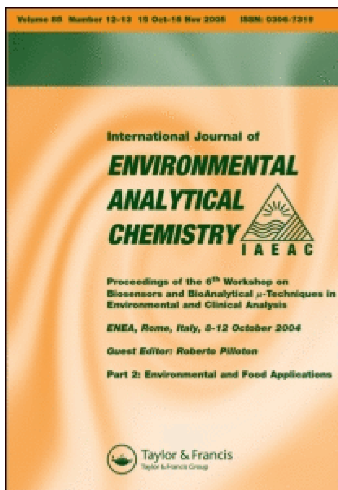
This article was downloaded by:
On: *17 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Detection of ricin toxin in water using immunoassays

Junqi Yue[a]; Lifeng Zhang[a]; Zhaoguang Yang[a]
[a] Centre for Advanced Water Technology, Public Utilities Board, 608575 Singapore

Online publication date: 22 September 2010

To cite this Article Yue, Junqi , Zhang, Lifeng and Yang, Zhaoguang(2009) 'Detection of ricin toxin in water using immunoassays', International Journal of Environmental Analytical Chemistry, 89: 8, 821 — 833
To link to this Article: DOI: 10.1080/03067310903029822
URL: http://dx.doi.org/10.1080/03067310903029822

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

Intern. J. Environ. Anal. Chem.
Vol. 89, Nos. 8–12, 15 July–15 October 2009, 821–833

Taylor & Francis
Taylor & Francis Group

Downloaded At: 13:41 17 January 2011

Detection of ricin toxin in water using immunoassays

Junqi Yue*, Lifeng Zhang and Zhaoguang Yang

Centre for Advanced Water Technology, Public Utilities Board, 80/82 Toh Guan Road East, #C4-03, 608575 Singapore

(*Received 31 July 2008; final version received 8 May 2009*)

Two types of immunoassays were employed to determine ricin toxin through the substitute of A chain sub-unit in water. Protocol for detecting ricin by commercial enzyme-linked immunosorbent assay (ELISA) kits was established and evaluated. Final results were obtained by this assay in six hours and the detection limit was found to be less than $3\,ng\,mL^{-1}$ based on ricin A chain. Cross-reactivity from ricin B chain was minimal. The validated assay was successfully applied to the determination of ricin toxin in water for the purpose of water security. The results showed that water samples were free from this biotoxin. In order to reduce the assay time and enhance the ability of securing water quality, a fibre optic biosensor RAPTOR^{TM} has been applied as another platform to rapidly analyse ricin toxin in water. Polyclonal anti-ricin antibody was captured on the waveguide through incubation. Four waveguides were assembled into each coupon, which could provide multiplex analysis in one assay run. Cy5-labelled antibody was used as detector antibody and characterised by MALDI-TOF mass spectrometry. Results showed that the detection limit was 10 and $60\,ng\,mL^{-1}$ of ricin A chain in deionised water and tap water, respectively. Based on readily assembled coupon, the assay run could be completed and the results reported in less than 15 minutes. Thus, the biosensor has been shown to be a promising technology for rapid and sensitive detection of biotoxins in water. In the future, more research work will be carried out to further improve the sensitivity and enhance reproducibility of the assay for the detection of ricin toxin.

Keywords: ricin toxin; ricin A chain; immunoassay; ELISA; biosensor; antibody; MALDI-TOF mass spectrometry

1. Introduction

Ricin is a toxic protein derived from the seeds of castor bean plant. It consists of two polypeptide chains (chain A and chain B) bridged through a disulfide bond. Ricin A chain expresses toxicity by inhibiting protein synthesis, with the facilitation of B chain which is the pure agglutinin. Oral LD_{50} of ricin for human was estimated to be more than $1\,mg\,kg^{-1}$ of body weight [1]. Although the lethal toxicity of ricin is approximately 1000-fold less than botulinum toxin [2], the worldwide ready availability of castor beans and the ease with which toxin can be produced give it significant potential as a biological weapon. Ricin is listed as schedule I controlled substance. Since September 11, five ricin incidents have been reported in the USA and the UK, some of which were suspected of being a terrorist attack.

*Corresponding author. Email: yue_junqi@pub.gov.sg

ISSN 0306–7319 print/ISSN 1029–0397 online
© 2009 Taylor & Francis
DOI: 10.1080/03067310903029822
http://www.informaworld.com

Ricin can be delivered to the target site in the form of powder, mist or a pellet. It can be dissolved in water, which renders the aqueous system a potentially vulnerable medium in terms of a terrorist using a biothreat agent such as ricin. To disarm the potential biothreat attack on site instantly so as to secure water quality and public health, it becomes critical to develop a rapid and sensitive method to detect ricin in water.

One of the earliest methods for the detection of ricin was radioimmunoassay (RIA) [3,4]. The major drawbacks were its lengthy incubation time, the problems of handling and disposing of radioisotopes. Enzyme-linked immunosorbent assay (ELISA) is a widely accepted method for the detection of specific proteins [5–8]. However, the assay time is still too long (>6 hours) to meet the requirement of rapidly diagnosing a potential terrorist attack involving a biothreat agent. To bring about fast detection of ricin and other biothreat agents, different immunoassay platforms based on ELISA were investigated, such as planar array immunosensor equipped with a charge-coupled device (CCD) [9,10], flow-based microarray platform [11] and fluorescence-based optic fibre biosensor [12–17], among which similar sensitivities were achieved.

