Quantitative Metabolome Analysis Using Capillary Electrophoresis Mass Spectrometry

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A new approach for the comprehensive and quantitative analysis of charged metabolites by capillary electrophoresis mass spectrometry (CE−MS) is proposed. Metabolites are first separated by CE based on charge and size and then selectively detected using MS by monitoring over a large range of m/z values. This method enabled the determination of 352 metabolic standards and its utility was demonstrated in the analysis of 1692 metabolites from *Bacillus subtilis* extracts, revealing significant changes in metabolites during *B. subtilis* sporulation.

Keywords: metabolomics • metabolic pathways • capillary electrophoresis • mass spectrometry • *Bacillus subtilis* • sporulation • glycolysis • TCA • nucleotide • CoA

Introduction

High-throughput and comprehensive analysis of intracellular metabolites can reveal the connection of biochemical networks and provide a systems-level understanding of the cell. Metabolomics, which can be defined as the measurement of the level of all intracellular metabolites, has become a powerful new tool for gaining insight into cellular function. Proteins and metabolites are the main effectors of phenotype and, thus, the functional entities within the cell. Tracking metabolite changes under different conditions not only provides direct information on metabolic phenotypes but is also complementary to gene expression and proteome analysis.[1−4] Moreover, metabolome analysis is indispensable for studies of human diseases, metabolic engineering of plant and microorganisms, and *in silico* simulation of living cells.[5,6]

Despite its importance, only a limited number of methodologies have been developed for metabolome analysis. This is primarily due to the characteristics of most metabolites that display high polarity, nonvolatility, poor detectability, and overall similar properties. In addition, the fact that over 1000 different metabolic substrates exist in a cell complicates the analysis. Most of the pioneering work in large-scale metabolite analysis was performed by gas chromatography mass spectrometry (GC−MS).[7,8] Although the GC−MS method demonstrates outstanding performance, it is limited by the need for multiple derivatization procedures for each chemical moiety, and the fact that nonvolatile compounds cannot be determined. Recently, direct infusion analysis approaches using NMR[9] and other mass spectrometry methods such as Fourier transform ion cyclotron resonance mass spectrometry

(FT-ICRMS)[10] or electrospray ionization mass spectrometry (ESI−MS)[11] have been developed for metabolome profiling. Although these infusion techniques enable the instantaneous acquisition of metabolic snapshots, there are still some important drawbacks. In NMR, sufficient amounts of samples must be prepared, and quantification is difficult. Infusion techniques using MS lack accuracy and precision for quantification due to ion suppression effect[12,13] and often cannot separate a number of isomers. Therefore, the development of quantitative and high-resolution metabolome analysis methods remains one of the most demanding challenges.

Capillary electrophoresis mass spectrometry (CE−MS) has recently emerged as a powerful tool for the analysis of charged species.[14−18] The major advantages of CE−MS are that this methodology exhibits extremely high resolution and that almost any charged species can be infused into MS. Previous work in our laboratory has shown that CE−MS techniques are quite useful for the determination of several anionic metabolites.[19,20]

Here, we propose CE−MS methods for metabolome analysis that cover a wide (70−1027) m/z range. Using this system, we successfully determined 352 standard molecules derived from known metabolic pathways, and the methods were readily applied to the analysis of 1692 compounds from *Bacillus subtilis* extracts. The results provide information about how changes in metabolites are implicated in sporulation.

Experimental Section

Reagents. 1-Adamantanamine and p-coumarate were purchased from ICN Biomedicals (Aurora, OH); L-Albizziine from BACHEM (Bubendorf, Swizerland); L-cystathionine from Toronto Research Chemicals (Ontario, Canada); piperazine-1,4-bis(2-ethanesulfonic acid) (PIPES) from Dojindo (Kumamoto, Japan); and methionine sulfone from Avocado (Lancashire, UK). All other reagents were obtained from common com-

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mercial sources. Individual stock solutions at a concentration of 10 mM or 100 mM were prepared in Milli-Q water, 0.1N HCl or 0.1N NaOH. The working standard mixture was prepared by diluting these stock solutions with Milli-Q water just prior to injection. All chemicals used were of analytical or reagent grade. Water was purified with a Milli-Q purification system (Millipore, Bedford, MA).

Bacterial Strains and Growth Conditions. The *Bacillus subtilis* 168 strain (*trpC2*, laboratory stock) was used and cultured at 37 °C in modified 2× SG medium (1.6% nutrient broth, 0.1% KCl, 1 mM MgSO$_4$, 1 mM Ca(NO$_3$)$_2$, 1 μM FeSO$_4$, 10 μM MnCl$_2$, and 0.1% glucose). Growth was monitored by measuring optical density at 660 nm, and cells were collected at $T_{-0.5}$ (0.5 h before T_0), T_0 (corresponding to the end of exponential growth) and T_2 (2 h after T_0), respectively.

