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Aflatoxin Screening by MALDI-TOF Mass Spectrometry

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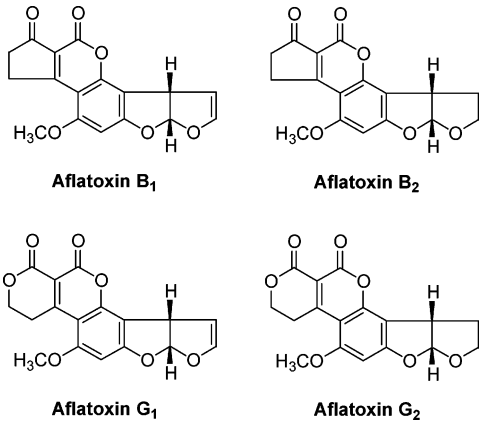
Efficient detection of aflatoxins B_1, B_2, G_1, and G_2 has been performed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry using a UV-absorbing ionic liquid matrix to obtain "matrix-free" mass spectra and addition of NaCl to enhance sensitivity via Na^+ cationization. Using ionic α-cyano-4-hydroxycinnamic acid (Et_3N−α-CHCA) as the matrix, matrix-free mass spectra in the *m/z* range of interest are acquired, and the B_1, B_2, G_1, and G_2 aflatoxins are readily detected with an LOD as low as 50 fmol. The technique is fast, requires little sample preparation and no derivatization or chromatographic separation, and seems therefore to be suitable for high-throughput aflatoxin screening. It should be easily extended to other micotoxins and provide an attractive technique to control the quality of major crops subjected to huge world commercial trades such as peanuts, corn, and rice as well as to monitor bioterrorism threats by micotoxin poisoning.

The broad success of matrix-assisted laser desorption/ionization (MALDI)[1] is related mainly to its ability to ionize relatively heavy or polar molecules such as peptides and proteins.[2] In MALDI, the analyte is co-crystallized with a solid ultraviolet-absorbing organic acid matrix that vaporizes upon laser radiation, carrying with it the analyte.[3] MALDI is not so widely applicable, however, to relatively light molecules owing to severe chemical noise from matrix ions in the low *m/z* range. Matrix-free laser desorption/ionization has been applied, but its use is restricted owing to rapid analyte degradation due to direct exposure to laser radiation.[4] An interesting approach used in MALDI to minimize matrix interference is to use a UV-absorbing ionic liquid as the matrix.[5,6] Ionic liquids are easily prepared and have many beneficial properties for MALDI, including the broad ability to dissolve organic, inorganic, and polymeric substances, good thermal stability, and low vapor pressures. The pioneering study of Gross and co-workers[6] demonstrated the suitability of ionic liquids as MALDI matrixes. We have also applied ionic liquids as the MALDI matrix for rapid screening of low molecular weight chemicals by thin-layer chromatography and "on-spot" MALDI-TOF MS analysis.[5]

Chart 1



Aflatoxins (Chart 1) are carcinogenic mycotoxins that can be ingested via contaminated food or inhaled via dust from mold-infected products.[7] Mycotoxin poisoning is more serious in developing countries where the climatic conditions and the agricultural and storage practices facilitate fungal growth and toxin production. Peanuts (and their products) is the crop most susceptible to aflatoxin contamination.[8] Herein we report that high-throughput MALDI-TOF MS screening of low molecular weight aflatoxins is efficiently performed by using a UV-absorbing ionic liquid as the matrix to nearly eliminate matrix noise and by adding NaCl for enhanced ionization via Na^+ cationization.

EXPERIMENTAL SECTION

The Et_3N−α-cyano-4-hydroxycinnamic acid (α-CHCA) ionic liquid matrix was prepared by adding triethylamine to a solution of α-CHCA in acetonitrile, with subsequent reflux for 1 h, and by

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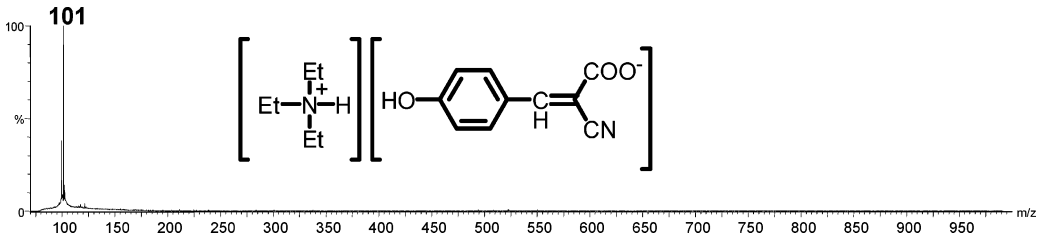

Figure 1. Blank MALDI-MS for the $Et_3N-\alpha$-CHCA ionic liquid matrix.