A commercial available fluorescence-based optic fibre immunosensor, RAPTOR™ (Research International, Monroe, WA), is claimed to be designed for withstanding the rigours of field-testing [18]. It provides a platform for rapid detection of various types of pathogens [15,19,20] and biotoxins [15,21]. In principle, this biosensor employs the format of sandwich immunoassay, with the capture antibody being immobilised on the surface of optical fibre and fluorescent dye labelled antibody as the detector antibody. The injection-molded polystyrene fibres are assembled in coupon with four fluidic channels, one fibre in each channel, thus allowing for measurement of replicates of the same analyte or simultaneous detection of multiple different analytes. The geometry of the optical fibre or waveguide is designed in such a way that total internal reflection (TIR) occurs while light from a diode laser is travelling through it [22]. The resultant evanescent wave (EW) due to TIR decays exponentially with distance and primarily excites fluorophores bound near the surface of the waveguide. The emitted fluorescent signal is detected and related to the concentration of analytes.

Downloaded At: 13:41 17 January 2011

While intact ricin is highly toxic, the sub-unit A chain alone is incapable of entering the cell and is thus not poisonous [4,23]. It should be interesting to note whether the ELISA kits for intact ricin can also be utilised to detect non-toxic sub-unit ricin A chain or B chain. In this report, the commercial ELISA kits (Tetracore, Rockville, MD) for the analysis of ricin toxin were evaluated and found to be able to detect ricin A chain in buffer with similar sensitivity to intact ricin, but not B chain. Thus, ricin A chain acted as a substitute for the detection of intact ricin by ELISA kits in a safer manner. Using the antibody from the same source as ELISA kits, a rapid and sensitive method based on the optic fibre biosensor RAPTOR™ was developed to detect ricin toxin through its substitute ricin A chain in water. Although the previous model of the biosensor Analyte 2000™ from the same manufacturer was used to analyse ricin in water [24], the current model has achieved a certain improvement in fluidic control and optics design. Polystyrene optical fibre replaces silica fibre in the current model. Therefore, it is necessary to conduct a thorough study on the detection of ricin toxin in water based on this new system. We systematically investigated factors affecting the performance of the biosensor. The two immunoassays, namely ELISA kits and biosensor, use the same source of capture polyclonal antibody so that the results obtained by these two assays were subjected to being fairly compared. We have employed a novel and accurate method using MALDI-TOF mass spectrometry to characterise the labelled antibody.

2. Experimental

2.1 *ELISA kits method*

2.1.1 *ELISA kits, ricin toxin and buffer*

Both ricin A chain and B chain were purchased from Sigma (St. Louis, MO, USA). ELISA kits for detection of ricin were obtained from Tetracore (Rockville, MD, USA). The microplates were pre-coated with positive and negative capture antibodies (both with $10\,\mu g\,mL^{-1}$ in PBS) in alternating rows. The assay was conducted as recommended by the manufacturer. Blocking/dilution buffer: PBS (10 mM Phosphate, 0.15 M NaCl), 0.1% (v/v) Tween-20, 5% (w/w) dry skim milk, pH 7.4. Wash buffer (PBST): PBS, 0.1% (v/v) Tween-20. Pre-coated positive capture antibody: goat polyclonal anti-ricin antibody; pre-coated negative capture antibody: normal goat IgG; detector antibody: mouse anti-ricin monoclonal, $2.0\,mg\,mL^{-1}$; conjugate antibody: goat anti-mouse IgG-HRP), $0.4\,mg\,mL^{-1}$; ABTS 2-part peroxidase substrate (component A and B). Microplate reader: SPECTRAmax 340 PC 384 (Molecular Devices, Sunnydale, CA, USA).

2.1.2 *ELISA protocol*

Detailed ELISA protocol was provided by the supplier. In general, all incubation proceeded at 37°C and the microplate was washed four times with PBST after each incubation step. First, pre-coated microplate was blocked by adding $150\,\mu L$ of blocking buffer to each well for 1 h. Subsequently, along with drinking water samples, sample spiked with ricin A chain or B chain and dilution buffer as negative control, a series of ricin A chain and B chain standards diluted in blocking/dilution buffer were added to the microplate, being allowed to incubate for another 1 h. Afterwards, detector antibody at $10\,\mu g\,mL^{-1}$ was incubated for 1 h, followed by incubation of conjugate antibody at $0.08\,\mu g\,mL^{-1}$ for another 1 h. Finally, ABTS substrate was introduced to the wells of microplate to allow for colour development. Absorbance was read at 405 nm every 10 min during 90 min of incubation.

Downloaded At: 13:41 17 January 2011

2.1.3 *Water samples*

The ELISA kits have been used for the detection of ricin toxin (through substitute ricin A chain) in tap water or surface water samples. All samples were collected in amber bottles and kept at 4°C prior to analysis. Determination of water samples spiked with ricin A chain supported validity of the assay.

2.2 *Immunoassay based on fibre optic biosensor*

2.2.1 *Antibody, buffer, reagents and biosensor consumables*

Polyclonal goat anti-ricin IgG ($5.3\,mg\,mL^{-1}$ in PBS with 0.1% NaN_3, pH 7.4) was obtained from Tetracore. Blocker™ Casein in PBS (1% w/v, pH 7.4) was purchased from Pierce (Rockford, IL, USA). Bovine Serum Albumin (BSA) was purchased from Sigma (St. Louis, MO, USA). Coating buffer comprises 0.1 M sodium carbonate at pH 9.6. Blocking buffer consists of $1\,mg\,mL^{-1}$ Casein and $1\,mg\,mL^{-1}$ BSA in PBS with 0.1% Triton X-100. Wash buffer is composed of 10 mM phosphate at pH 7.2, 0.05% (v/v) Triton X-100 and 0.01% (w/v) sodium azide. Cy5 labelling kits were purchased from GE Healthcare (Little Chalfont, Buckinghamshire, UK). Micro Bio-Spin P-30 Tris

chromatography column was obtained from Bio-Rad (Hercules, CA, USA). The biosensor consumables, including polystyrene optic fibres, coupon card, covering tapes and barcode labels, a batch of pre-assembled coupons for the detection of ricin along with labelled reagents, were all supplied by Research International (Monroe, WA, USA).

