Understanding the sorption mechanisms of aflatoxin B1 to kaolinite, illite, and smectite clays via a comparative computational study

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ABSTRACT

In current adsorption studies of biotoxins to phyllosilicate clays, multiply weak bonding types regarding these adsorptions are not well known; the major attractive forces, especially for kaolinite and illite, are difficult to be identified as compared to smectite with exchangeable cations. Here, we discriminated the bonding types of aflatoxin B1 (AFB1) contaminant to these clays by combined batch experiment with model computation, expounded their bonding mechanisms which have been not quantitatively described by researchers. The observed adsorbent-to-solution distribution coefficients (K_d) of AFB1 presented in increasing order of 18.5–37.1, 141.6–158.3, and 354.6–484.7 L/kg for kaolinite, illite, and smectite, respectively. Normalization of adsorbent-specific surface areas showed that adsorption affinity of AFB1 is mainly dependent on the outside surfaces of clay aggregates. The model computation and test of ionic effect further suggested that weakly electrostatic attractions ($((\text{Si}/\text{Al}-\text{OH})_2 \cdots (\text{O}=\text{C})_2)$) are responsible for AFB1–kaolinite adsorption (K_d , 18.5–37.1 L/kg); a moderate electron–donor–acceptor attraction ($((\text{C}=\text{O})_2 \cdots \text{K}^+ \cdots (\text{O}-\text{Al})_3)$) is related to AFB1–illite adsorption (K_d , 141.6–158.3 L/kg); a strong calcium-bridging linkage ($((\text{C}=\text{O})_2 \cdots \text{Ca}^{2+} \cdots (\text{O}-\text{Si})_4)$) is involved in AFB1–smectite adsorption (K_d , 354.6–484.7 L/kg). Changes in Gibbs free energy (ΔG°) suggested that the computed result is reliable, providing a good reproduction of AFB1–clay interaction.

Keywords: Sorption; Aflatoxin B1; Bonding types; Quantum computation; Clays

1. Introduction

Aflatoxins (AFs) are classified as naturally biotoxins produced by *Aspergillus flavus* and *Aspergillus parasiticus*, and have been implicated in the etiology of cancers due to their high toxicity, mutagenicity, and carcinogenicity [1]. AFs produced or deposited in the soil environment can reduce the quality of soils by destroying the biological productivity, environmental function, and organism health [2, 3]. Previously reported concentrations of AFs in the soil environment are in the range of 1.9–881.4 nmol/kg soil [4]; once these mycotoxins are taken up by crops, they can be transported from the root into edible portions of the plant [5], subsequently contaminating feeds [6], grains [7, 8], and even dairies [9]. Therefore, the environmental behavior of AFs in cultivated soil has raised worldwide scientific, political, and economic concerns [10, 11].

Clay minerals in the soil environment can sequester and detain AFs, and the many groups of AFs can be easily adsorbed onto smectite [12, 13]. The major bonding between adsorbed aflatoxin B1 (AFB1) and smectite has been shown to be likely due to ion–dipole interactions and/or cation/carbonyl oxygen coordination [14]. Other study suggested that electrostatic attraction in AFB1 played the most important role in the bonding of the toxin to smectite [15]. There are still much dispute about major bonding mechanisms, as there is confusion in the various mechanisms controlling the adsorption of AFB1 to smectite. For example, especially for the kaolinite and illite clays with a low cation exchange capacity (CEC), the major bonding mechanisms of aflatoxin to them are not well understood. Due to their abundance and importance (kaolinite ~35% and illite clays 11–50% in desert and latosol-like soils [16]), weak bonding types dominating AFB1-to-clay sorption need to be discriminated before evaluating the effectiveness of mycotoxin adsorption.

Because of variety of functional groups, AFB1 may be adsorbed to the surface of clays by many ways. Chemically, AF molecules consist primarily of two major functional groups (Fig. 1a, b): two

furan rings relating to toxicity [17] and a highly substituted coumarin relating to carcinogenicity [18]. The latter contains an organic lactone, two adjacent carbonyls, a five-carbon ring, and a phenyl group. Carbonyl groups are considered to be rich in excess electrons, thus they interact strongly with electron-deficient or positively charged species [14]. Since the secondary kaolinite and illite clays contain negligible exchangeable cations and less interlayer space compared to smectite, AFs cannot be bonded to their surface through known carbonyl cation electron–donor–acceptor (EDA) interactions. Therefore, a quantitative discrimination of the multiple molecular attractions between AFB and clays is necessary for understanding the sequestration of AFB to various clays.

