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pH and dissolution of crystalline style in some bivalve molluscs of Porto Novo coastal waters

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Abstract. A comparative study of the pH of crystalline style, digestive diverticula and stomach fluid in six bivalve species, *Anadara rhombea*, *Crassostrea madrasensis*, *Meretrix meretrix*, *M. casta*, *Katelysia opima* and *Donax cuneatus* revealed that the digestive diverticula are more acidic while crystalline style is either slightly acidic or nearly neutral. The significance of this finding is discussed. *In vitro* dissolution of crystalline style of all the six species in buffer solutions of varying pH from 3·6 to 9·0 was observed and the study indicated that the optimum pH for style dissolution is around 8·0 in all species excepting *Donax cuneatus* where some dissolution was recorded only at pH 9·0. Based on the physical changes observed in the style, a mechanism of style dissolution has been put forward.

Keywords. Bivalve molluscs; crystalline style; style dissolution; *Anadara*; *Crassostrea*; *Meretrix*; *Katelysia*; *Donax*.

1. Introduction

The crystalline style is a long, transparent rod of glycoprotein, lodged in the midgut of most bivalves and some gastropods aiding in the processes of feeding and digestion through its dissolution. Dissolution of the crystalline style had been shown to be pH dependent by Yonge (1925, 1926), Venugopalan (1955), Mathers (1974, 1976) Mathers *et al* (1979) and Alyakrinskaya (1977, 1979). Yonge (1925, 1926) proposed a theory that the style was the most acidic part of the gut in bivalves and that the dissolution of the style in the stomach was effected by the higher pH of the stomach content. On the other hand, Purchon (1971) and Morton (1973) found that the style was not the most acidic part of the gut and that the style pH was near neutral or slightly acidic. Further, the pH in different regions of the gut was reported to be variable (Mathers 1974; Owen 1974). Therefore, the present study was undertaken to clarify the actual situation in the bivalve species of Indian coastal waters.

2. Materials and methods

The bivalve species, *Anadara rhombea* (Born), *Crassostrea madrasensis* (Preston), *Meretrix meretrix* (Linnaeus), *M. casta* (Chemitz) and *Katelysia opima* (Gmelin) were collected from the mud flats of Vellar estuary joining Porto Novo sea shore (11°29′ N lat. and 79°46′ E long.) and *D. cuneatus* from the marine intertidal zone. The pH of aqueous solutions of the crystalline style, the digestive diverticula and the stomach

content was measured in a digital pH meter (Toshniwal Cat. No. CL 46) to the nearest 0·01 pH following the method of Mathers (1974). *In vitro* dissolution of the style was observed in buffer solutions with the pH range from 3·6 to 9·0 and in distilled water, sea water and estuarine water at room temperature (34° C). The time required for the complete dissolution was recorded following the method of Mathers (1974). Changes occurring in the style during dissolution was observed under a microscope. Three estimations were made at each pH and the mean dissolution time (t) and the rate of dissolution ($1/t$) were calculated.

3. Results

Table 1 presents the mean pH of the crystalline style and the digestive diverticula in six species and that of stomach fluid on four species. The mean pH was either slightly acidic or neutral. It is interesting to note that pH of any of these three regions of the gut was not stable but variable within a limited range (table 1) and the digestive diverticula was the most acidic part in all the species tested. The relationship between the pH of the medium and the rate of style dissolution ($1/t$) was studied on all the six species (figure 1). In general, at low pH (3·6 to 4·6) the styles were stable for a prolonged period with low rate of dissolution. The rate of style dissolution was rather slow at pH range of 5 to 7. Faster dissolution was observed at pH 8·0 in all species tested excepting *D. cuneatus*. At this optimum pH, a high rate of dissolution was recorded in *C. madrasensis* (3 min) and in *A. rhombea* (9 min). The pattern of style dissolution in *D. cuneatus* was quite distinct from that of other species tested in that the style of this species was highly resistant to dissolution in the pH range of 3·6 to 8·0. At pH 9·0 the style dissolved at a slow rate (175 min) which was close to the dissolution time in sea water (215 min).

