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Short communication

Capillary ion chromatography–mass spectrometry for simultaneous determination of glucosylglycerol and sucrose in intracellular extracts of cyanobacteria

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ABSTRACT

A capillary ion chromatography–mass spectrometry (MS) method was proposed to determine glucosylglycerol (GG), sucrose, and five other carbohydrates. MS conditions and make-up flow parameters were optimized. This method is accurate and sensitive for simultaneous analysis of carbohydrates, with mean correlation coefficients of determination greater than 0.99, relative standard deviation of 0.91–2.81% for eight replicates, and average spiked recoveries of 97.3–104.9%. Limits of detection of sodium adduct were obtained with MS detection in selected ion mode for GG (0.006 mg/L), sucrose (0.02 mg/L), and other carbohydrates (0.03 mg/L). This method was successfully applied to determine GG and sucrose in intracellular extracts of salt-stressed cyanobacteria.

1. Introduction

Cyanobacteria can naturally synthesize and accumulate some soluble sugars, such as trehalose, sucrose, glucosylglycerol (GG), and glycinebetaine, to compensate hyperosmotic stresses; these characteristics signify the osmotic and protective functions of cyanobacteria [1]. GG and sucrose are highly significant compounds among carbohydrates because of their extensive applications in health, food industry, pharmacy, and cosmetics. For example, sucrose is one of the basic food additives that are mostly abundant in sugarcane and beet. GG also serves as a protein drug stabilizer [2] and cosmetic additive [3] because of its stabilizing effect on protein structure and its antioxidative property. Moreover, GG has been found as a bioactive compound in some Japanese foods [4]. GG applications have been patented by some Japanese firms [5]. Thus, many studies focus on sugar biosynthetic pathway modification for production rate improvement [6,7] and GG accumulation by heterotrophic bacteria, such as *Pseudomonas* [8] and *Stenotrophomonas* [9] strains, or cyanobacteria with moderate salt tolerance, such as *Synechocystis* sp. PCC 6803 [1] and *Synechococcus* sp. PCC 7002 [3].

High-performance liquid chromatography (HPLC) has been presented by A. Schoor et al. [10] to determine cyanobacterial osmolyte GG. However, in their report, reversed-phase chromatographic and ion-moderated partition chromatographic (Ca^{2+}) (IMPC) column separation was restricted to samples containing insignificant amounts of sucrose and glucose, respectively. Moreover, lactose, GG, and glucose cannot be easily isolated on IMPC columns. Amino-bonded silica and acetonitrile–water mixtures provide excellent GG separation from disaccharides; however, separation from important hexoses remains limited. Isomer pairs of sucrose and trehalose have also been co-eluted on Aminex analysis columns [11]. Afterward, few studies have reported about osmolyte GG and sucrose determination.

High-performance anion-exchange chromatography (HPAEC) with pulsed amperometric detector has been recognized as a highly efficient tool for carbohydrates analysis. This method requires

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no pre- or post-column derivatization and offers superb resolution and sensitivity [12]. Therefore, our co-worker, Wei Du and Xiaoming Tan have used HPAEC method with CarboPac® MA1 analytical column to measure GG and glucose in genetically engineered cyanobacteria [4,13]. Nevertheless, fructose and lactose are co-eluted on MA1 column; trehalose, GG and mannitol are co-eluted on PA10 column respectively. Furthermore, the characteristics of some unknown carbohydrates are not easily derived from its retention time. Therefore, online mass spectrometry (MS) detection is required not only because of rapid and reliable identification and peak conformation based on mass-to-charge ratio, but also because of additional separation and sensitivity [14,15]. However, ion chromatography application in conjunction with MS has been hampered by high-salt requirement for effective ion chromatography (IC). With the development of electrolytic suppression technique, the hamper was settled down, and IC coupled with MS significantly contributed to applications in the environment, food, biotechnology, and life sciences [16,17]. However, few studies have emphasized carbohydrate quantification. Many recent reports demonstrated that capillary IC can significantly improve separation efficiency and sensitivity [18–21]. Additionally, the split-flow is not needed when IC tandems MS detector with much smaller diameter columns and lower flow rate. Thus, this finding serves as a new foundation for the applicability of capillary IC in biological analysis [22].

In the present study, we coupled capillary IC with electrospray ionization–tandem MS (ESI–MS-MS) to achieve an acute and highly sensitive detection for GG, sucrose, and five other carbohydrates, which may exist in cyanobacteria. ESI interface parameters, including ionization voltage, ion source gas pressure, nebulization temperature, DP voltage, and make-up flow parameters, were optimized. Isomer pairs of fructose, glucose, trehalose, lactose, and sucrose are well separated which are not distinguished by MS; and pairs of fructose and lactose, trehalose, GG and mannitol are well identified which are co-eluted on IC column. Moreover, lower limits of detection (LODs) of sodium adducts were determined using MS detection in selected ion mode (SIM) for GG (0.006 mg/L), sucrose (0.02 mg/L), and other carbohydrates (0.03 mg/L) with relative standard deviation (RSD) for eight replicates of 0.91% to 2.81%. The proposed method was successfully used to determine sucrose and GG in the intracellular extracts of the wild-type cyanobacterium *Synechocystis* sp. PCC 6803 (WT-6803) under salt-stressed conditions.

