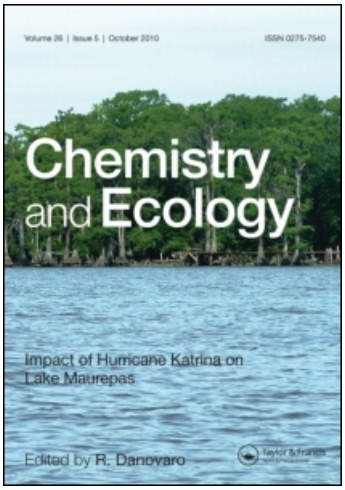

Chemistry and Ecology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713455114

Morphological integrity and toxicological properties of *Pelagia noctiluca (Scyphozoa) nematocysts*

A. Marino[a]; R. Crupi[b]; G. Musci[b]; G. La Spada[a]
[a] Department of General Physiology and Pharmacology, University of Messina, Italy [b] Department of Microbiological, Genetic and Molecular Sciences, University of Messina, Italy

To cite this Article Marino, A. , Crupi, R. , Musci, G. and La Spada, G.(2006) 'Morphological integrity and toxicological properties of *Pelagia noctiluca (Scyphozoa) nematocysts*', Chemistry and Ecology, 22: 4, S127 — S131
To link to this Article: DOI: 10.1080/02757540600677757
URL: http://dx.doi.org/10.1080/02757540600677757

Chemistry and Ecology
August 2006, Vol. 22 (Supplement 1), pp. S127–S131

Taylor & Francis
Taylor & Francis Group

Morphological integrity and toxicological properties of *Pelagia noctiluca (Scyphozoa) nematocysts*

A. MARINO†, R. CRUPI‡, G. MUSCI‡ and G. LA SPADA*†

†Department of General Physiology and Pharmacology, University of Messina, Italy
‡Department of Microbiological, Genetic and Molecular Sciences, University of Messina, Italy

(Received 7 March 2005; in final form 7 March 2006)

Isolated nematocysts of the Scyphozoan *Pelagia noctiluca* became diaphanous when incubated at low pH for 1 h or left at room temperature for a few hours. Diaphanous nematocysts were unable to undergo discharge when triggered by proper physico-chemical stimuli. The aim of the present study was to investigate the relationship between the morphological features of diaphanous nematocysts and the haemolytic power of their crude venom, obtained by sonication on ice. Nematocysts stored at −20 °C in neutral medium were used as a control. Our results show that the haemolytic power of crude venom from isolated nematocysts kept at low pH or at room temperature decreased significantly with respect to the control. From this study, it can be deduced that a neutral pH and low temperature conditions are recommended to store nematocysts properly in order to use them in toxicological investigations.

Keywords: Crude extract; Haemolysis; Nematocysts; Wall collapse; *Pelagia noctiluca*

1. Introduction

The phylum Cnidaria is a source of biologically active compounds, like cytolytic toxins, contained in specialized intracellullar organelles termed nematocysts. Toxins can be introduced into prey organisms or aggressors by an explosive exocytotic process termed discharge, which typically occurs when an adequate chemical and/or mechanical stimulus is applied. The discharge takes place by rapid eversion of the inner coiled thread, which in turn either adheres to or penetrates into the prey, injecting the venomous compounds contained in the capsular fluid. Owing to the complexity of this mostly undisclosed process, many studies have been performed on isolated nematocysts, which retain their discharging capacity even when stored at −20 °C for long periods [1–3].

A relevant aspect of nematocysts physiology deals with the toxicology of the capsular fluid, which has been widely investigated so far by means of a large number of biological assays mainly on Anthozoa due to their availability and ease of maintenance in aquaria [4–6]. Studies on Scyphozoa, on the other hand, are less well represented in the literature, mostly because their availability depends on cyclic blooming periods.

*Corresponding author. Email: glaspada@unime.it

Chemistry and Ecology
ISSN 0275-7540 print/ISSN 1029-0370 online © 2006 Taylor & Francis
http://www.tandf.co.uk/journals
DOI: 10.1080/02757540600677757

In recent years, a notable increase in the population of the holoplanctonic epipelagic scyphomedusa *Pelagia noctiluca* has occurred in the Strait of Messina. *P. noctiluca* is very dangerous to bathers, as accidental contact with its tentacles or oral arms can trigger a complex symptomatology, characterized by pain in the initial site of contact, followed often by skin lesions of varying size and persistance. For this reason, *P. noctiluca* bloomings are of prime interest to countries bordering the Mediterranean Sea, especially in tourist resort areas.

