IMMUNSENSOR DEVELOPMENT FOR FOOD AND ENVIRONMENTAL ANALYTICAL APPLICATIONS

Theses of Ph.D. Dissertation

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1. INTRODUCTION

Mycotoxin contamination of cereals present an important risk on human and animal health all over the world. Mycotoxin produced by fusarium fungi are the most common in Hungary, within that deoxynivalenol contamination is the main problem. Due to global warming and to industrial agriculture the fusarium head blight in cereals causes an enormous economic loss. Use of the contaminated grains as feed results in also a significant relapse in the output of stock raising, while food made from contaminated grains have adverse effects on human health.

Thanks to the rapid development of industry and to the industrial agriculture and farming the use of different chemicals, pharmaceutics and pesticides are increased, which raise concerns not only on food safety but raise serious environmental issues as well. In the last decade endocrine disruptors (EDCs) have come under the spotlight, which can cause serious disorders in the endocrine system causing fertility problems. Aquatic animals particularly sensitive to their presence. In most of the cases pesticides, plastics and pharmaceutics were found to be responsible for these effects.

Rapid determination of the concentration and the presence of contaminants are surpassingly important both food safety and environmental point of view. The analysis of numerous samples are still challenging, sith in several cases conventional analytical methods can not be applied, because they are slow, expensive, require complex sample pretreatment and well trained professionals, therefore important the development of new techniques allowing rapid detection of contaminants and they are suitably sensitive and selective. Application of biosensors, including label-free immunosensors, could solve those analytical problems, therefore it is an important and current research area.

2. OBJECTIVES

The aim of my PhD work was to develop immunosensors for food and environmental analytical applications. During the investigation my goals were to develop an immunosensor suitable for DON determination from wheat, and a sensor for detection of Vtg from carp (*Cyprinus carpio*) and frog (*Bombina bombina*) samples, used as a biomarker to detect surface water contamination with EDCs. In the course of this the most important tasks were the preparations of the immonoreagents necessary for the sensor development, investigation of the possible application of direct and indirect measuring methods, optimization of the measuring system and prove the applicability of the established sensor by analyses of real samples. The results obtained by the OWLS method were compared to those of the ELISA reference method used in food- and environmental analysis to verify the reliability. With the established method my aim was to develop a new technique which is fast, simple and allows quantitative analysis of a large number of samples with high sensitivity and selectivity.

3. MATERIALS AND METHODS

For DON determination certified (by GC–MS, LOD: 0.01 mg kg^{-1}) DON-free wheat flour was applied which was spiked with different concentrations of DON standard in the range of 0–100.0 mg kg^{-1} and used as samples.

For Vitellogenin determination the male and female carps (*Cyprinus carpio*) were derived from Aranyponty Inc. (Rácegres, Rétimajor) ecological fish farm. For the determination of frog vitellogenin the fire bellied toads were raised under laboratory conditions from eggs collected in natural habitat by Double Delta Ltd. (Berettyóújfalu).

For Vitellogenin determination lipovitelin (Lpv) protein was used as immunogen / antigen because it is easier to isolate and the Vtg and Lpv from the same species give 95% cross-reactivity with each other.

OWLS measurements were carried out using amino functionalized integrated optical waveguide sensors (chips) type OW 2400 (MicroVacuum Ltd., Budapest). OWLS 120 instrument produced by MicroVacuum Ltd. was used for the measurement. The instrument was controlled by the software BioSense 2.2 (MicroVacuum Ltd, Budapest). As a light source a linearly polarized laser light (632.8 nm) was used. The experiments were performed in a flow-injection analyzer (FIA) system in order to maintain the steady operational parameters. The sensors were used in a flow through sensor holder. The steady flowrate was maintained by a Gilson Minipulse 3 peristaltic pump. The temperature was controlled by the OWLS TC heating/cooling unit. The injections were carried out by using a Rheodyne injector equipped with a 200 µl sample loop. The results were evaluated by the MicroVacuum Ltd. BioSense 2.6 software.

