



Interaction of zearalenone-14-sulfate with cyclodextrins and the removal of the modified mycotoxin from aqueous solution by beta-cyclodextrin bead polymer

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ABSTRACT

The xenoestrogenic mycotoxin zearalenone is a common food contaminant produced by *Fusarium* strains. The modified mycotoxin zearalenone-14-sulfate (Z14S) is formed in both fungi and mammals during the biotransformation of zearalenone. Cyclodextrins (CD) are cyclic oligosaccharides which can form host-guest type complexes with some mycotoxins, including zearalenone, zearalenols, and zearalenone-14-glucoside. As a result of the complex formation, the fluorescence signal of these mycotoxins strongly increases. Furthermore, CD polymers seem to be suitable for the extraction of some mycotoxins from aqueous solutions and beverages. In this study, the interaction of Z14S with CDs and soluble CD polymers was examined with fluorescence spectroscopy and molecular modeling. Furthermore, the removal of Z14S from aqueous solution by β -CD bead polymer (BBP) was also tested. Our results demonstrate the formation of stable Z14S-CD complexes ($K = 0.1$ to 5.0×10^4 L/mol). Dimethyl- β -CD (DIMEB) produced the most stable complexes with Z14S at pH 5.0 and 7.4. At pH 10.0, the binding constant of Z14S-DIMEB complex decreased and quaternary ammonium- β -CD showed similar affinity toward the mycotoxin than DIMEB. In addition, Z14S was successfully removed from aqueous solutions by BBP. Considering the above-listed observations, besides the parent mycotoxins, some of their modified/masked derivatives can also interact with CDs.

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1. Introduction

Zearalenone is a toxic secondary metabolite of *Fusarium* species, which frequently contaminate grains (especially maize) and other commodities (e.g., fruits, meat, pastry, and beverages) [1,2]. The consumption of zearalenone-contaminated food and feed can result in the development of reproductive disorders in animals and humans, due to the xenoestrogenic effect of the mycotoxin and its metabolites [2–4]. Zearalenone is highly biotransformed by mammals, plants, and fungi, during which reduced (zearalenols, zearalanone, and zearalanols) and conjugated metabolites are formed [4,5].

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Mycotoxin conjugates produced by plants are designated as masked mycotoxins, while other compounds formed by structural modification of the parent mycotoxins are referred to modified mycotoxins [5,6]. Zearalenone-14-sulfate (Z14S; also termed as zearalenone-4-sulfate; Fig. 1) is produced by different strains (e.g., *Aspergillus oryzae*, *Fusarium* and *Rhizopus* species) as a fungal metabolite or by sulfotransferase (SULT) enzyme during the phase II metabolism of zearalenone in mammals [4,5]. Generally, the sulfate conjugation results in less toxic derivatives, due to the increased hydrophilicity of the metabolite formed. However, Z14S can be degraded partly to the more toxic parent compound by the human intestinal microbiome [7]. Therefore, we need to consider Z14S as a similarly toxic compound to zearalenone, and should take into consideration during the health risk assessment of the exposure [5].

Cyclodextrins (CDs) are cyclic glucose oligomers joined through 1–4 glycosidic linkage. CDs possess a lipophilic internal cavity and a hydrophilic external part, the latter provides them high aqueous solubility [8,9]. Since CDs can entrap nonpolar molecules in their internal cavity,

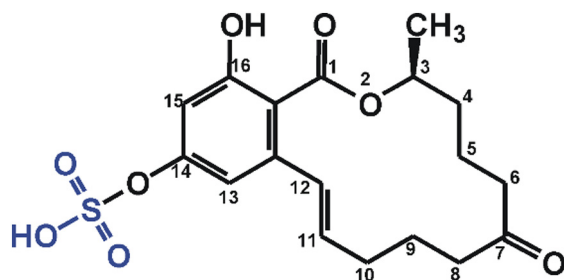


Fig. 1. Chemical structure of zearalenone-14-sulfate (Z14S).

they are commonly utilized by pharmaceutical, cosmetic, and food industries. The three most frequently applied CDs are α -, β -, and γ -CDs, containing six, seven, and eight D-glucopyranose units, respectively [9]. The complexation of guest molecules by CDs is strongly influenced by the chemical modification of the host [9,10]. As it has been reported, CDs can interact with some mycotoxins (and their derivatives), including aflatoxins, citrinin, dihydrocitrinone, ochratoxin A, zearalenone, zearalenols, and zearalenon-14-glucoside [11–17]. Typically, the complex formation of fluorescent mycotoxins with CDs increases their fluorescence signal, which may be beneficial from the analytical point of view [18,19]. Furthermore, based on previous studies, CD polymers seem to be suitable for the removal of certain mycotoxins (e.g., patulin, ochratoxin A, alternariol, zearalenone, and zearalenols) and masked mycotoxins (e.g., zearalenone-14-glucoside) from aqueous solutions, including beverages (such as fruit juices, wine, or beer) [17,20–23].

Despite the fact that modified mycotoxins commonly appear in grains and in other commodities, we have only limited information regarding their interactions with CDs. Since the masked mycotoxin zearalenone-14-glucoside forms complexes with CDs [17], it is reasonable to hypothesize that other modified mycotoxins may also be entrapped by CDs. Therefore, in this study, the complex formation of Z14S with native and chemically-modified CDs and soluble CD polymers (pH 5.0–10.0) was investigated employing fluorescence spectroscopy and molecular modeling. Furthermore, the removal of Z14S from aqueous solution by insoluble β -cyclodextrin bead polymer (BBP) was also investigated. Our results demonstrate that, similarly to zearalenone, Z14S also formed stable complexes with native and chemically-modified CDs and BBP successfully removed the modified mycotoxin from aqueous solution.

2. Materials and methods

2.1. Reagents

Zearalenone-14-sulfate ammonium salt (Z14S) was obtained from ASCA GmbH (Berlin, Germany). Z14S stock solution (5000 μ M) was prepared in spectroscopic grade ethanol (96 v/v%; Reanal, Budapest, Hungary) and stored at -20°C . Cyclodextrins, including β -CD (BCD), γ -CD (GCD), randomly methylated β -CD (RAMEB), heptakis-2,6-di-O-methyl- β -cyclodextrin (DIMEB), 6-monodeoxy-6-monoamino- β -cyclodextrin (MABCD), (2-hydroxy-3-*N,N,N*-trimethylamino)propyl- β -cyclodextrin (QABCD), (2-hydroxypropyl)- β -CD (HPBCD), sulfobutyl- β -cyclodextrin (SBCD), epichlorohydrin cross-linked soluble BCD polymer (BCD content: 70 m/m%), epichlorohydrin cross-linked soluble QABCD polymer (QABCD content: 60 m/m%), and insoluble (water-swellaable) β -cyclodextrin bead polymer (BBP; cyclodextrin-epichlorohydrin cross-linked bead polymer; BCD content: 50 m/m%) were provided by CycloLab Cyclodextrin Research and Development Laboratory, Ltd. (Budapest, Hungary).

2.2. Spectroscopic measurements

Fluorescence spectra were recorded at 25°C in the presence of air, using a Hitachi F-4500 fluorimeter (Tokyo, Japan). In spectroscopic studies, sodium acetate (0.05 M, pH 5.0), sodium phosphate (0.05 M, pH 7.4), and sodium borate (0.05 M, pH 10.0) buffers were applied. Fluorescence emission spectra of Z14S were examined in the presence of increasing CD concentrations, applying 320 nm excitation wavelength.

Binding constants (K ; unit: L/mol) of Z14S-CD complexes were determined based on the graphical application of Benesi-Hildebrand equation [13]:

$$\frac{I_0}{(I-I_0)} = \frac{1}{A} + \frac{1}{AK[CD]^n} \quad (1)$$

where I_0 and I are the fluorescence emission intensity of Z14S in the absence and presence of CDs, respectively. A is a constant, n is the number of binding sites, and $[CD]$ is the concentration (unit: L/mol) of the host molecule.

2.3. Molecular modeling studies

The molecular modeling approach relied on a combination of pharmacophoric analysis of CD cavity integrated with docking studies to provide a plausible architecture of binding. The 3D structures of β -CD, γ -CD, and DIMEB derived from the crystallographic structures recorded in the Cambridge Crystallographic Data Center (CCDC) database (<https://www.ccdc.cam.ac.uk/structures>) having accession code WEWTOJ, LAJLALO2, and ZULQAY, respectively. The 3D coordinates of Z14S were retrieved from PubChem (<https://pubchem.ncbi.nlm.nih.gov>; CID = 45359500, accessed on November 21, 2019). The consistency of atom and bond types assignment of Z14S and CDs were checked with the Sybyl software (version 8.1; www.certara.com), as previously reported [24]. Z14S was computed in the deprotonated form and the 3D structure was energetically minimized using Powell algorithm (with a coverage gradient of ≤ 0.05 kcal/(mol \times Å) and a maximum of 250 iterations) before the analysis, as it has been reported [17].

The description of CD sites was carried out using the FlapSite tool of the FLAP software (Fingerprint for Ligand And Protein; <https://www.moldiscovery.com>) while the GRID algorithm was used to investigate the corresponding pharmacophoric space [25,26] in agreement with previous studies [27]. The GOLD software [28] was used to perform all the docking simulations as it previously proved to be reliable in predicting the binding architectures of host-guest type complexes [17,29,30]. GOLD setting reported by Dellaflora and co-workers was used [29]. As exception, ten poses were generated for each compound in each CD and the best-scored pose according to the GOLDScore scoring function was considered to represent the most probable architecture of binding, in agreement with previous works [17,31–33].

