red. This change of colour is typical of orthodiphenols which act as precursors in quinone-tanning.

DISCUSSION

The observations made on the byssus threads of all the species examined are in agreement with those given by Brown (1952). All the byssus threads have been found to end in a root region and the roots are attached to the stem part. The threads are attached to the substratum by means of the attachment disc. Tamarin *et al.* (1976) reported that the attachment disc is the only adhesive interface between the byssus and the external environment. The thread when first formed is white, then slowly turns yellow and then becomes brown. This is due to the phenomenon of tanning. *Pinna vexillum* has a stem which is bifurcated. Pujol (1967) stated that the byssus of *Pinna nobilis* corresponds only to the stem of *Mytilus edulis.*

In the present study it has been revealed that the posterior corrugated root region is the extensible part in a byssus thread. Mercer (1952) has also shown that the byssus threads are extensible. However, the degree of extensibility varies from fibre to fibre. Brown (1952) stated that the proximal region of the byssus thread is the most extensible but Allen *et al.* (1976) are not in agreement with this statement.

According to Tamarin (1975), the byssus stem is formed by the secretions of the cells of the musculo-glandular region at the base of the stem generator and that secretion pressure in the gland forces it out. Brown (1952) is of the opinion that the formation of the thread is associated with periodic secretory activity at the mouth of the byssus gland and the newly secreted material does not fuse with that previously secreted. From our observations it has been revealed that as the material is being added from the glands, the stem increases in length. The threads are formed in the groove and the attachment disc is

formed by the depression of the groove. As the stem increases in length, the threads attached to it are carried far away from the groove region.

SCHLOSSBERGER (1856) and KRUKENBERG (1881 & 1882) analysed the byssus threads of *Mytilus* and concluded them to be conchiolin. Based on the sulphur content of the material, FAURÉ-FRÉMIET & BOUDONY (1938) stated it to be keratin. On boiling the byssus threads in water, they exhibited contractions. BROWN (1952) has shown that either alteration in shape or dissolution in boiling water indicates the presence of weak linkages easily broken by thermal agitation.

In the present study it has been shown that the byssus threads are not keratin because they are insoluble in aqueous alkaline sodium sulfide (BROWN, 1952). It could be quinone-tanned protein because they dissolved in aqueous sodium hypochlorite. BROWN (*op. cit.*) pointed out that quinone-tanning of the byssus threads may be occurring during their formation. The change of colour of the byssus threads to green in dilute ferric chloride solution and the subsequent change to reddish mauve in a solution of sodium carbonate is characteristic of orthodiphenols. This observation is in accordance with BROWN (*op. cit.*), who stated that orthodiphenols act as precursors in quinone-tanning. Quinone-tanning has been reported in the byssus and periostracum of *Mytilus edulis* and byssus of *Dreissena* (BROWN, 1950).

The presence of quinone-tanning in these byssus threads is further confirmed by the histochemical reactions of the white, phenol and enzyme glands whose secretions lead to the formation of the byssus threads. The white gland and the phenol gland supply phenolic proteins which are acted upon by the polyphenol oxidase from the enzyme gland leading to the formation of the byssus thread. The tanning of the byssus thread takes place by an 'auto-quinone' mechanism (ANISA BANU, 1977). This is in direct support of SMYTH's (1954) findings that the byssus thread is a phenolic protein, which under enzyme action becomes tanned by the combination of the amino portion and the quinone nucleus of adjacent molecules. He termed this process auto-quinone tanning.

The function of the byssus is to secure the post-larva to a substratum when it is undergoing metamorphosis. In younger forms it fixes them for shorter or longer periods. The strength and extensibility of the byssus threads and their distribution allows the bivalves to withstand both the pounding of the waves and the drag of the advancing and receding tides. The byssus threads are generally present in a circle around the base of the foot and help the animal to withstand wave action from any direction. The animal may remain fixed in one place for many months and the high chemical resistance of the byssus keeps it from decaying. When the mussel moves it either breaks the byssus threads or pulls them off entirely by strong contractions of the byssus muscles.

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