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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.

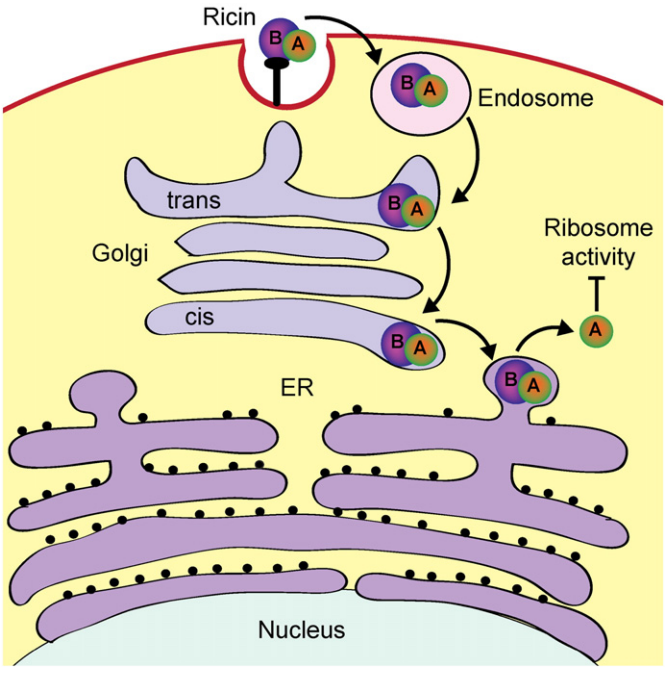

Fig. 1. Intracellular uptake of ricin and ribosome inactivation. Ricin can be internalized through clathrin-dependent and clathrin-independent endocytosis; once inside the cell ricin proceeds from the early endosome into the *trans*-Golgi network. Ricin then progresses from the Golgi into the endoplasmic 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

Table 2
Notable ricin detection methods that cannot detect biological activity.

Ricin detection method	Ricin enrichment method	Sample matrix	LOD	Time	Reference
Sandwich-type ELISA	Antibody immobilized to silicone rods	Buffer and body fluids	10–100 fmol	10 h	Koja, 1980
Colorimetric and chemiluminescence ELISA	Antibody conjugated to microtiter plate	Buffer and body fluids	0.1–0.5 ng/ml	3–24 h	Poli, 1994
Fiber optic-based biosensor	Antibody conjugated to optical fiber	Buffer and river water	0.1–1 ng/ml	20 min	Narang, 1997
Colloidal immunochromatographic assay	Antibody conjugated nitrocellulose membrane	Buffer	0.1–50 ng/ml	10 min	Shyu, 2002b
Inca bioanalytical microarray platform	Antibody conjugated to IncaSlide	Buffer and milk	0.5–1 ng/ml	90 min	Weingart, 2012
xMAP microspheres immunoassay	Antibody conjugated to xMAP microspheres	Buffer and milk	0.01–0.03 ng/ml	2.5 h	Simonova, 2012
DNA aptamer and Raman scattering technique	Aptamer conjugated to magnetic particles	Buffer and beverage	25 ng/ml	Not reported	Lamont, 2011
SPR	Antibody conjugated to SPR chip	Buffer and environmental samples	0.1 ng/ml	15 min	Tran, 2008
Hand-held SPR	Antibody conjugated to SPR chip	Buffer	200 ng/ml	10 min	Feltis, 2008
Localized SPR	β-lactoside-coated Au nanoparticle SPR chip	Buffer	30 ng/ml	7.5 min	Nagatsuka, 2013
SPR	Glycans conjugated to SPR chip	Buffer	10 pg/ml	5 min	Uzawa, 2008
SPR	sdAb conjugated to SPR chip	Buffer	0.7 ng/ml	2–6 min	Anderson, 2013
sdAb-QD fluoroimmunoassay	sdAb conjugated to 96-well plate	Buffer	1 ng/ml	Not reported	Anderson, 2013
Microring resonator array	sdAb conjugated to microring resonator array	Buffer	300 pM	15 min	Shia, 2013
Lectin pull-down	Galactose-magnetic iron-oxide nanoparticles	Buffer and serum	2–4 ng/ml	3 h	Liu, 2011
Sandwich-type glyco immunoassay	Lactose chip	Buffer	80 ng/ml	20 min	Huebner, 2013
Immuno-PCR	Antibody conjugated to microtitration plate	Ground beef, milk, and egg	0.01–0.1 ng/ml	Not reported	He, 2010
Nano LC–MS	Lactose-immobilized monolithic spin column	High protein solution	8 ng/ml	5 h	Kanamori-Kataoka, 2011
Immunocapture and MALDI-TOF/MS	Antibody conjugated to magnetic particles	Buffer and milk	50 ng/ml	6 h	Duriez, 2008