Metabolite Extraction. The development of efficient metabolite extraction procedures from cells must meet several requirements. First, rapid enzyme inhibition and efficient metabolite extraction are necessary to quantify intercellular metabolites because turnover of some metabolites occurs rapidly. Second, samples should be enriched to facilitate detection. Third, simultaneous extraction of both cationic and anionic metabolites is desirable. Finally, metabolites should be dissolved in low conductivity solutions to achieve maximum performance of CE−MS.[21] A method meeting these requirements was developed by modifying a previously described procedure.[19] A culture medium volume of 10 mL was passed through a 0.45 μm pore size filter. Residual *B. subtilis* cells on the filter were washed with Milli-Q water and then plunged into 1 mL of methanol, containing internal standards (5.6 μM methionine sulfone for cations and 5.6 μM PIPES for anions), to inactivate enzymes. After a 5-min incubation at room temperature, 1 mL of chloroform and 380 μL of Milli-Q water were added to the solution, and the mixture was thoroughly mixed to remove phospholipids liberated from cell membranes, which can adsorb on the capillary wall and reduce CE performance. The separated 1 mL methanol−water layer was centrifugally filtered through a Millipore 5-kDa-cutoff filter to remove proteins. The filtrate was lyophilized and dissolved in 20 μL of Milli-Q water before CE−MS analysis. Overall, this procedure resulted in a 500-fold enrichment of metabolites.

Instrumentation. All CE−MS experiments were performed using an Agilent CE Capillary Electrophoresis System equipped with an air pressure pump, an Agilent 1100 series MSD mass spectrometer and an Agilent1100 series isocratic HPLC pump, a G1603A Agilent CE−MS adapter kit and a G1607A Agilent CE−ESI−MS sprayer kit (Agilent Technologies). System control, data acquisition and MSD data evaluation were performed via a G2201AA Agilent ChemStation software for CE−MSD.

CE−MS Conditions for Cationic Metabolites. Separations were carried out on a fused silica capillary (50 μm i.d. × 100 cm total length) using 1 M formic acid as the electrolyte. Sample was injected with a pressure injection of 50 mbar for 3 s (3 nL). The applied voltage was set at +30 kV. The capillary temperature was maintained at 20 °C using a thermostat and the sample tray was cooled below 5 °C. 5 mM ammonium acetate in 50% (v/v) methanol−water was delivered as the sheath liquid at 10 μL/min. ESI−MS was conducted in the positive ion mode and the capillary voltage was set at 4000 V. A flow of heated dry nitrogen gas (heater temperature of 300 °C) was maintained at 10 L/min. In MS with selective ion monitoring (SIM), sets of 30 protonated [M+H]$^+$ ions were
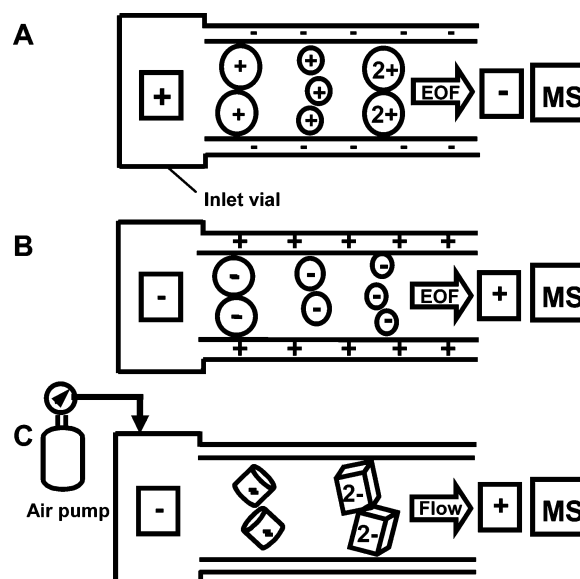

Figure 1. Schematic of the various CE−MS methods. (**A**) Cationic metabolites, (**B**) Anionic metabolites, and (**C**) Nucleotides and CoA compounds.

analyzed successively to cover the whole range of m/z values from 70 through 1027.

