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Anthracylmethyl crown ethers as fluorescence sensors of saxitoxin

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Abstract

Anthracylmethyl crowns **1** and **2** show up to 30-fold increase in fluorescence emission intensity in the presence of saxitoxin in ethanol, and sixfold enhancement in 80% ethanol. The binding constant between saxitoxin and **1** is 3880 M^{-1} in ethanol and 1100 M^{-1} in 80% ethanol. © 1999 Elsevier Science Ltd. All rights reserved.

Saxitoxin (STX) and its hydroxylated and sulfonated structural analogs[1] are bis-guanidium ions that are produced by a number of dinoflagellates (genera *Alexandrium, Pyrodinium, Gonyaulax,* and *Gymnodinium*) as well as by some bacteria, blue-green algae, and red algae, in both freshwater and marine environments.[2–5] Blooms of these species are responsible for some of the so-called 'red tides', more correctly described as 'harmful algal blooms' (HABs), since red coloration in the water is not always apparent. When a STX-producing HAB occurs near shellfish beds (in either freshwater or marine environments), the toxins are concentrated in filter-feeding shellfish such as clams, oysters, and mussels. Consumption of saxitoxin-containing shellfish produces a syndrome known clinically as paralytic shellfish poisoning (PSP), which has been documented in North America since the 1700s (ship's logs from Cook's exploration of the Pacific Northwest contain the first report). Although the cause of PSP was discovered in the early 20th century,[6] the structure of saxitoxin was not elucidated until 1975.[7,8] The most severe symptom of PSP is respiratory paralysis; the LD_{50} (oral, monkey) is 364 μg/kg, with severe symptoms occuring in humans after ingestion of 124 μg and death from less than half a milligram.[9] The mortality rate for humans (worldwide) suffering from PSP can be quite high. For example, the overall mortality was 7–9% in several studies many years ago,[10–12] but in a 1987 outbreak on the Pacific coast of Guatemala, the mortality was 14% overall and 50% for children under the age of 6.[13]

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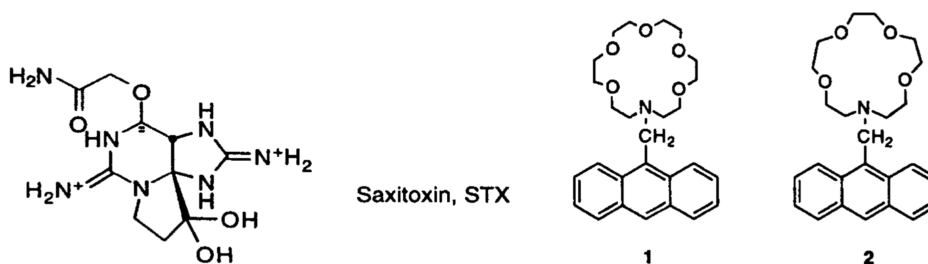

STX detection programs may use a number of techniques,[14] including HPLC with post-column derivatization,[15–17] methods employing molecular pharmacology,[18–20] capillary-zone electrophoresis coupled with either laser fluorescence[21] or ion spray mass spectroscopy,[22] and HPLC-linked mass spectroscopy. [23,24] But routine screening of multiple samples on a regular basis requires inexpensive high-throughput methods for detection and quantitation of toxins in seawater and meat. The current benchmark technique is mouse bioassay.[25] We have undertaken a program to develop chemical sensors for saxitoxin, and report our preliminary findings herein.

Fluorescence sensing of inorganic cations and polyatomic anions using the principles of host–guest chemistry has received considerable attention, with several remarkable advances having been made.[26–29] Examples of detection of organic cations by fluorescence signaling is less common.[30,31] Utilization of fluorescence signaling in sensing devices has a number of advantages, including its high sensitivity of detection — in some cases down to the single molecule level, on–off switchability, subnanometer spatial resolution with submicron visualization and submillisecond temporal resolution.[26–29] This field is quite large, with many designs of fluorescence-signaling molecules having been developed. An excellent overview is provided in a very recent review.[29]

Since saxitoxin is a bis-guanidinium ion, and since guanidiniums are known to bind to crown ethers,[32] we tested the fluorescence signaling of two anthracene-modified crowns, **1** and **2**.[33,34] Aminomethylanthracenes, in general, fluoresce poorly as free bases because the nitrogen lone pair quenches the excited state by electron transfer, known as photoinduced electron transfer (PET). [29] Tying up the lone pair with a proton or an alkali cation 'turns off' the PET, thereby 'turning on' the fluorescence of the anthracene.[33,34] Our hope was that, as part of its binding, saxitoxin would deliver one of its eleven potential hydrogen-bond donating atoms to the benzylic nitrogen and 'turn off' the PET, and 'turn on' the fluorescence. The 18-crown-6 derivatives, **1** and **2**, were initially prepared by de Silva for the detection of alkali cations and protons.[33,34] Both fluoresce strongly in water, so our experiments were done in either absolute or 80% ethanol.

Irradiation of **1** or **2** at either 366 or 386 nm produces only weak fluorescence with three maxima at approximately 394, 417, and 441 nm. Addition of a drop of concd HCl to a 10^{-6} M solution of **1** in ethanol results in an 80-fold enhancement of fluorescence intensity at 417 nm, as expected.[33,34] Fig. 1 shows the emission spectra of **1** and **2** (10^{-6} M) in the absence and presence of 10^{-3} M STX. With **1**, there is a 30-fold enhancement of fluorescence at 417 nm; the increase in total fluorescence intensity is 25-fold. With **2**, a 20-fold fluorescence enhancement was evident at 418 nm; total fluorescence was enhanced by 21-fold.