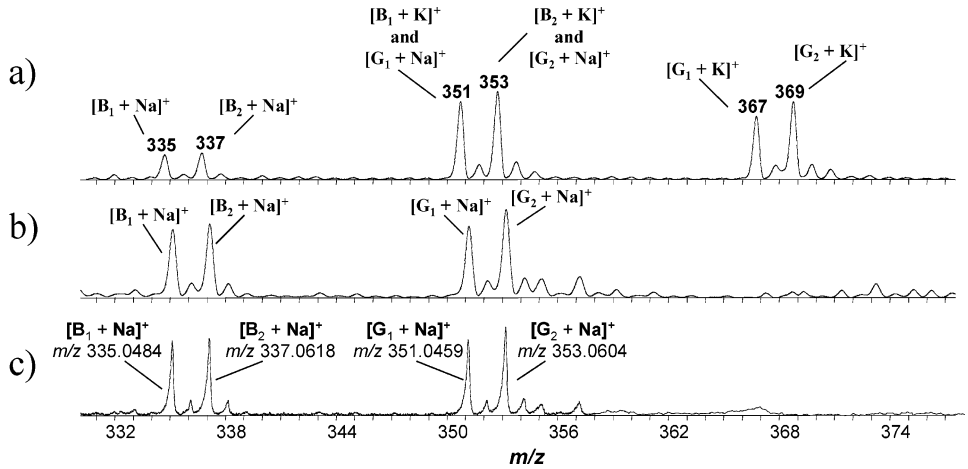

Figure 2. MALDI-TOF mass spectrum using the $Et_3N-\alpha$-CHCA ionic liquid as the matrix of (a) an equimolar mixture of aflatoxins B_1, B_2, G_1, and G_2 (25 pg of each analyte); (b) an equimolar mixture of aflatoxins B_1, B_2, G_1, and G_2 (25 pg of each analyte) spiked with 1.0 μL of a NaCl solution (10 mM); (c) aflatoxins detected as their Na^+ adducts from fungus-contaminated peanuts. For (c), the TOF analyzer was calibrated for high-accuracy *m/z* measurements.

removing the solvent under vaccum. The aflatoxins standards were purchased from Sigma and used as received. Real samples were obtained by using peanuts deliberately contaminated with *Aspergillus flavus* and *Aspergillus parasiticus*. These peanuts were then extracted by weighing 50.0 g of dust peanuts and adding 270 mL of MeOH followed by 30.0 mL of KCl (4% aqueous solution). The mixture was stirred for 5 min and then filtered. An aliquot of 150 mL was transferred to a beaker, followed by addition of 150 mg of $CuSO_4$, 10 g of diatomaceous earth (Celite), and 150 mL of water. The mixture was extracted with chloroform (2 × 10 mL) and concentrated under vacuum with volume reduction to 10 mL.[9] MALDI-TOF MS were acquired on a Micromass instrument using the reflectron and positive ion modes. The main settings were as follows: pulse voltage, 2450 V; delay extraction, 100 ns; acelerating voltage, 15 kV; reflectron voltage, 2 kV. Spectra were generated by summing up 10 single spectra on the *m/z* 50–1000 range by shooting the laser at random positions on the target spot doped with a 1.0 μL of a 10 mM NaCl solution.

RESULTS AND DISCUSSION

To evaluate matrix noise, particularly in the *m/z* range in which the aflatoxin ions should be detected, we acquired a mass spectrum on a blank spot with 1.0 μL of the $Et_3N-\alpha$-CHCA matrix (Figure 1). Fortunately, just a single ion from the matrix is detected, that is, Et_3NH^+ of *m/z* 101, which is too light to interfere with aflatoxin detection.

