Development of platform for analysis of mycotoxin interactions with biomembranes

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INTRODUCTION

Mycotoxins are a major risk due to fungi contaminated vegetable food products and also due to accumulation of mycotoxins in animal food products from feeding a contaminated fodder. To generate its toxic effect, the particular toxin has to pass the barrier of cytoplasmic membrane. Therefore, it can be anticipated that the toxins, usually bearing amphiphilic qualities, has an ability to pass or disrupt plasmatic membrane. In the presented work, the selected mycotoxins were left to interact with prepared phospholipid biomembrane vesicles with mixed phospholipid composition and negative surface net charge. The interaction was evaluated using the method of liposomal electrokinetic chromatography. The preliminary data proved that the interaction intensity is connected to particular composition of the biomembrane as well as it is connected to the chemical composition of the solution (mainly pH) in which the interaction takes part in. By selection and optimization of the interaction system we were able to separate and identify Ochratoxin A from mixture of compounds.

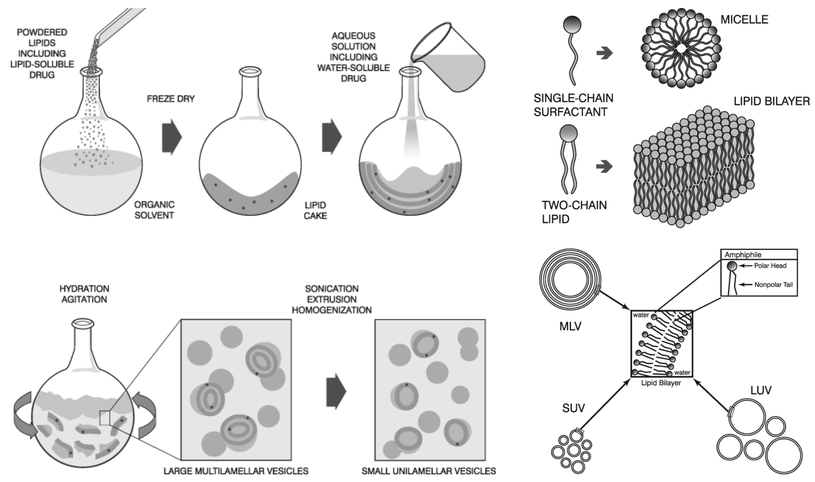
METHODS

Capillary electrophoresis

Liposome electrokinetic chromatography

- 50 µm i.d. fused silica capillary with polyimide coating
- *Vesicles* – 0.15 mM 1-palmitoyl-2-oleoyl-glycero-3-phosphocholine/1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-L-serine (80/20 molar ratio) small unilamellar vesicles prepared by 20 min sonication at 37 kHz/100% power setting; mean hydrodynamic radius of approximately 30 nm vesicles were dissolved in phosphate buffer of ionic strength of 10 mM and pH of 3, 6, 7,4 and 11.
- Detection – diode array detector at 200 nm, 4 Hz sampling rate, 0.5 Hz for full spectra

Vesicle preparation



Credit: Avanti polar lipids, Inc.; part of Merck KGaA

Inoculation of grain with Ochratoxin producing fungi

Used organisms: *Aspergillus ochraceus Wilhelm and Aspergillus melleus Yukawa* (Czech Collection of Microorganisms designation F-269 and 8002, respectively), both expected to produce Ochratoxin A nad B

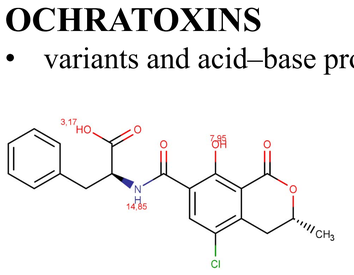
- 10 ml of SAB cultivating medium (5 replicas) inoculated by the fungi → 20 days at temperature 30 °C
- 25 g rye + 25 ml water inoculated with the fungi → 20 days at temperature 30 °C

Extraction:

- 50 ml of SAB cultivating medium → extracted 3 times with 100 ml of chloroform
- 25 g fungi covered rye grain was collected in mortar and 25 ml of water was added. Thin smooth mixture was produced and, subsequently, it was filtered through a double layer of filter paper. Approximately 15 ml of the filtrate was recovered. The filtrate was extracted three times with 30 ml of chloroform with 1% HCl.
- The chloroform was further evaporated under vacuum and the resulting film was dissolved in 20 ml of methanol. The methanolic extract was further split in halves and evaporated under gentle stream of air to dryness. 1 ml of water or methanol was used for the final dissolution.

