Peanut lectin adsorption and interlayer expansion of clays.

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ABSTRACT

Peanut seeds contain the globular protein, peanut seed lectin (PNA). A lectin is a protein of non-immune origin that agglutinates cells and/or precipitates complex carbohydrates. Lectins are widely distributed in nature and occur in edible legumes, such as kidney beans, soy beans, lima beans, peanuts, and many other plants that are common in human diets. Peanut lectin is only toxic to people with a peanut allergy and can be used as a surrogate for the highly toxic castor seed lectin, ricin. The adsorption of purified peanut lectin to clay minerals was examined using batch adsorption isotherms. X-ray diffraction was used to measure interlayer expansion of clays due to PNA adsorption. Treatment of 30 mg of Na-montmorillonite with 10 mg of PNA at pH 5 expanded the basal spacing from 1.24 to 2.65 nm. At pH 7, 115 mg PNA/g montmorillonite was adsorbed. The effect of pH on both PNA adsorption and interlayer clay expansion will also be examined.

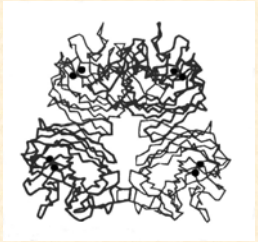
INTRODUCTION

Simulants are used to minimize exposure to toxins. A toxin simulant is particularly useful in the design and testing of new experimental apparatus and techniques. Simulants can be used in experiments that would be hazardous using the actual toxin. However, to be effective, experiments conducted using a toxin simulant should yield comparable results to experiments using the actual toxin. Hence, an ideal toxin simulant should have many properties in common with the toxin.

Peanuts contain the allergenic protein, peanut seed lectin (PNA). A lectin is a protein of non-immune origin that agglutinates cells and/or precipitates complex carbohydrates. Lectins are widely distributed in nature and occur in edible legumes, such as kidney beans, soy beans, lima beans, peanuts, and many other plants that are common in human diets. PNA is a tetrameric globular protein consisting of four subunits that each contain 236 amino acids with a total molecular weight of 110,000 (Figure 1). The protein can be grown into orthorhombic crystals that contain one tetrameric molecule in the asymmetric unit cell with dimensions of a = 12.93 nm, b = 12.69 nm, and c = 7.69 nm (Banerjee et al., 1994). The relatively non-toxic PNA might be used as a simulant for more toxic materials. The PNA protein is low in basic amino acids with 2.5 times as many acidic as basic amino acids (Lotan et al., 1975). The extremely toxic ricin lectin is a 65,000 molecular weight globular protein with about equal numbers of basic and acidic amino acids (Figure 2).

The objective of this study was to examine the adsorption and interlayer expansion of clays by peanut seed lectin.. Previous research has shown that the ricin toxin is strongly adsorbed to clays and produces interlayer expansion of smectites. The extent of peanut seed lectin adsorption and interlayer expansion of clays should reveal the suitability of this lectin as a ricin simulant.

Figure 1. Alpha-carbon backbone representation of peanut lectin, a tetrameric molecule of M_w =110,000. Subunits 1 and 3 shown as thin lines, subunits 2 and 4 shown as thick lines, circles represent metal ions. (After Banerjee et al., 1994).



MATERIALS AND METHODS

A sample of the Clay Minerals Society (CMS) reference Wyoming montmorillonite (SWy-2) was obtained and the <2μm fraction separated. Pure peanut seed lectin (PNA) was obtained from Sigma-Aldrich. Peanut seed extracts containing PNA were prepared by extracting raw peanuts with phosphate buffered saline solution (PBS). The peanut seed extracts were purified by centrifugation, filtration, precipitation with saturated $(NH_4)_2SO_4$, and dialysis. The purified peanut seed extracts contained ~35% PNA. The purified peanut seed extract was used in the adsorption isotherms. The pure Sigma-Aldrich PNA was used for standards and to prepare an oriented slide of SWy-2 for X-ray diffraction.

Batch adsorption isotherms of PNA to SWy-2 were prepared by adding 1.5 mg of PNA to 1 to 20 mg of SWy-2 in 15-mL centrifuge tubes. Blanks were used to correct for losses due to PNA precipitation, hydrolysis, or adsorption to the container. The blank concentrations were used as the initial concentrations for calculating the amount adsorbed. The pH was controlled by the addition of pH 4, pH 6, pH 7.4, and pH 10 buffer solutions. An enzyme-linked immunoassay technique (ELISA) was used to measure PNA concentrations in standards and in isotherm equilibrium solutions. Rabbit anti-PNA antibody, horse-radish peroxidase-labeled goat anti-rabbit antibody, and other ELISA reagents were obtained from Sigma-Aldrich Chemical Company.