fingerprints. Of these, ricin immuno-based detection methods, such as enzyme-linked immunosorbent assays (ELISA) and handheld lateral flow immunochromatographic devices (LFID) (Dayan-Kenigsberg et al., 2008; Fulton and Thompson, 2007), are heavily represented in the literature and have advantages in specificity, simplicity, and analysis time. Surprisingly, some of the earliest ELISA methods developed using colorimetric and chemi-luminescence detection are still competitive in terms of detection limits (Koja et al., 1980; Poli et al., 1994; Shyu et al., 2002a). The use of fluorescence-based fiber optics (Narang et al., 1997), colloidal gold particles (Shyu et al., 2002b), and electrochemiluminescence (Garber and O'Brien, 2008), has significantly improved assay times without sacrificing the sensitivity associated with the classical ELISAs. Variations in the solid phase surface of the immunoassay have been also investigated by using magnetic microspheres (Yu et al., 2000) and gold-coated magnetoelastic sensor surfaces (Ruan et al., 2004; Shankar et al., 2005). The use of microspheres generated an increase in collective surface area, providing improvements in sensitivity and assay time, while a magnetoelastic sensor surface also helped to reduce total assay time. The use of microarrays, which include panels of antibodies for simultaneous detection of a variety of antigenic targets of interest, allowed multiplexed detection of ricin in parallel with other harmful toxic agents, such as cholera toxin, staphylococcal enterotoxins A and B, *Bacillus globigii*, botulinum toxin A, *Yersinia pestis*, and heat labile toxin of *Escherichia coli*, and provided dramatic improvements in assay utility and flexibility (Delehanty and Ligler, 2002; Garber et al., 2010; Simonova et al., 2012; Wadkins et al., 1998; Weingart et al., 2012). In other works, the toxin capture antibodies used as receptors were substituted by DNA/RNA aptamers (Haes et al., 2006; Kirby et al., 2004; Lamont et al., 2011), single domain antibodies (Anderson et al., 2013; Shia and Bailey, 2013; Stine et al., 2005), and sugar-conjugated materials (Huebner et al., 2013; Liu et al., 2011). By bypassing traditional antibodies, improvements can be made in regard to reagent stability and storage life. Finally, by combining sample enrichment steps (using ricin-specific antibodies, aptamers, or sugar-conjugated materials) with enhanced detection technologies, such as surface plasmon resonance (SPR) (Blome et al., 2010; Feltis et al., 2008; Nagatsuka et al., 2013; Tran et al., 2008; Uzawa et al., 2008), polymerase chain reaction (PCR) (He et al., 2010; Lubelli et al., 2006), or mass spectrometry (MS) (Duriez et al., 2008; Kanamori-Kataoka et al., 2011), an added level of specificity and sensitivity can be achieved. Despite the many advantages associated with immunochemical methods for ricin detection, a major disadvantage exists in the inability to distinguish between biologically active and inactive ricin. The inability to identify biological activity prevents these detection methods from assessing the actual toxic potential of a bioweapon threat.

Methods that can identify biologically active ricin

An effective method for ricin detection should address whether a sample contains biologically active ricin capable of exerting a toxic

Table 3
Notable ricin detection methods that can detect biological activity.