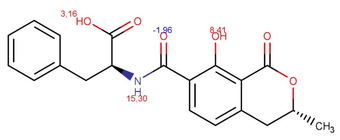
OCHRATOXINS

- variants and acid–base properties with simulation of hydrophobicity



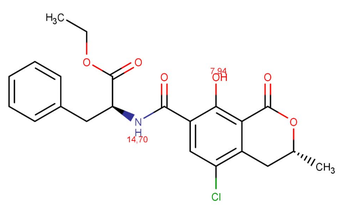
Ochratoxin A (OTA)

- the most toxic and abundant compound from the group
- nephrotoxic, carcinogenic, teratogenic, hepatotoxic, neurotoxic, slightly mutagenic and immunosuppressive agent



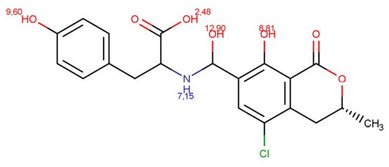
Ochratoxin B (OTB)

- lower abundance and toxicity compared to OTA
- cytotoxic to kidney and liver cells
- it is rapidly metabolized and excreted from the organism



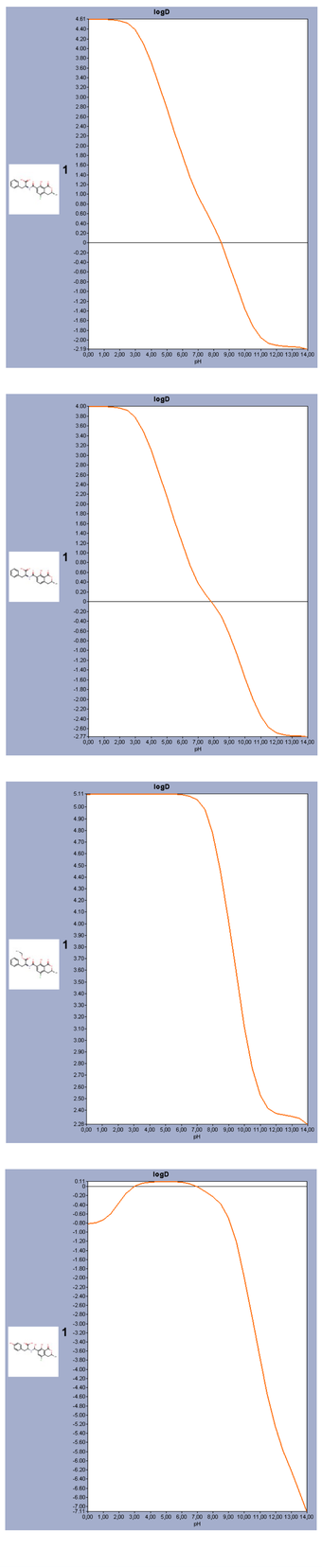
Ochratoxin C (OTC)

- rare abundance, incomplete toxicity data
- formed by esterification of OTA
- supposedly more toxic than OTA in chicken embryo toxicity test



Ochratoxin TA (OTTA)

- very rare abundance, incomplete toxicity data
- synthesis based on tyrosine rather than phenylalanine
- supposedly less toxic than OTA in chicken embryo toxicity test



The acid-base and logD calculations were performed using Marvinsketch 18.11 (ChemAxon, Chemaxon Ltd., Hungary)

