機能情報解析部門　Division of Biomedical Informatics

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◇研究目的

メタボローム解析などの生命科学分野では，高分解能，高感度な質量分析が必須の化学分析となっている。しかし，このような質量分析の高性能化にもかかわらず，マススペクトルで検出された生体内物質のうち同定される化合物の割合は 1-3 % にすぎない。これは生体内物質のマススペクトルを収集したデータベース（MSDB）が無いからである。

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，MSDB 化することによって，和漢医薬の化学成分の同定率を改善することを目的とする。これによって生薬の（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り，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 MSDB 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・検出した，溶出時間分割マススペクトルを収集する。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) **Abe M, Kubo A, Yamamoto S, Hatoh Y, Murai M, Hattori Y, Makabe H, Nishioka T, & Miyoshi H : Dynamic function of the spacer region of acetogenins in the inhibition of bovine mitochondrial NADH-ubiquinone oxidoreductase (complex I). Biochemistry, 47(23), 6260-6266, 2008.**

Abstract: Studies of the action mechanism of acetogenins, the most potent and structurally unique inhibitors of bovine heart mitochondrial complex I (NADH-ubiquinone oxidoreductase), are valuable in characterizing the inhibitor binding site in this enzyme. Our previous study deepened our understanding of the dynamic function of the spacer region of bis-THF acetogenins [Abe, M., et al. (2005) Biochemistry 44, 14898-14906] but, at the same time, posed new important questions. First, while the two toxophores (i.e., the hydroxylated THF and the gamma-lactone rings) span a distance shorter than that of the extended 13 carbon atoms [-(CH 2) 13-], what is the apparent optimal length of the spacer for the inhibition of 13 carbon atoms? In other words, what is the functional role of the additional methylene groups? Second, why was the inhibitory potency of the mono-THF derivative, but not the bis-THF derivative, drastically reduced by hardening the spacer covering 10 carbon atoms into a rodlike shape [-CH 2-(C identical withC) 4-CH 2-]? This study was designed not only to answer these questions but also to further disclose the dynamic functions of the spacer. We here synthesized systematically designed acetogenins, including mono- and bis-THF derivatives, and evaluated their inhibitory effects on bovine complex I. With regard to the first question, we demonstrated that the additional methylenes enhance the hydrophobicity of the spacer region, which may be thermodynamically advantageous for bringing the polar gamma-lactone ring into the membrane-embedded segment of complex I. With regard to the second question, we observed that a decrease in the flexibility of the spacer region is more adverse to the action of the mono-THF series than that of the bis-THF series. As a cause of this difference, we suggest that for bis-THF derivatives, one of the two THF rings, being adjacent to the spacer, is capable of working as a pseudospacer to overcome the remarkable decrease in the conformational freedom and/or the length of the spacer. Moreover, using photoresponsive acetogenins that undergo drastic and reversible conformational changes with alternating UV-vis irradiation, we provided further evidence that the spacer region is free from steric congestion arising from the putative binding site probably because there is no receptor wall for the spacer region.

2) **Ishihara A, Hashimoto Y, Tanaka C, Dubouzet JG, Nakao T, Matsuda F, Nishioka T, Miyagawa H, & Wakasa K: The tryptophan pathway is involved in the defense responses of rice against pathogenic infection by serotonin production. Plant Journal. 54, 481-495, 2008**

Abstract: The upregulation of the tryptophan (Trp) pathway in rice leaves infected by Bipolaris oryzae was indicated by: (i) enhanced enzyme activity of anthranilate synthase (AS), which regulates metabolic flux in the Trp pathway; (ii) elevated levels of the AS (OASA2, OASB1, and OASB2) transcripts; and (iii) increases in the contents of anthranilate, indole, and Trp. The measurement of the contents of Trp-derived metabolites by high-performance liquid chromatography coupled with tandem mass spectrometry revealed that serotonin and its hydroxycinnamic acid amides were accumulated in infected leaves. Serotonin accumulation was preceded by a transient increase in the tryptamine content and by marked activation of Trp decarboxylase, indicating that enhanced Trp production is linked to the formation of serotonin from Trp via tryptamine. Feeding of radiolabeled serotonin to inoculated leaves demonstrated that serotonin is incorporated into the cell walls of lesion tissue. The leaves of a propagating-type lesion mimic mutant (sl, Sekiguchi lesion) lacked both serotonin production and deposition of unextractable brown material at the infection sites, and showed increased susceptibility to B. oryzae infection. Treating the mutant with serotonin restored deposition of brown material at the lesion site. In addition, the serotonin treatment suppressed the growth of fungal hyphae in the leaf tissues of the sl mutant. These findings

indicated that the activation of the Trp pathway is involved in the establishment of effective physical defenses by producing serotonin in rice leaves.

3) Sato S, Arita M, Soga T, Nishioka T and Tomita M : Time-resolved metabolomics reveals metabolic modulation in rice foliage. BMC Systems Biology 2 51doi:10.1186/1752-0509-2-51, 2008.