Many of AFB molecules, including environmentally relevant AFB1, B2, G1, G2, M1, M2, P1, Q, H1, GM, B2a, and aflatoxicol, have the similar molecular moieties/groups, e.g., electrophilic C–H in unsaturated carbon rings, electron-donating carbonyl, oxygen-containing moieties, and H-bonding moieties. The surface chemistry of clays might have a considerable effect on the sequestration and detainment of AFB via the discriminated multiple bonding interactions. The representative AFB1, known with the highest toxicity, was used in the present study. The objective was to investigate the interaction between AFB1 and clays, and in particular, to explore how AFB1, by resorting to its typical moieties/groups, bond to the reference illite, kaolinite, and smectite clays via the discriminated attractions. First, a group of batch sorption experiments are performed to evaluate the adsorption affinities of different adsorbate–adsorbent combinations. Second, the computation, visual isosurfaces of AFB1–clay interactions, and change in Gibbs free energy (ΔG°) are presented to discriminate the bonding types, and examine the association of physical bonding strengthen/energy with AFB-to-clay sequestration. Both the amount of ions released from the interlayer space of clays and the effect of model cations on adsorption are comprehensively considered to characterize the bonding mechanisms.

2. Materials and Methods

2.1. Materials

Three reference clays (kaolinite, illite, and smectite) were used to prepare adsorbents for the sorption experiments. The kaolinite and smectite clays were purchased from Sigma-Aldrich (St. Louis, MO, USA). The illite clay was sampled from Liaocheng, Liaoning Province, in northeast China. After the clays were crushed in an agate mortar and screened using a 37.4- μm sieve, clay grains were mixed with ultrapure water (1:10, w/v). The suspensions were stirred for 20 min at 100 revolutions per minute (rpm), followed by a 24-h standing. The liquid supernatants were removed out by a siphon. The subsided clays were dried by a vacuum freeze drying apparatus at -65°C for one week. The dried clays were stored at room temperature for later analysis. The physicochemical properties of the reference clays, including particle size, cation exchange capacity (CEC), surface charge (ξ potential), BET specific surface area (BET-SSA), and light scattering-based specific surface area (LS-SSA) are provided in the Supplemental Table S1. Particularly, aqueous clays (10 g/L) were used to measure their physicochemical properties. Both size and light scattering-based specific surface area of clay particles were measured by a laser grain size analysis (Zetasizer, UK) [19], after a low-power ultrasound for 10 min (2.0 W/cm, 40 KHz). The ξ potential was determined by a zeta analyzer (Zetasizer Nano, UK). The BET-SSA was analyzed by a Brunauer-Emmett-Teller method [20, 21]. Here, the light scattering method was used to measure the outside surface of clay aggregates. The BET method was used to determine the outside and interstitial surface area of clay aggregates. The AFB1, used as a model biotoxin, was purchased from Sigma-Aldrich. Selected properties of the solutes including the molecular weight (MW), octanol–water partition coefficient ($\log K_{ow}$), and water solubility (S_w) are also listed in Table S1 [18]. Ultrapure water produced using

a Milli-Q gradient system (electric conductivity $18.2 \text{ M}\Omega \times \text{cm}$; Millipore, Bedford, MA, USA) was used to carry out all experiments.

