Application Note
#CBRNE - 1849601

Detection of ricin in different food and environmental matrices using the pBDi

Growing concern about ricin, a potent biologic toxin, as a terrorist weapon, demands a reliable, rapid and sensitive identification of ricin in various sample matrices. The portable BioDetector integrated (pBDi) performs sensitive detection of ricin exhibiting a detection limit of 0.5 ng mL^{-1}. Ricin is selectively identified using the pBDi Toxin Test Kit 1. The selective identification procedure means that closely related non-toxic lectins and abrin are not detected. Finally, we showed identification of ricin in different *Ricinus communis* extracts and in various food and environmental matrices.

Introduction

The potential use of ricin by terrorist groups and the ricin findings in the US in 2003 and 2013, the justified need to have rapid, sensitive and specific detection systems for toxins available. The glycoprotein ricin is a toxic lectin present in seeds of *Ricinus communis*, commonly known as the castor plant. Worldwide, one million tons of castor beans are processed annually in the production of castor oil; the waste mash contains up to 5% ricin per weight [1]. Therefore, ricin can be produced in large quantities without sophisticated or expensive technologies. As a biological warfare agent (BWA) it could be used for the contamination of food, contained water supplies or aerosolized for inhalation.

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Ricin is a highly toxic protein inhibiting protein synthesis, with a typical mouse LD_{50} in the order of 2 µg kg^{-1} of body weight. The human LD_{50} is estimated in the range of 3-30 µg kg^{-1} by inhalation or ingestion, respectively [1]. Therefore, ricin is classified as a Schedule 1 controlled substance under the Chemical Weapons Convention and a Category B substance according to the Centers for Disease Control and Prevention (CDC). Currently, there is no specific medicine available to treat ricin exposure [1]. Early detection and rapid identification of terrorist, aggressor and state actor released bioagents are critical to minimise casualties and effect a timely and efficient response and containment.

Experimental

Material

Seeds of different *Ricinus communis* varieties and of *Abrus precatorius* were obtained from Sandeman Seeds (Lalongue, France). Agglutinin from *Ricinus communis*, concanavalin, lectin from *Helix pomatia*, and soybean lectin, bentonite and kaolin were purchased from Sigma-Aldrich (Steinheim, Germany). Purified ricin, abrin and ricin toxoid were purchased from Toxin Technology, Inc. (Sarasota, FL, USA), whereas ricin B chain was obtained from Biozol (Eching, Germany). Liquid, solid, and semisolid foods were purchased from local grocery stores.

For ricin detection the pBDi with the pBDi Control software and the pBDi Toxin Test Kit 1 (Bruker Daltonik, Germany) were used. The portable BioDetector integrated (pBDi) is a self-contained identification platform for automated detection of up to six biothreat agents [2]. For the sample preparation of multiple sample matrices, the pBDi Sample Preparation Kit (Bruker Daltonik, Germany) was used.

Detection of ricin in extracts from *Ricinus communis* seeds

Ricin was extracted from *Ricinus communis* seeds using the acetone precipitation method according to Colburn *et al.* [3]. Abrin was isolated from Abrus precatorius using an analogue procedure. Extracts were diluted 10^6 fold with assay buffer prior analysis using pBDi Toxin Test Kit 1.

Detection of ricin in multiple matrices

Reference sample of purified ricin (5 ng mL^{-1}) was prepared in 1 mL sample buffer. For liquid food samples such as cola, water or milk, a 1 mL sample and 1 mL sample buffer was mixed. The sample was spiked with 5 ng mL^{-1} ricin (final concentration) and incubated for 2 h at room temperature. The samples were then filtered via a 0.8 µm syringe filter to remove solid particles. Subsequently, 0.5 mL to 1.0 mL filtrate was used for toxin detection with the pBDi.
For solid and semisolid samples approx. 0.5 - 1 g sample was mixed with 5 mL sample buffer and a final concentration of 5 ng ml^{-1} ricin was added. Further processing is analogue to the sample preparation procedure for liquid samples.

Results

Because of the high toxicity of ricin (LD_{50} 0.7 - 2 µg per kg body weight in mice) [1] sensitivity of the assay must be exceptional to prove the usefulness of the detection platform. Using pBDi Toxin Test Kit 1 and pBDi, sensitivity of ricin detection was tested applying different dilutions of ricin in assay buffer (Figure 1). The detection limit is defined as lowest measured value above the mean blank value plus three times standard deviation. For ricin a detection limit of 0.5 ng mL^{-1} was achieved, meaning the detection of small amounts of this particular toxic protein can be achieved in large volumes such as drinking water or food samples. For comparison, the oral, half-lethal dose for a 10 kg child in 100

