Chemical and Conformational Study of the Interactions Involved in Mycotoxin Complexation with β-D-Glucans

Alexandros Yiannikouris,*,‡ Gwénaëlle André,⊥ Laurent Poughon,§ Jean François,# Claude-Gilles Dussap,§ Georges Jeminet,|| Gérard Bertin,‡ and Jean-Pierre Jouany†

Alltech-France, 14 Place Marie-Jeanne Bassot, 92300 Levallois-Perret, France, Unité de Biochimie Structurale, Institut Pasteur, 75224 Paris, UR1213 INRA, Unité de Recherches sur les Herbivores, Centre de Clermont-Theix, 63122 St-Genès Champanelle, France, Laboratoire de Génie Chimique et Biologique, CUST, Université Blaise Pascal, Clermont-Ferrand II, 24 avenue des Landais, 63174 Aubière Cedex, France, UMR-CNRS 5504, UR-INRA 792, INSA, 135 avenue de Rangueil, 31077 Toulouse Cedex 4, France, and UMR-CNRS 6504, Laboratoire de Synthèse et Étude des Systèmes à Intêret Biologique, Université Blaise Pascal, Clermont-Ferrand II, 63177 Aubière Cedex, France

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In a previous paper we reported that β-D-glucans isolated from *Saccharomyces cerevisiae* could adsorb zearalenone, reduce its bioavailability in the digestive tract, and protect animals against its adverse effects. We have now investigated, in vitro, the kinetics of the interaction between other mycotoxins and β-D-glucans from several sources at three pH values found along the digestive tract (3.0, 6.0, and 8.0). Acid and neutral conditions gave the highest affinity rates for aflatoxins B1 > deoxynivalenol > ochratoxin A and involved both the (1 → 3)-β-D-glucans and the (1 → 6)-β-D-glucans. Alkaline conditions, owing to their destructuring action on glucans, were favorable only for the adsorption of patulin. Using molecular mechanics, we found that hydroxyl, ketone, and lactone groups are involved in the formation of both hydrogen bonds and van der Waals interactions between aflatoxins B1, deoxynivalenol and patulin, and β-D-glucans. Differences in the binding capacity of the mycotoxins are due to their specific physical and chemical characteristics.

Introduction

No technology is currently available that will totally eliminate mycotoxin contamination from the food and the feed chain. The lack of practical solutions to control mycotoxin contamination in the field or in harvested products has prompted us to investigate methods for directly protecting animals fed on contaminated feeds, thereby protecting the consumers of edible animal products.[1] There have been reports of adsorbents that can be used to reduce the bioavailability of mycotoxins in the digestive tract and alleviate their adverse effects on animals. Organic compounds such as yeast[2−5] and bacterial[6−8] cell walls have been studied for their ability to complex with several mycotoxins without harming the environment or reducing the bioavailability of certain nutrients. Recent studies[9−13] have indicated that *Saccharomyces cerevisiae* cell walls can be added to contaminated food or feeds to bind mycotoxins selectively. This allows a portion of the toxins to pass through the digestive tract without any negative effect on animals or carry-over to edible animal products such as milk, eggs, or meat. The β-D-glucans, and specifically (1 → 3)-β-D-glucans moderately branched with (1 → 6)-β-D-glucan chains from the cell wall of *S. cerevisiae* have shown affinity for mycotoxins. Recent evidence shows that these carbohydrates are able to adsorb, in vitro and at physiological temperature (+ 37−39 °C), up to 50% of zearalenone (ZEN, 6-(10-hydroxy-6-oxo-*trans*-1-undecenyl)-β-resorcylic acid lactone), a nonsteroid estrogenic mycotoxin produced by numerous *Fusarium* species.[14] ^{1}H NMR spectroscopy and molecular modeling have established that the hydroxyl, lactone, and ketone groups of the ZEN molecule, plus the hydroxyl groups of the (1 → 3)-β-D-glucan single helix, are engaged in hydrogen bonding, whereas a van der Waals stacking interaction occurs between the phenyl moiety of ZEN and the two opposite β-D-glucopyranose moieties of (1 → 3)-β-D-glucans. In addition, (1 → 6)-β-D-glucan side chains exhibit stabilizing effects by enhancing the van der Waals interactions with ZEN. Chains of (1 → 3)-β-D-glucans are organized in helical chains of six β-D-glucopyranose units per turn of the helix. This structure can form highly relaxed triple-helix and/or single-helix organizations depending on environmental conditions. Our current experiment was designed to extend our study to other mycotoxins of major importance, using in vitro techniques to quantify their adsorptiveness by yeast cell wall components and identify the potential chemical links between yeast cell wall components and mycotoxins by molecular mechanics.

Aflatoxin B1 (AFB1), a decapolyketide derived from acetate, is a carcinogenic metabolite (classified in group 1 by IARC)[15] produced primarily by *Aspergillus flavus* and *A. parasiticus*. A large number of commodities are susceptible to AFB1 production especially when stored under conditions of high moisture and temperature.[14,16] This mycotoxin is a potent liver toxin that can be lethal when consumed in large doses, and it induces cancer by chronic exposure. Deoxynivalenol (DON), a member of the trichothecene group, is synthesized by *Fusarium* spp and measurably contaminates more than 75% of cereals worldwide.[16] DON together with other trichothecenes such as diacetoxyscir-

* Author for correspondence. Phone: + 33 (0)1 34 38 98 98. Fax: + 33 (0) 1 34 38 98 99. E-mail: ayiannikouris@alltech.com.
† UR1213 INRA, URH.
‡ Alltech-France.
§ CUST, UBP.
⊥ UBS, Institut Pasteur.
|| UMR-CNRS 6504, UBP, SEESIB.
UMR-CNRS 5504, UR-INRA.

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Table 1. Chromatographic Conditions for the Analysis of Several Mycotoxins by HPLC

mycotoxin	column	mobile phase[a]	flow (mL/min)	UV detection (nm)	fluorescent detection Ex (nm)	fluorescent detection Em (nm)
AFB1[a,40]	C_{18}Nucleosil (150 × 4 mm, 3μm, 100Å) ODS II (Macherey-Nagel, Germany)	A: H_2O (55%) B: ACN/MOH (3/2, v/v) (45%)	0.8−1	220	340	450
OA[a,41,42]		A: H_2O/glacial acetic acid (99/1, v/v) (52%) B: ACN (48%)		220	340	450
PAT[a,43,44]		A: H_2O/TFA (1000/1, v/v) (80%) B: ACN (20%)		280	no detection	
DON[a,45]	C_8Nucleosil (150 × 4 mm, 5μm, 100Å) ODS (Macherey-Nagel, Germany)	H_2O/THF (76/24, v/v)		220	no detection	

[a] AFB1 = aflatoxin B1; ACN = acetonitrile; DON = deoxynivalenol; MOH = methanol; OA = ochratoxin A; PAT = patulin; TFA = trifluoroacetic acid; THF = tetrahydrofuran.

penol, T-2 toxin, their hydroxy and thiol derivatives, and fusaric acid are implicated in synergic mechanisms inducing enhanced toxicological action.[17−21] DON is hematotoxic and neurotoxic and has immunosuppressive effects. Patulin (PAT, 4-hydroxy-4H-furo[3,2c]pyran-2-6H)-one), a tetrapolyketide, is a potentially carcinogenic metabolite (classified in group 2 by IARC)[22] and is produced by several species of the genera *Aspergillus*, *Penicillium*, and *Byssochlamys*. PAT is mainly found in apple and apple products but has also been extracted from other rotten fruits, moldy feeds,[14] maize silage, and mature cheese.[23] PAT has been shown to be toxic for animals through its mutagenic, carcinogenic, and teratogenic properties.

