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SHORT COMMUNICATION

Comparative x-ray microanalysis of the sporocyst wall of Aggregata octopiana and Aggregata eberthi (Protista: Apicomplexa)

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Sporocyst walls of the coccidian parasites Aggregata eberthi and A. octopiana, were subjected to semiquantitative x-ray elemental microanalysis. Peaks above background level were obtained for Na, P, S and Si in both species, with also a peak for Ca in A. eberthi which was not detectable in A. octopiana. Whilst the amounts of Na, P and S can be considered similar for both species, the relative percentage of Si was 3 times higher in A. eberthi than in A. octopiana. This technique may be useful for distinguishing between the sporocysts of these two species.

Key words: Aggregata octopiana; Aggregata eberthi; Sporocyst wall; X-ray elemental microanalysis, Ria de Vigo (Galicia, Spain).

Introduction

Heavy infections by the intracellular cimeriorin coccidia Aggregata eberthi Labbé, 1899 and Aggregata octopiana Schneider, 1875 have been demonstrated in European waters in the digestive tracts of the common cuttlefish Sepia officinalis and the common octopus Octopus vulgaris respectively (Dobell 1925; Gestal 2000). Both species of Aggregata have been studied by scanning and transmission electron microscopy, as well as by atomic force microscopy (Porchet-Henneré and Richard 1971; Gestal et al. 1999b; Gestal et al. 2002), but the elemental composition of the sporocyst wall has not been analysed.

Microanalysis of the elemental composition of hard structures has been employed in diagnosis and comparison between species (Chapman 1985; Shinn et al. 1995), and could be useful in distinguishing between the sporocysts of the two species of Aggregata.

Material and methods

Sporocysts of A. eberthi and A. octopiana were taken from Sepia officinalis and Octopus vulgaris, respectively, caught in the Ria de Vigo (Galicia, Spain). After removal of the digestive tract, the caecum was dissected and thoroughly washed in saline solution. Parasitised host tissues were homogenised and mature sporocysts were isolated, purified, suspended in distilled water and stored at 4°C (Gestal et al. 1999a). Before analysis purified sporocysts were precipitated on Isopore filters, fixed in 70% ethanol, dehydrated in an ethanol series, critical pointdried in CO_2 (Polaron E3000) and coated with amorphous carbon (Polaron CA508). Sporocysts were mounted on carbon stubs to avoid contamination with other elements, and analysed under a Phillips XL-30 scanning electron microscope coupled to an EDX EDAX DX 4I energy disperse detector. The microscope was operated at 10 kV and the electron beam focussed to a spot diameter of 1 µm. Measurements lasting 100 s were taken in an energy range from 0 to 10 keV.

Results and discussion

X-ray microanalysis of the sporocyst wall revealed several peaks above the background noise, as shown in the examples in Figure 1. The mean relative composition of the relevant elements in the sporocyst wall of each species is given in Table 1.

With the exception of Ca, evident in A. eberthi but below background in A. octopiana, the same elements were present in both species, with peaks of Na, P, S and Si. Peaks of C, N and O, always present in organic material, were also observed, even though they were not quantifiable by this technique. Different relative percentages of each element were observed in sporocyst walls of the two species. The quantities of Na, S and P can be considered to be in similar relative proportions for both species, but a major difference was observed in the relative percentage of Si, which was 3 times higher in the sporocyst wall of A. eberthi than in A. octopiana.

Light microscopy of fresh material had previously shown that A. eberthi sporocysts presented a higher resistance to compression on slides, and also to the isolation and purification processes, than those of A. octopiana (unpubl. data). There appears to be a link between mechanical strength and Si concentration, although the sporocyst wall of A. octopiana is thicker than that of A. eberthi. The sporocyst wall of A. octopiana also shows a rough surface with numerous spiny projections, in contrast to the completely smooth sporocyst wall of A. eberthi (Gestal et al. 1999b). Silicon provides strength in skeletal structures of diatoms and sponges, whose Si content depends on environmental factors (Hartman 1981). Bone and the chitin of crustacean shells also have Si as an important component. The Si observed in the sporocyst wall of Aggregata could similarly have a structural function, and contribute to its strength. The significance of P in structural function and strength has been noted by Shinn et al. (1995), who also pointed out the presence of S in the

keratin-like substance of sclerites in Monogenea.