2.2. Batch sorption

Sorption was performed in 22-mL polytetrafluoroethylene (PTFE)-lined screw cap glass vials receiving 100 mg of clays. A suspension of clays (0.1 g) was prepared by adding ultrapure water (10 mL), followed by the addition of an AFB1 solute to obtain the desired concentrations (0–0.5 mg/L) using a micro-injector (Agilent Technologies, Palo Alto, CA, USA). The added benzene/acetonitrile (98/2) carrier with the AFB1 solute was kept below 0.1% (v/v) for the AFB1–clay suspension to minimize cosolvent effects on sorption. The samples were shaken in an orbital shaker incubated at $25 \pm 0.5^\circ\text{C}$ for 3 days, which was demonstrated to be sufficient to reach apparent sorption equilibrium (no further uptake) on the basis of kinetic studies (data not shown). At the end of the sorption experiments, the samples were centrifuged at $3000 \times g$ for 20 min and the supernatants were collected from an aliquot of the aqueous phase. The solute AFB1 was analyzed directly via high-performance liquid chromatography (HPLC) with a $4.6 \times 150\text{-mm}$ HC- C_{18} column (LC-20AT; Shimadzu, Otsu, Japan). Isocratic elution was performed with a fluorescence detector (RF-10AxL; Shimadzu) at an excitation of 365 nm and an emission of 435 nm. The control and data analysis were performed at a flow rate of 1 mL/min and temperature of 40°C under the conditions of acetonitrile and water ($v/v = 45/55$). Calibration curves included at least seven standards over the concentration range of the sorption samples. Adsorbed mass was assumed to equal the difference between added mass and mass in the solution aqueous phase. The sample pH was unadjusted and varied slightly (7.0 ± 0.4) at sorption equilibrium, as measured at the end of the sorption experiments.

In addition, after the isothermal adsorption equilibrium, the release of interlayer cations in

swelling clays, including Ca^{2+} and Na^+ from smectite, K^+ from illite, and Ca^{2+} and K^+ released from kaolinite, was investigated. And a separate experiment was performed to test the effect of metal ions to the sorption equilibrium. The experimental details could be seen in the ionic effect of supplementary material.

2.3. Computation

According to the physicochemical properties of the clays, and considering the workload for the computation and dominance of clay surfaces in AFB1 adsorption, monolayer models for the three clays (around 240 atoms) were constructed using CrystalMaker Software [22]. Kaolinite minerals had a simple composition and structure being composed of aluminum, silicon, oxygen, and hydroxyl ions, while their structure was a 1:1 layer clay, in which one sheet consisted of aluminum octahedra and the other of silicon tetrahedra [23]. The structure of illite consisted of repeated tetrahedron–octahedron–tetrahedron (TOT) layers. The interlayer spaces were mainly occupied by poor potassium cations that were responsible for the absence of swelling, even though the structure consisted of TOT layers. Thus, in the illite structure, silicon in the tetrahedra was commonly replaced by Al^{3+} , the K^+ was near the aluminum-oxygen tetrahedra to neutralize the charges. The structure of smectite clays was a 2:1 layer of clay, in which two sheets consisted of silicon tetrahedra and one sheet comprised aluminum octahedra. The yellow-labeled Mg^{2+} replaced Al^{3+} in the aluminum octahedral, and its charged outside surface could adsorb and accommodate the Ca^{2+} from the interlayer spaces. The geometry of AFB1 was structured using the ChemBioOffice Software 13.0 (PerkinElmer, Waltham, MA, USA). All of the constructed geometries, including the typical crystal models and AFB1, were initially optimized using the Semi-Empirical Method PM6 (Gaussian 09 program [24]), followed by both an optimization and frequency analysis using DFT at the B3LYP/6-31G(d,p) [24]. Solvent (water) effects were implicitly taken into consideration. The

calculated molecular structures were imported for later topological analysis and graphic representations of the distribution of electron density.

Topological analysis and graphic representations of the distribution of electron density were performed to discriminate bonding types using the Multiwfn program [25], in discriminating bonding types of supplementary material. The ΔG° used to compare the bonding strength were calculated by either the adsorbent-to-solution distribution coefficient (K_d) or a computer simulation. Gibbs free energies (ΔG°) for these adsorptions were obtained by the K_d of AFB1 to clays. K_d is also calculated at the DFT b3lyp/6-31g(d) level to corroborate the reliability for calculating the AFB1–clay bonding, in the change in Gibbs free energy (ΔG°) of supplementary material.