Ricin detection method	Ricin enrichment method	Sample matrix	LOD	Time	Reference
[^{3}H]-adenine release from DNA	N/A	Buffer	1.4–14 μg/ml	40 min	Brigotti, 1998
Luminescent adenine release assay	N/A	Buffer	1.6–200 ng/ml	30 s–20 min	Sturm, 2009
[^{3}H]-borohydride labeling	N/A	Buffer	400 ng/ml	15 min	Ling, 1994
qPCR rRNA depurination assay	N/A	Buffer, detergents, and juice	7 ng/ml	3 h	Melchior, 2010
Immunoaffinity and LC–MS adenine release assay	Antibody conjugated to magnetic beads	Water and milk	0.1–0.3 ng/ml	4–24 h	Becher, 2007
Isotope dilution MS and deadenylase activity assay	Antibody conjugated to magnetic beads	Water, milk, and juice	0.6 ng/ml	4–6 h	McGrath, 2011
Serum peptide profiling by MALDI-TOF/MS	Magnetic beads	Serum	1 μg/ml	Not reported	Zhao, 2012
Cellular GFP cytotoxicity assay	N/A	Medium	1 ng/ml	6 h	Halter, 2009
Cellular GFP cytotoxicity assay	N/A	Medium, juice, and food paste	0.2 ng/ml	72 h	Rasooly, 2012
[a]SensoLyte AMC Caspase 3/7 assay	N/A	Buffer	0.1 ng/ml	6 h	Jetzt, 2009
Cellular impedance cytotoxicity assay	N/A	Medium, milk, juice, baby food	0.2–14 ng/ml	3.5–60 h	Pauly, 2012
[a]High-throughput cytotoxicity assay	N/A	Medium	1 nM	27 h	Stechmann, 2010
[a]High-throughput cytotoxicity assay	N/A	Medium	5 ng/ml	48 h	Wahome, 2010
Mouse bioassay	N/A	Buffer	<7.5 μg/ml	24 h	Zhan, 2003

[a] Denotes that the assay did not determine a limit of detection and the LOD value listed is only the reported concentration value used in the assay.