The discharge of *Pelagia noctiluca* holotrichous nematocysts, employed to ascertain the physiological integrity of such organoids, has been thoroughly investigated by our group [7, 8]. These studies showed that the anatomical integrity of the capsule is a prerequisite for the discharge to occur. In particular, exposure of nematocysts to acid pH media for short periods, or to neutral media for 1–2 h at room temperature, induces the collapse of the wall, with consequences both on the morphology of nematocysts, which become diaphanous, and on their discharge ability.

In this study, we investigate the relationship between the morphological features of isolated nematocysts of *Pelagia noctiluca* and the toxic power of their capsular fluid. We aimed to verify whether the well-known haemolytic power of the capsular fluid is retained after wall collapse of the isolated nematocyst. The recent blooming of *Pelagia noctiluca* in the Strait of Messina has allowed us to study toxicological features in fresh and stored isolated capsules.

2. Materials and methods

2.1 *Isolation of nematocysts*

Holotrichous-isorhiza nematocysts were isolated from oral arms of *Pelagia noctiluca* and used immediately or stored at $-20\,^{\circ}C$ [9]. Briefly, the excised oral arms were submerged in cold distilled water for 2 h in order to induce the detachment of the epidermis and the osmotic rupture of nematocytes, so that the nematocysts could be delivered undischarged in water. The suspension was washed repeatedly in distilled water and filtered through a plankton net (40, 60, and 100 μm mesh, respectively) to remove most of the tissue debris.

2.2 *Production of collapsed nematocysts*

Aliquots of the suspension of isolated nematocysts were either treated with 1 mM HCl (pH 3) solution for 1 h or left in distilled water at room temperature for a few hours. The percentage of diaphanous nematocysts was calculated by observation under a light microscope (Leica DMLS), connected to proper image-acquisition hardware.

2.3 *Crude venom extraction*

The crude venom was extracted by sonication on ice (Sonoplus 70 mHz, 30×, 20 s) of a population of isolated untreated nematocysts (90 nematocysts μl^{-1}) and, alternatively, an equal amount of nematocysts collapsed by acidic treatment or by storage at room temperature as specified above, and successively resuspended in the control medium (0.9% NaCl, 10 mM phosphate, pH = 7.4). The sonicated suspension was clarified by centrifugation at 3000 rpm for 10 min (ALC refrigerated centrifuge) to discard any broken capsules and tubules, and the crude venom was then used for the haemolytic assays. The protein content of crude extract from a suspension of 90 nematocysts μl^{-1} was measured using the BCA test (Pierce).

2.4 *Haemolytic assay*

The crude venom from both untreated and collapsed nematocysts was assayed upon fresh human red blood cells (0.05% suspension in physiological buffer, 0.9% NaCl, 10 mM phosphate, pH 7.4). After incubation at 37 °C for 1 h, the supernatant was spectrophotometrically read ($\lambda = 414$ nm). Haemolysis was calculated as a relative percentage with respect to maximal lysis obtained by treated nematocysts with distilled water, taking into account the background lysis of untreated red blood cells in physiological buffer. Statistical analysis was performed by Student's *t* test, and measures were described as the mean ± S.E. of at least 10 experiments.

3. Results and discussion

The crude extract obtained from normal resting nematocysts (population of 90 nematocysts μl^{-1}) had a protein content of 1.6 $\mu g\ \mu l^{-1}$, and induced almost 100% haemolysis on a 0.05% suspension of human erythrocytes after 1 h of incubation (figure 1). On the other hand, a much lower (albeit still significant) degree of haemolysis was induced by the crude extract from collapsed capsules, with pH-collapsed nematocysts (figure 1A) being less active than spontaneously collapsed capsules (figure 1B). Interestingly, the extraction of the capsular fluid by sonication turned out to be more difficult when collapsed capsules were used, suggesting that major structural changes in wall characteristics may occur upon wall collapse and consequent diaphanization.

