Journal of Photochemistry & Photobiology, B: Biology 170 (2017) 16–24

Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology, B: Biology

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

Interaction of mycotoxin zearalenone with human serum albumin

Miklós Poór [a,b,*], Sándor Kunsági-Máté [c,d], Mónika Bálint [b,e], Csaba Hetényi [b,f], Zsófia Gerner [c], Beáta Lemli [c,d]

[a] Department of Pharmacology, University of Pécs, Faculty of Pharmacy, Szigeti út 12, Pécs H-7624, Hungary
[b] Department of Pharmacology and Pharmacotherapy, University of Pécs, Medical School, Szigeti út 12, Pécs H-7624, Hungary
[c] Department of General and Physical Chemistry, University of Pécs, Ifjúság útja 6, Pécs H-7624, Hungary
[d] János Szentágothai Research Center, Ifjúság útja 20, Pécs H-7624, Hungary
[e] Department of Biochemistry, Eötvös Loránd University, Pázmány sétány 1/C, Budapest H-1117, Hungary
[f] MTA-ELTE Molecular Biophysics Research Group, Hungarian Academy of Sciences, Pázmány sétány 1/C, Budapest H-1117, Hungary

ARTICLE INFO

Article history:
Received 29 August 2016
Received in revised form 19 March 2017
Accepted 21 March 2017
Available online 27 March 2017

Keywords:
Zearalenone
Human serum albumin
Albumin-ligand interaction
Fluorescence spectroscopy

ABSTRACT

Zearalenone (ZEN) is a mycotoxin produced mainly by *Fusarium* species. Fungal contamination of cereals and plants can result in the formation of ZEN, leading to its presence in different foods, animal feeds, and drinks. Because ZEN is an endocrine disruptor, it causes reproductive disorders in farm animals and hyperoestrogenic syndromes in humans. Despite toxicokinetic properties of ZEN were studied in more species, we have no information regarding the interaction of ZEN with serum albumin. Since albumin commonly plays an important role in the toxicokinetics of different toxins, interaction of ZEN with albumin has of high biological importance. Therefore the interaction of ZEN with human serum albumin (HSA) was investigated using spectroscopic methods, ultrafiltration, and molecular modeling studies. Fluorescence spectroscopic studies demonstrate that ZEN forms complex with HSA. Binding constant (K) of ZEN-HSA complex was quantified with fluorescence quenching technique. The determined binding constant ($\log K = 5.1$) reflects the strong interaction of ZEN with albumin suggesting the potential biological importance of ZEN-HSA complex formation. Based on the results of the investigations with site markers as well as docking studies, ZEN occupies a non-conventional binding site on HSA. Considering the above listed observations, we should keep in mind this interaction if we would like to precisely understand the toxicokinetic behavior of ZEN.

1. Introduction

The foodborne mycotoxin zearalenone (ZEN) is produced by several *Fusarium* species [1]. ZEN occurs principally in cereals (e.g. maize, barley, rye, wheat, and sorghum), however, other sources of the mycotoxin were described as well, such as nuts, edible oils, spices, milk, beer, and drinking water [1–3]. The wide occurrence and high thermal stability of ZEN (it is stable up to 150 °C) are making very difficult its eradication from the food chain [4]. ZEN is an endocrine disruptor molecule because of its xenoetrogenic effect on animals and humans [5]. The effect of ZEN on estrogen receptors leads to reproductive disorders in farm animals as well as hyperoestrogenic syndromes in humans [1,6]. In addition, further toxic impacts of ZEN are also described in previous studies (e.g. haematotoxic, hepatotoxic, or genotoxic effects) [1,7].