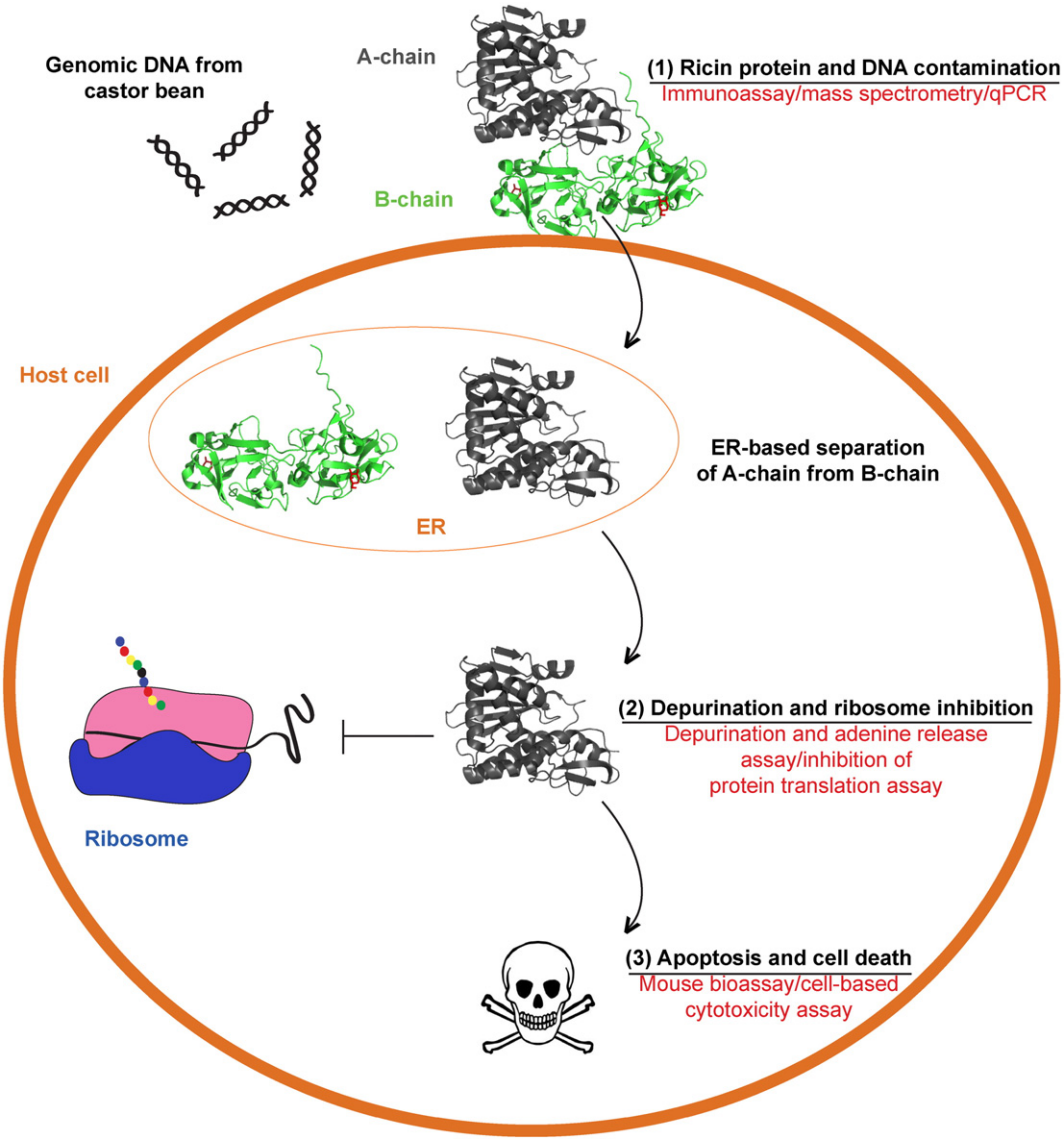

Fig. 2. Ricin detection methods in relation to biological steps that occur during ricin intoxication. (1) Ricin protein and DNA contamination present in a sample can be readily detected by immuno-based assays, mass spectrometry, and qPCR techniques. (2) Depurination and adenine release assay detect ricin enzymatic products. Assays that monitor inhibition of protein translation can detect ribosome inactivation by ricin. (3) Mouse bioassays and cell-based cytotoxicity assays can detect ricin triggered cell death. Ricin X-ray structures (PDB # 3RTI) were generated using PyMol software.

effect. Because B-chain glycan binding is necessary for cell penetration and A-chain enzymatic activity is required for ribosome inactivation, ricin is highly toxic only if: (1) both A- and B-chains are present, (2) the B-chain retains its glycan-binding ability, and (3) the A-chain is catalytically active.

Ricin A-chain (RTA) enzyme activity can be measured using in vitro cell-free assay methods that detect the immediate products of the RTA-catalyzed reaction, namely the release of free adenine or the formation of depurinated reaction products from ribosomal RNA or other RIP substrate molecules. Alternatively, the RTA-dependent inhibition of protein synthesis from poly-U RNA or with specific mRNA transcripts can be monitored in vitro using fluorography (Harley and Beevers, 1982; May et al., 1989; Olsnes et al., 1975) or with coupled in vitro transcription/translation systems with detection of luciferase activity or green fluorescent protein reporter gene expression (Mei et al., 2006).

Ricin can be formally classified as a ribosomal RNA *N*-glycosidase [EC 3.2.2.22] because its cytotoxic activity is related to its ability to depurinate a single adenosine located in the SRL of 28S rRNA. However, ricin can also be described as polynucleotide:adenosine *N*-glycosidase because ricin catalyzes depurination reactions with alternative adenosine-containing RNA and DNA substrates. Thus, monitoring the release of free adenine provides a convenient means for assessing the catalytic activity of ricin.