2.2.2 *Immobilisation of antibody on fibres and coupon assembly*

Capture antibody was immobilised onto fibre optic probes by passive adsorption. Injection-moulded, polystyrene fibre optic probes were pre-cleaned with 1 : 1 isopropanol/water and air-dried. Then, the distal ends of fibres were blackened using a fine marker (Zebra™, waterproof) to prevent reflection of excitation light. Probes then were inserted into 20 µL Eppendorf tips (0.5–20 µL) pre-filled with 100 µL of anti-ricin antibody solution in 0.1 M carbonate buffer (pH 9.6) and sealed with Critoseal® tube sealant (Fisher Scientific) at the tips. For negative control probes, only carbonate buffer was used for incubating. After overnight incubation at 4°C, unbound antibody was rinsed off the probes with distilled water three times. The probes were mounted into the channels of coupon through the holes in the edge and UV cured glue NOA68® (Norland Products, Cranbury, NJ, USA) was applied at each corner of the probe flanges inside the coupon slot. The adhesive was cured under UV lamp for 45 minutes. The cover tape was applied to seal the coupon channels on the surface. The coupon was then coded by blackening a certain combination of squares of the label pasted on the back of the coupon, which corresponds to a specific assay recipe preprogrammed and loaded into the RAPTOR™.

Downloaded At: 13:41 17 January 2011

2.2.3 *Preparation of fluorescent-labelled antibody*

Two scales of labelling reaction, 1 mg and 50 µg of antibody, were carried out. The antibody labelling kit was used for labelling 1 mg of polyclonal antibody (same as capture antibody) according to the manufacturer's instruction. Briefly, 200 µL of antibody (1 mg) mixed with coupling buffer (sodium carbonate, pH 9.6) was added to one vial of Cy5 bisfunctional dye. The reaction proceeded in the dark for 30 minutes with additional mixing every 10 minutes. Subsequently, Cy5-labelled antibody was separated from non-conjugated dye by gel filtration column (Bio-Gel P-10 column, Bio-Rad) contained in the kit.

One vial of dye was dissolved in 5 mL of acetone. Aliquots of 100 µL, 200 µL or 300 µL were transferred into individual 2 mL centrifuge tubes, dried under nitrogen and stored at –20°C. Aliquots in 10 µL (50 µg) of antibody were also stored in centrifuge tube at –20C. Prior to labelling, antibody and dye were taken out from the freezer and allowed to equilibrate to room temperature. 50 µg of antibody was diluted by adding 40 µL of PBS into the tube, and 5 µL of coupling buffer supplied in the labelling kit was added to the antibody solution (pH 9.6). Then the mixture was transferred to different aliquots (100 µL, 200 µL and 300 µL) of dye previously stored. Reactions proceeded for 30 minutes in the dark with intermittent mixing. The labelled antibody was separated from free dye on micro Bio-Spin P-30 Tris column by centrifugation after buffer being exchanged to PBS. The separated labelled antibody had a volume of 100–150 µL and was diluted to 0.5, 1 or 1.5 mL to achieve different concentrations.

2.2.4 *Characterisation of labelled antibody by UV-vis spectroscopy and MALDI-TOF spectrometry*

UV-vis measurement was accomplished on HP 8453 UV-vis spectrometer (Agilent Technologies, CA) with standard cell. Sample concentration was adjusted so that the absorbance at 650 nm fell between 0.5 and 0.7 unit. Dye-to-protein molar ratio was calculated based on absorbance at 280 and 650 nm as described by the manufacturer.

MS spectra were obtained on a MALDI-TOF mass spectrometer (Waters Micromass MALDI Micro MX, Manchester, UK). Matrix was sinapinic acid with a concentration of 10 mg mL^{-1} in 4 : 6 acetonitrile : 0.1%TFA (aq). Untagged pure antibody and purified labelled antibody through micro Bio-Spin column were diluted in 0.1% TFA (aq) to achieve the concentration of 0.1 to 1 pmol μL^{-1}. Salt was eliminated by eluting Bio-Spin column with deionised water instead of PBS buffer. The diluted sample was then pre-mixed with matrix in 1 : 3 ratio and 1 μL of this mixture was applied on each designated well, air dry prior to analysis. The TOF analyser was operated in linear mode. Mass scan range was from 10,000 to 200,000 Da. Data collection and processing was controlled by MassLynx software (Waters, MA, USA).

2.2.5 *Assay procedure and data processing*

Downloaded At: 13:41 17 January 2011

As-prepared coupon was fit into compartment on the RAPTOR^{TM} and was identified through barcode. Four reagent vials containing 1 mL of labelled antibody each were connected to corresponding channels. Pre-programmed recipe defined automatic fluidics control, data collection schedule and data analysis parameter. Standard assay recipe took 10 min to complete, including 7 min of sample incubation and 90 s of binding and interrogation by detector antibody. High sensitivity recipe required 12–15 min of assay time. Deionised water acted as the blank was introduced into the channels to incubate the probes, and initial reading (signal I) was taken. Reagent was then pumped into the channels and the non-specific binding rate was recorded as the background assay rate. After the reagent being returned to the vial, the probes were rinsed with wash buffer, and a final reading (signal II) was taken. The background wash delta was obtained by subtracting signal I from signal II. Similarly, the binding rate and wash delta were obtained for water sample containing analyte. The signal increase or wash delta for the blank is considered the noise, representing non-specific binding. Cut-off value is defined as the noise plus three times of standard deviation. Signal increase for sample containing analyte is the difference between signal II of sample and signal I of blank. If the signal increase is bigger than the cut-off value, the positive result will be reported.

