Quantitation of aflatoxin B₁ in foodstuffs by enzyme-linked immunosorbent assay

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上野郁子*¹, 祓川貴美江*², 上野芳夫*²: 酵素免疫法による Aflatoxin B₁ の定量とその食品中の Aflatoxin B₁ 検出への応用

Abstract

Aflatoxin B₁ at levels down to pg was quantitated by the enzyme-linked immunosorbent assay method. For application of this method, however, clean-up procedures of aflatoxin B₁ from crude extracts were found to be critical. Aflatoxin B₁ was not detected by the immunoassay method in the commercial samples of peanuts, pine nuts, cashew nuts, pistachio nuts, corn and fish feeds.

Immunoassay methods are now widely used as valuable tools for microdetermination of biological materials and they are highly specific as well as sensitive. Radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA) methods for quantitation of aflatoxin B₁ (AFB₁) have been developed¹⁾⁻⁴⁾. However, these methods have not yet been used for survey of AFB₁ contamination in foodstuffs. We modified the ELISA method in order to quantitate AFB₁ in peanut samples artificially or naturally contaminated with AFB₁. The method was also applied to other commercial foodstuffs and fish feeds.

Materials and Methods

AFB₁ of Makol and ³H-AFB₁ of Moravek (10 Ci/mmol) were used. Anti-AFB₁ antibody (rabbit serum IgG) and AFB₁-peroxidase conjugate were prepared according to the method of Chu and Ueno²⁾ and Pestka et al.⁴⁾, respectively. Preparation of anti-AFB₁ IgG-coated polystyrene microwells (Fig. 1) and quantitation of AFB₁ by ELISA (Fig. 2) were carried out according to the method of Pestka et al.⁴⁾ except that O-phenylenediamine was used as substrate of the peroxidase.

Samples of peanut meal spiked with AFB₁ or ³H-AFB₁ were prepared by immersion of the peanut meal into a methanol solution of known amounts of AFB₁ or ³H-AFB₁ followed by evaporation of the solvent under reduced pressure below 50°C. Peanut meal samples naturally contaminated with AFB₁ were kindly supplied by Dr. K. Maeda of the Mycotoxin Research Association, Japan.

Five foodstuffs (10 samples of peanuts, 5 of pine nuts, 6 of cashew nuts, 2 of pistachio nuts, and 3 of corn) were obtained from food shops in Tokyo and Chiba Prefecture. Two samples of fish feeds were obtained from Nihon Haigo Shiryō (one for

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(J. J. Pestka et al. 1980).

Fig. 1. Preparation of anti-AFB₁ IgG-polystyrene microwells.

Fig. 2. Procedures of ELISA.

Fig. 3. Extraction and clean-up of AFB₁ in peanut meal.

trout and another for carp). The purchased samples were ground into meal in our laboratory, and one gram each of the meal samples was used for the assay.

AFB₁ was extracted with two different solvent systems and cleaned up using prepacked mini-columns as follows: (1) extracted with a mixture of methanol, 1% NaCl aqueous solution and n-hexane (55:45:100) according to the method of Kanshoku No. 128⁵⁾ and cleaned up with an extrelut column (Merk) according to the method of Schweighardt et al.⁶⁾ (1/50 scale), as shown in Fig. 3; (2) extracted with a mixture of CHCl₃-water (10:1) and cleaned up using a sep pak column (Waters Associations), according to the method of Minamizawa et al.⁷⁾ (extraction, 1/25 scale; clean-up, 1/10 scale), or extracted with the aqueous CHCl₃ and cleaned up using the extrelut column. In the method (2), CHCl₃ layer was separated by centrifugation at 3000 rpm for 20 minutes instead of filtration.

Recovery of ³H-AFB₁ from peanut meal samples was tested at two steps, i.e., before and after the clean-up by column, by counting the radioactivities of the peanut extracts in aquasol II (NEN) using a liquid scintillation counter (Beckman LS 9000).

Results and Discussion

With standard AFB₁ solution in methanol, the concentration-response curve was a straight line in concentrations from 10 to 330 pg/assay of AFB₁ as shown in Fig. 4. Cross-reactivity tests between AFB₁ and related compounds indicated a high specificity of the anti-AFB₁ IgG for AFB₁ (Fig. 4). Samples of peanut meal spiked with either 10 ppb or 33 ppb of AFB₁ were processed as shown in Fig. 3, and 2.8±0.5 ppb (n=5) and 10.8±1.9 ppb (n=5) of AFB₁ were detected by the ELISA, respectively. These values corresponded to 28.0 and 32.7% of the original contents of AFB₁, respectively. Peanut meal samples with known amounts of AFB₁ were extracted with the aqueous CHCl₃ and AFB₁ was cleaned up by either the extrelut or the sep pak (1/10 scale) column. The data are shown in Table 1.