Abstract: BACKGROUND: To elucidate the interaction of dynamics among modules that constitute biological systems, comprehensive datasets obtained from "omics" technologies have been used. In recent plant metabolomics approaches, the reconstruction of metabolic correlation networks has been attempted using statistical techniques. However, the results were unsatisfactory and effective data-mining techniques that apply appropriate comprehensive datasets are needed. RESULTS: Using capillary electrophoresis mass spectrometry (CE-MS) and capillary electrophoresis diode-array detection (CE-DAD), we analyzed the dynamic changes in the level of 56 basic metabolites in plant foliage (Oryza sativa L. ssp. japonica) at hourly intervals over a 24-hr period. Unsupervised clustering of comprehensive metabolic profiles using Kohonen's self-organizing map (SOM) allowed classification of the biochemical pathways activated by the light and dark cycle. The carbon and nitrogen (C/N) metabolism in both periods was also visualized as a phenotypic linkage map that connects network modules on the basis of traditional metabolic pathways rather than pairwise correlations among metabolites. The regulatory networks of C/N assimilation/dissimilation at each time point were consistent with previous works on plant metabolism. In response to environmental stress, glutathione and spermidine fluctuated synchronously with their regulatory targets. Adenine nucleosides and nicotinamide coenzymes were regulated by phosphorylation and dephosphorylation. We also demonstrated that SOM analysis was applicable to the estimation of unidentifiable metabolites in metabolome analysis. Hierarchical clustering of a correlation coefficient matrix could help identify the bottleneck enzymes that regulate metabolic networks. CONCLUSION: Our results showed that our SOM analysis with appropriate metabolic time-courses effectively revealed the synchronous dynamics among metabolic modules and elucidated the underlying biochemical functions. The application of discrimination of unidentified metabolites and the identification of bottleneck enzymatic steps even to non-targeted comprehensive analysis promise to facilitate an understanding of large-scale interactions among components in biological systems.

4) Mitsuno H, Sakurai T, Murai M, Yasuda T, Kugimiya S, Ozawa R, Toyohara H, Takabayashi J, Miyoshi H & Nishioka T : Identification of receptors of main sex-pheromone components of three Lepidopteran species. European Journal of Neuroscience 28 (5), 893-902, 2008.

Abstract: Male moths discriminate conspecific female-emitted sex pheromones. Although the chemical components of sex pheromones have been identified in more than 500 moth species, only three components in Bombyx mori and Heliothis virescens have had their receptors identified. Here we report the identification of receptors for the main sex-pheromone components in three moth species, Plutella xylostella, Mythimna separata and Diaphania indica. We cloned putative sex-pheromone receptor genes PxOR1, MsOR1 and DiOR1 from P. xylostella, M. separata and D. indica, respectively. Each of the three genes was exclusively expressed with an Or83b orthologous gene in male olfactory receptor neurons (ORNs) that are surrounded by supporting cells expressing pheromone-binding-protein (PBP) genes. By two-electrode voltage-clamp recording, we tested the ligand specificity of Xenopus oocytes co-expressing PxOR1, MsOR1 or DiOR1 with an OR83b family protein. Among the seven sex-pheromone components of the three moth species, the oocytes dose-dependently responded only to the main sex-pheromone component of the corresponding moth species. In our study, PBPs were not essential for ligand specificity of the receptors. On the phylogenetic tree of insect olfactory receptors, the six sex-pheromone receptors identified in the present and previous studies are grouped in the same subfamily but have no relation with the taxonomy of moths. It is most likely that sex-pheromone receptors have randomly evolved from

ancestral sex-pheromone receptors before the speciation of moths and that their ligand specificity was modified by mutations of local amino acid sequences after speciation.

5) Ichimaru N, Murai M, Kakutani M, Kako NJ, Ishihara A, Nakagawa Y, Nishioka T, Yagi T, & Miyoshi M. : Synthesis and Characterization of New Piperazine-Type Inhibitors for Mitochondrial NADH-Ubiquinone Oxidoreductase (Complex I). Biochemistry, 47, 10816–10826, 2008.

