Matrix Design for Matrix-Assisted Laser Desorption Ionization: Sensitive Determination of PAH–DNA Adducts

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Two matrices, 4-phenyl-α-cyanocinnamic acid (PCC) and 4-benzyloxy-α-cyanocinnamic acid (BCC), were identified for the determination of polycyclic aromatic hydrocarbon (PAH) adducts of DNA bases by matrix-assisted laser desorption ionization. These matrices were designed based on the concept that the matrix and the analyte should have structural similarities. PCC is a good matrix for the desorption of not only PAH-modified DNA bases, but also PAHs themselves and their metabolites. Detections can be made at the femtomolar level. *(J Am Soc Mass Spectrom 1994, 5, 1021–1025)*

The success of matrix-assisted laser desorption ionization (MALDI) for the sensitive detection of an analyte depends on the nature of the matrix. Rational design of a matrix requires understanding of the mechanism of MALDI. The mechanism is not fully understood. Nevertheless, empirical criteria for matrix selection have been suggested [1]. Two important ones, in our view, are (1) high molar absorptivity and (2) miscibility with the analyte in the solid phase.

The question to be answered is how does one assure miscibility in the solid state? We propose that miscibility is best achieved when the analyte and matrix molecules have similar structures. In this paper, we expand upon the guidelines proposed earlier [1] and describe the design and evaluation of a matrix molecule for the specific problem of desorbing polycyclic aromatic hydrocarbon (PAH) adducts of DNA bases. Our argument at this stage is qualitative, and we do not address quantitatively the solubility of an analyte in the matrix.

Some PAH materials are carcinogenic [2] because they are metabolically activated to form electrophiles that attack nucleophilic sites on DNA bases. Three mechanisms of activation of PAHs have been proposed: (1) formation of diol epoxide [3], (2) formation of a radical cation [4], and (3) formation of benzylic esters [5]. Investigations of the relative importance of these mechanisms in in vitro and in vivo studies require sensitive detection methods at the low picomole and mid-femtomole level, respectively [6].

One method for detection that also gives structural information is fast-atom bombardment (FAB) coupled with tandem mass spectrometry [6–8]. Unfortunately, the combination lacks sensitivity because there is inefficiency of FAB desorption and interference from matrix-derived ions. Improvements can be made by derivatizing the modified base [9] and by using coaxial continuous-flow FAB [10].

MALDI ionization may offer high sensitivity for modified DNA bases as it has for peptides [11] and proteins [12]. Encouragement comes from the successful application of MALDI coupled with time-of-flight mass spectrometry to other molecules such as polymers [13–15] and oligosaccharides [16]. For DNA and RNA, success has been realized with both large [17] and small [18] oligonucleotides, nucleotides [19], and one modified nucleoside, which was detected at the femtomolar level [20]. MALDI coupled with Fourier transform mass spectrometry also has been demonstrated for the determination of modified nucleosides produced by attack of PAH diol epoxides on DNA, but the required quantities of material thus far are in the 20-pmol range [21, 22].

Experimental

The synthesis of the PAH-modified DNA bases was published elsewhere [23]. Biphenyl carboxylic acid, anthracene-9-carboxylic acid, and 4-hydroxy-α-cyano-

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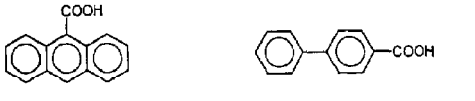
1044-0305/94/$7.00

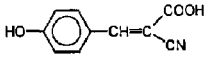
Received June 9, 1994
Revised July 13, 1994
Accepted August 8, 1994

cinnamic acid (4HCC) were purchased from Aldrich Chemical Co. (St. Louis, MO). 4-Benzyloxy-α-cyanocinnamic acid (BCC) (mp 204–205 °C) was synthesized by reacting benzyl bromide (1.21 g) with 4HCC (0.9 g) in methanol. Potassium hydroxide (0.8 g) was added to deprotonate the 4HCC. 4-Phenyl-α-cyanocinnamic acid (PCC) was synthesized following the literature procedure [24], mp 243–244 °C (lit. [24] mp 243–245 °C; see structures).



