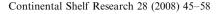


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Brevetoxin abundance and composition during ECOHAB-Florida field monitoring cruises in the Gulf of Mexico

Richard Pierce*, Michael Henry, Patricia Blum

Mote Marine Laboratory, 1600 Ken Thompson Parkway, Sarasota, FL 34236, USA

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Abstract

This project was undertaken to provide information about the composition and fate of brevetoxins in concert with the multidisciplinary study, ECOHAB-FL, of *Karenia brevis* blooms in the Gulf of Mexico. Brevetoxin composition was provided for water samples collected during and in the absence of *K. brevis* blooms from November 1998, through September 2002. The identity and concentration of the most abundant brevetoxins were determined using high performance liquid chromatography with ultraviolet diode array detection (HPLC-DAD). The analytical methods changed in 2002 to the use of a mass spectrometer for brevetoxin identification and quantitation. The most abundant brevetoxins observed during blooms were PbTx-1, -2 and -3. PbTx-2 was the most abundant toxin observed in viable bloom situations with an abundance of *K. brevis* cells. Starting with the 2000 cruises, a distinction was made between intracellular toxins (inside viable *K. brevis* cells) and extra-cellular brevetoxins from intra-cellular to extra-cellular toxins. The most abundant intra-cellular toxin was PbTx-2, whereas the most abundant brevetoxin recovered from the extra-cellular (dissolved) fraction in the water was PbTx-2. The abundance of PbTx-3 relative to PbTx-3 in the water after cell death.

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1. Introduction

Brevetoxins are a suite of neurotoxic, polycyclic ether compounds produced by the dinoflagellate, *Karenia brevis* (Poli et al., 1986; Baden et al., 1995). Although always present in low concentrations in the Gulf (Tester and Steidinger, 1997; Landsberg,

*Corresponding author. Tel.: +1941 388 4441; fax: +1941 388 4312.

E-mail addresses: rich@mote.org (R. Pierce),

mhenry@mote.org (M. Henry), pcblum@mote.org (P. Blum).

2002), high concentrations of *K. brevis* produce sufficient neurotoxins to cause massive fish kills, neurotoxic shellfish poisoning (NSP) and respiratory irritation in marine mammals and humans (Kirkpatrick et al., 2003). This project was undertaken to provide information on the production and fate of brevetoxins associated with *K. brevis* blooms in the Gulf of Mexico. Brevetoxin analyses were performed on water samples collected during monthly transects from Sarasota Bay, Florida to 50 km out in the Gulf of Mexico, both during and in the absence of red tide blooms. Water samples also

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were collected during annual. long-term cruises to investigate the fate of brevetoxins during and following red tide blooms, from November 1998, through September 2002. During the monitoring cruises, a distinction was made between intracellular toxins (inside viable K. brevis cells) and extra-cellular toxins (dissolved brevetoxins outside of the cell). Viable K. brevis cells contain as many as 10 different neurotoxins comprising the suite of brevetoxins (Bourdelais et al., 2005). When the cells lyse, these toxins are released into the water column and the composition changes (Pierce et al., 2001, 2003). The ability to assess the fate and effects of brevetoxins in the marine environment requires that we distinguish between intra-cellular and extracellular toxins providing information on transport mode of exposure and toxicity.

Extra-cellular brevetoxins is an operational definition based on the toxins passing through a $0.8 \,\mu\text{m}$ filter (Pierce et al., 2001). These toxins may be dissolved or in association with small particles or micelles. Due to the hydrophobic nature of these molecules they are only slightly soluble in water and when released into the water they tend to adsorb to surfaces such as suspended particles and bubbles in the water rendering them available for transport to the benthic environment in association with particles that have settled, as well as upward transport to the air/water interface adsorbed to the surface of bubbles, with subsequent incorporation into marine aerosol as the bubbles burst (Pierce et al., 1990, 2006).

An important observation from the field studies was the change in composition of the major brevetoxins from intra-cellular to extra-cellular toxins. The most abundant intra-cellular toxin was PbTx-2, whereas the most abundant brevetoxin recovered from the extra-cellular (dissolved) fraction in the water was PbTx-3. The abundance of PbTx-3 relative to PbTx-2 generally increased as a bloom aged, indicating the conversion of PbTx-2 to -3 as cells lysed, and the persistence of PbTx-3 in the water after cell death. PbTx-3 also has been observed as the primary brevetoxin recovered from marine aerosol that affects marine mammals and human respiratory function (Pierce et al., 2003, 2006). The process by which the PbTx-2 aldehyde is reduced to to the PbTx-3 alcohol is not well understood, but is presumed to be driven by enzymatic activity (Poli et al., 2000).

Understanding changes in concentrations and composition of the primary brevetoxins is impor-

tant because of their different toxicity and rate of metabolism/depuration in marine organisms. K. brevis cell concentrations of 1 million cells/L have been observed to cause immediate fish mortality, whereas exposure to 500,000 cells/L required several hours for death to occur indicating the accumulative effect of chronic, sublethal exposure (R. Pierce, unpublished data). PbTx-3 was found to be 10 times more toxic than PbTx-2 when ingested orally by mice (Baden and Mende, 1982), yet both PbTx-2 and-3 exhibited similar toxicity (LC-50 of $14/\mu g/L$) to the minnow, Gambusia affinis (Rein et al., 1994). Manatee deaths resulted from consumption of brevetoxin-contaminated seagrasses even after the bloom had terminated (Flewelling et al., 2005). Thus, in some instances the primary factor for manatee intoxication was not the cell density of a red tide bloom to which manatees were exposed, rather the duration and intensity of exposure to seagrasses on which the manatees were subsequently grazing.

Shellfish exposed to *K. brevis* concentrations as little as 5000 cells/L accumulate sufficient amounts of brevetoxins to cause NSP (Baden et al., 1995). Analysis of contaminated oyster tissue showed that PbTx-3 remained intact in oyster tissue for several days following exposure, while PbTx-2 was rapidly metabolized (Poli et al., 2000; Plakas et al., 2002; Pierce et al., 2006). Therefore the toxicity of NSP-contaminated shellfish may be a function of the amount of extra-cellular PbTx-3 in the water as well as the concentration of live *K. brevis* cells to which they are exposed.

In many instances the first sign of a low-level red tide bloom is respiratory irritation experienced along the shore. People have complained of such irritation from exposure to brevetoxin-containing marine aerosol produced in surf water containing cell densities as low as 10⁴ cells/L (Pierce et al., 2006), below fish kill or visual detection levels. Both PbTx-2 and -3 were found to have similar adverse effects on asthmatic sheep when introduced as an aerosol into their lungs at 1 pg/mL concentrations, indicating problems associated with even very low-level exposure (Abraham et al., 2005).

2. Materials and methods

2.1. Sarasota area transects

Transects were run from New Pass in Sarasota Bay, to 30 miles off shore, with stations every Download English Version:

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