RESULTS

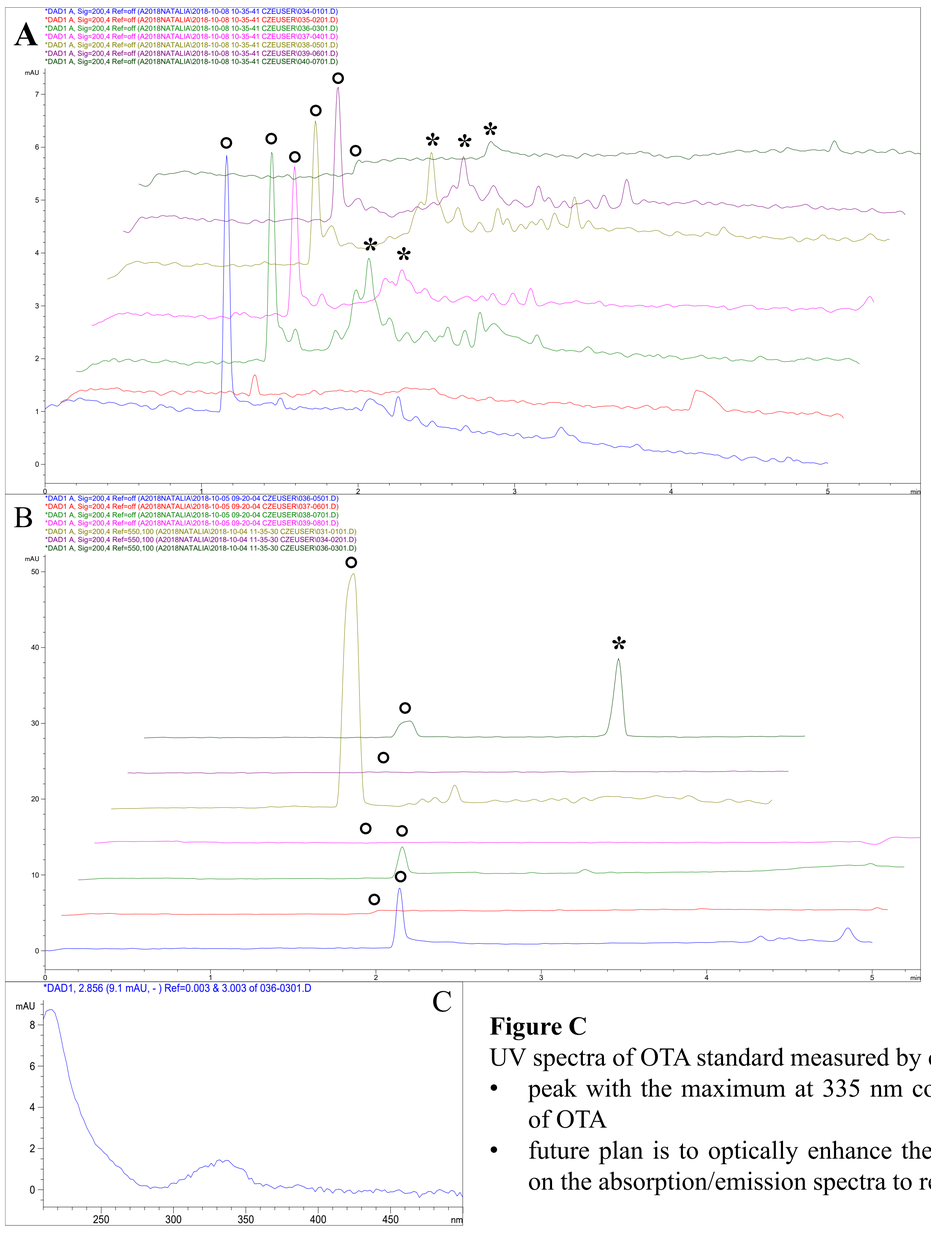


Figure A

Liposome electrokinetic chromatography analysis of mycotoxin contamination of the 20 days old fungi inoculated matrices.

- SAB medium shows a very few peaks compared to rye grain.

Legend:
F269 H_2O extract from SAB medium
F269 MeOH extract from SAB medium
8002 H_2O extract from rye
8002 MeOH extract from rye
F269 H_2O extract from rye
F269 MeOH extract from rye
OTA standard 10 µg/ml
* - OTA
° - Electroosmotic flow

Figure B

Capillary zone electrophoresis analysis of mycotoxin contamination of the 20 days old fungi inoculated matrices.

- peaks detected with liposome pseudostationary phase are missing in the plain buffer.

Legend:
see above

Figure C

UV spectra of OTA standard measured by capillary electrophoresis DAD detector

- peak with the maximum at 335 nm coheres with the absorption/emission spectra of OTA
- future plan is to optically enhance the capillary electrophoresis instrument based on the absorption/emission spectra to reach higher sensitivities

Merits of the analysis and future challenges

- The liposomes proved not only to be suitable as a pseudostationary separation phase but also as a dissolving matrix for keeping the amphiphilic compounds in the aqueous solution.
- pH value of 6 provided the most stable system for separation of the extracts.
- Posphocholine/phosphoserine composed liposomes showed low retention factor.
- The liposome composition has to be optimized to reach higher retention factors and, therefore, provide better separation of the analytes in extracts.
- Sensitivity of the instrument has to be increased in order to cope with legislation limits by optimization of injection, utilization of stacking of the sample, and optical enhancements of the detector.
- Broader portfolio of mycotoxin standards has to be utilized to identify higher number of the compounds in the extracts.

Acknowledgements: This work was supported by the Programme for research and mobility support of starting researchers of the Czech Academy of Sciences number MSM200311602, by the Ministry of the Interior of the Czech Republic (Project No.VI20172020069), and by institutional support RVO:68081715.

ISBN 978-80-270-4852-6