Study on the analytical application of matrix-assisted laser desorption/ionization mass spectrometry-imaging technique for visualization of polyphenols

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（マトリックス支援レーザー脱離イオン化質量分析イメージング法を用いたポリフェノール検出のための分析化学的研究）

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Thesis Summary

Tea polyphenols, which contain monomeric and condensed catechins, have been consumed worldwide owing to potential health-benefits such as prevention of cardiovascular diseases, diabetes, and cancers. In order to evaluate bioavailability of polyphenols including their metabolism for elucidating the bioactive mechanism(s), analytical tools such as liquid chromatography-mass spectrometry (LC-MS), and matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) techniques have been developed and applied. Based on sensitivity, selectivity, and tolerance for impurity of MALDI-MS, its current application in imaging technique has been well established and extensively used to visualize drugs and food compounds, which could provide the spatial localization of the analytes in biological tissue without any staining or labelling preparations. However, application of MALDI-MS in detection of polyphenols is restricted due to lack of appropriate matrix reagents. Thus, the present study primarily aimed to developed suitable matrix for MALDI-MS detection of polyphenols. The MALDI-MS imaging technique using the developed matrix, then, was established for investigating intestinal absorption and metabolism of polyphenols.

In order to obtain MALDI-MS detection of polyphenols, a matrix screening based on a strategy for compelling subtraction of a proton from polyphenol molecule was performed. Epigallocatechin-3-*O*-gallate (EGCG) and theaflavin-3'-*O*-gallate (TF3'G), common bioactive polyphenols in green and black tea, respectively, were selected as the target polyphenols. It was demonstrated for the first time that a dihydropyridine photobase generator, nifedipine (5 mg/mL), successfully achieved the MS detection of EGCG and TF3'G compared to some common matrices. Of other dihydropyridines investigated, only nifedipine facilitated the MS detection of EGCG and TF3'G, indicating that the nitrosopheny pyridine photobase derived from nifedipine under ultraviolet (UV)-irradiation at 355 nm is crucial for deprotonation of polyphenols in negative MALDI-MS. The reduced MS detection of 5-*O*-methylnaringenin among mono-methylated naringenin derivatives indicated that nifedipine might preferably subtract a proton from the 5-position OH group in the A ring of the flavonoid skeleton. The enhanced MS detection by nifedipine was extensively observed for a variety of polyphenols including flavonols, flavones, flavanones, flavonones, chalcone, stilbenoid, and phenolic acid, suggesting that nifedipine must be a novel MALDI matrix reagent for the analysis of polyphenols.

According to the finding of successful MALDI-MS detection of polyphenols by the novel matrix, nifedipine, an *in situ* MALDI-MS imaging technique using nifedipine was establish to elucidate absorption and metabolism processes of polyphenols in intestine. An absorbable epicatechin-3-*O*-gallate (ECG) and non-absorbable TF3'G were used as representative bioactive polyphenols in intestinal transport experiments (each at 50 μmol/L for 60 min) using Sprague-Dawely rat jejunum membrane mounted onto an Ussing chamber system. Interestingly, the nifedipine-aided MALDI-MS imaging with a matrix additive, phytic acid, firstly visualized that the non-absorbable TF3'G specifically located only in the apical region of the intestinal membrane, whereas absorbable ECG distributed in the whole membrane. The visualized regions of TF3'G and ECG were reduced in phloretin and estrone-3-sulfate (inhibitors of monocarboxylic acid transporter, MCT, and organic anion transporting polypeptides, OATP, respectively), suggesting that the both polyphenols could incorporate into the intestinal membrane via MCT and OATP transport routes. The visualized regions of TF3'G and ECG were expanded in cyclosporine A, a non-specific ATP-binding cassette (ABC) efflux transporter inhibitor, indicating that both polyphenols were efflux back to the apical compartment via ABC transporters. Furthermore, the present MALDI-MS imaging simultaneously visualized phase II metabolites of ECG such as methylation, sulfation, and their combination conjugates, but not those of TF3'G, which indicated that TF3'G was stable against phase II metabolism in the intestinal membrane.

In conclusion, the present study demonstrated that nifedipine, could act as a novel matrix reagent for negative MALDI-MS of polyphenols through the generated photobase-induced proton subtraction. The nifedipine/phytic acid-aided MALDI-MS imaging technique, in combination with the inhibitor-aided intestinal transport experiments must be a novel and powerful analytical strategy for evaluation of intestinal processes for absorption and metabolism of polyphenols.