The surface of the waveguide sensor was modified by liquid phase silanization with γ-aminopropyltriethoxysilane (APTS) by merging the sensor into the silane solution.

The biomolecules were immobilized onto the sensor surface with glutaraldehyde during the development of the sensors suitable for DON and carp Vtg determination. In the case of frog Vtg determination the amino groups of the APTS modified sensor surface were derivatized into carboxyl groups with succinic anhydride treatment and the biomolecules were immobilized by using 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDC) and n- hydroxysuccinimide (NHS) reagents to them.

The statistical and graphical evaluation of the data were made by EXCEL (Microsoft Office professional Edition 2003) and by ORIGIN Scientific Graphing and Analysis Software (version7).

4. RESULTS

- Changes in the thickness of the added layer during immobilization, the dependence of sensor response on molecular mass and concentration of the analyte, the sensor response time, the accuracy and also the impact of the thickness of the applied cuvette on the sensor response were investigated in modelling studies.

Results of the DON immunosensor development:

- DON-ovalbumin and DON-bovine serum albumin conjugates were prepared for the immunization and for the sensor development. The protein fractions obtained were verified by isoelectric focusing.

- DON specific polyclonal serum was developed by immunization in rabbits, and the purified IgG fractions were made from the serum by salting out. The antibody activity against the antigen was investigated by indirect ELISA system.

- Determination of DON applying direct measuring method was investigated with OWLS sensor.

- OWLS based competitive immunosensor was developed for DON determination. After optimization of the operational parameters spiked wheat flour samples were investigated.

Results of the Vitellogenin immunosensor development:

- Application of the direct measuring method for carp (*Cyprinus carpio*) and fire bellied toad (*Bombina bombina*) derived vitellogenin determination was investigated.

- Competitive immunosensor was developed for carp vitellogenin determination. Ponty vitellogenin meghatározására kompetitív immunszenzort fejlesztettem ki. After optimization of the operational parameters Vtg content in blood and liver from male and female carps was determined.

- Competitive immunosensor was developed for vitellogenin determination in fire bellied toad. Vitellogenin content was investigated in liver, heart, blood and gonad tissue samples from male and female toads. The results obtained by the OWLS immunosensor were compared with those of the ELISA method.

5. NOVEL SCIENTIFIC RESULTS

- In modelling studies it was demonstrated that the surface modification and immobilization steps are reproducible during measurements. Dependence of sensor response on molecular mass and concentration of the analyte was investigated during immobilization. It was concluded that the sensor response is proportional to the molecular mass and the sensor response changes linearly with the concentration of the analyte.

- Competitive label-free immunosensor was developed for DON determination in wheat samples. The measuring range of the established sensor was two orders of magnitude lower than that obtained in the direct OWLS method and about one order of magnitude lower than obtained in a similar immunosensor by using SPR detection. After sample preparation, the recovery of the DON mycotoxin from the spiked wheat flour was 91.6-123.0%. Results indicate that the developed competitive label-free immunosensor is suitable for DON determination in wheat.

- Competitive immunosensor with OWLS detection was successfully developed for detection of fish (carp, *Cyprinus carpio*) vitellogenin. For the sensor development Lpv protein was applied, which is easier to isolate and gives 95% cross-reactivity with the Vtg from the same species. With the established sensor the vitellogenin content in blood from male and female carp was investigated. According to my results it can be stated that the developed immunosensor method is suitable for monitoring elevated Vtg in male carp due to possible exposure to water-contaminating endocrine disrupting chemicals.

- Selective, competitive OWLS immunosensor was developed for vitellogenin determination in fire bellied toad (*Bombina bombina*) for the first time. Vitellogenin content was measured in liver, heart, blood and gonad samples of male and female toads. The results were compared with those of the ELISA reference method. According to my results it can be stated that the sensitivity of the developed sensor is one magnitude higher than the sensitivity of the ELISA method, therefore the established immunosensor is suitable for monitoring the contamination of aquatic habitats with endocrine disrupting chemicals.

6. PUBLICATIONS

Articles in journals:

Journals with impact factor:

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