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Communication

Surface Plasmon Spectroscopic Detection of Saxitoxin

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Abstract: For the surface-optoelectronic study of Saxitoxin sensing, we fabricated self-assembled calix[4]arene derivative monolayers as the recognition-functional interfaces on a gold surface. An interaction study between Saxitoxin and calix[4]arene derivative monolayers were performed using surface plasmon resonance (SPR) spectroscopy. Among three calix[4]arene derivatives, calix[4]arene crown ether SAM showed the highest sensitivity to Saxitoxin. The detection limit of this system is three orders of magnitude lower than that of the mouse bioassay which is the current benchmark for Saxitoxin detection.

Keywords: Saxitoxin, surface plasmon resonance, calixarene, self-assembled monolayer (SAM).

1. Introduction

Harmful algal booms (red tides) produce a wide variety of secondary metabolites, but Saxitoxin (STX) is virtually isolated among them in being capable of causing human mortality by consumption of tainted shellfish. STX and its congeners are known as paralytic shellfish poisons (PSPs). Currently, governments of many countries monitor shellfish beds for the presence of saxitoxin by using one of several tests, the most reliable test is mouse bioassay. New approaches include insect bioassay, tissue biosensors, molecular pharmacology, neurophysiology, whole-cell bioassay, HPLC with postcolumn

oxidation of the C4-C12 bond [1-4]. However, most methods have low sensitivity and many pre-required treatment steps for analysis.

STX is a bis-guanidinium dication and its molecular structure was shown in Figure 1. Several crystal structures of 18-crown-6 and its aza analogues with guanidinium ions show hydrogen bonding with crown heteroatoms. R.E. Gawley et al. fabricated Aza-18-crown-6 ether sensor that showed excellent fluorescence enhancement [5]. Calix[4]arene and calix[6]arene derivatives were found for their guanidinium ion selectivity based on CHEMFETs [6]. However, to our knowledge, there has no report about STX sensing using calix[n]arene derivative receptor yet.

Surface plasmon resonance (SPR) allows the user to study the interaction between immobilized recognition molecular and analytes in solution without labeling of the analyte. Recently, a novel method based on SPR, immobilization by recognition monolayers on noble metals have been utilized to detect small molecules and to obtain better reproducibility [7, 8]. These approaches of small molecule measurement have been proved to increase the sensitivity compared to other techniques. The SPR technique and careful construction of appropriate SAM are very useful methods to the development of more efficient biosensing interface.

Our research purpose here is a SPR based fundamental study of STX binding properties on the calixarene derivative recognition monolayer system in order to make high sensitive detection of STX. For this purpose, three calixarene derivatives containing ethylester (**1**), carboxylic acid (**2**), and crownether (**3**) at the lower rim were applied to recognition layer for STX. Self-assembly technique was utilized to construct a well–characterized recognition monolayer. The characterization of calixarene SAMs has been showed detailly in our previous work [9]. A molecular interaction study between calix[4]arene derivative SAMs and STX was investigated by SPR to detect low concentration up to 1.0×10^{-9} M on **3** SAM. The refractive index of the surface interfacial layer was calculated by the fitting of SPR curve with the Fresnel equations, which was found increased derived by the binding of STX onto the **3** SAM.

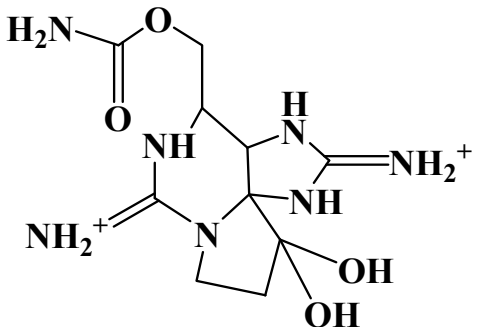


Figure 1. Molecular structure of saxitoxin.

2. Experimental

2.1. Chemicals and Reagents

STX was bought from National Research Council in Canada. The syntheses of Calix[4]arene derivative **1** (ethylester) and **2** (carboxylic acid) were carried out from the literature with minor modification [10]. Calix[4]arene crown ether derivative **3** was obtained from Proteogen Co. (Seoul,

Korea). The structure of calix[4]arene derivatives was shown in Figure 2. Other reagents were bought from Sigma Chemicals (St. Louis, Mo) and Aldrich Chemical (Milwaukee, WI, USA). Milli-Q grade (> 18.2 mQ / cm) water was utilized for the preparation of the buffer solution.

