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Short Communication

Identity between cytolytins purified from two morphos of the Caribbean sea anemone *Stichodactyla helianthus*

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Abstract

Stichodactyla helianthus is a sea anemone relatively abundant along Cuban coasts appearing in two morphos with different colors in their tentacles: green or brownish, probably due to their association with algal symbionts. Traditionally, the brownish morpho has been used as a source of sticholysins I and II, the most characterized cytolytins from this anemone, but the green morpho is the most abundant along the western coasts of Havana. The present work is aimed to establish if the cytolytins purified from the green morpho (StIg and StIIg) are similar to those purified from brownish anemones (StI and StII). Following the same chromatographic procedure used to purify the toxins from morphos, the electrophoretic mobilities, amino acid compositions, amino terminal sequences and molecular masses were practically identical between analog cytolytins. In conclusion, homologous sticholysins purified from the green and brownish variants of *Stichodactyla helianthus* are the same molecular entities. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Hemolysin; Cytolysin; Sticholysin; Sea anemone; Morphos; *Stichodactyla helianthus*

Stichodactyla helianthus is a sea anemone relatively abundant along coastal waters of the Caribbean region. It exists in two morphos that differ in the color of tentacles that can be green or brownish. This difference is probably due to their association to zooxantelles (Dunn, 1981). Weis and Levine (1996) have reported a similar association between the sea anemone *Anthopleura elegantissima* and the photosynthetic dinoflagellate *Symbiodinium californium*, in which the algal symbionts are housed in vacuoles within animal endodermal cells. In this case, animals occurring in sun-exposed locations harbor anemone within their endodermal cells (symbiotic animals), giving them a deep brown color,

whereas animals occurring in caves of rock crevices lack symbiotic algae and appear white (aposymbiotic animals).

As other coelenterates, the sea anemone *Stichodactyla helianthus* produces many toxic polypeptides located within intracellular specialized organelles called nematocysts and employed on the tentacles for defense against predators or in order to immobilize preys (Macek, 1992). The toxicity of these products has been known for a long time (Devlin, 1974; Bernheimer and Avigad, 1976). Among them, some neurotoxins affecting sodium and potassium channels, protease inhibitors, phospholipases and hemolytic factors have been purified to homogeneity and analyzed (Blumenthal and Kem, 1983; Kem and Dunn, 1988; Kem et al., 1989; Pazos et al., 1993a,b; Castañeda et al., 1995; Delfín et al., 1996; Pazos et al., 1998). Cytolytins are water-soluble polypeptides exhibiting the unique property of inserting and accommodating spontaneously into membranes. Due to their lytic capacity and the possibility to address them to specific tissues, cytolytins have been evaluated as promising anti-tumor agents (Avila et al.,

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Abbreviations: StI and StII, cytolytins from brownish morpho of *Stichodactyla helianthus*; StIg and StIIg, cytolytins from green morpho of *Stichodactyla helianthus*; HPLC, high performance liquid chromatography; ESI-MS, electrospray ionization mass spectrometry

1988; Pederzoli et al., 1995). Anticoagulant properties have also been reported (Díaz et al., 1992).

Characterization of cytolytins purified from *Stichodactyla helianthus* revealed that they are single-chain 20 kDa basic polypeptides (Kem and Dunn, 1988), that increase ion membrane permeability by forming oligomeric pores comprising three or four toxin molecules whose effective hydrodynamic radius have been estimated to be around 1 nm (Tejuca et al., 1996; De los Rios et al., 1998; De los Rios et al., 1999).

Traditionally, the brownish variant of *Stichodactyla helianthus* has been used in our laboratory as a source of sticholysins I and II, the most characterized cytolytins from this anemone (Tejuca et al., 1996; Pazos et al., 1998; Alvarez et al., 1998; Lanio et al., 2001; Alvarez et al., 2001), but green morpho is the most abundant, along the western coasts of Havana (unpublished data). The present work is aimed to establish if the cytolytins purified from the green morpho are the same molecules to those from the brownish anemones.