Saxitoxin is a fairly acidic molecule, with a pK_a of 8.24 in water,[8,35] so one explanation for the fluorescence enhancement could be proton transfer to the crown. If the enhancement was due to acid–base chemistry, any acid of comparable acidity would produce the same effect. 2-Bromophenol has a pK_a of 8.45,[36] so it was tested in a similar way. In the presence of a 1000-fold excess of 2-bromophenol, no appreciable change in fluorescence emission was observed. Conceivably, low fluorescence of protonated

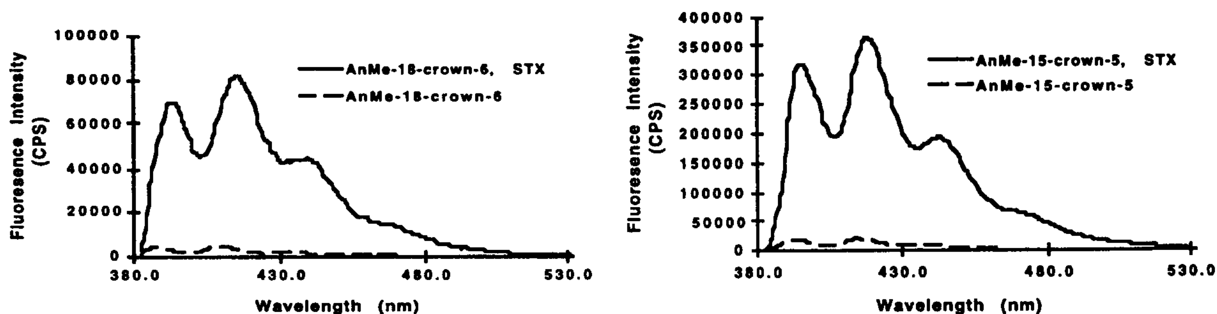

Figure 1. Fluorescence emission spectra of **1** (left) and **2** (right), 10^{-6} M, in the absence (dashed line) and presence (solid line) of 10^{-3}M STX, in absolute ethanol. Irradiation at 366 nm

1 could be due to quenching by the bromine atom of the phenol, but a control experiment showed that 2-bromophenol had no effect on the fluorescence of anthracene at the same concentrations.

To further probe the binding of STX and anthracyl crowns, we examined the fluorescence response of **1** and **2** in 80% ethanol solution, in the presence of ammonium phosphate buffer at pH 7.1; we also examined the effect of guanidinium hydrochloride on fluorescence emission of **1**. Ammonium phosphate buffer (10^{-4} M, pH 7.1) showed an insignificant effect on the intensity of fluorescence emission of either **1** or **2**, which is interesting since ammonium ions are known to bind to crown ethers. Apparently, if the ammonium ion is associated with the crown in this solvent, the binding does not involve hydrogen bonding to the nitrogen atom. Saxitoxin enhances the flourescence intensity of **1** by sixfold in this buffered solvent system. This smaller enhancement compared to the effect in absolute ethanol is mostly due to the increased fluorescence of the crown in the presence of water. The emission spectrum of 10^{-6} M **1** in the presence of 10^{-2} M guanidium hydrochloride in 10^{-4} M buffer is superimposable on the curve obtained in buffer alone, indicating that more than a guanidinium ion is required for enhancement.

By comparing the fluorescence intensities in the absence and presence of saxitoxin, the binding constant, β, can be calculated according to a Scatchard-type analysis.[37] Intensities were measured at [**1**]=10^{-5} M and [STX]=10^{-3} M to 10^{-4} M. The binding constant for the STX·**1** complex is 3880 M^{-1} in absolute ethanol and 1100 in 80% ethanol.

X-Ray crystal structures of 18-crown-6 and aza analogs with urea, uronium ions, amides, and amidines exhibit mainly two types of hydrogen bonding.[38-40] Terminal -NH_2 groups (ureas, amides, amidines) bridge alternating heteroatoms (1,7 positions of the crown), whereas -HNC(=X)NH- groups (ureas, amidines) bridge 1,4-heteroatoms. With either of these crowns, there are far too many degrees of freedom to predict conformation reliably, and the stoichiometry of binding is not known. Nevertheless, modeling studies (Macromodel,[41] MM2* force field) suggest that such a mode of binding could occur here, with the more acidic guanidinium (in the 5-membered ring) being delivered to the benzylic nitrogen of the crown, and the electrostatic forces holding the other guanidinium close to the crown. Testing of this hypothesis will require further studies.

In summary, saxitoxin has been shown to bind to monoaza 18-crown-6 and 15-crown-5 ethers having a pendant anthracylmethyl group. In binding, the toxin inhibits the normal photoinduced electron transfer of these molecules, permitting a strong enhancement of fluorescence intensity, as high as 30-fold. Presumably, this enhancement occurs by hydrogen bond donation from one of the toxin's eleven hydrogen bond donor-groups to the benzylic nitrogen. Studies are underway to elucidate the mechanism of binding, and to design a superior sensor.

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