2.2 Formation of calix[4]arene derivative SAMs on a gold chip

A microscope cover glass (18 × 18 × 0.15 mm, with a refractive index of 1.515, Matsunami, Japan) with gold layer was used as a substrate for the formation of calix[4]arene derivative SAMs. The gold film (thickness ≈ 50 nm) was deposited on the cover glass by the sputter coating system (E5000, Polaron Co., U.K.) under conditions of 2.0×10^{-2} mbar and 20 mA for 135 s. The sputtered Au substrate was rinsed using distilled water, methanol and acetone, sequentially. Then, the gold chip was dried in a nitrogen stream softly and ready to use. Calix[4]arene derivative **1** and **3** were prepared to 0.1 mM in chloroform:methanol = 1:9 (v/v) solution mixture for the formation of their SAMs on gold surface. The SAM was formed by immersing the gold chips into solution for 6 h. After the immobilization process, the sensor chip was rinsed with chloroform-methanol solution mixture and methanol and then dried under N_2 stream [9].

The monolayer of calix[4]arene derivative **2** was converted from **1** SAM through the hydrolysis of ethylene group by treatment of LiOH for 3 h (Figure 2) [9]. LiOH solution was prepared to 0.1 M in methanol:DW = 1:1 (v/v) mixture.

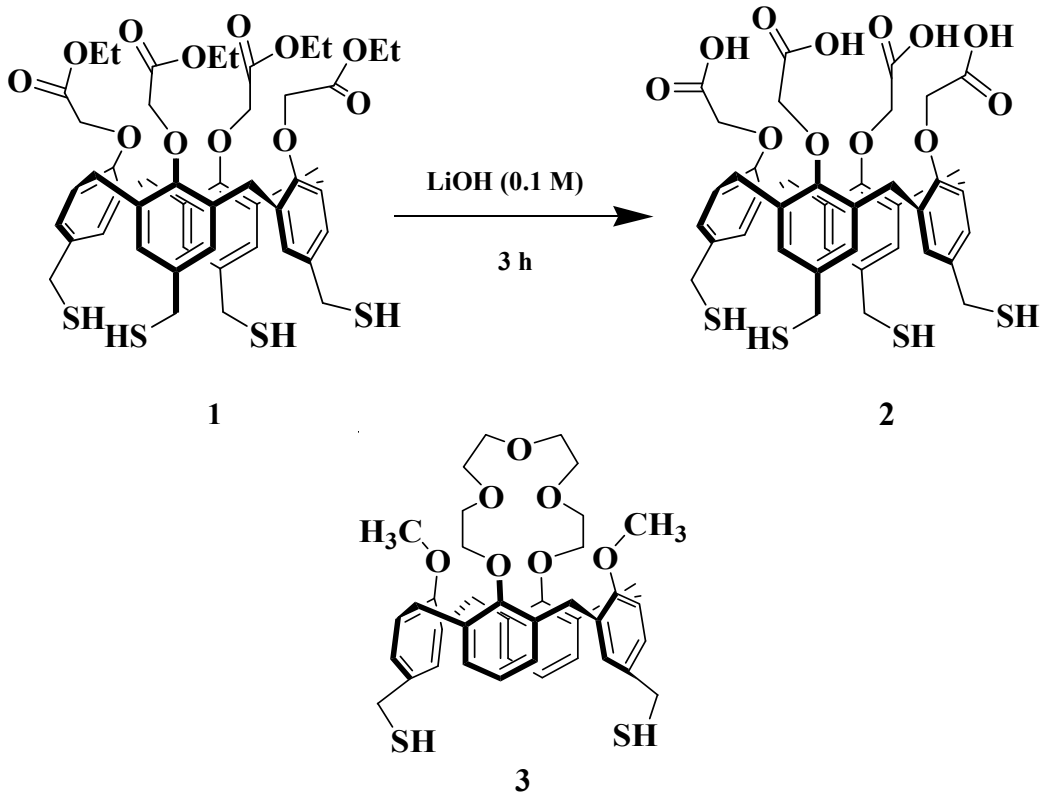


Figure 2. Molecular structure of calix[4]arene derivatives used in this study.