The RTA-catalyzed release of unlabeled free adenine from intact ribosomes or from natural and synthetic DNA and RNA substrates has been quantified using reverse phase HPLC with UV (Chen et al., 1998) or MS detection (Hines et al., 2004) or with pre-column derivatization using chloroacetaldehyde and fluorescent detection (Zamboni et al., 1989). Although conversion of free adenine to the fluorescent derivative 1,N(6)-ethenoadenine using chloroaldehyde involves an additional step, it provides a significant increase in sensitivity compared to UV detection and the ethenoadenine product exhibits improved retention and chromatographic resolution from other reaction components.

The RTA-catalyzed release of [^{3}H]-adenine from labeled pBR322 plasmid DNA fragments has been quantified using liquid scintillation counting (Brigotti et al., 1998). RTA-dependent adenine release from natural and synthetic polynucleotide RIP substrates has also been detected using coupled enzyme reactions monitored colorimetrically (Heisler et al., 2002) or with chemiluminescence (Brigotti et al., 1998; Sturm and Schramm, 2009). Many RTA activity assay methods utilize

intact ribosomes because the highest reaction rates are observed with this type of substrate. In 1987, Endo and Tsurugi (1987) reported that the enzyme catalytic constant for RTA-catalyzed reactions with rat liver ribosomes (k_{cat} 1777 min^{-1}) was much greater than the catalytic constant measured for reactions with purified rat liver 28S rRNA (k_{cat} 0.02 min^{-1}). In some cases, however, RTA assay methods using alternative DNA or RNA substrates exhibit offsetting enhancements in sensitivity gained by the use of radiochemical or chemiluminescent detection methods and by coupling the RTA-dependent reaction with additional enzyme-catalyzed reactions which can be measured with greater sensitivity. Moreover, because multiple adenines may be released from a variety of sites within larger polynucleotide substrates such as total RNA, herring sperm DNA, or pBR322 fragments, assay designs that measure the more general polynucleotide:adenosine *N*-glycosidase activity of RTA may exhibit an additional gain in sensitivity when compared to assay designs that monitor only the single adenine released from the 28S rRNA SRL GAGA target sequence. For this reason, adenine release assays for RTA that monitor its general polynucleotide:adenosine *N*-glycosidase activity in acidic buffers (e.g., pH 4–5) take advantage of the increased reaction rates and relaxed substrate specificity observed under these conditions. Furthermore, it has been shown that some synthetic substrates can be depurinated efficiently by ricin. Notably, Sturm and Schramm (2009) utilized a coupled multi-enzyme/luciferase-based assay system to measure adenine release from intact yeast 60S ribosomes (k_{cat} 460 min^{-1}), rabbit 80S ribosomes (k_{cat} 2310 min^{-1}), and synthetic stem–loop oligonucleotide substrates. They found that a 14-base a synthetic oligonucleotide substrate designated A14 2-dA exhibited reaction kinetics (k_{cat} 1110 min^{-1}) that were intermediate between those of the two types of intact ribosomes they tested. Higher substrate concentrations are typically used to overcome the higher K_m values associated with most synthetic stem–loop oligonucleotide substrates for detecting RTA activity. But this minor disadvantage is balanced by the benefits of low cost, simplicity of use, and ease of storage associated with oligonucleotide substrates for RTA.

Depurinated RNA or DNA reaction products generated by ricin enzyme activity have been detected using agarose gel electrophoresis (Endo and Tsurugi, 1988), radiolabeling with [^{3}-H]-borohydride (Ling et al., 1994), [^{32}P]-labeled primer extension (May et al., 1989), quantitative real-time PCR (Melchior and Tolleson, 2010), hybridization to a cleavage-sensitive oligonucleotide with electrochemiluminescent