CE−MS Conditions for Anionic Metabolites. A cationic polymer coated SMILE (+) capillary was obtained from Nacalai Tesque (Kyoto, Japan) and used as the separation capillary (50 μm i.d. × 100 cm total length). The electrolyte for the CE separation was 50 mM ammonium acetate solution, pH 8.5. Sample was injected with a pressure injection of 50 mbar for 30 s (30 nL). The applied voltage was set at −30 kV. ESI−MS was conducted in the negative ion mode and the capillary voltage was set at 3500 V. In MS with SIM, sets of 30 deprotonated [M−H]$^-$ ions were analyzed successively to cover the whole range of m/z values from 70 through 1027. Other conditions were the same as in cationic metabolite analysis.

CE−MS Conditions for Nucleotides and Coenzyme A Compounds. Separations were carried out on a GC capillary, poly-(dimethylsiloxane) (DB-1) (50 μm i.d. × 100 cm total length) (Agilent Technologies). The electrolyte for the CE separation was 50 mM ammonium acetate, pH 7.5. The applied voltage was set at −30 kV, and a pressure of 50 mbar was added to the inlet capillary during the run to maintain a conductive liquid junction at the capillary outlet.[20] Other conditions were the same as in anionic metabolite analysis.

Results and Discussion

CE−MS Methods for Metabolome Analysis. To achieve high-throughput and quantitative analysis of over 1000 metabolites, a novel strategy was developed: (i) Metabolites were roughly separated by CE and detected by MS. (ii) Isomers were at least partially separated to allow identification by MS. (iii) To detect complete sets of metabolites, m/z values were monitored from approximately 70 through 1000 m/z by MS. (iv) To improve throughput, three CE−MS systems (Figure 1) were employed in parallel, for cationic metabolites, anionic metabolites, and nucleotides, respectively.

In CE, all cations migrate toward the cathode, whereas all anions move to the opposite direction. Therefore, in principle all charged species can be analyzed using only two CE−MS configurations. Figure 1A illustrates the cation analysis system,

where cations are separated based on their charge and size on a fused-silica capillary by CE and then detected by MS. To analyze all cations simultaneously, a very low pH electrolyte, 1 M formic acid (pH 1.8),[15] was used to confer a positive charge on all cationic metabolites, making them amenable to MS analysis. A total of 174 cationic metabolite standards such as amino acids, amines, and nucleosides, which are listed in the COMPOUND section in LIGAND database,[22] were purchased and then analyzed by CE−MS. All cations were successfully determined as the protonated molecular weight form with SIM mode (Supporting Information Table 1).

Subsequently, the development of a CE−MS method for anionic metabolites was investigated. Analysis of anions by CE−MS was performed in negative mode, where the inlet of the capillary is at the cathode and the outlet at the anode. Under normal conditions, because the CE−MS system does not possess an outlet vial, electroosmotic flow (EOF)[21] movement toward the cathode (opposite to MS direction) creates a gap in the liquid phase at the capillary exit, resulting in a current drop.[19] This problem was overcome by reversing the EOF[19] using a SMILE(+),[23] cationic polymer coated capillary, as illustrated in Figure 1B. At first, 50 mM ammonium acetate (pH9.0) was used as the running electrolyte, but a current drop was observed after fewer than 30 runs using this buffer. We assumed that this was caused by hydrolysis of the coated capillary under alkaline condition. Because decreasing the pH resulted in a loss of resolution of important isomers such as fructose 6-phosphate and fructose 6-phosphate, a pH 8.5 solution was selected and its utilization enabled successive anion analysis without current drop. A total of 124 anionic metabolite standards including carboxylic acids, phosphorylated carboxylic acids, phosphorylated saccharides, and phosphorylated compounds were determined by this CE−MS method (Supporting Information Table 1). However, the detection sensitivity for anions was several times lower than that of cations in CE−MS. To obtain sufficient sensitivity, we found it necessary to increase the injection volume up to 30 nl for anionic samples.

Using the above anion-analysis system, significant adsorption of multivalent ions (e.g., nucleotides and CoA compounds) on the cationic-coated capillary was observed. To prevent adsorption and allow precise quantification, a pressure-assisted CE−MS technique using a noncharged polymer coated capillary[20] (Figure 1C) was used. After optimization, 41 different nucleotides including cyclic nucleotides, deoxy nucleotides, and CoA compounds could be simultaneously determined (Figure 2). Although the migration times for most of the compounds were very close, they could be selectively separated and identified by MS. Altogether, the three CE−MS methods allowed the analysis of a total of 352 metabolic standards (See the Supporting Information Table 1). The sensitivity and linearity of these methods were validated for 50 key metabolites.[15,19,20] The detection limits for all compounds were in the range of 0.3 to 11 μmol/L at a signal-to noise ratio of 3. The calibration curves showed correlation coefficients between 0.991 and 0.999 at 10, 20, 50, and 100 μmol/L.

