DETECTION OF RICIN WITH BIOSENSORS AND ELISA: A REVIEW

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Summary

The main aim of this work is to summarize the information about the most common methods for detection of ricin. Ricin is a phytotoxin produced by castor plant, Ricinus communis. It is known as ribosome-inactivating protein because it inhibits the proteosynthesis. It can be abused for terrorist actions or it can cause very serious intoxication, either accidental or murder and suicide. Therefore there is an urgent need to have a reliable, specific and selective detection method. This paper offers information about enzyme-linked immunosorbent assay (ELISA) and biosensors. The magnetoelastic immunosensor and the fiber-optic based biosensor are represented here. Because ricin is a protein, the most suitable methods are based on immunological principles. Biosensors have similar arrangement as immunoassays but they are constructed for the quick detection and screening. Thus they are faster and do not require difficult conditions, such as trained personal, incubation time etc. The detection limit for ricin offered by immunoassays is much lower than by biosensors. Biosensors can be used also for some other toxins, such as Staphylococcal enterotoxin B and Yersinia pestis F1 antigen.

Key words: Ricin; ELISA; Biosensors; Toxin analysis.

Detekce ricinu s použitím biosenzorů a ELISA: přehled

Souhrn

Hlavním cílem této práce je shrnout informace o nejběžněji používaných metod pro detekci ricinu. Ricin je fytotoxin produkovaný rostlinou zvanou skočec obecný, Ricinus communis. Je znám jako protein blokující proteosyntézu blokací ribozomů. Jako toxin může být zneužit k teroristickým akcím, ale také může způsobit velmi vážnou otravu, a to buď náhodnou nebo úmyslnou (vražda, sebevražda). Proto je zde urgentní potřeba detekčních metod, které jsou spolehlivé, specifické a selektivní. Tato práce nabízí informace o enzymatické imunoanalýze (ELISA) a biosenzorech. Z biosenzorů jsou zde uvedeny magnetoelastic imunosenzor a fiber-optic based biosenzor. Jelikož ricin je protein, nejvhodnější metody jsou založeny na imunologických principech. Uspořádání biosenzorů je podobné jako u imunoanalytických metod, ale jsou konstruovány pro rychlou detekci a záchyt toxinů. Jsou proto rychlými metodami nevyžadujícími náročné podmínky, jako je požadavek vyškoleného personálu, nebo např. inkubační doby. Detekční limit je u imunoanalýz podstatně nižší než u biosenzorů. Biosenzory mohou být použity také pro detekci stafylokokového enterotoxinu B a antigen F1 bakterie Yersinia pestis.

Klíčová slova: Ricin; ELISA; Biosenzory; Analýza toxinů.

Introduction

Ricin, well known phytotoxin, is the product of the castor plant. It is a 66-kDa globular glycoprotein that makes up 1% to 5% by weight of the bean of castor plant, *Ricinus communis.* Ricin is a ribosome--inactivating protein (7). It means that it has the ability to inhibit the proteosynthesis with irreversible inactivation of bigger subunit 60S of ribosome (2, 5, 9). The literature contains also the information about attempted suicide by ingesting castor beans or accidental intoxication of adults and children. Some authors discussed over 700 human intoxications dating back to the late 1800s (9). There were also some murders in history. Ricin is very stable in aerosolized form and can be produced in large quantity or amount relatively easy or easily. The ricin poisoning has no effective treatment or specific vaccine hence is very dangerous (6). The procedures and methods are not easy because ricin is a protein.

Nearly only methods usable for this manner of analysis are those based on immunological principles (2, 4, 9, 11). There are not many possibilities of instrumental arrangement through difficulty of the procedure. It is also not possible to make *post mortem* analysis due to quick ricin destruction in human body. Therefore analyses are limited for diagnosis of poisoning only. If we have suspicion of death or murder due to ricin we cannot prove it. This paper summarizes three utilizable methods for ricin detection in biological material. The first one is ELISA with colorimetric and chemiluminiscence detection. The next two types of biosensors have been considered. Those are magnetoelastic ricin immuno-sensor and fiber optic-based biosensor.