Sensitivity of ricin detection

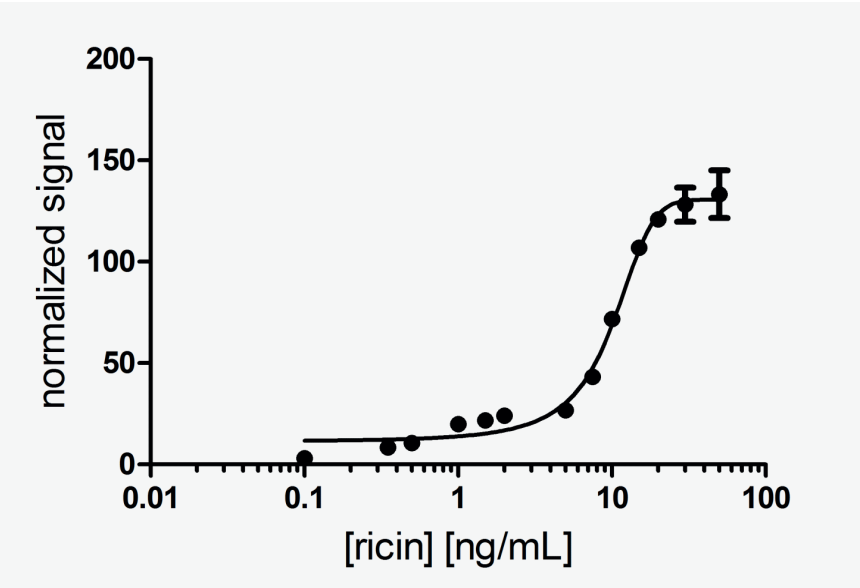

Figure 1: Sensitivity of ricin detection using pBDi and pBDi Toxin Test Kit 1. Different concentrations of ricin in assay buffer were applied for electrochemical measurement. The determined detection limit for ricin was 0.5 ng mL^{-1}.

mL food matrix is 700 ng mL^{-1} ricin [4].

Beside sensitivity selectivity of ricin detection is critical for generating valid results. Because ricin is a plant lectin, ricin-specific antibodies can potentially cross-react with other plant lectins like pea lectin, concanavalin A, soybean lectin or abrin. Figure 2 shows the signals of ricin, ricin B chain, ricin toxoid as well as other lectins and bioterrorism relevant proteotoxins (BoNT/A, BoNT/B, BoNT/F, SEB) using the pBDi Toxin Test Kit 1. Signals of positive control electrodes are defined as 100% and signals of each target electrode are standardized to positive control. Neither the different lectins nor the closely related plant toxin abrin resulted in a significant signal. In contrast, ricin and the highly homologous *R. communis* agglutinin showed significant signals (26.8% and 19.9%, respectively). An inactivated ricin protein (ricin toxoid) showing no toxic effect resulted in a slightly decreased electrochemical signal compared to native ricin (15.9%).

The fact that ricin can be extracted relatively easily from castor bean seeds makes the compound a potential threat agent for terrorist use. The reliable detection of ricin isolated from different *R. communis* varieties is of utmost importance since it has been reported that horticultural varieties of *R. communis* behave differently when characterized by liquid chromatography/ mass spectrometry [5]. Therefore, ricin was extracted from seeds of *R. communis, R. communis zanzibariensis, R. communis carmencita red, R. communis carmencita pink, R. communis Blue Giant, R. communis Green Giant and R. communis sanguineus* by a relatively simple laboratory protocol [3] and analyzed with the toxin chip (Figure 3). Similarly, abrin was extracted from seeds of Abrus precatorius. All crude extracts from *Ricinus communis* seeds produced a significant signal on the ricin specific electrode positions, whereas no signals were obtained on electrode positions for the other toxins. In contrast, no significant signal is produced by applying pure buffer or crude extract from *Abrus precatorius*.

Selectivity of ricin identification

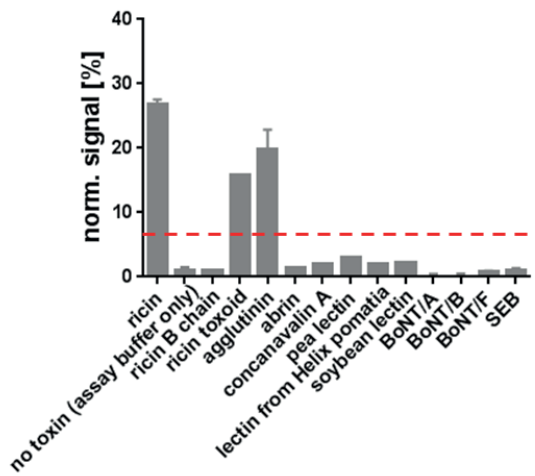

Figure 2: Selectivity of ricin detection using pBDi and pBDi Toxin Test Kit 1. 5 ng mL^{-1} ricin, ricin B chain, ricin toxoid or agglutinin were used, respectively, whereas for the closely-related lectins (pea lectin, concanavalin A, lectin from *Helix pomatia*, soybean lectin, abrin) at least 100-fold excess (>50 ng mL^{-1}) were applied for electrochemical measurement. Furthermore, buffer without toxin (no toxin) was applied. Results were defined as positive when the normalized signal exceeds the threshold value (dashed red line).

