Sugar Bags II – Molecules for Mycotoxin Capture

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Objectives

- Development of a novel mycotoxin-adsorbing agent, based on physical inclusion of different mycotoxins in cyclodextrins (CD)
- CD are proven to include different mycotoxins depending on the ring-size which is usually composed of six, seven or eight glucose molecules (α-, β- or γ-CD)
- Increase the stability of cyclodextrins resp. inclusion complexes in the intestinal tract of animals by chemical modification of the outer surface
- Computer simulations have shown that inclusion of Aflatoxin B_1, Ochratoxin A, Deoxynivalenol and Zearalenone in different CD is possible (Figure 1)

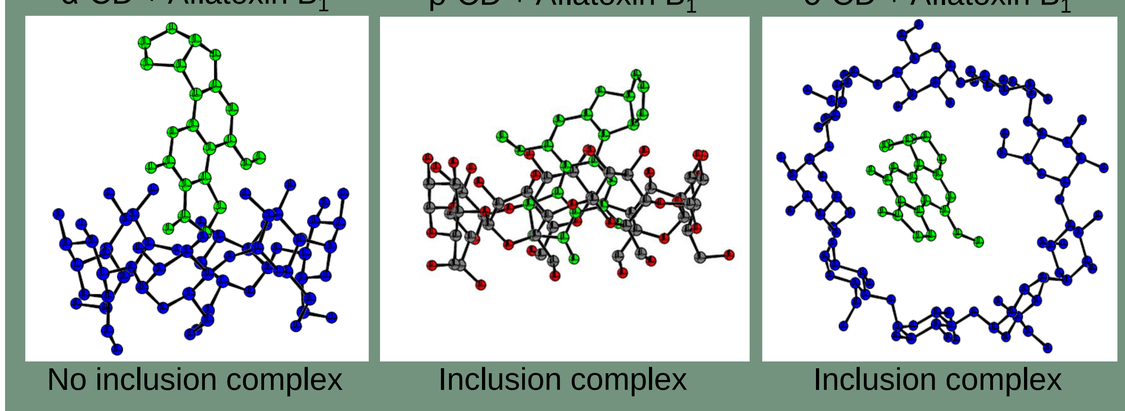

Figure 1: Computer simulation of inclusion complexes with Aflatoxin B_1 and different CD [1]

Analytics

- A quantitative method for high-performance thin-layer chromatography was validated
- With this method a simultaneous detection of Deoxynivalenol (DON) and CD in one sample is possible

