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A portable and chromogenic enzyme-based sensor for detection of abrin poisoning

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ABSTRACT

A first of its kind portable, colorimetric detection system has been developed for the rapid diagnosis of abrin poisoning. Abrin, a natural biotoxin that is homologous to ricin yet more lethal, has high potential for becoming a weapon of bioterrorism given its ease of production. Using an immobilization strategy that implements non-natural amino acids for site-specific conjugation, we have created a reusable N-methyltryptophan oxidase based magnetic bead system that is capable of detecting L-abrine, a marker for abrin poisoning, at concentrations as low as 4 μM in mock urine. Furthermore, we propose that this detection strategy may be readily adaptable for sensing other targets of interest. This unique diagnostic test for abrin poisoning has demonstrated key benefits of portability and simple visual readout. These significant advantages can thus provide the potential for more rapid assessment and corresponding poison management if dedicated toxicology laboratories are not an option.

1. Introduction

L-abrine, otherwise known as N-methyl L-tryptophan, is an acute toxic alkaloid and chemical marker for abrin, a lethal albumin found in *Abrus precatorius* seeds (jequirity pea or rosary pea). Abrin itself is highly homologous to the more well-known biological warfare agent ricin, and both are class II ribosome inactivating proteins (Garber, 2008; Tang et al., 2007). Abrin has been found to be more toxic than its counterpart ricin, with a human lethal dose of 0.1–1 μg/kg (Dickers et al., 2003) as compared to 5–10 μg/kg for ricin inhalation (Bradberry et al. 2003), and due to its easy cultivation and cheap preparation should be closely monitored as a threatening biological agent. A number of effective sensors have been constructed for detection of ricin and abrin (Feltis et al., 2008; Pradhan et al., 2009; Shankar et al., 2005; Strehlitz et al., 2008; Yang et al. 2011; Zhou et al., 2007,2012); however, we find that no tests exist yet to confirm if a person has been poisoned by exposure to abrin (CDC, 2013). Therefore, an unmet need remains for the accurate diagnosis of abrin exposure in order to offer effective poison management and treatment. A strong indication for abrin poisoning is the presence of the chemical maker L-abrine, which can survive metabolism in significant amounts making it detectable in human urine (Johnson et al., 2009). Because L-abrine makes up 1% of the dry weight of *A. precatorius* seeds, its higher abundance than abrin itself makes this alkaloid a particularly useful marker for detection of abrin poisoning (Owens and Koester, 2008). The analogous presentation of the alkaloid marker ricine in the urine of persons poisoned by ricin has inspired us to develop a unique system for the sensitive detection of L-abrine as a first step toward detection of abrin poisoning.

Here we describe the first reusable, chromogenic sensor for the detection of L-abrine using a novel approach that may be adaptable for detection of other biomarkers of interest. In practice, alkaloid based biomarkers are generally quantified for poison assessment using expensive and complex processing steps of solid-phase extraction, high-performance liquid chromatography method, followed by mass spectrometry, though the detection limits obtainable are impressive (Johnson et al., 2009). Fast access to such facilities for accurate diagnosis may not be an option particularly in the areas known for indigenous growth of the jequirity pea; hence, a more widely deployable resource for the rapid diagnosis of abrin poisoning would offer a significant benefit. In the following work, we present a detection system for a chemical marker of abrin which is reusable, portable, and also does not require complicated analytical skills, since it is colorimetric. In addition, our unique approach avoids the use of expensive antibodies or equipment, but instead we implement a naturally occurring enzyme from *Escherichia coli* with recognition capabilities for the abrin biomarker, L-abrine, thereby offering the possibility for large scale sensor production.

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The enzyme N-methyl tryptophan oxidase (MTOX) is a key component of our sensor due to the fact that it will oxidize L-abrine into the primary alpha-amino form of tryptophan and simultaneously generate H_2O_2 as a by-product (Wagner et al., 1999). The key formation of H_2O_2 allows us to provide simple visual detection by exposure to the commonly used reporter enzyme horseradish peroxidase when in the presence of a chromogenic substrate, such as tetramethylbenzidine (TMB) (Wang et al., 2011). Colorimetric style readout sensors have been popularized in ELISA assays among others chromogenic sensors which have demonstrated to be easily observable by the naked eye without the need for expensive analytical equipment (Jaworski et al., 2011; Jung et al., 2006; Lee et al., 2013; Li et al., 2013; Son et al., 2012; Wang et al., 2009). Quantification can be achieved in our system through a simple set of control samples of known L-abrine content. Because this approach relies on a simple readout technique, it may be readily adopted for widespread use. Using this detection system with MTOX, we were able to detect L-abrine at concentrations as low as 4 μM. Since we utilize MTOX immobilized onto the surface of magnetic beads in our approach, we are able to use a simple magnet to extract the sensor after detection, allowing reusability of the sensor over 5–6 cycles with minimal effects to the sensitivity of L-abrine detection. As with other magnetic particle based systems (Harjanto et al., 2013; Lee et al., 2012), isolation and reuse of our enzymatic sensor can be carried out by applying a simple magnet. Furthermore, we find that the heat stability of the enzymatic components of our sensing system allows for portability, thereby offering a significant advantage over existing approaches to poison diagnosis that typically use large immovable equipment. In addition, our sensing strategy proved to be equally as effective in mock urine samples. Due to the fact that urine samples can contain H_2O_2 background in varying concentration depending on individual health (Halliwell et al., 2004), we have devised a simple scheme to simply eliminate H_2O_2 background prior to MTOX detection of L-abrine.

