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Matrix-assisted laser desorption/ionization mass spectrometry for the analysis of polyamines in plant micro-tissues using cucurbituril as a host molecule

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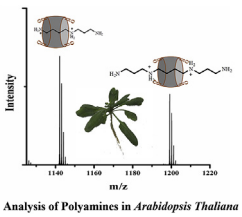
HIGHLIGHTS

- A cucurbit[n]uril assisted MALDI MS strategy was presented.
- Cucurbit[6]uril was employed as a selective mass shifting reagent of MALDI MS.
- Quantitation of endogenous polyamines was realized in plant micro-tissues (~μg FW).
- High throughput analysis of polyamines in plant was accomplished within 10 min.

GRAPHICAL ABSTRACT



Analysis of Polyamines in *Arabidopsis Thaliana*

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ABSTRACT

In this study, a matrix-assisted laser desorption/ionization (MALDI) mass spectrometry (MS) strategy using cucurbit[*n*]uril (CB[*n*]) as a host molecule is proposed for the analysis of low molecular weight (LMW) compounds in complex samples. As a proof-of-concept, CB[6] was selected as the host molecule, and endogenous polyamines in plant tissue were chosen as the target analytes. Due to the molecular recognition and mass shifting properties of CB[6], the ionic signals associated with polyamines were moved to the higher mass region (>1000 Da) after specifically binding to CB[6], while signal interference derived from the conventional organic matrix and the complex sample matrix remained in the low mass region because of the incompatibility of their molecular size with CB[6] cavities. The strategy not only facilitated the analysis of LMW compounds in complex samples by MALDI MS, but also offered high throughput by accomplishing the entire analytical procedure within 10 min. The detection of polyamine concentration showed good linearity in the range of 0.02–10.0 ng/μL with correlation coefficients (R) greater than 0.9915. The limits of detection were 8.8–28.8 pg. The good reproducibility and reliability of the method were demonstrated by excellent intraday and interday precisions with relative standard deviations less than 7.9%, and the recovery ranged from 92.1% to 117.1%. Finally, the good sensitivity of the method allowed for the quantitative analysis of endogenous polyamine concentrations in various micro-tissues of *Arabidopsis thaliana* (20.0–740.0 μg fresh weight for each sample).

1. Introduction

Matrix-assisted laser desorption/ionization time-of-flight mass

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spectrometry (MALDI-TOF MS) is a soft ionization tool introduced in the 1980s for analyzing macromolecules, and is known for its high-throughput data acquisition, good sensitivity, simplicity of operation, and low sample consumption [1–3]. These intrinsic advantages have prompted scientists to explore the potential of MALDI MS for the analysis of low molecular weight (LMW) compounds (<500 Da) [4]. Nevertheless, efforts to identify and quantify the concentrations of LMW compounds using MALDI MS are usually limited due to interfering signals in the low mass region (0–500 Da) derived from conventional organic MALDI matrices and complex organic samples [5].

Two approaches are commonly employed to reduce or circumvent the interference associated with conventional organic matrix-derived peaks obtained by MALDI MS in the low mass region. The first option involves selecting matrix materials that consist of high molecular weight organic compounds or inorganic materials, including porous silicon [6], carbon-based materials [7–11], metal nanoparticles [12,13], metal oxides [14], and phosphorus [15]. These materials are relatively stable under laser irradiation and therefore less prone to generate interfering peaks in the 0–800 Da range [16]. The second option involves labeling LMW compounds with a large molecular weight tag, such as peptides [17], fullerene derivatives [18], and other synthetic derivatization reagents [19–21], which can react with LMW analytes with certain functional groups and shift the molecular weight of the target compounds to the high mass region (>1000 Da), and thus altogether avoid interference with signals in the low mass region. However, these approaches are only applicable when applied to analytes in standard samples or relatively clean samples, which merely require distinguishing compounds according to differences in molecular weight. However, complex samples, such as plant samples and human body fluids, inherently contain a variety of interfering matrix components with molecular weights similar to those of the target LMW analytes, which greatly hinders the detection ability of MALDI MS.