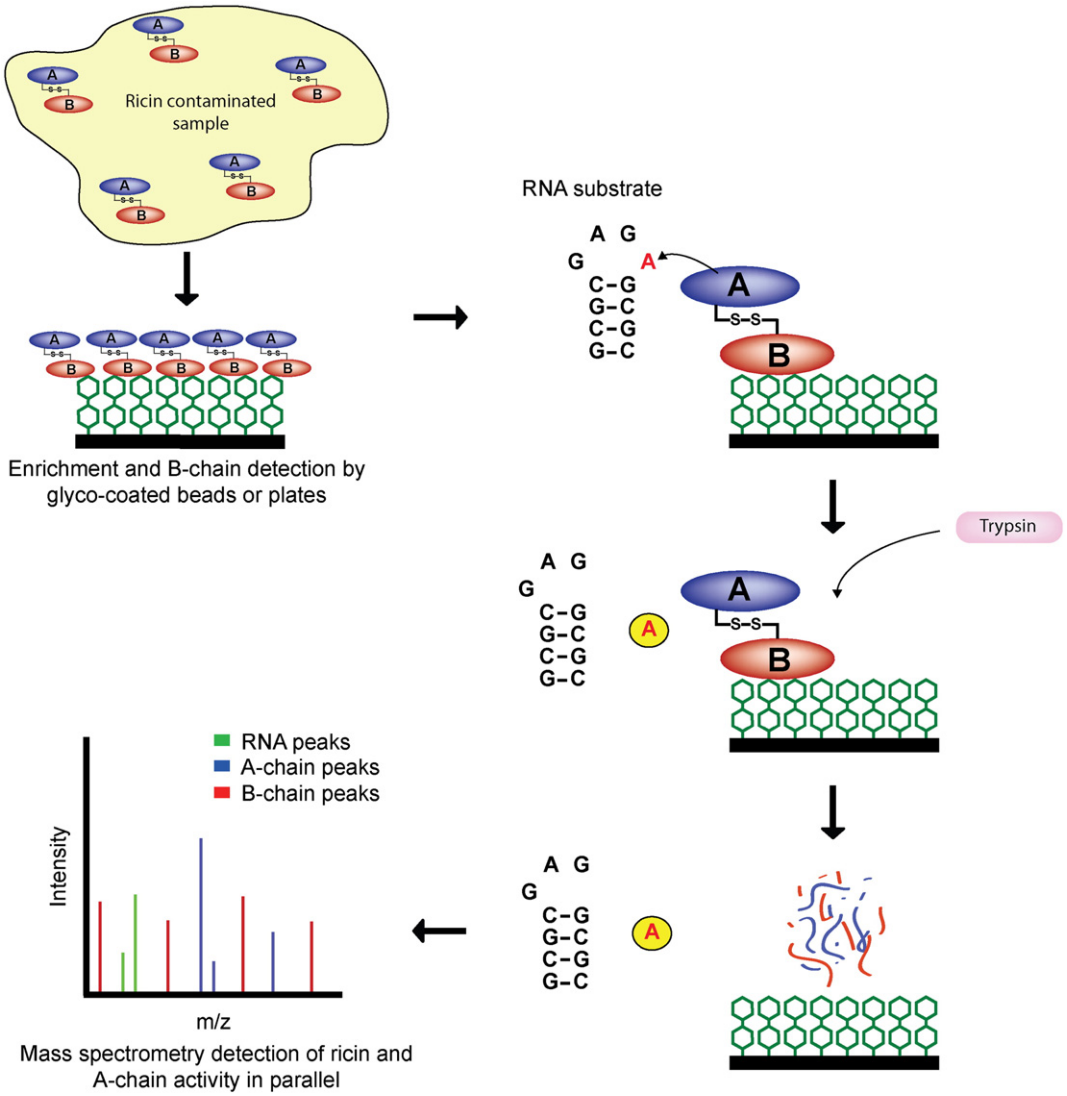

Fig. 3. Proposed future ricin detection method. Ricin can be enriched from a contaminated sample using glyco-coated beads or plates, based on the lectin binding properties of ricin B-chain. Enriched ricin can then be incubated with a 12-mer RNA substrate that mimics the GAGA sequence in the α-sarcin/ricin loop (SRL) of 28S rRNA. A sample containing active ricin A-chain will deadenylate the substrate and release free adenine into the solution. After-trypsin mediated protein digestion, mass spectrometry analysis of the sample will allow for a paralleled identification of ricin peptide fingerprints and deadenylated RNA substrate. Together this methodology allows for detection of fully functional and active ricin in a simplified experimental setting.