Metabolome Analysis of *B. subtilis*. The proposed CE−MS methods were applied to the analysis of metabolites ranging from 70 to 1027 m/z values in *B. subtilis* extracts. Because a large number of metabolites are present at different concentrations in *B. subtilis*, it was necessary to limit the MS scan range to a window of 30 m/z to maximize detection sensitivity. This narrow m/z scanning technique allowed a severalfold increase

in sensitivity and detection of a large number of metabolites. To cover the necessary mass range, each sample was analyzed successively 33 times using an automatic injection sequence, while varying the m/z detection range between 70 and 1027 in both cation and anion modes. Figure 3 shows the results for cations extracted from exponentially growing *B. subtili*s cells ($T_{-0.5}$) for a 101−150 m/z value range as obtained by this method. The peak contents were identified by comparing their molecular weights and migration times with metabolite standards. Over the range of 101−150 m/z, and among the corresponding 45 peaks we could identify several metabolites such as GABA, Ser, Pro, and Val. Quantification was performed by comparing their peak areas against a calibration curves generated using internal standardization-techniques. For unidentified compounds, the relative migration times of all peaks were calculated by normalization with that of methionine sulfone (internal standard) and compounds were designated systematically (e.g., UC73, 0.4799, where UC73 means Uncharacterized Cation of protonated molecular weight 73, and 0.4799 relative migration time). Peaks, which were clearly recognized as isotopic molecular ions, were excluded. Normally, fragmented ions formed during MS analysis should be removed, but in the present CE−MS method very few fragmentations were observed.[15]

Although complete analysis took over 16 h, the whole procedure is highly automated and could reveal up to 1053 cationic metabolites including 70 identified and 37 other catalogued ions from exponentially growing *B. subtili*s cells (Supporting Information Table 2). In addition, the anionic CE−MS method yielded 637 anions including 78 important metabolites involved in glycolysis, the TCA cycle, and pentose phosphate pathways (Supporting Information Figure 1 and Supporting Information Table 3). Finally, several nucleotides and CoA compounds were analyzed by pressure-assisted CE−MS system (data not shown).[20] Overall, a total of 1692 metabolites, including 150 identified and 83 assigned (see below) compounds from exponentially growing *B. subtili*s cell extracts, were determined using the three CE−MS methods. Table 1 shows the corresponding data. The sensitivities of these methods were extremely high, revealing the presence of as little as 40 zepto moles of adenine and 350 atto moles of Glu per cell. The relative standard deviations (% RSD) ($n = 5$) for metabolite quantification for the whole procedure, including metabolite extraction through CE−MS analysis for 63 identified metabolites, were generally between 2% and 40% for peak areas, except for the smallest peaks where the % RSD was larger. The % RSD for identified metabolites were better than 2.7% for relative migration times. These results indicate that relative migration times can help to define unidentified peaks.

Identification of Unknown Metabolites. Because most cellular metabolites are not commercially available, identification of unknown peaks remains a problem. We thus developed a procedure to characterize unidentified peaks by combining CE−MS results together with bioinformatics analysis. First, from the measured molecular weight and the ^{13}C contributions, the number of carbons was estimated, and the chemical formula was obtained. Subsequently, candidates were selected using the LIGAND database that contains information about known metabolites obtained from various metabolic studies. Finally, the compound identity was predicted by considering its charge, electrophoretic mobility[24] and isotopic contributions. For example, since the ^{13}C contribution of UA105, 1.068 was measured as 3.1% by CE−MS, the number of carbon was

Table 1. Quantification of *B. subtilis* 168 Metabolites at $T_{-0.5}$ Phase and Overall Reproducibility from Metabolite Extraction through CE−MS Analysis

