Folia biologica (Kraków), vol. **57** (2009), No 3-4
doi:10.3409/fb57_3-4.139-142

Aluminium Deposition in Hydras

Goran KOVAČEVIĆ, Mirjana KALAFATIĆ and Karlo HORVATIN

Accepted April 20, 2009

KOVAČEVIĆ G., KALAFATIĆ M., HORVATIN K. 2009. Aluminium deposition in hydras. Folia biol. (Kraków) **57**: 139-142.

The aim of this research was to explore, compare and explain the appearance, purpose and possible distribution of aluminium depositions in symbiotic and aposymbiotic hydra species. Al deposition in treated hydras appeared pink as single and multiple aluminium depositions or as clusters in the shape of globular or spot-like structures inside the cytoplasm of the hydra cells. Areas of aluminium deposits were also present. Endosymbiotic algae in the green hydra were occasionally coloured pink. Authors suggest that the mesoglea represented a buffer of some sort as the depositions were almost completely absent in some parts of the body such as the gastroderm of brown hydras or the ectoderm in some concentrations of green hydras, or the mesoglea may have the ability to dispose of all the present aluminium and the occurrence and distribution of these depositions may be a mechanism of cellular detoxification and might be species specific.

Key words: Green and brown hydra, aluminium depositions, endosymbiotic alga.

Goran KOVAČEVIĆ, Mirjana KALAFATIĆ, Faculty of Science, University of Zagreb, Division of Biology, Department of Zoology, Rooseveltov trg 6, HR-10000 Zagreb, Croatia.
E-mail: goran@zg.biol.pmf.hr
Karlo HORVATIN, Vladimir Prelog Science School, Ulica grada Vukovara 269, HR-10000 Zagreb, Croatia.

Hydras are simple freshwater invertebrates, members of the phylum Cnidaria, class Hydrozoa, order Hydroida, suborder Hydrina, family Hydridae (HOLSTEIN & EMSCHERMANN 1995). Hydras have a simple cylindrical body with an adhesive foot at one end and a mouth surrounded by six to eight tentacles at the other. The body comprises two cellular layers: the ectoderm and the gastroderm, separated by the mesoglea. The green hydra forms an endosymbiotic relationship with individuals of unicellular green algae that reside in the gastrodermal myoepithelial cells of the hydra host (Douglas 1994). Hydra has been a useful experimental subject in ecotoxicological and evolutionary research for more than 250 years (HABETHA *et al.* 2004; KALAFATIĆ & KOPJAR 1994; TREMBLEY 1744).

Although aluminium (Al) is one of the most abundant chemical elements in the Earth's crust and is considered as a potential neurotoxicant, its toxicity has not been studied substantially (EXLEY 2003; HERMANN 2001). This silvery metal is essentially insoluble in water (MACDONALD & MARTIN 1988) and the concentration of its ions in most natural waters remains extremely low (<0.1 mg/l). However, its solubility dramatically rises as the acidity of the solvent water increases (GOENAGA & WILLIAMS 1988). The contemporary dosage of aluminium in freshwater experiments varies from lower levels (25, 50 mg/l) to as high as 500 mg/l of a specific aluminium containing compound (for example $Al_2(SO_4)_3.18H_2O$) in hydra research or 1500 mg/l in planarian research, where the LC_{50} value for brown hydra is less than 475 mg/l, between 475-480 mg/l for green hydra (KOVAČEVIĆ *et al.* 2007) and 1100 mg/l for planarians (KOVAČEVIĆ *et al.* 2009).

Morphometry has become an interesting and helpful tool in biological analyses in the last 15 years. It has been used in different fields of biological applications (GRIBBEN *et al.* 2001; NAKAHARA *et al.* 2003).

Endosymbiosis is one of the most important and most interesting subjects in evolutionary biology and symbiotic associations are of wide significance in evolution (MARGULIS & SAGAN 2002; SECHBACH 2001). The aim of this work was to explore, detect and compare the distribution and purpose of aluminium depositions in the symbiotic green and the aposymbiotic brown hydra species.