3. Results and discussion

3.1. Adsorption isotherms

The sorption isotherms for the AFB1–kaolinite, AFB1–illite, and AFB1–smectite clays were fitted using the Freundlich model: $q = K_F C_w^n$, where q ($\mu\text{mol/kg}$) and C_w ($\mu\text{mol/L}$) are the equilibrium concentrations of adsorbate on the adsorbent and in the aqueous solution, respectively, K_F ($\mu\text{mol}^{1-n} \text{L}^n/\text{kg}$) is the Freundlich affinity coefficient, and n (unitless) is the Freundlich linearity index (larger departures from $n = 1$ imply more nonlinear sorption). The sorption isotherms of AFB1 to the three clays are shown in Fig. 2, and fitting parameters are summarized in Table 1. The different sorbate–sorbent combinations had varying degrees of linearity, with n values of 1.1 (AFB1 to illite), 1.2 (AFB1 to smectite), and 1.5 (AFB1 to kaolinite). For all adsorbents, the adsorption of AFB1 was much more nonlinear than the reported adsorption of aromatics ($n = 0.82\text{--}1.0$ for phenanthrene soil [26]). A clear trend was observed wherein the sorption isotherms of AFB1 to the three clays appeared to be slightly upwardly concave ($n > 1$), which suggests that interactions

between AFB1 and clays were weak [27] compared to the previously reported partition ($n = 1$) or upward convex curve-related ($n < 1$) sorption [28]. In Table 1, the K_d was calculated as the ratio of q versus C_w at each datum. Within the tested concentration ranges (0–0.5 mg/L), the adsorption coefficient (K_d) was within a gradient range of 18.5–37.1 L/kg for AFB1 on kaolinite, 141.6–158.3 L/kg for AFB1 on illite, and 354.6–484.7 L/kg for AFB1 on smectite (Table 1). Overall, the K_d values for AFB1–smectite adsorption were significantly larger than those for AFB1–kaolinite and AFB1–illite.

The outer surfaces of clay aggregates are responsible for AFB1 adsorption. K_d is used both for the weight-based partition coefficient (L/kg) and the surface area normalized partition coefficient (L/m²). Here, we used two normalizations of surface area to explore the availability of interstitial spaces in clay aggregates. The light scattering-based specific surface area (LS-SSA) is related to the outside surface of flocculation; the Brunauer–Emmett–Teller (BET)-based specific surface area (BET-SSA) is linked to the outside surface and interstitial spaces of clay aggregates. Fig. 3a shows normalized AFB1–clay isotherms produced using the LS-SSA. The observed adsorbent-to-solution distribution coefficient (K_d) followed the order of smectite (0.19–0.26 L/m²), illite $(9.6–10.7) \times 10^{-2}$ L/m², and kaolinite $(4.5–9.0) \times 10^{-3}$ L/m² (Table 2). Fig. 3b shows that normalized adsorption (K_d) of AFB1 by the BET-SSA followed an order of decrease: smectite $(4.64–6.35) \times 10^{-2}$ L/m², illite $(2.01–2.25) \times 10^{-2}$ L/m², and kaolinite $(1.27–2.54) \times 10^{-3}$ L/m². By comparing the two normalizations, the normalized K_d of AFB1 to the three clays by LS-SSA was noted to be 0.5–1.0 orders of magnitude greater than that observed using the BET-SSA, which is consistent with the outside surface area accessibility of the adsorbent. This may indicate that the entire outside surface area of the clays was potentially available for AFB1 adsorption, while the interstitial spaces in clay aggregates were not completely available for AFB1 adsorption due to the molecular sieving effect

[29, 30].

In addition, after samples were processed by a vacuum freezing drying technique (-65°C , 7 days), another piece of evidence provided by X-ray diffraction (XRD) (Fig. 4) shows an unchanged interlayer spacing before and after the adsorption of smectite to AFB1 (0–1.6 mmol/L). This suggested that interlayer spacing of the smectite was not affected by the adsorption, which might be due to the low AFB1 concentration[14] in the present study.

