Chemical Physics xxx (2012) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Chemical Physics

journal homepage: www.elsevier.com/locate/chemphys

Cyclodextrin-supported organic matrix for application of MALDI-MS for forensics. Soft-ionization to obtain protonated molecules of low molecular weight compounds

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ARTICLE INFO

Article history:
Available online xxxx

Keywords:
Cyclodextrin
Protonated molecule
Forensic science
Drug identification

ABSTRACT

A mass measurement technique for detecting low-molecular-weight drugs with a cyclodextrin-supported organic matrix was investigated. By using cyclodextrin-supported 2,4,6-trihydroxyacetophenone (THAP), the matrix-related peaks of drugs were suppressed. The peaks of protonated molecules of the sample and THAP were mainly observed, and small fragments were detected in a few cases. Despite the Na^+ and K^+ peaks were observed in the spectrum, Na^+ or K^+ adduct sample molecules were undetected, owing to the sugar units of cyclodextrin. The advantages of MALDI-MS with cyclodextrin-supported matrices as an analytical tool for forensic samples are discussed. The suppression of alkali adducted molecules and desorption process are also discussed.

1. Introduction

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) has been extensively used for the mass analysis of biomolecules and synthetic polymers [1,2]. Laser desorption/ionization (LDI) is a soft-ionization process producing mainly singly charged molecular-weight related ions. The advantages of MALDI include its ability to analyze high mass molecules and rapidly obtain accurate mass spectra. These features are important in the field of forensic science, and it has recently discovered that LDI-MS is an effective system. MALDI-MS has also been applied to detect the toxic protein ricin [3,4] and some additives in the adhesives [5,6]. However, conventional sample preparation for MALDI usually produces matrix-related peaks and fragments, which suggests that MALDI-MS may not be ideal for low-molecular-weight compounds. On the contrary, MALDI-MS can easily confirm low-molecular-weight compounds in the mass range between 200 and 1500 Da, such as small peptides [7]. Although for compounds in the mass range smaller than 200 Da, alternative sample preparations or matrices are favorable, even though there are few reports that show MALDI imaging of a drug [8].

Surface-assisted laser desorption/ionization (SALDI) was introduced as a possibility to overcome these problems. Various metal-, silicon-, and carbon-based nanostructured materials as well as nanoparticles have been used as SALDI matrices in the analysis of low-molecular-weight compounds [9–14]. SALDI has been used in forensic sciences [15]. For example, polymeric lubricants in a criminal case can be detected by desorption/ionization on porous silicon-mass spectrometry (DIOS-MS) using porous silicon, which is the most recognized SALDI-MS system [16]. Also, the rapid analysis of drugs was developed using SALDI with nanostructured Si [17]. Au nanoparticles were used to visualize fingerprints by using SALDI-MS imaging, [18] and oligonucleotide analysis was carried out by using magnetic nanoparticles [19]. However, SALDI-MS system usually shows reduced soft-ionization ability as revealed using a thermometer ion [12]. Thermometer ions are *para*-substituted benzylpyridium ions which have been used for internal energy calibration of ions produced in ESI and MALDI sources.

Recently, we have developed an effective organic matrix for suppressing the formation of matrix-related ions during MALDI-MS analysis. A typical organic matrix, 2,4,6-trihydroxyacetophenone (THAP), was mixed with cyclodextrin (CD) and was put into its cavity. Then, fragment ions and alkali adducted ions of THAP were significantly suppressed and only the mass peak of the protonated THAP was detected [19,20]. Adding a sugar to the THAP decreased the amount of fragmentation for DNA samples [21].

In this study, we have applied this type of CD-supported organic matrix in the field of forensic sciences. Nine examples, including

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http://dx.doi.org/10.1016/j.chemphys.2012.10.003

testosterone and diazepam, were used for investigating of the feasibility of this CD-supported matrix. The advantage of the CD-support matrix and their ionization mechanism is discussed.

2. Experimental

2.1. Materials

α-Cyclodextrin (αCD), β-cyclodextrin (βCD) (Sigma), and 2,4,6-trihydroxyacetophenone (THAP, 168 Da) (Wako Chemical), analyte molecules, acetaminophen (151 Da, antipyretic analgesic), caffeine (194 Da, alkaloid), lidocaine (234 Da, local anesthetic), amitriptyline (277 Da, antidepressant), diazepam (284 Da, anticonvulsant), testosterone (288 Da, steroid hormone), and sildenafil (474 Da, erectile dysfunction therapy) were used as received. Water was purified with a Milli-Q system (>18 MΩ).

2.2. Preparation of cyclodextrin-supported organic matrix

In this study, THAP was selected as an organic matrix. In our previous study, we compared THAP and α-cyano-4-hydroxycinnamic acid (CHCA) as matrix compounds to be incorporated into the cavities of CD. THAP showed a better result for detection of substance P [19].

Preparation of the CD-supported organic matrix was carried out as reported previously [19,20]. First, αCD was dissolved into water (10 mmol dm^{-3}). THAP was dissolved into an acetonitrile: water mixed solvent (7/3 vol/vol) (ca. 75 mmol dm^{-3}) containing trifluoroacetic acid (TFA, 0.1% by volume). To prepare the αCD-supported organic matrix, 120 μL of aqueous αCD and 20 μL of THAP solution were mixed in a microtube with ultrasonification. In this situation, one molecule of THAP is incorporated in one CD. The βCD-supported organic matrix was also prepared in a similar manner.

