Analytical Research

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Biosensors and multiple mycotoxin analysis

Mycotoxins are toxic fungal metabolites that can occur in primary food products such as nuts, cereals and fruits as a result of mould growth. Some mycotoxins have been proved strong carcinogenic agents like aflatoxin B1; others are under suspicion to have carcinogenic effects. Currently a few hundred mycotoxins are known, the most prominent being aflatoxin, deoxynivalenol, zearalenone, ochratoxin, fumonisin and patulin.
There is an increasing awareness of the hazards imposed on both human and animal health by mycotoxins present in food and feed. Legislation regarding the allowed levels of mycotoxins present in food and feed products and in raw materials is expected. Therefore, a need exists for a reliable, economical and easy to use assay for the measurement of the mycotoxin contents, especially in the raw materials for food and feed production.
The assay that is presented here is designed as a multi-analyte inhibition assay with a single, straightforward clean up procedure, in which the principle of detection is based on surface plasmon resonance (SPR).

Principle of the technique

The assay is designed as an inhibition assay. A fixed amount of mycotoxin specific antibody is mixed with a sample containing an unknown amount of mycotoxin. The antibody and mycotoxin form a complex. The sample is then passed over a sensor surface to which mycotoxins have been immobilised. The amount of non-complexed antibodies is determined as they bind to the immobilised mycotoxin on the sensor surface.

Development of the sensor

For the development a BIACORE surface plasmon resonance (SPR) device was used. Toxins were immobilised on the sensor surface of a CM-5 sensor chip. For the individual toxins, separate immobilisation procedures adapted from the procedures described by Jönsson (1) Thouvenot (2) and Xiao (3) *et al*. were developed. Sample extracts and reference solutions were mixed 1:1 with an antibody mixture and injected in the four mycotoxin flowcells for simultaneous detection of four mycotoxin levels.

Results

Figure 1 shows a typical calibration curve for one of the mycotoxins measured. This calibration of deoxynivalenol is the result of six separate measurements. Figure 2 shows the correlation of the multi-analyte SPR sensor and the standard GC-HPLC mycotoxin determinations for several analysis.

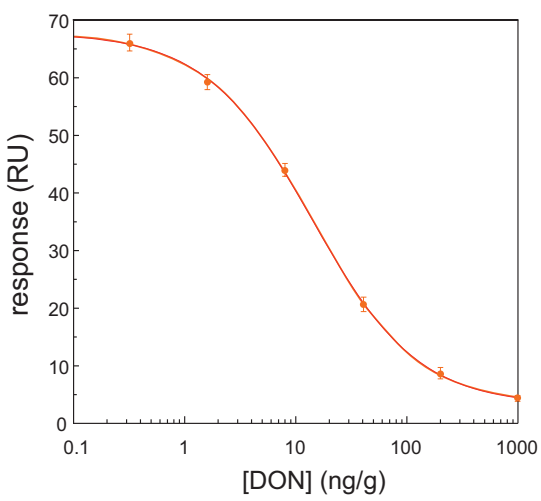

Figure 1. Calibration curve of DON in 4.2% acetonitril.

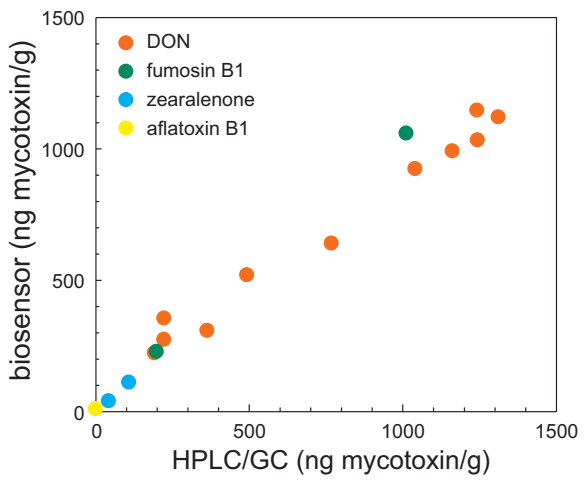

Figure 2. Correlation between the biosensor and standard HPLC-GC analyses for several mycotoxins.

Conclusion

A biosensor for the simultaneous detection of four mycotoxins has been developed. Extraction and clean up of the sample require approximately 15 minutes. Additionally, the measurement takes 10 minutes, including regeneration of the sensor chip surface, making a total of approximately 25 minutes for the simultaneous determination of four mycotoxins in a single sample.

The *Fusarium* and *Aspergillus* toxins tested can all be detected in the relevant concentrations (see table 1). Future research will be focussed on the development of easy to apply biocoatings for use in small, low cost SPR-equipment, which can be used in the field and/or at intake facilities.

Table 1. Legal or advisory levels, the detection limit and the reproducibilityof the multi analyte assay.

Mycotoxin	levels[1] (ng/g)	detection limit (ng/g)	RSD (%)
aflatoxin B1	2	0.2	2-10
zearalenone	200	0.01	16-19
ochratoxin A	3	0.1	4-9
fumonisin B1	1000	50	2-8
deoxynivalenol	500	0.5	2-5

[1]*Depending on matrix*

References

1. Jönsson, U. Real time biospecific interaction analysis using surface plasmon resonance and a sensor chip technology. BioTechniques 1991; 11: 620-627.
2. Thouvenot, D and Morfin, RF. Radioimmunoassay for zearalenone and zearalenol in human serum: production, properties and use of porcine antibodies. Appl. Environ. Microb. 1983; 45: 16-23.
3. Xiao, H, Clarke, J.R. , Marquardt, R.R. and Frohlich, A.A. Improved methods for conjugating selected mycotoxins to carrier proteins and dextran for immunoassays. J. Agri. Food Chem. 1995 49: 2029-2097.

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