3.2. Discrimination of bonding types

The adsorption of AFB1 to the three clays was calculated on the basis of structured models of AFB1 and clay minerals. Multiple interactions between AFB1 and clays can be seen in Fig. 5. The results can be considered to be a good reproduction of the AFB1–clay interactions. The gradient isosurfaces in Fig. 5a, c, and e are colored according to the corresponding values of $\text{sign}(\lambda_2)\rho$ in Fig. 5b, d, and f, respectively, which were found to be a good indicator of bonding strength. We can utilize the sign of λ_2 to distinguish bonded ($\lambda_2 < 0$, such as H-bonding) from nonbonded ($\lambda_2 > 0$) interactions (a more negative value indicates a stronger bonding strength), whereas the density itself provides information regarding the strength [31]. Values near zero indicate a weak van der Waals (VDW) electrostatic attraction.

The gradient isosurfaces suggest that AFB1 bonded to kaolinite via a weak H bonding between AFB1 and kaolinite (Fig. 5a). The H-bonding attraction is classified into the vdWs electrostatic interaction. The green isosurfaces between AFB1 and kaolinite suggest that the AFB1 bonded to kaolinite through a pair of weak $(\text{Si/Al-OH})_2 \cdots (\text{O}=\text{C})_2$ bonds (dotted lines between atoms represent a bonding site). Fig. 5b shows the corresponding plots of the reduced density gradient (RDG) versus the electron density multiplied by the sign of the second Hessian eigenvalues. The low-gradient spikes (-0.01 to 0.01 a.u.) for $(\text{Si/Al-OH})_2 \cdots (\text{O}=\text{C})_2$, corresponding to light green isosurface in Fig.

5a, quantitatively indicate a vdWs electrostatic attraction.

Visual gradient isosurfaces in Fig. 5c indicate a EDA interaction between AFB1 and illite interaction via K^+ bridge. Green isosurfaces show that AFB1–illite interaction via K^+ is moderate as compared to AFB1-kaolinite (weak) and -smectite (strong, later introduced), and strength of attraction falls into the scope of vdWs' interaction; but the EDA interaction may be associated with coordination of K^+ to electronegative oxygen atoms in AFB1 and illite. It is obviously observed that the AFB1 bonded to illite through the (AFB1) $C=O \cdots K^+ \cdots O-Al$ (illite); the bonding site in the illite clay is near substitutive $Al^{3+}-O$ tetrahedron. The weak attraction was also confirmed by the corresponding low-gradient spikes at $-0.01 \text{ — } -0.02$ a.u. (Fig. 5d). The formation of their weak EDA attraction can be attributed to the negatively charged surface of illite clay due to the isomorphous replacement ($Al^{2+} \rightarrow Si^{4+}$) [32], as supported by the ξ potential of Supplemental Table S1. These results confirm the above conclusion in which the observed K_d values for the AFB1-to-illite adsorption are significantly larger than those for the AFB1-to-kaolinite adsorption which is associated with electrostatic interaction (weak H-bonding).

The evidence from visual gradient isosurfaces was also used to verify the enhanced AFB1-to-smectite adsorption by the release of Ca^{2+} (Fig. 5e) from the interlayer space. The blue isosurfaces between AFB1 and Ca^{2+} suggest that the Ca^{2+} was synchronously bonding to two carbonyls. Meanwhile, Ca^{2+} was also attracted to the four oxygen atoms of the Si–O ring on the surface of smectite clays. Two low-gradient spikes at $-0.02 \text{ — } -0.04$ a.u. further demonstrate the formation of a $(C=O)_2 \cdots Ca^{2+} \cdots (O-Si)_4$ bridging linkage via a strong EDA coordination reaction on the surface of the smectite, which is consistent with previously published studies [14]. The enhanced interaction between AFB1 and smectite by Ca^{2+} is dependent on the major cation-bridging linkage related to the $(C=O)_2 \cdots Ca^{2+} \cdots (O-Si)_4$, secondary weaker $C=O \cdots Si$, $O=C \cdots O$, and aromatic $C \cdots O$ bonding. This

reveals that the observed increasing distribution coefficient (K_d , L/kg) of AFB1 to kaolinite, illite, and smectite clays can be attributable to vdWs electrostatic attraction, H-bonding attraction, and calcium bridging linkages, respectively.

