Detection of Fumonisins by ELISA Method

Ayumu Nagahara*, Satoshi Fukuda**, Mamoru Kikuchi** and Susumu Kumagai***

Abstract

Abbreviation BSA, bovine serum albumin; OVA, ovalbumin; KLH, keyhole limpet hemocyanin; i.p., intraperitoneal; ELISA, enzyme-linked immunosorbent assay; CI-ELISA, competitive indirect enzyme-linked immunosorbent assay; CD-ELISA, competitive direct enzyme-linked immunosorbent assay; FB1 (or FB2), fumonisin B1 (or B2); PBS, phosphate-buffered saline.

Key words: fumonisin, ELISA, monoclonal antibody, corn

Introduction

Fumonisins are mycotoxins originally isolated from a culture of Fusarium moniliforme, one of the most commonly occurring fungi on corn1,2). Fumonisin B1, which is the major fumonisin in both culture and naturally contaminated samples, has been shown to cause equine leukoencephalomalacia3) and porcine pulmonary edema4), and to have cancer-promoting activity in rats5). Hepatocellular carcinoma was induced in rats by giving them fumonisin B1-containing feed6). Corresponding to the cancer-promoting and carcinogenic activities of the toxin, epidemiological studies have shown possible involvement of fumonisins in human esophageal cancer7). Analytical methods such as thin-layer chromatography (TLC)8,9), gas chromatography-mass spectrometry (GC-MS)10), liquid secondary ion mass spectrometry (Liquid-SIMS)10), and high-pressure liquid chromatography (HPLC)11,12) have been used for the detection of fumonisins. These methods require extensive extraction, sample cleanup, or derivatization procedures. Therefore, these methods are not suitable for routine screening of large numbers of samples.

Immunoassays have recently been described as alternative methods for detection of fumonisins13,14). In general, Low molecular weight compounds, such as fumonisins, have to be conjugated to carriers for immunization. Here we report the production of stable hybridomas by fusion of a mouse myeloma cell line, Sp-2, with mouse splenocytes immunized with fumonisin B1-ovalbumin conjugate (FB1-OVA) or fumonisin B1-keyhole limpet hemocyanin conjugate (FB1-KLH) together with Freund’s complete and incomplete adjuvants, and the characterization of the anti-fumonisin monoclonal antibodies.

* Environmental Planning Department, Kikkoman Corporation, 350 Noda, Noda City, Chiba 278-0037, Japan
** Research and Development Division, Kikkoman Corporation, 399 Noda, Noda City, Chiba 278-0037, Japan
*** Department of Biomedical Food Research, National Institute of Infectious Diseases, Toyama 1-23-1, Shinjuku-ku, Tokyo 162-8640, Japan
Methods

Fumonisin B₁(FB₁) was extracted from culture of Fusarium moniliforme. FB₁ was conjugated, using glutaraldehyde, with ovalbumin(OVA) and keyhole linpet hemocyanin(KLH) for use as an immunogen (FB₁-OVA and FB₁-KLH, FB₁/protein ratio was 4 : 5 (w/w))\(^{15}\). FB₁ was also conjugated using m-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS) to BSA (FB₁-BSA, molar ratio of FB₁/BSA was 70 : 1)\(^{16}\).

Eight-week-old BALB/C female mice were immunized by intraperitoneal (i.p.) injections, two with FB₁-OVA and two with FB₁-KLH (50 μg/injection) with Freund's complete and incomplete adjuvants. At the final injection (30 days later), each high-titer mouse was given by i.p. injection of 100/μl of conjugate in PBS without adjuvant. Four days after the last injection, the mice were killed and spleens were removed aseptically and transferred to plastic Petri dishes containing Eagle's minimum essential medium (MEM). Myeloma cells (Sp-2) and spleen cells were fused with polyethylene glycol (PEG)\(^{17,18}\). The fused cells were suspended in hypoxanthine-aminopterin thymidine (HAT) medium containing thymocytes (5×10⁶ cells/ml) as feeder cells and pipetted into 96-well culture plate. After HAT selection, supernatants from hybridoma cells were examined for the reactivity with FB₁-BSA by ELISA and also measured for the specificity toward FB₁ by competitive indirect ELISA(CI-ELISA). The same method of ELISA and CI-ELISA for antisera were used to the case of culture supernatants from hybridoma cells. After successive single-cell cloning by limiting dilution methods, five hybridoma cell lines producing anti-FB₁ monoclonal antibodies were obtained. Four of the five hybridomas (OVA4A41, OVA4A48, OVA8D26 and OVA8D29) were obtained from the mouse immunized by FB₁-OVA and one hybridoma (KLH17C10) was obtained from the mouse immunized by FB₁-KLH.

