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Full Length Article

Fluorescence spectroscopic investigation of the interaction of citrinin with native and chemically modified cyclodextrins

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ABSTRACT

Citrinin (CIT) is a nephrotoxic mycotoxin produced by several *Aspergillus*, *Penicillium* and *Monascus* species. CIT is unavoidable contaminant of different foods and drinks due to its wide occurrence and high thermal stability. For this reason, development of new, more sensitive analytical methods and decontamination strategies has high importance. In our study, the complex formation of CIT with native and chemically modified cyclodextrins was investigated using fluorescence spectroscopy. Furthermore, thermodynamic and molecular modeling studies were also performed for the deeper understanding of these host-guest interactions. Our results show that among the tested compounds methylated β-cyclodextrins form the most stable complexes with CIT and these derivatives cause the highest fluorescence enhancement of CIT as well. These observations recommend that some of the chemically modified derivatives show more favourable properties than the native cyclodextrin, and suggesting more promising analytical applicability and higher affinity as potential toxin binders.

1. Introduction

Mycotoxins are toxic secondary metabolic products of various fungi. The foodborn mycotoxin citrinin (CIT; Fig. 1) is produced mainly by *Aspergillus*, *Penicillium* and *Monascus* species [1,2]. Due to the wide environmental occurrence of fungi, mycotoxins are unavoidable contaminants of foods, drinks and animal feeds. CIT appears principally in maize, rye, oats, barley, wheat and rice [1,2]. The high thermal stability of CIT makes also more difficult of its eradication from the food chain: It is decomposed at 175 °C by dry heating and at 140 °C in presence of water; however, the resulting decomposition products are as toxic as or more toxic than CIT itself [3]. CIT is a nephrotoxic mycotoxin with a complex mechanism of action (e.g. induction of cell cycle arrest, oxidative stress and apoptosis, etc.) [4–7]. Several analytical techniques are applied for the quantitative determination of CIT; HPLC-FLD (high-performance liquid chromatography with fluorescence detection) methods are commonly applied because of the strong fluorescence of CIT under acidic conditions [8–10].

Cyclodextrins (CDs) are extensively studied host molecules built up from glucopyranose unites [11]. CDs have a conical structure with a hydrophobic interior as well as a hydrophilic exterior space [12]. The internal cavity can include different guest molecules and the stabilities of these host-guest complexes can be considerably increased by various chemical modifications [13]. Mycotoxins commonly form stable complexes with native or chemically modified cyclodextrins [14–17]. This complex formation is usually associated with the fluorescence enhancement of fluorescent mycotoxins (e.g., aflatoxin and zearalenone), resulting in the possibility to apply CDs in order to develop more sensitive fluorescent analytical methods [17–19]. Furthermore, recent studies also highlighted that mycotoxins can be effectively extracted from aqueous solutions by CD polymers [20,21], suggesting that CD technology may be suitable to decontaminate different toxin-exposed drinks. The interaction of CIT with native β-cyclodextrin was also described [22]; on the other hand, we did not find any other references regarding CIT–CD interactions during our literature survey.

In this study, the interaction of CIT with native α-, β- and γ-cyclodextrins as well as with chemically modified β-cyclodextrins was investigated using steady-state fluorescence spectroscopy. Because previously only the native β-cyclodextrin was studied, our main goal was to identify more appropriate CDs which form more

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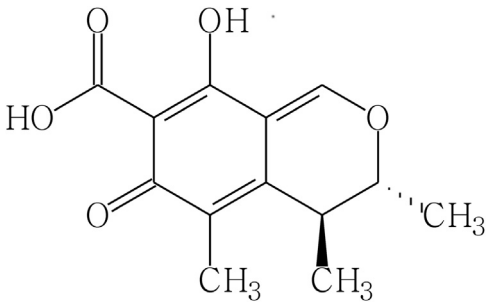

Fig. 1. Chemical structure of the mycotoxin citrinin.

stable complexes with CIT and cause stronger fluorescence enhancement of CIT compared to β-cyclodextrin. Our results demonstrate that both of these requirements were fulfilled by some of the tested CD derivatives.

2. Materials and methods

2.1. Reagents

All applied reagents were of analytical or spectroscopic grade. Citrinin (CIT) was purchased from Sigma-Aldrich; 5000 μM stock solution was prepared in ethanol (Reanal, spectroscopic grade) and stored at 4 °C protected from light. Cyclodextrins, namely α-cyclodextrin (ACD), β-cyclodextrin (BCD), hydroxypropyl-β-cyclodextrin (HPBCD), sulfobutylated-β-cyclodextrin (SBCD), randomly methylated-β-cyclodextrin (RAMEB), heptakis-2,6-di-*O*-methyl-β-cyclodextrin (DIMEB) and γ-cyclodextrin (GCD) were obtained from CycloLab Cyclodextrin Research & Development Laboratory, Ltd.

