Journal of Photochemistry and Photobiology B: Biology 151 (2015) 63–68

Contents lists available at ScienceDirect

Journal of Photochemistry and Photobiology B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

Interactions of zearalenone with native and chemically modified cyclodextrins and their potential utilization

Miklós Poór [a,*], Sándor Kunsági-Máté [b,c], Nikolett Sali [c,d], Tamás Kőszegi [c,d], Lajos Szente [e], Beáta Peles-Lemli [b,c]

[a] Department of Pharmacology and Pharmacotherapy, Toxicology Section, University of Pécs, Szigeti út 12, Pécs H-7624, Hungary
[b] Department of General and Physical Chemistry, University of Pécs, Ifjúság útja 6, Pécs H-7624, Hungary
[c] János Szentágothai Research Center, Ifjúság útja 20, Pécs H-7624, Hungary
[d] Institute of Laboratory Medicine, University of Pécs, Ifjúság útja 13, Pécs H-7624, Hungary
[e] CycloLab Cyclodextrin Research & Development Laboratory, Ltd., Illatos út 7, Budapest H-1097, Hungary

ARTICLE INFO

Article history:
Received 22 May 2015
Received in revised form 7 July 2015
Accepted 9 July 2015
Available online 11 July 2015

Keywords:
Zearalenone
Cyclodextrin
Fluorescence spectroscopy
Fluorescence enhancement
Detoxification

ABSTRACT

Zearalenone (ZEA) is a widespread xenoestrogenic mycotoxin produced by several *Fusarium* species. ZEA can cause reproductive disorders in farm animals and hyperoestrogenic syndromes in humans; therefore, development of more sensitive analytical methods (to quantify the mycotoxin) as well as strategies for prevention of its toxic impacts is highly important. In this study, the interactions of ZEA with native and chemically modified cyclodextrins (CDs) were investigated using fluorescence spectroscopy. Furthermore, *in vitro* experiments on liver cells were also performed to test the potential effect of CDs on toxin uptake. Our results demonstrate that ZEA forms stable complexes with CDs (log*K* values are approximately 3.7–4.7) resulting in the considerable elevation of its fluorescence signal. In addition, some of the CDs show ability to inhibit the cellular uptake of ZEA, suggesting their potential suitability to develop new CD-based preventive/detoxification strategies against ZEA in the future.

1. Introduction

Zearalenone (ZEA; previously called as F-2 toxin) is a mycotoxin produced by several *Fusarium* species (e.g. *F. culmorum*, *F. graminearum*, *F. cerealis*, etc.) [1]. ZEA occurs mainly in maize; however, other crops such as barley, rye, wheat and sorghum are also often contaminated with this mycotoxin [1,2]. Because of its wide occurrence and high thermal stability (ZEA is heat stable up to 150 °C) its complete eradication from the food chain does not seem feasible [3]. Chemically ZEA (3,4,5,6,9,10-hexahydro-14,16-dihydroxy-3-methyl-1H-2-benzoxacyclotetradecin-1,7(8H)-dione) is a macrocyclic β-resorcylic acid lactone (see in Fig. 1). Despite that ZEA is a non-steroidal compound, it has xenoestrogenic effect on animals and humans [4]; therefore ZEA is belonging to the endocrine disruptor family. Because of its estrogenic activity, ZEA is able to cause reproductive disorders in farm animals and in some cases hyperoestrogenic syndromes in humans [1,5]. Furthermore, other toxic (e.g. haematotoxic, hepatotoxic, genotoxic, etc.) effects are also attributed to ZEA [1,6,7].