Anthracene-9-carboxylic acid

Biphenyl carboxylic acid



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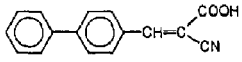

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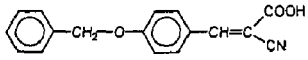

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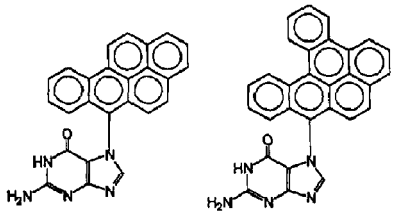

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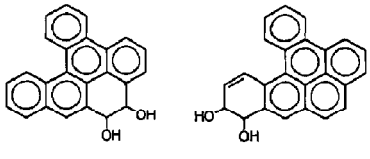

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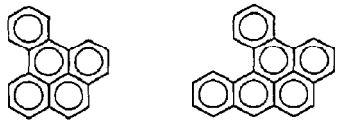

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PCC and BCC were characterized by ^{1}H NMR [δ 7.40–7.55 (*m*, 3H), δ 7.75–7.80 (*d*, 2H), δ 7.87–7.95 (*d*, 2H), δ 8.17–8.22 (*d*, 2H), δ 8.37 (*s*, 1H) for PCC and δ 5.26 (*s*, 1H), δ 7.27 (*d*, 2H), δ 7.38–7.43 (*m*, 3H), δ 7.50–7.52 (*d*, 2H), δ 8.09–8.12 (*d*, 2H), δ 8.24 (*s*, 1H), for BCC] with a GE Omega spectrometer (General Electric, Fremont, CA) operating at 300 MHz. Negative-ion FAB that used 3-nitrobenzyl alcohol as matrix produced abundant $[M - H]^-$ ions of m/z 278.0821 (calculated for $C_{17}H_{12}NO_3$, 278.0817) and 248.0710 (calculated for $C_{16}H_{10}NO_2$, 248.0712) for BCC and PCC, respectively. The compounds were pure at least as could be determined by thin-layer chromatography and melting point. The spectra were obtained with a Kratos MS-50 three-sector mass spectrometer (Kratos Analytical, Ramsey, NJ) equipped with an argon FAB gun.

The MALDI-TOF experiments were carried out on a BenchTOF Bruker instrument (Bruker-Franzen Analytik GMBH, Bremen, Germany). A nitrogen laser beam (337 nm, 20-kW peak laser power, spot size of 1 mm^2, and 1.25-ns pulse width) was used to desorb the samples. The ions were accelerated with a potential of 25 kV.

Samples of benzo[*a*]pyrene-6-*N*7-guanine (BP-6-*N*7-Gua), **1** [25], dibenzo[*a,l*]pyrene-10-N^7-guanine (DBP-N^7-Gua), **2** [26], dibenzo[*a,l*]pyrene-10-*N*7-adenine [26],and dibenzo[*a,l*]pyrene-8,9-diol (DBP-8,9-diol), **3** [27] were obtained from synthesis as previously described. Dibenzo[*a,l*]pyrene-11,12-diol (DBP-11,12-diol), **4**, was purchased from CHEMSYN Science Laboratories (Lenexa, KS), benzo[*e*]pyrene (B[*e*]P), **5**, was purchased from Sigma Chemical Co. (St. Louis, MO), and dibenzo[*a,l*]pyrene (DBP), **6**, was obtained from the National Cancer Institute Chemical Carcinogen Repository (Bethesda, MD) (see structures). Stock solutions (11 pmol/μL for BP-6-*N*7-Gua, 2.5 pmol/μL for DBP-N^7-Gua, 100 nmol/μL for dibenzo[*a,l*]pyrene-10-*N*7-adenine, 10 pmol/μL for DBP-8,9-diol, 10 pmol/μL for DBP-11,12-diol, 1 nmol/μL for B[*e*]P, and 10 pmol/μL for DBP) were made by dissolving the analyte in methanol. Saturated solutions of the matrices were made in a mixture (40:60) of acetonitrile and water (1% trifluoroacetic acid). Samples were prepared by mixing 1 μL of matrix and variable volumes (up to 1 μL) of analyte on the probe tip and allowing them to crystallize prior to irradiation with the laser.