2.2. Fluorescence spectroscopic measurements

Fluorolog τ3 spectrofluorometric system (Jobin-Yvon/SPEX) was applied for fluorescence measurements. All analyses were performed at 25 °C except thermodynamic studies. During the measurements 1 μM CIT was applied in absence and presence of increasing CD concentrations (0–10 mM). Fluorescence spectra were recorded using 330 nm and 505 nm as excitation and emission wavelengths, respectively.

Binding constants of CIT–CD complexes were determined by the Benesi–Hildebrand equation, assuming 1:1 stoichiometry:

$$\frac{F_0}{(F-F_0)}=\frac{1}{A}+\frac{1}{A*K*[\mathrm{CD}]^n} \quad (1)$$

where K is the actual binding constant, F_0 is the initial fluorescence intensity of CIT (in absence of CDs), F is the fluorescence intensity of CIT in presence of CDs, with concentration [CD], while A is a constant and n is the number of binding sites ($n=1$).

To get insights into the formation details of CIT–BCD or CIT–DIMEB complexes the measurements were repeated and the binding constants were determined at five different temperatures: 20 °C, 25 °C, 30 °C, 35 °C and 40 °C. Thermodynamic parameters were determined for the formation of CIT–BCD and CIT–DIMEB complexes using the van't Hoff equation:

$$\ln K=-\frac{\Delta G}{RT}=-\frac{\Delta H}{RT}+\frac{\Delta S}{R} \quad (2)$$

where ΔG is the Gibbs free energy change, ΔS is the entropy change and ΔH is the enthalpy change associated with the complex formation.

2.3. Molecular modeling studies

In the case of DIMEB, for each glucose units, two methyl groups were used to replace hydrogens of OH groups at the specified positions i.e. of the OH groups on the C-2 and C-6 carbon atoms. In the case of RAMEB, where the methyl substituents at the synthesis were placed randomly, all the OH groups were considered equivalent at the preparation of the model structure, thus at the lower rim four methyl groups were used to replace the hydrogen atoms of OH groups at C-6 , while at the upper rim eight at C-2 and/or C-3 positions.

According to the carried out experimental studies, where aqueous solutions were used and pH=2.0 was applied (phosphate buffer), the citrinin molecule as having $pK_a=2.3$ [22] was considered to have neutral charge. Its structure was optimized in vacuum by B3LYP/6-31G(d) density functional and basis set. The initial structures of the three CDs (BCD, RAMEB and DIMEB) were obtained from molecular dynamics simulation with explicit water molecules using the MM+ force field in the HyperChem 8.0 program. In the cases of CIT and the CDs, the atomic charges for the subsequent molecular docking studies were calculated using the B3LYP/6-31G(d) method and basis set by performing natural population analysis (NPA).

The docking studies to determine the possible positions and orientations of CIT within the cavity of cyclodextrins and the associated binding affinities (thus approximating ΔG of the complexation – where van der Waals and electrostatic interactions, hydrogen-bond formations, desolvation and also entropy change at the complex formation are considered in the applied model) were carried out using the Vina 1.1.2 [23] program. The necessary .pdbqt input files were prepared using the OpenBabel program.

3. Results and discussion

3.1. Effect of pH on fluorescence emission spectrum of CIT

In order to determine the optimal pH range for our measurements, 0.05 M phosphate buffers were prepared (pH=2.0, 2.5, 3.0 and 4.0). Under the applied conditions, CIT has excitation and emission wavelength maxima at 330 nm and 505 nm, respectively. In agreement with previous studies [22] we also observed that the decrease of pH results in the fluorescence enhancement of CIT (Fig. 2). Since the pK_a value of CIT is 2.3 in water [22], this phenomenon can be attributed to the elevation of the highly fluorescent nonionic form of CIT, which predominates at pH 2.0. At

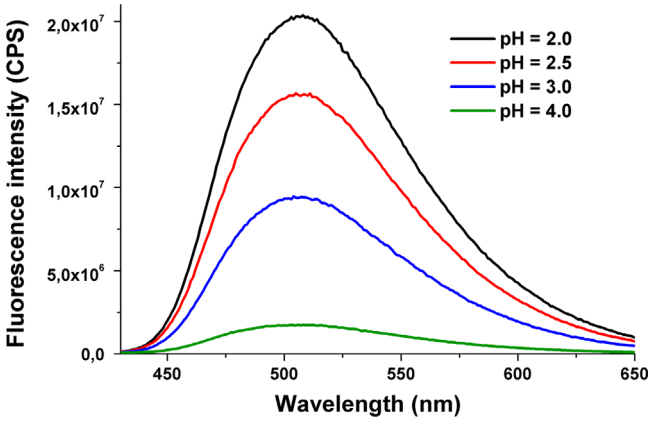

Fig. 2. Fluorescence emission spectra of citrinin in 0.05 M phosphate buffer at pH 2.0, 2.5, 3.0, 4.0 [$\lambda_{exc}=330$ nm].