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An affinity improved single-chain antibody from phage display of a library derived from monoclonal antibodies detects fumonisins by immunoassay

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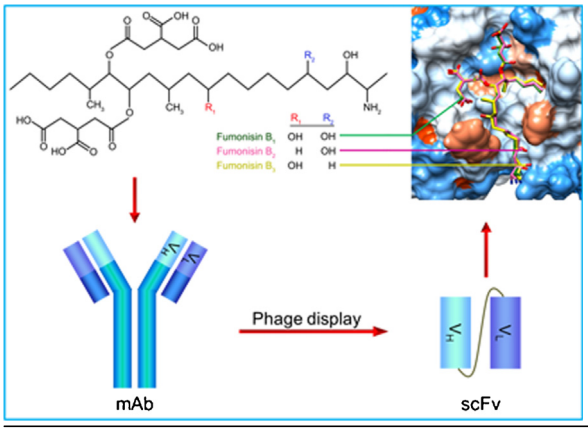
HIGHLIGHTS

- An affinity-improved scFv antibody was compared to its parent monoclonal antibody.
- scFv antibody-based detection of three fumonisin toxins in agricultural samples.
- Good agreement of novel antibody-based fumonisin detection and a chemical method.
- Favorable modeling of the scFv antibody structure complementary to fumonisins.

GRAPHICAL ABSTRACT



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ABSTRACT

Fumonisin B analogs, particularly FB_1, FB_2, and FB_3, are major mycotoxins found in cereals. Single-chain fragment variable (scFv) antibodies represent a promising alternative immunoassay system. A phage-displayed antibody library derived from four monoclonal antibodies (mAbs) generated against FB_1 was used to screen high binding affinity scFv antibodies; the best candidate was designated H2. Surface plasmon resonance measurements confirmed that the H2 scFv displayed a 82-fold higher binding affinity than its parent mAb. Direct competitive enzyme-linked immunosorbent assay demonstrated that the H2 antibody could competitively bind to free FB_1, FB_2, and FB_3, with an IC_{50} of 0.11, 0.04, and 0.10 μM, respectively; it had no cross-reactivity to deoxynivalenol, nivalenol and aflatoxin. Validation assays with naturally contaminated samples revealed a linear relationship between the H2 antibody-based assay results and chemical analysis results, that could be expressed as $y = 1.7072x + 5.5606$ ($R^2 = 0.8883$). Homology modeling of H2 revealed a favorable binding structure highly complementary to the three fumonisins. Molecular docking analyses suggested that the preferential binding of the H2 scFv to FB_2 was

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due to the presence of a hydrogen radical in its R1 position, leading to a proper electrostatic matching and hydrophobic interaction. The H2 scFv antibody can be used for the rapid, accurate, and specific detection of fumonisin contamination in agricultural samples.

1. Introduction

Fumonisins are a series of long-chain polyhydroxyl alkylamines esterified with two tricarballylic acid moieties (Fig. 1) that were first identified in 1988 from *Fusarium verticillioides* MRC826 isolated from maize [1]. 21 phytopathogenic *Fusarium* species are known to frequently infect maize and other cereals in the field and during storage and produce fumonisins; *F. verticillioides* and *Fusarium* proliferatum are the most problematic fumonisin producing species [2–4]. There are dozens of fumonisin analogs and isomers; these are separated into five groups: A, B, C, D, and P [3,5]. Among these, the fumonisin B analogs FB_1, FB_2, and FB_3, are the most abundant naturally occurring mycotoxins. FB_1 is the most prominent and is usually found at the highest levels in maize and other cultivated crops [3,6]. FB_2 is produced massively in higher quantities than FB_1 by some newly identified *Fusarium* species that infect maize [4]. FB_1 can disrupt sphingolipid metabolism, leading to many animal diseases such as porcine pulmonary edema and equine leukoencephalomalacia [7,8]. FB_1 is considered to stimulate the occurrence of human esophageal cancer [9–11] and liver cancer [11,12]. The International Agency for Research on Cancer has classified FB_1 as a class 2B toxic compound, possibly carcinogenic to humans [13]. Fumonisins are heat-tolerant and can be stably present during food/feed processing and thus enter into food/feed chains [6]. To prevent these mycotoxins from entering food/feed chains and avoid the consumption of fumonisin-contaminated products, the rapid and accurate detection of fumonisins is essential.