With the aim of improving our understanding of the chemical and structural factors involved at a molecular level in the β-D-glucan and mycotoxin complexing mechanisms, progress has been made in molecular modeling investigations that enable us to draw conclusions about the accessibility of AFB1, DON, and PAT molecules inside the polysaccharide chain and help us to elucidate the nature of the interaction and identify the most important functional groups involved in the docking process.

Materials and Methods

Mycotoxin Quantification. AFB1, PAT, DON, and OA (Sigma-Aldrich Chemie GmbH, Germany) were quantified by isocratic HPLC on a gold 126 solvent module and a gold 168 UV diode array detector (Beckman Coulter Inc., CA) coupled to an RF-530 fluorescence detector (Shimadzu Corp., Japan). Conditions of analysis are presented in Table 1. Twenty microliters of each sample was injected through a six-port sample valve. Each analysis was duplicated.

In vitro Technique to Estimate the Mycotoxin Complex-Forming Capacity of Several Pure β-D-Glucans. The original in vitro technique used to study the complex-forming capacity of β-D-glucans from yeast cell walls for ZEN was described by Yiannikouris et al.,[10] and applied to alkali-soluble and -insoluble β-D-glucans extracted from four *S. cerevisiae* strains,[11] *wt292*, *fks1*, *mnn*9, and *sc1026* (Alltech Inc., Nicholasville, KY). For each test, 1 mg/mL of adsorbent, evaluated using a separative and quantitative analysis of sugars for yeast cell wall extracts, as described elsewhere,[11] was placed in tubes with 2, 5, 10, 20, 40, 60, 80, or 100 μg/mL of previously solubilized mycotoxins. The influence of pH was tested with three model (1 → 3)-β-D-glucans: soluble laminarin and insoluble curdlan (Sigma-Aldrich Chemie GmbH, Germany), insoluble pachyman with side chains containing (1 → 6)-β glucosidic linkages (Megazyme International Ireland Ltd); and one model insoluble (1 → 6)-β-D-glucan, pustulan (Calbiochem, Merck Bioscience GmbH, Germany) in 1 mL of three media to evaluate the influence of pH related to animal physiology: citrate buffer (0.05 M; pH 3.0), succinate buffer (0.05 M; pH 6.0), and Tris-HCl buffer (0.05 M; pH 8.0). The tubes were shaken at 39 °C on a rotary shaker at 640 rpm for 1.5 h and then centrifuged at 5000*g*. The supernatant fraction was then collected for free toxin analysis. Each mycotoxin concentration, adsorbent and medium condition was tested in triplicate. The amount of bound toxin was calculated by subtracting the amount of free toxin found in the experimental tubes from the amount found in control tubes with no adsorbent.[9]

Data Processing and Curve Fitting.[9−11] The amount of bound mycotoxin was plotted as a function of the amount of added mycotoxin according to the Hill sigmoid equation (HMN):[9] $T_{bound} = \{[T^{max}_{bound}(T_{total})^n]\}/\{[KD_{total} + (T_{total})^n]\}$ using DataFit 7.1 software (Oakdale Engineering). Biological parameters such as the association constant per site (K_D), the maximal amount of toxin bound (T^{max}_{bound}), the saturation point (K_{sat}), the cooperativity coefficient (n), and the affinity rates (A) were computed for each β-D-glucan. Our integrated approach consisted of adding to the equation the amount of β-D-glucans originating from the different yeast cell walls to evidence the interaction between β-D-glucan content and adsorption properties for mycotoxins.

In Silico Molecular Mechanics Investigations.[24] Molecular modeling was carried out on Silicon Graphics computers with Accelrys packages (Accelrys. Inc, San Diego, CA). Molecular displays and energy minimizations were performed using InsightII, Biopolymer, Analysis, Docking, and Discover modules. For all calculations, the CFF91 force field adapted to polysaccharide/protein or polysaccharide/ligand interaction studies was selected with the steepest descent minimization.[25,26]

Construction of the (1 → 3)-β-D-Glucan Single Helical Chain and (1 → 6)-β-D-Glucan Side Chain. (1 → 3)-β- and (1 → 6)-β-D-glucans were constructed from the laminaribiose and gentobiose molecule as discussed in a previous work.[24] Their most probable conformations were driven by the binding of two glucosyl moieties. The bonding is fully described by the set of [φ, ψ] and [φ,ψ,ω] dihedral angles, respectively, which are responsible for a given geometry characterized by a distinct potential energy value. Dimeric fragments were explored by the rotation of these angles from −180° to +180° with an increment of 10° and the potential energy evaluation at every step within a zero iteration minimization procedure. The low energy conformations were built and minimized within a consistent 10 000 iteration procedure and further elongated to a 5868 Da helical polymer. The energy value was approximately 46.0 kcal/mol for chains of (1 → 3)-β-D-glucans and ranged from 46.6 to 53.8 and from 56.4 to 59.2 kcal/mol for the (1 → 3)-β-D-glucan helix respectively branched respectively with the

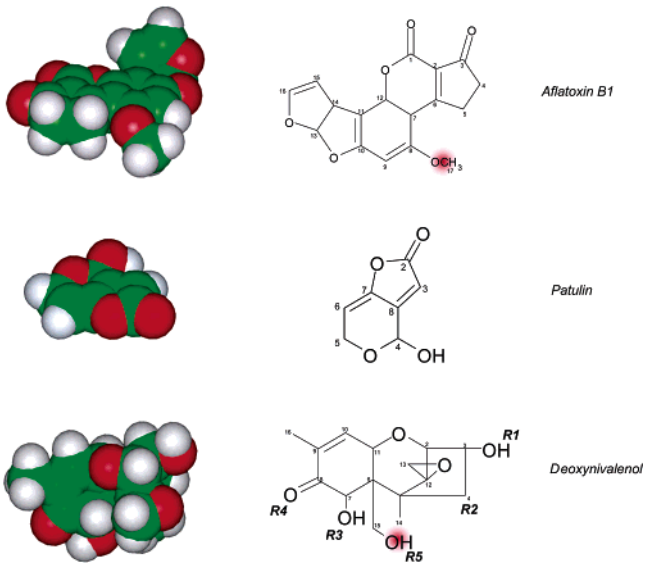

Figure 1. Computer-generated models using the Insight II program of the energy-minimized conformations of AFB1, PAT, and DON molecules. Degrees of freedom are shown in gray.

