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Concentrations of Cd, Cu, Pb and Zn in Different Parts of the Byssus of Green-lipped Mussel *Perna viridis* (Linnaeus)

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Abstract: The concentrations of Cu, Cd, Pb and Zn were analysed in different parts (root, byssus stem, byssal thread and attachment plaque) of the byssus of green-lipped mussel *Perna viridis*. Foot and total soft tissue were analysed for comparison. Heavy metal distributions in different byssus portions were observed. The attachment plaque recorded the highest levels of Cd (2.22 $\mu g\ g^{-1}$), Cu (17.48 $\mu g\ g^{-1}$), Pb (11.92 $\mu g\ g^{-1}$) and Zn (73.94 $\mu g\ g^{-1}$) when compared to the other parts of byssus, foot and total soft tissue. Different protein composition is believed to be the reason for the different heavy metal levels found in the different parts of the byssus.

Key words: *Perna viridis,* byssus, heavy metals

Introduction

Mussels attach to a substratum by means of a byssus, which is an extracellular, collagenous structure secreted by the foot. From the survival point of view, a secure attachment of the mussel byssus to the hard substratum is crucial to its survival (Harris, 1990). This is because sedentary mussels inhabiting coastal zones with intertidal waves must withstand the hydrodynamic forces generated by the strong waves (Bell and Gosline, 1996).

The mussel byssus is composed of three distinct parts (Fig. 1). According to Brown (1952) based on his observation on the byssus of *Mytilus edulis*, the first part is a root, which is embedded in the byssal gland at the basal region of the foot and links the entire structure to the byssal retractor muscles. The second part is a stem which extends from the root and supports each byssal thread. The third part is a byssal thread which extends from the stem in many directions and attaches to the substratum. Each byssal thread is formed within the groove of the mussel foot by a process resembling polymer injection-molding (Waite, 1992). The byssal threads are often comparable to tendons on the basis of their construction namely anisotropically oriented bundles of collagen fibrils (Qin *et al.,* 1997). From our observation, the three distinct portions of the byssus of *Perna viridis* are similar to those of *M. edulis* namely the root, stem and byssal thread.

The byssal thread is a complex structure which can be divided into three parts (Fig. 1). The first part is a proximal portion representing approximately one-third of the total thread length. The second part is a distal portion, which is smoother, narrower and approximately twice the length of the proximal portion. The third part is an adhesive plaque which attaches the thread distally to a substratum. Although there have been some detailed reports on the biochemical compositions of mussel byssus (Qin and Waite, 1995, 1998; McDowell *et al.*, 1999) there is no

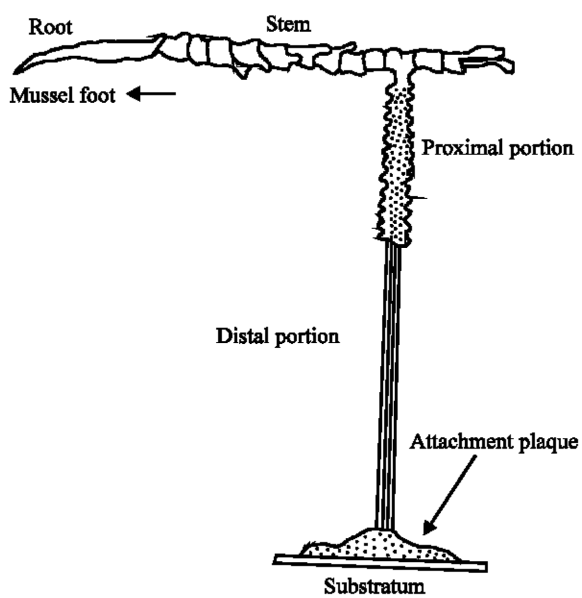

Fig. 1: A schematic of a single byssal thread from the green-lipped mussel *Perna viridis*, showing the locations of the different morphological regions

information available on heavy metal levels in the different parts of a mussel byssus especially of the green-lipped mussel *Perna viridis*. Our previous papers on *P. viridis* have reported on the distributions of heavy metals in the different soft tissues (Ismail *et al.*, 1999; Ismail and Yap, 2000). We found that heavy metal levels were elevated in total byssus when compared to the soft tissues. In addition, it has been shown that the byssus of *Mytilus* species could act as an excretion route for heavy metals (George *et al.*, 1976; Unlu and Fowler, 1979; Szefer *et al.*, 1997, 1999).

Knowing that the byssus is composed of the root, stem, byssal thread and attachment plaque, the question of 'the distribution of heavy metals in these different parts of the mussel byssus' has become an interesting one. Since the byssus consists of collagen fibers and maintains a continuous growth, heavy metals might be excreted through the byssus and show different levels of heavy metals in the byssus. Therefore, this study reports on the concentrations of Cu, Cd, Pb and Zn in the root, stem, byssal thread (proximal and distal portions) and the attachment plaque of the byssus of *P. viridis*.

Materials and Methods

Mussels were collected from an unpolluted coastal water at Pasir Panjang. They were frozen at –10°C until analysis. The samples were thawed at room temperature and the byssus was carefully pulled out from the mussel foot. The whole mussel byssus was rinsed several times in DDW and 5% of concentrated HNO_3 (69%) to get rid of suspended solids attached onto the byssus. Under a dissecting microscope, the byssus was carefully dissected into its root, stems, byssal thread and attachment plaque, using a small stainless steel scissors. A schematic view of a mussel byssus from the root to the attachment plaque is given in Fig. 1. Since these different byssus portions were small, the roots, stems, byssal threads and attachment plaques of about 20 individuals (shell length: 7-9 cm) were pooled so that there were enough of the different parts for metal analysis. For comparative purposes, the foot where the byssus root projected from and the total soft tissue were also analysed.