		RSD ($n = 5$) (%)	
compd	mole/cell (amol)[a]	peak area	relative migration time
Gly	5.8	20	1.5
L-β-Ala	0.14	28	2.7
L-Ala	24	13	1.3
GABA	0.12	27	1.7
L-Ser	4.5	23	0.83
L-Pro	3.8	3.0	0.48
L-Val	4.1	19	0.83
L-Homoserine	2.1	2.0	0.84
L-Thr	13	6.3	0.33
Creatine	2.5	30	1.4
L-Ile	2.2	24	0.61
L-Leu	6.3	21	0.74
L-Hydroxyproline	0.17	29	0.13
L-Ornitine	0.27	60	2.1
L-Asn	0.43	57	1.3
L-Asp	11	11	0.2
Adenine	0.04	63	1.8
Tyramine	0.37	25	1.6
Spermidine	0.12	53	2.9
L-Lys	0.12	33	2.0
L-Gln	190	8.4	0.49
L-Glu	350	4.0	0.47
L-Met	1.4	15	0.51
L-His	0.32	16	2.0
L-Phe	1.2	44	0.37
L-Arg	1.7	32	2.1
L-Citrulline	1.6	8.5	0.55
Tyramine	0.3	50	0.32
L-Carnosine	1.1	50	2.2
Cytidine	0.12	26	0.91
Adenosine	0.06	55	0.84
Pyruvate	3.2	33	0.28
Lactate	23	22	0.18
Fumarate	0.73	25	0.44
Succinate	3.2	6.9	0.31
Malate	0.16	16	0.32
2-Oxoglutarate	1.2	24	0.30
Phosphoenol pyruvate	1.7	30	0.31
Dihydroxyacetone phosphate	2.5	26	0.07
Glycerol 3-phosphate	3.1	22	0.06
3-Phosphoglycerate	9.1	16	0.20
Citrate	0.36	27	0.69
Erythrose 4-phosphate	1.1	12	2.5
Ribulose 5-phosphate	1.7	30	0.10
Ribose 5-phosphate	0.45	25	0.32
Glucose 1-phosphate	1.1	29	0.14
Fructose 6-phosphate	2.6	38	0.17
Glucose 6-phosphate	1.4	49	0.18
6-Phosphogluconate	0.24	28	0.18
Fructose 1,6-diphosphate	1.9	51	0.18
CMP	0.29	23	0.93
AMP	1.3	47	0.78
GMP	0.14	42	0.85
CDP	0.07	52	0.89
ADP	0.72	49	0.43
GDP	0.05	55	0.93
CTP	0.15	37	0.89
ATP	0.83	36	0.61
GTP	0.12	49	0.63
NAD	4.5	8.9	0.72
NADP	0.26	23	0.64
NADPH	0.06	53	0.72
Acetyl CoA	0.67	36	0.59

[a] The quantity of each metabolite per cell was calculated using the number of cells per ml culture, which was determined as 1.0×10^8 by colony forming unit (CFU) on LB plates.
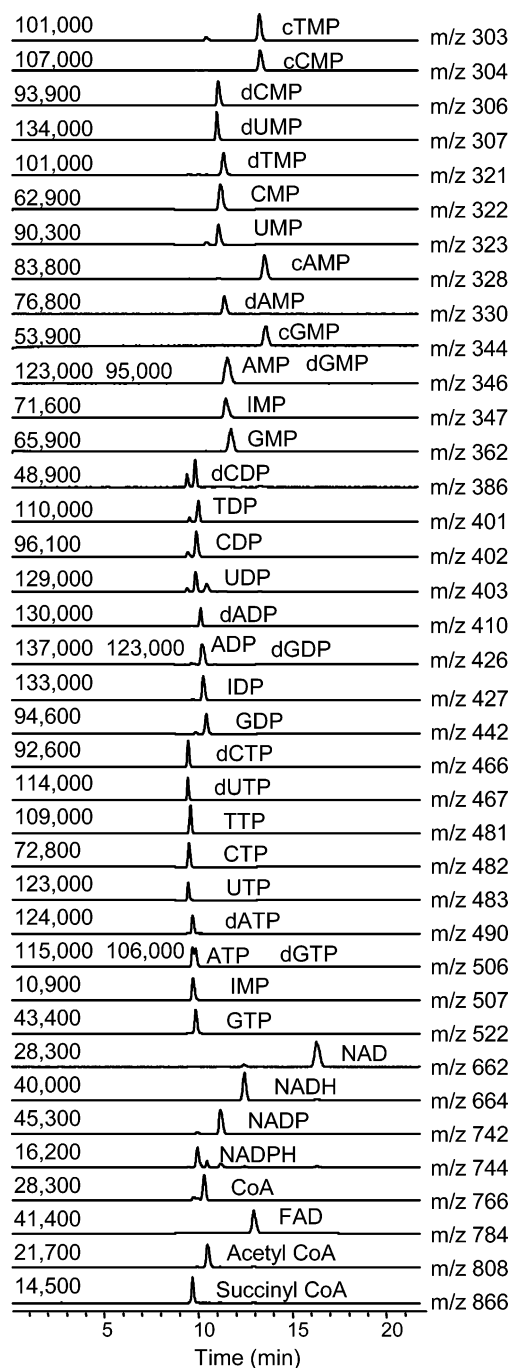
Figure 2. Determination of a standard mixture (100 μM each) of nucleotides and CoA compounds by pressure assisted CE−MS. Experimental conditions are described in the Experimental Section. The numbers in the upper left corner are the abundances associated with the tallest peak in the electropherogram, for each *m/z*.

