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Communications

Phosphate-containing Metabolite Enrichment with TiO_2 Micro-tips

Hyun Ju Yoo[†,‡,*] **and Kristina Hâkansson**[‡]

[†]*Metabolomics Core Lab, Biomedical Research Center, Asan Institute of Life Sciences, Seoul 138-736, Korea*
[*]*E-mail: yoohyunju@amc.seoul.kr*
[‡]*Department of Chemistry, University of Michigan, Ann Arbor, MI 48105, USA*
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Metabolites are essential to living cells. Metabolite levels represent integrative information of cellular function, and define the phenotype of a cell or tissue in response to genetic or environmental changes. mRNA levels do not always correlate with protein levels, and translated protein may or may not be enzymatically active. Therefore, changes observed in the transcriptome or proteome do not always correspond to phenotypic alterations. Thus measurement of the metabolites synthesized by a biological system is very important to assess genetic function and also aids the understanding of the proteome.[1] Metabolomics is the study of the full arsenal of endogenous small molecules in biological systems, with the ultimate goal of identifying biomarkers and enzymatic pathways related to human disease. The size of the metabolome is extraordinary.[2] In addition, metabolites have wide variations in chemical (*e.g.*, molecular weight, polarity, acidity) and physical (*e.g.*, volatility) properties, which make metabolome analysis highly challenging. Thus, targeted metabolite analysis would be valuable to selectively detect a few members of compound classes within a cell or tissue. Enrichment of specific subsets of small molecules from biological systems would greatly benefit targeted approaches.

Mass spectrometry coupled with liquid chromatography (LC/MS) is preferred in metabolomics, due to the reduction of sample complexity and additional information for metabolite identification. However, polar metabolites are poorly retained with commonly used C18 columns. Incomplete separation of these metabolites may cause ion suppression even in LC/MS due to co-elution. Thus, metabolite enrichment can be beneficial to metabolomics due to the removal of co-eluted unwanted analytes. Recently, chemoselective probes based on functional groups have been used to enrich amine, acid, aldehyde, and thiol-containing compounds among polar metabolites.[3,4] However, this approach alters metabolite structure as well as tandem mass spectra. Thus, current metabolite databases cannot be used.

TiO_2 has been applied to enrich phosphopeptides from complex biological matrices.[5,6] To the best of our knowledge, this is the first study in which TiO_2 was used to enrich phosphate-containing metabolites. Phosphate-containing metabolites are important in life activities such as carbon metabolism and energy conversion. Here, we present enrichment of phosphate-containing metabolites with TiO_2 micro-tips.

Standard metabolite mixture including 7 phosphate-containing metabolites and 8 other polar metabolites was used to explore signal enhancement after using TiO_2 tips. Concentrations of non phosphate-containing metabolites are much higher (~2-100 times) than those of phosphate-containing metabolites to test signal enhancement. Phosphate-

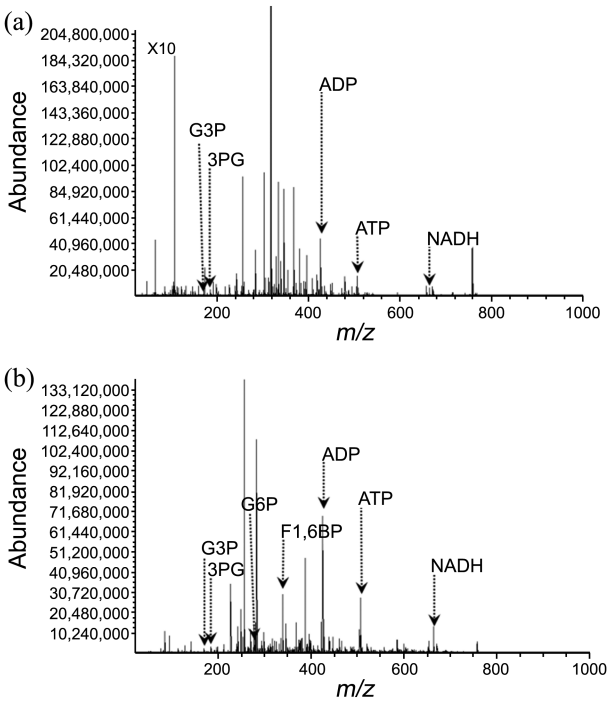

Figure 1. Mass spectra of standard metabolite mixture (Table S1) before (a) and after enrichment (b). This mixture contains glycerol-3-phosphate (G3P), 3-phosphoglyceric acid (3PG), glucose-6-phosphate (G6P), fructose-1,6-bisphosphate (F1,6BP), ADP, ATP, and NADH. "× 10" indicates that y-axis is zoomed in by 10 times. Standard mixture composition is shown in Table S1.