2.4. Extraction of Z14S from aqueous buffer by BBP

To investigate the removal of Z14S by BBP, the mycotoxin (2 μ M in 1.5 mL volume) was incubated with increasing amounts of the bead polymer (0.0, 1.0, 2.5, 5.0, 10.0, and 20.0 mg/1.5 mL) in sodium acetate buffer (0.05 M, pH 5.0; 40 min, 1000 rpm, 25°C). Then BBP was sedimented by pulse centrifugation (4000g, 6 s, room temperature) and the mycotoxin content of the supernatant was determined by high-performance liquid chromatography (HPLC; see in Section 2.5).

Using the same experimental conditions, increasing mycotoxin concentrations (0.2, 2.5, 5.0, 7.5, and 10.0 μ M in 1.5 mL volume) were added to standard amount of BBP (2.0 mg/1.5 mL), after which the Z14S content of the supernatants were quantified. Data were evaluated applying the Langmuir and Freundlich sorption isotherms [21,23]. The Langmuir

equation was described as:

$$q_e = \frac{Q_0 K_L C_e}{1 + K_L C_e} \quad (2)$$

where q_e is the bound amount of Z14S (mg/g BBP), Q_0 represents the maximum amount of Z14S bound (mg/g BBP), C_e denotes the free amount of Z14S (mg) in the solution at equilibrium, and K_L is the Langmuir equilibrium constant (L/mg). The Freundlich equation was described as:

$$q_e = K_F C_e^{1/n} \quad (3)$$

where n and K_F are the heterogeneity index and the Freundlich constant, respectively.

2.5. HPLC analysis

Z14S concentrations in the supernatants were quantified by an integrated HPLC system (Jasco, Tokyo, Japan), which includes an autosampler (AS-4050), a binary pump (PU-4180), and a fluorescence detector (FP-920). A 20- μ L volume of samples was driven through a Phenomenex Security Guard™ (C18, 4.0 \times 3.0 mm) guard column joined to a Kinetex (C18, 250 \times 4.6 mm, 5 μ m) analytical column. The isocratic elution was performed with 1.0 mL/min flow rate at room temperature. The mobile phase contained methanol, acetonitrile, and distilled water (6:35:59 v/v%). Z14S was detected at 465 nm (λ_{ex} = 330 nm), and the chromatograms were evaluated with ChromNAV software.

2.6. Statistics

Statistical analyses were performed with one-way ANOVA test (IBM SPSS, New York, NY, USA). The level of significance was set to $p < 0.01$.

3. Results and discussion

3.1. Fluorescence excitation and emission spectra of zearalenone-14-sulfate

First, the fluorescence excitation and emission spectra of Z14S were recorded in different buffers (pH 5.0, 7.4, and 10.0; Fig. 2). The excitation wavelength maximum of Z14S was noticed around 330 nm under weakly acidic (pH 5.0) and physiological (pH 7.4) circumstances. However, at pH 10.0, a slight red shift of the maximum (330 nm \rightarrow 334 nm) and a significant decrease in the fluorescence signal were noticed (Fig. 2A). Furthermore, at pH 5.0 and 7.4, the emission spectra of the

mycotoxin were similar, while a blue shift of the wavelength maximum (465 nm \rightarrow 458 nm) and the decreased emission signal were observed at pH 10.0 (Fig. 2B). Since the protonation state can influence the absorption and fluorescence spectra of the fluorophores [34–36], these spectral changes are likely resulted from the deprotonation of the mycotoxin at the highest pH tested (similarly to zearalenone, zearalenols, and zearalenone-14-glucoside [17,37,38]): The phenolic hydroxyl group of Z14S on C16 may lose its proton under alkaline conditions.

3.2. Effects of cyclodextrins on the fluorescence emission signal of zearalenone-14-sulfate

Fluorescence spectra of Z14S were also examined in the presence of native β - and γ -CDs at pH 5.0. CDs induced a blue shift in the excitation wavelength maximum of Z14S (data not shown); therefore, emission spectra were recorded using 320 nm excitation wavelength. In the presence of CDs, the fluorescence emission signal of the mycotoxin was considerably increased and a slight blue shift of the emission wavelength maximum (465 nm \rightarrow 460 nm) was observed (Fig. 3). Typically, the inclusion of organic fluorophores by β -CDs results in a blue shift in their fluorescence emission wavelength maxima [34,35]. This fact and the observation that CDs alone did not exert significant fluorescence under the applied conditions suggest the formation of host-guest type Z14S-CD complexes. The fluorescence enhancement observed can be explained by the decreased quenching effect of water molecules due to the inclusion of Z14S in the nonpolar CD cavity [39,40]. BCD induced higher fluorescence enhancement of the mycotoxin (Fig. 3B) and formed more stable (approximately three-fold; see details later in Section 3.3) complexes with Z14S than GCD. Thus, Z14S favors the smaller cavity of BCD vs. GCD, similarly to zearalenone [37]. Interestingly, the masked mycotoxin zearalenone-14-glucoside showed the opposite [17]. In the following experiments, BCD and its chemically modified derivatives were studied further.

