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Short communication

Rapid and high-throughput determination of endogenous cytokinins in *Oryza sativa* by bare Fe_3O_4 nanoparticles-based magnetic solid-phase extraction

Bao-Dong Cai[a], Jiu-Xia Zhu[a], Qiang Gao[a,b], Dan Luo[c], Bi-Feng Yuan[a], Yu-Qi Feng[a,*]

[a] *Key Laboratory of Analytical Chemistry for Biology and Medicine (Ministry of Education), Department of Chemistry, Wuhan University, Wuhan 430072, China*

[b] *Engineering Research Center of Nano-Geomaterials of Ministry of Education, Department of Chemistry, China University of Geosciences, Wuhan 430074, China*

[c] *Shimadzu Global COE for Application & Technical Development, Shimadzu International Trading (Shanghai) Co., Ltd, Shanghai 200052, China*

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ABSTRACT

A rapid method was developed for determination of endogenous cytokinins (CKs) based on magnetic solid-phase extraction (MSPE) followed by ultra-performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS). We illustrated the hydrophilic character of bare Fe_3O_4 nanoparticles that were directly used as a MSPE sorbent for rapid enrichment of endogenous CKs from complex plant extract. To the best of our knowledge, this is the first report of bare Fe_3O_4 directly used as efficient extraction sorbent to enrich target CKs based on hydrophilic interaction. Under the optimized conditions, a rapid, sensitive and high-throughput method for the determination of 16 CKs was established by combination of MSPE with UPLC–MS/MS. Good linearity was obtained with correlation coefficients (r) from 0.9902 to 0.9998. The limits of detection (LODs) and quantification (LOQs) ranged from 1.2 pg mL^{-1} to 391.3 pg mL^{-1} and 4.1 pg mL^{-1} to 1304.3 pg mL^{-1}, respectively. 16 CKs could be successfully determined in spiked sample with 80.6–117.3% recoveries and the relative standard deviations (RSDs) were less than 16.6%. Finally, 10 endogenous CKs were successfully quantified in 50 mg *Oryza sativa* sample using the developed MSPE-UPLC–MS/MS method.

1. Introduction

Cytokinins (CKs) are a class of adenine-derived signaling molecules with an isoprenoid or aromatic side chain which play a crucial role in the regulation of plant growth and development [1–3]. In the last few decades, many significant progresses have been achieved on the biosynthesis, transportation and signal perception of CKs in plants [3–7]. Whereas, the molecular mechanism of how CKs regulate the growth and development of plant is rarely known [3]. In this respect, it is extremely important to explore the homeostasis of CKs with various forms in plant at different growth periods. Therefore, rapid and high-throughput methods for endogenous CKs analysis will greatly facilitate the functional studies of CKs.

As endogenous CKs in plant are typically present at very low concentration (pmol per gram fresh weight, pmol g^{-1} FW) and complex matrices would heavily affect the accurate quantification, the sample pretreatment process is indispensable before instrumental analysis. Up to now, various sample preparation methods have been developed to enrich CKs from crude extract, such as liquid–liquid extraction (LLE) [8,9], solid-phase extraction (SPE) [10–13], immunoaffinity purification (IAP) [14–16], polymer monolith microextraction (PMME) [17] and magnetic solid-phase extraction (MSPE) [18]. Among these techniques, SPE was widely used to purify CKs due to many kinds of commercially available sorbents, such as hydrophobic sorbents, cation exchange sorbents and mix-mode sorbents. Whereas, acceptable results normally required the combination of two or more SPE steps [19] or several materials [20], which makes the analytical process tedious and limits the high throughput analysis. Compared with the traditional SPE, MSPE can be rapidly completed with vortexing to accelerate the mass transfer of analytes through increasing the interfacial area between the solid sorbent and sample solution. And the magnetic sorbent can be readily isolated from suspensions with the external magnetic field. However, the synthetic procedures of these functional magnetic materials were relatively tedious [21]. There are few reports

* Corresponding author. Tel.: +86 27 68755595; fax: +86 27 68755595.
E-mail address: yqfeng@whu.edu.cn (Y.-Q. Feng).

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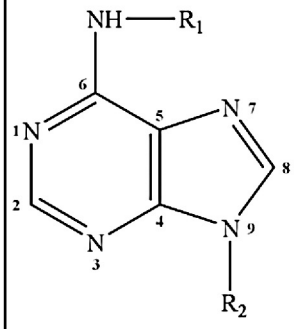
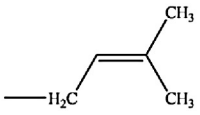
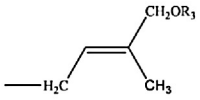
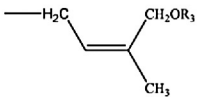
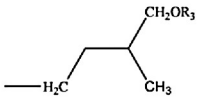
Table 1
Structures, names and abbreviations of naturally occurring CKs.



