Qualitative and quantitative analysis of hemolytic toxins from dinoflagellates specifically associated with fish kills by mass spectrometry

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Introduction:
Dinoflagellates are motile, unicellular protists found in many aquatic environments and capable of causing harmful blooms, sometimes referred to as “red tide.” Karlotoxins and amphidinolins are hemolytic polypeptide toxins (>1000 Da) produced by Karlodinium and Amphidinium dinoflagellate species, respectively, that have been associated with fish kills throughout the world (2-4). Many species, even strains of the same species, seem to make unique toxin structures. However, very little genomic data exist to delineate strains of these species, particularly due to their very large genomes (1). The goal of this research was to develop a comprehensive mass spectrometric methodology to identify and define primary chemical structures of polypeptide toxins for support of applied attribution studies and basic dinoflagellate biology studies.

Methods:
Previously archived extracts and newly acquired water samples, from areas in which fish kills were observed, were analyzed. All samples were extracted using the method previously published by Bachvaroff, et al. (5). Eluents were injected onto a Phenomenex (Torrance, CA) Kinetix core shell C8 column (2.1 mm i.d. x 100 mm, 2.6 um particle) and subjected to a ten minute, linear, acetonitrile-water gradient from 20% to 95% organic composition. A hybrid 3D ion trap-time of flight mass spectrometer coupled to an analytical flow HPLC with an online degasser and diode array detector (Shimadzu, Columbia, MD) was used for the analyses. Source and collision parameters were optimized by direct infusion. A data dependent acquisition tandem MS strategy was employed for sample screening, followed by targeted tandem experiments to achieve optimal spectral quality. Ultra-high resolution, accurate mass spectra were acquired on a hybrid linear ion trap – 21 T Fourier transform-ion cyclotron resonance mass spectrometer, including several tandem experiments with various activation methods.

Results:

![Figure 1](image1)

**Figure 1.** Representative polypeptide dinoflagellate toxin structures. A.) karlotoxin 2, made by *K. venecicum* and. B.) amphidinol 18, made by *A. catenula*.

![Figure 2](image2)

**Figure 2.** ESI-TOF mass spectra of toxin extracts from four water samples after possible dinoflagellate fish kills. (blue box) Two spectra from Karlodinium species. (red box) Two spectra from Amphidinium species. The last spectrum is from a sulfated analogue of amphidinol.

![Figure 3](image3)

**Figure 3.** ESI (+) FT-ICR broad mass spectra of two karlotoxins purified from a *K. venecicum* isolate (GM2) from the East China Sea.

![Figure 4](image4)

**Figure 4.** CID interpretation from FT-ICR MS/MS data for a KnTxs2 variant obtained after a fish kill in China. All product ions’ m/z values were determined at <150 ppb mass accuracy.

![Figure 5](image5)

**Figure 5.** KnTxs2 variant dissociation using A.) trap CID @ NCE = 50% and. B.) UVPD @ 1115 μL (red box). Additional ions detected after UVPD.

![Figure 6](image6)

**Figure 6.** A.) Hemolytic data from water sample extracts of a karlotoxin variant. B.) Quantitative measures of dinoflagellate toxin levels in water samples and sample information from a recent fish kill event.

Discussion and Conclusions:

- Comprehensive LC-MS/MS methods were developed to identify and quantify dinoflagellate polypeptide toxins in water samples.
- Tandem, accurate mass spectra from both IT-TOF and FT-ICR instruments were used to confidently assign empirical formulae and primary structures.
- For a newly discovered karlotoxin, CID performed with ultra-high mass accuracy detection allowed for localization of functional groups previously assigned to other loci by NMR spectroscopy.
- Multiple dissociation techniques provided more complete coverage for structural inferences.
- Fish kills presumed to be associated with coincident dinoflagellate blooms were confirmed by accurate mass LC-MS/MS detection of hemolytic toxins.
- Toxic levels were quantified by LC-ADAD area under the curve for characteristic absorption maxima.
- Hemolytic data supported the conclusion that a karlotoxin was responsible for a recent fish kill in the Gunpowder River, MD, USA.

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References: