Study on the analytical approach of anthocyanins on intestinal absorption by MALDI-MS imaging technique

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Title：Study on the analytical approach of anthocyanins on intestinal absorption by MALDI-MS imaging technique
(MALDI-MS イメージング法を用いたアントシアニン類の腸管吸収動態解明のための分析化学的研究)

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Thesis Summary

Most studies on the physiological effects of food compounds such as anthocyanins have focused on evaluating the bioactivity and their mechanisms *in vitro* and *in vivo*. However, there are few reports on food compounds' bioavailability such as intestinal absorption, blood circulation, and tissue-accumulation in target organs. One of the factors would be considered as the lack of an analytical assay capable of accurate evaluation of their bioavailability. Thus, the present study aimed to clarify the bioavailability such as intestinal absorption, metabolism, and tissue accumulation of food compounds like anthocyanins using *in situ* MALDI-MS imaging technique. The aim of the present study was then to establish a novel MALDI-MS imaging system to visually and quantitatively assess the tissue accumulation of food compounds.

Firstly, *in situ* MALDI-MS imaging method that can visualize intestinal absorption/metabolism dynamics of food compounds was used to evaluate the absorption process of anthocyanins, including an acylated cyanidin (cyanidin-3-(2"-xylose-6"-feruloyl-glucose-galactoside, Cy3XFGG), in rat intestinal tissue. As a result, Cy3XFGG was clearly visualized in rat jejunum tissue sections with 60-min of transport. The present results also exhibited that the MS image and intensity of Cy3XFGG in the jejunum tissue sections were diminished by inhibition of organic anion-transporting polypeptide (OATP) 2B1. This indicates that Cy3XFGG can be absorbed into the intestinal membrane via the OATP 2B1 in its intact form. Moreover, in this study, conjugated Cy3XFGG (glucuronidated, sulfated, methylated, and their combination forms) was not detected in the Cy3XFGG-transported intestine tissue sections, while Cy as a degraded form of Cy3XFGG was slightly visualized in Cy3XFGG-transported tissue sections. Thus, it was demonstrated for the first time that acylated anthocyanins can be incorporated and transported across the intestinal tissue in their intact form with relatively strong resistance against phase I/II metabolism.

Secondly, this study focused on establishing a novel quantification approach for MALDI-MS imaging by applying MS ionizable fluorescent compounds for nondestructive fluorescent monitoring of the sprayed matrix amount, and additives to improve the homogeneity of the matrix crystal. As a result, among fluorescent reagents (R6G, 7-methoxycoumarin-3-carboxylic acid, naphthalene, and 6-carboxyfluorescein) used in this study, R6G was added to 1,5-DAN solution as MS ionizable fluorescent reagent to compensate matrix spray amount and normalize MS intensity. Furthermore, *O*-DNB was used as a matrix additive to

form uniform matrix crystals on tissue sections. At the spraying condition using R6G in 1,5-DAN solution containing *O*-DNB, reproducibility (RSD = 3.1%) and linearity (0.5–75 pmol/mm^2, R^2 = 0.9972) of relative MS intensity of ferulic acid (FA) were significantly improved compared to those of conventional cycle-number-fixed spraying (40 cycles, RSD = 31.1%, R^2 = 0.9349). Then, tissue-accumulated FA was visually determined to be 3.5, 3.0, and 0.2 μmol/g tissue in rat kidneys at 15, 30, and 60 min after the oral administration (50 mg/kg), respectively. A linear correlation was observed between quantified accumulation levels by the present MALDI-MS imaging and those by LC–MS (R^2 = 0.9906, n = 3). Therefore, successful quantitative capability of MALDI-MS imaging of tissue-accumulated food compounds was achieved via the formation of fluorescence-assisted reproducible matrix crystals by R6G (the MS normalizing standard) in the 1,5-DAN solution containing *O*-DNB.

In conclusion, the present study has demonstrated that an intact form of acylated anthocyanin can be absorbed into the intestine via OATP 2B1 by *in situ* MALDI-MS imaging, and the MALDI-MS imaging system with fluorescence-assisted spraying method was established as visible and quantitative evaluation technique for the bioavailability of food compounds, such as intestinal absorption, metabolism, and tissue-accumulation. In the future, it is highly expected that the present qMALDI-MS imaging system will be a powerful analytical method to evaluate the bioavailability of functional compounds in the development of functional foods