estimated at three.[25] We then searched for three-carbon anions in the COMPOUND section in the LIGAND database, and the result yielded $C_3H_6O_2S$ (methylthioacetate) and $C_3H_6O_4$ (glycerate) as matching candidates. Because methylthioacetate contains sulfur, it should display 4% of ^{34}S isotopic contribution.[25] However, no isotopic ion of ^{34}S was detected by CE−MS. We, therefore, excluded $C_3H_6O_2S$ (methylthioacetate) and concluded that the unknown compound was glycerate. Validation of this identity assignment technique was experimentally tested with CE−MS to analyze 20 predicted com-
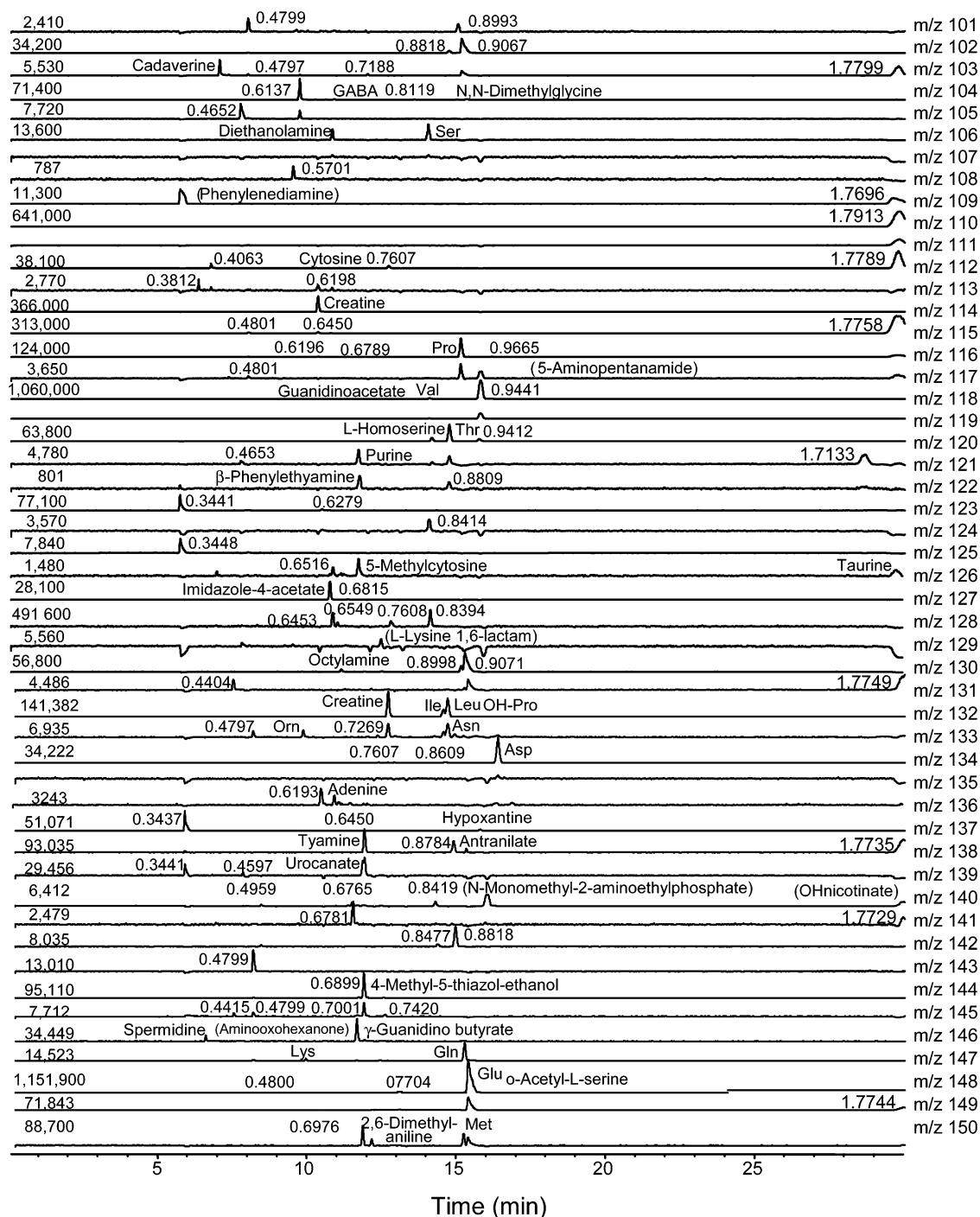
Figure 3. Selected ion electropherograms for cationic metabolites at $T_{-0.5}$ of *B. subtilis* 168 in the range of 101 to 150 *m/z*. Analytical conditions are described in the Experimental Section. The numbers in the upper left corner of each trace are the abundances associated with the tallest peak in the electropherogram, for each *m/z* and the numbers on top of peaks are relative migration times normalized with methionine sulfone (IS).

pounds including glycerate, tiglate, hypoxanthine and homocarnosine. Among these, 16 predictions were shown to be accurate (80%). When this identification procedure was applied to all other unknown peaks, an additional 60 compounds were identified and 23 new chemical formulas were derived.