R_1	R_2	R_3	Compound & abbreviation
	H	H	isopentenyladenine (iP)
	R	H	isopentenyladenine riboside (iPR)
	G	H	isopentenyladenine riboside 9-glucoside (iP9G)
	H	H	*trans*-zeatin (tZ)
	R	H	*trans*-zeatin-riboside (tZR)
	–	H	*trans*-zeatin 7-glucoside (tZ7G)
	G	H	*trans*-zeatin 9-glucoside (tZ9G)
	H	G	*trans*-zeatin *O*-glucoside (tZOG)
	H	H	*cis*-zeatin (cZ)
	R	H	*cis*-zeatin-riboside (cZR)
	G	H	*cis*-zeatin 9-glucoside (cZ9G)
	H	G	*cis*-zeatin *O*-glucoside (cZOG)
	H	H	dihydrozeatin (DHZ)
	R	H	dihydrozeatin riboside (DHZR)
	G	H	dihydrozeatin 9-glucoside (DHZ9G)
	H	G	dihydrozeatin *O*-glucoside (DHZOG)

H, hydrogen; R, *β*-*D*-ribofuranosyl; G, *β*-*D*-glucopyranosyl. In tZ7G, *β*-*D*-glucopyranosyl group is substituted at N^7.

to directly use bare Fe_3O_4 as MSPE sorbent for the enrichment of target analyst [22].

Inspired by the existence of lots of polar hydroxyl group on the surface of bare Fe_3O_4 nanoparticles, we proved the hydrophilic character of bare Fe_3O_4 nanoparticles in the current work. And then we employed the easily prepared bare Fe_3O_4 nanoparticles as a magnetic sorbent based on hydrophilic interaction to directly enrich CKs from crude plant extract (Table 1). With the use of MSPE method, the modification of the surface of bare Fe_3O_4 is not necessary, which favorably facilitates the application of Fe_3O_4. In the proposed MSPE, CKs can be efficiently enriched from crude plant extract with one-step purification based on hydrophilic interaction, which significantly simplified the procedure of sample preparation and largely improved the analysis throughput. Subsequently, 16 CKs, including four pairs of isomer (t/cZ, t/cZR, t/cZ9G, t/cZOG), were well separated by ultra-performance liquid chromatography (UPLC) in 11 min and detected by tandem mass spectrometry (MS/MS). Finally, by employing the developed MSPE-UPLC–MS/MS method, 10 endogenous CKs were distinctly detected in 50 mg *Oryza sativa* sample.

2. Materials and methods

2.1. Chemicals and reagents

CK standards: N^6-isopentenyladenine (iP), isopentenyladenine riboside (iPR), N^6-isopentenyladenine-9-glucoside (iP9G), *trans*-zeatin (tZ), *trans*-zeatin-riboside (tZR), *trans*-zeatin-7-glucoside (tZ7G), *trans*-zeatin-9-glucoside (tZ9G), *trans*-zeatin-O-glucoside (tZOG), *cis*-zeatin (cZ), *cis*-zeatin-9-glucoside (cZ9G), *cis*-zeatin-riboside (cZR), *cis*-zeatin-O-glucoside (cZOG), dihydrozeatin (DHZ), dihydrozeatin riboside (DHZR), dihydrozeatin-9-glucoside (DHZ9G), dihydrozeatin-O-glucoside (DHZOG), and stable isotope-labeled standards: $[^2H_6]$iP, $[^2H_6]$iPR, $[^2H_6]$iP9G, $[^2H_5]$tZ, $[^2H_5]$tZR, $[^2H_5]$tZ9G, $[^2H_3]$DHZ, $[^2H_3]$DHZR, $[^2H_5]$tZ7G, $[^{15}N_4]$cZ, $[^2H_3]$DHZ9G, $[^2H_5]$ tZOG, $[^2H_7]$DHZOG were all purchased from Olchemim (Olomouc, Czech Republic). The stable isotope-labeled CKs were used as internal standards (I.S.).

Ferric chloride ($FeCl_3{\cdot}6H_2O$), sodium acetate (NaAc), ethylene glycol (EG), 1,2-ethylenediamine (ETH) and ethanol (EtOH) were all purchased from Sinopharm Chemical Reagent (Shanghai, China). Acetonitrile (ACN, HPLC grade) was obtained from Tedia Co. (Fairfield, OH, USA). Tetraethylorthosilicate (TEOS) was purchased from the Chemical Plant of Wuhan University (Wuhan, China). Ultrapure water was purified with Milli-Q system (Milford, MA, USA).

2.2. Plant materials

Oryza sativa was grown in greenhouse at 30 °C under 16 h light/8 h dark photoperiods according to previously described protocol [23]. Seven-day-old *Oryza sativa* was harvested, weighted, immediately frozen in liquid nitrogen, and stored at −80 °C until needed.

2.3. Synthesis of Fe_3O_4 and Fe_3O_4@SiO_2 magnetic nanoparticles

Fe_3O_4 and Fe_3O_4@SiO_2 magnetite nanoparticles were synthetized according to our previously reported method (see supporting information) [24].