Both mean values of background assay rate and wash delta plus respective three standard deviations could be utilised to set the limit of detection. The two parameters are consistent with each other. In this study, only wash delta was applied to analyse the data.

3. Results and discussion

3.1 *Sensitivity of ELISA kit*

The ELISA kits supplied by Tetracore were intended to detect intact ricin toxin in water samples. In terms of safety, the possibility of applying sub-units of ricin to examine the reliability and validity of the kits was investigated. Standard ricin (both A chain and B chain) solution was prepared in dilution buffer, with the concentration ranging from

0.7 to 710 ng mL^{-1} for ricin A chain and 4.6 to 1165 ng mL^{-1} for ricin B chain. Twelve water samples were tested at the same time. Dynamic reading was collected on the microplate reader. It was found that the colour development nearly achieved completion after 60 min, while the manufacturer recommended reading being taken at 30 min of incubation. The assay results of various concentrations of standard ricin A chain and B chain are shown in Figure 1. The positive cut-off is determined by adding three times the standard deviation to the mean of triplicate negative control (dilution buffer containing no antigen). Based on the result for negative control, the detection limit was found to be less than 3 ng mL^{-1} for the detection of ricin A chain, representing the same level for the detection of intact ricin (data was provided by the manufacturer). On the other hand, the ELISA kit hardly responded to ricin B chain, indicating minimal cross-reactivity of this sub-unit. The responses of all the water samples tested were below the detection limit. The confidence of the results was strengthened by applying alternative +/− capture antibody format along the row of the microplate.

Ricin A chain was spiked in DI water and blocking buffer at two concentrations (9 and 36 ng mL^{-1}) to examine the recovery. From the response curve of standards of ricin A chain (Figure 1), the recovery for the spiked samples was determined to be 69–92% in dilution buffer and 90 –135% in DI water.

Downloaded At: 13:41 17 January 2011

3.2 *Investigation of biosensor*

3.2.1 *Characterisation of labelled antibody by MALDI-TOF mass spectrometry*

Initially 1 mg of polyclonal antibody was labelled with one vial of Cy5 dye supplied in the labelling kit, following the protocol instructed by the manufacturer. Due to the high cost

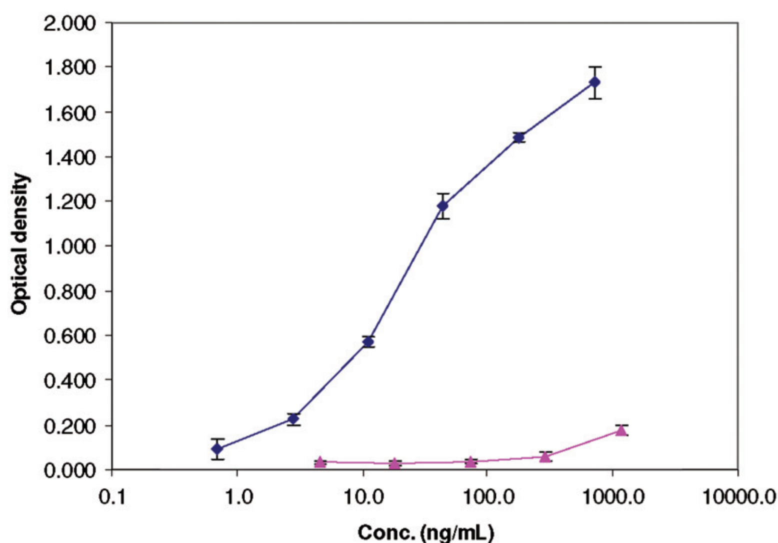


Figure 1. Optical density of standards of ricin A chain (◆) and ricin B chain (▲) in dilution buffer upon 60 min of colour development by ELISA kits protocol. Error bars represent the standard deviation of triplicates at each concentration of diluted standard. The optical density of negative control (not shown) had a mean value of 0.0728 , with the SD of 0.0315 ($n = 3$).

of high-quality antibody, labelling reaction was scaled down to 50 μg of antibody, which greatly facilitated the optimisation of the reaction. In order to improve the sensitivity of the bioassay using the biosensor, optimisation of dye-to-protein (D/P) ratio is critical. Conventionally, D/P ratio was determined by UV spectroscopy based on the absorbance at 280 nm for protein and 650 nm for Cy5 dye. In a typical UV-vis spectrum of Cy5-labelled antibody, while the absorbance unit for Cy5 at 650 nm was in the optimal range (0.5 – 0.7 AU), the absorbance at 280 nm for protein was below 0.1. Such small value of absorbance led to increased uncertainty of measurement and elevated inaccuracy of result for D/P ratio.

MALDI-TOF spectrometry has been increasingly used for characterising large molecules by generating molecular ion [25,26]. This technique offers high sensitivity ($<1\,\text{pmol}\,\mu\text{L}^{-1}$), requires only a few microliters of sample for analysis and produces molecular weight of target analyte almost instantly. In this study, we explored MALDI-TOF spectrometry to accurately determine D/P ratios of labelled antibody for the first time. MALDI-TOF mass spectra were obtained for both untagged and labelled antibodies. Figure 2 shows the typical TOF MS spectrum for pure untagged antibody. The molecular ion $[M+H]^+$ of untagged antibody at m/z 145427.8 is clearly displayed. The highest peak at m/z 73311.1 represents the doubly charged molecular ion $[M+2H]^{2+}$. Thus the molecular weight of untagged antibody was determined to be 145428 Da. An unresolved shoulder peak at $m/z \sim 13500$ was 85% less intense than the peak of antibody molecular ion, therefore did not hinder clarification of target peak. Another peak of lower intensity at m/z 121450.6 (corresponding doubly charged peak m/z 61416.9) was well separated, but no attempt was made to identify it. The spectrum of BSA was used for mass calibration.