Moreover, we have considered the solvent (water) effects on AFB1-clay interactions by using an implicit solvent model (water cavity), when the molecular structures were computed by the software. It is well known that water molecule was likely to combine with K^+ or Mg^{2+} (hydrated ions). This combination possibly influence the AFB1-to-illite and -to-smectite adsorption in some weak interactions. Thus, an explicit water molecule was added in the model to assess the possible effect. The computed results were exhibited in Fig. S2. It can be observed from the Fig. S2a and c that oxygen atom in water molecule bonded to K^+ and Ca^{2+} , showing the formation of hydrated metals. In the presence (Fig. S2) and absence (Fig. 5) of an explicit water molecule, two low-gradient spikes at 0 — -0.02 a.u from the Fig. S2b were almost the same as that from the Fig. 5c; the low-gradient spikes from Fig. S2d were similar to that from Fig. 5f (0 — -0.04 a.u). They suggested that comparing to moderate and strong EDA coordination between metals and electronegative oxygen atoms in AFB1 and clays, the hydrated metals via a weak $H_2O \cdots metal$ interaction cannot obviously caused the change in bond types of AFB1-metal-clays.

ΔG^0 -based $\ln K_d$ (■) was calculated at the density functional theory (DFT) b3lyp/6-31g(d,p) level. The ΔG^0 -based $\ln K_d$ scatter in areas of $\ln K_d$ -based ΔG^0 was found to correspond to smectite, illite, and kaolinite, suggesting that the results of the computation regarding the model were reliable and provided a good reproduction of the AFB1-clay interaction. Furthermore, the $\ln K_d$ -based ΔG^0 for the adsorption of AFB1 to the three clays appears as an orderly distribution of AFB1-smectite > AFB1-illite > AFB1-kaolinite (a minus in the x -coordinate represents a spontaneous process), suggesting that the bond energies increased in the order of vdWs electrostatic attraction (weak

C=O $\cdots$ Si, O=C $\cdots$ O in AFB1–kaolinite adsorption), weak EDA attraction ((C=O) $_2\cdots$ K $^+\cdots$ (O-Al) $_3$ in AFB1–illite adsorption), and calcium bridging linkage ((C=O) $_2\cdots$ Ca $^{2+}\cdots$ (O-Si) $_4$ in AFB1–smectite adsorption). In addition, the $\ln K_d$ -based ΔG^0 plot indicates that all sites were likely to involve a spontaneously physisorption mechanism because the ΔG^0 was lower than 20 kJ/mol [33]. Note that the reported B3LYP basis set established previously was not suitable for systems with long-range interactions, including the dispersion force between nonpolar molecules [34, 35]. In this study, ΔG^0 from computation (ΔG^0 -based $\ln K_d$) coincided with tested adsorption data ($\ln K_d$ -based ΔG^0), suggesting that the vdWs electrostatic attractions were generated from the excellent local polarity in AFB1 molecules/clays, rather than from the dispersion force between weak-/non-polar molecules.

3.3. Adsorption associated with exchangeable cations

The release/leaching of cations in clays is spontaneous process. The release/leaching of Ca $^{2+}$ in smectite clay will be conducive to the combination of AFB1 on clay surface via Ca $^{2+}$ bridge. In order to expound this question, the amount of interlayer cations released from clays with a surface adsorption of AFB1 is shown in Fig. 7a. The amount of Ca $^{2+}$ released from smectite with a surface adsorption of AFB1 displayed an entirely different pattern. The amount of Ca $^{2+}$ released from the AFB1–smectite combination displayed a more than 21-fold increase compared to the K $^+$ released from AFB1–illite, and the K $^+$ and Ca $^{2+}$ released from AFB1–kaolinite (control) when the AFB1 was greater than 7×10^{-3} $\mu\text{mol/L}$. The amount of Ca $^{2+}$ released from smectite gradually increased from 0.64 to 0.85 $\mu\text{mol/L}$ as the AFB1 increased from 0 to 1.6×10^{-2} $\mu\text{mol/L}$, but the K $^+$ and Ca $^{2+}$ released from the kaolinite (control) and illite, respectively, remained below 0.03 $\mu\text{mol/L}$. The enhanced Ca $^{2+}$ release in AFB1–smectite suggests that the interlayer Ca $^{2+}$ in smectite can be rapidly released from interlayer sites to the aqueous phase [36] and sequestered to carbonyl to form the detected Ca $^{2+}$ –AFB1 complex [37], which will accelerate the dissolution of Ca $^{2+}$ due to the