Material and Methods

A comparative subacute static toxicity test was performed using individuals of the green (*Hydra*

viridissima Pallas, 1766; strain S1J-J1) and brown (*Hydra oligactis* Pallas, 1766; strain S1M-K1) hydra. Animals were collected from Zagreb lakes Maksimir and Jarun. The collected animals were grown in culture under stable laboratory conditions in aerated aquarium water (photoperiod 10 hrs light/15 μmol/m^{2}s, 14 hrs dark).

Five animals of each species were treated under laboratory conditions (21.5^{o}C) with one of the following 6 concentrations (25, 50, 80, 100, 250 and 475 mg/l) of aluminium sulfate (AL) $Al_2(SO_4)_3$. $18H_2O$ (Kemika, Croatia) in aerated aquarium water in glass dishes, 6 cm in diameter, 3.5 cm height. The experiment lasted for 72 hrs and the results were compared to the control groups of green and brown hydra. The experiments were repeated three times.

Standard histological methods were used (Bouine fixative, dehydration, paraplast, light microscope). Sections were differentially stained with 0.2% acid eriochrome cyanine (EC) for 22 min and immediately washed out in running hot tap water, dehydrated in a series of ethanol solutions starting with 70% acid ethanol (1%) (PEARSE 1972; modified by KOVAČEVIĆ *et al.* 2006). Using a sample of 200 cells per group, we determined the percentage of cells containing aluminium depositions. We measured the surface area of those depositions in comparison to the area of cells containing them (mean$\pm$standard deviation). Morphometric analysis was performed using Lucia G DXM1200 version 4.81 software. Micrographs were made using the Reichert and Nikon Eclipse E600 microscope, Nikon DXM1200 camera.

Results and Discussion

Al depositions in treated hydras appear pink in the form of single depositions, multiple (2-4 separate) depositions, clusters or areas (Tables 1 & 2) of aluminium deposits (KOVAČEVIĆ *et al.* 2006). More than 4 single indistinguishable depositions bound together were regarded as clusters. Single, multiple and clustered aluminium depositions occurred in the shape of globular- or spot-like structures inside the cytoplasm and seemed not to be membrane-bound. Areas of aluminium deposits were regions that consisted of cells full of aluminium (Fig. 1). Earlier descriptions maintained that Al depositions are found both in the ectoderm and gastroderm. No depositions are found in the mesoglea. Brown hydra contained depositions in the ectodermal cellular layer and green hydra contained depositions mostly in the gastrodermal cellular layer including endosymbiotic algae (KOVAČEVIĆ *et al.* 2006). Therefore, the occurrence of these depositions seems to be species specific.

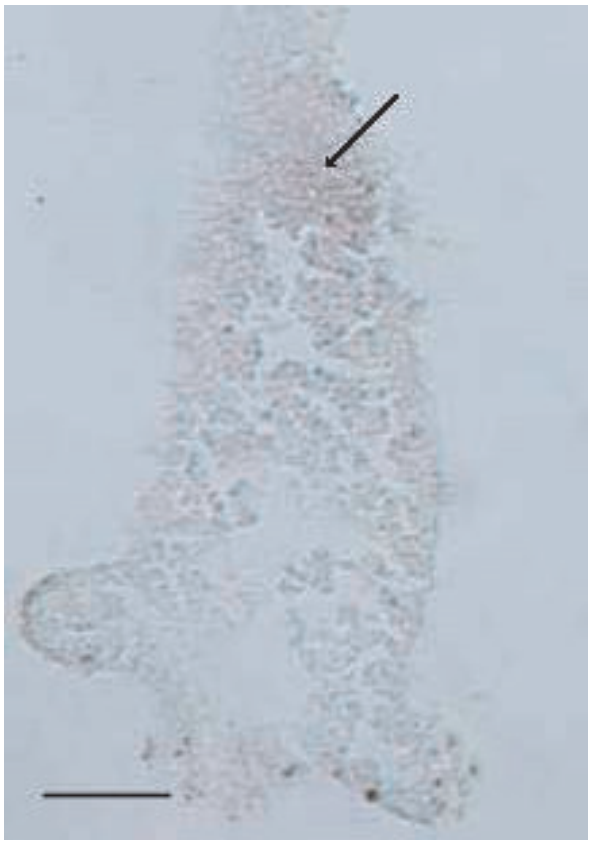

Fig 1. Green hydra (*Hydra viridissima* Pallas, 1766) treated with 250 mg/l Al. Foot of the animal full of aluminium depositions (pink coloration, arrow). Bar=50 μm.

