機能情報解析部門

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◇研究目的

当部門では，和漢医薬に含まれる生体内物質の高分解能マススペクトルを収集・解析し，データベース化する。国内外の和漢医薬の化学成分を分析している研究者や研究グループとの間でマススペクトルを共有することを視野にいれて，物質同定に必要な情報学的ツール類の開発に重点を置いた研究をおこなう。マススペクトルのデータを共有することによって，質量分析によって検出された化学成分の同定が容易になるだけでなく，生薬の（微量成分を含む）化学組成と薬理活性との関係をより詳細に解析することが可能になることによって，薬理機構の解明に寄与するものと期待される。

◇研究概要

Ⅰ）薬理活性を有する代謝物質の高分解能マススペクトルの収集とデータベース化

和漢医薬学総合研究所でこれまでに測定された，植物に含まれる二次代謝物質のマススペクトルの収集とデジタル化をおこなってデータベース化をすすめる。また，本研究所で高分解能 IT-TOFMS を用いて新たに測定した生薬のマススペクトル MS^n を解析し，プロダクトイオンの化学構造情報化をおこなうことによって，生薬成分のマススペクトルの同定に必要な「化学構造とマススペクトル MS^n との関係」を解析・蓄積する。

Ⅱ）和漢医薬試料の LC-高分解能 MS^2 で測定したマススペクトルのデータベース化

生薬の抽出物を LC-高分解能 MS^2 で測定した，溶出時間分割マススペクトルを収集し，MassBank レコード形式でデータベース化する。これらのマススペクトルは，化合物を同定できたものだけでなく，同定できなかったマススペクトルも収集する。代謝物質を同定できなかったマススペクトルは，MS^2 を代謝物質の化学構造のタグとしてデータベース化する。薬用植物それぞれ，およびそれらの混合した生薬について，異なる抽出法，抽出条件，異なるブレンド法で作成した試料をマススペクトル MS^2 で測定したものを試料ごとに比較できるようにする。このような比較をとおして，試料の薬理効果と二次代謝物質の組成との関係を明らかにする。

◇原著論文

1) **Murai M., Sekiguchi K., Nishioka T., and Miyoshi H. : Characterization of Inhibitor Binding Site in Mitochondrial NADH-Ubiquinone Oxidoreductase by Photoaffinity Labeling using a Quinazoline-Type Inhibitor. Biochemistry, 48: 688-698, 2009.**

Abstract: The diverse inhibitors of bovine heart mitochondrial complex I (NADH-ubiquinone oxidoreductase) are believed to share a common large binding domain with partially overlapping sites, though it remains unclear how these binding sites relate to each other. To obtain new insight into the inhibitor binding domain in complex I, we synthesized a photoreactive azidoquinazoline {[(125)I]-6-azido-4-(4-iodophenethylamino)quinazoline, [(125)I]AzQ}, in which a photolabile azido group was introduced into the toxophoric quinazoline ring to allow specific cross-linking, and carried out a photoaffinity labeling study using bovine heart submitochondrial particles. Analysis of the photo-cross-linked proteins by peptide mass fingerprinting and immunoblotting revealed that [(125)I]AzQ specifically binds to the 49 kDa and ND1 subunits with a frequency of approximately 4:1. The cross-linking was completely blocked by excess amounts of other inhibitors such as acetogenin and fenpyroximate. Considerable cross-linking was also detected in the ADP/ATP carrier and 3-hydroxybutyrate dehydrogenase, though it was not associated with dysfunction of the two proteins. The partial proteolysis of the [(125)I]AzQ-labeled 49 kDa subunit by V8-protease and N-terminal sequencing of the resulting peptides revealed that the amino acid residue cross-linked by [(125)I]AzQ is within the sequence region Thr25-Glu143 (118 amino acids). Furthermore, examination of fragment patterns generated by exhaustive digestion of the [(125)I]AzQ-labeled 49 kDa subunit by V8-protease, lysylendopeptidase, or trypsin strongly suggested that the cross-linked residue is located within the region Asp41-Arg63 (23 amino acids). The present study has revealed, for the first time, the inhibitor binding site in complex I at the sub-subunit level.