① and ② conformations of the (1 → 6)-β-D-glucan side chains (three glucosyl moieties), as demonstrated elsewhere.[24]

Mycotoxin Conformations. The AFB1, DON, and PAT molecules were studied by molecular mechanics taking into account all of the experimental data available in the literature.[27] AFB1 displayed a degree of freedom on the orientation of the O-methyl group located on C(17). The predominance of five- and six-membered carbon rings in the AFB1 molecule induced a relative rigidity of the overall structure. PAT did not display any degree of freedom in its spatial conformation. DON molecular conformation was driven by the rigidity of the epoxy and basic tetracyclic scirpenol ring system that is common to all trichothecenes. However, DON exhibited a degree of freedom on the hydroxyl group of the C(15) (Figure 1). No constraints were applied to the three molecules and their stability was assessed by minimization in 10 000 iterations.

Formation of the [Mycotoxin + β-D-Glucan] Complexes. AFB1, PAT, and DON molecules were manually positioned in one of the cavities offered by the helical (1 → 3)-β-D-glucan polymer outside or inside the (1 → 6)-β branched fragment. All of the possible spatial orientations of the mycotoxins in interaction were investigated; translations plus rotations as well as up and down positioning were thus carefully explored within a 10 000 iteration minimization. Steric clash between entities was avoided.

Results and Discussion

Adsorption of Mycotoxins by Alkali-Soluble and Alkali-Insoluble Fractions of β-D-Glucans from Yeast Cell Walls Estimated in Vitro and Plotted with Hill's Model. The *Mnn9* and *wt292* yeast strains exhibited large amounts of β-D-glucans in theirs cell wall, 80 and 42%, respectively, compared with the *fks1* and *Sc1026* strains (around 30%).[10] *sc1026*, *fks1*, and *mnn9* presented high alkali-insoluble β-D-glucan contents respectively 28.7, 28.2, and 74.5% of the whole yeast cell wall, whereas *wt292* contained only 1.7%. Conversely, *wt292* contained 42.0% of alkali-soluble β-D-glucans, whereas other strains had less than 3%.

As shown in Table 2, the R^2 values were between 0.980 and 1.000 (RSD below 0.855 μg/mL), showing the reliability of the HMN equation for estimating the adsorption efficacy of yeast strains. The T^{max}_{bound} values obtained for AFB1 and to a lesser extent OA over-passed the experimental domain used in this study, the HMN model allowing the extrapolation of these values. The high values were due to the low cooperative characteristics and higher standard deviation values obtained for T^{max}_{bound}. For all of the mycotoxins tested, the values of T^{max}_{bound}, K_D, and *A* were maximal for the *wt292* and *mnn9* strains. Thus, the adsorption efficacy was closely related to the β-D-glucan content of the yeast cell walls. However, some differences were recorded that were attributable to the stereochemical, electrical charge, solubility, nature, and size differences among mycotoxins. Affinities for the 3D-structure of the binding sites offered by β-D-glucans accordingly varied among toxins: AFB1 > DON > ZEN > PAT > OA. Previous work showed[11] that ZEN adsorption was also governed by the amount of β-D-glucans in the cell wall, with marked differences between strains (*A* = 50% for *mnn9*). In the same way, DON exhibited its maximum affinity for strains *wt292* and *mnn9*, for which *A* was respectively 94.8 and 84.0%. The differences between the efficacy of strains toward DON (between 50 and 95%) were characterized by an intensive adsorption at very low amounts of DON but a rapid saturation of the adsorptive sites, measured by the low T^{max}_{bound} values, below 19.2 μg/mL. AFB1 exhibited an affinity of 94.8% for the *wt292* strain and 72.5% for *mnn9*. Thus, AFB1 adsorption seemed not only to be due to the amount of β-D-glucans but was favored by the more flexible cell wall conformation of the *wt292* strain as suggested by its lower chitin content. PAT, owing to its small molecular size, was presumably able to penetrate deeply inside the β-D-glucan network, but had a lower specificity of interaction than AFB1, ZEN, or DON giving a maximum affinity of 37.0% with the *mnn9* strain.

Table 2. Evaluation of the Biological Parameters for the Adsorption of AFB1, PAT, DON, and OA Using Hill's Equation (HMN) Integrating the Amount of Soluble and Insoluble Alkali Fraction of β-D-Glucans Extracted from Four Strains of *Saccharomyces cerevisiae*

biological parameter	strains	AFB1 [a]	PAT [a]	DON [a]	OA [a]
percentage of β−D-glucans implicated in the adsorption [b]	wt292	42.1	44.5	43.1	43.1
	fks1	3.4	45.3	21.5	21.9
	sc1026	1.9	43.0	19.7	20.1
	mnn9	35.2	109.9	48.1	49.1
K_D (μg/mL)	wt292	3250.5	84.4	10.1	1476.5
	fks1	368.9	82.7	8.8	768.2
	sc1026	559.0	85.5	10.5	828.6
	mnn9	2260.1	150.5	11.1	1651.0
T^{max}_{bound} (μg/mL)	wt292	6177.7	45.1	19.2	402.6
	fks1	278.0	43.6	8.9	187.3
	sc1026	502.7	45.9	13.5	204.6
	mnn9	1169.0	111.3	18.5	458.9
adsorption (%) = $T^{max}_{bound}/(2K_D)$	wt292	94.8	26.7	94.8	13.6
	fks1	37.7	26.4	50.5	12.2
	sc1026	45.0	26.9	64.4	12.4
	mnn9	72.5	37.0	84.0	13.9
R^2 (HMN)	wt292	1.000	0.995	0.999	0.989
	fks1	0.995	0.995	0.999	0.994
	sc1026	1.000	0.999	0.998	0.980
	mnn9	0.999	0.994	0.999	0.997
RSD (μg/mL)	wt292	0.411	0.630	0.320	0.382
	fks1	1.327	0.738	0.585	0.422
	sc1026	0.418	0.785	0.459	0.135
	mnn9	0.688	0.855	0.508	0.490

[a] AFB1 = aflatoxin B1; DON = deoxynivalenol; OA = ochratoxin A; PAT = patulin. [b] The percentage of β-D-glucans was calculated as follows: % β-D-glucans = [(% alkali-soluble β-D-glucans) + (*a* x % alkali-insoluble β-D-glucans)].[11]

Table 3. Evaluation Using Hill's Equation (HMN) of the Affinity of Adsorption in Percent (*A*) of Four Model β-D-Glucans for the Adsorption of AFB1, PAT, DON, and OA in Several pH Conditions

β-D-glucan	pH	AFB1	PAT[a]	DON	OA[a]
laminarin	3.0	7.2	7.3	28.3	n.s.
	6.0	46.0	7.6	31.0	7.1
	8.0	*b*	49.3	*b*	*b*
curdlan	3.0	16.4	2.4	31.1	7.4
	6.0	49.3	9.4	21.8	35.0
	8.0	8.0	32.2	*b*	*b*
pachyman	3.0	2.0	9.6	33.6	14.2
	6.0	55.8	7.8	31.8	14.0
	8.0	*b*	51.5	*b*	16.1
pustulan	3.0	8.5	2.8	32.7	8.8
	6.0	47.8	6.5	55.4	38.5
	8.0	*b*	53.4	*b*	*b*

[a] Parameters of the adsorption were determined using logarithmic and exponential regression curves since HMN was not suited to our experimental data. [b] Adsorption was too low to be evaluated by a regression model.