3.3. Interaction of zearalenone-14-sulfate with native and chemically modified cyclodextrins in different buffers

Emission spectrum of Z14S was recorded in the presence of increasing CD concentrations in different buffers. At pH 5.0, β -CDs induced 14- to 21-fold increase in the emission signal of the mycotoxin at 460 nm (Fig. 4 and Table 1). Monoamino- (MABCD) and dimethyl- β -CDs (DIMEB) caused stronger while other derivatives resulted in weaker fluorescence enhancement than the native BCD (Fig. 4). Binding constants of Z14S-CD complexes were determined using the Benesi-Hildebrand equation (Eq. (1)). Benesi-Hildebrand plots showed excellent fitting ($R^2 = 0.990$ – 0.999) with the 1:1 stoichiometry model

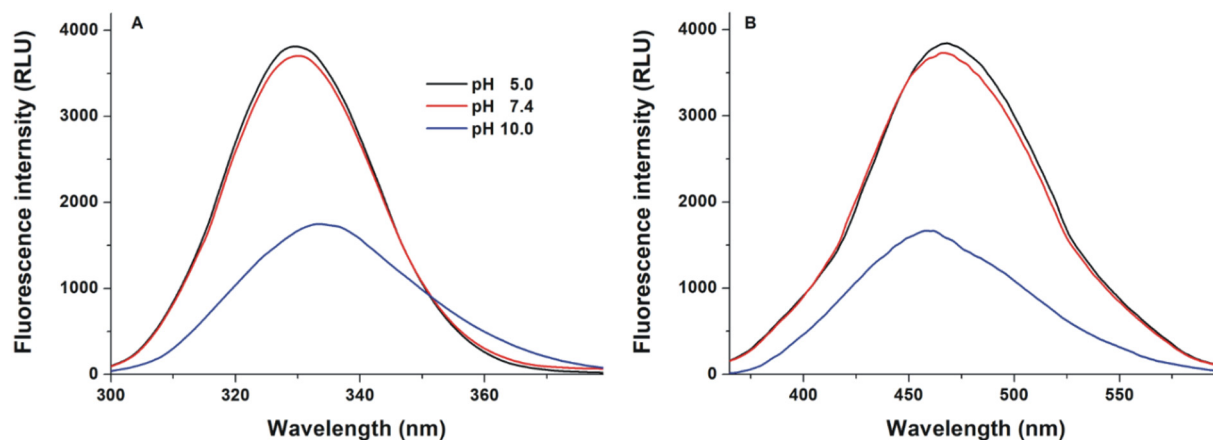


Fig. 2. Fluorescence spectra of Z14S (25 μ M). Excitation (A; pH 5.0 and pH 7.4: λ_{em} = 465 nm; pH 10.0: λ_{em} = 458 nm) and emission (B; pH 5.0 and pH 7.4: λ_{ex} = 330 nm; pH 10.0: λ_{ex} = 334 nm) spectra of the mycotoxin in different buffers (0.05 M sodium acetate, pH 5.0; 0.05 M sodium phosphate, pH 7.4; 0.05 M sodium borate, pH 10.0). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

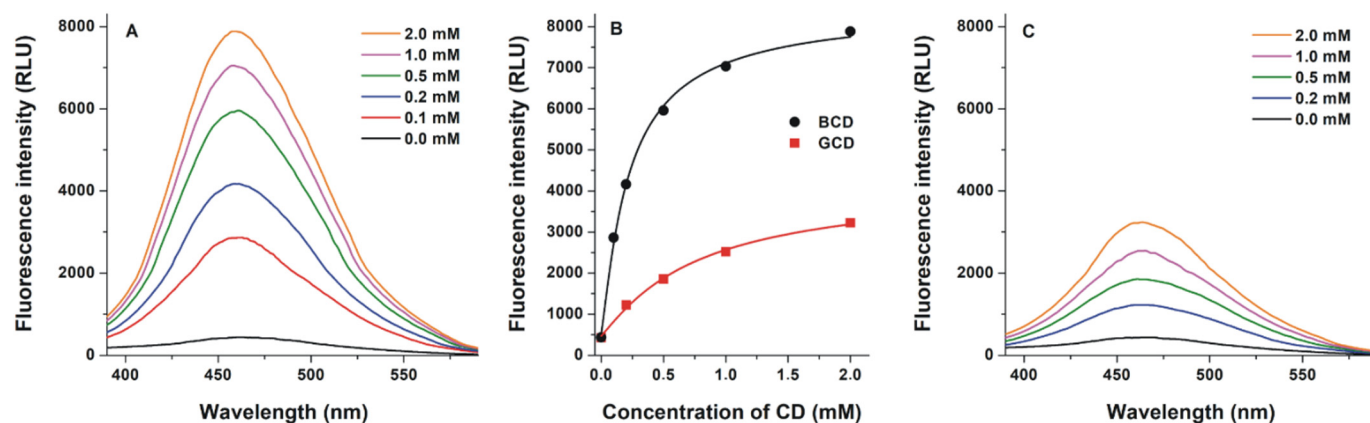


Fig. 3. Fluorescence emission spectra of Z14S (1 μM) in the presence of increasing BCD (A) and GCD (C) concentrations (0.0–2.0 mM) in 0.05 M sodium acetate buffer (pH 5.0; λ_{ex} = 320 nm). (B) BCD- and GCD-induced increase in the fluorescence of Z14S (λ_{ex} = 320 nm, λ_{em} = 460 nm).

