THE CHARACTERIZATION OF CLAYS AND CETYLPYRIDINIUM-EXCHANGED CLAYS FOR THEIR ABILITY TO ADSORB ZEARALENONE

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Abstract

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The adsorption of zearalenone (ZEN) onto Ca-montmorillonite (STx-1), Na-montmorillonite (SWy-2), acid-activated montmorillonite (AAM), clinoptilolite and cetylpyridinium (CP) exchanged clays was studied. The results indicated that CP-exchanged clays were more effective than unmodified base clays in adsorbing ZEN, i.e. STx-1 (7.4±2.3 mg/g) < clinoptilolite (7.6±1.1 mg/g) < AAM (10.0±0.0 mg/g) < SWy-2 (11.9±0.4 mg/g), whereas the extent of ZEN adsorption into modified clay products did not follow the order seen in the based clays. The adsorption efficiency of CP-exchanged clays is in the order of CP*STx-1 (56.9±1.1 mg/g) = CP*AAM (56.9±1.8 mg/g) > CP*SWy-2 (47.9±2.6 mg/g) > CP*clinoptilolite (15.5±1.1 mg/g). Three CP-exchanged clays were further characterized by isotherm studies. The results indicated that the isotherm shapes of CP-exchanged clays exhibited a S-shape. The isotherm plot is concave up to the inflection point and then plateaus, which suggests multiple, specific binding sites and saturation at each site. It is important to rigorously test and thoroughly characterize potential mycotoxin binding agents both in vitro and in vivo to provide an understanding of the mechanism of adsorption, in order to modify or develop adsorbents for enhanced products and multi-mycotoxin binding agents.

Keywords: zearalenone, adsorption, clay, cetylpyridinium-exchanged clays

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Introduction

Zealarenone (ZEN) or F-2 toxin is an important mycotoxin in temperate and warm regions of the world. The toxin is an estrogenic substance produced by Fusarium fungi. ZEN occurs in maize and small grains like barley, wheat, sorghum, millet and rice (Miller, 1995). ZEN causes alterations in the reproductive systems of several animals. The toxin produces a potent hyperestrogenic response in susceptible animal species. Swine are the most affected, other animals, such as cattle, poultry and laboratory animals, are also affected, but to a lesser extent. Symptoms of ZEN poisoning include precocious sexual development in gilts, uterine enlargement, swollen vulva and mammae, anestrous, pseudopregnancy, reduced litter size and reduced viability of newborn pigs (Pier, 1981). In pregnant gilts, ZEN causes embryo loss and the retention of corpora lutea in affected pregnant gilts (Long and Diekman, 1986). In boars, ZEN exposure resulted in inflammation of the prepuce, smaller testes, epididymitis, reduced vesicular gland weights, cessation of spermatogenesis and reduced libido. Also, ZEN causes a reduction in the conception rate of dairy heifers (Diekman and Green, 1992).

Methods for the inactivation of mycotoxins in contaminated feedstuffs are critically needed. Recently, a reduction in the bioavailability of ZEN by adding adsorbents to contaminated feedstuffs has been considered as a cost-effective and practical strategy for the animal production industry to pursue. Several adsorbents, including clays and modified clays are able to detoxify
Mycotoxins (Alegakis et al., 1999; Huwig et al., 2001). The modified clays that by altering morphological properties and binding capacities by exchanging with organic cations, have been recently studied extensively for their ability to remove toxins in the environment including mycotoxins (Ake et al., 2001; Pal and Vanjara., 2001; Tomasevic-Canovic et al., 2003). Probably, the most favorable approach for the study of mechanisms of adsorption of toxins onto the surfaces of sorbents, is through the use of isotherm analysis (where the amount of toxin adsorbed is plotted against the concentration of toxin in the external phase, at a constant temperature and usually under equilibrium concentration). Previous studies have utilized and developed multi-tiered methods to the in vitro prescreening of clay-based adsorbents for the adsorption of mycotoxins, including isothermal analysis (Lemke et al., 2001). These methods have allowed an insight into the molecular mechanisms of sorbents and enabled a comparison of critical similarities and differences. Various sorbents and clay minerals possess a distinct structure which can bind mycotoxins in different ways. Important structural differences provide unique functional and chemical characteristics. The objective of this study was to compare the ability of clays (Ca-montmorillonite (STx-1), Na-montmorillonite (SWy-2), acid-activated montmorillonite (AAM), clinoptilolite) and these clays exchanged with cetylpyridinium chloride (CP) to adsorb ZEN in vitro and include isotherm studies to characterize the toxin adsorption of the modified clay products.