detection (Cho et al., 2009), and LC–MS (Bevilacqua et al., 2010; Kalb and Barr, 2009; McGrath et al., 2011). Notably, by complementing ricin-specific sample enrichment steps with RTA enzyme activity assays, ricin could be selectively identified while confirming A-chain activity in parallel (Becher et al., 2007; Bevilacqua et al., 2010; He et al., 2010; May et al., 1989), allowing distinction from nontoxic monomeric type 1 RIP toxins. In addition to anti-ricin antibodies, ricin-specific RNA aptamers (Haes et al., 2006; Kirby et al., 2004; Lamont et al., 2011) and carbohydrate compounds (Huebner et al., 2013; Liu et al., 2011) have also been evaluated for recovering ricin from suspect contaminated samples (Blome and Schengrund, 2008; Stine et al., 2005; Uzawa et al., 2008). Carbohydrate ligands selectively bind to the ricin B-chain. Therefore, ricin sample enrichment steps that target its B-chain also aid in discriminating between heterodimeric type 2 RIP toxins and monomeric type 1 RIP toxins, because the latter only possess the A-chain activity and lack the cell-binding activity provided by type 2 RIP toxin B-chains.

Animal or cell based-toxicity assays can confirm functional B-chain and active A-chain by assessing animal survival time and cell death. Specifically, mouse bioassays have been used for ricin detection either by monitoring survival time (Fodstad et al., 1976; Zhan and Zhou, 2003) or by serum peptide profiling (Zhao et al., 2012). Using in vitro cell-based assay systems, either inhibition of protein synthesis (Halter et al., 2009; Rasooly and He, 2012; Saenz et al., 2007; Stechmann et al., 2010) or cell viability (Jetzt et al., 2009; Oda et al., 1997; Pauly et al., 2012; Wahome et al., 2010) can be monitored in order to detect biologically active ricin. One disadvantage of these assays is selectivity, because other harmful toxins may elicit a similar cytotoxic effect.

Conclusions and perspectives on future ricin detection methods

Although many of the ricin detection methods discussed in this review provide for robust, sensitive, and quantitative ways to detect ricin, detection assays that can distinguish between biologically active and inactive ricin are essential for evaluating the lethality of a bioterrorism threat and also for monitoring site decontamination procedures. However, many of the biological assays discussed here that can detect toxicity and A-chain activity have limitations in selectivity and cannot distinguish ricin from other harmful toxins. For these reasons, it is necessary to utilize an integrated approach in the development of an ideal ricin detection method. The optimal assay design would have a rapid and efficient enrichment step, an A-chain activity checkpoint, and a selectivity step that can distinguish ricin from other bioactive toxins. Indeed, streamlined assays have been developed for ricin, where ricin is enriched from samples through the use of ricin specific antibodies (Kalb and Barr, 2009; McGrath et al., 2011). However, this format is unable to confirm B-chain lectin functionality and, therefore cannot guarantee the sequestered ricin is capable of penetrating a host cell membrane and exert toxicity. A viable approach for sample enrichment would be the use of sugar-conjugated materials that exploit the lectin binding properties of the ricin B-chain. Several glycosphingolipids and synthetic sugars that bind to ricin with high affinity have been identified (Blome and Schengrund, 2008; Stine et al., 2005; Uzawa et al., 2008). After sample enrichment, development of mass spectrometry methods that could simultaneously identify ricin peptide fingerprints and in vitro RNA substrates that have been deadenylated by active ricin A-chain would allow for quick and sensitive detection of biological active ricin. Together, in a few very efficient steps, this assay could detect functional B-chain necessary for cell penetration, confirm A-chain activity required to inactivate the ribosome, and selectively identify ricin as the harmful toxin (Fig. 3). Additionally, a robust cell-based assay that can detect ricin cytotoxicity can be used as a confirmatory test. Ideally, this cellular cytotoxicity can be detected using sensitive methods such as fluorescence or luminescence based molecular probes.

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