It was not possible to use calibration plots to determine the limits of detection because the signals produced by MALDI were highly variable. As an expedient, the lowest amounts of analytes required for reproducible observation of their molecular ions (signal-to-noise ratio of at least 3:1) for three sample loadings were taken as detection limits. These limits were established by loading progressively lower and lower amounts of analyte. Fifty to sixty laser shots were signal-averaged for each spectrum. A blank of each matrix was run to identify the matrix peaks that might confound an analyte peak. Data acquisition was controlled by using the Bruker SUN data system and XTOF software.

Results and Discussion

The structures of the PAH-modified DNA bases (for example benzo[*a*]pyrene-6-*N7*-guanine (BP-6-*N7*-Gua), **1**, and dibenzo[*a,l*]pyrene-10-N^7-guanine (DBP-N^7-Gua), **2** have nonpolar aromatic ring systems on one end and a polar nitrogen heterocycle on the other. In an attempt to match the nature of the matrix with that of the analyte, we selected a set of aromatic compounds—biphenyl carboxylic acid, anthracene-9-carboxylic acid, 4-benzyloxy-α-cyanocinnamic acid (BCC), and 4-phenyl-α-cyanocinnamic acid (PCC)—as candidate matrices for this class of PAH-modified bases. These matrix candidates are similar in structure to the adducts because they have a polar group on one end of the molecule and aromatic rings on the other. The matrix, 4-hydroxy-α-cyanocinnamic acid (4HCC), one of the common MALDI matrices used for desorbing peptides, is included in this study for comparison. Note that 4HCC is a molecule that has polar groups on both ends, and it has been used with good success to detect peptides, which possess a number of polar groups, at the femtomolar level. Metabolites such as dibenzo[*a,l*]pyrene-8,9-diol (DBP-8,9-diol), **3**, and dibenzo[*a,l*]pyrene-11,12-diol (DBP-11,12-diol), **4**, and the PAHs, benzo[*e*]pyrene (B[*e*]P), **5**, and dibenzo [*a,l*]pyrene (DBP), **6**, do not contain the polar DNA base, and an electrophilic matrix, which may promote radical cation formation, may be indicated for these substances.

We have observed that PAH-modified bases do desorb from 4-hydroxy-α-cyanocinnamic acid (4HCC) matrix. This matrix, however, does not provide detection limits low enough for analyzing samples for in vitro studies. The MALDI-TOF mass spectrum of benzo[*a*]pyrene-6-*N7*-guanine (BP-6-*N7*-Gua) adduct (see Figure 1) obtained by using 11 pmol of the analyte, shows an abundant $[M + H]^+$ ion of m/z 402. The detection limit (see Experimental Section) for this adduct was found to be 275 fmol (see Table 1). The detection limit, however, for the dibenzo[*a,l*]pyrene-10-N^7-guanine (DBP-N^7-Gua) adduct was found to be higher—2.5 pmol (see Table 1). Thus, the use of the 4HCC matrix leads to variable detection limits for adducts depending on the hydrocarbon moiety. Another drawback associated with use of the 4HCC matrix for screening HPLC fractions from in vitro experiments is that the PAH metabolites **3** and **4**, which are two major components, are not detected with MALDI. Moreover, the 4HCC matrix is not suitable for determination of PAHs themselves because relatively large amounts of analyte are required (see Table 1). A reasonable hypothesis is that these disadvantages can be overcome by modifying the structure of 4HCC.

