LIPASE ACTIVITY IN THE CRYSTALLINE STYLE OF SOME BIVALVE MOLLUSCS

P. SHAHUL HAMEED
Centre of Advanced Study in Marine Biology, Parangipettai 608 502, India

THE crystalline style is a long conical rod of glycoprotein housed in the midgut of most bivalves and a few gastropods[1]. It is considered as a primary structure that brings about extracellular digestion in the lumen of stomach by releasing digestive enzymes (carbohydrases and lipases)[2]. George[3] first demonstrated qualitatively the lipase activity in the crystalline style of an oyster *Crassostrea virginica* and a mussel *Modiolus desmessus*. Lipase activity of bivalve crystalline style has been quantified only in a very few species[4–6]. However, no report is available on the bivalve species of peninsular India. Hence the present study is initiated on the lipase activity of the crystalline style of five intertidal bivalve species, *Crasssostrea madrasensis* (Preston), *Meretrix meretrix* (Linnaeus), *M. casta* (Chemnitz), *Katelysia opima* (Gmelin) and *Donax cuneatus* (Linnaeus).

Specimens of *C. madrasensis*, *M. meretrix*, *M. casta* and *K. opima* were collected during the high tide regime from mudflats of Vellar estuary and those of *D. cuneatus* from the intertidal zone of Porto Novo sea shore (11° 29′ N lat. and 79° 49′ E long.). The styles were removed by dissection, washed with distilled water and blotted dry. Aqueous extract of the style was prepared by homogenizing it with distilled water using a all glass homogenizer. After centrifugation for 15 min. at 3000 rpm, the supernatant was used as enzyme extract, the strength of the extract being 10%.

The style extract (1 ml) was incubated with 3 ml of olive oil and 1 ml of Tris buffer (pH 8) for 6 hr at 30°C. The amount of fatty acids released by the lipase was estimated by titrating the reaction mixture after adding 3 ml of 95% ethyl alcohol, against 0.05 N. NaOH solution using thymolphthalein as indicator. The difference between the volume of NaOH consumed by the reaction mixture and control mixture was taken as a measure of the lipase activity. The experimental procedure is based on the method of Tietz *et al*[7]. Control experiments were also run with heat inactivated style extract (boiled for 10 min). The results were expressed as volume of NaOH consumed per ml of style extract (ml NaOH/ml). Five estimations were made for each species and the results averaged. For comparison, the lipase activity of digestive glands of all the five species was also estimated.

It is evident from table 1 that a maximum rate of lipid hydrolysis was recorded in the style extract of *D. cuneatus* (0.9 ml NaOH/ml) and a minimum rate in *K. opima* and *C. madrasensis* (0.3 ml NaOH/ml). The lipase activity in *M. casta* (0.5 ml NaOH/ml) was slightly higher than the activity in *M. meretrix* (0.4 ml NaOH/ml). The lipolytic activity found in the digestive glands of all species tested in the present study was weaker than that in the crystalline styles of their respective species.

Lipid digestion involves two groups of enzymes, lipases and esterases which are characterized by their preference towards the type of substrates[8]. Lipases prefer carboxylic esters containing long chain fatty acids with more than ten carbon atoms and they form a fine emulsion in water whereas esterases readily hydrolyze lipids with short chain fatty acids and form a true solution with water. Since olive oil, the substrate used in the present study, contains long chain fatty acids and it forms an emulsion with water, the enzymes for lipolytic activity detected in the style is confirmed to be a lipase. The presence of

Table 1 *Lipase activity in the crystalline style and digestive glands of bivalve species (average of five estimations)*

Species	Crystalline style		Digestive glands	
	Mean (ml. NaOH/ml.)	Range (Style extract)	Mean (ml. NaOH/ml.)	Range (Style extract)
C. madrasensis	0.30	0.27–0.35	0.10	0.05–0.15
M. meretrix	0.38	0.35–0.45	0.23	0 20–0.29
M. casta	0.53	0.35–0.60	0.30	0.25–0.36
K. opima	0.30	0.25–0 35	0 16	0.12–0.20
D. cuneatus	0 85	0 82–0.88	0.35	0.30–0.38

lipolytic activity in the crystalline style of five species analyzed agreed with earlier studies[3–6].

Payne[6] also found that lipase of crystalline style was more active that that of digestive glands in a littoral bivalve *Scrobicularia plana* and conversely esterase activity was more pronounced in the digestive gland than in the style. Further, it has been reported that the most prominent carbohydrases detected in the style were amylase, glycogenase, cellulase and laminarinase[9–11] which degraded sugars of high molecular weight. Hence it is evident that crystalline style of bivalve mollusca, in general, is equipped with enzymes that hydrolyze high molecular weight substance (lipids with long chain fatty acids and polysaccharides) and initiates the extracellular digestion in the stomach while the final breakdown and absorption take place intracellularly in the digestive glands[6, 9, 12].

The author is thankful to Dr A. L. Paulpandian for guidance and UGC, New Delhi for a fellowship.

10 October 1986

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AN OVIPOSITIONAL ATTRACTANT ISOLATED FROM NATURAL BREEDING WATER OF *MANSONIA UNIFORMIS*

B. AYYACHAMY DANIEL, CHERIYAN THOMAS and R. S. PRASAD
Department of Zoology, University of Kerala, Kariavattam, Trivandrum 695 581, India.

SEVERAL studies, in recent years, have demonstrated that a number of diverse natural aquatic factors (microorganisms, decomposing organic matter of several types and biophysical parameters) play a prominent role in attracting and stimulating gravid females of mosquitoes to oviposit at a particular site. A scan through the review of Maire[1] reveals that most of these studies have been carried out on different species of mosquitoes belonging to four genera only—*Aedes*, *Anopheles*, *Culex* and *Psorophora*. The most recent reports on oviposition site selection of gravid female mosquitoes are those of Ahmadi and McClelland[2], Laurence and Pickett[3], Maire[4,5] and Maire and Langis[6].

Iyengear[7,8], Laurence[9], and Laurence and Samarawickrema[10] emphasized the importance of aquatic vegetation and topographical marker features as factors deciding oviposition site selection by *Mansonia* mosquitoes. Recently, Gass *et al*[11] pointed out the significance of homogeneous aquatic plant species coupled with egg cluster densities and visible water bodies in attracting *Mansonia* species towards oviposition site. Ikeshoji[12], however, based on his experiments with *Mansonia annulifera* on forced oviposition in tapwater, suggested the role of some chemical factors present in field water as oviposition stimulant for these mosquitoes.

The present paper reports the results of some laboratory investigations into the isolation of certain chemical atractant(s)/stimulant(s) of *Mansonia uniformis*.

The experimental mosquitoes, *Ma. uniformis*, were held in small cages as described by Sasikumar *et al*[13]. Water samples (100 ml) were offered in small aluminium bowls. As a substratum for ovipositing, 1 mm thick thermocol pieces (expanded polystyrene) of 10 mm^2 were made to float on water. The room temperature during the study ranged from 26–28°C. The experimental and control colonies were examined once every 24 hr for 3 consecutive days and the egg clusters, as and when laid, were counted and removed.