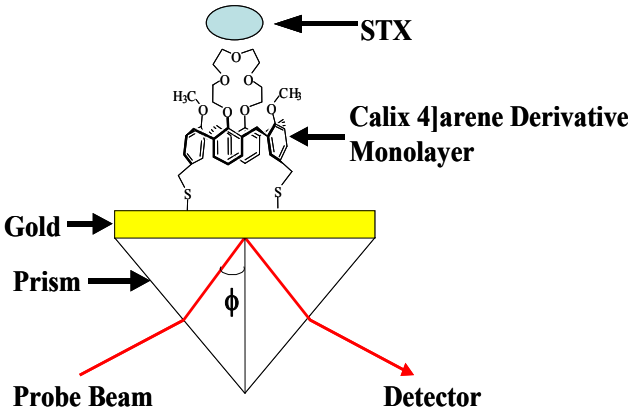
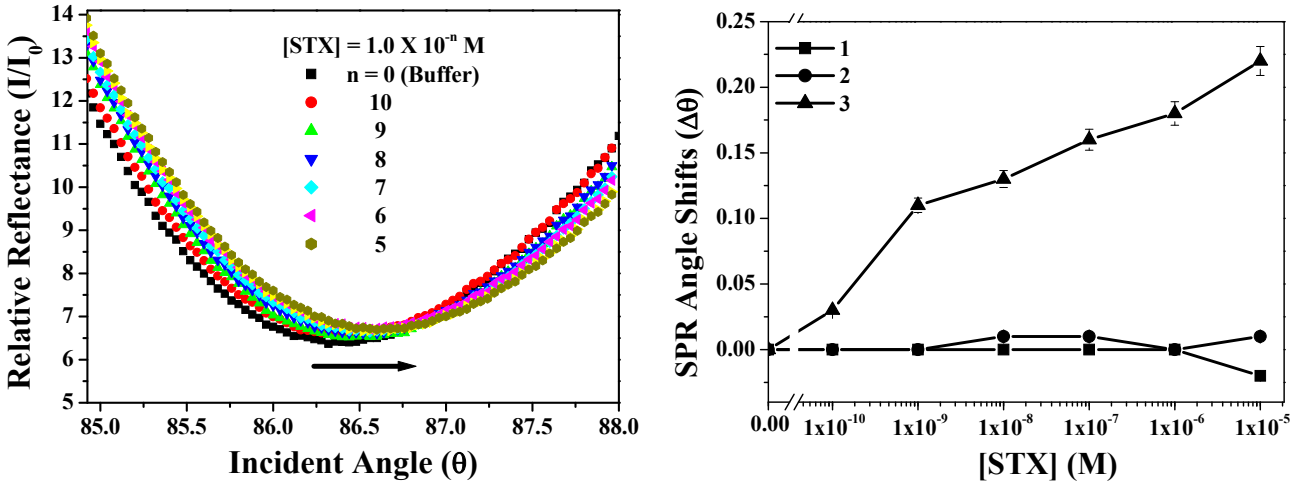


Figure 3. Schematic diagram of the sensor chip configuration.

2.3 SPR spectroscopic measurement

The SPR spectroscopic measurements for the interactions between STX and the SAM were performed by homemade SPR system based on the traditional Kretschmann configuration (Figure 3) [9]. A laser diode (LD, λ_{max} = 675 nm) was used as the light source. The reflected intensity of light through the polarizer and the prism was measured with the photodiode detector (ANDO Electric Co. Ltd., AQ-1976, Kanagawa, Japan). The incident angle into the prism varied with the motorized rotary stage and its controller (Suruga Seiki, D80, Shizuoka, Japan). The signal from photodiode was converted through a signal process board (K-MAC Co., Spectra View 2000, Taejeon, Korea) and could be interfaced with a computer. The angle resolution of the SPR system that was determined by the resolution of motorized rotary stage was 0.004 degree.

Molecular interaction between the calix[4]arene derivatives and the STX was measured by the batch method in a Teflon sample chamber. SPR spectra were recorded at concentration of STX ranging from 1.0×10^{-10} to 1.0×10^{-5} M (pH =7.4, 0.01 M phosphate buffer, containing 0.15M NaCl).



(a) (b)

Figure 4. (a) SPR spectra of **3** SAM in the presence of various concentrations of STX; (b) relative SPR angle shifts of calix[4]arene derivatives corresponding to the presence of various concentration of STX.

3. Results and discussion

To compare binding properties, STX was injected onto different calix[4]arene derivative SAMs modified chip surface. In case of calix[4]arene **3** SAM, as the concentration of STX increased, the SPR angle shifts that resulted from molecular interaction between the calix[4]arene **3** SAM and the STX gradually increased (Figure 4 (a)). However, in case of **1** and **2,** no response was shown to STX, which indicates they have weak or no interactions with STX (Figure 4 (b)).

The calix[4]arene binds with STX through the π–π and van der Waals interactions for all three derivatives[11]. The upper rims of **1** (ethylester) and **2** (carboxylic acid) have very small binding affinity with STX, which results little refractive index change of interfacial layer. However, **3** SAM bound to STX may involve in the strong hydrogen-bonding of the crown-like loop [5]. It was well known that calix[4]arene crown ether binding to cations and completely encapsulated the cation [12]. Gawley et al. presented the possible mode of binding between crown ether and STX, which shows the several hydrogen bonds between the guandinium of STX and crown ether oxygens.