ELISA (Enzyme Linked Immunosorbent Assay)

Radioimmunoassay was the first analytical immunoassay developed for detection of ricin. This assay utilized ^{125}I-labeled ricin (2). This method was time-consuming because it required overnight incubation and the problems with handling and disposal of radioisotopes were connected with this method. First ELISA (enzyme-linked immunosorbent assay) utilized rabbit antiserum. The detection limit of ricin in rabbit body was 40 ng/ml (1). Subsequently ELISA based on affinity-purified rabbit antiserum was developed. Using this method the detection limit decreased to 10 ng/ml (9). The most sensitive ELISA was developed and based on utilization an avidin/biotin immunoperoxidase complex (to amplify the signal). The authors reported the detection limit of 200 pg/ml of tissue extract (9).

Poli et al. (9) reported the ELISA with the detection limit of 100 pg/ml. This method used affinity--purified goat antibodies. The ricin was detectable in phosphate-buffered saline, human serum and human urine. This kind of ELISA can be configured either to a colorimetric or chemiluminiscence image.

The goat antibodies were produced by hyperimmunization of goats. The hyperimmunization was provided with formalin-treated ricin toxoid. And the affinity-purified anti-ricin IgG was isolated. The standard curves for ricin concentrations were prepared. Then analysis was performed with coating of wells of microtiter plates with the antibody and subsequent washing. The optical density at 405 nm was measured on a Bio-Tek EL 311 microtiter plate reader (Bio-Tek Instruments, Winooski, VT, U.S.A.) (9). In the event of the chemiluminiscence detection, the special microtiter plates were used (Microfluor WHT, Dynatech Labs) (9).

Magnetoelastic ricin immunosensor

Because the radioactive and non-radioactive immunoassays are relatively time-consuming and requiring trained personal (10), Ruan et al. suggested to use the biosensors for detection of toxins, in our case ricin (11). This biosensor is constructed to detect ricin in aqueous media such as water, blood or serum. Magnetoelastic immunosensor for detection of ricin is based on the effect of magnetic field and measuring of the response. Magnetoelastic thin-film sensors can be considered the magnetic analog of an acoustic bell: in response to an externally applied magnetic field impulse the sensors ring like a bell, emitting both magnetic flux and acoustic energy with a characteristic resonant frequency. The magnetic flux can be detected remotely, external to the test area, using a pick-up coil, or the acoustic energy by a microphone. By monitoring changes in the characteristic resonant frequency of the sensor, multiple environmental parameters can be measured including temperature, pressure, velocity of ambient medium and, when immersed in a liquid, viscosity, liquid density, and surface tension. In combination with mass changing, chemically responsive layers, such as polymers or ceramics, remote query chemical sensors can be made (Fig. 1).

The instrument consists of the source of magnetic field, the coil with the sensor and detector. The magnetoelastic sensor platform, coupled with chemical or biological sensitive coatings enables the sensitive and selective detection of various chemical and biological substances, and then also toxins such as ricin. By means of time varying magnetic field the oscillations occur. Those oscillations have specific fundamental resonance frequency. The vibrations generate acoustic waves and magnetic flux, which can be detected (11). Ruan et al. presented the specialized magnetoelastic biosensor configuration for detection of ricin. This technique is based on solution using an enzyme catalytic precipitation system associated with antigen-antibody reaction. The anti-ricinus communis agglutinin I&II was placed on the goldcoated sensor surface and sandwich immunoassay using alkaline phosphatase conjugated rabbit anti-ricin was used. Mentioned antibody was