2. Experimental

2.1. Instrumentation

An ICS-5000 system (Thermo Scientific Dionex, Sunnyvale, CA, USA), equipped with two pumps exhibiting capillary flow capability and 18 MΩ water regenerant (flow rate, 0.03 mL/min), an EG eluent generator, an ACES300 suppressor, and an AS-AP autosampler, were used in this study. A 1200 HPLC system (Agilent Technologies, Forest Hill, Victoria) was used to pump 0.01 mL/min make-up flow of 0.25 mmol/L sodium acetate in acetonitrile–water (volume ratio, 9:1). Chromeleon software (Thermo Fisher Scientific, San Jose, California) was used for system control. MS was performed using AB 4000Q-Trap mass spectrometer (AB Sciex, Toronto, Canada).

2.2. Chromatographic and MS conditions

IC separation was performed using a capillary column (CarboPac PA-20, 0.4 mm × 150 mm, Thermo Fisher Scientific) and a guarder (0.4 mm × 35 mm, Thermo Fisher Scientific). KOH/H_2O eluent flow rate was 0.01 mL/min at 30 °C. Gradient profile was described as follows: 0 min to 30 min, 3 mmol/L; 30.1 min to 35 min, 80 mmol/L; 35.1 min to 40 min, 3 mmol/L. Injection volume was 0.4 μL.

Table 1
Ion monitoring modes.

Substance	Quantitative ion	Ion mode	DP voltage (V)
Trehalose	365.2	+	110
GG	277.2	+	100
Mannitol	205.2	+	70
Sucrose	365.2	+	110
Glucose	203.2	+	60
Fructose	203.2	+	60
Lactose	365.2	+	110

ESI–MS was operated in SIM mode at 550 °C, curtain gas pressure: 10.0 psi, ion source gas 1 pressure: 20.0 psi, ion source gas 2 pressure: 50.0 psi, and 4.5 kV positive voltage. Table 1 presents the ion monitoring modes.

2.3. Materials and sample preparation

All solutions were prepared in 18 MΩ water (Milli-Q) with a 0.22 μm nylon membrane filter. Sugar standards (glucose, sucrose, lactose, trehalose, and fructose) were purchased from Sigma–Aldrich (St. Louis, MO, USA). HPLC-grade acetonitrile was purchased from Burdick and Jackson (Morristown, New Jersey, USA). GG was purchased from Bitop AG (Stockumer, Germany). Mannitol was purchased from Amresco (Solon, USA), and sodium acetate (>99.0%) was purchased from Sigma–Aldrich (St. Louis, MO, USA).

Cultures were sampled obtaining 1.5 mL of aliquots centrifuged at 6000 rpm for 10 min to obtain cell pellets and supernatants. Pelleted cells were suspended in 1.8 mL of 80% ethanol (v/v) and subsequently heated at 65 °C for 4 h, leading to nearly complete low-molecular-mass compound extraction. After centrifugation at 10,000 rpm for 5 min, supernatants were transferred to a clean tube and subsequently dried at 55 °C under N_2 stream [7]. Dry residues were dissolved with water and filtered using a 0.22 μm nylon membrane prior to capillary IC.

To detect the recovery rates, we selected a non-salt-stress culture as matrix sample. After obtaining the pelleted cells, two levels (0.50 and 1.00 mg/L) of standards were added. Pretreatment was then performed as described above.

2.4. Method validation

The proposed method was validated for precision, sensitivity, linearity, recovery, and reproducibility. Repeatability (RSDs) was determined by replicate measurements of 0.50 mg/L standard mixtures, the relative standard deviations were assessed of 0.5 mg/L standards mixtures within (n = 8) and among (n = 6) days. And reproducibility was determined using six replicates at 0.10, 0.50, and 1.00 mg/L concentrations. Linear regressions of calibration curves were calculated by plotting peak area ratios against standard concentrations. The recovery represented the mean of two levels (0.50 and 1.00 mg/L) of spiked recoveries.

3. Results and discussion

3.1. Optimization of MS condition and make-up flow parameters

SIM parameters (ionization voltage, nebulization temperature, ion source gas 2 pressure, and DP voltage) were optimized by infusing 1.00 mg/L standard solution into the ESI source through an infusion pump set at 0.01 mL/min. We tested different ionization voltages (2.5, 3.0, 3.5, 4.0, 4.5, 5.0, and 5.5 kV), nebulization tem-