![Structure of Fumonisins](image)

Fig. 1 Structure of Fumonisins.
(1) fumonisin B₁; (2) fumonisin B₂.
Fig. 2 Specificity of Anti-Fumonisin Monoclonal Antibodies produced by Selected Hybridomas.
Specificity was analyzed by CI-ELISA. Each data point represents the average value of triplicate measurements. ○, fumonisin B₁; □, fumonisin B₂.
Table 1 Reactivity of Anti-Fumonisin Monoclonal Antibodies from Selected Hybridomas as measured by Cl-ELISA

<table>
<thead>
<tr>
<th>Hybridoma</th>
<th>Amount required for 50% inhibitiona (ng/ml)</th>
<th>Cross-reactivityb (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fumonisin B₁</td>
<td>Fumonisin B₂</td>
</tr>
<tr>
<td>OVA4A41</td>
<td>255</td>
<td>49.5</td>
</tr>
<tr>
<td>OVA4A48</td>
<td>239</td>
<td>43.8</td>
</tr>
<tr>
<td>OVA8D26</td>
<td>65.3</td>
<td>50.9</td>
</tr>
<tr>
<td>OVA8D29</td>
<td>71.9</td>
<td>48.2</td>
</tr>
<tr>
<td>KLH17C10</td>
<td>85.9</td>
<td>89.8</td>
</tr>
</tbody>
</table>

aData were estimated from Fig. 2.

bCross-reactivity defined as (ng/ml of fumonisin B₁ required for 50% inhibition)/(ng/ml of fumonisin B₂ required for 50% inhibition)×100.

Fig. 3 Effects of acetonitrile concentration of solvent determined by CD-ELISA with monoclonal antibodies produced by FB₁-BSA conjugate.

Fig. 4 Effects of acetonitrile concentration of solvent determined by CD-ELISA with monoclonal antibodies produced by FB₁-KLH conjugate.
Results and Discussion

The selected hybridoma cells were cultivated in Iscove's modified Dulbecco's medium

![Graph showingpercent inhibition vs concentration of FB1 in ng/ml](image)

Fig. 5 Detection of FB₁ by CD-ELISA with monoclonal antibodies produced by FB₁-KLH conjugate.

Contents of Food Checker for Fumonisin

1. Antibody coated micro-well
2. 48 wells for concentrate
3. Positive control (Fumonisin B₁)
4. Enzyme conjugated *Fumonisin*
5. Substrate solutions
6. Stopping reagent
7. Washing solution(20x concentrated)

Photo Contents of FOOD CHECKER for FUMONISIN developed by KIKKOMAN CORPORATION.
(IMDM) containing 20% fetal bovine serum (FBS). The specificities of antibodies were characterized by CI-ELISA with respect to FB1, FB and tricarballylic acid which is an analogue of the characteristic side-chains of fumonisins at position C14 and C15 (Fig. 1). The subclass of secreted antibodies were identified with a mouse monoclonal antibody isotyping kit (Amersham, UK). The isotype of all monoclonal antibodies produced by five selected hybridomas were IgG1 with κ light chain. Specificities of the antibodies are shown in Fig. 2 and Table. The concentration of 50% inhibition doses of fumonisin B1 and B2 for antibodies from five hybridoma clones ranged from 65 to 255 ng/ml for FB1 (mean, 144) and 44 to 86 ng/ml for FB2 (mean, 56). Reactivity was not observed with tricarballylic acid. Figure 2 shows the response curves of FB1. Response ranges of FB1 were from 10 to 1000-5000 ng/ml. Cross-reactivity of our antibodies ranged from 100 to 550% for FB2 (mean, 290%), however Azcona-Olivera et al. previously reported the value of 38% for the cross-reactivity of FB2 measured by competitive direct ELISA. These results suggest that our high cross-reactive antibodies (produced by OVA4A41 and OVA4A48) may recognize a different region as antigenic determinant. The antibody secreted by clone KLH7C10 reacted almost to the same degree with FB1 and FB2. This property is desirable, since it enables simultaneous detection of both fumonisins.

This time, we used OVA and KLH as the carriers and also used BSA. FB1-BSA conjugate was also used for immunization and we got hybridomas from this (data not shown). Twenty five % of acetonitrile effected to FB1-BSA antibodies but not effected to FB1-KLH antibodies by competitive direct ELISA (CD-ELISA) shown in Fig. 3 and 4. Figure 5 indicate the CD-ELISA standard carve of FB1 in PBS and diluted corn extract with FB1-KLH antibody. Diluted corn extracts did not effect to the activity of FB1-KLH antibody.

We investigate the easy and rapid detection system for fumonisin using our FB1-KLH antibody. The name of this system is “FOOD CHECKER for FUMONISIN” shown in Photo. This ELISA system will be useful for detection of fumonisins in feeds and foods.

References