equilibrium of interlayer Ca^{2+} shifting toward aqueous Ca^{2+} . Conversely, the amount of Na^+ released from the interlayer space of smectite clays mediated by an AFB1 adsorption can be obtained from the Supplemental Fig. S3, which suggests that although the Na^+ in smectite could also be easily released from interlayer sites to the aqueous phase, sequestering carbonyl to form the complex was difficult and resulted in a negligible change in the aqueous Na^+ as AFB1 increased. In addition, the amount of K^+ released from the interlayer space of illite clays was very low due to its strong electrostatic interlayer attraction [38, 39]. The low level of K^+ and Ca^{2+} dissolution from kaolinite clays can be attributed to the absence of exchangeable cations (K^+ and Ca^{2+}).

The aqueous Ca^{2+} and K^+ were selectively added to the adsorbate–adsorbent solution to further expound the effect of correspondingly stimulated interlayer cations on the AFB1 adsorption. The adsorption of kaolinite to AFB1 in the presence of Ca^{2+} and K^+ was also investigated as a control experiment. On the basis of Fig. 7b, the observed K_d values for AFB1 on smectite increased from 410 to 920 L/kg as the Ca^{2+} increased up to 65 mmol/L. The K_d values for AFB1 on illite decreased from 410 to 100 L/kg, when K^+ rose from 0 to 64 mmol/L. The effect of the added $\text{K}^+/\text{Ca}^{2+}$ on AFB1–kaolinite can be neglected, because the K_d values were less than 40 mmol/L.

In addition, we found that the exchangeable Ca^{2+} in smectite was more appropriate to serve as the coordination center than the K^+ in illite, although the illite clays had the strongest negatively charged surface (Supplemental Fig. S1). The main reason is that Ca^{2+} has an empty valence electron orbit, which will allow the Ca^{2+} –AFB1 to adsorb to the surface of electronegative smectite via a cation-bridging linkage, whereas K^+ binds to either the carbonyl of AFB1 or the surface of electronegative illite. Here, it is worthy that compared with previous studies [14, 15], in the present study, the adsorption did not mainly occur in the interstitial spaces and interlayer, associated with the surface adsorption. As observed in Fig. 7a, the surface adsorption of AFB1 to smectite caused

the release/leaching of Ca^{2+} in the interlayer, whereas free Ca^{2+} may mediate the increase in surface adsorption (Fig. 7b). The mutually reinforcing relationship between them may imply that release/leaching of Ca^{2+} in the interlayer of smectite ought to make a greater contribution to AFB1 sequestration in natural environments.

4. Conclusions

The most striking observation in this study is that bonding types for the sorption of AFB1 to kaolinite, illite, and smectite clays can be well discriminated, and we confirmed that observed adsorbent-to-solution adsorption coefficients (K_d) of AFB1 to three clays are dominated by different bonding types between them. Furthermore, the findings suggest that weakly electrostatic attractions $((\text{Si-OH})_2 \cdots (\text{O}=\text{C})_2)$ are responsible for AFB1–kaolinite adsorption (low K_d , 18.5–37.1 L/kg), that a moderate EDA interaction $((\text{C}=\text{O})_2 \cdots \text{K}^+ \cdots (\text{O-Al})_3)$ is related to AFB1–illite adsorption (middle K_d , 141.6–158.3 L/kg), and that a strong calcium-bridging linkage $(\text{C}=\text{O})_2 \cdots \text{Ca}^{2+} \cdots (\text{O-Si})_4$ is involved in AFB1–smectite adsorption (high K_d , 354.6–484.7 L/kg). Overall, considering the abundance and importance of the three clays, especially for kaolinite and illite, our findings suggest that the bonding types for the sorption of AFB1 to kaolinite, illite, and smectite clays can be well discriminated using computation assisted with batch sorption experiment, which will help to understand the environmental behavior of relative fungi biotoxin contaminants involved in phyllosilicate clays.

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Figure Legends

Fig. 1. Chemical structure of major functional moieties (a) and surface electrostatic potential (b, ESP) of AFB1. Figure 1a: Arabic numerals 1–6, two furan rings; 7–17, substituted coumarin. Figure 1b: blue regions represent the negative potential surface of oxygen-containing groups.