Table 1. The refractive index (RI) of the interfacial layer according to the interaction between **3** SAM and the different concentration of STX.

[STX] (M)	1.0×10^{-10}	1.0×10^{-9}	1.0×10^{-8}	1.0×10^{-7}	1.0×10^{-6}	1.0×10^{-5}
RI	1.323	1.365	1.387	1.405	1.426	1.440

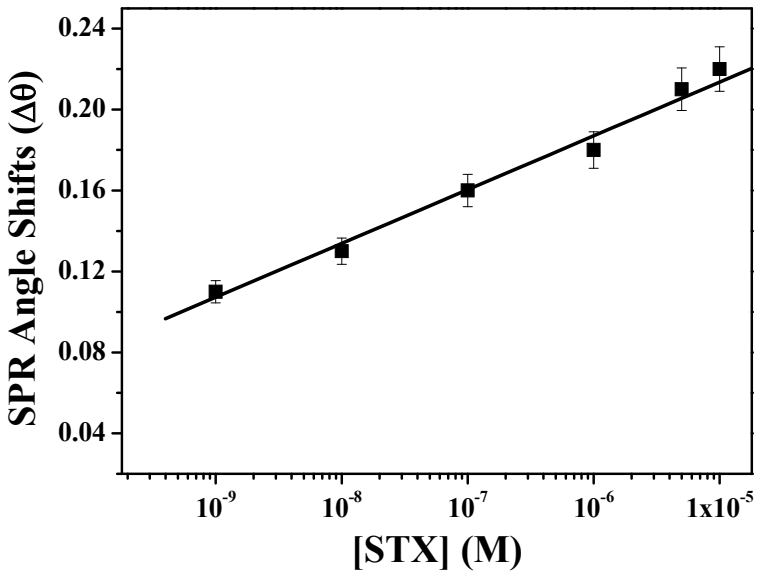


Figure 5. Relative SPR angle shifts of **3** SAM corresponding to various concentration of STX. Linear regression shows a working range of 1.0×10^{-9} - 1.0×10^{-5} M ($r^2 = 0.994$).

SPR is a collective electron excitation that may exist at the interface of two media with different dielectric constants. Since the dipole moment of a molecule was a measuring factor of charge

distribution of the overall molecule, the conformation of calix[4]arene crown ether ionic complex with STX was able to affect its dipole moments. The dipole moment is intimately related to the dielectric constant κ through

$$P = (\kappa - 1)\varepsilon_0 E \quad (1)$$

where P is the total polarization of a sample, $\kappa = \varepsilon/\varepsilon_0$ (where ε and ε_0 are the permittivity of the material and the permittivity of vacuum respectively) and E is the local electric field vector. Note that for optical frequencies, the dielectric constant is related to the refractive index n via

$$\kappa = n^2 \quad (2)$$

The dipole moments of STX binding calix[4]arene crown ether SAM derived variation of dielectric constant, consequently, resulting in distinguishable of RI change and a greater SPR angle shifts (eq. 1 and 2). To further study the RI change on the sensor surface, the RI was determined through four layer theoretical simulation by the Fresnel equation with experimental SPR data. We reported in our previous work on our SPR-based RI calculation method [8]. Table 1 presents the RI changes via computer simulation when STX was bound on the **3** SAM. It was observed that the RI was linearly increasing by the gradual change of STX concentration. However, **1** and **2** SAM showed no RI change even when presenting the 1.0×10^{-5} M of STX (data not shown).

The SPR angle change for the increase in STX concentration from zero (buffer) to 1×10^{-5} M is 0.22 degree. The linear detection range using calix[4]arene crown ether is found to be 1.0×10^{-9} - 1.0×10^{-5} M with $r^2 = 0.994$ (Figure 5). With regard to the low detection limit of conventional mouse bioassay and chemosensor, which stays at the micromolar level, these experimental results are quite remarkable [1, 5].

4. Conclusion

We have constructed a calix[4]arene derivative monolayers using a SAM method. To make the high sensitivity of STX detection, three different calix[4]arene derivative monolayers were applied to a STX recognition system coupled with SPR spectroscopy. Among three derivatives, calix[4]arene crown ether SAM has been shown to bind to STX, even at very low STX concentration. In binding, the RI change of the interfacial recognition layer induces the SPR angle shift, permitting a sensitive detection of STX at a range of 1.0×10^{-9} - 1.0×10^{-5} M. This RI increase may result from the conformation change of calix[4]arene induced by the guanidinium ion bond on the low rim of crown ether. Consequently, these results reveal that a well-designed molecular recognition system based on SPR spectroscopy is very useful for the study of small molecular interaction. Studies are underway to elucidate the mechanism of binding, and to design a superior sensor.

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