Cellular and Molecular Biology [TM] **53**, OL994-OL1002
DOI 10.1170/113

ISSN 1165-158X
2007 Cell. Mol. Biol.[TM]

THE UNUSUAL TOXICITY AND STABILITY PROPERTIES OF CRUDE VENOM FROM ISOLATED NEMATOCYSTS OF *Pelagia Noctiluca (Cnidaria, Scyphozoa)*

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Received February 26th, 2007; Accepted May 31th, 2007; Published September 17th , 2007

Abstract – We have firstly investigated the toxicological activity by hemolytic assay of crude extract obtained by sonication of holotrichous isorhiza isolated nematocysts of the Scyphozoan *Pelagia noctiluca*, collected in the Strait of Messina. The hemolytic activity was both time- and dose-dependent on fish, rabbit, chicken and human red blood cells. At lowest doses rabbit and chicken erythrocytes were the most sensitive, whereas those of eel were the most resistant to the crude extract. Different storage conditions, such as -20 °C, -80 °C for up to 6 months and lyophilization, did not affect the stability of crude venom. Moreover, neither treatment at 4 °C, 20 °C and 37 °C for different time periods ranging between 30 min and 24 h, nor harsh thermal treatment at 80 °C and 100 °C affected the hemolytic power. The crude venom resulted even stable towards proteolysis and alkaline pH values.

Key words: Crude venom, hemolytic assay, nematocysts, *Pelagia noctiluca*

INTRODUCTION

Cnidarians nematocytes are specialized stinging cells, used in the capture of prey as well as for defense against predators. The *phylum* Cnidaria represents a widely explored source of marine bioactive compounds. This *phylum* has provided a great number of natural products, which include proteins and secondary metabolites with toxic and/or biomedical properties. Within Cnidaria, sea anemones and jellyfish have developed a variety of biologically active compounds, including some potent toxins.

For Hydrozoan, Scyphozoan and Anthozoan species, there is evidence that toxins are delivered by nematocysts, characteristic stinging organelles contained in nematocytes.

Abbreviations: C, centrifuged sample; **NC**, not centrifuged sample; **R**, resuspended sample; **BCA**, bicinchonic acid assay; **SDS**, sodium dodecyl sulphate;

Unlike pelagic Cnidaria, sedentary sea anemones have proved to be less harmful for humans, due to a lower incidence of stinging and sting severity. Anyway, the major part of peptides and proteins produced by Coelenterates act as neurotoxins or cytolysins (3, 5, 8, 14).

A relevant blooming of the pink jellyfish *Pelagia noctiluca* has occurred in the last few years, with invasion of large areas of the Mediterranean Sea, including the Strait of Messina (Sicily, Italy). The accidental contact with specimens of *Pelagia noctiluca* causes the discharge of thousands of nematocysts. Once inside the skin the nematocyst ejects a thread that in turn either adheres to or penetrates into the prey, injecting the venomous substances contained in the capsular fluid. The resulting allergic-like reaction is accompanied by pain, redness, swelling and sometimes even fever. The toxin of *Pelagia noctiluca* is not lethal, but the

impact on public health and on tourism is relevant.

The morphology of different types of nematocytes of *Pelagia noctiluca* has been investigated by Avian and coworkers (2). *Pelagia noctiluca* is a Scyphozoa equipped with eight long tentacles covered by stinging cells and four oral arms arising from beneath its pulsing umbrella. In the oral arms there are warts of holotrichous isorhiza nematocysts (fig. 1), following Mariscal classification (25). On the other hand, the functional aspects of the discharge mechanism of *Pelagia noctiluca* nematocysts have been thoroughly investigated by Salleo and coworkers in the last two decades (29, 30, 31, 32, 33), contributing to elucidate some aspects of this special and exciting phenomenon. However, the biochemical properties and the possible mechanism(s) of action of the various toxins stored in the capsular fluid are still poorly understood.

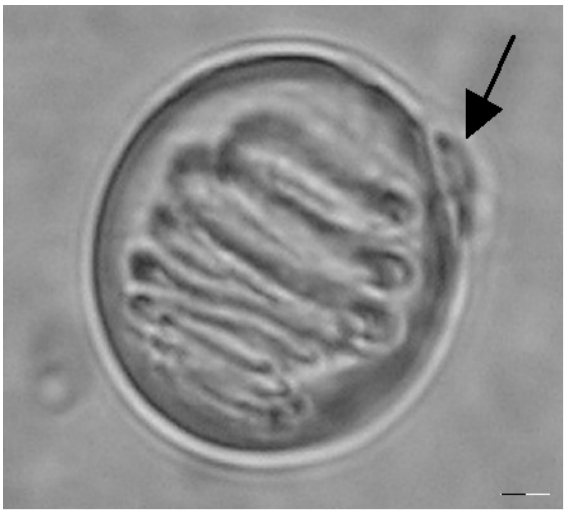

Figure 1. Light microscope picture of an isolated holotrichous isorhiza nematocyst from *Pelagia noctiluca.* Note the coiled tubule inside the capsule and the operculum (arrow). Bar= 15 µm.

