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Potentiometric chemical sensors for the detection of paralytic shellfish toxins

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ABSTRACT

Potentiometric chemical sensors for the detection of paralytic shellfish toxins have been developed. Four toxins typically encountered in Portuguese waters, namely saxitoxin, decarbamoyl saxitoxin, gonyautoxin GTX5 and C1 &C2, were selected for the study. A series of miniaturized sensors with solid inner contact and plasticized polyvinylchloride membranes containing ionophores, nine compositions in total, were prepared and their characteristics evaluated. Sensors displayed cross-sensitivity to four studied toxins, i.e. response to several toxins together with low selectivity. High selectivity towards paralytic shellfish toxins was observed in the presence of inorganic cations with selectivity coefficients ranging from 0.04 to 0.001 for Na^+ and K^+ and $3.6*10^{-4}$ to $3.4*10^{-5}$ for Ca^{2+}. Detection limits were in the range from 0.25 to 0.9 $\mu molL^{-1}$ for saxitoxin and decarbamoyl saxitoxin, and from 0.08 to 1.8 $\mu molL^{-1}$ for GTX5 and C1&C2, which allows toxin detection at the concentration levels corresponding to the legal limits. Characteristics of the developed sensors allow their use in the electronic tongue multisensor system for simultaneous quantification of paralytic shellfish toxins.

1. Introduction

Marine toxins are chemical compounds biosynthesized by a few phytoplankton species that cause negative impacts on marine organisms and, in severe cases, mortality of fish, birds and mammals. In addition, filter-feeding bivalves can accumulate toxins in their tissue and provoke human poisoning when consumed [1]. Proliferation of toxic phytoplankton leading to shellfish poisoning are called toxic algal blooms [2,3]. As occurrence of toxic algal blooms is unpredictable, routine monitoring of the presence of marine toxins in bivalves and toxic phytoplankton species in seawater near bivalve catching and production areas are necessary. To address this need, monitoring programs have been established in several coastal countries. EU monitoring programs currently include three groups of toxins, divided according to the symptoms in humans: diarrheic shellfish toxins (DSTs), paralytic shellfish toxins (PSTs) and amnesic shellfish toxins (ASTs), and also some other lipophilic toxins [4,5]. The occurrence of PSTs in Portuguese coastal waters is less frequent compared to the other types of toxins [6], however they are of particular concern due to the life-threatening neurological symptoms they can cause in humans. In severe cases respiratory paralysis and death may occur, with overall mortality estimated to be about 8.5 −9.5% [7,8].

According to the EU legislation, the official reference method for the detection of PSTs is the Liquid Chromatography (LC) with Fluorimetric Detection (FLD) [9,10]. As LC-FLD is a laboratorial technique involving the use of expensive apparatus, which must be operated by highly skilled personnel, development of less costly and less complex assays and probes for marine toxins detection is of practical interest.

Several biosensors and immunoassays have been proposed for individual PSTs' detection, along with nerve cell and sodium channel based assays [11]. Antibody-based assays and biosensors can achieve very low limits of detection, but usually only for a small number of known PSTs, since the antibodies employed have a low cross-reactivity. Additionally, antibodies require an animal host for their production. Nerve cell and sodium channel based methods are of particular interest as they produce toxicity estimate of PSTs, which was found to be well correlated with animal tests. Furthermore, nerve cell and sodium-channel based methods have higher selectivity than animals as the latter can respond to other contaminants besides PSTs. Direct measurements of toxicity instead of concentration is also more relevant in the monitoring as toxins are always present as a mixture of compounds with toxicity varying up to two orders of magnitude. However, both nerve cells and sodium channels involve laborious preparation procedures, have long response times and, most importantly, lack stability,

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which leads to low reliability and reproducibility of measurements [12,13].

Chemical sensors represent an interesting alternative to the methods described above, mainly due to their robustness and low cost. However, there are only few reports on the chemical sensors for PST detection. Detection of one of the PSTs, saxitoxin, using surface plasmon resonance sensor with calix [4] arene derivatives as a recognition element was reported in [14]. Aza and diaza crown ethers modified with anthracene and coumaril moieties were proposed as receptors for fluorescent detection of saxitoxin in a series of publications [15–19]. It is important to note that these works were mostly of exploratory nature and only one study [14] reports a calibration curve. Furthermore, development of the sensors for PSTs detections, including chemical sensors, biosensors, immunoassays, etc., targeted mainly only one toxin – saxitoxin, which is the most common PST worldwide. However, typical profile of PSTs detected in bivalves in Portuguese coast differ and comprises mainly decarbamoylated and N-sulfocarbamoylated toxins [6,20,21].

The purpose of the present work was the development of potentiometric chemical sensors for PSTs typically present at Portuguese coast. No previous reports of potentiometric chemical sensors for PST detection nor chemical sensors for detection of PSTs other than saxitoxin were reported.

2. Materials and methods

2.1. Reagents

Sodium hydrogen phosphate and dihydrogen phosphate, aniline, tris(hydroxymethyl) aminomethane (BioPerformance Certified) were from Sigma Aldrich, ethanol, sodium hydroxide, hydrochloric acid, sulfuric acid, sodium nitrate, potassium nitrate and calcium nitrate were from Panreac, tetrahydrofuran (Chromasolv) was from Fisher. All reagents were p.a. (for analysis) unless stated otherwise. High molecular weight polyvinyl chloride (PVC), dibutyl phthalate (DBP), potassium tetrakis(4-chlorophenyl)borate (KTPB), tridodecylmethylammonium chloride (TDMACl) and ionophores were from Fluka. Screen-printed electrodes (SPE) with gold working and auxiliary electrodes and silver reference electrode were from DropSens (Spain). Ultrapure water produced by Merck Millipore Water System (18 $M\Omega cm^{-1}$) was used for solution preparation and sensor washing.