Finally, OA exhibited poor adsorptive efficacy, below 14%, and thus had a low affinity despite T_{bound}^{max} reaching 458.9 μg/mL of toxin. The T_{bound}^{max} values categorized the optimal efficacy in favor of the *mnn9* and *wt292* strains. As stated earlier for ZEN,[11] these results confirmed the major role played by the 3D-structure of β-D-glucans in the mycotoxin adsorption process. Thus, a complex structure involving random coils, and single or/and triple helix conformations for β-D-glucans favored adsorption by increasing binding site accessibility. In addition, the alkali-insoluble β-D-glucan fractions, which had more complex structures, exhibited greater *A* values than the simplest alkali-soluble fraction. Adsorption of AFB1 ranged from 22.8 to 59.7% and from 48.4 to 82.3% for the alkali-insoluble and alkali-soluble fractions of β-D-glucans, respectively: from 35.8 to 64.3% and from 76.6 to 101.7% for DON; from 6.5 to 8.3% and from 15.6 to 16.2% for OA; from 38.9 to 39.3% and from 69.0 to 99.0% for PAT.

In Vitro Adsorption of Mycotoxins by Several Pure Model β-D-Glucans Differing in Their Chemical Structure. The R^2 values obtained in acid conditions (pH 3.0 and 6.0) were in the range of 0.819 (RSD = 0.847 μg/mL) to 0.998 (RSD = 0.270 μg/mL) for AFB1 and 0.880 (RSD = 0.303 μg/mL) to 1.000 (RSD = 0.054 μg/mL) for DON. The HMN equation closely fitted the binding curves of these two toxins. However, logarithmic and exponential regression curves were preferred for PAT and OA. A mean R^2 value of 0.968 for the former and 0.939 for the latter were then obtained (data not shown). As presented in Table 3, AFB1 and OA exhibited poor adsorptive values in acid pH conditions, whereas the affinity was between 46 and 56% and 14 and 39%, respectively, in near-neutral conditions. DON had a fairly constant affinity at pH 3.0 and 6.0 of around 30% and, like AFB1 and DON, no affinity at pH 8.0. Only PAT had a high adsorption efficacy in alkaline conditions (near 50%) and failed to form a stable interaction at pH 3.0 and 6.0. The evaluation of the adsorption also took into account the low stability of the PAT molecule at pH 8.0.

Among β-D-glucans, curdlan and pachyman gave acceptable results for the adsorption of all the mycotoxins, as they had a (1 → 3)-β-D-glucan complex structure that made them insoluble, confirming the results obtained in an earlier study with ZEN.[12] Laminarin, made of soluble (1 → 3)-β-D-glucans, gave comparable results with AFB1 and DON. Pustulan, which is a pure insoluble (1 → 6)-β-D-glucan molecule organized in single or triple helix chains, was highly efficient for the adsorption of DON and PAT, suggesting a marked involvement of this glucan structure in the interaction with the two toxins, *A* being equal to 55.4 and 53.4%, respectively. Conversely, adsorption of AFB1 was not significantly influenced by the structure of β-D-glucans, indicating that the single helical structure of β-D-glucans was of prime importance in its binding process. The low adsorption capacity observed for OA with all of the tested glucans indicated that limiting factors other than the 3D-structure and the nature of β-D-glucans were involved.

These results concerning the adsorptive properties of β-D-glucans toward mycotoxins prompted us to study the chemical mechanisms of the interaction and undertake further investigations by molecular modeling.

In Silico Molecular Mechanics Investigations of Mycotoxins. Three stable, semi-relaxed conformations for the AFB1 molecule were found after minimization in 10 000 iterations in a vacuum, depending on the orientation of the O-methyl group located on C(17). The A, B, and C structures displayed low-energy conformations of 22.25, 21.91, and 20.98 kcal/mol, respectively (Figure 1). However, the experiments for the docking of AFB1 inside a β-D-glucan molecule were conducted using the conformation exhibiting the lowest potential energy value reflecting the highest molecular stability, i.e., the C conformation (Figure 1). The C conformation was characterized by the orientation of the O−C(17)H_3 group outside the molecule and in the plane of the aromatic and pyran rings of the AFB1 molecule. Despite the absence of potential intramolecular hydrogen bonds, the AFB1 molecule seemed to adopt a very stable molecule conformation due to the five- and six-membered cycles, which stiffen and stabilize the general conformation of the toxin. Thus, the molecular geometry of AFB1 in solution and in the solid state may be essentially the same. The PAT molecule had no degree of freedom, leading to a single stable conformation with a potential energy value of 4.28 kcal/mol (Figure 1). The DON molecule, like the AFB1 molecule, exhibited one degree of freedom corresponding to the hydroxyl group located on C(15). The rest of the overall structure was nicely stabilized by the basic tetracyclic scirpenol ring system corresponding to the C(2) to C(5) carbon atoms, the two six-membered rings, and the epoxy group. Thus, three stable semi-relaxed structures of DON were found after minimization, corresponding to the three possible orientations of the −C(15)-H_2−OH group, with energy values of 179.4, 175.2, and 169.8 kcal/mol, respectively (Figure 1).

In silico Molecular Docking of Mycotoxins inside the β-D-Glucan Structure. The complete set of energy values including the β-D-glucan energy, the AFB1, PAT and DON energies, the complex energy, and the docking energy are given in Tables 4−6. Among the numerous complexes, no selection could be made in any particular case on the assessment of a single energy value, whether it is the complex energy, the β-D-glucan energy, the toxin energy, or the docking energy. None of these energy values was pertinent enough in itself to form an ultimate criterion of selection. Each complex had to be compared with its counterpart over its full set of energies, and only after careful examination of the whole set of values could a soundly based selection be made.

AFB1 Interaction with β-D-Glucans. As indicated in previous work,[24] the docking of the AFB1 molecule inside the β-D-glucan structure can be approximated by a two-step mechanism: (i) AFB1 is trapped inside the single helix of the (1 → 3)-β-D-glucan chain and (ii) the branched (1 → 6)-β-D-glucan chain covers the toxin molecule and maintains it inside the helix. Thus, the branched (1 → 6)-β-D-glucans can be considered as an

Table 4. Most Stable Energy Values Obtained after Minimization with Insight II Software for the Docking of AFB1 (with Different Positionings Indicated by a Letter) to the Single Helix of (1→3)-β-D-Glucan Chain and the Single Helix of (1 → 3)-β-D-Glucan Branched with Three β-D-Glucopyranose Units in (1 → 6)-β (Conformations ① and ②)

AFB1	energy (kcal/mol)					
				docking energy		
conformation of β−D-glucans	complex energy	glucan energy	toxin energy	VdW	elect	total
3 [a]	26.7	45.3	21.5	−36.3	−3.8	−40.1
3 + ①6 [b]/ A [c]	26.7	47.3	21.5	−35.7	−3.5	−39.2
3 + ②6 [b]/ F [c]	41.1	52.9	21.2	−31.6	−1.4	−33.0

[a] 3 = (1 → 3)-β-D-glucan single helix. [b] 3 + 6 = (1 → 3)-β-D-glucan single helix + (1 → 6)-β-D-glucan side chain. [c] Arbitrary annotation characterizing the position of the mycotoxin inside the β-D-glucan structure.

