（続紙 1 ）

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論文題目	Fission yeast and human blood metabolomic comparison with focus on age related compounds 分裂酵母とヒト血液のメタボローム比較		

（論文内容の要旨）

Metabolomics, a modern branch of chemical biology, provides qualitative and quantitative information about the metabolic states of organisms or cells at the molecular level. It aims to profile small molecules present in living organisms, and is now recognized as an important tool for studying metabolic regulation in a systematic way, together with transcriptomic and proteomic analyses. Here the degree candidate reports non-targeted, metabolomic analyses of human blood, using liquid chromatography-mass spectrometry (LC-MS).

The candidate compared the blood metabolome to the previously reported metabolome of the fission yeast, *Schizosaccharomyces pombe* (*S. pombe*). The two metabolomic datasets were highly similar: 101 out of 133 compounds identified in human blood (76%) were also present in *S. pombe*, and 45 out of 57 compounds enriched in red blood cells (RBCs) (79%), were also present in the fission yeast. The most abundant metabolites were ATP, glutathione, and glutamine. Apart from these three, the next most abundant metabolites were also involved in energy metabolism, anti-oxidation, and amino acid metabolism such as NAD^+, UDP-glucose, ergothioneine, and ophthalmic acid. The degree candidate identified fourteen new blood compounds: citramalate, GDP-glucose, trimethyl-histidine, trimethyl-phenylalanine, trimethyl-tryptophan, trimethyl-tyrosine, UDP-acetyl-glucosamine, UDP-glucuronate, dimethyl-lysine, glutamate methyl ester, N-acetyl-(iso)leucine, N-acetyl-glutamate, N2-acetyl-lysine, and N6-acetyl-lysine. Eight of these were enriched in RBCs. Of the fourteen newly identified blood metabolites, ten were also detected in *S. pombe*; and ten of the fourteen metabolites were methylated or acetylated amino acids. For the metabolites in common between RBCs and fission yeast, it may be possible to investigate their physiological roles using yeast genetics.

Comparison of blood metabolome profiles from 30 human volunteers revealed that about a third of the detected compounds were variable between individuals. Furthermore, in comparing profiles of young and elderly volunteers, the candidate found different levels of six unidentified peaks, three of which are possibly trimethylated compounds between the age groups. Among identified compounds, the candidate found different levels of sugar derivatives, methylated nucleotides, vitamins, and antioxidants that were differentially present between the age groups. The candidate also performed short-term (58 hours) fasting experiments in human volunteers and showed that one antioxidant increased during the fasting. This antioxidant, reported as an oxidative stress marker, as well as a compound showing different abundance between the age groups, is an interesting metabolite for further studies.

Highly abundant metabolites conserved between red blood cells and *S. pombe* exist, which seem to be implicated in structural maintenance, energy production and protection against various stresses. Distinct metabolomic patterns from the individual human blood samples indicate that certain compounds may be specific to age or other conditions.

（続紙 2 ）

（論文審査の結果の要旨）

Red blood cells (RBCs) deliver oxygen to tissues and remove carbon dioxide. Numerous blood metabolomic analyses have been previously reported, mostly of serum and plasma. On the other hand, metabolomics of whole blood or RBCs have not been well investigated except for a handful of reports. The degree candidate performed extensive non-targeted, metabolomic studies of human blood, mainly RBCs, by using liquid chromatography-mass spectrometry. Throughout the thesis, experimental procedures were meticulously carried out, as shown by the fact that the sample preparations were well controlled and the data were quantitatively analyzed.

On top of the massive data, the candidate compared the blood metabolome to that of vegetative cells of the fission yeast, which has been used as a model organism to study comprehensive metabolic patterns under defined genetic and physiological conditions, and showed significant conservation in metabolites between these evolutionarily distant cells. The high similarity of these two metabolomes raises the possibility that the fission yeast may be a useful model to understand the role of some metabolites, such as small anti-oxidants (ophthalmic acid, ergothioneine, and glutathione), which are enzymatically synthesized in the fission yeast. The conservation also motivates scientists of the relevant fields to address physiological roles of ten novel compounds discovered in human blood (UDP-acetyl-glucosamine, citramalate, dimethyl-lysine, GDP glucose, trimethyl-histidine, N-acetyl-glutamate, N-acetyl isoleucine, N2-acetyl-lysine, N6-aceltyl-glutamate, and glutamate methyl ester) by taking advantage of the power of the fission yeast genetics.

The candidate further extended his human study to explore the variability in blood metabolites between individuals, whether any of the identified compounds showed different levels and distributions between young and old persons, and finally whether levels of any compounds changed during short-term fasting. Thus this thesis reveals individual variability and age-related differences of some metabolites in blood, which would provide new insights for human biology.

As such, the doctoral thesis includes a number of novel and interesting findings about metabolites of human RBCs and fission yeast, which potentially make important contributions to life science. The thesis demonstrates a wide range of advanced knowledge and research competency of the candidate. In conclusion, the thesis satisfies the thesis requirement for the degree of Doctor of Philosophy in Life Sciences.

The Ph.D. thesis defense was held on July 31, 2014. In the thesis defense, the doctoral degree candidate gave an oral presentation of his own research, and then the examiners and the expert member interviewed and questioned the degree candidate about the doctoral thesis and the presentation. After the defense, the judgments of examiners and the expert member were discussed in deliberation and it was concluded that the candidate passed the examination.

論文内容の要旨及び審査の結果の要旨は、本学学術情報リポジトリに掲載し、公表とする。特許申請、雑誌掲載等の関係により、学位授与後即日公表することに支障がある場合は、以下に公表可能とする日付を記入すること。**（ただし、学位規則第８条の規定により、猶予期間は学位授与日から３ヶ月以内を記入すること。）**

要旨公開可能日：平成 26 年 12 月 23 日