FAB tandem mass spectra of aflatoxins, sterigmatocystin and their analogs†

D. UYAKUL*^{1,2}, M. ISOBE*^{1,3}, and T. GOTO*¹

Duangchan UYAKUL*^{1,2}, 磯部 稔*^{1,3}, 後藤俊夫*¹: Aflatoxin sterigmatocystin 類
の FAB タンデムマススペクトル†

Summary

The positive Fast Atom Bombardment (FAB) tandem mass spectrometry as an effective technique was applied for identification and confirmation of 20 mycotoxins such as aflatoxins B₁, B₂, G₁ and G₂, and sterigmatocystin and its related toxins. The fab ms/ms technique demonstrated to be a good method for analyzing these mycotoxins with less than one microgram. It resolved complex mixture of mycotoxins even with the same molecular weight.

Mycotoxins produced by *Aspergillus versicolor* (Vuillemin) Tiraboschi, *Emericella nidulans* (Eidam) Vuill. and *Bipolaris* sp. are mainly sterigmatocystin, versicolorin and averufin.^{1,2} The above toxins were reported as biosynthetic precursors of aflatoxins.¹⁾ The toxins are potential contaminants of foodstuffs and animal feeds. The health hazards associated with these toxins have posed serious problems for animals and humans spread out in the world. Natural contamination often occurs in trace amount. Accurate methods are required in determining the specific mycotoxins. For the analyses of mycotoxins, minicolumn, thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) become very useful techniques. Enzyme-linked immunosorbent assay (ELISA) has been introduced for aflatoxin analyses³⁻⁶⁾. Recently, mass spectrometry (MS) proved to be an extremely valuable method to provide unambiguous identification of mycotoxins. Recent publications, using various techniques of mass spectrometry such as field desorption (FD)⁷⁾, electron impact (EI)⁸⁾ and negative ion chemical ionization (NICI)⁹⁾, can be used in the identification of mycotoxins at a low level. However, it is necessary that samples must be extensively cleaned-up before analysis. In case of many mycotoxins or interferences having coincident molecular ion with other mycotoxin, the above techniques do not provide simultaneous specification of the mycotoxin. In this study, using positive Fast Atom Bombardment tandem mass spectrometry (FAB-MS/MS), we have demon-

† A paper presented during the 7th International Symposium on Mycotoxins and Phycotoxins, August 16-19, 1988 at Tokyo, Japan.

*¹ Laboratory of Organic Chemistry, School of Agriculture, Nagoya University (Chikusa, Nagoya 464) 名古屋大学農学部生物有機化学研究室 (〒464 名古屋市千種区不老町)

*² A PhD candidate on study leave from Mycotoxin Laboratory, Division of Food Analysis, Department of Medical Sciences, Yodse, Bangkok, Thailand under the scholarship grant of the Ministry of Education, Science and Culture (Monbusho).

*³ To whom correspondence should be addressed.

strated the identification of 20 mycotoxins including four pairs of mycotoxins with the same molecular weights.

Materials and Methods

Chemical

Aflatoxins were secured from Makor Chemicals LTD, Jerusalem, Israel. Sterigmatocystin-related compounds were obtained from Prof. Hamasaki of Tottori University, Japan¹⁾.

Mass Spectrometry

A JEOL JMS-DX 705L quadrupole doublet (QPD) mass spectrometer was used for FAB-MS/MS (tandem MS) analysis. It involves two JEOL JMS-DX300 instruments which are

Table 1. FAB-MS/MS of aflatoxins

Aflatoxins	molecular weight	parent ion M+1	daughter ions				
			-Me 15	-H ₂ O 18	-C=O 28	-CO ₂ 44	others
Aflatoxin B ₁	312	313	298		285	269	241, 213
Aflatoxin B ₂	314	315	300		287	271	259, 243 229
Aflatoxin G ₁	328	329		311	300 (301)*	283 (285)*	268, 243 226
Aflatoxin G ₂	330	331		313	303	287	268, 228

Condition of the FAB MS/MS: Instrument JEOL model DX705 L. 1st MS ionization positive FAB/Xe, acceleration 3 kV, matrix=glycerin+3-nitrobenzylalcohol, sample amount <1 µg. 2nd MS collision with He at 10 mmHg.

Data process: average of accumulation 10 times.

* found in another measurement.

combined together to become EB-EB through an interface with a He-collision chamber and a QPD to obtain daughter ions caused by the collisional activation. A mixture of glycerin and 3-nitrobenzylalcohol was employed as matrix in FAB measurements. Mycotoxin (less than

Table 2. FAB-MS/MS of sterigmatocystin and its analogs

Sterigmatocystins	Molecular Weight	M+1	daughter ions				
			-Me 15	-H ₂ O 18	-C=O 28	-CO ₂ 44	others
Demethylsterigmatocystin	310	311		294 (293)*	282 (283)*		265, 241 (269, 255)*
Sterigmatocystin	324	325	310		297	281	268, 255, 239
Dihydrosterigmatocystin	326	327	312		299	283	271, 255
O-Methylsterigmatocystin	338	339	324		306 (311)*	295	278 265
5-Methoxysterigmatocystin	354	355	340		325(-OMe) (324)*		297, 281
5,6-Dimethoxysterigmatocystin	384	385	370		355(-OMe) (354)*		337, 312
Sterigmatin	310	311			283		269

1 μg) was dissolved in 10 μl solvent (dioxane, chloroform or dimethyl sulfoxide). Aliquot of 2 μl mycotoxin solution was mixed with matrix (ca 10 μl). The mixture was introduced into the first mass spectrometer which positively ionized the sample with FAB. The sample was irradiated with Xenon gun at 10^{-6} torr for ionization in an electric field with 3 kV acceleration. The molecular ion ($M+1$) of each mycotoxin was selected from the first mass system. It was simultaneously collided with He gas to produce daughter ions which were subsequently analyzed by the second mass system. The data of each measurement were averaged by accumulation about 10 times.

Results and Discussion

In ordinary FAB mass analysis, mycotoxins show the molecular ions at $m/z=M+1$ in appreciable abundance. The molecular ion peaks are sufficiently stable for longer than 10 min

Table 3. FAB-MS/MS of versicolorin and its analogs

Versicolorins	molecular weight	parent ion $M+1$	daughter ions				
			-Me 15	-H ₂ O 18	-C=O 28	-CO ₂ 44	others
Versicolorin A	338	339		321	311		227
Deoxyversicolorin A	322	323		305	295	279	265
6,8-O-Dimethylversicolorin A	366	367	352		339		324(-Me-CO), 311
Versicolorin B racemate Versicolorin C	340	341		323	313
6,8-O-Dimethylversicolorin B	368	369		351	338(-OMe)	325	313

Table 4. FAB-MS/MS of averufin and its analogs

Mycotoxins	molecular weight	parent ion M+1	daughter ions			
			-H ₂ O 18	-C=O 28	-CO ₂ 44	others
Averufin	368	369	351		326 (325)*	311, 299
6,8-Dimethylaverufin	396	397	379	366(-OMe)	353	339, 325, 313
Nidurufin	384	385	367			309
Norsolorinic acid	370	371	353			339, 299