Fig. 4. Standard curve of AFB₁ by ELISA and specificity of antibody to AFB₁.

ELISA was performed by the procedure shown in Fig. 2. Sterig.: sterigmatocytin; M₂: aflatoxin M₂; M₁: aflatoxin M₁; G₂: aflatoxin G₂; AFL: aflatoxicol; G₁: aflatoxin G₁; B₂: aflatoxin B₂; B₁: aflatoxin B₁.

Table 1. AFB₁ quantities determined by ELISA in the samples of peanut meal with known amounts of AFB₁ after extraction with aqueous CHCl₃ and clean-up by prepacked mini-column.

in peanut meal	AFB ₁ contents (ppb)	
	determined by ELISA	
	After clean-up by Extrelut	After clean-up by Sep pak
33*	10.6 (32.1)	9.1 (27.6)
10*	2.7 (27.5)	—
7.8*	—	2.75(35.3)
1130**	—	295 (26.1)
26.7**	3.05(11.4)	8.6 (32.2)
4**	1.45(36.3)	1.11(27.6)

* Peanut meal spiked with known amount of AFB₁.

** Peanut meal contaminated naturally with AFB₁ and AFB₁ contents were determined in the Mycotoxin Research Association, Japan.
() % of the original AFB₁ contents in the peanuts.

Table 2. Radioactivities recovered from the samples of peanut meal spiked with ^3H -AFB₁ after the extraction and clean-up procedures.

Extraction and clean-up method Solvent	Column	^3H -AFB ₁ contents (ppb)	Recovery (%)	
			Before clean-up	After clean-up
MeOH-1% NaCl aq.-n-Hexane (55:45:100, v/v)	10 ml Extrelut	100*	58 (n=2)	31 (n=2)
CHCl ₃ -H ₂ O (10:1, v/v)	6.6 ml Sep pak(Silica)	10**	102 (n=2)	39 (n=2)+
"	"	10**	—	50 (n=2)**
"	"	5***	94±7 (n=5)	68±13 (n=5)**
"	"	1***	88±6 (n=5)	49±13 (n=5)**

* 705 dpm/ng ^3H -AFB₁, ** 950 dpm/ng ^3H -AFB₁ and *** 1040 dpm/ng ^3H -AFB₁ were used.

Solvents in the scales + 1/10 the original and ** the original volumes were used.

In both artificially and naturally contaminated peanut meal samples, the AFB₁ quantities determined by the ELISA were less than 40% of the original contents. Therefore, recovery tests with ^3H -AFB₁ were carried out at two steps before and after the clean-up by column. The results are shown in Table 2. The extraction method using aqueous CHCl₃ was efficient enough to recover AFB₁ at levels down to one nanogram, but the extraction method with methanol-saline and the clean-up method by the extrelut column did not give quantitative recovery. After the clean-up by sep pak using solvents in the scale 1/10 the original 39% of the original ^3H -AFB₁ was recovered. With the solvents in the original scale around 50% of the original radioactivities was recovered. These data indicated that AFB₁ at the levels less than one nanogram could not be completely recovered by the method.

In this experiment it was observed that unknown materials extracted from the samples along with AFB₁ inhibited the color development in the O-phenylenediamine-peroxidase reaction. The inhibitory degree of the color development varied with the source of the samples examined. Therefore, we prepared a standard curve for each specimen of the samples by adding graded amounts of AFB₁ to the methanolic extract obtained from a specimen of the

same source which had not been contaminated by the toxin.

Under these experimental conditions we surveyed AFB₁ contamination in commercial samples of foodstuffs and fish feeds. AFB₁ was not detected in any of these samples by the ELISA.

The present data suggest that we need more efficient clean-up methods for AFB₁ in the extract from small amounts of foodstuffs in surveying AFB₁ contamination by the immunoassay methods. Development of an easy clean-up method is now under way in our laboratory.

References

- 1) Langone, J. J., Vunakis, H. V.: J. Natl. Cancer Inst., **56**, 591 (1976).
- 2) Chu, F. S., Ueno, I.: Appl. Environ. Microbiol., **33**, 1125 (1977).
- 3) Lawellin, D. W., Grant, D. W., Joyce, B. K.: Appl. Environ. Microbiol., **34**, 94 (1977).
- 4) Pestka, J. J., Gaur, R. K., Chu, F. S.: Appl. Environ. Microbiol., **40**, 1027 (1980).
- 5) Minamizawa, M., Sugimoto, T., Kino, N.: Proc. Jap. Assoc. Mycotoxicol. No. 11, 23 (1980).
- 6) in Shokuhin eisei shyo roppo, 1983, pp. 1289. Shinnihon Hoki Shuppan LTD., Japan.
- 7) Shweighardt, H., Bohm, J., Leibetseder, J. in Ernährung/Nutrition Vol. 2, 3 (1978) (Abstract in data of Merk).