Materials and Methods

Chemicals and reagents: Zearalenone was purchased from Sigma Chemical Co. (St. Louis, MO). Cetylpyridinium chloride (CP) was purchased from Aldrich Chemical Co. (Milwaukee, WI). Ca-montmorillonite (STx-1) and Na-montmorillonite (SWy-2), acid-activated montmorillonite (AAM), clinoptilolite) and these clays exchanged with cetylpyridinium chloride (CP) to adsorb ZEN in vitro and include isotherm studies to characterize the toxin adsorption of the modified clay products.
an incubator, at 25°C. After shaking, the samples were centrifuged (International Centrifuge, Model UV, International Equipment Co., Needham, MA) at 2000 rpm for 30 min, to separate the clay from the supernatant. The supernatant from the samples was analyzed for absorbance at 236 nm, to determine the concentrations of ZEN remaining in solution.

**Isotherm studies:** The isotherm method used in these studies was modified from one described previously published study (Lemke et al., 1998). A stock solution of ZEN was prepared as described earlier. Each isotherm study consisted of triplicate 5 ml solutions of ZEN made from dilutions of the stock. These yielded approximate concentrations of 0.2, 0.4, 0.8, 1.2, 1.6, 2.0, 2.4, 3.0, 3.2, 3.6, and 4.0 µg/ml. These concentrations were achieved by adding an appropriate amount of working solution to 16x125 ml, disposable, borosilicate test tubes and then adding the complimentary amount of purified water, to make a total volume of 5 ml. Approximately 10 mg of clay was weighed in a 16x125 ml, disposable, borosilicate test tube. Purified water was then added to the clay to make 2 mg/ml suspension. The suspension of clay was vortexed to achieve homogeneity. From this suspension, 50 µl (containing 100 µg of clay) was pipetted out with an autopipetter to each 5 ml test sample. Four controls were also prepared: a purified water control containing no toxin, a toxin control containing 0.2, 4 µg/ml ZEN, without clay and a control containing 5 ml purified water and 100 µg of clay. The samples and controls were capped and placed on an electric shaker running at 1,000 rpm (for 24 hr) and held in an incubator at 25°C. After shaking, the samples were centrifuged at 2000 rpm, for 30 min, to separate the clay from the supernatant. The supernatant was analyzed for absorbance at 236 nm using a UV-Visible spectrophotometer to determine the concentrations remaining in solution.

**Data calculations and curve fitting:** The UV/Visible absorption data was entered into an Excel spreadsheet (Microsoft, Redmond, WA) to calculate the amount of ZEN left in solution (CW), and the amount adsorbed (q) for each data point. The data was then transferred to a Table Curve 2D v 3 (Jandel Scientific, San Rafael, CA) to fit the modified Freundlich isotherm equation so as to determine the distribution constant (Kd) (Grant et al., 1998; Pimpukdee et al., 2002).

**Statistical analysis:** ZEN adsorption data were represented as the arithmetic mean±SEM of the three replicates per sample. Means showing significant differences in ANOVA were compared using Duncan’s multiple range test (Ott, 1993). All statements of differences were based on a significance of p < 0.05.

**Results**

Figure 1 shows the binding capacities of clays and cetylpyridinium-exchanged clays, for ZEN, under equilibrium adsorption conditions. Binding capacities of these clays ranged from 7.38±2.30 mg/g to 56.90±1.09 mg/g. The lowest average adsorption of ZEN from solution was STx-1 (7.38±2.30 mg/g) whereas the highest average adsorption of ZEN from solution were CP*STx-1 (56.90±1.09 mg/g) and CP*AAM (56.90±1.80 mg/g). SWy-2 and AAM demonstrated similar binding with an average adsorption of 11.90±0.41 mg/g and 10.00±0.00 mg/g, respectively. CP*clinoptilolite (15.48±1.09 mg/g) showed about two times increased adsorption capacity as compared to clinoptilolite (7.62±1.09 mg/g).