The molecular weights of untagged and labelled antibody with various conditions were listed in Table 1. The mass increase of labelled antibody over untagged antibody was

Downloaded At: 13:41 17 January 2011

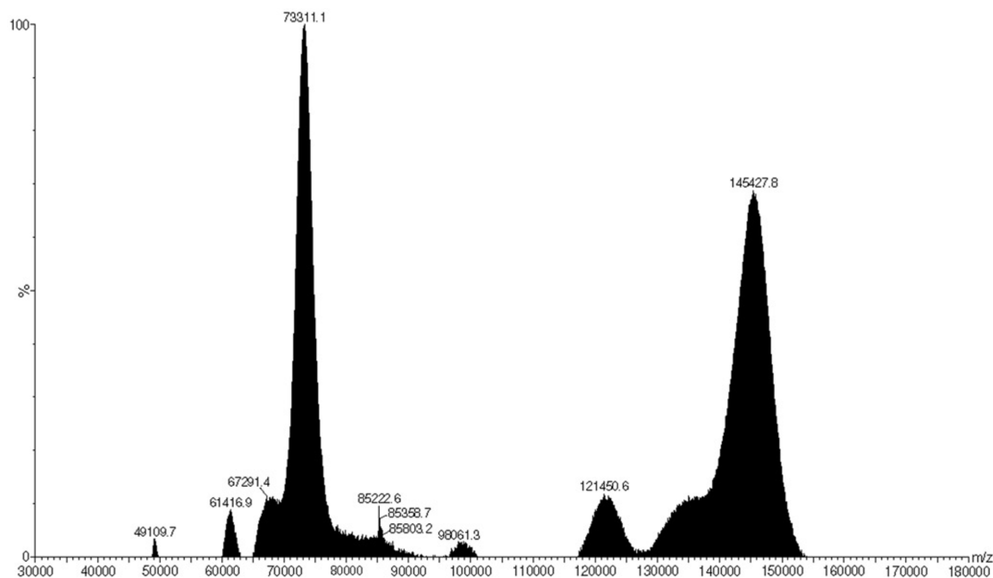


Figure 2. MALDI-TOF Mass spectrum of pure untagged antibody, obtained in linear mode. The mass spectrum of BSA was used for mass calibration. It shows molecular ion $[M+H]^+$ m/z of 145427.8 and doubly charged molecular ion $[M+2H]^{2+}$ m/z 73311.1 for the untagged antibody.

Table 1. D/P ratios determined by MALDI-TOF mass spectrometry, with 50 μg of pure antibody reacted with various amounts of Cy5.

Analyte	Molecular weight (Dalton)	Mw(label) - Mw(untag)	D/P ratio	Remark
Untagged antibody	145,428	–		
Labelled antibody 1	146,400	972	1.0	reacted with 2/100 of one-vial dye
Labelled antibody II	147,621	2193	2.2	reacted with 4/100 of one-vial dye
Labelled antibody III	149,763	4193	4.3	reacted with 6/100 of one-vial dye
Cy5	975	–		known molecular weight

obtained, attributed to coupling of antibody with dye. Dividing mass increase by molecular weight of Cy5 dye resulted in the corresponding D/P ratio. The utilisation of mass differences compensated for confined mass accuracy (0.05%) of TOF MS to certain extent when calculating D/P ratios. As shown in Table 1, the D/P ratios were determined to be 1.0, 2.2 and 4.3 for the reactions with 2, 4 and 6% of one-vial dye, respectively.

Downloaded At: 13:41 17 January 2011

3.2.2 *Effect of concentration of capture antibody on response of biosensor*

To examine the influence of concentration of capture antibody during waveguides incubation on the performance of the biosensor, three coupons were prepared, each containing four waveguides incubated in 10, 50, 100 and 200 μg mL^{-1} of capture antibody, respectively. Considering the volume (100 μL) of the capture antibody solution in the incubation container, these concentrations would translate into masses of 1, 5, 10 and 20 μg correspondingly. The waveguides of one coupon were incubated for 48 hours, while those of the other two coupons were incubated for 24 hours only. Commercial labelled antibody was utilised as the detector reagent during the assay run. After baseline was taken, each coupon was first interrogated by deionised water as the blank, followed by 200 and 1000 ng mL^{-1} of ricin in water sequentially. Figure 3 shows average fluorescence signal increase of each concentration of coated waveguides on three coupons. The results indicated that signal increase obtained from 5 and 10 μg of capture antibody were quite close for spiked samples, both higher than that from the waveguide incubated with 1 μg, but lower than 20 μg. It should be noted that the signal increase for DI blank was also lowest for 1 μg of capture antibody in Figure 3.

3.2.3 *Optimisation of D/P ratio*

Four parallel waveguides coated with capture antibody (100 μg mL^{-1}) under same condition were inserted in one coupon. Three reagents with different D/P ratios at same concentration plus one commercial reagent obtained from Research International were introduced to each of the four channels. Two concentrations of ricin A chain in DI water, 200 and 1200 ng mL^{-1}, were tested. As shown in Figure 4, among the three D/P ratios, the signal increase with D/P ratio of 4 was biggest for both concentrations of ricin, indicating the sensitivity with D/P of 4 was the highest. The commercial labelled antibody, of which concentration was not determined, had even better sensitivity than the one with D/P ratio of 4. Data shown in Figure 4 also implied D/P ratio could go higher in order to enhance sensitivity. Anderson [21] found that the optimal D/P ratio was between 2 and 4, which was in good agreement with our result.