In the current study we have examined the hemolytic power of the crude extract from nematocysts of *Pelagia noctiluca* (Scyphozoa), collected in the Strait of Messina during the past bloomings, in order to define its general properties. In particular we aimed to clarify toxicological features exclusively contributed by the capsular fluid of isolated nematocysts and not deriving from other tissue components.

MATERIALS AND METHODS

Chemicals

All chemicals were purchased from Sigma Aldrich (Milan, Italy).

Nematocysts preparation and crude venom extraction

Specimens of *Pelagia noctiluca* were collected in the Strait of Messina. Immediately after capture, the specimens of *Pelagia noctiluca* were transported alive to the laboratory and nematocysts were isolated as described by Salleo and coworkers (29). Briefly, the oral arms were excised within two hours and singly suspended in cold distilled water for 2 hours at 4 °C. The treatment with distilled water lead to detachment of the epidermis and to osmotic rupture of nematocytes, with consequent delivery of undischarged nematocysts. The naked oral arms were gently removed and the nematocysts suspension was repeatedly washed in distilled water, filtered through a plankton net of less than 0.1 mm mesh to discard debris, and centrifuged in a refrigerate centrifuge (ALC PK 120R) at 4,000 g for 5 min. Thus nematocysts classified as holotrichous isorhizas according to Mariscal (25) were obtained.

Since the holotrichous isorhizas settle down more rapidly than smaller ones and debris, most of the latter could be easily removed from the sample. The pellet was finally resuspended and stored at -20 °C or -80 °C. Before use, the samples were thawed and filtered again. In the present paper we have exclusively employed nematocysts from oral arms (holotrichous isorhiza).

The crude extract was prepared from isolated, clean nematocysts of *Pelagia noctiluca*, without other cellular components. Holotrichous isorhiza nematocysts from *Pelagia noctiluca* samples were thawed and resuspended in 0.01 M phosphate buffer containing 0.9% NaCl (pH 7.4; osmotic pressure=300mOsm/Kg_{H_2O}) in order to have a suspension of ca. 90 nematocysts/µl after counting in a Bürker chamber. After sonication on ice (Sonoplus, 30 times, 20 sec at 20 kHz) the crude extract was separated from crushed capsules by refrigerated centrifugation at 4,000 g for 10 min. The obtained venom was kept at 4 °C until use or stored at –20 °C or -80 °C, unless otherwise stated.

Hemolytic assays

For the hemolytic tests performed on a single nematocyst, fresh human erythrocytes were used, while either human, rabbit, chicken or eel red blood cells were employed for standard spectrophotometric hemolytic assays.

Hemolytic tests were performed essentially as described elsewhere (22). Briefly, human, rabbit or chicken erythrocytes were centrifuged from citrated blood, washed three times with isotonic buffer (0.01 M phosphate, pH 7.4, containing 0.9 % NaCl) and resuspended in the same buffer to a final concentration of either 5 % for the single nematocyst tests, or 0.05 % for the spectrophotometric assay. A Ringer solution (concentration in mM: NaCl 133, KCl 3.2; $MgCl_2$ 1.4; $CaCl_2$ 2.5; $NaHCO_3$ 20; KH_2PO_4 0.8; glucose 20) was employed for eel red blood cells.

In order to directly observe the hemolytic activity of a single isolated nematocyst, a slide was prepared with human red blood cells (5 % suspension in an isotonic buffer). An equal volume of isolated undischarged capsules was added together with two volumes of the discharging agent (605 mM NaSCN isosmotic solution containing

10mM Ca^{2+}). A cover slip was put in place and hemolysis around discharged nematocysts was then observed under a light microscope (Leica DMLS, x200). Control conditions were set up by treating 5 % red blood cells suspension with the discharging agent in the absence of nematocysts.

For spectrophotometric assays, aliquots of a 0.05 % suspension of human, rabbit, chicken or eel red blood cells were incubated at desired temperature (either 4 °C or 20 °C or 37 °C) with the proper amount of crude toxin and gently shaken for one hour. They were then centrifuged (3,000 g, 5 min) and the optical absorbance of the supernatant at 414 nm was measured. The lytic power of the venom was expressed as percent of the optical absorbance observed after maximal lysis induced by addition of 1 volume of distilled water (properly corrected for dilutions). The supernatant of untreated red blood cells in isotonic buffer was taken as a spectrophotometric blank.

Miscellaneous

Kinetics of hemolysis was monitored incubating the crude venom aliquots with red blood cells for various periods of time (in min: 1, 3, 5, 10, 30, and 60) at 4 °C, 20 °C and 37 °C, respectively. Stability of crude venom was verified either by different storage conditions as thawing at -20 °C and -80 °C or by lyophilization, or by heat treatment at 40 °C, 60 °C, 80 °C and 100 °C, respectively. Variations of pH value of the crude extract were obtained by suspending the nematocysts in 0.5 M acetate (pH 4.5, and 5.5), 0.1 M phosphate (pH 6.5, 7.5, and 8.5) or 0.1 M borate (pH 9.5, and 10.5).

Protein concentration of the crude extracts was measured by the bicinchonic acid assay (Pierce BCA Protein Assay kit). Denaturing SDS-PAGE on 7.5 % polyacrylamide gels was performed according to Laemmli (17). Molecular weight markers were from Sigma (Milan, Italy).