(Fig. S1). The β-CDs examined, formed stable complexes with Z14S (logK = 3.6 to 4.7) at pH 5.0 (Table 1). Hydroxypropyl- (HPBCD) and sulfoethyl-substitution (SBCD) did not affect the affinity of β-CD toward Z14S; therefore, these derivatives were not tested further. MABCD and QABCD complexes showed approximately two-fold higher binding constants vs. Z14S-BCD. Furthermore, the methylated β-CDs, namely DIMEB and RAMEB formed the most stable complexes with the mycotoxin (approximately 11- and 5-fold compared to BCD, respectively) (Table 1). As it has been reported, the masked mycotoxin zearalenone-14-glucoside formed less stable complexes with BCD (logK = 2.8) than Z14S [17]; however, the binding constants of Z14S-BCD (logK = 3.6) and zearalenone-BCD (logK = 4.0) [37] complexes were similar at pH 5.0. Furthermore, the stability of Z14S complexes with RAMEB and DIMEB is practically identical to zearalenone complexes; however, SBCD is less while QABCD is more suitable for the entrapment of Z14S compared to zearalenone [37].

The interaction of Z14S with BCD, RAMEB, DIMEB, MABCD, and QABCD were also investigated at pH 7.4 and 10.0. The absolute fluorescence intensities of Z14S-CD complexes were the highest at pH 5.0 (see in Fig. 4). Furthermore, the relative enhancement in the fluorescence of Z14S by CDs is demonstrated in Table 1. At pH 7.4, the CD-induced relative increase in fluorescence was similar to observed at pH 5.0. Despite the fact that the absolute intensities of Z14S-CD complexes were the

lowest at pH 10.0 (see representative spectra in Fig. S2), the relative enhancement was significantly higher under these conditions (Table 1). The only exception was Z14S-QABCD complex, which showed poor emission signal at pH 10.0 (Fig. S2, bottom right). Under alkaline circumstances, the phenolic hydroxyl group of Z14S likely becomes deprotonated which results in a blue shift in its emission maximum and the significant decrease in its emission signal (see in Fig. 2). CDs tested (except QABCD) behaved similarly and likely favor the protonated form of the mycotoxin. Because the same emission wavelength maximum (approximately at 460 nm) was observed at each pH examined, it is reasonable to hypothesize that most of the CDs formed stable complexes with the same (protonated) form of Z14S. This hypothesis is supported by the following observations: (1) the relative increase in the fluorescence is larger at pH 10.0 due to the higher fluorescence signal of protonated Z14S (since CDs produce more stable complexes with this form, the equilibrium changes in favor of the protonated Z14S); (2) the higher relative fluorescence enhancement accompanied with significantly lower binding constant at pH 10.0, because CDs form complexes with the protonated mycotoxin which is presumably not the dominant form under alkaline conditions. On the other hand, QABCD seems to favor the deprotonated form based on the following observations (Fig. S2): (1) At pH 10.0, the emission wavelength maximum of Z14S-QABCD (447 nm) is different from the other CD complexes tested; (2) Z14S-QABCD complex has much lower emission signal than other complexes at pH 10.0, despite the fact that it was an effective enhancer at pH 5.0 and 7.4; (3) Z14S-QABCD shows the highest stability at pH 10.0. Since QABCD contains cationic substituents (tetraalkylammonium moieties), it is reasonable to hypothesize that its ionic interaction with the deprotonated mycotoxin may stabilize the formed complex.

3.4. Molecular modeling studies

Z14S showed a similar mode of binding regarding β- and γ-CD (Fig. 5), even though the capability to get sunk within the diverse cavities was found dependent on the internal radius of CDs. Specifically, the interaction with either BCD or DIMEB was more superficial, while it was deeper within the GCD (whose internal radius is higher in comparison to that of BCD and DIMEB). The analysis of the binding poses showed that Z14S arranged the aliphatic ring within the hydrophobic core of CD cavity, while it used the sulfate and keto groups to engage the large border of the CDs with polar contacts. The binding poses calculated in the light of the affinity to CDs observed experimentally (DIMEB >> BCD > GCD) suggested the stark importance of hydrophobic interactions to stabilize the Z14S-CD complexes. Indeed, the 2-O-methylation regarding DIMEB was thought providing an additional