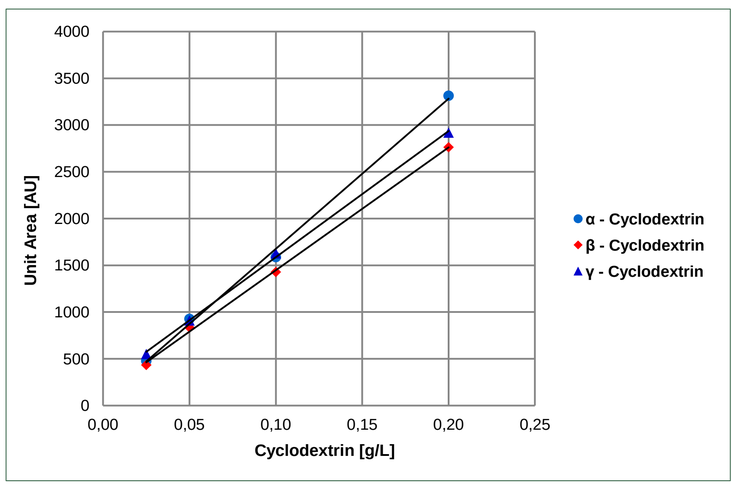

Figure 2: Calibration curve α-, β- or γ-CD

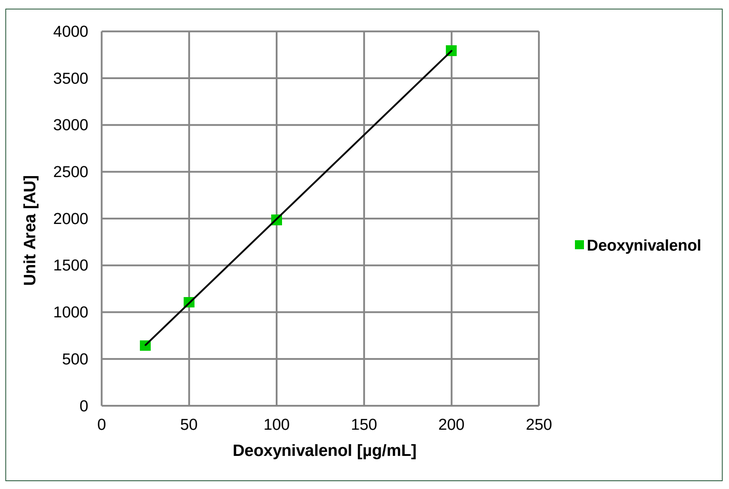

Figure 3: Calibration curve Deoxynivalenol

- Silicagel 60 RP-18W, Solvent system Methanol and Chloroform/Aceton/2-Propanol
- Calibration curve for CD is corresponding to a concentration range of 0.025 - 0.20 g/L
- Calibration curve for DON is corresponding to a concentration range of 25 - 200 µg/mL
- For both calibration a coefficient of variation < 5% was calculated (see Table 1)

Table 1: Overview Validation Parameter

Parameter	α-Cyclodextrin	β-Cyclodextrin	γ-Cyclodextrin	Deoxynivalenol
Detection limit [g/L]	0,01	0,01	0,01	0,01
Quantification limit [g/L]	0,025	0,025	0,025	0,025
Coefficient of variation CV [%]*	2,99	2,80	3,26	2,34
Accuracy [%]*	101	101	98	99
Linearity [R^2] **	0,9986	0,9986	0,9985	0,9972

*Calculated from six validation runs ; **Calculated from three calibrations curves

Inclusion complex

- Experiments have shown that a mixture of β- and γ-cyclodextrins is able to include the mycotoxins Aflatoxin B1, Ochratoxin A and Zearalenone up to 98%
- Inclusion complex formations was followed via fluorescence- and ultrafast laser spectroscopy
- Inclusion complex formation of DON was followed by HPTLC
- Until now only a few experiments with different concentration ratio were performed

Table 2: Reduction of DON with α-, β- and γ-CD

DON:CD	10 min	30 min
1:10	11%*	14%*
1:100	14%*	31%*
1:400	26%*	31%*

*reduction of DON

- Experiments indicate a 40% reduction of DON after 60 min with a 1:100 DON:CD ration (Figure 4)

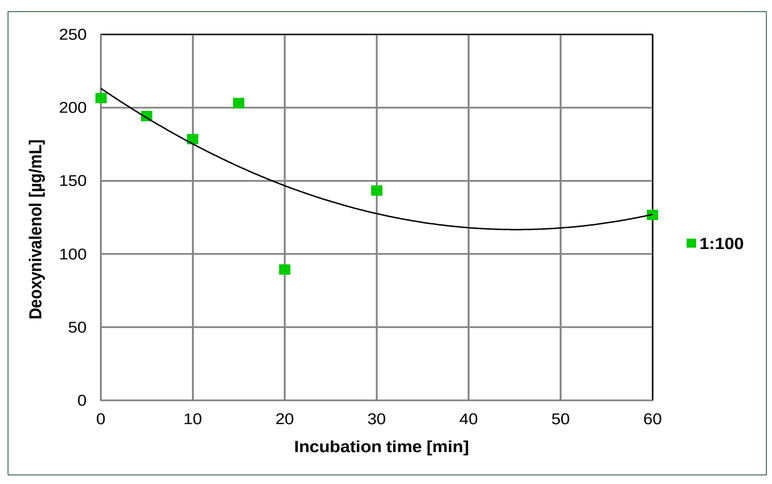

Figure 4: Reduction of DON concentration over 60 min

Outlook

- Chemical modification of Cyclodextrin should be done to increase the stability of Cyclodextrins against chemical and enzymatic degradation
- Possible methods of modification are cross-linking of Cyclodextrins and chemical or enzymatic modification of the primary alcohol groups on the outer surface of Cyclodextrins
- Laboratory scale tests on stability of reactants and the binding efficacy of mycotoxin-detoxifying agent are under investigation with a gastro intestinal tract model
- The investigation with a dynamic, computer-controlled in vitro gastro intestinal model of selected animals is planned
- The inclusion complex characterization should be done by nuclear magnetic resonance spectroscopy

Realized at:
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Literature: [1] Prof. Dr. Pablo Wessig, Institut für Chemie der Universität Potsdam