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Research review paper

Ricin detection: Tracking active toxin

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ABSTRACT

Ricin is a plant toxin with high bioterrorism potential due to its natural abundance and potency in inducing cell death. Early detection of the active toxin is essential for developing appropriate countermeasures. Here we review concepts for designing ricin detection methods, including mechanism of action of the toxin, advantages and disadvantages of current detection assays, and perspectives on the future development of rapid and reliable methods for detecting ricin in environmental samples.

Contents

Introduction

Ricin is a naturally occurring toxin found in the seeds of the castor plant (*Ricinus communis*), which is globally cultivated and processed in large quantities. Ricin has been used as a biothreat agent in the past and has gained national attention due to its remarkable toxicity. The toxicity associated with ricin has long been established with over 700 human intoxications reported, dating as far back as the late 1800s (Balint, 1974). A summary of notable accounts can be found in Table 1, including a recent case report of a fatality due to the ingestion of an herbal product containing a lethal level of castor bean powder (Assiri, 2012). In many of the other recent examples provided, anti-government and terrorist groups were involved in the attempted use of ricin as a bioweapon. Its history of use as a weapon has led to ricin being categorized by the US Centers for Disease Control and Prevention (CDC) as a category B biothreat agent (CDC Strategic Planning Workgroup, 2000; Department of Health and Human Services, 2005) and its possession, transfer, and use are subject to domestic and international regulations (Department of Health and Human Services, 2005; Depositary Governments, 1972).

Ricin is a type 2 ribosome-inactivating protein (RIP) composed of A- and B-polypeptide chains covalently linked via a single interchain disulfide bond (S–S). Ricin gains cellular entry through the lectin binding properties associated with the 34 kDa ricin B-chain (RTB). X-ray crystallography (Rutenber and Robertus, 1991) and other biophysical methods (Blome and Schengrund, 2008; Gustafson, 2003; Houston and Dooley, 1982) have revealed that ricin preferentially binds to the abundant galactose-containing glycoproteins and glycolipids that line the surface of the cell. Using cultured HeLa cells and [^{125}I]-labeled ricin, Sandvig et al. (1976) detected approximately 3.3×10^7 toxin binding sites per cell with an association constant of 2.6×10^7 M^{-1}. Ricin bound to the cell surface is internalized via endocytic vesicles which facilitate its retrograde transport through the Golgi and endoplasmic reticulum, after which it is extruded into the cytosol where the A-chain of ricin re-natures and then attacks and inactivates ribosomes with high efficiency

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Table 1
Notable incidents involving the biological toxin ricin.

Date and location	Summary of incident
Washington, DC, US (2013)	Shannon Richardson was arrested for sending ricin laced letters to politicians including president of the United States of America Barack Obama and Mayor Michael Bloomberg.
Washington, DC, US (2013)	Envelopes addressed to president of the United States of America Barack Obama and Senator Roger Wicker were intercepted and found to be contaminated with ricin.
Abha, Saudi Arabia (2012)	A lethal ricin poisoning due to ingestion of an herbal medicine mixture containing ricin.
Georgia, US (2011)	Four members of a domestic militia group were arrested for plotting to make and use over 10 lb of ricin.
Washington, US (2009)	Patrons and employees at several gay bars in the city of Seattle were threatened with ricin poisoning.
Nevada, US (2008)	Roger Von Bergendorff was arrested for possession of a large amount of ricin, firearms, and anarchist propaganda.
London, UK (2003)	Six members suspected to be involved with the "Chechan network" were arrested and found to possess traces of ricin, castor beans, and ricin purification equipment.
South Carolina and Washington, DC, US (2003)	Letters and packages laced with ricin were intercepted at several postal mail facilities in South Carolina and Washington, D.C. Ricin contamination was also detected at United States Senator Bill Frist's mailroom.
Iraq (2002)	The Sunni militant group, Ansar al-Islam, was reported to be testing aerosolized ricin on animals.
Washington, US (2002)	Kenneth Olsen was arrested for possession of 1 g of ricin.
Michigan, US (1998)	Dwayne Kuehl was arrested for the attempted use of ricin against a city official.
Michigan, US (1998)	Four members of the North American Militia were arrested and charged for weapons and conspiracy. During the investigation, a videotape was found which described how to purify ricin from castor beans in a cooking-show format.
Wisconsin, US (1997)	Thomas Leahy was found to possess 0.67 g of ricin mixed with nicotine and some type of solvent. He also was believed to be attempting to lace razor blades with ricin.
Missouri, US (1995)	Michael Farrar was food poisoned with castor beans by his wife Debora Green, which led to several heart and brain surgeries.
Arkansas, US (1995)	Thomas Lavy was caught and found to possess an astonishing 130 g of ricin.
Minnesota, US (1994–1995)	A tax-protesting militia group was the first to be convicted for possession of ricin under the 1989 Biological Weapons Anti-Terrorism Act.
Texas, US (1982)	William Chanslor was convicted of attempting to euthanize his wife by ricin intoxication.
Virginia, US (1981)	Boris Korczak, a CIA double agent, was shot with a ricin laced pellet which penetrated his kidney.
London, UK (1978)	Georgi Markov, a Bulgarian dissident, was assassinated through the use of a ricin laced bullet fired from an umbrella-like gun.

