Spectroscopic detection of Saxitoxin: an alternative to mouse bioassay

Péter Kele,[a] Jhony Orbulescu,[b] Robert E. Gawley[c] and Roger M. Leblanc*[b]

Received (in Cambridge, MA, USA) 10th January 2006, Accepted 30th January 2006
First published as an Advance Article on the web 23rd February 2006
DOI: 10.1039/b600383d

Herein, we report the surface modification of quartz with a coumaryl–aza-crown-6 derivative to detect Saxitoxin using fluorescence enhancement through Photoinduced Electron Transfer and the sensitivity with this system approaches the limit of the mouse bioassay which is the current benchmark for Saxitoxin detection.

Saxitoxin (Fig. 1) and a range of structurally related derivatives produced by three marine dinoflagellate genera (*Alexandrium, Gymnodinium*, and *Pyrodinium*) are responsible for paralytic shellfish poisoning (PSP) in humans.[1] Where such algal blooms precede or coincide with the harvest and consumption of filter-feeding bivalve mollusks, serious human illness or fatalities may occur.[2,3] Manifestations of Saxitoxin poisoning in mammals include oral paresthesia followed by cardiovascular dysfunction and respiratory paralysis. The symptoms of PSP can be attributed to Saxitoxin (STX) and its congeners binding to voltage-gated sodium channels thereby blocking the influx of Na^+ ions. This binding, subsequently, prevents nerve cells from producing action potentials, which inhibits impulse conduction in excitable tissues, *e.g.* muscle, peripheral nerve and brain tissues.[4]

Traditionally PSP toxins have been determined by a mouse bioassay.[5] This method relies upon the time of death as the determinant of the amount of the toxin present in the shellfish. The detection limit by mouse bioassay is about 40 μg per 100 g shellfish, which corresponds to injection of an approximately micromolar solution of the toxin. The use of this procedure has drawn much criticism regarding both the number of animals used and the high cost of the experiment. In spite of these drawbacks the mouse bioassay is still the most reliable way to detect and quantify PSP toxins in shellfish. Other tests, including immunological assays (ELISA), HPLC-MS and membrane potential measurements, have also been developed.[6–10] Although these techniques are able to detect STX and are much more sensitive than the mouse bioassay, their high cost and/or time consuming nature are disadvantages for their implementation in standardized protocols.

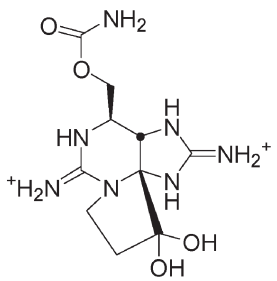

Fig. 1 Structure of Saxitoxin.

Fluorescent sensor molecules that possess a crown ether receptor unit were found to be effective for signaling the presence of Saxitoxin.[11] These sensing molecules belong to the *Photoinduced Electron Transfer* (PET) sensor family.[12] Our recent work with coumarin based PET sensors has shown that the toxin can be detected in buffered aqueous solution in the presence of Na^+, K^+, and Ca^{2+} ions.[13]

Moreover, these coumarin sensors showed larger fluorescence enhancement in the presence of the toxin than the anthracyl derivatives.[14–17] Monolayer studies with an amphiphilic coumaryl–crown derivative revealed that detection of STX is possible even in cases when only one layer of the sensing molecules is present on the solid substrate, *e.g.* a Langmuir–Blodgett film on quartz substrate.[18] Repeated exposure of solutions to these films, however, leads to the loss of the sensor molecules from the surface as they are only physically adsorbed on the substrate. Here we report results with a sensor monolayer that is covalently bound on a quartz substrate and used for Saxitoxin detection. A bromoalkyl modified coumaryl–crown derivative was bound on thiol modified quartz substrate by thioether formation (Fig. 2).[19]

The UV-Vis absorption spectrum of the modified quartz slide showed the characteristic band for coumarin at 332 nm (Fig. 3). The surface density of the sensor molecules can be estimated by using the corresponding form of the Lambert–Beer equation (eqn (1)).[20]

$$\rho = A\varepsilon^{-1} \quad (1)$$

For calculation, the absorbance was divided by two as the surface density refers only to one side of the quartz substrate.

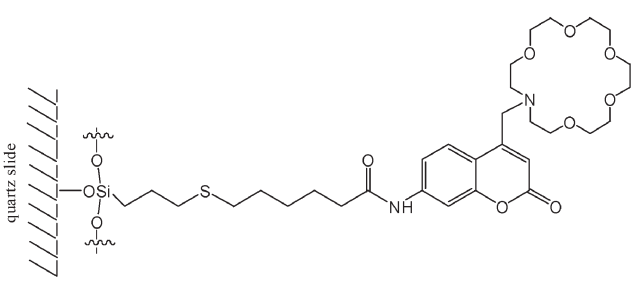

Fig. 2 Covalently attached coumaryl–crown sensor on quartz surface.