Table 5. Most Stable Energy Values Obtained after Minimization with Insight II Software for the Docking of PAT to the (1 → 3)-β-D-Glucan Single Helix and the (1 → 3)-β-D-Glucan Helix Branched with Three β-D-Glucopyranose Units in (1 → 6)-β (Conformations ① and ②)

PAT	energy (kcal/mol)					
				docking energy		
conformation of β-D-glucans	complex energy	glucan energy	toxin energy	VdW	elect	total
3[a]	18.9	44.5	0.4	−20.6	−5.5	−26.1
3 + ①6[b]/A[c]	28.0	51.1	4.6	−17.8	−9.9	−27.7
3 + ②6[b]/B2[c]	19.7	51.8	4.1	−10.3	−5.2	−7.0
3 + ②6[b]/B3[c]			0.2	−9.8	−7.0	−16.8

[a] 3 = (1 → 3)-β-D-glucan single helix. [b] 3 + 6 = (1 → 3)-β-D-glucan single helix + (1 → 6)-β-D-glucan side chain. [c] Arbitrary annotation characterizing the position of the mycotoxin inside the β-D-glucan structure.

Table 6. Most Stable Energy Values Obtained after Minimization with Insight II Software for the Docking of DON to the (1→3)-β-D-Glucan Single Helix and the (1 → 3)-β−D-Glucan Helix Branched with Three β−D-Glucopyranose Units in (1 → 6)-β (Conformations ① and ②)

DON	energy (kcal/mol)					
				docking energy		
conformation of β-D-glucans	complex energy	glucan energy	toxin energy	VdW	elect	total
3 [a]	181.4	45.0	169.9	−20.8	−3.6	−24.4
3 + ①6 [b]/ A [c]	185.5	51.0	169.8	−29.8	−4.9	−34.7
3 + ②6 [b]/ A [c]	186.1	48.2	170.9	−28.5	−4.4	−32.9
3 + ②6 [b]/ C [c]	199.2	56.3	169.4	−21.0	−5.5	−26.6
3 + ②6 [b]/ E [c]	194.5	57.0	168.8	−16.5	−14.7	−31.2
3 + ②6 [b]/ F [c]	201.1	58.0	169.3	−22.7	−3.6	−26.3

[a] 3 = (1→3)-β-D-glucan single helix. [b] 3 + 6 = (1→3)-β-D-glucan single helix + (1→6)-β-D-glucan side chain. [c] Arbitrary annotation characterizing the position of the mycotoxin inside the β-D-glucan structure.

enlarged binding site expanding outside the main (1 → 3)-β-D-glucan chain. Several positions testing the different orientations of the toxin inside the (1 → 3)-β-D-glucan single helix were found and gave energy values ranging from − 36.3 to −41.0 kcal/mol. We note that among the various positions of the AFB1 molecule during the setting up of the docking minor differences between the energy values were found. This may allow for an easier penetration and adsorption of the AFB1 into the β-D-glucan helix. The lowest docking energy value obtained (−41.0 kcal/mol) was the sum of −38.3 kcal/mol due to van der Waals interaction and −2.7 kcal/mol for an electrostatic contribution (Table 4). The latter value accounted for the occurrence of a single hydrogen bond during interaction. Considering both the position of the glucose units inside the helical structure of (1 → 3)-β-D-glucans and the AFB1 positioning, there was strong evidence that the hydrogen bond involved the hydroxyl group located on C(6) of the fifth β-D-glucopyranose residue and the oxy-methyl group of AFB1 molecule. Thus, the orientation of this latter group, which was the only degree of freedom of the structure in the plane of the pyran and aromatic groups of the AFB1 molecule, was of prime importance in the interaction with the (1 → 3)-β-D-glucan single helix. However, the stacking interaction due to van der Waals bonds played a major role in the binding strength of AFB1, accounting for 93.4% of the total docking energy. The stacking interaction involved the AFB1 core structure made of two aromatic cycles and the first and fifth glucosyl residues of the (1 → 3)-β-D-glucan chain (identified in Figure 2) and is responsible for the stability of the chemical complex resulting from the inclusion of a molecule of AFB1 in the (1 → 3)-β-D-glucan helix.

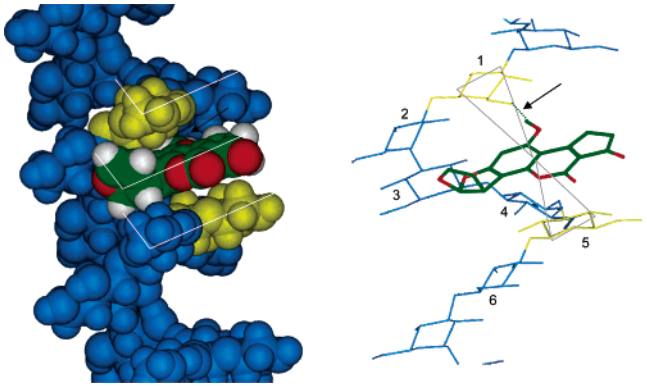

Figure 2. Computer-generated models (Insight II) of the energy-minimized structure of the docking of the most favorable conformation of AFB1 into the single-helix of (1→ 3)-β-D-glucan chain. Arrows indicate hydrogen bonds involved in the interaction. Lines highlight the steric complementarities between AFB1 and (1 → 3)-β-D-glucan geometry. van der Waals interactions are indicated.

The branching of three β-D-glucopyranose residues in a (1 → 6)-β position to the (1 → 3)-β-D-glucan helix did not influence the energy values, the stability of the interaction remaining the same for conformation ① (−39.2 kcal/mol) or even decreasing for conformation ② (−33.0 kcal/mol; Figure 3). Also, there were no significant changes in electrostatic or in van der Waals energies after addition of the (1 → 6)-β-D-glucan side chain. Thus, the (1 → 3)-β-D-glucan single helix was the critical structure in establishing the interaction with AFB1, leading to a maximum affinity independently of the presence or absence of side chains of (1 → 6)-β-D-glucans. The addition of two molecules of AFB1 at an adjacent binding site did not alter the chemical interaction already described between the molecules of toxin and the helical (1 → 3)-β-D-glucan structure.