Chromatography of crude venom on ConA-Sepharose was perfomed according to standard procedures. The resin was equilibrated in 0.01 M phosphate buffer containing 0.9 % NaCl. After passage of crude venom, the resin was washed with 10 volumes of the same buffer (collected in 1 ml fractions) and bound proteins were eluted with two volumes of phosphate buffer containing NaCl 1 M and α-methylmannoside 1M at room temperature for 60 min, repeated twice.

All results are expressed as the mean of at least 10 experiments ±SE.

RESULTS

Evaluation of the protein content of crude extracts from sonication of nematocysts suspensions containing ca. 90 nematocysts/µl gave a protein content of 1.6±0.4 µg/µl. The SDS-PAGE pattern of a representative crude extract is shown in Fig. 2. A number of well defined bands –over 10 by visual inspection- were observed, with molecular weights ranging from 145.6 kDa to 13.8 kDa.

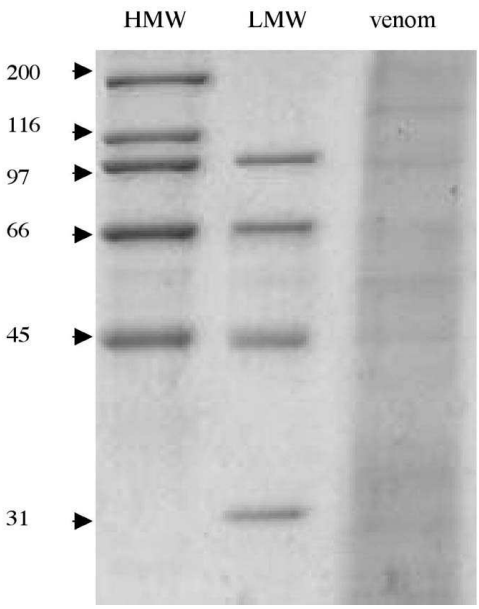

Figure 2. SDS-PAGE analysis of *P. noctiluca* crude venom. Molecular weight markers are shown in the left and middle lane, with the following values: 200, 116.3 kDa (HMW, high molecular weight) and 97.4, 66.2, 45, 31 kDa (LMW, low molecular weight).

We tested at first the hemolytic activity of single discharged nematocysts from *Pelagia noctiluca*. Isolated holotrichous nematocysts, induced to discharge by SCN^- treatment, showed powerful hemolytic activities when directly observed under light microscope, as shown in Fig. 3. Immediately after the onset of discharge, erythrocytes began swelling, and a progressively expanding lytic area started to appear at the tip and successively on either side of the evaginated section of the tubule. Within 5 min all surrounding red blood cells had turned into ghosts.

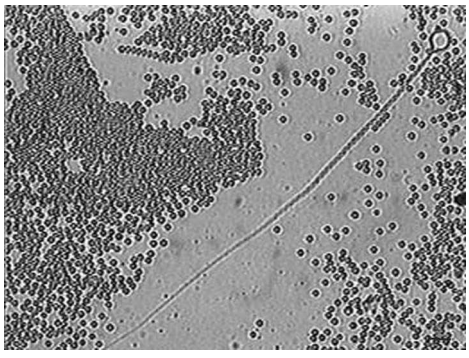

Figure 3. Hemolysis of red blood cells induced by the *P. noctiluca* crude venom of a discharged nematocyst (100x magnification).

A comparable lytic area formed around partially discharged nematocysts: even if the tubule was not properly evaginated, the released liquid was hemolytically active and erythrocytes ghosts were produced all around within a time comparable to that needed for hemolysis by a discharged capsule.

In order to better characterize the hemolytic power of *P. noctiluca* toxins, the crude extract from a 90 nematocysts/µl suspension of holotrichous isorhizas was used in a classical spectrophotometric assay. As shown in Fig. 4, the crude venom induced a dose-dependent hemolysis on erythrocyte suspensions from a number of different sources, including eel, rabbit, chicken and human red blood cells, with the hemolytic power differing among the various bloods. In particular, a dose of 0.1µg/µl, corresponding to 5 % (v/v), induced a notable (ca. 90 %) hemolysis on rabbit and chicken erythrocytes, but only 35 % and 41% hemolysis on human and eel erythrocytes, respectively. On the other hand, an essentially complete hemolysis was observed at the maximal dose tested (0.5 µg/µl) corresponding to 20 % (v/v), irrespective of the blood used.

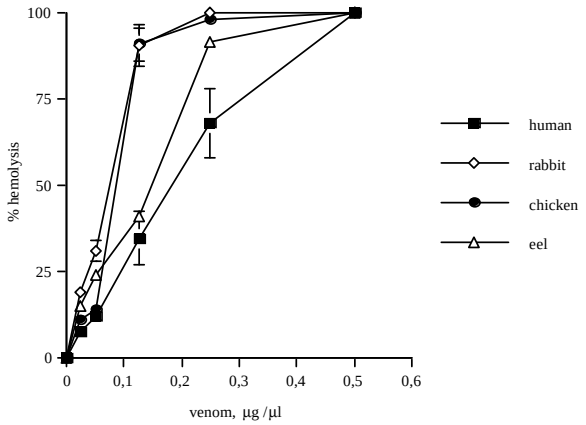

Figure 4. Percent of hemolysis induced by *P. noctiluca* crude venom on red blood cells from different species.

