

Observations on the mucopolysaccharides in the mantle of *Barbatia obliquata* (Gray) (Arcidae)

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Abstract. In *Barbatia obliquata* the mantle edge is divided into three folds as in most other lamellibranchs. Two types of gland cells are noticed in the mantle; epithelial and sub-epithelial. The epithelial glands include mucous glands, mucoprotein glands and lipid glands. The epithelial mucous glands secrete metachromatic, alcianophilic sulphated mucopolysaccharides. The sub-epithelial mucous glands are classified into two categories, according to their histochemical reactions. Some of them secrete non-metachromatic, alcianophilic sulphated mucopolysaccharides and the others secrete mucosubstances which are carboxylated.

Keywords. Mucopolysaccharides; mantle; *Barbatia obliquata*; Arcidae.

1. Introduction

Details of mantle mucins have been studied by several workers (Beedham 1958; Hillman 1968, 1969; Beedham and Owen 1965; Kale and Patil 1976, 1977). However, the nature of gland cells in the mantle of *Barbatia obliquata* (Gray) (Arcidae) has not been studied. A comparison of the mantle organization and the nature of gland cells of this species, with that of other bivalves is thus felt necessary. Hence in the present investigation the nature of mucopolysaccharides in *B. obliquata* and their distribution and function have been studied.

2. Material and methods

Specimens of *B. obliquata* were collected from Rishikonda, Waltair coast and were maintained in the laboratory. The animals were opened and the mantle was cut into small pieces and fixed in Susa, Zenker, formol calcium and other routine fixatives. For general histological visualization sections (10-12 μ m) were stained with Azan, Delafield's hematoxylin and eosin, and Ehrlich's hematoxylin. All histochemical procedures are adopted from Pearse (1968) (tables 1 and 2).

3. Results

A transverse section of mantle edge of *B. obliquata* depicts the following structure. The mantle edge is longitudinally divided into three folds: an outer, a middle and an inner fold (figure 1). The groove in which periostracum is inserted lies between the middle and inner folds. In all, two types of gland cells were noticed in the mantle of *B. obliquata*. The epithelial gland cells occur in the general outer epithelium, the sub-epithelial gland

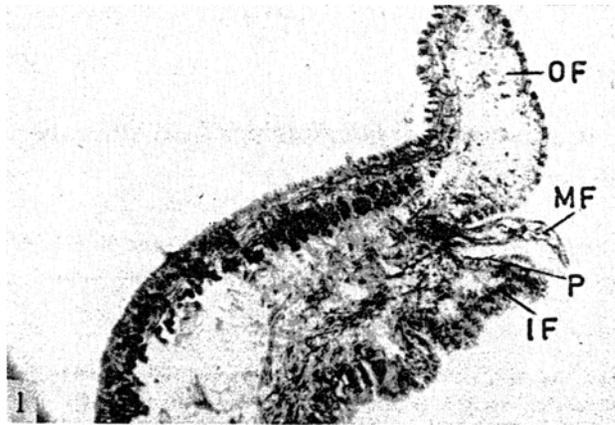


Figure 1. TS of mantle showing the topography of three folds (Azan).

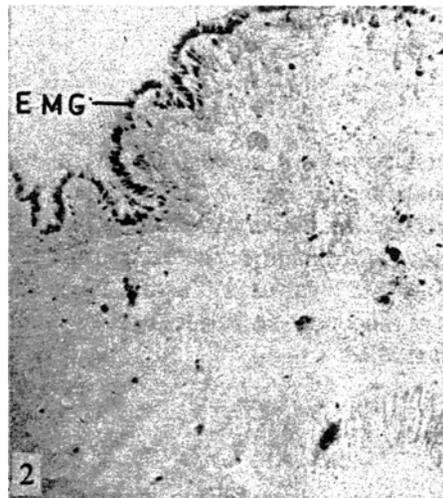


Figure 2. Epithelial mucous glands of mantle displaying sulphated mucopolysaccharides (Azure A pH 1).

cells lie in the connective tissue below the general epithelium as well as in the folds. The epithelial gland cells are spindle-shaped and do not take any colour with Azan. These cells have been found to secrete acid mucopolysaccharides as shown below.

The sub-epithelial glands are of three types (a) large pitcher-shaped glands staining dark blue to purple with Azan, (b) spherical cells staining red with Azan and (c) irregular-shaped cells staining blue or colourless with Azan. At the base of the middle fold lying in the connective tissue is a gland opening into the periostracal groove.

With periodic acid/Schiff (PAS) reaction the epithelial mucins gave negative reaction.

With Azure A at pH 1 (figure 2) these glands exhibited metachromatic red colour

indicating the presence of sulphated mucopolysaccharides. These glands also showed metachromasia at pH 2 and this can be attributed to the presence of sulphated mucopolysaccharides only. With toluidine blue the epithelial gland cells stained metachromatically thereby indicating their acidic nature.