PAT Interaction with β-D-Glucans. The different orientations of the PAT molecule inside the (1 → 3)-β-D-glucan single helix were investigated. Five positions were evaluated for their energy minima and ranged between −23.5 and −26.1 kcal/mol. Thus, the docking strength did not differ significantly between the various positions of the PAT molecule. It may be hypothesized that its small molecule size, its low steric bulk and the very planar conformation of its structure enable PAT to interact in several ways and penetrate very deeply inside the single helical structure of β-D-glucans. However, only two positions were able to create electrostatic interaction leading to the formation of

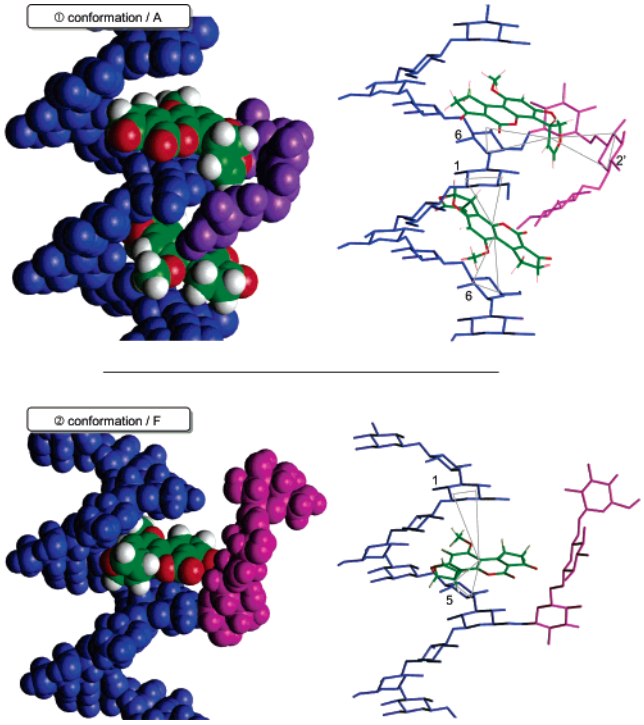

Figure 3. Computer-generated models (Insight II) of the docking of AFB1 into the (1 → 3)-β-D-glucan chain branched with three β-D-glucopyranose moieties of (1 → 6)-β-D-glucan side chain, in conformations ① and ②, respectively.

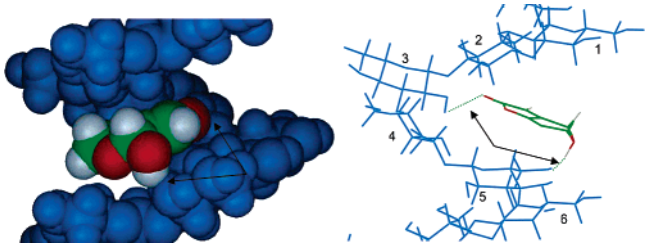

Figure 4. Computer-generated models (Insight II) of the energy-minimized structure of the docking of the most favorable conformation of PAT into the (1→ 3)-β-D-glucan chain. Arrows indicate hydrogen bonds involved in the interaction.

two hydrogen bonds and also giving the lowest values for the total docking energy. The most stable conformation obtained (−26.1 kcal/mol) resulted from a −20.6 kcal/mol van der Waals interaction and a −5.5 kcal/mol electrostatic contribution (Table 5). These hydrogen bonds involved the hydroxyl group and the lactone group respectively located on C(4) and C(2) of the PAT molecule and the hydroxyl groups on C(4) of the β−glucopyranose residues of the single helix (Figure 4).

The branching of three β-glucopyranose residues in conformation ① onto the (1 → 3)-β-D-glucan single helix was able to generate another favorable binding site for PAT. Again, due to its low steric bulk, the toxin penetrated deeply inside the cavity formed by the (1 → 6)-β-D-glucan branch, leading to a sharp decrease in the energy (−27.7 kcal/mol) compared with the interaction of PAT with (1 → 3)-β-D-glucan taken alone. A hydrogen bond was formed accounting for an electrostatic energy value of 9.9 kcal/mol between the hydroxyl group located on C(4) of PAT and the C(6)−O-C′(1) group of the β-(1,6) linkage of the two external β-glucopyranose residues (Figure 5). The value obtained for the energy measurement of van der Waals bonds, 17.8 kcal/mol, accounted for the interaction between the lactone ring and the proximity of the β-glucopyranose rings. The addition of the side chains of (1 → 6)-β-

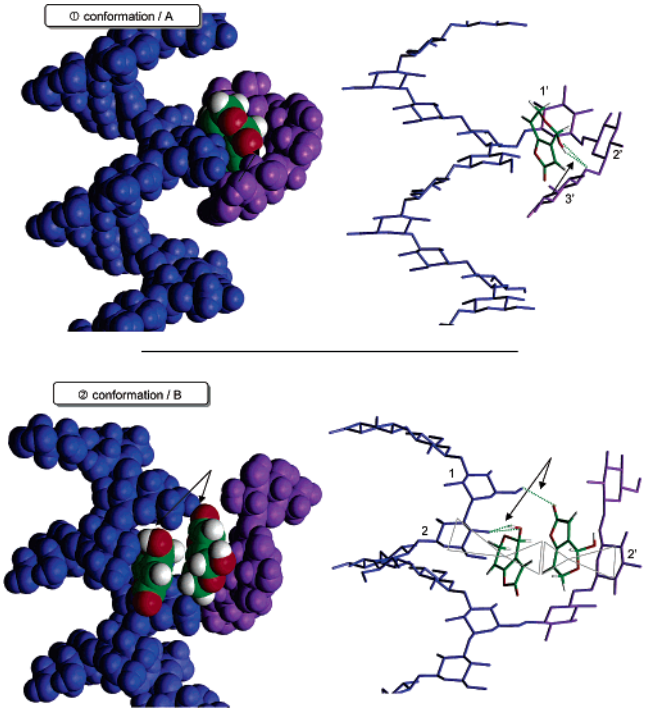

Figure 5. Computer-generated models (Insight II) of the docking of PAT into the (1→ 3)-β-D-glucan chain branched with (1 → 6)-β-D-glucans, in conformations ① and ②, respectively. van der Waals interactions are indicated.

D-glucan in conformation ② created large cavities where two head-to-tail molecules of PAT could be inserted. The energy value of each van der Waals interaction between PAT molecules and either (1 → 3)-β- or (1 → 6)-β-D-glucan chains amounted to 10 kcal/mol. In addition, the head-to-tail conformation resulted in van der Waals interaction between the two molecules of PAT, involving their 2H-pyran ring. Each of the two molecules of PAT was able to form hydrogen bonds with the (1 → 3)-β-D-glucans of the single helix. The latter interaction involved the hydroxyl group on C(4) of PAT that was closest to the (1 → 3)-β-D-glucan chain, and the lactone group of PAT interacted with the hydroxyl groups located on C(4) and C(6) of two adjacent β-glucopyranose units from the side chain of (1 → 6)-β-D-glucans. The latter was less stable than the interaction obtained from conformation ①, the total docking energy being −15.6 and −16.8 kcal/mol, respectively. The docking of two molecules of PAT at the same interaction site led to a change in the conformation of PAT, from an energy value of 4.1 to 0.2 kcal/mol and thus produced an increase in the stability of the PAT conformation.