Detection of ricin from plant seeds

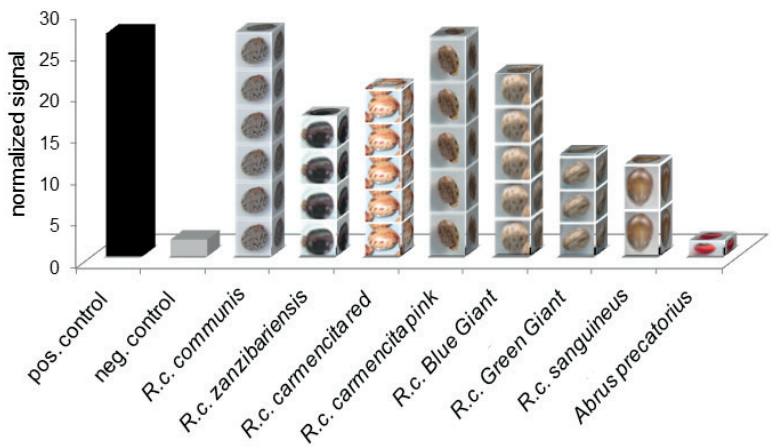

Figure 3: Identification of ricin extracted from seeds of different varieties of *Ricinus communis*. As negative controls buffer without toxin (neg. control) and crude extract from Abrus precatorius seeds were applied, whereas 5 ng mL^{-1} ricin was applied as positive control (pos. control).

The pBDi Toxin Test Kit 1 is suitable for selective detection of *Ricinus communis* extracts, which are possible samples in connection with bioterrorist activity.

One likely scenario of a potential bioterrorism attack focuses on toxins as food and water contaminants. As such, we tested the pBDi Toxin Test Kit 1 for identification of ricin in several liquid, solid and semisolid sample matrices (Figure 4). Each sample matrix was spiked with 5 ng mL^{-1} ricin, which is tenfold above the detection limit for ricin in buffer solution. As negative control, background was measured with no toxin in buffer. As reference the ricin-specific signal for 5 ng mL^{-1} ricin in buffer solution was determined. It is obvious that the signals for ricin in presence of sample matrices varied compared to ricin in buffer. However, ricin was detected in all sample matrices and a normalised signal was within a range of +/- 10 % compared to ricin in buffer. 5 ng mL^{-1} ricin added to honey showed the most significant signal decrease, yet was still detectable. Clearly, matrix effects appear to play a major role in assay performance, probably by masking antibody-binding epitopes. These results demonstrated the usability of the pBDi with the pBDi Toxin Test Kit I for sensitive detection of BWA relevant toxins in various complex sample matrices.

Conclusion

We have explored the performance of pBDi together with pBDi Toxin Test Kit 1 to detect ricin, a highly toxic and potential biological terrorist agent. pBDi allows highly sensitive and selective detection of ricin even in complex matrices. Furthermore, ricin was successfully identified in crude extracts of *Ricinus communis* seeds. Therefore, pBDi Toxin Test Kit 1 and pBDi is optimal for rapid and reliable detection of ricin in case of a bioterroristic attack.

Ricin detection in solid and fluid samples

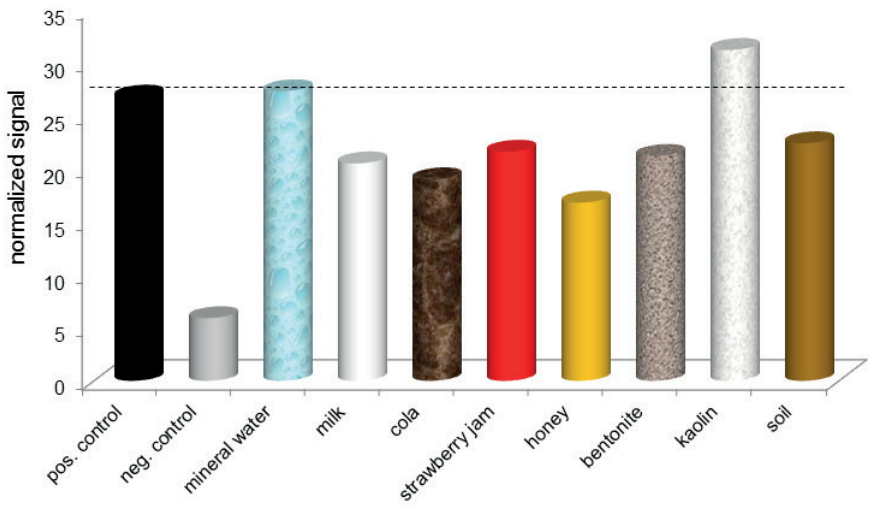

Figure 4: Detection of 5 ng mL^{-1} ricin in various sample matrices. As negative control buffer without toxin (neg. control) was applied, whereas as positive control 5 ng mL^{-1} ricin in buffer (pos. control) was tested.

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