The hemolytic effect was also time-dependent, and even the lowest dose (1% v/v) on the less responsive human blood induced total hemolysis when the incubation was prolonged to two hours. A better kinetic characterization was achieved by incubating the low-responsive human erythrocytes with the highest dose of crude venom for different times within a period of 60 min. The experiment was carried out at three different temperatures (4 °C, 20 °C and 37 °C) and the results are shown in Fig. 5. Surprisingly, the incubation temperature did not significantly affect the extent of hemolysis at any time. A second interesting feature of the kinetics of hemolysis emerged in that the shape of the curve appeared biphasic at all temperatures. The possible causes of both phenomena will be discussed later.

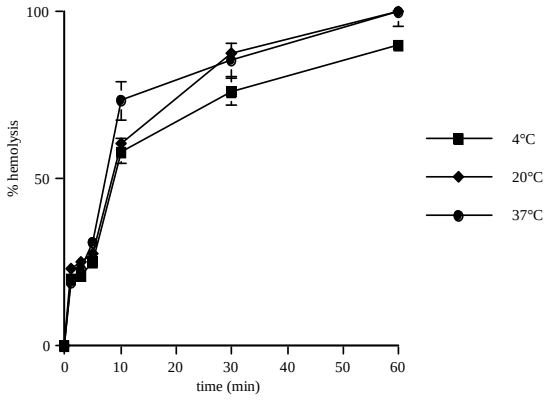

Figure 5. Percent of hemolysis induced on human red blood cells by *P. noctiluca* crude venom preincubated for various periods of time at the indicated temperatures.

Temperature variations were also used to assess the thermal stability of the crude venom. The first experiment was done to assess the stability of the venom to different storage conditions, which included storage at -20 °C or -80 °C for up to 6 months. The stability of the crude venom was not compromised by these storage conditions, as shown by its unaltered hemolytic power (Table 1). Also, lyophilization of the venom was found to be an excellent method to preserve its functional properties as a hemolytic agent (Table 1).

Table 1. Effect of different storage conditions on the hemolytic power of *P. noctiluca* crude venom.

Crude extract	***% hemolysis***
Control*	100%
-80°C **	97%±2.1
-20°C **	95%±3.1
Lyophilized **	98%±1.0

* Tests performed immediately after extraction.
** Tests performed after periods ranging between a week and six months of storage

A second approach to investigate the thermal stability of the nematocysts extract of *P. noctiluca* was to expose the freshly extracted venom to temperatures of 4 °C, 20 °C and 37 °C for 30 or 60 min, and for 3, 6 and 24 h before testing it at the highest 20 % v/v dose upon a 0.05 % human red blood cells suspension. Neither of these treatments significantly affected the hemolytic power of the crude extract (data not shown).

Higher incubation temperatures were therefore tested, namely 40, 60, 80 and 100 °C for 10, 30 and 60 min. Since a minute precipitate formed at these highest temperatures, an aliquot of each sample was centrifuged to obtain a clear supernatant. The non-centrifuged heat-treated crude extract (NC), as well as the centrifuged supernatant (C) and the pellet resuspended in an equal volume of isotonic buffer (R) were then assayed as usual upon a 0.05 % human red blood cells suspension. The results are shown in Fig. 6. Clearly, treatment at 40 °C did not impair the hemolytic activity of the crude venom, that induced a full 100% hemolysis before and after centrifugation (cfr. NC and C samples). Indeed, some toxin possibly aggregated and precipitated, though maintaining its functional effects, as some hemolytic activity could also be recovered in the resuspended pellet (see sample R). It should not surprise the observation of the hemolytic activity found in the resuspended pellet in addition to a 100% activity in the centrifuged supernatant, since the dose of crude venom used in the assay was above that sufficient to induce total hemolysis under our conditions.