[a]*Department of Chemistry, Eötvös Loránd University, Pazmany Peter setany 1/A, VI. Emelet 646 szoba, Budapest, 1117, Hungary*
[b]*Department of Chemistry, University of Miami, 1301 Memorial Drive, Coral Gables, FL 33124, USA. E-mail: rml@miami.edu; Fax: +1(305) 284-6367; Tel: +1(305)284-2194*
[c]*Department of Chemistry and Biochemistry, University of Arkansas, Fayetteville, AR 72701, USA*

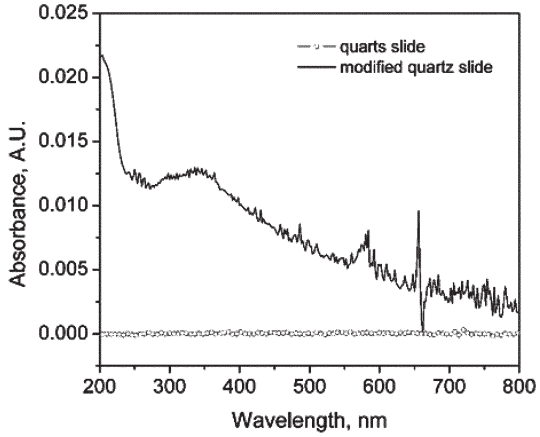

Fig. 3 Absorbance spectrum of PET sensor modified quartz slide.

Furthermore, the extinction coefficient for acetamidocoumaryl–aza-crown (ε = 16 398 M^{-1} cm^{-1}) was used.[13] This gave a surface density of 2.2 molecules per 100 $Å^2$. In comparison, the limiting molecular area obtained from a close packed monolayer of a similar amphiphilic coumaryl–aza-crown at the air–water interface is 2.6 molecules per 100 $Å^2$.[18] This implies 85% surface coverage by the covalently attached sensor.

The coverage of the surface was also determined by ellipsometry.[21] Fig. 4A shows the ellipsometric contrast image of the modified quartz substrate from which the two-dimensional and three-dimensional thickness maps were generated (Figs. 4B, 4C) (optical model: refractive index, 1.55; extinction coefficient, 0.05). The average thickness of the modified slide was estimated to be 20 $\pm$ 1 Å. This value, when compared with the energy minimized molecular model (25.5 Å) gives ~78% coverage, in approximate agreement with the absorbance results.[22]

The covalently modified quartz slide was placed under a bifurcated optical fiber and the fluorescence changes in the presence of different Saxitoxin concentrations were tested (λ_{Exc} = 332 nm, λ_{Em} = 415 nm; Fig. 5).

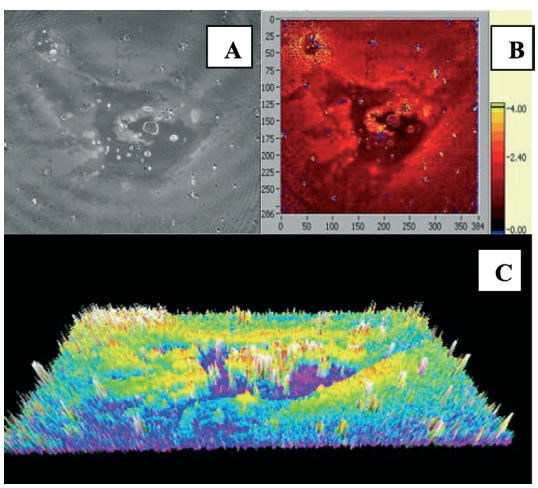

Fig. 4 Ellipsometric contrast image of the surface of coumaryl–crown modified quartz slide (A); the respective thickness-map in 2D (thickness values are in nm) (B); lighter areas indicate higher surface thickness (C).

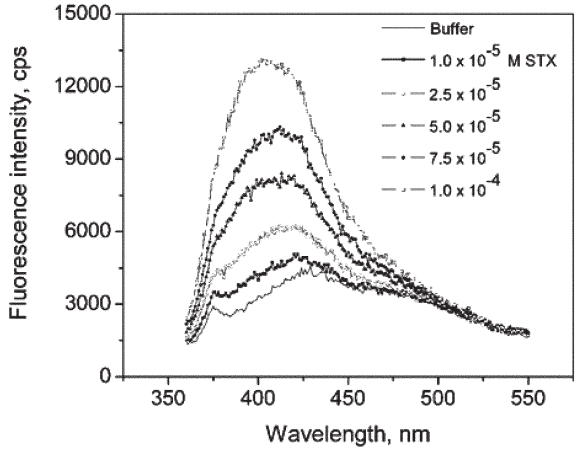

Fig. 5 Fluorescence changes of the self-assembled monolayer in the presence of Saxitoxin.

Saxitoxin was administered from 10^{-6} to 10^{-4} M concentrations in 0.01 M phosphate buffer (pH = 7.4; [NaCl] = 0.137 M and [KCl] = 0.0027 M). Fluorescence enhancement was observed with a detection limit of 10^{-5} M STX concentration.