A novel aspect of our sensing system is the fact that it is created through the controllable immobilization of the L-abrine recognizing enzyme, MTOX, onto the surface of magnetic particles, which we perform in a unique manner. To ensure a stable and reusable sensor, the interaction between the enzyme and magnetic support has to be sufficiently secure, and the enzyme should be attached in a directional manner as not to mask the catalytic site. Many effective strategies exist for enzyme immobilization, including non-specific adsorption, attachment of substrate binding domains, encapsulation, and ionic linkages among others (Hwang et al., 2011; Lee et al., 2005; Mateo et al., 2000; Takahashi et al., 2000; Vepari and Kaplan, 2006); however, in this work we have utilized an immobilization approach which allows for site-specific attachment of our MTOX enzyme onto a magnetic bead support in a controlled manner.

This unique strategy for site-specific MTOX attachment onto the magnetic beads makes use of an un-natural amino acid, formylglycine, which we produce at a single controlled location on the MTOX enzyme. The un-natural aldehyde functional group present on the formylglycine residue that we produce is biologically unique in comparison to the other 20 naturally occurring amino acids; hence, we are able to provide a site-specific reactive site for covalently coupling the MTOX enzyme to the surface. This is a distinct advantage over existing coupling approaches which typically use non-specific adsorption or generalized bioconjugation reactions. In order to introduce the un-natural formylglycine amino acid at a controlled, specific location, we utilize a selective Formylglycine Generating Enzyme (FGE), which is known to be capable of converting a sulfhydryl group into a reactive aldehyde functional group at a specific location (Frese and Dierks, 2009). The exact creation of the un-natural amino acid occurs at a conserved (C-X-P-X-R) protein sequence recognized by FGE, which has recently been utilized by the Bertozzi group for protein engineering (Rabuka et al., 2012). By genetic modification of the MTOX enzyme, we are able to incorporate this (C-X-P-X-R) sequence at a defined location. After purification of the MTOX enzyme and conversion into the reactive aldehyde-bearing form by incubation with FGE, we were able to simply mix the MTOX enzyme with our amine-coated magnetic beads in order to provide a direct covalent bond in the form of an imine linkage which may be further stabilized by reduction with sodium borohydride. The potential modularity of this approach stems from the site specific conversion strategy, in that other proteins could also be easily immobilized onto the magnetic scaffold if they, too, were expressed with the (C-X-P-X-R) tag. Our detection scheme comprising an immobilized H_2O_2 generating oxidase may offer an adaptable approach for detection of other chemical compounds if other oxidase enzymes having different chemical specificities were to be immobilized. In the following work we provide a detailed discussion of the concepts and results for our demonstrated reusable and portable colorimeteric sensor for detection of L-abrine poisoning, which should provide a good source for researchers to explore a range of other immobilized enzymes for their particular applications of interest.

2. Material and methods

2.1. Cloning and expression of CXPXR tagged MTOX

Double stranded DNA for the MTOX gene insert was first obtained by 50 μL colony PCR from the genome of *E. coli* K12 using the following primers and an annealing temperature of 58 °C (mtoxforward: 5′-ACTAGACTGCAGATGAAATACGATCTCATCATTATTGGCAGC-3′; mtoxreverse: 5′-GTACCTGGATCCTTATTGGAAGCGGGAAAGCCTGAATG-3′). The resulting PCR product contained a 5′ pstI site and a 3′ bamHI flanking the MTOX gene. After column purification of the PCR product using a Qiagen spin kit, the insert MTOX DNA was then digested using pstI and bamHI enzymes (New England Biolabs, USA) followed by an additional column purification step. The DNA was eluted from the column in 20 μL of water, and 7 μL of this insert DNA was mixed with 1 μL of cut expression vector DNA (vector already contains CXPXR tag DNA as described in detail in the supplementary information) 1 μL of ligase buffer and 1 μL of T4 DNA ligase (New England Biolabs, USA). The mixture was incubated at 22 °C for 2 h followed by heat shock transformation into 50 μL of chemically competent BL21 (DE3) cells. The cells were then plated onto LB agar-ampicillin petri dishes (100 μg/mL ampicillin) and grown overnight at 37 °C. On the following day, colonies were picked and sequenced to confirm insertion of the MTOX DNA to yield the expression vector bearing CXPXR tagged MTOX (see Supplementary material for sequence details).

A colony having the confirmed sequence was then grown under shaking condition in 5 mL LB-ampicillin (100 μg/mL) overnight at 37 °C. The following day 500 μL of the culture was transferred to a flask containing 1 L of LB-ampicillin and was again placed in the 37 °C shaker. Once the optical density at 600 nm of the cell culture reached 0.25, it was removed from the shaker and placed at room temperature for 5 min followed by induction by adding 100 μL of 1 M IPTG. The flask was then placed in an 18 °C shaker for 18 h to allow expression of CXPXR tagged MTOX in high yield. The liquid culture was then aliquoted and centrifuged at 6500 rpm for 20 min at 4 °C, and the supernatant was discarded. Next, 100 mL of sterile water was to resuspend the pellet, and the sample was centrifuged again using the same conditions followed by discarding of the supernatant. The samples were placed in a −80 °C