The epithelial mucins are intensely alcianophilic at both pH 1 and 2.5 (figure 3), further confirming the acidic nature. The intensity of alcianophilia is reduced to some extent after active methylation/AB, but is restorable after saponification/AB.

With the combined technique of AB (pH 1 and 2.5)/PAS these gland cells stained blue. The dark purple staining of these glands with aldehyde fuchsin and AF/AB indicates that the material is sulphated. When exposed to alcian blue/safranin technique the epithelial gland cells stained red suggesting their acidic nature (table 1).

Below the general outer epithelium there are mucous glands of smaller sizes whose reactions resemble those of the epithelial mucins suggesting the nature of their secretions being acid sulphated mucosubstances. Below these a row of mucoprotein gland cells is present. These seem to open to the exterior by narrow necks. These cells secrete mucoprotein as evidenced by their PAS positivity and positive reactions with all protein tests.

At the margins in the three folds a number of gland cells are distributed in the connective tissue. In all the three folds, glands which secrete mucoproteins are distributed. In the third or inner fold these are especially concentrated in large numbers.

In addition, in the first fold beneath the outer epithelium lie few cells which secrete sulphated mucopolysaccharides. Beneath the inner epithelium lie a row of cells. The secretion of these cells is positive to PAS, non-metachromatic and alcianophilic at AB (pH 1 and 2.5). The alcianophilia is abolished on methylation and is restored by subsequent saponification/AB. These secretions become purple with the combined technique of AB/PAS and retain alcianophilia when exposed to AF/AB. Thus their carboxylated nature is evident.

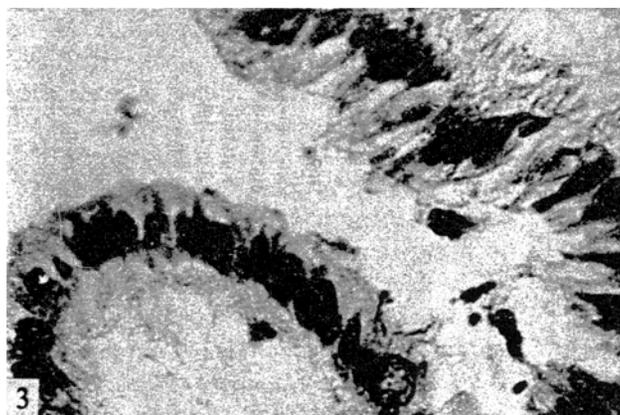


Figure 3. ts of mantle showing epithelial mucous glands stained with AB pH 2.5.

Abbreviations: EMG—Epithelial mucous glands; IF—Inner fold; MF—Middle fold; OF—Outer fold; P—Periostracum.

Table 1. Histochemical reactions for epithelial mucocytes in the mantle.

Histochemical tests applied	Results	Remarks	Reference
Periodic acid/Schiff (PAS)	Negative	Absence of vicinal hydroxyl groups	McManus (1946)
Azure A—pH 1	γ -metachromatic	Sulphated mucosubstances	Spicer (1960)
pH 2	β -metachromatic	Sulphated mucosubstances	Spicer (1960)
Toluidine blue	Metachromatic	Sulphated mucosubstances	Kramer and Windrunt (1955); (cf Pearse (1968))
Alcian blue (AB) pH 1	Blue	Sulphated mucopolysaccharides	Pearse (1968)
AB pH 2.5	Blue	Weakly acidic sulphated mucopolysaccharides, hyaluronic acid	Pearse (1968)
Methylation at 37°C/AB	Blue	Sulphated mucosubstances	Spicer (1960)
Mild methylation/ saponification/AB	Alcianophilia enhanced		
Methylation at 60°C/AB	Residual alcianophilia	Highly sulphated polymers containing alcianophilic groups (Spicer 1960, 1965)	Spicer (1960)
Active methylation/ saponification/AB	Alcianophilic		
AB pH 1/PAS	Blue	Sulphated mucosubstances	Mowry and Winkler (1956)
AB pH 2.5/PAS	Blue	Weakly acidic sulphated mucosubstances	Mowry and Winkler (1956)
Aldehyde fuchsin (AF)	Purple	Sulphated mucosubstances	Spicer and Meyer (1960)
AF/AB	Purple	Sulphated mucosubstances	Spicer and Meyer (1960)
AB/safranin	Light red	Acidic mucosubstances	Spicer (1960)

In the second or middle fold along with the mucoprotein secreting gland cells some cells secreting carboxylated mucosubstances have been noticed.

In the inner most or third fold mucous cells secreting strongly sulphated mucopolysaccharides, which resemble the epithelial mucins in histochemical reactions, are present. But these cells do not exhibit any metachromasia with toluidine blue or Azure A indicating that they may not be as acidic as epithelial mucins. Similar type of cells lie beneath the inner epithelium of mantle facing the mantle cavity (table 2).