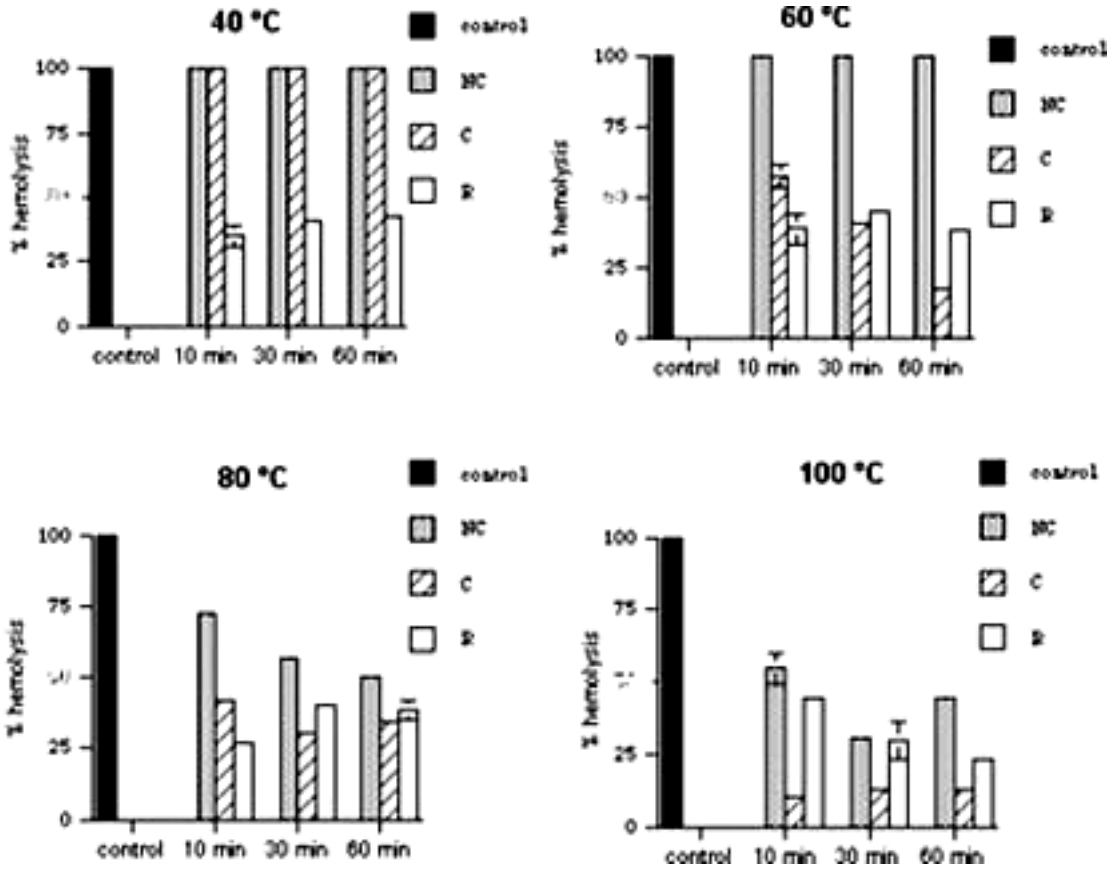

Figure 6. Percent of hemolysis induced on human red blood cells by *P. noctiluca* crude venom preincubated for various periods of time at the indicated temperatures. The venom was centrifuged after incubation at the specific temperature, and supernatants (C), resuspended pellets (R) and venom as such (NC) were tested.

At temperatures higher than 40 °C the crude venom suffered a progressive loss of potency, especially at the longest incubation time. Nevertheless, it is evident from the figure that the toxins contained in the crude extract were highly stable, as strikingly shown by the almost 50 % hemolytic power retained by the extract incubated for 60 min at 100 °C. Again, the hemolytic activity was somewhat distributed into the C and R fractions, indicating that, while

heat was certainly able to induce aggregation and eventually sedimentation of the toxins, these latter remained, at least in part, active.

A final assay on the stability of *P. noctiluca* crude venom was run by assessing the effect of pH on the hemolytic activity of crude venom. To this end, the capsular fluid of holotrichous isorhizas was extracted in different buffers with pH values between 4.5 and 10.5. The venom was kept at room temperature for 15 min and, after restoring pH to neutrality, it was hemolytically tested as usual. As shown in Fig. 7, the hemolytic activity was gradually inhibited by preincubation at pH 6.5 and lower, whereas neutral and alkaline buffers had no effect.

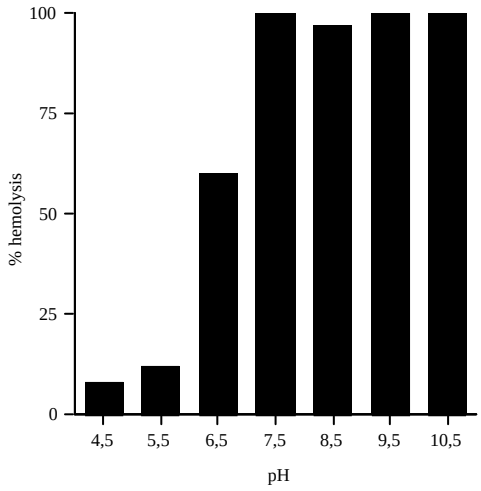

Figure 7. Hemolytic power of *P. noctiluca* crude venom extracted and incubated for 15 min in buffer at different pH values.

The thermal and pH stability of *P. noctiluca* venom seemed unusual for proteic toxins, therefore experiments were set up to ascertain the macromolecular nature of the cytolytic principles contained in the capsular fluid of this Coelenterate. Attempts to deplete the crude extracts by protein precipitation with trichloroacetic acid were apparently successful, however they were run before ascertaining the acid instability of the toxins reported in Fig. 7. On the other hand, the protein content of the various aliquots subjected to heat treatment and centrifugation (see Fig. 6) closely paralleled the percent of observed hemolytic power strongly suggesting this latter to be due to the proteic fraction of the extracts. Clear-cut evidence came from a simple filtration assay using Amicon microconcentrators with a molecular cut-off of 30 kDa. Most, if not all, of the cytolytic power was found in the retained fraction with molecular mass higher than 30 kDa, strongly corroborating the macromolecular nature of the toxic substances.