Although in our solution work a lower concentration was detected (5×10^{-7} M),[13] the sensor monolayer is promising based on its simplicity and nano-size dimension. The surface modification was repeated ten times and from the absorbance data a surface coverage between 70–80% was obtained. The variation in surface coverage is given by the surface defects that are different from slide to slide. The sensitivity of the assay is within one order of magnitude of the mouse bioassay with our experimental design using only one side of the modified slide. Approaching this sensitivity would make it possible to fabricate an inexpensive and reusable nanosensor device, which could serve as a substitute for the currently used standard that requires the use of animals.

This work was supported by the National Science Foundation (RML, CH-0416095), US Army Research Office (RML, DAAD19-03-1-0131) and, in part, by the US EPA Science to Achieve Results (STAR) program (REG, R82959902 and GR83238201). Péter Kele thanks the Hungarian Ministry of Education and the Hungarian Scientific Fund for financial support (BÖ125/2003, F048739). The authors thank Dr Mykhaylo Dukh for his assistance under EPA R826655.

Notes and references

1 S. Hall, G. Strichartz, E. Moczydlowski, A. Ravindran and P. B. Reichart, in *Marine Toxins: Origin, Structure and Molecular Pharmacology*, ed. S. Hall and G. Strichartz, American Chemical Society, Washington, DC, 1990, vol. 418, pp. 29–65.
2 G. L. Boyer, C. F. Wichmann, J. Mosser, E. J. Scantz and H. K. Scnoes, Toxins isolated from Bay of Fundy scallops, in *Toxic Dinoflagellate Blooms*, ed. D. L. Taylor and H. H. Sallinger, Elsevier/North Holland, New York, 1979, p. 373.
3 D. M. Anderson, *Sci. Am.*, 1994, **271**, 62.
4 W. A. Catterall, C. S. Morrow and R. P. Hartsborne, *J. Biol. Chem.*, 1979, **254**, 11379.
5 AOAC Official Methods 959.08, Paralytic Shellfish Poison: biological method, in *Official Methods of Analysis of AOAC International*, ed. W. Horwitz, AOAC International, Gaithersburg, MD, 17th edn, 2000.
6 Y. Oshima, *J. AOAC Int.*, 1995, **78**, 528.
7 J. F. Lawrence and B. Wong, *J. Chromatogr., A*, 1996, **755**, 227.

8 C. J. Mirocha, W. Cheong, U. Mirza and Y. B. Kim, *Rapid Commun. Mass Spectrom.*, 1992, **6**, 128.
9 M. C. Louzao, M. R. Vieytes, J. M. V. Baptista de Sousa, F. Leira and L. M. Botana, *Anal. Biochem.*, 2001, **289**, 246.
10 R. A. Nicholson, G. H. Li, E. Buenaventura and D. Graham, *Toxicon*, 2002, **40**, 831.
11 R. E. Gawley, S. Pinet, C. M. Cardona, P. K. Datta, T. Ren, W. C. Guida, J. Nydick and R. M. Leblanc, *J. Am. Chem. Soc.*, 2002, **124**, 13448.
12 A. P. de Silva, H. Q. N. Gunaratne, T. Gunnlaugsson, A. J. M. Huxley, C. P. McCoy, J. T. Rademacher and T. E. Rice, *Chem. Rev.*, 1997, **97**, 1515.
13 P. Kele, J. Orbulescu, T. L. Calhoun, R. E. Gawley and R. M. Leblanc, *Tetrahedron Lett.*, 2002, **43**, 4413.
14 J. Orbulescu, P. Kele, A. Kotschy and R. M. Leblanc, *J. Mater. Chem.*, 2005, **15**, 3084.
15 R. E. Gawley, Q. H. Zhang, P. I. Higgs, S. P. Wang and R. M. Leblanc, *Tetrahedron Lett.*, 1999, **40**, 5461.
16 R. E. Gawley, M. Shanmugasundaram, J. B. Thorne and R. M. Tarkka, *Toxicon*, 2005, **45**, 783.
17 S. P. Wang, Q. H. Zhang, P. K. Datta, R. E. Gawley and R. M. Leblanc, *Langmuir*, 2000, **16**, 4607.
18 P. Kele, J. Orbulescu, T. L. Calhoun, R. E. Gawley and R. M. Leblanc, *Langmuir*, 2002, **18**, 8523.
19 P. Kele, PhD Thesis, University of Miami, USA, 2002.
20 S. Flink, F. C. J. M. van Veggel and D. N. Reinhoudt, *Chem. Commun.*, 1999, 2229.
21 Imaging ellipsometry measurements were performed by Nanofilm, Göttingen, Germany using an I-Elli2000 instrument.
22 Geometry optimized model was achieved using Hyperchem 7.03 for Windows (evaluation version), Hypercube, Inc., 2002.