

values above 2.58 form 0.18% of total tests made. Thus only 0.27% of the d/σ values are clearly significant and all the others are non-significant *i.e.* the differences between the two sets of values compared are statistically of no importance for the characters under consideration.

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These results thus confirm the earlier general conclusion of Seshappa and Chakrapani (1984 a, b, 1987) that populations of the Malabar sole *C. semifasciatus* were very largely homogeneous on the basis of samples examined in the period of sampling. The data of individual centres also show similar significance.

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HISTOCHEMICAL LOCALIZATION OF SULPHATED POLYSACCHARIDES IN THE TISSUES OF THE BLOOD CLAM *ANADARA RHOMBEA*

ABSTRACT

Histochemical analyses of sulphated polysaccharides were made in mantle, palp, foot, gill and adductor muscle of the blood clam *Anadara rhombea*. Specific staining procedures such as PAS, alcian blue pH 2.5 and 1.0, aldehyde fuchsin, combined alcian blue pH 2.5/PAS, alcian blue pH 1.0/PAS, aldehyde fuchsin/alcian blue pH 2.5 were followed. The mantle, palp, foot, gill and adductor muscle have contained PAS positive, sulphated acid polysaccharides.

Introduction

IT IS well known that either natural or synthetic sulphated polysaccharides have blood anticoagulant property. Histochemistry is a more sensitive method for localizing sulphated polysaccharides than the biochemical analysis of tissues. In the present study different histochemical methods were used to locate sulphated polysaccharides in mantle, palp, foot, gill and adductor muscle of the blood clam *Anadara rhombea*.

The first author thanks ICMR for Junior Research Fellowship.

Materials and methods

The blood clam *Anadara rhombea* were collected from the Vellar Estuary (11° 29' N, 79° 46' E). The shells were opened and the tissues amputated gently. The tissues were then fixed in buffered neutral formalin (Bullock *et al.*, 1976). After fixation for 24 hr, small

portions of the tissues were dehydrated and embedded in paraffin. Sections were cut at 5 μ and stained. The histochemical methods used were given in Table 1.

sulphated polysaccharides. Whereas with alcian blue pH 2.5 and the combined stain with PAS, the tissues showed weak alcianophilia. This shows the presence of little amount of

TABLE 1. *Histochemical methods used to demonstrate different polysaccharides in the tissues of Anadara rhombea*

Method	Specific histochemical results	Procedures used
Periodic-acid Schiff (PAS)	Oxidation of Vicinyl hydroxyl groups followed by formation of coloured complex (red) with Schiff reagent	McManus (1948)
1% Alcian blue in 3% HAC (pH 2.5)	Acid mucins-blue.	Pearse (1968)
1% Alcian blue in 1.0 N-HCl (pH 1.0)	Sulphated mucins-blue.	Pearse (1968)
Aldehyde fuchsin	Sulphated mucins purple or blue-purple. Nonsulphated acid mucins stain weakly or not at all.	Spicer and Meyer (1960)
Alcian blue pH 2.5/PAS	Sulphate-free, sialic acid containing mucins-blue.	McManus and Mowry (1960)
Alcian blue pH 1.0/PAS	Sulphate-free, sialic acid containing mucins-red.	Mowry and Winkler (1956)
Aldehyde fuchsin/Alcian blue pH 2.5	Sulphated mucins-purple or blue-purple. Non-sulphated acid mucins stain weakly or not at all.	Spicer and Meyer (1960)

TABLE 2. *Histochemical reaction of tissues*

Stains	Colour intensity					Reaction
	Mantle	Palp	Foot	Gill	Adductor	
PAS	2	3	1	3	2	Red
Alcian blue pH 2.5	2	1	2	1	2	Weak blue
Alcian blue pH 1.0	3	2	2	2	3	Blue
Aldehyde fuchsin	3	2	3	2	3	Purple
Alcian blue pH 2.5/PAS	2	1	1	1	1	Some cells blue
Alcian blue pH 1.0/PAS	3 (blue) 1 (red)	1 (blue) 1 (red)	2 (blue) 1 (red)	2 (blue)	1 (red)	Most cells blue, Some cells red
Aldehyde fuchsin/Alcian blue pH 2.5	1 (purple) 2 (blue)	2 (purple) 1 (blue)	3 (purple)	2 (purple) 1 (blue)	1 (purple) 1 (blue)	Most cells purple, Some cells blue

Results

The results of the histochemical analyses are presented in Table 2. The intensity of stain taken by the tissues was visually estimated with 3 representing strong, 2 moderate and 1 weak staining activity. All the tissues showed positive reaction with PAS, although the intensity varied in different tissues. The tissues were alcianophilic in both alcian blue pH 1.0 and in the combined alcian blue pH 1.0/PAS procedures. This indicates the presence of highly

non-sulphated polysaccharides. With aldehyde fuchsin, a stain for sulphated mucosubstances, the mantle, palp, gills and adductor muscle showed positive reaction. In the combined aldehyde fuchsin/alcian blue pH 2.5 most cells were red and only few cells stained blue. This reaction was strong in the foot and indicating the presence of highly acidic sulphated group. Thus the results of single and combined procedures used in this study suggested that the major components of the tissues were sulphated acid polysaccharides.

Discussion

The mantle, palp, foot, gill and adductor of the blood clam *Anadara rhombea* stained red with PAS technique showing the presence of glycoprotein. Alcianophilia at varying pH indicates the acidic nature of the polysaccharide. All the tissues showed strong to moderate reaction with alcian blue pH 1.0 and aldehyde fuchsin and indicated the presence of highly sulphated polysaccharides. In the combined aldehyde fuchsin/alcian blue pH 2.5, most cells were purple and only a few cells blue. This shows that the major component is sulphated polysaccharide. It is further evidenced by combined alcian blue pH 1.0/PAS reaction where most cells were blue and a few red. However, a weak alcianophilia in alcian blue pH 2.5/PAS and aldehyde fuchsin/alcian blue pH 2.5 showed the presence of little non-sulphated polysaccharides.

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Hillman (1969), Wada and Furuhashi (1971) have shown the presence of sulphate groups of acid mucopolysaccharides in the mantle fold of adult bivalve *Mercenaria mercenaria*. Cranfield (1974) has also demonstrated PAS — reactive sulphated acid polysaccharide in the mantle folds of oyster larvae (*Ostrea edulis*). In the present study all the tissues of *Anadara rhombea* showed the presence of sulphate groups of acid polysaccharide which are similar in properties to heparin or mucoitin sulphate (Kent and Whitehouse, 1955). Holick *et al.* (1985) have shown that a sea clam has 63,840 units of heparin like activity per kilogram tissue and pointed out that the sulfur contents of the mucopolysaccharides are important for biological activity. As the tissues of *A. rhombea* contain a highly sulphated acid polysaccharide they may be a potent source of heparin like substances.

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