The data were fitted to a modified Freundlich isotherm to determine the distribution constant (Kd). The coefficient of determination (r²) and the distribution constant (Kd) are displayed in the graph (Figure 2-4). CP*SWy-2 had highest Kd value (42,913) as compared to Kd values of CP*STx-1 (23,263) and CP*clinoptilolite (29,519), which suggests that the CP*SWy-2 is more effective for removal ZEN in solution. Also, the modified Freundlich isotherm gave a good fit for CP*SWy-2 (r² = 0.9478) but not for CP*STx-1 (r² = 0.7104) and CP*clinoptilolite (r² = 0.8827). This study showed that experimental adsorption isotherms obtained for ZEN on CP*exchanged clays, may be classified, according to the Giles classification (Giles et al., 1974), as a S-shape
Figure 1  Average zearalenone adsorption onto clays and cetylpyridinium-exchanged clays. Means with the same letter are not significantly different by Duncan’s multiple range test at p < 0.05. The data represent mean ± standard error for triplicates in each sample.

Figure 2  Adsorption isotherm for ZEN on CP*STx-1. The data represents the mean adsorption to clay ± SD from three replicate experiments. Data were fitted using the modified Freundlich isotherm equation; values of Kd and r² are displayed in the graph.
Figure 3  Adsorption isotherm for ZEN on CP*SWy-2. The data represents the mean adsorption to clay ± SD from three replicate experiments. Data were fitted using the modified Freundlich isotherm equation; values of Kd and $r^2$ are displayed in the graph.

Figure 4  Adsorption isotherm for ZEN on CP*clinoptilolite. The data represents the mean adsorption to clay ± SD from three replicate experiments. Data were fitted using the modified Freundlich isotherm equation; values of Kd and $r^2$ are displayed in the graph.
subgroup max (Figure 2), a S-shape subgroup 3 (Figure 3), and a S-shape subgroup 2 (Figure 4), for CP*STx-1, CP*SWy-2 and CP*clinoptilolite, respectively.

**Discussion**

The montmorillonite is a dioctahedral smectite and the isomorphous substitution in montmorillonite occurs in the octahedral sheet rather than in the tetrahedral sheet. The large cation exchange capacity (CEC) of smectites arises from the substitution of Al$^{3+}$ for Si$^{4+}$ in the tetrahedral sheets and the substitution of divalent cations, such as Mg$^{2+}$, Ca$^{2+}$, and Fe$^{2+}$, for trivalent cations such as Al$^{3+}$ and Fe$^{3+}$ in the octahedral sheets. Montmorillonites swell in water, and the extent of the swelling is influenced by the hydration of the interlayer cations. Ca-montmorillonites, for example, can swell when immersed in water to give a basal spacing corresponding to the inclusion of three to four layers of water. Na-montmorillonites swell more than Ca-montmorillonites when immersed in water and in many instances the mean layer separations of these Na-exchanged clays can amount to hundreds of Å units. Molecular sorption on montmorillonites may involve chemical as well as physical effects. The large internal surface area of montmorillonite (up to 8 x 10^5 m$^2$ kg$^{-1}$) provides most of the adsorption surface. In addition, the external surface area of montmorillonite is generally greater than that of other clay minerals, due to its small particle size. The most important chemical properties of montmorillonites include high CEC, ion selectivity and molecular sorption. The most important physical properties of montmorillonites are expansion and collapse, retention of large quantities of water, high cohesion and adhesion, small particle size and an extremely large specific surface area (Barrer, 1978). The chemical structures of montmorillonite and AAM are different in that montmorillonites, activated in acids, yield products with a highly amorphous SiO$_2$ content and a new microstructure with pores that have greater adsorption properties. However, our results, indicated that montmorillonite and AAM were little different in their adsorption of ZEN.