We performed a detailed morphometric analysis and quantified the results measured from the body and the foot of the brown hydra (Table 1) and the green hydra (Table 2). Green hydra cells, being much smaller as well as having smaller, occasionally indistinguishable depositions compared to those of brown hydra cells, were much more difficult to measure and determine the occurrence of depositions. Therefore, the green hydra depositions at a concentration 50 mg/l AL were not measurable. Higher concentrations of Al also yielded a greater cell surface area covered by increasing quantities of aluminium deposition in all forms in the brown hydra. Cellular layers containing the depositions were not as damaged. The pink coloration was mildly contrasted and could barely be detected at lower concentrations. The intensity of the pink coloring rose with increasing concentration. We assumed that the appearance and distribution of these depositions could represent a mechanism of cellular detoxification. The aluminium did not seem to have dissolved inside the cells. It was packed into visible depositions that were possibly excreted with mucous, not presenting a greater threat to the organism. At the highest concentrations this mechanism may have ceased to operate and the animals could not resist the high impact of

Table 1

Fraction of cells (%) containing aluminium depositions in brown hydra

Body [mg/l] Al	25	50	80	100	250
single depositions	1.74	7.42	7.37	12.98	9.00
multiple depositions	0	0	0	1.08	22.00
clusters	0	0	0	2.70	0
Total	1.74	7.42	7.37	16.76	31.00
Foot					
single depositions	6.37	14.81	8.88	25.00	7.14
multiple depositions	0	0	0	0	25.00
clusters	0	0	0	0	0
Total	6.37	14.81	8.88	25.00	32.14

Table 2

Fraction of cells (%) containing aluminium depositions in green hydra

Body [mg/l] Al	25	50
single depositions	9.14	16.18
multiple depositions	1.71	0
clusters	0.57	0
Total	11.42	16.18
Foot		
single depositions	10.64	–
multiple depositions	2.13	–
clusters	0.00	–
Total	12.77	–

– not measurable

the metal. At some point the rate of detoxification became possibly too slow for the growing quantities of aluminium to be processed. Endosymbiotic algae in the green hydra were only occasionally colored pink in the lowest concentration, in 250 mg/l AL half of the algae and in 475 mg/l AL all of the algae were colored.

Since the results show depositions only in the cellular layers of the hydras (KOVAČEVIĆ *et al.* 2006), we suggest that the mesoglea represents a buffer of some sort as the depositions were almost completely absent in some parts of the body such as the gastroderm of brown hydras or the ectoderm in some concentrations of green hydras. On the other hand, the mesoglea may have the ability to dispose of all the present aluminium. It is a gel-like substance consisting of fibrilar matter similar to collagen and elastin. It makes possible the migration of food and cells during regeneration (NIDARIĆ 1970).

Except for the detoxification mechanism of excretion of the compressed aluminium depositions through mucus (KOVAČEVIĆ *et al.* 2007), high amounts of mucous could also inhibit gaseous exchange and osmoregulation and therefore affect physiology. Detoxification mechanisms in other organisms include transformation of aluminium into non-reactive or colloidal forms of aluminium reducing toxicity (KROGLUND *et al.* 2001), localization in excretory granules of snails (BROOKS & WHITE 1995) or bioaccumulation in lysosomes (REBOREDA & DAVIES 2006).

The foot of hydra appeared to contain more depositions than the body of hydra (Fig. 1). We conclude that with the effect of Al on this fraction of hydra, the viability of the whole organism could be disturbed. In our former paper we reported the effect of Al on the morphology and behavior of both green and brown hydras, including mortality, reproduction, tentacle reduction, reactions to mechanical stimuli, deformations, mucous secretion and migration in an experimental dish (KOVAČEVIĆ *et al.* 2007), in which almost all of the parameters were concentration dependent.