The samples were digested in concentrated nitric acid (69%). They were placed in a hot-block digestor first at low temperature for one hour and than they were fully digested at high temperature (140°C) for at least 3 h. The digested samples were then diluted to a certain volume with double distilled water. After filtration, the prepared samples were determined for Cu, Cd, Pb and Zn by using an air-acetylene flame atomic absorption spectrophotometer (AAS) Perkin-Elmer Model 4100. The data were presented in $\mu g\ g^{-1}$ fresh weight basis.

To avoid possible contamination, all the glassware and equipment used were acid-washed and the accuracy of the analysis was checked with the blanks and the standard addition testing procedure. The percentages of recoveries for the heavy metal analyses were 103% for Cd, 95% for Cu, 90% for Pb and 110% for Zn. The one-way ANOVA Student-Newman-Keuls (SNK) test (Day and Quinn, 1989) was used to elucidate for the significant differences ($P< 0.05$).

Results

Comparisons of levels of Cd, Cu, Pb and Zn in the different parts of byssus, foot and total soft tissue of *P. viridis* are shown in Table 1. The levels of Cd (2.22 $\mu g\ g^{-1}$), Cu (17.48 $\mu g\ g^{-1}$) and Pb (11.92 $\mu g\ g^{-1}$) in the attachment plaque were found to be significantly ($P< 0.05$) higher than those found in other parts of byssus (root, stem and byssal thread), foot and total soft tissue. The total soft tissue was found to have the lowest for the levels of Cd (0.16 $\mu g\ g^{-1}$), Cu (1.53 $\mu g\ g^{-1}$) and Pb (1.48 $\mu g\ g^{-1}$) among the different parts and foot studied. Highest level of Zn (73.94 $\mu g\ g^{-1}$) was also found in the attachment plaque when compared to other parts of the byssus while the lowest Zn level (16.88 $\mu g\ g^{-1}$) was found in the foot.

Discussion

The different heavy metal distributions in the different parts of byssus might be due to their differences in both ultrastructure formation and protein composition (Bairati and Vitellaro-Zuccarello, 1976; Benedict and Waite, 1986). Bell and Gosline (1996) reported that the mechanical behaviours of the proximal, distal and attachment portions were different due to differences in the structure and composition of the different portions of a byssal thread. The differential heavy metal distributions in the byssus gave rise to the question of why and how these distributions occurred in the different parts of a byssus, apart from the soft tissues (Ismail *et al.*, 1999). The high metal concentrations found at the attachment plaque are believed to be due to the collagen-like fibers having a unique protein composition. The actual protein composition for *P. viridis* is unknown. The attachment plaque is considered as the natural glue that helps the survival of the green-lipped mussel. According to Grayson (1983) the adhesive plaque of bivalves is a substance that is capable of holding materials together by substrata. Several attempts had been made to commercialize the adhesive properties of these substances. For example, the adhesive properties were used for cellular attachment and as mucoadhesives for drug delivery (Deacon *et al.*, 1998). Waite (1990) suggested that the attachment strategies of marine

Table 1: Student-Newman-Keuls (SNK) Comparisons of Cd, Cu, Pb and Zn Concentrations (Means μg g^{-1} fresh weight) in different parts in the byssus of *P. viridis*

Different portion	ATT plaque	BYS thread	Stem	Root	Foot	TST
Mean Cu	17.48	14.91	14.52	6.21	2.63	1.53
Different portion	ATT Plaque	Foot	Root	BYS thread	Stem	TST
Mean Cd	2.22	0.71	0.57	0.56	0.22	0.16
Different portion	ATT plaque	Root	BYS thread	Foot	Stem	TST
Mean Pb	11.92	7.17	4.47	4.36	2.94	1.48
Different portion	ATT plaque	BYS thread	Root	Stem	TST	Foot
Mean Zn	73.94	51.15	34.35	21.54	19.32	16.88

Note: Means not differing significantly at $P< 0.05$ are indicated by a line under the corresponding values. ATT- attachment, TST- total soft tissues: BYS-byssal.

mussels rely on 3, 4- dihydroxyphenyl-L-alanine (DOPA)-containing proteins. The properties on how the protein can increase the heavy metal levels may be one of the ways for detoxification in *P. viridis*. Since the attachment plaque is a newly formed compartment of the byssal thread and therefore it can show current exposure of *P. viridis* to heavy metals in the environment.

Since the byssus of *P. viridis* had higher metal contents than the foot and the total soft tissue, it is believed that the hard-tanned protein in the byssus (Ikuta, 1986) could accumulate and bind with heavy metals at higher affinities resulting in the high metal concentrations. Thus, the byssus of *P. viridis* could be considered as one of the important secretion routes for heavy metals. Unlu and Fowler (1979) reported that the byssus of *M. galloprovincialis* played an important role in the elimination of arsenic from the mussels. George *et al.* (1976) also found that a major proportion of iron was excreted by transfer to the byssus of *M. edulis*. Szefer *et al.* (1999) found that the byssus of *M. edulis* was suitable to monitor Hg contamination in the coastal water.

To our knowledge, only the different biochemical and protein compositions of mussel byssus explain why there was a differential distribution of Cd, Cu, Pb and Zn in the different parts of the byssus of *P. viridis*. Whether or not metal-binding proteins such as metallothioneins and lysosomes (both of which are usually reported to be present in the soft tissues of bivalves) might have roles in the metal distributions of the different parts of the mussel byssus, would be an interesting subject for future studies.

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