Clinoptilolite is one of the zeolite group minerals, it is non expandable clay. Zeolite is a type of hydrous aluminosilicate belonging to the tectosilicates in which the SiO$_4$ tetrahedra form a 3-dimensional cage-like framework. In the zeolite structure, some Si$^{4+}$ ions are replaced by Al$^{3+}$, which results in a net negative charge, that needs to be balanced by exchangeable cations. The approximate chemical formula of clinoptilolite is (Ca, Na, K)$_6$A$_6$Si$_{30}$O$_{72}$.24H$_2$O (Li, 1999). Clinoptilolite’s framework consists of four channels. Three channels are formed with 8-membered rings of oxygen and one channel with 10-membered rings. The dimensions of the open channels in clinoptilolite are 0.89 x 0.35 nm$^2$ for the 10-member ring allowing exchangeable cation to enter and exit freely, but too small for ZEN sorption. Thus, the sorption of ZEN on clinoptilolite is limited to the external surface.

Our results indicated that cetylpyridinium-exchanged clays were more effective than unmodified base clays in adsorbing ZEN. Natural clays contain exchangeable metal ions (Ca$^{2+}$, Na$^+$, K$^+$), which make their surfaces hydrophilic; consequently they are quite ineffective in sorbing nonionic compounds from aqueous solutions. However, it has been found that if the metal ions are exchanged with large surfactant cations, such as long-chain cetylpyridinium (CP) or hexadecyltrimethylammonium (HDTMA), the clay surfaces become hydrophobic or organophilic. These clays are commonly referred to as organoclay and are very effective in sorbing organic pollutants (Mortland, 1970). This study suggests that natural clays exchanged with large surfactant cation (CP) greatly improved their adsorption abilities. It might be explained that CP interacts with clays and replaces the exchangeable cations on their surfaces, forming a stationary phase in the clay particles. The modification produces a change of surface property from hydrophilic to hydrophobic. In addition, as a result of the larger size of the CP cations, compared with that of the replaced cations, the interlamellar distance (basal spacing)
of the clay increases and additional space is produced in the clay particles, thus facilitating the entry of ZEN into interlayer region. Preliminary studies suggest that CP-exchanged clays can also be effective for the adsorption of bacteria (Herrera et al., 2000). Recently attempts have been made to modify natural clinoptilolite, with different amounts of octadecyldimethyl benzyl ammonium chloride and dioctadecyldimethyl ammonium chloride, for the effective adsorption of aflatoxin B1, zearalenone and ochratoxin A, with varying degrees of success (Tomasevic-Canovic et al, 2003).

Isotherm shapes are categorized into four types of curve, designated as H, L, C, and S, which represent different adsorption mechanisms (Giles et al., 1960). Generally, S isotherms have a concave shape at low concentrations and the amount adsorbed increases as a semilinear function of the concentration. The isotherm plot is concave, up until an inflection point and then plateaus. Subgroups are defined by sorption behavior at high concentrations. Subgroup 2 is characterized by one plateau, whereas, subgroup 3 has an inflection point due to a change from the plateau to a concave shape. Subgroup max is defined by the existence of a maximum. Isotherms of the S-shape have two mechanisms. First, solute-solute attractive forces on the surface may cause cooperative adsorption which lead to the S-shape. Secondly, the sorption of a solute may be inhibited by a competing reaction within the solution, such as a complexation reaction with a ligand (Hinz, 2001).

Clays with different structures are not all equal in their ability to adsorb ZEN. This article details the characterization of clays and modified clay products for the adsorption of ZEN. Further research could be made to modify natural based clays with chemical or physical treatments for producing mycotoxin binding agents with a higher adsorption capacity or multi-mycotoxin sequestering agents. It is important to rigorously test and thoroughly characterize potential mycotoxin binding agents in vitro and in vivo to provide a comprehensive understanding of the mechanism of adsorption. It will be useful for both the investigation and the modification of safe and effective clay products in order to prevent ZEN-induced diseases in animals and ZEN residues in food of animal origin.

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References