Changes in Metabolites during Sporulation. Nutritional limitations or increase in cell density leads *B. subtilis* to produce a dormant, environmentally resistant spore.[26] This phenomenon is universally accepted as a basic model of bacterial differentiation. The complex morphological changes that occur

during sporulation are thought to be highly controlled by metabolic networks.[27] However, no comprehensive metabolite profiling approach has been used to demonstrate alterations in large-scale metabolites. We thus took advantage of the above-described CE−MS methods for metabolite profiling in *B. subtilis* cells at different time points before and during spore formation stages The measured levels of both cationic and anionic metabolites at $T_{-0.5}$, T_0, and T_2 were normalized (Supporting Information Table 4) and a number of uncharacterized compounds such as UC190, 0.9287 (estimated to be
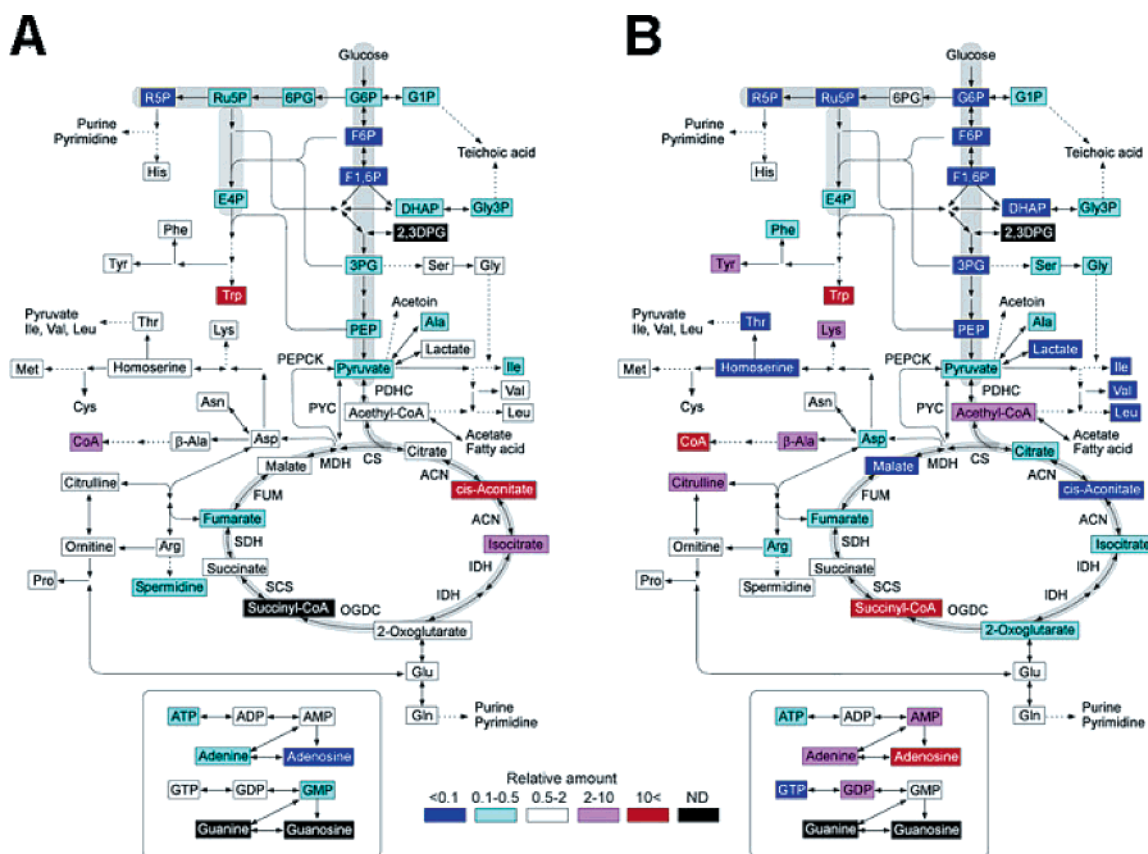
Figure 4. Metabolic profiling upon onset of sporulation, based on the simultaneous analysis of charged metabolites by CE−MS. (A) Changes in metabolite levels during the late logarithmic growth phase (T_0 versus $T_{-0.5}$). (B) Changes in metabolite levels in the early stage of sporulation (T_2 versus $T_{-0.5}$). Magenta and red boxes indicate metabolites whose levels increased 2−10-fold and more than 10-fold, respectively. Light blue and indigo boxes indicate the metabolites whose level was decreased to 0.1−0.5 and less than 0.1 of the original level, respectively. White boxes represent the metabolites whose levels remain approximately the same. Black boxes with white lettering indicate the undetected metabolites. The *B. subtilis* metabolic map was constructed based on ARM database (see http://www.metabolome.jp/).