All incidents were derived from reports in open sources.

(Sandvig et al., 2010) (Fig. 1). One molecule of ricin A chain can inactivate 1777 ribosomes per minute (Endo and Tsurugi, 1988). Ribosome inactivation is enzymatically accomplished by the 32 kDa ricin A-chain (RTA), which is a highly active N-glycosidase responsible for selectively deadenylating the first adenine in a GAGA sequence in the α-sarcin/ricin loop (SRL) of 28S rRNA (Endo and Tsurugi, 1987; Parikh et al., 2008). Removal of this adenine prevents mammalian elongation factor-2 from binding to the ribosome (Brigotti et al., 1989), which blocks protein synthesis and activates cell death pathways (Stirpe and Battelli, 2006; Walsh et al., 2013). Ultimately, the cytotoxicity of ricin can lead to organ failure and death.

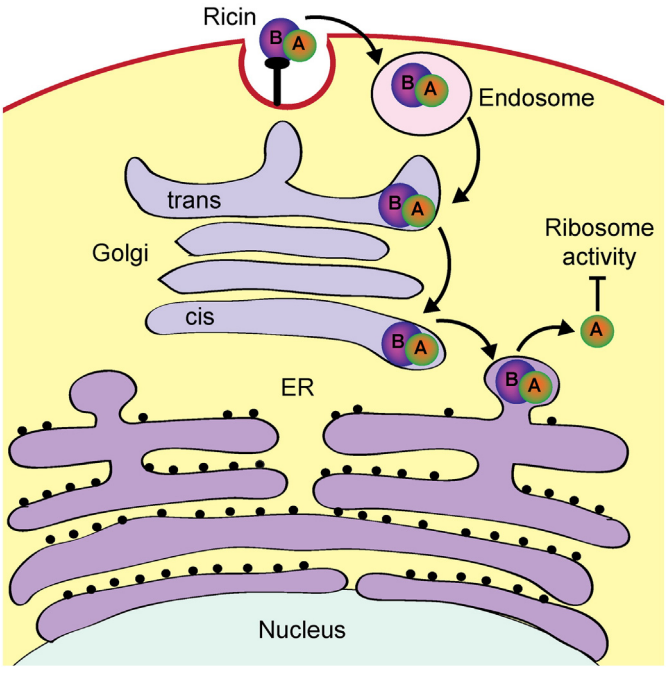

Fig. 1. Intracellular uptake of ricin and ribosome inactivation. Ricin can be internalized through clathrin-dependent and clathrin-independent endocytosis; once inside the cell ricin proceeds from the early endosome into the *trans*-Golgi network. Ricin then progresses from the Golgi into the endoplasmic reticulum (ER) through retrograde trafficking; its catalytic A-chain is released into the cytosol where it inactivates the ribosome.

This review summarizes current approaches for the detection of ricin that can aid in the development of countermeasures against a ricin biothreat. We divide these detection methods based on their ability to distinguish between biologically active and inactive ricin toxin. Based on our review, we provide a prospective on a future well-tailored ricin detection method.

Ricin detection methods

Many different approaches towards developing simple, reliable, and sensitive methods for ricin detection have been investigated. We describe and differentiate two distinct classes of ricin detection methods; those that detect biologically active ricin and those that do not (see Tables 2 and 3 and Fig. 2). It is critical for a ricin detection method to be capable of distinguishing between active and inactive ricin for several reasons. During the initial response to a suspected ricin exposure, information regarding the level of ricin bioactivity will greatly influence the emergency response plan necessary to protect public health, especially in the case of contaminated foods or food production facilities. In response to an intentional attack using ricin, assays that can detect biologically active ricin will be needed to aid in site decontamination and sample disposal by clarifying whether the toxic activity has been destroyed. Additionally, the availability of biological assays can facilitate the development of ricin-related therapeutic and medical countermeasure products. In this regard, appropriate biological assays for ricin are necessary to evaluate product quality and manufacturing consistency. A myriad of ricin containing therapeutics have been developed where the ricin toxin can be selectively delivered to diseases such as cancer, HIV, and graft versus host disease (GVHD) (Shapira and Benhar, 2010).

Methods that cannot identify biologically active ricin

Ricin detection methods have been developed that exploit the intrinsic physical and biochemical properties associated with the toxin, such as molecular weight, ionic charge, antigen epitopes, and genomic

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