Cucurbit[*n*]uril (CB[*n*]), as illustrated in Fig. 1, is a macrocyclic product of the condensation reaction between glycoluril and formaldehyde, and is one of the more interesting host molecules [22–24]. The CB family consists of a variety of homologues, which are denoted as CB[*n*], where *n* is 5, 6, 7, 8, 9, 10, etc., depending on the number of glycoluril units, and they exhibit distinct cavity sizes that determines that their association constants toward various analytes. The CB[*n*] molecule is able to bind with specific guest molecules through molecular recognition based on the selectivity associated with cavity size, and hydrophilic and ion-dipole interactions, which resultantly forms stable host-guest complexes with association constants log K of up to 15.7 [25]. Because the interaction between CB[*n*] and guest molecules is specific and similar to lock-and-key interactions [26], even guest molecules in a highly complex organic sample matrix can specifically bind to CB [*n*]. This feature can potentially circumvent the obstacle associated with sample matrix interference in the low mass range. Moreover, the molecular weights of, e.g., CB[6] and CB[7] are 996 and 1162 Da, respectively, which would conveniently shift guest LMW compounds complexed with either of these molecules to the high mass region in MALDI MS, and therefore circumvent interfering signals associated with conventional organic matrices. In addition, CB complexes are fully amendable to characterization by MS [27,28]. The above advantages suggest that CB[*n*] may be developed as useful reagents for the analysis of LMW compounds in complex samples by MALDI MS.

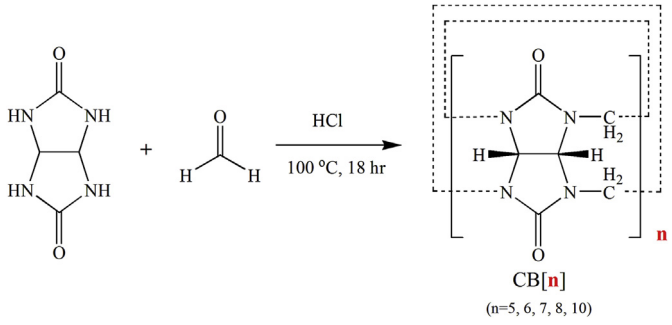

Fig. 1. The synthetic process and chemical structure of cucurbit[*n*]uril.

In the current study, we propose a CB[*n*]-assisted MALDI MS strategy for the analysis of LMW compounds in complex samples. As a proof-of-concept, polyamines in plant samples were selected as the target analytes. Their biological relevance is demonstrated by the fact that they are important plant growth regulators in a great number of plant growth and developmental processes. The distinct cavity sizes of CB[*n*] ensures that their association constants towards polyamines are distinct. The restrictions of portal size and cavity volume also determine that CB[6] and CB[7] can only form 1:1 inclusion complexes with guest molecules, while CB[8], CB[9] or], and CB[10] form 1:2 or 1:1:1 complexes with various guest molecules [25]. To avoid the generation of any 1:2 or 1:1:1 complexes and ensure the most stable CB-polyamine complexes, CB[6] and CB[7] were preliminarily selected and we compare their association constants with polyamines in Table 1 [25,29]. The data for representative polyamines spermidine (SPD) and spermine (SPM) show that CB[6] exhibits the highest log K values of 8.61 and 9.52, respectively. Therefore, CB[6] was selected as the host molecule. The formation of CB[6]-polyamine complexes produce a shift of the polyamine analytes into the high mass region, and can therefore be monitored by MALDI MS in the absence of interference caused by the other LMW molecules in the sample matrix, which remain in the low mass region. Further application of the strategy in complex samples was conducted through the analysis of polyamines in single organs of *Arabidopsis thaliana* by detecting the concentrations of CB[6]-polyamine complexes.

2. Materials and methods

2.1. Chemicals

We obtained CB[6] from J&K Chemical, Ltd. (Tianjin, China), and SPD (purity 99%), SPM (purity 98%), and α-Cyano-4-hydroxycinnamic acid (CHCA) were obtained from Aladdin Chemical Reagent Co. (Shanghai, China). In addition, d_8-SPD and d_8-SPM were purchased from Sigma-Aldrich (St Louis, USA), and formic acid (88%), sodium chloride (AR), and tris(hydroxymethyl)aminomethane (AR) were obtained from Sinopharm Chemical Reagent (Shanghai, China). Angiotensin II was purchase from Fine Peptide Co., Ltd (Wuhan, China). Purified water was prepared by a Milli-Q apparatus (Millipore, Bedford, MA, USA).

Table 1
Association constants (log K) of polyamine-CB[n] complexes for representative polyamines spermidine (SPD) and spermine (SPM) [25,29].

CB[*n*][a]	Association constants (log K)	
	SPD	SPM
CB[6]	8.61	9.52
CB[7]	5.30	7.60

[a] The molecular weights of CB[6] and CB[7] are 996.29445 and 1162.34352, respectively.