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Synopsis of Thesis

Title: Simultaneous profiling of polar lipids by supercritical fluid chromatography/tandem mass spectrometry with derivatization for lipidomics (誘導体化法適用超臨界流体クロマトグラフィー/質量分析による極性脂質の一斉プロファイリング)

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Chapter 1: General introduction

Lipidomics is a systems-level analysis of lipid species, their abundance, biological activities, and subcellular localization. Many studies have reported the aberrant metabolism of polar lipids in human diseases. To characterize the altered polar lipids: phospholipids (PLs), lysophospholipids (LPLs), and sphingolipids (SLs), an effective analytical method is needed. In particular, comprehensive analysis of polar lipids is hindered by the peak tailing and low detection sensitivity of acidic PLs: phosphatidylserine (PS) and phosphatidic acid (PA). Peak tailing must be advanced because it can decrease the detection sensitivity of compounds. To solve these issues, adjusted mobile phase or EDTA-prewashed column were used previously. However, the modification of chromatographic conditions was insufficient due to the low repeatability to treat and the needs to use the non-metal system.

In this study, the derivatization was applied for the modification of characteristics in polar lipids to advance the peak shape and sensitivity. The main factor of peak tailing was found as the metal-affinity of polar head groups. To block several functional groups in polar lipids, various derivatization can be used. In the selection of derivatizations, acylation and esterification were excluded due to their needs of additional extraction step and the removal of water. It can cause time-consuming and handy-error. Finally, at first, TMS silylation was used due to its simplicity and wide applicability to various functional groups. Upon silylation, the analysis of several polar lipids improved, but it was insufficient to derivatize phosphate group that has high metal-affinity to cause peak tailing. Secondly, TMSD methylation was used to derivatize phosphate for the advanced analysis of PLs, LPLs, and SLs. SFC/MS/MS was effective to analyze derivatized polar lipids with the increased hydrophobicity.

Chapter 2: Development of a polar lipid profiling by SFC/MS/MS with silylation

Of various derivatizations, TMS silylation which is highly reactive, simple and widely applicable to various functional groups containing reactive hydrogen (e.g. –OH, -COOH, -NH2) was used to advance the polar lipid profiling by SFC/MS/MS. By silylation, the metal-affinity of acidic PLs was suppressed by blocking the functional groups in polar head groups. In addition, the silylation increased the hydrophobicity of polar lipids so that their separation was also advanced in SFC using non-polar CO2. Especially, hydroxyl groups were silylated mainly, hence, the analysis of phosphatidylinositol (PI) was improved well. However, this method was insufficient to derivatize phosphate group that has high metal-affinity and to improve PS analysis. Thus, more effective method should be selected for polar lipid profiling.

Chapter 3: Development of a polar lipid profiling by SFC/MS/MS with methylation

Methylation is an effective method to derivatize the phosphate group for polar lipid profiling. Diazomethane is a conventional reagent for the methylation, but this reagent needs care due to its explosive and carcinogenic. Thus, in this study, trimethylsilyldiazomethane (TMSD) which is relatively safe, stable, and easy to treat, was used. Finally, 6 PLs, 6 LPLs, and 4 SLs were methylated by the optimized conditions, and the comprehensive profiling of polar lipids was achieved with sharp peaks. Advanced peak shapes provided the increased detection sensitivity. Detail and reliable quantification of various polar lipid species in mouse liver was obtained successfully.

Chapter 4: Conclusions

In this study, two derivatizations (silylation & methylation) were used for the advanced polar lipid profiling by SFC/MS/MS. Of two methods, methylation was better than silylation for the comprehensive profiling of polar lipids. However, in the analysis of PI, silylation was better than methylation. Finally, according to the purpose, silylation and methylation are expected to be useful for the polar lipid profiling. Simultaneous analysis is helpful for the detail characterization of various polar lipids. For the practical uses, high-throughput analysis is also effective when the samples are too many. In lipidomics, this method will be useful for the detail phenotype of polar lipids in the study of biomarker and drug development.

List of publications:

1. Lee J. W., Yamamoto T., Uchikata T., Matsubara A., Fukusaki E., Bamba T., Development of a polar lipid profiling method by supercritical fluid chromatography/mass spectrometry, Journal of Separation Science, 34, 3553-3560 (2011).
2. Lee J. W., Nishiumi S., Yoshida M., Fukusaki E., Bamba T., Simultaneous profiling of polar lipids by supercritical fluid chromatography/tandem mass spectrometry with methylation, Journal of Chromatography A, 1279, 98-107 (2013).

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論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

本論文では、誘導体化法適用超臨界流体クロマトグラフィー/質量分析を用いて生体試料内に存在する微量の極性脂質を同時に、そして精密分析ができるシステムを開発することを目標とした。

第一章では緒論として、リピドミックスの研究分野の重要性と分析手法の開発状況、そして極性脂質分析の重要性について紹介した。特に、極性脂質は細胞膜構成および信号伝達に大きく関与する主要脂質成分として、疾患試料内の定性、定量分析に関する多くの研究報告がなされてきた。しかし、既存の分析手法では PS や PA のような酸性リン脂質がピークテーリングするため、生体試料内に微量しか存在しない PS や PA の精密分析は困難であった。本研究では、極性脂質のピークテーリングの原因となる金属親和性等をなくしピーク形状を改善することを目的として、適切な誘導体化法を適用した多様な極性脂質の精密一斉分析法の開発に取り組んだ。

第二章では、トリメチルシリル（TMS）誘導体化法の適用について検討した。様々な誘導体化法の中で極性脂質の極性部分に適切な方法を選択し、高い反応性と複数の官能基への適用が可能な TMS 法を使用した。TMS 試薬の中で TMSI を選択し、これを用いて 10 種類の極性脂質(PG、PI、PA、LPC、LPE、LPG、LPI、LPA、SM、SoIP)の誘導体化を試みた。その結果、それぞれの極性脂質の極性官能基が誘導体化され、ピーク形状が改善された。さらに、構築した分析法を生体試料(ヒツジ血漿)の分析に適用した結果、これまでの方法より 27 個の極性脂質をあらたに検出することができ、本分析法の有用性を証明した。

第三章では、16 種類の極性脂質(PC、PE、PS、PG、PI、PA、LPC、LPE、LPS、LPG、LPI、LPA、SM、CerIP、SoIP、SaIP)を対象としてジアゾメタンによるメチル誘導体化法の適用について検討した。TMSD 試薬を使用したメチル化は酸性リン脂質のピークテーリングの原因となるリン酸基に対しても適用可能であり、高い反応性を有し誘導体化操作も簡便である。ほとんどの極性脂質がその構造にリン酸基を有していることから多くの脂質のピーク形状の改善が見られ、特に PS と PA や SoIP や SaIP などのスフィンゴ脂質において大幅な感度向上が認められた。。さらに、当該手法をマウス肝臓サンプルに適用した結果、これまでの方法を用いた場合と比べて 84 個の極性脂質があらたに検出されたことから、TMSD メチル化法の有用性が証明された。

第四章では、以上の研究成果と意義をまとめ、今後の課題と展望について記述した。

以上のように、本論文は誘導体化法を用いて酸性リン脂質のような生体内微量の極性脂質のピーク形状を改善して、精密一斉分析が可能な手法を開発し、その実用性についても生体サンプルにおいて確認している。よって本論文は博士論文として価値あるものと認める。