Albumin is the most abundant plasma protein in the blood. Human serum albumin (HSA) maintains the oncotic pressure and the pH in the human circulation as well as one of its very important functions is the complex formation with several endogenous (e.g., bilirubin and fatty acids) and exogenous (e.g., drugs and xenobiotics) compounds [8]. Thus, HSA commonly plays a crucial role in the pharmacokinetics and toxicokinetics of drugs and toxins, respectively [9,10]. Albumin is built up from three domains (I, II, and III), and each domain contains two subdomains (A and B). There are two main binding site on albumin for most of the drugs and xenobiotics, Sudlow's Site I (located on subdomain IIA) and Sudlow's Site II (on subdomain IIIA) [8]. However, recent studies highlighted that a third binding site on sundomain IB (Heme binding site) is also commonly involved [11]. Formation of stable non-covalent complexes of aflatoxin B1, citrinin, deoxynivalenol, and patulin with HSA were described in previous studies [12–15]. Furthermore, highly stable interaction of HSA with ochratoxin A was demonstrated by many studies as well [10]. Despite several toxicokinetic studies performed with ZEN investigating more species (including man) [16–20], we have no information regarding ZEN-albumin interaction.

In this study the interaction of ZEN with human serum albumin was investigated applying fluorescence spectroscopic methods as well as ultrafiltration and molecular modeling studies. Herein, we demonstrate that ZEN forms stable complex with albumin, occupying a non-conventional binding site on HSA. Our data suggest that ZEN-HSA interaction may play an important role in the toxicokinetics of ZEN.

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

http://dx.doi.org/10.1016/j.jphotobiol.2017.03.016
1011-1344/

2. Materials and Methods

2.1. Reagents

All of the applied reagents and solvents were of spectroscopic or analytical grade. Zearalenone (ZEN), human serum albumin (HSA; purity: >96%), bilirubin (BIL), ibuprofen (IBU), L-thyroxine, ochratoxin A (OTA), phenylbutazone (PBZ), and warfarin (WAR) were obtained from Sigma. 5000 μM stock solution of ZEN was prepared in ethanol (Reanal, spectroscopic grade) and stored at −20 °C. In order to mimic extracellular physiological conditions, measurements were performed in phosphate buffered saline (PBS, pH 7.4).

2.2. Spectroscopic Measurements

Fluorescence spectroscopic measurements were carried out by Fluorolog τ3 spectrofluorometric system (Jobin-Yvon/SPEX) and Hitachi F-4500 fluorescence spectrophotometer. UV-VIS spectra were recorded applying Specord Plus 210 (Analytic Jena AG, Jena, Germany) and HALO DB-20 (Dynamica, London, UK) spectrophotometers. All analyses were performed at 25 °C.

Fluorescence anisotropy (r) data were determined using the following equation:

$$r = \frac{(I_{VV} - G * I_{VH})}{(I_{VV} + 2 * G * I_{VH})} \quad (1)$$

where I_{VV} and I_{VH} are fluorescence intensities measured in vertical position of polarizer at pre-sample site and at vertical and horizontal position of post-sample polarizer, respectively; while G is the instrumental factor.

Considering the additive behavior of anisotropy, in a mixed system where both ZEN and HSA show fluorescence property as well as assuming absence of interaction between ZEN and HSA, the minimal value of fluorescence anisotropy of the samples can be calculated as the following:

$$r = f_{ZEN} * r_{ZEN} + f_{HSA} * r_{HSA} \quad (2)$$

where r is fluorescence anisotropy of the sample, f_{ZEN} and f_{HSA} are the fractions of ZEN and HSA in the solution, respectively; while r_{ZEN} and r_{HSA} are the anisotropies of ZEN and HSA alone, respectively. If measured fluorescence anisotropy values exceed the values calculated using Eq. (2), that reflects the interaction of ZEN with HSA.

Binding constant of ZEN-HSA complex was calculated applying Hyperquad2006 program package, assuming 1:1 stoichiometry [21,22].

To investigate the role of drug binding site I regarding ZEN-HSA complex formation, interaction of ZEN with warfarin and ochratoxin A was investigated in the presence of HSA, using our previously described models with minor modifications: (1) Increasing amounts of ZEN (0–10 μM) were added to 1 μM warfarin and 3.5 μM HSA (in PBS, pH 7.4) then fluorescence emission spectra were recorded using 317 nm excitation wavelength [13,23]; (2) Increasing amounts of ZEN or warfarin (0–50 μM) were added to 1 μM OTA and 1.4 μM HSA (in PBS, pH 7.4) then fluorescence anisotropy was determined applying 393 and 446 nm excitation and emission wavelengths, respectively [13,24].