Species of Aggregata have an extremely high host specificity, mainly in the definitive cephalopod host (Hochberg 1990). The difference in composition of the sporocyst wall in the two species of Aggregata could depend on the eating behaviour of the hosts. Although the diet of both species of cephalopod includes crustaceans, bony fish and molluscs, Octopus vulgaris usually drills a hole in the carapace of crabs and the shells of molluscs to obtain the flesh within before discarding the exoskeletons, whilst Sepia officinalis ingests much skeletal material from crustaceans and fish (Guerra and Nixon 1987; Castro and Guerra 1990). The difference in relative percentage of Si in the sporocyst wall may be of use in distinguishing between the sporocysts of these two species of Aggregata.

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References

Castro B. and Guerra A. (1990): The diet of Sepia officinalis (Linnaeus, 1758) and Sepia elegans (Blainville, 1827) (Cephalopoda: Sepioidea) from the Ría de Vigo (NW Spain). Sci. Mar. 54, 375–388.

Chapman D. (1985): X-ray microanalysis of selected coelenterate statoliths. J. Mar. Biol. Ass. U.K. 65, 617–627.

Dobell C. C. (1925): The life-history and chromosome cycle of Aggregata eberthi (Protozoa: Sporozoa: Coccidia). Parasitol. 17, 1–136.

Gestal C., Abollo E. and Pascual S. (1999a): Evaluation of a method for isolation and purification of sporocysts of the cephalopod coccidian parasite Aggregata Frenzel, 1885 (Apicomplexa: Aggregatidae). Iberus 17, 115–121.

Gestal C., Pascual S., Corral L. and Azevedo C. (1999b): Ultrastructural aspects of the sporogony of Aggregata octopiana (Apicomplexa, Aggregatidae), a coccidian parasite of Octopus vulgaris (Mollusca, Cephalopoda) from NE Atlantic coast. Europ. J. Protistol. 35, 417–425.

Gestal C. (2000): Epidemiología y patología de las coccidiosis en cefalópodos. Tesis doctoral. Universidad de Vigo. 157 pp.

Gestal C., Serra C., Guerra A. and Pascual S. (2002): Scratching the sporocyst surface: characterisation of European Aggregata species by atomic force microscopy. Parasitol. Res. 88, 242–246.

Guerra A. and Nixon M. (1987): Crab and mollusc shell drilling by Octopus vulgaris (Mollusca: Cephalopoda) in the Ría de Vigo (north-west Spain). J. Zool. Lond. 211, 515–523.

Hartman W.D. (1981): Form and distribution of silica in sponges. In: T. L. Simpson and B. E. Volcani (eds.):

Silicon and Siliceous structures in biological systems. pp. 453–493. Springer-Verlag, New York, Heidelberg, Berlin.

Hochberg F.G. (1990): Diseases of Mollusca: Cephalopoda. Diseases caused by protistans and metazoans. In: Kinne O (ed): Diseases of marine annimals, Vol III. Cephalopoda to Urochordata. pp 47–227. Biologische Anstalt Helgoland: Hamburg, Germany.

Porchet-Henneré E. and Richard A. (1971): La sporogenèse chez la Coccidie Aggregata eberthi. Etude en microscopie electronique. J. Protozool. 18, 614–628.

Shinn A.P., Gibson D.I. and Sommerville C. (1995): A study of the composition of sclerites of Gyrodactylus Nordmann, 1832 (Monogenea) using x-ray elemental analysis. Int. J. Parasitol. 25, 797–805.

Table 1. Relative elemental composition by weight of the A. octopiana and A. eberthi sporocyst wall.

Element	% Weight			
	A. eberthi		A. octopiana	
	Mean (n = 5)	Range	Mean (n = 5)	Range
Na	5.45	(4.18–5.96)	8.81	(7.22–10.165)
Si	70.84	(66.70–73.97)	23.18	(19.92–32.26)
P	12.45	(9.75–15.70)	29.52	(27.38–31.99)
S	9.04	(8.34–10.02)	38.42	(29.47–42.37)
Ca	2.42	(1.02–4.00)	Not detectable	Not detectable

Fig. 1. EDX spectrum of the composition of the sporocyst wall. A. Aggregata octopiana. B. Aggregata eberthi.