Fig. 2. Original isotherms of AFB1 to kaolinite, illite, and smectite clays plotted based on the sorbed concentration (q) versus the aqueous phase concentration (C_w).

Fig. 3. Normalized isotherms by light scattering-based specific surface area (LS-SSA) (a) and BET-based specific surface area (BET-SSA) of clays (b) for AFB1 ($q_N = q / BET$).

Fig. 4. X-ray diffraction (XRD) of smectite after the isothermal adsorption equilibrium.

Fig. 5. Gradient isosurfaces (a, c, and e) and the plots of reduced density gradient versus the electron density multiplied by the sign of the second Hessian eigenvalues (b, d, and f). AFB1–kaolinite (a and b), AFB1–illite (c and d), and AFB1–smectite (e and f). The C, O, Si, and H in AFB1 and clays are labeled by cyan, red, yellow, and white, respectively. The one Si^{4+} is replaced by Al^{3+} (cyan and smaller than C in size) in illite (c) ($Si^{4+} \rightarrow Al^{3+}$). One Al^{4+} is substituted by Mg^{2+} in the smectite crystal (e) ($Al^{4+} \rightarrow Mg^{2+}$), but it cannot be seen in Figure 5e because of the shade from other atoms.

Fig. 6. Plot of $\ln K_d$ -based ΔG^0 and ΔG^0 -based $\ln K_d$. Gibbs free energy (ΔG^0) was obtained by the K_d of AFB1 to clays ($\square$). K_d is also calculated at the DFT b3lyp/6-31g(d) level to corroborate the reliability for calculating the AFB1 – clay bonding ($\blacksquare$). The ΔG^0 at basis set is calculated from the energy difference before and after the reaction of AFB1–clays with AFB1 and clays ($\Delta G^0 = G_{AFB1-Clays} - (G_{AFB1} + G_{Clays})$). Solvent (water) effects were taken into consideration implicitly.

Fig. 7. Effect of cations on AFB1-to-clay adsorption. (a) Released interlayer cations by AFB1

adsorption. The release of Ca^{2+} and K^{+} in kaolinite clay was used only as a control. (b) The effect of correspondingly stimulated interlayer cations on AFB1-to-clay adsorptions. Error bars represent the standard deviation of triplicate samples.

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 6.

Fig. 7.

Table 1 Freundlich model parameters K_F for the isotherms measured for the sorption to kaolinite, illite, and montmorillonite clays, along with the lowest and highest clay-solution sorption coefficients (K_d , L/kg). Here, the K_d was calculated as the ratio of q versus C_w at each datum.

Clays	Adsorbates	K_F	n	R^2	K_d
Kaolinite	AFB1	35.8	1.5	0.994	18.5 – 37.1
Illite	AFB1	157.8	1.1	0.997	141.6 – 158.3
Smectite	AFB1	670.0	1.2	0.988	354.6 – 484.7

Table 2 Normalized Freundlich model parameters measured for AFB1 sorption to kaolinite, illite, and montmorillonite clays, along with the lowest and highest clay-solution adsorption coefficients (K_d , L/m²).

Normalized parameters by BET-based specific surface area (BET-SSA)					
Clays	Adsorbates	K_F	n	R^2	K_d
Kaolinite	AFB1	2.5×10^{-3}	1.5	0.994	$(1.27 - 2.54) \times 10^{-3}$
Illite	AFB1	2.2×10^{-2}	1.1	0.997	$(2.01 - 2.25) \times 10^{-2}$
Smectite	AFB1	8.8×10^{-3}	1.2	0.988	$(4.64 - 6.35) \times 10^{-2}$
Normalized parameters by light scattering-based specific surface area (LS-SSA)					
Clays	Adsorbates	K_F	n	R^2	K_d
Kaolinite	AFB1	8.7×10^{-3}	1.5	0.994	$(4.5 - 9.0) \times 10^{-3}$
Illite	AFB1	0.11	1.1	0.997	$(9.6 - 10.7) \times 10^{-2}$
Smectite	AFB1	0.36	1.2	0.988	0.19 – 0.26