Despite their proteic nature, the toxins were found to be highly stable towards different proteases. Neither trypsin, α-chymotrypsin, collagenase IV or papain, employed at a final dilution of 2 U/ml from a stock solution of 100 U/ml (in 0.1 M phosphate buffer, pH 7.5) were able to impair the haemolytic power of crude extracts.

In order to ascertain whether *P. noctiluca* toxins are glycoproteins, the crude venom was passed through ConA-Sepharose, and tested for hemolytic potency. As shown in Fig. 8, the hemolytic ability could only be found in the flow-through fraction. This immediately suggests that the crude venom of *P. noctiluca* contains more than one active toxin, and that one or more toxins selectively act on red blood cells.

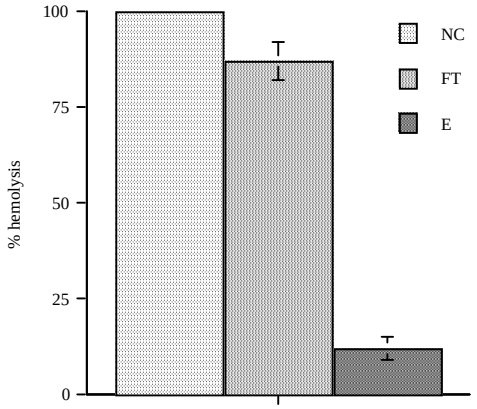

Figure 8. Hemolytic power of *P. noctiluca* crude venom chromatographed on ConA-Sepharose. Venom as such (NC), the flow-through (FT) and the fraction eluted with α-methylmannoside (E) were tested after proper dilution (1:10) on red blood cells.

DISCUSSION

Many investigations have been carried out on the biological activities of molecules of various species of Cnidaria. Venom of such animals is the strategy to survive in a specific environment so that it exhibits very potent biological effects. Among the various classes of venom isolated and characterized, most of them are of proteic nature. Unfortunately to date information about the

biological activities of crude venom from *Pelagia noctiluca* are poorly understood.

In the present study we have firstly focused on the characteristics and the cytolytic effect of crude extracts from isolated undischarged holotrichous isorhiza nematocysts of *Pelagia noctiluca*, that maintain their discharging capacity for a long time, as previously described (29). To this purpose, the crude extract was obtained by sonication of isolated nematocysts, a physical method without enzymes or discharging agents, thus excluding any possible interference from chemical extraction methods.

A salient characteristic is the strong hemolytic action of *P. noctiluca* crude venom. The assay performed on a slide with a single nematocyst, according to the method described by Klug et al. (15), reveals that an individual triggered capsule can induce hemolysis within 10 min with progressive expansion from the distal portion of the everted tubule. As shown above, undischarged damaged nematocysts release their content which induces hemolysis around the capsule. So, the release of hemolysin seems to be not dependent on a regular discharge and upon the complete eversion of the spines regularly located along the tubule, whereas in *Rhopilema nomadica* nematocysts the toxins are delivered via extended spirally arrayed spines (20).

The main point, however, is the extreme stability of the cytolytic components of *P. noctiluca* crude venom. As a matter of fact, our data show that not only storage at freezing temperature and lyophilization do not affect the venom, but even harsh thermal treatments, like incubation at 80-100 °C for 1 hour only partially affects its hemolytic power. Such feature appears peculiar if we consider the results obtained on other Coelenterates. For instance, we have recently reported that the hemolytic power of crude extract from *Aiptasia mutabilis* (Anthozoa) nematocysts decayed after 1 hour at 20 °C (22). Long and Burnett (19) interestingly observed that the hemolysis assay run with *Cyanea capillata* venom must be performed immediately after sonication and before lyophilization, otherwise hemolysin will be neutralized. It has been described that various toxins are inactivated by heat treatment at different temperatures. *Aiptasia pallida* venom showed a reduced hemolytic activity under exposure to 45 °C for 10 min (11), similar to *Carybdea alata* (7) and *Chironex fleckeri* (6) venoms. Higher temperatures inactivated hemolysins from *Physalia physalis*, *Bunodosoma caissarum* and *Actinia tenebrosa* (21, 10). Hemolytic activity of *Catostylus* venom was heat stable with 60% of activity being retained after treatment at 100 °C for one hour (16). On the other hand, there are other reports in the literature suggesting that the crude venoms from selected species can maintain their toxic power for a long time (3, 27). Thermal stability of *Pelagia noctiluca* crude venom could be a useful feature to led further toxicological studies.

Besides its thermal stability, *P. noctiluca* venom, although apparently of proteic nature, seems to be highly stable towards proteolysis. This is also fundamental when considering the advantages in time-requiring purification protocols. The proteolytic stability toward all tested proteases is unusual. Treatment with trypsin, collagenase or papain completely destroys the hemolytic activity in *Carybdea alata* (7), whereas *Rhopilema nomadica* venom is stable against trypsin, but sensitive to chymotrypsin (12), and extracts from *Carybdea marsupialis* are inactivated by trypsin and chymotrypsin, but not by papain and collagenase (28).

As a general feature, according to Lewis and Garcia (18) venom peptides, including those of *Pelagia noctiluca* nematocysts, once delivered, seem to be quite stable to counteract both chemical degradation in solution and enzymatic degradation due to proteases possibly contained in the released venom itself or in the prey tissues.