with very little fragmentation; thus, giving only limited information. The application of FAB-MS/MS to analyze mycotoxins is necessary to obtain wider information. The molecular ion (M+1) of each mycotoxin was selected from the first mass system and it was collided with He gas to produce daughter ions which were simultaneously analyzed by the second mass system. Structures of mycotoxins were analyzed and all of the daughter ions are shown in Tables 1, 2, 3 and 4. The main fragmentation behavior of aflatoxins B₁, B₂, G₁ and G₂ gave M+1-28 (-C=O) and M+1-44 (-CO₂) pattern. Aflatoxins B₁ and B₂ showed the same fragment ion at M+1-15 (-Me). Aflatoxins G₁ and G₂ also possessed the methoxy group of the same position with that of aflatoxins B₁ and B₂, but both of them (G₁ and G₂) showed the fragment ion at M+1-18 (-H₂O) instead of M+1-15 as shown in Table 1. Hence, the corresponding fragments in the mass spectrum of aflatoxin B group and aflatoxin G group follows a different pathway although the structures are closely related with each other. The fragmentation pattern of demethylation and decarbonylation was also found in strigatocystin and its derivatives as shown in Table 2. In the versicolorin group, the main daughter ions generated by dehydration and decarbonylation were mostly found as shown in Table 3.

Fig. 1. Tandem FAB mass spectra of 4 pairs of mycotoxins having the same molecular weights.

Averufin, nidurufin and norsolorinic acid were easily dehydrated during fragmentation as shown in Table 4.

The MS/MS analysis of mycotoxins is worthy of the identification and confirmation of the structures from a mixture. A particular mass ion can be selected from the molecular ion mixture formed by the first mass system and rendered fragmentation in the second mass system. The fragmentation spectrum is interpreted with that of an authentic to confirm the presence of such mycotoxin. When the normal mass spectrum does not supply all the necessary structural information, FAB-MS/MS was especially designed in determining the structures with the same molecular weight. We found that the fragmentation spectrum of averufin was clearly distinguishable from 6,8-O-dimethylversicolorin B. In addition nidurufin and 5,6-dimethoxysterigmatocystin; demethylsterigmatocystin and sterigmatin; versicolorin A and O-methylsterigmatocystin also provided unambiguous determination as shown in Fig. 1.

The FAB-MS/MS was demonstrated to be a very effective technique for unambiguous

analyses of mycotoxins even in the same molecular weight¹⁰.

Acknowledgements

The authors are indebted to Mr. Yamamoto, K., Mr. Tsubouchi, H. of Nagoya City Health Research Institute and Prof. Hamasaki, T., for providing the samples, and to Mr. Kitamura, S. for his technical help in mass analysis.

References

- 1) Hamasaki, T., Hatsuda, Y.: "Mycotoxins in Human and Animal Health," (Eds. Rodricks, J.V., Hesseltine, C.W., Mehlman, M.A.), p. 597-607 (1977), Pathotox Publishers, Illinois, U.S.A.
- 2) Aucamp, P.J., Holzapfel, C.W.: J. S. Afr. Chem. Inst., **23**, 40 (1970).
- 3) Pestka, J.J., Gaur, R.K., Chu, F.S.: Appl. Environ. Microbiol., **40**, 1027 (1977).
- 4) Haugen, A., Groopman, J.D., Hsu, I.C., Goodrich, G.R., Wogan, G.N., Harris, C.C.: Proc. Natl. Acad. Sci., U.S.A., **78**, 4127 (1981).
- 5) Pestka, J.J., Li, Y., Harder, W.O., Chu, F.S.: J. Am. Offic. Anal. Chem., **64**, 294 (1981).
- 6) Ueno, I.: Proc. Jpn. Assoc. Mycotoxicol., **21**, 24 (1985).
- 7) Sphon, J.A., Dreifuss, P.A., Schulten, H.R.: J. Am. Offic. Anal. Chem., **60**, 73 (1976).
- 8) Haddon, W.F., Wiley, M., Waiss, Jr. A.C.: Anal. Chem., **43**, 268 (1971).
- 9) Brumley, W.C., Neshelm, S., Trucksess, M.W., Trucksess, E.W., Dreifuss, P.A., Roach, J.A.G., Andrzejewski, D., Eppley, R.M., Pohland, A.E., Thorpe, C.W., Sphon, J.A.: Anal. Chem., **53**, 2003 (1981).
- 10) Uyakul, D., Isobe, M., Goto, T.: J. Am. Offic. Anal. Chem., **72**, 491 (1989).