The collapse occurs spontaneously at neutral pH at room temperature but appears to be kinetically favoured at acid pH values. As shown in figure 2, the collapsed capsules can be easily recognized under a standard light microscope, as the wall is less marked than in normal nematocysts, and the inner part of the organoid appears less structured. As expected, the phenomenon is totally irreversible: nematocysts collapsed at a low pH do not revert to their normal morphology when put into a neutral medium. SEM observations showed that collapsed capsules lack the outer rough layer of the wall, so that the underlying surface becomes visible (figure 3). It has been hypothesized that treatment at acid pH dissolves and/or detaches the glutamic acid-rich outer layer. It is less clear why this also occurs at neutral pH. Wall collapse does not occur under alkaline conditions. As a matter of fact, nematocysts spontaneously

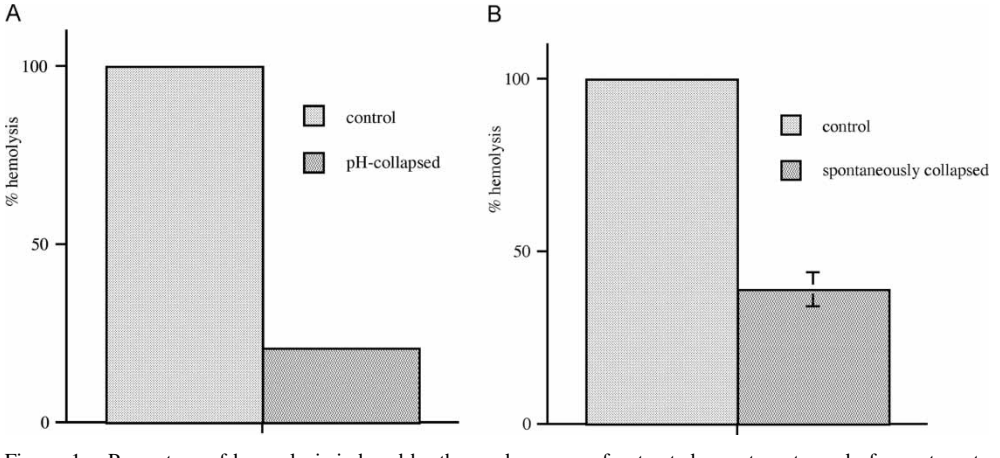

Figure 1. Percentage of haemolysis induced by the crude venom of untreated nematocysts, and of nematocysts collapsed at low pH (A) or at room temperature (B). The reduction in haemolytic power was statistically significant in both cases ($P < 0.001$).

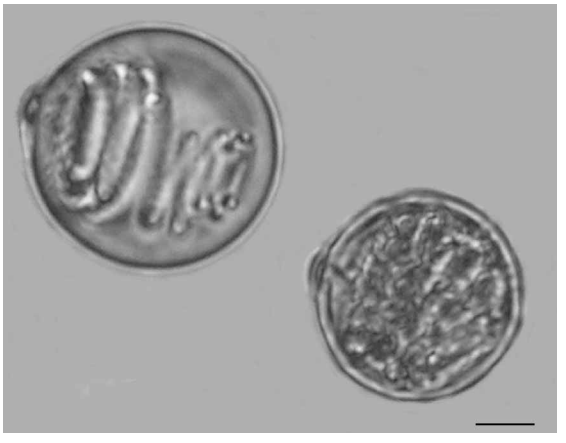

Figure 2. Light-microscope photomicrograph of a resting holotrichous isorhiza nematocyst (small arrow), isolated from oral arms of *Pelagia noctiluca*, and of a pH-collapsed nematocyst (large arrow). Bar: 15 μm.

discharge in highly alkaline media (pH > 11), thus excluding the possibility of investigating the toxic effect of capsular fluid from alkaline pH-treated nematocysts.

The structure controlling the intracapsular pressure could be placed either in the capsular fluid or in the wall itself: the collapse could be due to changes in the conformation of capsular fluid proteins, thus reducing the intracapsular pressure. As far as the wall features are concerned, it is worth remembering that capsule permeability is modified upon collapsing, when they become permeable to dyes of relatively high mass (up to 1300 Da) as well as Gram positive, at variance with the normal resting capsules [9].