The data on the style dissolution in distilled water (pH 7·0), estuarine water (pH 8·2) and sea water (pH 8·5) indicated that the style dissolved faster in natural media than in distilled water (table 2).

Observation on the mode of style of dissolution revealed two distinct patterns. In *D. cuneatus* the style underwent a process of lengthening before dissolution. In all other species dissolution was followed by the shortening of the style. In both cases the style had a central granular matrix around which concentric crystalline layers were found. During dissolution, the granular matrix was observed to be continuously flowing out largely through the anterior end and to a small extent through the posterior end.

4. Discussion

It is evident from the present study that the pH of the crystalline style of bivalve is either slightly acidic or neutral. Morton (1969, 1970, 1971) recorded that the pH of styles of *Dreissena* (pH 7·0) and *Ostrea* (pH 6·8) is approximately neutral whereas that of *Cardium* (pH 6·4) is slightly acidic. Similar results were also reported by Langton and Gabbott (1974) in *Ostrea*, Langton (1977) in *Mytilus* and Mathers (1974, 1976) in *Ostrea*, *Crassostrea* and *Pecten*. In a given individual, the digestive diverticula were always more acidic than its crystalline style. The pH of the style, digestive diverticula and stomach content were not stable but fluctuated within a range. Fluctuating pH in these three regions of the gut was also recorded by Mathers (1974, 1976) and Morton

Table 1. pH of style, digestive diverticula (DD) and stomach content of bivalves

Species	Style pH		DD pH		Stomach content pH	
	Mean[a]	Range	Mean[a]	Range	Mean[b]	Range
A. rhombea	6·39	5·99–6·98	5·83	5·63–6·06	6·2	5·75–6·65
C. madrasensis	7·17	6·88–7·52	6·43	6·31–6·79	6·4	6·41–7·10
M. meretrix	7·11	6·11–7·46	6·42	6·58–6·66	6·5	6·50–7·00
M. casta	7·37	7·08–7·57	6·56	6·76–7·32	—	—
K. opima	6·41	6·05–6·59	5·80	5·70–5·86	6·0	5·90–6·90
D. cuneatus	6·79	6·45–7·03	6·31	6·09–6·59	—	—

[a]Average of 10 estimates; [b]Average of 5 estimates;—not determined.

Table 2. Dissolution of crystalline style of bivalve species in natural media and distilled water

Species	Mean dissolution time (Minutes)		
	Estuarine water (pH 8·2)	Sea water (pH 8·5)	Distilled water (pH 7·0)
Anadara rhombea	53	—	174
Crassostrea madrasensis	12	—	16
Meretrix meretrix	65	—	180
Meretrix casta	23	—	203
Katelysia opima	44	—	174
Donax cuneatus	—	215	2580

(1969, 1970). The present work confirms the current concept that in bivalves, the most acidic part of the gut is the digestive diverticula and not the crystalline style; the pH of the crystalline style, digestive diverticula and stomach content fluctuates considerably and that the stomach pH is not stable and not buffered by style dissolution (Purchon 1971; Morton 1973; Owen 1974).

Bivalve crystalline style had long been known to dissolve at a faster rate in alkaline medium and stable in acidic medium (Yonge 1925, 1926; Venugopalan 1955; Mathers 1974, 1976; Mathers *et al* 1979; Kristensen 1972; Alyakrinskaya 1977, 1979). On the basis of the rate of dissolution, the crystalline styles of the bivalve species examined in the present work could be classed into two types: (i) rapidly dissolving styles and (ii) slowly dissolving styles. The rapidly dissolving styles were found to be housed in the style sac conjoined with midgut as observed in *A. rhombea*, *C. madrasensis*, *K. opima*, *M. meretrix* and *M. casta*. These estuarine bivalves had a high rate of dissolution at pH 8·0 (figure 1) and the complete dissolution time ranged between 3 minutes in *C. madrasensis* and 25 minutes in *M. meretrix*. On the other hand, *D. cuneatus* possessed a slowly dissolving style and its style sac is entirely separated from the midgut. Even at the pH of 9·0 its dissolution rate is very slow (22 hr). Probably the rate of dissolution depends on other factors as well such as the organic content of the style (Alyakrinskaya 1979).