Abstract: The mode of action of Deltalac-acetogenins, strong inhibitors of bovine heart mitochondrial complex I, is different from that of traditional inhibitors such as rotenone and piericidin A [Murai, M., et al. (2007) Biochemistry 46 , 6409-6416]. As further exploration of these unique inhibitors might provide new insights into the terminal electron transfer step of complex I, we drastically modified the structure of Deltalac-acetogenins and characterized their inhibitory action. In particular, on the basis of structural similarity between the bis-THF and the piperazine rings, we here synthesized a series of piperazine derivatives. Some of the derivatives exhibited very potent inhibition at nanomolar levels. The hydrophobicity of the side chains and their balance were important structural factors for the inhibition, as is the case for the original Deltalac-acetogenins. However, unlike in the case of the original Deltalac-acetogenins, (i) the presence of two hydroxy groups is not crucial for the activity, (ii) the level of superoxide production induced by the piperazines is relatively high, (iii) the inhibitory potency for the reverse electron transfer is remarkably weaker than that for the forward event, and (iv) the piperazines efficiently suppressed the specific binding of a photoaffinity probe of natural-type acetogenins ([(125)I]TDA) to the ND1 subunit. We therefore conclude that the action mechanism of the piperazine series differs from that of the original Deltalac-acetogenins. The photoaffinity labeling study using a newly synthesized photoreactive piperazine ([(125)I]AFP) revealed that this compound binds to the 49 kDa subunit and an unidentified subunit, not ND1, with a frequency of approximately 1:3. A variety of traditional complex I inhibitors as well as Deltalac-acetogenins suppressed the specific binding of [(125)I]AFP to the subunits. The apparent competitive behavior of inhibitors that seem to bind to different sites may be due to structural changes at the binding site, rather than occupying the same site. The meaning of the occurrence of diverse inhibitors exhibiting different mechanisms of action is discussed in light of the functionality of the membrane arm of complex I.

6) Horai, H. and Nishioka, T.: Automatic Generation of Structure of Phospholipids. Journal of Computer Aided Chemistry, 9, 55-61, 2008.

Abstract: An algorithm and a tool for automatic generation of structures of phospholipids are proposed. The input is a compact representation of the variable part of phospholipids in a systematic way. The output is a structure of the phospholipid represented in the MDL Molfile format. The output molfile describes not only the topological connectivity of atoms but also the 2D coordinate of each atom in order to draw the structure without any overlapping. The variation of phospholipids that the tool covers includes glycerophospholipids (phosphatidylcholines, phosphatidylethanolamines, phosphatidylglicerols, phosphatidylinositols and phosphatidylserines) and sphingophospholipids with arbitrary length and arbitrary number of double bonds at arbitrary positions and in arbitrary cis/trans isomerism.

◇総　説

1) 蓬莱尚幸，西岡孝明，“メタボローム解析のための質量分析スペクトル・データベース：MassBank”実験医学，26(7), 1155-1160, 2008.
2) 西岡孝明，“微生物のメタボローム解析”，「21 世紀の農学」第６巻（植田充美編集），京都大学出版会，207-248, 2008.
3) 西岡孝明，“質量分析法検出用低分子データベースの構築”，「科学技術・ 研究開発の国

際比較」2009 年版，JST 研究開発戦略センターが先端計測技術分野編集，発行。

◇学会報告　（∗：特別講演，シンポジウム，ワークショップ等）

1) 蓬莱尚幸，西岡孝明，有田正規 :“情報科学から見たスペクトルデータベース MassBank”，シンポジウム「データベースからひもとく質量分析情報学」，第 56 回質量分析総合討論会（日本質量分析学会主催），つくば国際会議場，つくば市，2008 年 5 月 14-16 日
2) Horai, H., Arita, M. and Nishioka, T. Comparison of ESI-MS in MassBank Database. 1st International Conference on BioMedical Engineering and Informatics, Sanya, Hainan, China, 2008 年 5 月 28 日～30 日.（査読あり）
3) Horai H, Arita M, & Nishioka T,“MassBank: Mass Spectral Database for Metabolome Analysis”, 56th ASMS Conference on Mass Spectrometry, (American Association for Mass Spectrometry 主催), Colorado Convention Center, Denver, Colorado, USA, 2008 年 6 月 1 日～5 日.
4) Horai H, Nishioka T. & Arita M,“MassBank: Mass Spectral Database for Metabolome Analysis”, 5th International Conference on Plant Metabolomics （口頭発表）, Pacifico Yokohama, Yokohama, Japan, 2008 年 7 月 15 日～18 日.（査読あり）
5) Sakurai T, Mitsuno H, Uchino K, Sezutsu H, Tamura T, Yokohari F, Nishioka T, and Kanzaki R.,“Activation of bombykol receptor neurons by ectopically expressed olfactory receptor triggers pheromone searching behavior in male silkmoths”, International Symposium on Olfaction and Taste (ISOT2008), San Francisco, USA, 2008 年 7 月 21 日～26 日.
6) Horai, H., Arita, M. and Nishioka, T. MassBank: A Mass Spectral Database for Metabolomics. 4th Annual Conference for Metabolomincs, Boston, USA, 2008 年 9 月 12 日～16 日.
7) 蓬莱尚幸，西岡孝明，有田正規，“MassBank: メタボローム解析のための質量分析スペクトルデータベース”，第 33 回日本医用マススペクトル学会，東京大学，東京都，2008 年 9 月 25 日～26 日.
8) 西岡孝明，“MassBank: Private library から構造推定まで”，第 68 回北陸質量分析談話会（日本医用マススペクトル学会北陸支部会），（招待講演），富山大学，富山市，2008 年 11 月 29 日.