DON Interaction with β-D-Glucans. DON, like other trichothecenes, was able to form chemical interactions with β-D-glucans despite its less stable stereochemistry (conformational energy around 179.0 kcal/mol) owing to the presence of a tetracyclic scirpenol ring when compared with other families of mycotoxins. This moderate stability of DON may be explained by the proximity of the six-, five-, and four-carbonated membered cycles and of the surrounding hydroxyl, epoxy, and ketone groups (Figure 1). However, despite the lack of similarities between the molecular geometry of β-D-glucans and DON, the toxin was able to enter the cavities of the helix of (1 → 3)-β-D-glucan, with or without the presence of the (1 → 6)-β-D-glucan side chain. Six positions of DON were evaluated for their energy minima, which ranged between 181.4 and 201.1 kcal/mol (Table 6). These rather high values of total energy of interaction were directly related to the high energy value of DON. The most

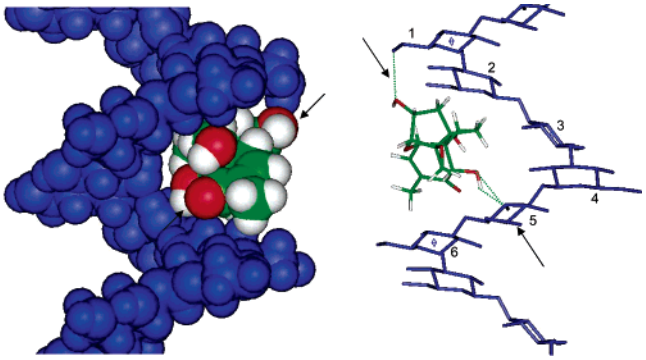

Figure 6. Computer-generated models (Insight II) of the energy-minimized structure of the docking of the most favorable conformation of DON into the (1→ 3)-β-D-glucan chain. Arrows indicate hydrogen bonds involved in the interaction.

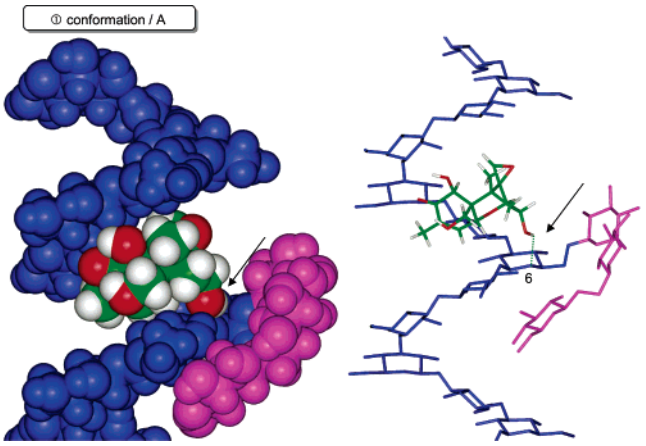

Figure 7. Computer-generated models (Insight II) of the docking of DON into (1→ 3)-β-D-glucan chain branched with (1 → 6)-β-D-glucans in conformation ①. Arrows indicate hydrogen bonds involved in the interaction.

stable conformation of the interaction is presented in Figure 6 and can be explained by the formation of hydrogen bonds between the two hydroxyl groups of the DON molecule located on C(3) and C(7) and the hydroxyl groups respectively borne by the C(6) and C(2) of the first and fifth β−glucopyranose residues. The value of the docking energy was −24.4 kcal/mol.

The addition of side chains of (1 → 6)-β-D-glucans in conformations ① and ② brought an increase in the stability of the chemical complexes formed between DON and (1 → 3)-β-D-glucans, the docking energy decreasing from −26.3 to −34.7 kcal/mol. The most stable docking complex was obtained with position A for conformation ① (Figure 7) and position A and E for conformation ② (Figure 8). Both A positions involved hydroxyl bonds between DON and the (1 → 3)-β-D-glucan chain with values for electrostatic bonds of −4.9 and −4.4 kcal/mol, respectively. In that case, the C(3)−OH of DON was bound to the O atom of the sixth β-D-glucopyranose residue in conformation ①. The C(7)−OH of DON interacted with the C(4)−OH of the second glucose unit in conformation ②. The (1 → 6)-β-D-glucan side chains reinforced the van der Waals interactions and stabilized the complexes formed between the β-D-glucans and DON as previously described for ZEN.[24] Interestingly, the E positioning of DON in the β-D-glucan structure in conformation ② induced the formation of two hydrogen bonds: (i) one between the hydroxyl group on C(3) of DON and the hydroxyl group on C(4) of the second glucose residue of the (1 → 3)-β-D-glucan chain and (ii) the other between the C(15)−OH group of DON and the −OH group of the third residue of the

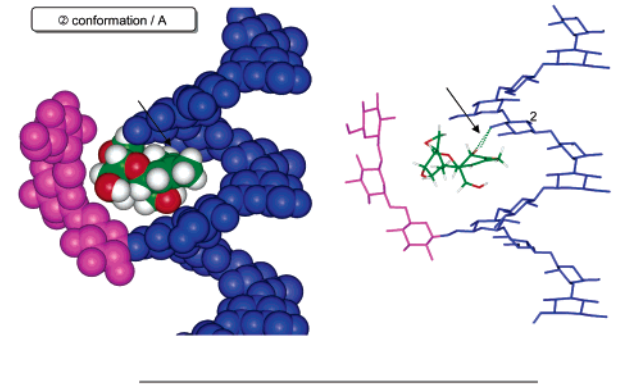

Figure 8. Computer-generated models (Insight II) program of the docking of DON into (1→ 3)-β-D-glucan chain branched with (1 → 6)-β-D-glucans in conformation , positions A and E. Arrows indicate hydrogen bonds involved in the interaction.

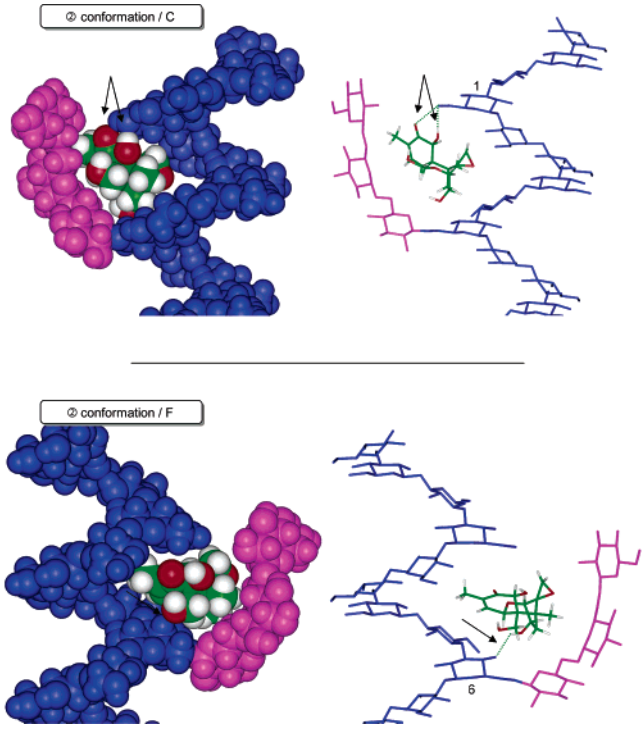

Figure 9. Computer-generated models (Insight II) of the docking of DON into (1→ 3)-β-D-glucan chain branched with (1 → 6)-β-D-glucans in conformation ②, positions C and F. Arrows indicate hydrogen bonds involved in the interaction.