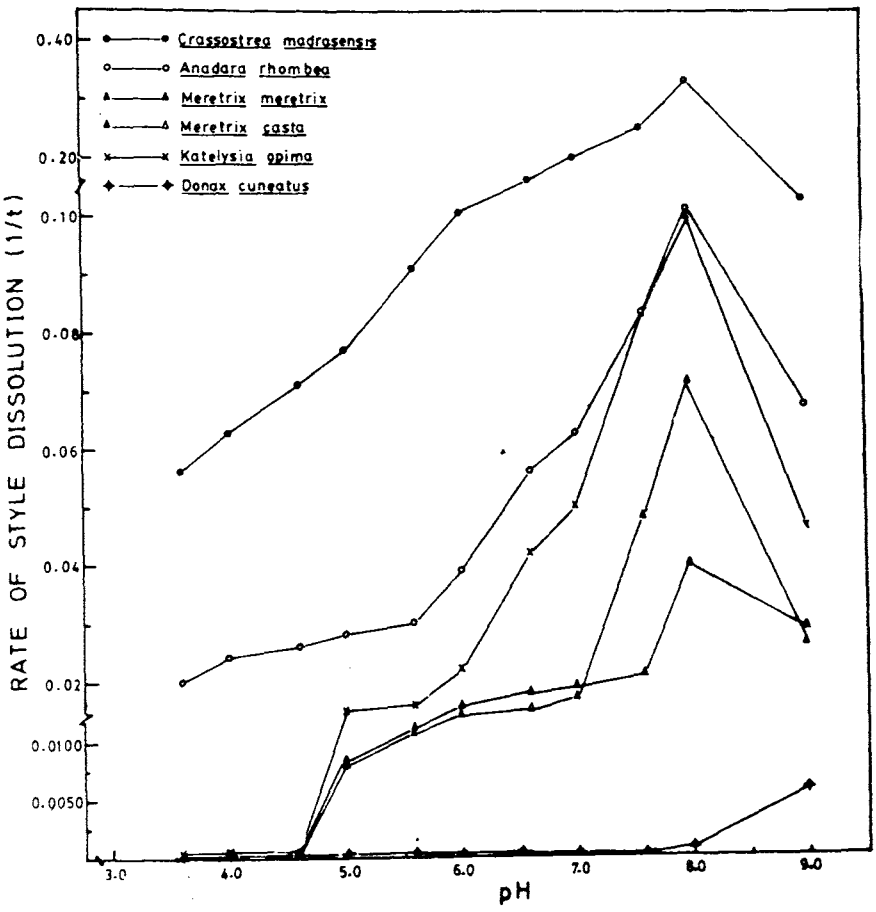

Figure 1. Relationship between the rate of style dissolution and pH.

The optimum pH for dissolution of the style coincided with the pH of the medium (table 2). It would seem that during feeding the entry of water into the stomach would raise the pH facilitating an increase in the rate of style dissolution and the consequent release of digestive enzymes. The inference is in conformity with the observations of Mathers (1974, 1976) in *Ostrea* and *Pecten*.

According to Yonge (1925, 1926, 1949) the dissolution of the style was thought to be restricted to its anterior end projecting into stomach lumen. But *in vitro* observations on the mode of style dissolution did not support this view. Dissolution of bivalve style is a physical phenomenon involving the entire style body. Kristensen (1972) reported that the outer crystalline layers of the style are relatively hard and more resistant to dissolution while the central core is hygroscopic. It is suggested that the dissolution of the style is brought about by the liquefaction of the crystalline layers into granular core through uptake of water. The actual transport of the dissolved materials was observed in the present study as an anteriorly directed streaming movement of the central granular matrix.

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