2) **Asai N., Nishioka T., Takabayashi J., and Furuichi T.: Plant volatiles regulate the activities of Ca^{2+}- permeable channels and promote cytoplasmic calcium transients in Arabidopsis leaf cells. Plant Signaling Behavior, 4: 294-300, 2009.**

Abstract: A variety of plant species emit volatile compounds in response to mechanical stresses such as herbivore attack. Although these volatile compounds promote gene expression leading to antiherbivore responses, the underlying transduction mechanisms are largely unknown. While indirect evidence suggests that the cytoplasmic free Ca^{2+} concentration ($[Ca^{2+}]c$) plays a crucial role in the volatile-sensing mechanisms in plants, these roles have not been directly demonstrated. In the present study, we used Arabidopsis leaves expressing apoaequorin, a Ca^{2+}-sensitive luminescent protein, in combination with a luminometer, to monitor $[Ca^{2+}]c$ transients that occur in response to a variety of volatile compounds and to characterized the pharmacological properties of the increase in $[Ca^{2+}]c$. When leaves were exposed to volatiles, $[Ca^{2+}]c$ was transiently raised. The $[Ca^{2+}]c$ increases induced by acyclic compounds were disrupted by Ruthenium Red, a potential plasma-membrane and endo-membrane Ca^{2+}- permeable channel inhibitor, but not by 1,2-bis(2-aminophenoxy) ethane-N,N,N',N'-tetraacetic acid (BAPTA), an extracellular Ca^{2+}-chelator, suggesting that acyclic compounds promote Ca^{2+}-release from intracellular stores. On the other hand, the electrophilic compound (E)-2-hexenal promoted Ca^{2+}-influx via ROS production by natural oxidation at the aquarius phase. In a gpa1-2 mutant lacking a canonical Gα subunit, the $[Ca^{2+}]c$ transients induced by all tested volatiles were not attenuated, suggesting that G-protein coupled receptors are not involved in the volatile-induced $[Ca^{2+}]c$ transients in Arabidopsis leaves.

3) **Muroi A., Ishihara A., Tanaka C., Ishizuka A., Takabayashi J., Miyoshi H., and Nishioka T.: Accumulation of hydroxycinnamic acid amides induced by pathogen infection and**

identification of agmatine coumaroyltransferase in Arabidopsis thaliana. Planta, 230; 517-527, 2009.

Abstract: Abstract Hydroxycinnamic acid amides (HCAAs) are secondary metabolites involved in the defense of plants against pathogens. Here, we report the first identification of HCAAs, p-coumaroylagmatine, feruloylagmatine, p-coumaroylputrescine and feruloylputrescine, in Arabidopsis thaliana rosette leaves infected with Alternaria brassicicola and the assignment of At5g61160 as the agmatine coumaroyltransferase (AtACT) that catalyzes the last reaction in the biosynthesis of the HCAAs. Feeding experiments with putative labeled precursors revealed that the four HCAAs were synthesized from hydroxycinnamic acids and agmatine or putrescine. AtACT gene function was identified from an analysis of a mutant that did not accumulate HCAAs. In wild-type Arabidopsis, AtACT transcripts markedly increased in response to A. brassicicola infection. Enzymatic activity that catalyzes the synthesis of the HCAAs was confirmed in vitro by using a recombinant AtACT expressed in Escherichia coli. The Atact mutant was susceptible to infection by A. brassicicola, indicating that HCAAs are responsible for defense against pathogens in A. thaliana.