Biphenyl carboxylic acid, a molecule with two benzene rings and lower polarity than 4-hydroxy-α-cyanocinnamic acid (4HCC), is expected to be a better matrix for the metabolites. Metabolite **3** was desorbed

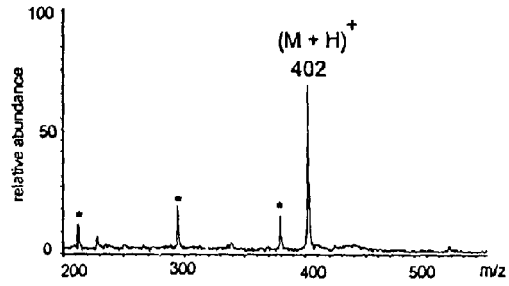

Figure 1. MALDI-TOF mass spectrum of 11 pmol of BP-6-*N7*-Gua obtained by using the 4HCC matrix. The $[M + H]^+$ ion is of m/z 402. The asterisks (*) indicate matrix ions.

to give a high abundance of radical cations when biphenyl carboxylic acid was the matrix. A modified base, dibenzo[*a,l*]pyrene-10-*N7*-adenine, however, could not be detected for a 10-pmol loading. Lack of detectability of the modified base may be due to the low molar absorptivity for the biphenyl carboxylic acid (λ_{max} = 265 nm) at the wavelength of the incident laser beam (337 nm), whereas the radical cation is detected readily because it desorbs directly (i.e., without matrix assistance).

Anthracene-9-carboxylic acid, another molecule that contains aromatic rings and has two strong UV absorption bands near 337 nm (327 and 347 nm) gave effective desorption of both adducts and metabolites. The limits of detection are, at best, 10 pmol. The rigid ring system of anthracene-9-carboxylic acid may prevent optimal crystallization.

On the basis of the foregoing observations, we sought a better MALDI matrix for PAH metabolites and PAH–DNA adducts. Two candidates were 4-benzyloxy-α-cyanocinnamic acid (BCC) and 4-phenyl-α-cyanocinnamic acid (PCC). These two matrix molecules have more than one aromatic ring, and both possess polar and nonpolar ends. Moreover, BCC and PCC have strong UV absorption bands at 334 and 332 nm, respectively, which are nearly resonant with the wavelength of the commonly used nitrogen laser. The difference between the two matrices BCC and PCC is the terminal or monosubstituted benzene ring. The inclusion of carbon and oxygen atoms to form BCC introduces additional degrees of freedom (i.e., rotations about single bonds) into the molecule, and that gives more flexibility to BCC compared to PCC. These matrices were investigated more thoroughly than biphenyl carboxylic acid and anthracene-9-carboxylic acid.

The limits of detection for both benzo[*a*]pyrene-6-*N7*-guanine (BP-6-*N7*-Gua), **1**, and dibenzo[*a,l*]pyrene-10-N^7-guanine (DBP-N^7-Gua), **2**, are in the femtomolar range when 4-benzyloxy-α-cyanocinnamic acid (BCC) is the matrix (see Table 1). Figure 2a and b demonstrates that MALDI-TOF mass spectra of 2500 and 500 fmol of DBP-N^7-Gua, respectively, are obtained read-

Table 1. Detection limits (in femtomoles) for PAH–DNA adducts, PAHs, and their metabolites

Matrix		4HCC	BCC	PCC
BP-6-*N7*-Gua	(1)	275	55	55
DBP-N^7-Gua	(2)	2500	500	200
DBP-8,9-diol	(3)	n.d.[a]	n.d.[a]	1000
DBP-11,12-diol	(4)	n.d.[a]	n.d.[a]	500
B[*e*]P	(5)	5000	500	500
DBP	(6)	2000	250[b]	200

[a] Not detectable
[b] This sample was prepared as the others except approximately 50 ion-exchange resin beads (H^+ form) were added to the solution of matrix and analyte on the probe tip.

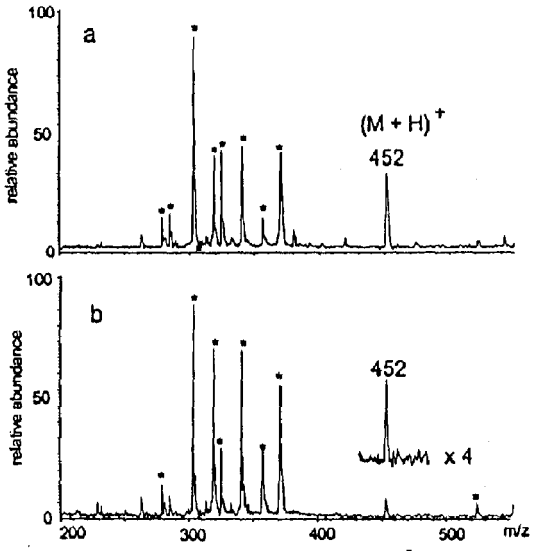

Figure 2. MALDI-TOF mass spectra of DBP-N^7-Gua obtained by using the BCC matrix. (**a**) 2500 fmol and (**b**) 500 fmol. The $[M + H]^+$ ion is of m/z 452. The asterisks (*) indicate matrix ions.