2.3. Mass measurement

For the analytes we have selected some important low-molecular-weight drugs in the field of forensic medicine. The concentration of analyte molecules in the solution was kept at ca. 10 mmol dm^{-3}.

MALDI mass measurements were performed with an Axima-CFR time-of-flight mass spectrometer (Shimadzu/Kratos, Manchester, UK) equipped with a pulsed nitrogen laser at 337 nm. All spectra in this study were obtained by the positive reflectron mode. The laser power (LP) was adjusted to obtain satisfactory spectra of the specimens. In most cases, the LP indicated by the spectrometer was 60, which was corresponding to the laser energy of 23.4 mJ cm^{-2}. Otherwise, the values are indicated in Figure caption.

3. Results and discussions

Fig. 1a shows the LDI mass spectrum of the THAP (168 Da) organic matrix. Many THAP-related peaks such as the protonated molecule, fragmentation, and alkali metal ion (Na^+ and K^+) adducts, are observed in this spectrum. In contrast, CD-supported THAP significantly suppressed the formation of fragment ions and alkali metal ion adducts as indicated in Fig. 1b. The vertical axes of the spectra were normalized with the highest intensity. As discussed in our previous studies [19,20], the peak intensity measured with the CD-supported matrices is in the same order as that obtained with the free matrices. Therefore, we can conclude that the suppression of alkali metal adducts is due to CD molecules. No peak corresponding to CD was observed. Sugar molecules are usually detected by positive ion mode MALDI-MS in the presence of alkali cations. Using 266 nm laser (forth-harmonic generation of a Nd^{3+}:YAG laser) irradiation, a similar mass spectrum of THAP was observed. The spectrum shown in Fig. 1a indicates that soft-ionization of THAP itself cannot be realized by LDI, and that CD

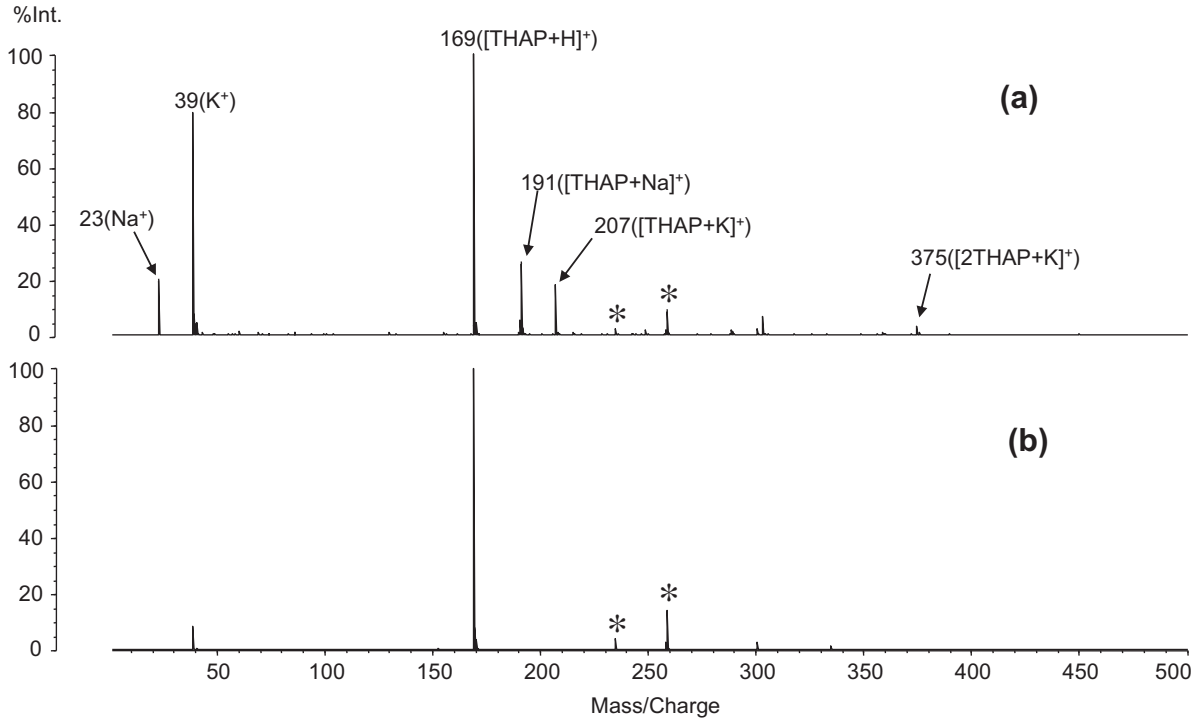

Fig. 1. Laser desorption/ionization (LDI) mass spectra of (a) THAP and (b) αCD-supported THAP. The protonated ion and alkali metal ion adducts can be observed in (a). CD-supported THAP shows only the protonated ion. No peaks of CD can be observed. The peaks indicated with an asterisk denote contaminations.

Please cite this article in press as: T. Yonezawa et al., Chem. Phys. (2012), http://dx.doi.org/10.1016/j.chemphys.2012.10.003