2.3. Thermodynamic Studies

The thermodynamic parameters, enthalpy and entropy changes associated to the complex formation between ZEN and HSA molecules were calculated as follows: the logarithms of binding constants (log K) at different temperatures were plotted against the reciprocal temperature according to the van't Hoff equation. The enthalpy change was derived from the slope while the entropy change was derived from the intercept of the line fitted to the data measured at different temperatures:

$$\log K = \frac{-\Delta H}{2.303 \cdot \mathrm{R}} \cdot \frac{1}{T} + \frac{\Delta S}{2.303 \cdot \mathrm{R}} \quad (3)$$

where ΔH and ΔS reflect the enthalpy and entropy change of the association reaction, while R is the gas constant and T refers the temperature.

2.4. Ultrafiltration

Ultrafiltration studies were performed using the procedure described earlier [13]. In order to remove glycerol, Amicon Ultra-4 centrifugal filter units (10 kDa molecular weight cut-off; from Merck Millipore) were washed twice with 3 ml water then with 3 ml PBS. Samples (2.5 ml) were transferred into the filter after which centrifuged at 7500g (fixed-angle rotor) for 10 min at 25 °C. Thereafter, WAR concentrations were quantified in the filtrate based on the fluorescence intensity of WAR (λ_{exc} = 309 nm, λ_{em} = 389 nm) using a calibration curve (0.1–1.0 μM). No spectral interferences were observed during these experiments because both ZEN (possibly because of its adsorption by the filter) and HSA (because of the large size of the protein) are unable to reach the filtrate. Regardless of the concentration used (0.2–1.0 μM WAR concentrations were tested), constant recovery of WAR was observed (80.3 ± 2.1%).

2.5. Structural Calculations

2.5.1. Ligand Preparation

The zearalenone molecule was built in Maestro [25]. The raw structure was energy minimized, using the semi-empirical quantum chemistry program package, MOPAC [26] and the PM6 parameterization. The gradient norm was set to 0.001. The energy minimized structure was subjected to force calculations. The force constant matrices were positive definite.

2.5.2. Target Preparation

An apo crystallographic structure (PDB code: 1ao6) was used as target a molecule in our calculations. Acetyl and amide capping groups were attached to the N- and C-termini, respectively, using the Schrödinger Maestro program package v. 9.6 [25]. As 1ao6 contains a homodimer structure, only chain A was used for calculations. Co-crystallized ions and water molecules were removed before minimizing the protein structure. The target molecule was minimized using a two-step protocol with the GROMACS [27] software package, including a steepest descent and a conjugate gradient step, using AMBER99-ildn force field [28]. Exit tolerance levels were set to 1000 and 10 kJmol^{-1} nm^{-1} while maximum step sizes were set to 0.5 and 0.05 nm, respectively. His464 was modeled in three forms, with proton at the epsilon, delta, and both positions, respectively.

2.5.3. Blind Docking

Using the optimized ligand and target structures, blind docking calculations were performed with AutoDock 4.2 [29] program package as described in our previous publications [13,30,31]. Gasteiger-Marsilli partial charges were added to both ligand and target atoms, using AutoDock Tools [29] and a united atom representation was applied for non-polar moieties. A grid box of 200 × 200 × 200 points, and 0.375 Å spacing was calculated and centered on the center of mass of the target by AutoGrid 4. Lamarckian genetic algorithm was used for global search. Flexibility at two active torsions was allowed on the ligand, number of docking runs was set to 100, numbers of energy evaluations and generations were 20 million [32]. The docked ligand copies were ordered according to AutoDock 4 scores [33], and subsequently clustered using a tolerance of 1.75 Å root mean square deviation (RMSD) between cluster