Acknowledgements

The presented results are a product of the scientific project “Molecular phylogeny, evolution and symbiosis of freshwater invertebrates” carried out with the support of the Ministry of Science, Education and Sport of the Republic of Croatia.

References

BROOKS A. W., WHITE K. N. 1995. The localization of aluminium in the digestive gland of the terrestrial snail *Helix aspersa*. Tissue Cell. **27**: 61-72.

DOUGLAS A. E. 1994. Symbiotic Interactions. Oxford University Press Inc., Oxford & New York.

EXLEY C. 2003. A biogeochemical cycle for aluminium? J. Inorg. Biochem. **97**: 1-7.

GOENAGA X., WILLIAMS D. J .A. 1988. Aluminum speciation in surface waters from Welsh upland area. Environ. Pollut. **52**: 138-149.

GRIBBENN P. E., CREESE R. G., HOOKER S. H. 2001. The reproductive cycle of the New Zealand venus clam *Ruditapes largillierti*. J. Shellfish Res. **20**: 1101-1108.

HABETHA M., ANTON-ERKSLEBEN F., NEUMANN K., BOSCH T. C. G. 2003. The *Hydra viridis/Chlorella* symbiosis. Growth and sexual differentiation in polyps without symbionts. Zoology **106**: 1-8.

HERMANN J. 2001. Aluminium is harmful to benthic invertebrates in acidified waters, but at what threshold(s)? Wat. Air Soil Pollut. **130**: 837-842.

HOLSTEIN T., EMSCHERMANN P. 1995. Cnidaria: Hydrozoa, Kamptozoa. Gustav Fischer Verlag, Stuttgart.

KALAFATIĆ M., KOPJAR N. 1994. Response of green hydra to the treatment with different pesticides under laboratory conditions. Z. Angew. Zool. **2**: 213-223.

KOVAČEVIĆ G., KALAFATIĆ M., HORVATIN K. 2006. Detection of aluminum depositions in green and brown hydra. Symbiosis **42**: 175-176.

KOVAČEVIĆ G., ELJEŽIĆ D., HORVATIN K., KALAFATIĆ M. 2007. Morphological features and comet assay of green and brown hydra treated with aluminium. Symbiosis **44**: 145-152.

KOVAČEVIĆ G., GREGOROVIĆ G., KALAFATIĆ M., JAKLINOVIĆ I. 2009. The effects of aluminium on the planarian *Polycelis feline* (Daly.) .Wat. Air Soil Pollut. **196**: 333-344.

KROGLUND F., TEIEN H. C., ROSSELAND B. O., SALBU B. 2001. Time and pH-dependent detoxification of aluminum in mixing zones between acid and non-acid rivers. Wat. Air Soil Pollut. **130**: 905-910.

MACDONALD T. L., MARTIN R. B. 1988. Aluminium ion in biological systems. Trends Biochem. Sci. **13**: 15-19.

MARGULIS L., SAGAN D. 2002. Acquiring Genomes: A Theory of the Origin of Species. Basic Books, New York.

NAKAHARA M., HANDA S., NAKANO T., DEGUCHI H. 2003. Culture and pyrenoid structure of a symbiotic *Chlorella* species isolated from *Paramecium bursaria*. Symbiosis **34**: 203-214.

PEARSE A. G. E. 1972. Histochemistry. Theoretical and Applied. Churchill Livingstone, Edinburg and London.

REBOREDA R., DAVIES M. S. 2006. Characterisation by X-ray microanalysis of metal granules in the mucus trails of *Littorina littorea* (Gastropoda) along a putative pollution gradient. Ecotoxicology **15**: 403-410.

SECHBACH J. (ed.) 2001. Symbiosis – Mechanisms and Model Systems, Kluwer Academic, The Netherlands.

TREMBLEY A. 1744. Mémoires por Servir à l'Histoire d'un Genre de Polypes d'Eau Douce, à Bras en Forme de Comes. Jean and Herman Verbeek, Leyden.

NIDARIĆ D. 1970. Comparison of the regeneration of the hypostome with the budding process in *Hydra littoralis*. Roux Arch. **166**: 45-53.