Solutions of PSTs, namely saxitoxin (STX), decarbamoyl saxitoxin (dcSTX) and N-sulfocarbamoyl toxins gonyautoxin GTX5 and C1&C2, were certified reference material from the Institute for Marine Biosciences, National Research Council, Halifax, Canada. When working with PSTs, long sleeved lab coat and non-permeable nitrile or latex gloves should be used. Toxin containing waste should be decontaminated using a 10% solution of sodium hypochlorite during 30 min and disposed of down the drain with plenty of water.

2.2. Sensor fabrication and potentiometric measurements

Potentiometric sensors with solid inner contact were fabricated using SPE. Firstly, surface of SPE working electrode was rinsed with ethanol and water and cleaned by cycling potential for 5 cycles between −0.2 and +1.2 V at 50 mV/s in 50 $mmolL^{-1}$ sulfuric acid. Solid contact was prepared by electropolymerization of aniline in deaerated aqueous solution of 50 mmol L^{-1} aniline in 1 mol L^{-1} hydrochloric acid by cycling potential for 100 cycles between −0.23 and +0.85 V at 50 mV/s. Sensors were washed with deionized water, conditioned for 2 h in 1 mmol L^{-1} hydrochloric acid and dried. All controlled-potential experiments were performed with an EZstat-Pro EIS (NuVant Systems Inc., Indiana, USA). Platinum wire served as the counter electrode and Ag/AgCl (KCl 3 $molL^{-1}$) served as a reference electrode.

Membrane mixtures were prepared by dissolving PVC (33%w/w), dibutyl phthalate (66%w/w), ionofore (1%w/w) and lipophilic salt (0.5%w/w) in tetrahydrofuran. Membrane compositions are listed in the Table 1. Membrane mixture was drop casted on the solid contact of the SPE and left to dry at room temperature. Prior to use, the sensors were conditioned in water for 2 h.

Table 1
Compositions of the sensing membranes. DBP - dibutyl phthalate, KTPB - potassium tetrakis(4-chlorophenyl)borate; TDMACl - tridodecylmethylammonium chloride.

Sensor	Ionophore	Lipophilic salt	Plasticizer
1	Calix [6] arene	KTPB	DBP
2	Calix [4] arene – 25,26,27,28-tetrol	KTPB	DBP
3	1,4,7,10,13-pentaoxa – 16-azacyclooctadecane	KTPB	DBP
4	1,4,10,13-tetraoxa – 7,16-diazacyclooctadecane	KTPB	DBP
5	Calix [6] arene-hexaacetic acid hexaethylester	KTPB	DBP
6	Octadecyl 4-formylbenzoate	KTPB	DBP
7	4,6,11,12-tetrahydro – 3-methyl – 1-phenyl-1H-pyrazolo[3′,4′:4,5]pyrimido [1,2-b]quinazolin – 5-ium tetrafluoroborate	KTPB	DBP
8	Octadecyl 4-formylbenzoate	TDMACl	DBP
9	4,6,11,12-tetrahydro – 3-methyl – 1-phenyl-1H-pyrazolo[3′,4′:4,5]pyrimido [1,2-b]quinazolin – 5-ium tetrafluoroborate	TDMACl	DBP

Calibration measurements were carried out in the solutions of STX, dcSTX, sodium, potassium and calcium nitrates on the background of 0.25 mmol L^{-1} Tris-HCl buffer and in the solutions of GTX5, C1&C2 and sodium chloride on the background of 1 mmol L^{-1} phosphate buffer. Both buffers had pH 7. Calibration measurements in sodium, potassium and calcium nitrate solutions were made in the concentration range from 1 $\mu molL^{-1}$ to 1 $mmolL^{-1}$.

Calibration solutions of PSTs were prepared by diluting toxin standards in buffer to the final concentrations from 0.1 to 6.8 μmol L^{-1}. Sensor response to pH was studied for pH range from 3 to 9. Measurements were started in the 1 mmol L^{-1} solutions of HCl with pH 3, to which 0.1 $mmolL^{-1}$ Tris-base solution was gradually added. Selectivity was evaluated using two solution method as described in [22] considering STX as a primary ion. Concentrations of STX and dcSTX were 2 μmol L^{-1}, of sodium and potassium were 1 mmol L^{-1} and calcium − 10 mmol L^{-1}.

Potentiometric measurements were carried out using custom-made high input impedance digital voltmeter (Sensor Systems LLC., St. Petersburg, Russia) connected to a PC for the data acquisition. Sensor potential was measured vs. SPE own pseudo-reference electrode. pH of the solutions was measured using combination pH glass electrode (Metrohm, Switzerland). Sensor potentials were recorded after stable readings were reached, typically after 5 min. At least three replicated calibration measurements were made. Between measurements, sensors were washed with deionized water until stable potential readings were reached. Typically about 30 min were necessary for potential recovery. When not in use, sensors were kept dry at room temperature and prior to measurements were soaked during 1 h in buffer solution.

3. Results and discussion

Selection of the ionophores for the sensors for the detection of PSTs was carried out taking into account literature data and properties of toxins. Paralytic shellfish toxins have two guanidinium moieties in their structure as shown in the Fig. 1 [8]. These guanidinium groups are basic and their pKa have been determined experimentally for STX and dcSTX: pKa of the group at C2 was found to be 8.22 and 8.10, respectively, and at C8 − 11.28 and 10.84, respectively [23]. Both of these toxins exist as doubly charged cations at pH below 7, uncharged species at pH above 13, and as mixture of forms in the pH range between 7 and 13, with