Cyclodextrins (CDs) are extensively studied molecules in the field of host–guest interactions. CDs have a conical structure with a hydrophobic interior and a hydrophilic exterior space [8]. The internal cavity can include a wide range of guest molecules; the stability and selectivity of these complexes are highly influenced by the chemical modification of native CDs [9,10]. The most abundant cyclodextrins are α-, β-, and γ-CDs (are built up from six, seven and eight glucopyranose unites, respectively). Previous studies highlighted that β-CDs are able to form stable complexes with different mycotoxins such as citrinin, aflatoxin B1, ochratoxin A and zearalenone [11–15]. The complex formation commonly results in the fluorescence enhancement of the fluorescent mycotoxins therefore some of these interactions are suitable to develop more sensitive fluorescent analytical methods [16]. Furthermore, the development of different applications in order to remove mycotoxins from aqueous solutions (e.g. from different drinks) is also possible [17]. Recent studies showed that the interaction of ZEA with β-CDs results in the extensive increase of its fluorescence, and revealed the potential analytical utilization of the complex formation, e.g. in the cases of high performance liquid chromatography (HPLC) and capillary electrophoresis (CE) with fluorescent

* Corresponding author at: Department of Pharmacology and Pharmacotherapy, Toxicology Section, University of Pécs, Medical School, Szigeti út 12, H-7624 Pécs, Hungary.
E-mail address: poor.miklos@pte.hu (M. Poór).

http://dx.doi.org/10.1016/j.jphotobiol.2015.07.009
1011-1344/

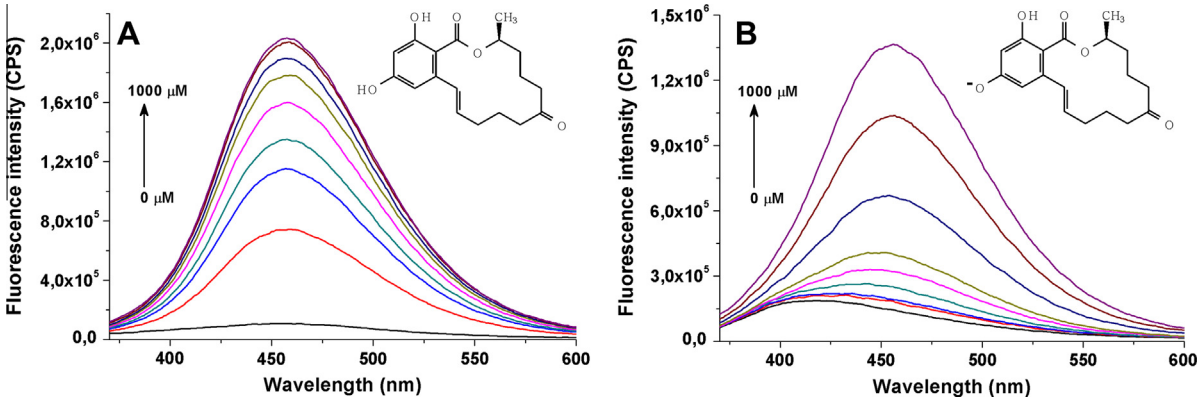

Fig. 1. Fluorescence emission spectra of 2 μM ZEA in 0.05 M ammonium acetate buffer at pH 5.0 (A) and 10.0 (B), in the presence of increasing DIMEB concentrations (0–1000 μM) [λ_{exc} = 315 nm].

detector (FLD) [15,18–21]. Complex formation of ZEA with β-cyclodextrin (BCD), hydroxypropyl-β-cyclodextrin (HPBCD) and heptakis-2,6-di-*O*-methyl-β-cyclodextrin (DIMEB) was proved and characterized by Dall'Asta et al. [15,18].

In our study, the interactions of zearalenone with native and chemically modified cyclodextrins were investigated using fluorescence spectroscopy. In the first step, the fluorescence behavior of ZEA was examined at different pH values. Thereafter, CD-mediated fluorescence enhancement of ZEA was tested as well as the stability constants of ZEA-CD complexes were determined. Finally, the toxic impact of ZEA alone and in presence of CDs was investigated on HepG2 liver cell line, in order to test our hypothesis that some of the CDs may be suitable to inhibit the cellular uptake of ZEA.