Figure 2. Ribbon depiction of ricin structure. Disulfide bond linking A and B chains shown at center right. Numbers identify positions of amino acids. A-chain sequential parts are shaded and B-chain lactose moieties denoted by the discs. After Montford et al. (1987).

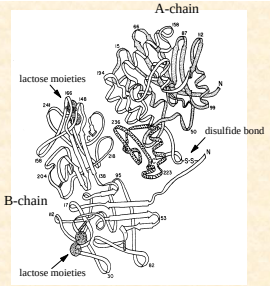


Figure 3. Peanut lectin adsorption and interlayer expansion of SWy-2.

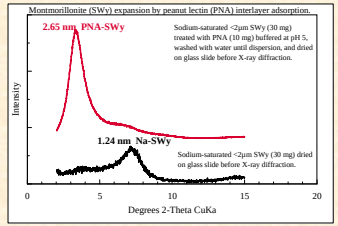


Figure 4. Ricin adsorption and interlayer expansion of SWy-2 (Jaynes et al., 2005).

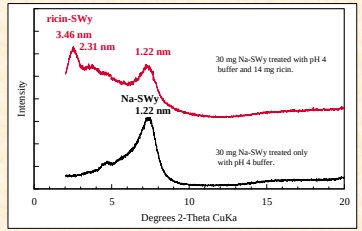


Figure 5. Effect of pH on peanut lectin adsorption to SWy-2.

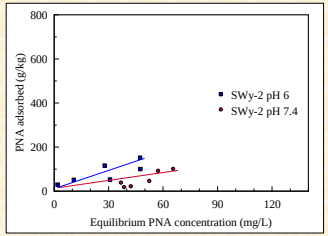
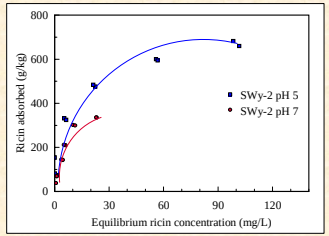


Figure 6. Effect of pH on ricin adsorption to SWy-2 (after Jaynes et al., 2005).



RESULTS AND DISCUSSION

Although PNA and ricin are both lectins and both globular proteins, the structures and molecular weights significantly differ (Figure 1 and 2). The adsorption of PNA to SWy-2 expanded the clay from 1.24 nm to 2.65 nm (Figure 3). However, ricin adsorption yielded greater interlayer expansion to 3.46 nm (Figure 4).

Blank recovery in the pH 4 and pH 10 adsorption isotherms for PNA were less than 10% and adsorption values were not calculated. Very low or very high pH conditions appear to destroy PNA. In contrast, ricin is stable from pH 4 to pH 10. Blank recoveries for the pH 6 and pH 7.4 PNA isotherms were about 60% and adsorption values were calculated. The adsorption of PNA at pH 6 was somewhat greater than at pH 7.4 (Figure 5). Protonation of the amine groups on the side chains of the basic amino acids in PNA at pH 6 might have contributed to greater adsorption. In contrast, ricin adsorption to SWy was much greater than PNA (Figure 6). Protonation of the far more numerous basic amino acids in ricin apparently contributed to much greater overall adsorption and greater differences in adsorption at pH 5 and pH 7.

Basinger (2005) measured breakthrough curves for the movement of PNA and ricin through soil columns. PNA breakthrough occurred in a few pore volumes, whereas, ricin breakthrough required many pore volumes and was only observed for the coarsest soil texture. The PNA and ricin breakthrough curves of Basinger (2005) are consistent with the adsorption isotherms in Figures 5 and 6. The adsorption of PNA to clay or soil particles is much lower than ricin adsorption.

There are several drawbacks in using PNA as a ricin simulant. There are significant differences in the properties of ricin and PNA. PNA has a different structure, a different amino acid composition, a larger molecular weight, and is not as stable as ricin. More importantly, PNA does not adsorb to clay or soil samples as readily as ricin. A better ricin simulant than PNA would be desirable. However, only a small number of lectins and antibodies for ELISA techniques are available.

CONCLUSIONS

Both peanut seed lectin (PNA) and ricin are globular proteins and lectins. Both lectins adsorb to clays and cause interlayer expansion of montmorillonite. However, PNA is not as strongly adsorbed as ricin and does not produce as much interlayer expansion. The acid/base stability of PNA is smaller than ricin. Clearly, the properties of PNA are significantly different than ricin. As a ricin simulant, PNA is useful but has great limitations. However, only small number of lectins and antibodies are available and a better ricin simulant might not be found.

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