4. Discussion

Various types of gland cells were reported in different bivalves. Beedham (1958), Sullivan (1960) and Saleuddin (1964a, b) described only one type of epithelial glands that secrete mucoproteins. Oldfield (1964) described two types of epithelial gland cells in the mantle of erycinids and montaculids. Trueman (1957) distinguished two types of gland cells in the mantle of *Arca noae*, one secreting the acid mucopolysaccharides and the other secreting polysaccharide protein complex. Sullivan (1960) described three types of subepithelial glands in the mantle of *Anadara trapezia*. Saleuddin (1964a, b) described altogether four types of gland cells in *Astarte* sp and *Cyprina islandica*. Hillman (1968, 1969) reported two types of gland cells in the mantle of *Mercenaria mercenaria*, one secreting glycosaminoglycans and the other secreting neutral mucopolysaccharides. Wada and Furuhashi (1973) analysed the mucous from the mantle of *Pinctada fucata* and *Chlamys nobilis* and described that it contains a relatively large amount of sulphated ester and a little amount of protein i.e. sulphomucopolysaccharide or sulphated polysaccharide protein complex. They attributed that this type of mucous is necessary to induce shell mineralization though it does not always induce it. Hillman (1961) stated that the heparin-like mucosubstances might aid in the handling of calcium by the clam *Mercenaria mercenaria*. He also speculated that it aids in handling of other ions like sodium and chloride. In general, it is known that acid mucopolysaccharides play an active role in the process of calcification by binding calcium ions probably as a metachelate complex (Beedham 1958; Saleuddin 1964b). Oldfield (1964) showed that some of the mucous secreting glands in the mantle of erycinids and montaculids secrete egg capsules as evidenced by similar results obtained by histochemical reactions of egg capsules and mantle mucous glands. In *B. obliquata* the mantle glands secrete acid mucopolysaccharides and mucoprotein whose functional role seems to aid in binding with calcium ions. Deposits of calcium were detected in the mantle of *B. obliquata* by the Alizarin red S technique and evidence of quinone tanned proteins is present as revealed by the presence of phenols and polyphenol oxidases. The gland at the base of the middle fold secretes lipid material as evidenced by the positive reaction to Sudan black B which is abolished in pyridine extracted sections. Part of this gland secretes mucoprotein. This type of gland was considered to secrete lipoprotein in *Solemya parkinsoni* by Beedham and Owen (1965) and they stated that this material aids in the lubrication of periostracum. We are in agreement with this in *B. obliquata* where the secretions are poured into the periostracal groove. On the whole, the mantle secretes mucopolysaccharides, lipid protein complexes, mucoproteins and there is presence of quinone tanned proteins. The role of these substances in the edification of the periostracum is thus clear.

Table 2. Histochemical reactions of sub-epithelial mucous glands in the mantle.

Histochemical tests applied	Results	Remarks	Reference
Periodic acid/Schiff (PAS)	Magenta	Vicinal hydroxyl groups	McManus (1946)
PAS/saliva	Magenta	No glycozen	Spicer <i>et al</i> (1967)
Acetylation/PAS	Magenta	No 1:2 glycol groups	Spicer <i>et al</i> (1967)
Deacetylation/PAS	Magenta	No 1:2 glycol groups	Spicer <i>et al</i> (1967)
Azure A—pH 1	Non-metachromatic	Non-sulphated mucosubstances	Spicer (1960)
	Metachromatic	Sulphated mucosubstances	
Toluidine blue	Non-metachromatic	Absence of strongly acidic mucosubstances	Kramer and Windrunt (1955) (<i>cf</i> Pearse (1968))
Alcian blue (AB) pH 1	Negative	Non-sulphated mucosubstances	Pearse (1968)
	Blue	Sulphated mucosubstances	
AB pH 2.5	Blue	Weakly acidic sulphated mucosubstances, hyaluronic acid (non-sulphated)	Pearse (1968)
Methylation at 37°C/AB	Alcianophilia abolished	Carboxylated mucosubstances	Spicer (1960)
	Alcianophilia retained	Sulphated mucosubstances	
Mild methylation/saponification/AB	Alcianophilia restored	Carboxylated mucosubstances	Spicer (1960)
Active methylation/AB	Alcianophilia abolished	Carboxylated and sulphated mucosubstances	Spicer (1960)
Active methylation/saponification/AB	Alcianophilia restored	Carboxylated mucosubstances	Spicer (1960)
AB pH 1.0/PAS	Alcianophilia not restored	Sulphated mucosubstances	
	Purple	Carboxylated mucosubstances	Mowry and Winkler (1956)
	Blue	Sulphated mucosubstances	
AB pH 2.5/PAS	Magenta	Carboxylated mucosubstances	Mowry and Winkler (1956)
	Purple	Sulphated mucosubstances	
Aldehyde fuchsin/AB	Blue	Carboxylated mucosubstances	Spicer and Meyer (1960)
AB/safranin	Purple	Carboxylated mucosubstances	
	Blue	Weakly acidic mucosubstances	Spicer (1960)

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