2. Materials and methods

2.1. Reagents

All of the applied reagents and solvents were of spectroscopic or analytical grade. Zearalenone (ZEA), bovine serum albumin (BSA), DMEM (Dulbecco's Modified Eagle's Medium) – high glucose (4500 mg/L), fetal bovine serum (FBS), DAPI (4′,6-diamidino-2-phenylindole), penicillin/streptomycin solution (all from Sigma–Aldrich), Coomassie Brilliant Blue G-250, spectroscopic grade methanol and acetonitrile (all from Reanal), β-cyclodextrin (BCD), hydroxypropyl-β-cyclodextrin (HPBCD), randomly methylated-β-cyclodextrin (RAMEB), heptakis-2,6-di-*O*-methyl-β-cyclodextrin (DIMEB), sulfobutylated-β-cyclodextrin (SBCD), (2-hydroxy-3-N,N,N-trimethylamino)propyl-β-cyclodextrin (QABCD), γ-cyclodextrin (GCD) and hydroxypropyl-γ-cyclodextrin (HPGCD) (all from Cyclolab Ltd.) were used as received. 5000 μM stock solution of ZEA was prepared in ethanol (Reanal, spectroscopic grade) and stored at 4 °C, protected from light. Spectroscopic measurements of ZEA in absence and presence of cyclodextrins were performed in 0.05 M ammonium acetate buffer (pH 5.0 and 10.0), in order to avoid the interferences of potential interaction of ZEA with alkali and/or alkaline earth metal ions.

2.2. Fluorescence spectroscopic measurements

Fluorolog τ3 spectrofluorometric system (Jobin–Yvon/SPEX) was applied for steady state fluorescence spectroscopic measurements. All analyses were performed in the presence of air at +25 °C. Binding constants (*K*) of ZEA-cyclodextrin complexes were determined by the Benesi–Hildebrand equation, assuming 1:1 stoichiometry:

$$\frac{I_0}{(I - I_0)} = \frac{1}{A} + \frac{1}{A * K * [H]^n} \quad (1)$$

where *K* is the binding constant, I_0 is the initial fluorescence intensity of ZEA (in the absence of cyclodextrins), *I* is the fluorescence intensity of ZEA in the presence of the host molecule with concentration [*H*], while *A* is a constant and *n* is the number of binding sites.

2.3. Tissue cultures

HepG2 cells (human liver, ATCC: HB-8065) were cultured in DMEM with 10% FBS, penicillin (100 U/mL) and streptomycin (100 μg/mL) in 25 cm^2 sterile plastic flasks (VWR). Cells were grown at 37 °C in humidified atmosphere, in the presence of 5% CO_2. Cells were plated into 24-well sterile plastic plates (VWR); and after attachment of cells (24 h), the medium was replaced with fresh one and HepG2 cells were treated with 0–100 μM ZEA in the absence and in the presence of CDs. Measurements were performed after 24-h incubation. All sterile work was carried out in an Aireguard-126300 (Nuaire) vertical laminar box.

2.4. Quantification of the number of living cells

In order to investigate the number of living cells, total DNA content/well was determined. The wells were washed twice with ice-cold PBS and the emptied wells were filled with 500 μL of 5% perchloric acid (PCA). Estimation of the number of the attached cells was performed by DAPI staining of PCA fixed cells. The wells were gently washed with McIlvaine's buffer (citric acid/Na_2HPO_4, pH 7.0) then 200 μL of a 10 μg/mL DAPI solution in the above buffer was pipetted into each well (24-well plates). Staining was performed at room temperature (30 min). After washing, 200 μL McIlvaine's buffer was pipetted into each well and the fluorescence of the samples was determined with a plate reader (Perkin Elmer EnSpire Multimode reader) applying 355 nm excitation and 460 nm emission wavelengths using area scan mode (cylindrical scan with 80 measuring points/well). Fluorescence intensity was expressed as the sum of the 80 measuring points. Unstained and untreated cells were used as blanks.

To confirm the results derived from DNA staining, quantification of total protein levels/well was also performed. Intracellular proteins from the cells in the wells were solubilized by 500 μL of 1 M NaOH (15 min incubation at room temperature). Total intracellular protein levels were quantified by the Bradford reaction with the measurement of absorbance at 595 nm, applying purified BSA as standard (concentration range: 20–100 mg/L). Then 20 μL