◇学会報告　（∗：特別講演，シンポジウム，ワークショップ等）

1) Horai H., Arita M., Nihei Y., and Nishioka T.: Merged Spectrum for Metabolite Identification in MassBank. 57th ASMS Conference on Mass Spectrometry and Allied Topics (By the American Society for Mass Spectrometry), 2009, 5, 31-6, 4, Philadelphia, PA, USA. (Refereed)
2) Nishioka T., Horai H., Arita M., and Kanaya S.: Development and Applications of MassBank, a Database Designed for Sharing Mass Spectra among Life-Science Research Communities. 18th International Mass Spectrometry Conference, 2009, 8, 30- 9, 4, Bremen, Germany. (Refereed)
3) Horai H., Arita M., Ojima Y., Nihei Y., Kanaya S., and Nishioka T.: Traceable Analysis of Multiple-Stage Mass Spectra through Precursor-Product Annotations. Proceedings of German Conference in Bioinformatics (GCB'09) (GI-Edition Lecture Notes in Informatics), 173-178, 2009, 9, 28- 30, Halle, Germany. (Refereed)
4) Horai H., Arita M., Nihei Y., Ikeda T., Ojima Y., and Nishioka T.: New Functions of MassBank: Mass Spectral Database for Metabolome Analysis －Peak Search by Formula, Web API and Database Maintenance－. 20th International Conference on Genome Informatics, 2009, 12, 14-16, Yokohama, Japan. (Refereed)
5) Horai H., Arita M., Nihei Y., Ikeda Y., Ojima Y., and Nishioka T.: MassBank: A Mass Spectral Database for Metabolomics. JSBi 20th Anniversary Special Session, 20th International Conference on Genome Informatics, 2009, 12, 14-16, Yokohama, Japan. (Invited lecture)
6) 池田 奨，二瓶義人，蓬莱尚幸，西岡孝明 ： Windows 版 MassBank を利用した個人用マススペクトルデータベース. 第 57 回質量分析総合討論会（日本質量分析学会主催），2009，5，13-15．大阪市.
7) 尾嶌雄也，二瓶義人，蓬莱尚幸，西岡孝明 ：MassBank におけるフラグメンテーションライブラリー．第 57 回質量分析総合討論会（日本質量分析学会主催），2009，5，13-15．大阪市.
8) 西岡孝明：マススペクトルを日本発のツールで解析，整理しよう．第 57 回質量分析総合討論会（日本質量分析学会主催），2009，5，13-15．大阪市.
9) 蓬莱尚幸，池田　奨，尾嶌雄也，二瓶義人，西岡孝明：マススペクトルを MassBank で公開，共有しよう. 第 57 回質量分析総合討論会（日本質量分析学会主催），2009，5，13-15．大阪市.
10) 西岡孝明，蓬莱尚幸：LipidBank とマススペクトルデータベース MassBank．第 51 回日本脂質生化学会，2009, 7, 30-31.名古屋市．（招待講演）
11) 西岡孝明，蓬莱尚幸，二瓶義人，池田　奨，尾嶌雄也，田中　聡，青島　健，小田吉哉：序論，第 132 回関東談話会（日本質量分析学会主催，JST-BIRD 共催），2009，9，29，横

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12) 蓬莱尚幸，二瓶義人，池田　奨，尾嶌雄也：MassBank の検索機能．第 132 回関東談話会（日本質量分析学会主催，JST-BIRD 共催），2009，9，29，横浜市.
13) 蓬莱尚幸，二瓶義人，池田　奨，尾嶌雄也：Windows 版 MassBank を用いたインハウスデータベースの構築．第 132 回関東談話会（日本質量分析学会主催，JST-BIRD 共催），2009，9，29，横浜市.
14) 蓬莱尚幸，有田正規，二瓶義人，池田　奨，尾嶌雄也，西岡孝明：マススペクトルデータベース MassBank の新機能 －Peak Search Advanced，Web API，データベース管理－．第 4 回メタボロームシンポジウム，2009，11，18-19 日，横浜市.
15) 西岡孝明，蓬莱尚幸，二瓶義人，池田　奨，尾嶌雄也：MassBank でマススペクトルを公開，共有する．第 32 回日本分子生物学会，ワークショップ「ファイトケミカルゲノミクスののための生物情報学」，2009，12，9-12，横浜市.