The chromatographic separation of the extracts on ConA-Sepharose followed by hemolytic tests showed that the venom is likely to contain multiple active components. In particular the glycosylated fractions seem not to be hemolytic.

The hemolytic power of crude extract is preserved at pH ranging between 6.5 and 10.5. A complete inhibition of hemolysis is observed at pH below 5.5. Consistent with previous findings (26), treatment of the stung skin with vinegar after accidental contact with *P. noctiluca* can be suggested as a first aid manoeuvre to immediately relieve the pain and limit the damage, as described by other authors (13, 4).

Our observations differ from those of Alvarez and coworkers (1) describing the loss of biological acitvity in venom from *Stichodactyla helianthus* pretreated at pH>11, while toxic power was unchanged under acid pH treatment. On the other, hand alkaline pH favoured the biological activity of crude extract from

Bunodosoma cavernata (9). Furthermore our previous findings on the cytotoxic activity of *Aiptasia mutabilis* nematocysts crude venom revealed that the optimal pH stability of the extract is around pH 7.5, while at extreme pH values (4.5 and 9.5) the stability is lost (23). In any case, the pH seems to be determinant in the binding and penetration of toxin in the red blood cell membrane. Interestingly, in a recent study, we observed that collapsed nematocysts at low pH not only lose their discharging activity but also the hemolytic power of the crude extract. It can be hypothesized that the excess of protons, besides destroying the external layer of the capsule wall, induce mayor conformational changes in the stored crude extract components which could undergo irreversible precipitation (24).

This study demonstrates the hemolytic power of crude venom from isolated nematocysts of *Pelagia noctiluca*, being effective upon red blood cells of different sources. Interestingly, the strong stability arising from our results allows further investigations concerning purification processes and toxin mechanism of action.

ACKNOWLEDGEMETS - This work was supported by University of Messina grants to G.L.S.

REFERENCES

1. Alvarez, C., Pazos, I.F., Lanio, M.E., Martinez, D., Schreier, S., Casallanovo, F., Campos, A.M. and Lissi, E., Effect of pH on the conformation, interaction with membranes and haemolytic activity of sticholysin II, a pore forming cytolysin from the sea anemone *Stichodactyla helianthus*. *Toxicon.* 2001, **39**: 539-553.
2. Avian, M., Rottini, L. and Bratina, F., Six types of cnidocysts in *Pelagia noctiluca* identified by means of light-scanning electron microscopy and videoanalyzer. *MAP Technical Reports Series.* 1991, **47**: 217-226.
3. Bruhn, T., Schaller, C., Schulz, C., Sanchez-Rodriguez, J., Dannmeier, C., Ravens, U., Heubach, J.F., Eckhardt, K., Schmidtmayer, J., Schmidt, H., Aneiros, A., Wachter, E. and Beress, L., Isolation and characterization of five neurotoxic and cardiotoxic polypeptides from the sea anemone *Anthopleura elegantissima*. *Toxicon.* 2001, **39(5)**: 693-702.
4. Burke, W.A., Cnidarians and human skin. *Dermatologic Therapy*. 2002, **15**: 18-25.
5. Cariello, L., De Santis, A., Fiore, F., Piccoli, R., Spagnuolo, A., Zanetti, L. and Parente, A., Calitoxin, a neurotoxic peptide from the sea anemone *Calliactis parasitica*: amino acid sequence and electrophysiological properties. *Biochemistry*. 1989, **28(6)**: 2484-2489.
6. Carrette, T.J., Cullen, P., Little, M., Peiera, P. L. and Seymour, J.E., Temperature effects on box jellyfish venom: a possible treatment for envenomed patients? *MJA.* 2002, **177(11/12)**: 654-655.
7. Chung, J. J., Ratnapala, L. A., Cooke, I. M. and Yanagihara, A. A., Partial purification and characterization of a haemolysin (CAH1) from Hawaiian box jellyfish (*Carybdea alata*) venom. *Toxicon.* 2001, **39**: 981-990.
8. Edwards, L. P., Whitter, E. and Hessinger, D. A., Apparent membrane pore-formation by Portuguese Man-of-war (*Physalia physalis*) venom in intact cultured cells. *Toxicon.* 2002, **40**: 1299-1305.
9. Eno, A.E., Konya, R.S. and Ibu, J.O., Biological properties of a venom extract from the sea anemone *Bunodosoma cavernata*. *Toxicon.* 1998, **36(12)**: 2013-2020.
10. Galettis, P. and Norton, R.S., Biochemical and pharmacological studies of the mechanism of action of tenebrosin-c, a cardiac stimulatory and haemolytic protein from the sea anemone *Actinia tenebrosa*. *Toxicon.* 1990, **28**: 695-706.
11. Grotendorst, G. R. and Hessinger, D. A., Purification and partial characterization of the phospholipase A_2 and co-lytic factor from sea anemone (*Aiptasia pallida*) nematocyst venom. *Toxicon.* 1999, **37**: 1779-1796.
12. Gusmani, L., Avian, M., Galil, B., Patriarca, P. and Rottini, G., Biologically active polypeptides in the venom of the jellyfish *Rhopilema nomadica*. *Toxicon.* 1997, **35 (5)**: 637-648.
13. Hartwick, R., Callanan, V. and Williamson, J. A. H., Disarming the box-jellyfish: nematocyst inhibition in *Chironex fleckeri*. *Med. J. Aust*. 1980, **1**: 15–20.
14. Hinds, M.G., Zhang, W., Anderluh, G., Hansen, P.E. and Norton, R.S., Solution structure of the eukaryotic pore-forming cytolysin equinatoxin II: implications for pore-formation. *J. Mol. Biol*. 2002, **315(5)**: 1219-1229.
15. Klug, M., Weber, J. and Tardent, P., Haemolytic and toxic properties of *Hydra attenuata* nematocysts. *Toxicon.* 1989, **27(3)**: 325-339.
16. Knorr, A., Effective *Catostylus mosaicus* venom on erythrocytes. *Toxicon.* 1990, **28**: 247.
17. Laemmli, U.K., Cleavage of structural proteins during the assembly of the head of the bacteriophage T4. *Nature.* 1970, **227**: 680-685.
18. Lewis, R.J. and Garcia, M.L., Therapeutic potential of venom peptides. *Nature rev*. 2003, **2**: 790-802.
19. Long, K. O. and Burnett, J. W., Isolation, characterization, and comparison of haemolytic peptides in nematocyst venoms of two species of jellyfish (*Chrysaora quinquecirrha* and *Cyanea capillata*). *Comp. Biochem. Physiol*. 1989, **94B (4)**: 641-646.
20. Lotan, A., Fishman, L. and Zlotkin, E., Toxin compartmentation and delivery in the Cnidaria: the nematocyst's tubule as a multihead poisonous arrow. *J. Exp. Zool*. 1996, **275 (6):** 444-451.
21. Malpezzi, E.L.A., Freitas, J.C., Muramoto, K. and Kamiya, H., Characterization of peptides in sea anemone venom collected by a novel pracedure. *Toxicon.* 1993, **31**: 853-864.
22. Marino, A., Musci, G. and La Spada, G., Haemolytic effects of crude venom from *Aiptasia mutabilis* nematocysts. *Chem. Ecol.* 2004, **20**, supplement 1: S451-459.
23. Marino, A., Valveri, V., Crupi, R., Muià, C., Rizzo G., Musci, G. and La Spada, G., Cytotoxicity of toxins from nematocysts of *Aiptasia mutabilis. Comp. Biochem. Physiol.* 2004, **139(4)**: 295-301.
24. Marino, A., Crupi, R., Musci, G. and La Spada, G., Morphological integrity and toxicological properties of *Pelagia noctiluca* (Scyphozoa) nematocysts. *Chem. Ecol.* 2006, **22**, Supplement 1: S127-S131.
25. Mariscal, R.N., Nematocysts. In: *Coelenterate Biology: Reviews and New Perspectives*, Muscatine, L. and Lenhoff,