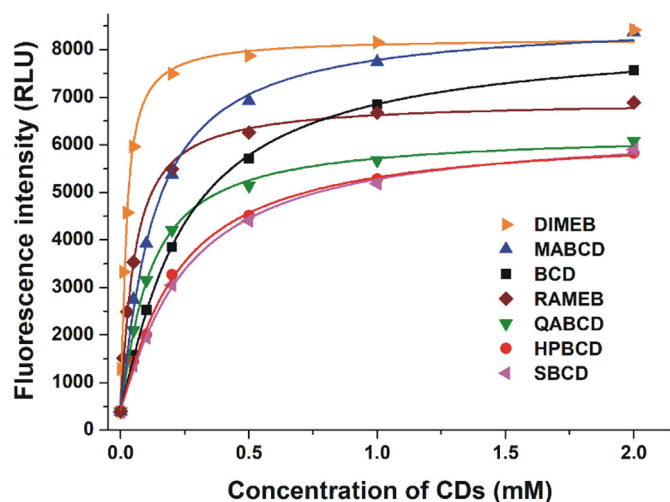


Fig. 4. Fluorescence emission intensities of Z14S (1 μM) in the presence of increasing CD concentrations (0–2 mM) in 0.05 M sodium acetate buffer (pH 5.0; λ_{ex} = 320 nm; λ_{em} = 460 nm).

Table 1

CD-induced relative increase in the fluorescence emission signal of Z14S (I/I_0 ; 1 μ M Z14S + 2.0 mM CD) in different buffers ($\lambda_{\text{ex}} = 320$ nm, $\lambda_{\text{em}} = 460$ nm). Decimal logarithmic values of binding constants (K ; unit: L/mol) of Z14S-CD complexes.

	$I/I_0 \pm \text{SEM}$			$\log K \pm \text{SEM}$		
	pH 5.0	pH 7.4	pH 10.0	pH 5.0	pH 7.4	pH 10.0
BCD	18.51 \pm 0.32	19.29 \pm 0.50	20.24 \pm 0.49	3.60 \pm 0.02	3.55 \pm 0.02	3.05 \pm 0.02
RAMEB	16.55 \pm 0.46	16.95 \pm 0.21	26.49 \pm 0.18	4.29 \pm 0.04	4.27 \pm 0.05	3.67 \pm 0.04
DIMEB	20.10 \pm 0.90	21.02 \pm 0.79	33.31 \pm 0.16	4.66 \pm 0.05	4.63 \pm 0.03	4.20 \pm 0.08
MABCD	21.03 \pm 0.11	21.76 \pm 0.41	25.10 \pm 0.66	3.90 \pm 0.03	3.99 \pm 0.05	3.04 \pm 0.03
QABCD	14.77 \pm 0.06	15.62 \pm 0.17	6.67 \pm 0.06	3.98 \pm 0.03	3.74 \pm 0.01	4.21 \pm 0.01
HPBCD	14.25 \pm 0.08			3.61 \pm 0.02		
SBCD	14.96 \pm 0.25			3.59 \pm 0.01		
GCD	7.51 \pm 0.05			3.14 \pm 0.01		

hydrophobic patch that could better stabilize the interaction with the aromatic ring of Z14S in comparison to either BCD or GCD. Concerning the diverse affinity between BCD and GCD observed experimentally, it could be partially explained by the differential sinking of Z14S within the two hydrophobic cavities. In particular, keeping in mind that keto group in position #7 and the 2-oxabicyclo group represent hydrophilic patches in the aliphatic ring of Z14S, the more superficial interaction with BCD is likely more favored than that within GCD, wherein both groups are deeper included within the hydrophobic core of GCD's cavity.

3.5. Interaction of zearalenone-14-sulfate with soluble β -cyclodextrin polymers

We also tested the interactions of the mycotoxin with soluble (epichlorohydrin cross-linked) BCD and QABCD polymers at pH 5.0 and 10.0 (the soluble polymer of DIMEB is not available). The fluorescence emission spectrum of Z14S (1 μ M) was recorded in the presence of increasing concentrations of soluble polymers. In order to apply comparable CD concentrations with the previous investigations, the polymer was normalized to its CD "monomer" content (0.0, 0.05, 0.1, 0.2, 0.5,

1.0, and 2.0 mM). Sometimes the CD polymers have higher affinity toward guest molecules due to the cooperativity of the monomers [22,41,42]. Therefore, the binding constants of the formed complexes were calculated based on the CD content of polymers applied (Eq. (1)), as it has been reported previously [22]. The complexes of Z14S with soluble BCD polymer showed 3.65 (\pm 0.04) and 2.74 (\pm 0.06) $\log K$ values at pH 5.0 and 10.0, respectively. These data are in good agreement with our previous results with the BCD monomer (Table 1). The soluble QABCD polymer formed slightly less stable complexes with Z14S at pH 10.0 ($\log K = 3.96 \pm 0.03$) than the QABCD monomer. However, approximately three-fold higher affinity of the polymer ($\log K = 4.48 \pm 0.01$) vs. the monomer was noticed at pH 5.0. These observations suggest that soluble QABCD polymer is suitable to form highly stable complexes with Z14S at both pH 5.0 and 10.0, in agreement with the data presented in Table 1.