In order to purify the cytolytins present in two morphos of *Stichodactyla helianthus*, we have employed the purification procedure previously reported by Lanio et al. (2001). Specimens of green and brownish morphos of the anemone were collected along the north coast of Havana City and Pinar del Río, and total extracts were obtained according to Gómez et al. (1986). Two cytolytic fractions were obtained from each morpho: StI and StII (brownish morpho) and StIg and StIIg (green morpho), eluting at similar ionic strength. The obtained profiles are similar to those previously reported by Lanio et al. (2001) working with an analogous fraction from the brownish morpho of *Stichodactyla helianthus*. SDS-polyacrylamide gel electrophoresis of toxins according to Laemmli (1970) showed a single band for each one with an apparent molecular mass of about 20 kDa. Interesting, the cytolytins of the green morpho (StIg and StIIg) exhibit the same electrophoretic mobilities as their respective analogues of the brownish variant (StI and StII).

In order to obtain four proteins completely pure for amino acid analysis, N-terminal sequencing and molecular mass determination, the fractions obtained by ionic exchange chromatography were submitted to a last purification step by HPLC (Pharmacia-LKB, Sweden) on a reversed phase column C4, (4.6 × 250 mm, Vydac). Elution was performed with an acetonitrile gradient from 25 to 85% in water containing 0.1% trifluoroacetic acid with a flow rate of 0.8 ml/min at 37 °C. Proteins were detected by absorbance measurements at 226 nm.

Amino acid analysis was performed after acid hydrolysis of the pure protein according to Allen's method (1989) using an automatic analyzer Alpha Plus 4151 (Pharmacia-LKB, Sweden). The results are very similar to those reported by Lanio et al. (2001) and show an almost identical amino acid composition for StI and StII obtained from both anemone variants. The N-terminal sequencing on the intact proteins was carried out by automated Edman degradation

of the proteins using a dual phase sequencer (model 810/816, Knauer, Germany) equipped with an on-line phenylthiohydantoin amino acid analyzer. The comparison of the N-terminal sequence of all samples confirmed the four substitutions found in StI in relation to StII, three of them non-conservative and one conservative, previously reported by Lanio et al. (2001) and as expected a similar situation is also observed for StIg in relation to StIIg.

Exact molecular weights were determined by ESI-MS. Measurements were carried out on an API III triple quadrupole MS system (PE Sciex Instruments, Toronto, Canada) using nanospray needles Econo12 (1 ± 0.5 μm) (New Objective Pico Tip™, USA). The instrument was calibrated with polypropyleneglycol solutions 425, 1000 and 2000 at concentrations of 0.4, 0.1, and 0.014 g/l, respectively. Samples were infused in an aqueous solution of 50% acetonitrile and 0.5% formic acid. Identical molecular masses were obtained, confirming the identity of cytolytins (19389.3 and 19389.8 Da for StI/StIg; 19280.0 and 19281.7 Da for StII/StIIg).

The hemolytic activity (HC50—the protein concentration necessary to lyse 50% of the cells in the assay) of StI/StIg and StII/StIIg were very similar (<30 ng/ml), when determined as described elsewhere (Lanio et al., 2001).

Weis and Levine (1996) demonstrated that differences in protein profiles between symbiotic and aposymbiotic *Anthopleura elegantissima* are due to differential protein production in host animal tissue and not to contamination from algal proteins that might be present in symbiotic animal homogenates. The differential protein synthesis between symbiotic and aposymbiotic states must be a reflection either of underlying differences in the regulation of gene expression or in post-translational modification of common protein. If any different total protein profile would exist between the two *Stichodactyla helianthus* morphos, this result would not include, for sure, the two cytolytins purified as previously described by Lanio et al. (2001). Further biological studies are needed to clarify the origin and importance of the existence of these two morphos for the Caribbean anemone. In summary, the two cytolytins purified from any of the investigated variants of *Stichodactyla helianthus* found along Havana's northern coast are the same molecular entities.

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