Optimal Aflatoxin Detection. MALDI-TOF MS was then performed for an equimolar mixture of four standard aflatoxins: B_1 (312 Da), B_2 (314 Da), G_1 (328 Da), and G_2 (330 Da). When a spectrum was acquired with no matrix addition, no analyte ions could be detected. But after spiking the MALDI spot with 1.0 μL of a 1% (w/v) acetonitrile solution of the $Et_3N-\alpha$-CHCA ionic liquid and evaporating the solvent, efficient ionization is achieved with little no-interfering matrix ions in the *m/z* range of interest. As Figure 2a shows, the main ions detected in the spectrum correspond to Na^+ and K^+ cationization of the four aflatoxins, that is, to the adduct ions of *m/z* 335 for $[B_1 + Na]^+$, *m/z* 337 for $[B_2 + Na]^+$, *m/z* 351 for both $[B_1 + K]^+$ and $[G_1 + Na]^+$, *m/z* 353 for both $[B_2 + K]^+$ and $[G_2 + Na]^+$, *m/z* 367 for $[G_1 + K]^+$, and *m/z* 369 for $[G_2 + K]^+$. Splitting of the analyte peak between both Na^+ and K^+ adducts and the isobaric interferences for the ions of *m/z* 351 and 353 compromise aflatoxin detection and quantitation. To eliminate these limitations, we spiked the MALDI spot containing the aflatoxin mixture and the ionic liquid matrix with different salt solutions hoping to obtain single-ion detection for each aflatoxin via favored cationization, which would also eliminate the isobaric interferences. The cations tested were K^+ (KCl solution), Ag^+ (AgCl solution), Zn^+ (ZnCl solution), and Na^+ (NaCl solution). The best results were obtained for NaCl spiking (Figure 2b); nearly just a single ion, the Na^+ adduct, was formed for each aflotoxin, that is, those of *m/z* 335 for $[B_1 + Na]^+$, *m/z* 337 for $[B_2 + Na]^+$, *m/z* 351 for $[G_1 + Na]^+$, and *m/z* 353 for

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$[G_2 + Na]^+$. Note also that all four ions display in the MALDI-TOF MS quite similar abundances (an equimolar mixture was used), which indicate similar dynamic ranges for the quantitation of each of the four aflatoxins. We also investigated the use of crown-ethers to sequester Na^+ and K^+ but with no success.

Screening of Aflatoxins in Peanuts. Following the optimized protocol just described for aflatoxin extraction and MALDI-TOF MS, we analyzed several samples of peanuts contaminated with the fungus *A. flavus* and *A. parasiticus.* The TOF mass analyzer was calibrated for high accuracy m/z measurements to improve selectivity. As Figure 2 illustrates for one of such samples, all four major aflatoxins were detected as a single Na^+ adduct: aflatoxin B1 (m/z 335.0484, calcd m/z 335.0532), B_2 (m/z 337.0618, calcd m/z 337.0688), G1 (m/z 351.0459, calcd m/z 351.0481), and G2 (m/z 353.0604, calcd m/z 353.0637).

Efficient screening of the major aflatoxins B_1, B_2, G_1, and G_2 can therefore be accomplished by MALDI-TOF MS using UV-absorbing ionic liquids such as $Et_3N-\alpha$-CHCA as the matrix to obtain matrix-free mass spectra and NaCl spiking to enhance detection via Na^+ cationization. Previous analyses of aflatoxins require either TLC separation with R_f identification with the inherent risk of false positives,[8] aflatoxins derivatization, and GC/MS analysis or liquid chromatography separation followed by APPI-MS,[10] APCI-MS,[11] or ESI-MS detection.[12] The technique we propose herein benefits from the superior features of MALDI-TOF MS analysis, that is, simplicity, speed, and ease of automation for high-throughput analysis with enhanced selectivity obtained when high-accuracy m/z measurements are performed. This method should be easily applied to monitor other related micotoxins such as ocratoxins. Since it greatly facilitates micotoxin screening, it could be useful to the quality control of the major crops subjected to huge world commercial trades such as peanuts, corn, and rice. This technique (could also be applied) seems also attractive to monitor bioterrorism threats by micotoxin poisoning.

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