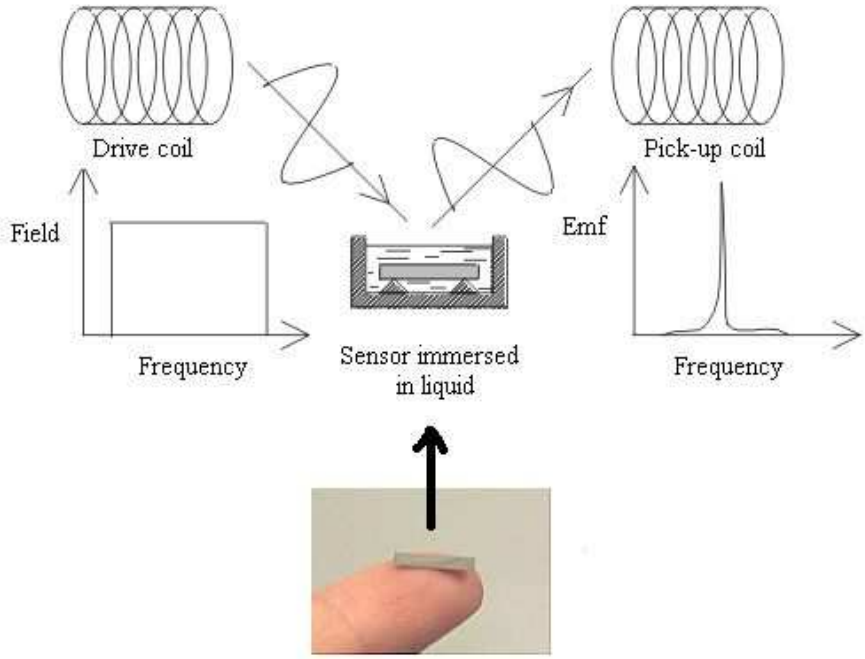

Figure 1: Remote query mechanism for magnetoelastic sensors. The magnetoelastic sensors are the magnetic analog of an acoustic bell. Under the influence of an alternating magnetic field, they resonate at a characteristic frequency which depends among other quantities, on the mass load on the sensor.

Figure 2: Fiber-optic biosensor from Naval Research Laboratory (Washington, DC) for environmental monitoring of hazardous chemical or biological materials and has undergone extensive field testing.

used as a secondary antibody. The response signal was generated through the enzyme catalytic oxidative hydrolysis process. 5-bromo-4-chloro-3-indolyl phosphate converted into an insoluble product that deposited in a surface of sensor shifting its resonance frequency and thereby enabling ricin quantification. The detection limit for this method was 10 ng/ml (11).

Fiber-optic based biosensor

Fiber-optic based biosensor is a new generation of sensors for use in control of many processes, which measure the chemical environment directly by means of a biological agent mainly enzymes so far. A biosensor may be a device or instrument comprising a biological sensing element coupled to a transducer (Fig. 2).

Narang et al. presented highly specific evanescent wave fiber-optic biosensor (4). For detection of ricin, a sandwich immunoassay scheme was used. Principally an anti-ricin IgG was immobilized onto the surface of an optical fiber. This was performed in two ways. First procedure was based on direct coating of the antibody to the silanized fiber, using a crosslinker. In the second method, the avidin-coated fibers were incubated with biotinylated anti-ricin IgG to immobilize the antibody using an avidin-biotin bridge. Goat anti-ricinus communis agglutinin I and II was used as a capture antibody. This antibody was placed into the capillary containing the avidin--coated fiber for 20 min followed by flushing with buffer. Cy5-labeled anti-ricin antibody was used for the detection. Before the Cy5-labeled anti-ricin antibody the fiber was exposed to ricin. Then the Cy5-labeled antibody was applied and flushed with buffer. After that the signal was noted. As the detector, the fiber-optic fluorimeter was used (4). The excitation sources were solid-state 635 nm laser diodes. Light from each diode was electronically pulsed at a frequency of 135 Hz to minimize interference from stray ambient light and channeled through a signal fiber to the tapered fiber probes. The evanescence wave-induced fluorescence was coupled back through the fiber probe to an array of high-numerical aperture plastic fibers that transfer this light to a photodiode. The photocurrent generated at the photodiode was amplified and detected at the laser diode pulsed frequency (4).

Using this method, authors noted the detection limit 100 pg/ml for ricin in buffer solution. This method was also tested for *Staphylococcal enterotoxin B* and *Yersinia pestis* F1 antigen (4).

Conclusions

There is a need to have a reliable, fast and specific analytical method to detect ricin for environmental monitoring, forensic use, and clinical diagnosis or to monitor the blood levels of this immunotoxin in people. Considering two types of methodical approaches (biosensor and classical immunoassays), we have to make following conclusions. The immunoassays are specific, selective and have low detection limit, but they are still time-consuming, require special techniques and trained personal. Therefore they are more expensive than biosensors. On the other hand, analysis using biosensors takes very short time and their cost is much lower comparing immunoassays. The detection limits of immunoassays can be 100 pg/ml. The biosensors offer little bit higher detection limit but combining with the immuno-techiques they can also offer the limit 100 pg/ml. Current trends for analysis of proteinaceous toxins such as ricin are based on mass spectrometry but these are characterized by sophisticated instrumentation and its high cost (3). Some biosensors, as a new technology of toxin detection have been developed with cooperation U.S. Army (13). These biosensors are also applicable for detection of anthrax, botulism, smallpox, plague, or cholera (12).

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