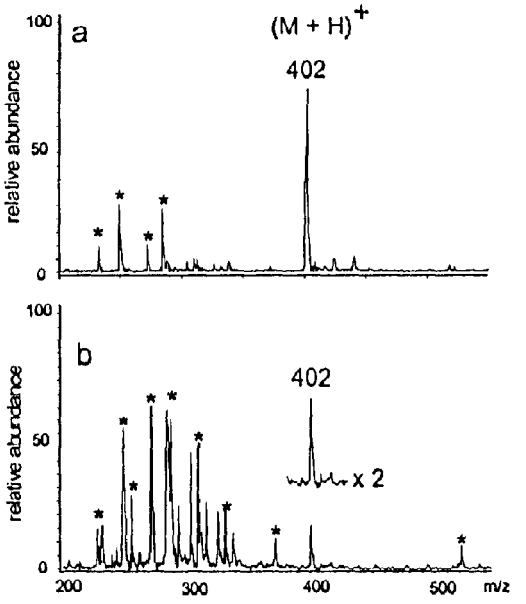

Figure 3. MALDI-TOF mass spectra of BP-6-*N7*-Gua obtained by using the PCC matrix. (**a**) 11000 fmol and (**b**) 55 fmol. The $[M + H]^+$ ion is of m/z 402. The asterisks (*) indicate matrix ions.

ily by using BCC as the matrix. Metabolites **3** and **4**, however, cannot be detected by using BCC matrix. The PAHs, benzo[*e*]pyrene (B[*e*]P) and dibenzo[*a,l*]pyrene (DBP), were determined by using BCC. The detection limits are 500 and 250 fmol for B[*e*]P and DBP, respectively (see Table 1), and these limits are better than those obtained when 4HCC was used.

When 4-phenyl-α-cyanocinnamic acid (PCC), which resembles biphenyl carboxylic acid on one end and 4-hydroxy-α-cyanocinnamic acid (4HCC) on the other, was employed as the matrix for the adducts **1** and **2**, detection limits of 55 and 200 fmol, respectively, were obtained (see Table 1). Figure 3a and b show the MALDI-TOF mass spectra of 11,000 and 55 fmol of benzo[*a*]pyrene-6-*N7*-guanine (BP-6-*N7*-Gua), respectively, obtained by using PCC as the matrix. Metabolites **3** and **4** of molecular weight 336 can be detected as radical cations by using this matrix. The detection limits for **3** and **4** are 1000 and 500 fmol, respectively (see Table 1). The aromatic rings in PCC are less flexible than those of 4-benzyloxy-α-cyanocinnamic acid (BCC), but more flexible than those of anthracene-9-carboxylic acid. Furthermore, PCC is a good matrix for detection of unreacted PAHs. The detection limits for PAHs **5** and **6** are 500 and 200 fmol, respectively (see Table 1).

In summary, we have identified two new matrices, 4-phenyl-α-cyanocinnamic acid (PCC) and 4-benzyloxy-α-cyanocinnamic acid (BCC), for the determination of PAH-modified DNA bases at the femtomolar level by MALDI-TOF. Additional improvements may be achieved by incorporation of the recently developed sample handling procedures of Vorm and Mann [28] and Xiang and Beavis [29]. The matrices developed here are also suitable for the detection of the PAHs themselves. We also wish to advocate the concept of designing the MALDI matrix to have structural simi-

larities to the particular analyte rather than selecting on an empirical basis.

Acknowledgment

This work was supported by the National Institutes of Health (Grant No. 1P01CA49210).

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