(1 → 6)-β-D-glucan side chain leading to an electrostatic energy value of −14.7 kcal/mol. Consequently, the share of the van der Waals interaction energy was then increased from −30.0 to −16.5 kcal/mol. Two other statistically relevant conformations of the interaction were found (Figure 9). The C positioning in conformation ② led to the formation of hydrogen

bonds, which involved a linkage of both the ketone group on C(8) of the five-membered carbon rings and the adjacent C(7)−OH group with the C(6)−OH group of the first glucose residue of (1 → 3)-β-D-glucan single helix, and a consequent electrostatic energy of −5.5 kcal/mol. The F positioning in conformation ② formed a hydrogen bond between the C(3)−OH of DON and the C(4)−OH of the sixth residue of β−glucopyranose. In both interactions, the docking energy and the total energy of the system were roughly −26.4 and +200.0 kcal/mol, respectively.

Comparison of the Docking Properties of Mycotoxins inside the β-D-Glucan Structure. Unlike ZEN[24] or AFB1, no geometrical complementarities were found between the 3D-structure of DON and the cavities created inside the (1 → 3)-β- and (1 → 6)-β-D-glucan chains. The chemical complexes made of DON and β-D-glucans were less stable than those involving AFB1, ZEN, or PAT since the value of the energy of the former, ranging from 168.8 to 170.9 kcal/mol, was higher than the energy of the three others, equal to +21.5, −20.0, and +4.0 kcal/mol, respectively (Table 6). The symmetry and geometrical similarities of both ZEN and AFB1 were highly advantageous in the setting up of van der Waals interactions, with highly stable energy values ranging from −43.7 to −35.7 kcal/mol. Owing to the small particle size of PAT and the presence of the pyran and furan rings only, the van der Waals contribution was lowered (−20 up to −9.8 kcal/mol) with this toxin. The results obtained with DON were closely related to the presence or absence of the (1 → 6)-β-D-glucan side chain, which allowed the strengthening of van der Waals interactions, leading to the energy value of −30 kcal/mol, which is close to that obtained with AFB1. This observation was consistent with the higher adsorption capacities estimated in vitro between DON and pustulan. Thus, (1 → 6)-β-D-glucans played a major role in the stability of the complexes formed between β-D-glucans and DON. The docking energy of DON due to van der Waals bonds was close to the energy found for PAT (around − 20.0 kcal/mol) when only the (1 → 3)-β-D-glucan fraction was considered. Again, the globular structure of the 3D-conformation of DON was the main limitative factor for its adsorption compared with AFB1 and ZEN, which both had a plane molecular structure, thus optimizing the capacity for an interaction with the glucose units in a turn of the (1 → 3)-β-D-glucan chains.

Interestingly, these calculations, together with the previous results obtained with ZEN[24] using molecular mechanics techniques, strongly evidence the geometrical, electrostatic and hydrophobic complementarities between the β-D-glucans, branched or unbranched, and the AFB1 structure, which can be extended to all of the aflatoxin metabolites exhibiting the same core dicarbonyl structure. Since the globular structure of DON is the same for all trichothecenes and considering the results found in our study, the chemical groups located in R_1 and to a lesser extent R_3 (Figure 1) were critical for the adsorption of the mycotoxin inside the β-D-glucan structure. Thus, we can reasonably extend our finding on DON to all of the trichothecenes with a hydroxyl group located on C(3) such as scirpenol, scirpendiol and derivatives, T-2 toxin and derivatives, nivalenol and derivatives, or fusarenon-X. Interestingly, T-2 toxin has an R_4 side structure made of a chain of isovaleroxyl, which could presumably act as an arm favoring the interaction inside the binding site of β-D-glucans. This particular structure could explain the higher adsorption capacity of T-2 relative to DON by yeast cell wall based adsorbents.[28] Some practical approaches to limit the bioavailability of mycotoxins using inorganic sorbents such as activated carbons and processed clays (HSCAS) were investigated by Phillips et al.[29−33] The authors used an isothermal analysis derived from isotherm equations (Langmuir, Freundlich, Toth, and various multiple combined transformed equations) and molecular modeling techniques to characterize and design clay-based materials of direct utility for enterosorption and inactivation of AFB1. Based on their findings, they indicated that the dicarbonyl group in AFB1 played a major role in its adsorption by HSCAS. In addition, aflatoxins interacted at multiple sites on the clay surface, especially those within the interlamellar region,[29,31,33,34] thus explaining their efficacy in the prevention of aflatoxicosis in animals.[35] However, their ability to react with other mycotoxins was negligible.[14]

Previous macromolecular in vitro experiments[9−11] and NMR experiments established that environmental conditions such as pH and nature of solvent could alter the geometry of β-D-glucans and decrease their complex-forming ability toward mycotoxins, thus explaining the poor adsorptive properties of model glucans obtained here at pH 8.0. As stated in earlier work,[24] the single helical conformation appeared to play the major role in the complexing process. Although the triple helical conformation has not yet been tested by molecular modeling, we can reasonably hypothesize that its compact structure will not allow an inner helix space large enough to accommodate any molecules other than water, except perhaps small molecules such as PAT. The triple helical structure would thus only display external surface adsorptive properties toward mycotoxins, which might be less stable in relation to environmental changes.

Conclusions

Combined with previous work,[9−13,24] this study demonstrates the major role played by the single helical conformation of (1 → 3)-β-D-glucans toward AFB1, DON and PAT complexation. As found previously with ZEN, the interaction between β-D-glucans and the mycotoxins described here is driven by steric complementarities enabling a marked involvement of van der Waals interaction causing some stacking effects as well as stable intermolecular hydrogen bonding involving the hydroxyl, lactone, and ketone groups commonly found on mycotoxins. (1 → 3)-β-D-Glucans were involved in both mechanisms, whereas (1 → 6)-β-D-glucans seemed to strengthen the van der Waals bonds and consequently to strongly stabilize the toxin−glucan interaction. However, the mycotoxins were not all equivalent in their ability to bind with β-D-glucans. Also, the environmental conditions such as pH were determining for the stability of the toxin−glucan complexes generated. The stereochemistry and hydrophobic properties of mycotoxins are of prime importance and account for the differences in their affinity for β-D-glucans. Unlike most inorganic sorbents, β-D-glucans can interact with a wide range of mycotoxins, which is of practical utility in the frequent cases of multicontamination found naturally. These theoretical findings for the 3D conformation of both toxins and β-D-glucans are in fully consistent with the literature.[27,37−39]

We note that in vivo application of β-D-glucans as feed additives can be considered only with insoluble yeast cell wall β-D-glucans with a selected complex spatial organization and densely organized structure that are not absorbed in the digestive tract and not degraded by enzymes such as β-D-glucanase.

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