L-homocitrulline using our compound identification approach), UC218, 0.8125 (β-alanyl-L-lysine) and UA289, 1.123 (sedoheptulose 7-phosphate) were found to increase or decrease dramatically during sporulation. Although a direct relationship between these compounds and sporulation has not been described, our results suggest a potential functional link.

B. subtilis cells undergo sporulation under conditions of glucose deprivation.[28] Interestingly, most metabolites in the glycolytic, pentose phosphate, and TCA pathways were markedly decreased in the early stage of sporulation (Figure 4). In particular, the level of F1,6P rapidly dropped more than 100-fold during sporulation. As F1,6P is a key factor in catabolite repression mediated by the transcriptional factors CcpA and CcpC,[27] it is possible that the decrease in F1,6P results in suppression of catabolite control and a subsequent expression of catabolic genes involved in sporulation.

Sonenshein previously showed that all inactivating mutations in the *B. subtilis* TCA cycle genes cause a defect in sporulation, thus suggesting that activation of the TCA cycle is indispensable for sporulation.[27] In our experiments, both *cis*-aconitate and isocitrate, intermediates in the TCA cycle, were shown to accumulate at T_0 (Figure 4A). Subsequently, the concentration of these metabolites, malate, and 2-oxoglutarate decreased, whereas acetyl CoA and succinyl CoA increased at T_2 (Figure 4B). This finding is in good agreement with previously reported studies regarding changes in enzyme and metabolite levels in

the TCA pathway[29] demonstrating the power of our CE−MS methods to monitor these metabolic responses simultaneously.

Transcriptional alterations of gene expression during sporulation have been previously measured using DNA array techniques.[30,31] The expression of most genes involved in these metabolic pathways were found to decrease during sporulation. On the other hand, the level of several metabolites such as *cis*-aconitate, isocitrate, CoA, acetyl CoA, succinyl CoA, Lys, and β-Ala were found to increase in our study. These results suggest that the metabolic consequences of gene expression changes cannot always be correctly predicted from transcriptome analysis most likely because metabolism may be regulated at other levels such as posttranscriptional control and modification of enzyme activity. Further metabolome research will thus be necessary to reveal these complex biological phenomena more precisely.

Conclusion

The CE−MS techniques we described enabled the comprehensive, direct, and sensitive analysis of charged species, and revealed the presence of 1692 compounds in *B. subtilis* cells including 150 that could be identified. Because less than 10% of those compounds could be readily identified, alternative methods using CE−MS are required and are now being developed. CE−TOFMS might help to determine the chemical

formula of unknown compounds and CE−MS/MS to provide structural information.

The proposed CE−MS methods enable the global determination of charged species, so that they can be used as universal tools for quantitative metabolome analysis. Metabolome data, along with transcriptome and proteome expression results, will provide important new information to elucidate the biological functions of uncharacterized processes. Meanwhile, this approach should be applicable to a wide range of fields for screening of metabolic abnormalities or as diagnosis marker in physiological samples, for analysis of drug metabolism and pharmacokinetics, and, of course, for global analysis of ionic components in biological matrixes.

Supporting Information Available: Supporting Information Table 1 (http://www.ttck.keio.ac.jp/IAB/microbiology/metabolome/sup/stab1.pdf). Supporting Information Table 2 (http://www.ttck.keio.ac.jp/IAB/microbiology/metabolome/sup/stab2.pdf). Supporting Information Table 3 (http://www.ttck.keio.ac.jp/IAB/microbiology/metabolome/sup/stab3.pdf). Supporting Information Table 4 (http://www.ttck.keio.ac.jp/IAB/microbiology/metabolome/sup/stab4.pdf). Supporting Information Figure 1 (http://www.ttck.keio.ac.jp/IAB/microbiology/metabolome/sup/sFigure 1.pdf). This material is available free of charge via the Internet at http://pubs.acs.org.

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PR034020M