Several chemical techniques for the detection and analysis of fumonisins are available, but these all require expertise and advanced facilities and are thus limited to use in laboratories [14]. Enzyme-linked immunosorbent assay (ELISA) technology provides a promising alternative, as it is portable and simple. Different polyclonal antisera [15,16] and monoclonal antibodies (mAbs) [17–21] have been developed for detecting fumonisin contamination in agricultural commodities. As compared to the labor-intensive and costly procedures of polyclonal and monoclonal antibody preparations, single-chain fragment variable (scFv) antibodies can be selected by phage display [22,23], expressed, and extracted simply through a bacterial expression system [24,25]. Moreover, scFvs can be isolated together with their coding sequences [22], evolved in vitro [24,26], engineered as variants, or fused genetically to other molecules [23,27,28]. However, the generation of recombinant antibodies from hybridoma cell lines is not easily accomplished due to high levels of aberrant mRNA molecules from rearranged, nonfunctional heavy- and light-chain genes [29,30]. ScFv antibodies directly cloned from monoclonal hybridoma cells often have lower binding affinities than their parent mAbs [20,31]. To date, five scFv antibodies against FB_1 have been reported. Three scFvs derived from hybridoma cell lines showed 10- to 100-fold less reactivity as compared with their parent mAbs [20,32]. One scFv antibody selected from the human synthetic antibody library had a K_D of only 4.08×10^{-7} M [33]. One recently reported scFv had a slightly lower affinity than its parent mAb, but displayed a poor cross-reactivity to FB_2 [34], which may not be satisfactory for sample detection, as the guidelines for fumonisins in human foods and animal feeds require the efficient detection of total fumonisins ($FB_1 + FB_2 + FB_3$) that invariably occur simultaneously in contaminated samples, with FB_2 being the predominant compound in some instances [4,6]. Thus, the generation of scFv antibodies that are highly reactive to fumonisins and that can be applied for immunoassay detection remains a challenge.

In this study, we combined hybridoma and phage display technologies to screen for fumonisin-specific recombinant antibodies with excellent properties. We firstly isolated fumonisin-specific hybridoma cell lines and then used them to construct a phage-displayed antibody library. Phage display coupled with highly stringent panning was used to isolate one high-affinity scFv antibody, designated H2. The objectives of this study were to characterize the features of the H2 scFv antibody and develop the H2 scFv-based assay for immunological detection of fumonisin contamination in agricultural products.

2. Experimental

2.1. Reagents and apparatus

Fumonisins (FB_1, FB_2, and FB_3), keyhole limpet hemocyanin (KLH), and bovine serum albumin (BSA) were purchased from Sigma–Aldrich (St. Louis, MO, USA). TRizol reagent, Oligo$(dT)_{12-18}$ primer, hypoxanthine–aminopterine–thymidine (HAT), and hypoxanthine–thymidine medium were purchased from Life Technologies (Grand Island, NY, USA). The composition of HBS-EP running buffer was 10 mM HEPES (pH 7.4) with 150 mM NaCl, 3 mM EDTA, and 0.005% (v/v) surfactant P20. 96-well cell culture plates were purchased from Corning (Acton, MA). Cells

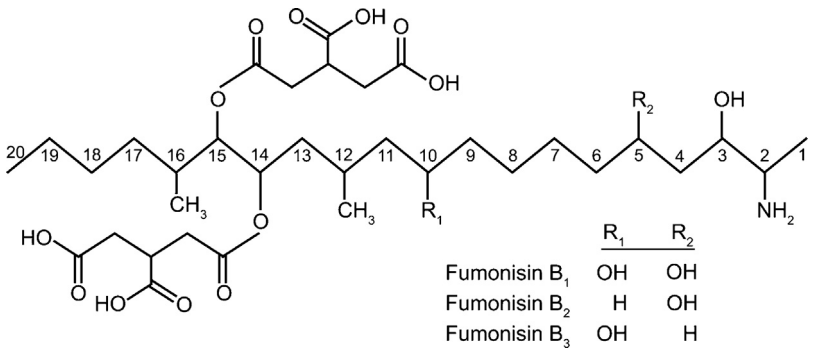

Fig. 1. Chemical structures of FB_1, FB_2, and FB_3.