3.6. Extraction of zearalenone-14-sulfate from aqueous solution by insoluble (water-swellaable) β -cyclodextrin bead polymer

As it has been demonstrated, insoluble CD polymers are suitable for mycotoxin removal from aqueous solutions, including certain beverages

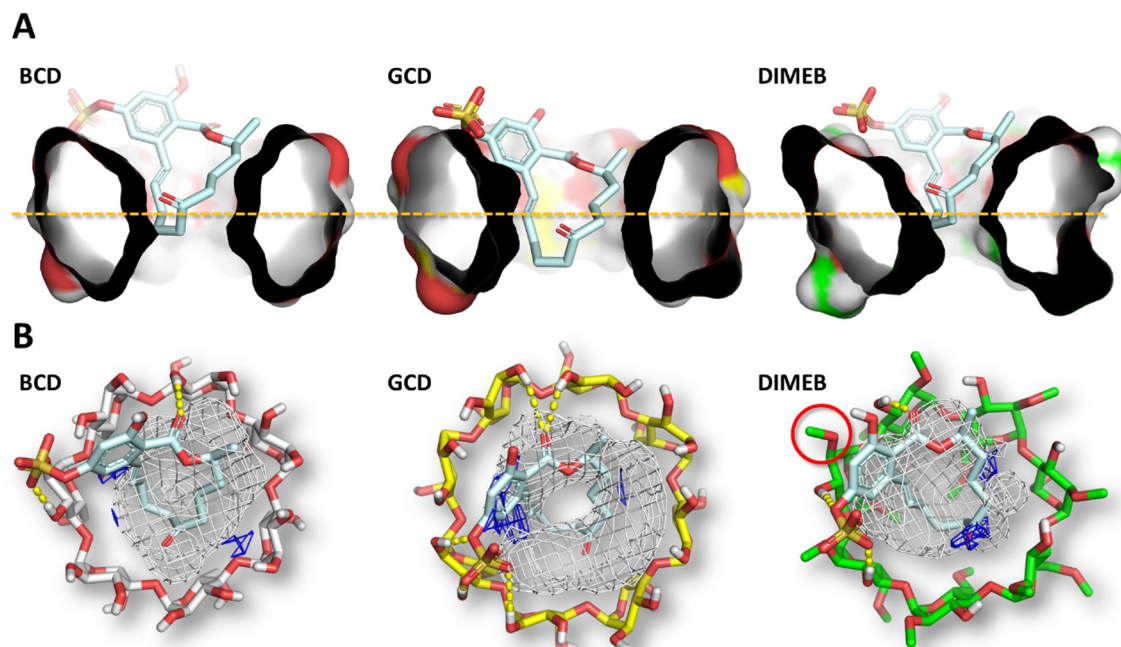


Fig. 5. Interactions of Z14S with BCD, GCD, and DIMEB. (A) Representation of the calculated complexes in cut surfaces (CDs) and sticks (Z14S). The dashed line serves as geometrical reference point and it indicates the centroid of hexose sugars of each CD: the inclusion of Z14S is deeper within GCD than within either BCD or DIMEB. (B) Representation of the calculated complexes in sticks. Yellow dotted lines indicate the formation of polar contacts while the grey, blue, and red mesh indicates regions sterically and energetically suitable to receive hydrophobic, hydrogen bond donor and hydrogen bond acceptor groups, respectively. The red ring indicates the position of the 2-O-methylation of DIMEB, which is thought favoring the interaction with the aromatic ring of Z14S. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

[21,23]. Zearalenone was successfully extracted from spiked corn beer [23] and the zearalenone-14-glucoside content of aqueous solution was also effectively decreased by BBP [17]. Some chemically-modified CDs have higher affinity toward Z14S than BCD; however, only the BCD polymer is available in insoluble (water-swellable) form. Therefore, the removal of Z14S by BBP was tested in 0.05 M sodium acetate buffer (pH 5.0). In a concentration-dependent fashion, BBP considerably decreased the Z14S content of the solution, resulting in 95% reduction of the mycotoxin concentration in the presence of 20.0 mg/1.5 mL BBP (vs. 1 μ M Z14S) (Fig. 6A). Based on these results, Z14S can be extracted by BBP with similar efficacy from aqueous solution than zearalenone [23].