Our results show that collapse of nematocysts, consequent to wall collapsing not only impairs the discharging ability of the organoids but also dramatically lowers the haemolytic power of the extracted crude venom. This is relatively easy to understand in acid pH-treated capsules, where the excess of protons can be hypothesized to induce major conformational changes in crude-extract components, which could undergo irreversible precipitation. Our observation that nematocysts spontaneously collapsed at neutral pH have also impaired discharging and haemolytic activity is intriguing and suggests that much more complex mechanisms are at the basis of the observed phenomenon. This is the first time that a possible

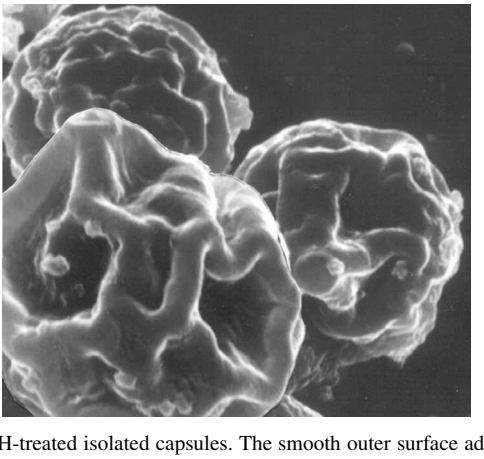

Figure 3. SEM picture of pH-treated isolated capsules. The smooth outer surface adheres to the inner content of the nematocyst, which becomes clearly visible (3000×).

link between the morphological integrity and nematocyst functionality has been related to toxicological investigations. In particular, our findings indicate that intact and well-stored (i.e. at $-20\,^{\circ}C$) isolated nematocysts can be used to perform the appropriate toxicological studies.

Preliminary results show that the crude venom of *P. noctiluca* is much more stable if extracted from fresh rather than collapsed capsules, suggesting that the loss of haemolytic power should be ascribed to phenomena occurring only when the fluid is in contact with the particulate components of the wall and/or the internal tubule. Further work is in progress to clarify this point.

References

[1] T. Holstein, P. Tardent. An ultrahigh-speed analysis of exocytosis: nematocyst discharge. *Science*, **223**, 830–833 (1984).
[2] A. Salleo, G. La Spada, M. Drago, G. Curcio. Hyposmotic shock-induced discharge in acontia of *Calliactis parasitica* is blocked by Gadolinium. *Experientia*, **50**, 148–152 (1994).
[3] G. La Spada, G. Sorrenti, A. Soffli, B. Montaleone, A. Marino, G. Musci. Thiol-induced discharge of acontial nematocytes. *Comp. Biochem. Physiol.*, **132B**, 367–373 (2002).
[4] G. Anderluh, P. Macek. Cytolytic peptide and protein toxins from sea anemones (Anthozoa: Actiniaria). *Toxicon*, **40**, 111–124 (2002).
[5] M.M. Monastyrnaya, T.A. Zykova, O.V. Apalikova, T.D. Shwets, E.P. Kozlovskaya. Biologically active polypeptides from the tropical sea anemone *Radianthus macrodactylus. Toxicon*, **40**, 1197–1217 (2002).
[6] A.M.Mayer, K.R. Gustafson. Marine pharmacology in 2000: antitumor and cytotoxic compounds. *Int. J. Cancer*, **105**, 291–299 (2003).
[7] A. Salleo. Discharge mechanism of the nematocysts of *Pelagia noctiluca*. In *Toxins and Drugs of Marine Animals to Pollutants*, L. Bolis, J. Zadunaisky, R. Gills (Eds), pp. 63–68, Springer, Berlin (1984).
[8] G. La Spada, A. Marino, G. Sorrenti. *Pelagia noctiluca* 'blooming' in the Strait of Messina: preliminary studies on the applicability of two methods for isolating nematocytes. *Mar. Ecol.*, **23** (Suppl. 1), 220–227 (2002).
[9] A. Salleo, G. La Spada, G. Falzea, M.G. Denaro. pH-induced collapse of the capsular wall in isolated nematocysts of *Pelagia noctiluca. Cell. Mol. Biol.*, **30**, 91–94 (1984).