Downloaded At: 13:41 17 January 2011

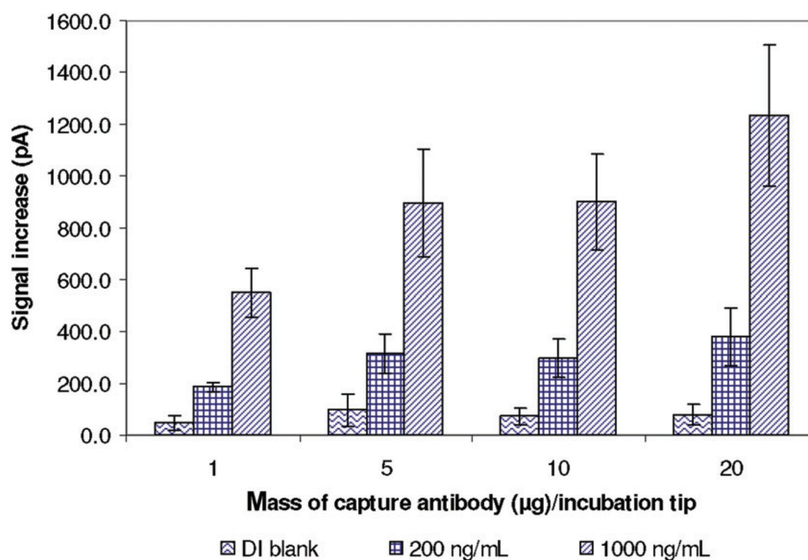


Figure 3. Fluorescent signal increase corresponding to waveguides incubated with different amount of capture antibody, upon challenge with $200\,\mathrm{ng\,mL^{-1}}$ and $1000\,\mathrm{ng\,mL^{-1}}$ of ricin A chain. Signal increase for blank is also shown. The average of three separate waveguides is presented.

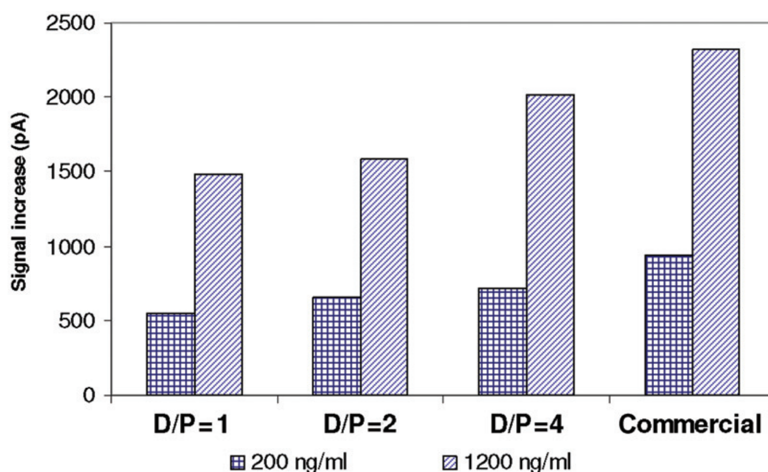


Figure 4. Dependence of fluorescence signal increase on Dye/Protein (D/P) ratio, with two concentrations of ricin A chain (200 and $1200\,\mathrm{ng\,mL^{-1}}$) tested. Assay was performed on one coupon containing four identical waveguides.

3.2.4 *Effect of concentration of labelled antibody on sensitivity of biosensor*

The concentration of labelled antibody (detector antibody) is a critical factor influencing the sensitivity of the biosensor. Detector antibody at the concentration as low as $12.5\,\mu\mathrm{g\,mL^{-1}}$ was applied during a number of initial trials, resulting in extremely poor sensitivity, essentially no response to the analyte. Figure 5 illustrates how signal increase drops rapidly as the concentration of labelled antibody decreases from 100 to 50 then