In order to describe quantitatively the interaction of Z14S with BBP, increasing Z14S concentrations were added to standard amount of BBP (see details in Section 2.4) in sodium acetate buffer (0.05 M, pH 5.0). The binding ability of BBP was evaluated using the Langmuir (Eq. (2)) and Freundlich (Eq. (3)) isotherms (Fig. 6B). The Langmuir affinity constant (K_L) was 0.096 ± 0.051 L/mg and the Q_0 value was 9.53 ± 4.49 mg/g. The Freundlich model showed a 0.827 ± 0.019 (mg/g) \times (L/mg) $^{1/n}$ Freundlich constant (K_F) and a 0.895 ± 0.045 1/ n value. K_L and K_F values of Z14S-BBP interaction were lower compared to zearalenone-BBP ($K_L = 0.60$ L/mg, $K_F = 1.16$ (mg/g) \times (L/mg) $^{1/n}$) [23], suggesting the slightly lower adsorptive capacity of BBP regarding Z14S vs. the parent mycotoxin. The n value suggests the relatively homogenous sorption of Z14S to BBP.

4. Conclusions

In summary, the interaction of the modified mycotoxin Z14S with native and chemically-modified CDs as well as with soluble CD polymers was investigated. Furthermore, the extraction of Z14S from aqueous solution by insoluble β -CD bead polymer was examined. Z14S form similarly stable complexes with CDs than zearalenone, and also favors β - vs. γ -CDs. CDs induced strong increase in the fluorescence signal of Z14S: MABCD, DIMEB, and BCD proved to be the most effective enhancers. The methyl derivatives (DIMEB and RAMEB) formed the most stable complexes with Z14S at pH 5.0 and 7.4. Furthermore, at pH 10.0, Z14S-QABCD showed similarly high stability to the Z14S-DIMEB complex. Soluble BCD and QABCD polymers demonstrated comparable binding ability regarding Z14S than CD monomers. BBP successfully decreased the Z14S content of aqueous solution, suggesting its possible suitability as mycotoxin binder. Based on the above-listed observations, CD technology seems to be a promising tool to make more sensitive the fluorescence detection of Z14S as well as to develop

mycotoxin binders which can entrap modified mycotoxins besides the parent compound.

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CRediT authorship contribution statement

Zelma Faisal: Methodology, Investigation, Writing - original draft, Writing - review & editing. **Eszter Fliszár-Nyúl:** Methodology, Investigation. **Luca Dellafora:** Methodology, Investigation, Writing - original draft, Writing - review & editing. **Gianni Galaverna:** Methodology, Investigation. **Chiara Dall'Asta:** Methodology, Investigation. **Beáta Lemli:** Methodology, Formal analysis, Investigation. **Sándor Kunsági-Máté:** Methodology, Formal analysis, Investigation, Funding acquisition. **Lajos Szente:** Conceptualization, Resources. **Miklós Poór:** Conceptualization, Methodology, Writing - original draft, Writing - review & editing, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.molliq.2020.113236>.

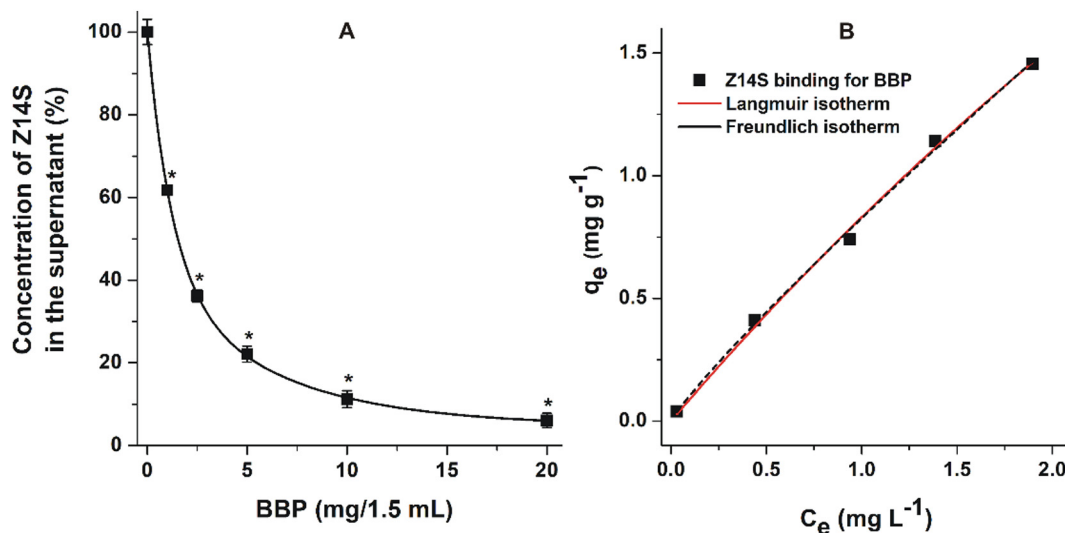


Fig. 6. (A) Removal of Z14S (1 μ M in 1.5 mL volume) from aqueous solution (0.05 M sodium acetate buffer; pH 5.0) by β -cyclodextrin bead polymer (BBP; incubation: 40 min, 1000 rpm, 25 $^{\circ}$ C; * $p < 0.01$). (B) Langmuir (solid line) and Freundlich (dashed line) isotherms for the Z14S-BBP interaction.

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