H. L (eds), Academic Press. New York, London, 1974, pp. 129-178.
26. Mianzan, H.W., Fenner, P.J., Cornelius, P.F.S. and Ramirez, F.C., Vinegar as a disarming agent to prevent further discharge of the nematocysts of the stinging hydromedusa *Olindias sambaquiensis. Cutis.* 2001, **68(1)**: 45-48.
27. Minagawa, S., Ishida, M., Nagashima, Y. and Shiomi, K., Primary structure of a potassium channel toxin from the sea anemone *Actinia equina*. *FEBS Letters*. 1998, **427**: 149-151.
28. Rottini, G., Gusmani, L., Parovel, E., Avian, M. And Patriarca, P., Purification and properties of a cytolytic toxin in venom of the jellyfish *Carybdea marsupialis*. *Toxicon.* 1995, **33 (3)**: 315-326.
29. Salleo, A., Discharge Mechanism of the nematocysts of *Pelagia noctiluca*. In: *Toxins, Drugs and Pollutants in Marine Animals*, Bolis, L., Zadunaisky, J. and Gilles, R. (eds.), Springer Verlag. Berlin, New York, Tokyo, 1984, pp. 63-68.
30. Salleo, A., La Spada, G., Falzea, G. and Denaro, M.G., Discharging effectiveness of lyotropic anions on nematocysts of *Pelagia noctiluca*. *Mol. Physiol*. 1983, **6**: 19-26.
31. Salleo, A., La Spada, G., Denaro, M.G. and Falzea, G., Effects produced by SCN^- and thioglycolate on isolated nematocysts of *Pelagia noctiluca*. *Cell. Mol. Biol.* 1986, **32**: 661-666.
32. Salleo, A., La Spada, G., Denaro, M.G. and Falzea, G., Dynamics of release of free calcium during the discharge of holotrichous isorhiza nematocysts of *Pelagia noctiluca*. In: *The biology of nematocysts*, Hessinger, D.A. and Lenhoff, H. (eds), Academic Press, New York, 1988, pp. 551-555.
33. Salleo, A., La Spada, G. and Barbera, R., Gadolinium is a powerful blocker of the activation of nematocysts of *Pelagia noctiluca*. *J. Exp. Biol.* 1994, **187**: 201-206.