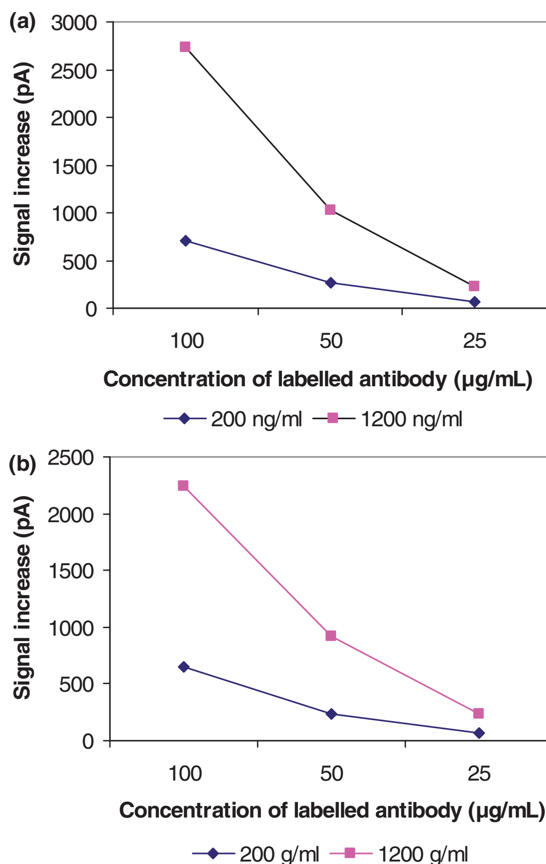


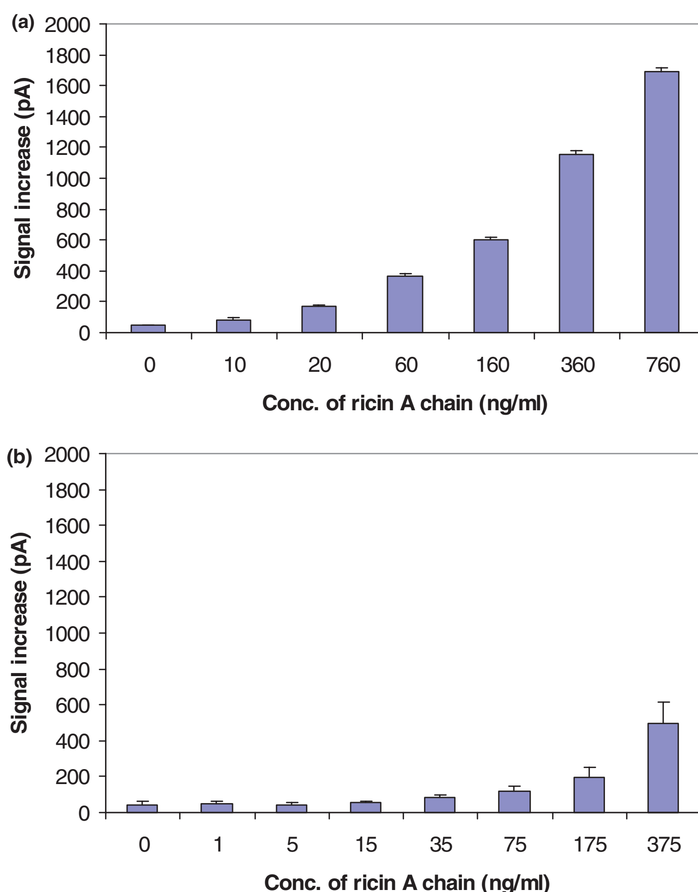
Figure 5. Effect of concentration of labelled antibody on the sensitivity of biosensor, (a) with D/P ratio of 4, and (b) D/P ratio of 2. Two spiked water samples at 200 ng mL^{-1} and 1200 ng mL^{-1} of ricin A chain were tested using coupon containing four identical waveguides incubated with 10 µg of capture antibody.

Downloaded At: 13:41 17 January 2011

25 µg mL^{-1}, which explained why the initial trials using 12.5 µg mL^{-1} failed. Figure 5(a) for D/P ratio of 4 and Figure 5(b) for D/P of 2 gave consistent results. Again, two concentrations of ricin A chain in water were tested to substantiate the results.

3.2.5 *Sensitivity comparison between home-made and commercial coupons*

Using commercial labelled reagent at same concentration, a series of standard ricin A chain solutions in DI water were tested in a sequence of low to high concentrations on both home-made and commercial ricin coupons, respectively. Figure 6 illustrates fluorescent signal increase with varying ricin A chain concentrations. Based on the wash delta approach, detection limit was found to be 10 ng mL^{-1} in DI water for the home-made coupon, while the commercial ricin coupon had a poorer sensitivity. For equivalent concentrations (60 vs. 75 ng mL^{-1}, 160 vs. 175 ng mL^{-1} and 360 vs. 375 ng mL^{-1}), fluorescent signal increases using the home-made coupon were more than twice those of the commercial coupon. Moreover, the reproducibility of self-made coupon was much better



Downloaded At: 13:41 17 January 2011

Figure 6. Sensitivity comparison between home-made coupon (a) and commercial coupon (b). Same commercial labelled antibody at same concentration was applied for both home-made coupon and commercial coupon. Error bars represent the standard deviation of average fluorescent signal increase for three identical waveguides incubated with 100 $\mu g\,mL^{-1}$ of capture antibody.

than commercial coupon. This is probably because the commercial coupon was prepared under less optimal conditions than the home-made coupon. Therefore, although the biosensor provides a platform for the analysis of toxins such as ricin, much effort has to be made by the individual researcher in order to achieve best sensitivity and robustness for real application. In addition, the manufacturer of RAPTOR^{TM} only supplies fully assembled ricin coupons. For the detection of other bio-threat agents using the biosensor, users must prepare the specific coupons on their own.

Although the sensitivity of biosensor was slightly lower than ELISA kit, the assay using biosensor platform was much faster than ELISA kit. Results were obtained in less than 15 minutes with biosensor, while it took more than 6 hours to get the result with ELISA kit. In addition, this compact device is portable and applicable in field. The sensitivity of biosensor can be improved by applying avidin-biotin chemistry. Anderson [21] found that the sensitivity could be further improved by employing Alexa Fluor 647 as a labelling reagent. Another advantage is that this biosensor is configured with a four-channel coupon, enabling simultaneous analysis of multiple target analytes.

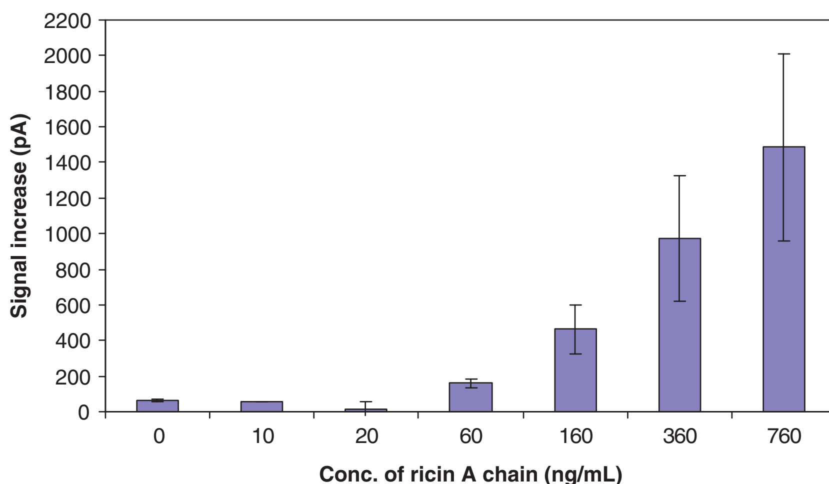


Figure 7. Fluorescent signal increase for real water samples spiked with ricin A chain at a series of concentrations using home-made coupon. Error bars represent the standard deviation of average fluorescent signal increase for three identical waveguides incubated with $100\,\mu g\,mL^{-1}$ of capture antibody.