Table 1. Signal enhancement (%) of phosphate-containing metabolites from three separate experiments using standard metabolite mixture (Table S1). Signal enhancement was calculated by (signal intensity after TiO_2 treatment/signal intensity before TiO_2 treatment) × 100. RSD means relative standard deviation. For G6P and F1,6BP, signal intensity after treatment was used for calculation due to the lack of signal before treatment

Metabolites	Signal enhancement (%)			Average	RSD (%)
Glycerol-3-phosphate	33	38	44	38	15
3-Phosphoglyceric acid	66	94	95	85	19
Glucose-6-phosphate	*2,885,087	*2,479,701	*3,299,893	*2,888,227	*14
Fructose-1,6-diphosphate	*29,974,670	*36,533,976	*39,101,096	*35,203,247	*13
ADP	156	173	175	168	6
ATP	176	228	244	216	16
NADH	203	109	133	148	33

containing metabolites were observed at low abundances in mass spectra before enrichment. On the other hand, metabolites which do not contain phosphate groups were dominantly observed. However, signal magnitudes of the most phosphate-containing metabolites increased after enrichment due to the reduced ion suppression from removal of other abundant metabolites shown in Figure 1 and table S2. Glucose-6-phosphate and fructose-1,6-bisphosphate were not detected before enrichment, but were very abundantly observed after enrichment. ADP and ATP showed increased signal abundance after enrichment. NADH also showed increased signal abundance after TiO_2 micro-tips enrichment.

Signal enhancement generally increased with the number of phosphate groups of phosphate-containing metabolites. Signal abundances of glycerol-3-phosphate and 3-phosphoglyceric acid containing one phosphate were not increased after enrichment in Figure 1. However, 3-phosphoglyceric acid in different metabolic composition was observed only after TiO_2 treatment (Figure S2). Glucose-6-phosphate was detected only after enrichment shown in Figure 1, but its signal abundance was decreased in different metabolic composition (Figure S2). Signal abundances of fructose-1,6-bisphosphate, ADP, and ATP containing two or three phosphate groups were increased significantly in various metabolic composition. NADH also has two phosphate groups, but its signal enhancement was not comparable to fructose-1,6-bisphosphate, ADP and ATP. The two phosphate groups in NADH are located at the center of the molecule and their interaction with TiO_2 may be hindered due to steric effect. So the less accessibility of phosphate groups to TiO_2 might cause rather low signal enhancement of NADH comparing to fructose-1,6-bisphosphate, ADP and ATP.[7] On the other hand, ADP, ATP, and fructose-1,6-bisphosphate have terminal phosphates which should be able to freely interact with TiO_2. The enrichment performance of phosphate-containing metabolites with terminal phosphate groups resulted in reproducible enrichment.

Metabolites not containing phosphate groups were observed very abundantly before TiO_2 treatment, due to their higher amount or better ionization efficiencies. These metabolites should cause ion suppression and hinder detection of phosphate-containing metabolites. However, their signal intensities were significantly reduced after TiO_2 treatment. Most of the non phosphate-containing metabolites did not bind to TiO_2 and remained in loading solution (Figure S2).

In conclusion, signal abundances of phosphate-containing metabolites increased after enrichment using TiO_2 micro-tips. Enrichment performance was affected by the number and the location of phosphates as well as the different ionic environment caused by different metabolic composition of sample solution. Metabolite enrichment is expected to maximize the number of observed phosphate-containing metabolites from complex biological systems and to expand the dynamic range for detection of these metabolites.

Supporting Information. Supporting information including brief method and result is available.

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