Downloaded At: 13:41 17 January 2011

3.2.6 *Application to real water samples*

When home-made coupon was applied to analyse tap water samples spiked by ricin A chain, the detection limit was elevated to $60\,ng\,mL^{-1}$ as shown in Figure 7. Comparing the data in Figure 6(a) with Figure 7, one would find that the fluorescent signal increases for DI water and tap water at each same concentration of antigen were quite comparable. The slight increase in detection limit in tap water might arise from matrix effect. Narang [24] developed a fibre optic biosensor to determine ricin toxin in water, and observed 10-fold increase in detection limit in river water as compared to PBS buffer solution. Detection of tap water samples was also performed with the commercial coupon. The sensitivity was lower than that achieved using the home-made coupon (data not shown). More water samples with different matrices will be analysed using a home-made coupon in the future to investigate possible matrix effects.

4. Conclusion

The protocol of ELISA for the detection of ricin through its sub-unit A chain in water has been well established and validated, with the detection limit below $3\,ng\,mL^{-1}$ in blocking buffer. The ELISA kits employed were highly specific to ricin A chain, with minimal cross-reactivity from B chain. Biosensor assay, on the other hand, has the advantage of a much shorter assay time, being portable and applicable in the field. Factors including concentration of capture antibody, D/P ratio of labelled antibody and concentration of labelled antibody all have influence on the performance of the biosensor. The home-made coupon was found to have better sensitivity than the commercial coupon for ricin A chain. Under optimal conditions, the detection limit of the biosensor was $10\,ng\,mL^{-1}$ in DI water, increased to $60\,ng\,mL^{-1}$ in tap water using the home-made coupon.

Labelled antibody was successfully characterised by a novel approach MALDI-TOF MS spectrometry for the first time.

Acknowledgement

The authors would like to thank Dr. Guo Zhongxian for the assistance in characterising antibodies by MALDI-TOF MS technique.

References

[1] J. Audi, M. Belson, M. Patel, J. Schier, and J. Osterloh, JAMA **294**, 2342 (2005).
[2] D.V. Kamboj, A.K. Goel, and L. Singh, Def. Sci. J. **56**, 495 (2006).
[3] A. Godal, S. Olsnes, and A. Phil, J. Toxicol. Environ. Health. **8**, 409 (1981).
[4] S. Ramakrishnan, M.R. Eagle, and L.L Houston, Biochim. Biophys. Acta **719**, 341 (1982).
[5] N. Koja, T. Shibata, and K. Mochida, Toxicon. **18**, 611 (1980).
[6] M.A. Poli, V.R. Rivera, J.F. Hewetson, and G.A. Merrill, Toxicon. **32**, 1371 (1994).
[7] H.F. Shyu, D.J. Chiao, H.W. Liu, and S.S. Tang, Hybridoma Hybridomics **21**, 69 (2002).
[8] E.A. Garber, R.M. Eppley, M.E. Stack, M.A. McLaughlin, and D.L. Park, J. Food Prot. **68**, 1294 (2005).
[9] R.M. Wadkins, J.P. Golden, L.M. Pritsiolas, and F.S. Ligler, Biosens. Bioelectron. **13**, 407 (1998).
[10] F.S. Ligler, C.R. Taitt, L.C. Shriver, K.E. Sapsford, Y. Shubin, and J.P. Golden, Anal. Bioanal. Chem. **377**, 469 (2003).
[11] J.B. Delehanty and F.S. Ligler, Anal. Chem. **74**, 5681 (2002).
[12] G.P. Anderson, K.D. King, K.L. Gaffney, and L.H. Johnson, Biosens. Bioelectron. **14**, 771 (2000).
[13] K.D. King, J.M. Vanniere, J.L. LeBlanc, K.E. Bullock, and G.P. Anderson, Environ. Sci. Technol. **34**, 2845 (2000).
[14] G.P. Anderson and C.A. Rowe-Taitt, Proc SPIE **4206**, 58 (2001).
[15] C.C. Jung, E.W. Saaski, D.A. McCrae, B.M. Lingerfelt, and G.P. Anderson, IEEE Sens. J. **3**, 352 (2003).
[16] T. Geng, J. Uknalis, S. Tu, and A.K. Bhunia, Sensors **6**, 796 (2006).
[17] H. Wei, Y. Zhao, Y. Bi, H. Liu, Z. Guo, Y. Song, J. Zhai, H. Huang, and R. Yang, Sens. Actuators, B **123**, 204 (2007).
[18] G.P. Anderson, C.A. Rowe-Taitt, and F.S. Ligler, Proc. first Conf. Point Detect. Chem. Biol. Defense, Williamsburg, VA, October, 2000.
[19] M.F. Kramer and D.V. Lim, J. Food Prot. **67**, 46 (2004).
[20] V. Nanduri, G. Kim, M.T. Morgan, D. Ess, B-K Hahm, A. Kothapalli, A. Valadez, T. Geng, and A.K. Bhunia, Sensors **6**, 808 (2006).
[21] G.P. Anderson and N.L. Nerurkar, J. Immunol. Methods **271**, 17 (2002).
[22] C.R. Taitt, G.P. Anderson, and F.S. Ligler, Biosens. Bioelectron. **20**, 2470 (2005).
[23] M.S. Lewis and R.J. Youle, J. Biol. Chem. **261**, 11571 (1986).
[24] U. Narang, G.P. Anderson, F.S. Ligler, and J. Burans, Biosens. Bioelectron. **12**, 937 (1997).
[25] M. Thevis, R.R.O. Loo, and J.A. Loo, J. Am. Soc. Mass Spectrom. **14**, 635 (2003).
[26] A. Nazabal, R.J. Wenzel, and R. Zenobi, Anal. Chem. **78**, 3562 (2006).

Downloaded At: 13:41 17 January 2011