機能情報解析部門

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◇研究目的

近年のクロマトグラフィーや質量分析の進歩は著しく，生体内にある高分子から低分子までを高分離かつ高分解能で化学分析することを可能にしている。その結果，化学分析によって得られるデータは膨大になり，もはや手作業でデータ処理をおこなうことが困難になっている。

当部門では，和漢医薬に含まれる代謝物質の高分解能マススペクトルデータを収集，整理，データベース化して公開することによって代謝物質と薬理機能との関係を明らかにする。

◇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

これまでに和漢医薬学総合研究所で測定された二次代謝物質のマススペクトルのデジタル化と収集，新たに高分解能 IT-TOFMS で測定したマススペクトルのデータベース化をおこなう。

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

試料の抽出物を LC-高分解能 MS で測定した，溶出時間分割マススペクトルを収集し，データベース化する。マススペクトルを未同定の二次代謝物質のタグとして利用することによって，異なる試料間に同一の未知二次代謝物質の有無，多少を知ることができる。このような比較をすることによって，試料の薬理効果と二次代謝物質との関係を明らかにする。

◇原著論文

1) **Ichimaru, N., Yoshinaga, N., Nishioka, T., & Miyoshi, H. :Effect of stereochemistry of Deltalac-acetogenins on the inhibitory effect on mitochondrial complex I (NADH-ubiquinone oxidoreductase). Tetrahedron, 63: 1127-1139, 2007.**

Abstract: Δ lac-Acetogenins are a new type of inhibitors of bovine heart mitochondrial complex I (NADH-ubiquinone oxidoreductase). We synthesized a series of Δlac-acetogenins in which the stereochemistry around the hydroxylated tetrahydrofuran (THF) ring moiety was systematically modified, and examined their inhibitory effect on complex I. The present results revealed that the inhibitory effects of the bis-THF ring analogs are much more potent than those of the mono-THF ring analogs and that the stereochemistry around the bis-THF ring moiety significantly influences the inhibitory effect. The profiles of the structure–activity relationship observed for Δlac-acetogenins were entirely different from those for natural-type acetogenins.

2) **Heinzle, E., Matsuda, F., Miyagawa, H., Wakasa, K., & Nishioka, T. :Estimation of metabolic fluxes, expression levels and metabolite dynamics of a secondary metabolic pathway in potato using label pulse feeding experiments combined with kinetic network modeling and simulation. The Plant Journal, 50: 176-187, 2007.**

Abstract: In this paper we present a method that allows dynamic flux analysis without a priori kinetic knowledge. This method was developed and validated using the pulse-feeding experimental data obtained in our previous study (Matsuda et al., 2005), in which incorporation of exogenously applied l-phenylalanine-d5 into seven phenylpropanoid metabolites in potato tubers was determined. After identification of the topology of the metabolic network of these biosynthetic pathways, the system was described by dynamic mass balances in combination with power-law kinetics. After the first simulations, some reactions were removed from the network because they were not contributing significantly to network behaviour. As a next step, the exponents of the power-law kinetics were identified and then kept at fixed values during further analysis. The model was tested for statistical reliability using Monte Carlo simulations. Most fluxes could be identified with high accuracy. The two test cases, control and after elicitation, were clearly distinguished, and with elicitation fluxes to N-p-coumaroyloctopamine (pCO) and N-p-coumaroyltyramine (pCT) increased significantly, whereas those for chlorogenic acid (CGA) and p-coumaroylshikimate decreased significantly. According to the model, increases in the first two fluxes were caused by induction/derepression mechanisms. The decreases in the latter two fluxes were caused by decreased concentrations of their substrates, which in turn were caused by increased activity of the pCO- and pCT-producing enzymes. Flux-control analysis showed that, in most cases, flux control was changed after application of elicitor. Thus the results revealed potential targets for improving actions against tissue wounding and pathogen attack.

3) **Ishii, N., Nakahigashi,K., Baba, T., Robert, M., Soga, T., Kanai, A., Hirasawa, T., Naba, M., Kenta Hirai, K., Hoque, A., Ho, P.,Y., Kakazu, Y., Sugawara, K., Igarashi, S., Harada, S., Masuda, T., Sugiyama, N., Togashi, T., Hasegawa, M., Takai, Y., Yugi, K., Arakawa, K., Iwata, N., Toya, Y., Nakayama, Y., Nishioka, T., Shimizu, K., Mori, H., and Tomita, M. :Multiple High-Throughput Analyses Monitor the Response of E. coli to Perturbations. Science, 316: 593-597, 2007.**

Abstract: Analysis of cellular components at multiple levels of biological information can provide valuable functional insights. We performed multiple high-throughput measurements to study the response of Escherichia coli cells to genetic and environmental perturbations. Analysis of metabolic enzyme gene disruptants revealed unexpectedly small changes in messenger RNA and proteins for most disruptants. Overall, metabolite levels were also stable, reflecting the rerouting of fluxes in the metabolic network. In

contrast, E. coli actively regulated enzyme levels to maintain a stable metabolic state in response to changes in growth rate. E. coli thus seems to use complementary strategies that result in a metabolic network robust against perturbations.

4) **Murai, M., Ishihara, A., Nishioka, T., Yagi, T. and Miyoshi, H. :The ND1 Subunit Constructs the Inhibitor Binding Domain in Bovine Heart Mitochondrial Complex I. Biochemistry, 46: 6409-6416, 2007.**

Abstract: Abstract: The inhibitor binding domain in bovine complex I is believed to be constructed by multisubunits, but it remains to be learned how the binding positions of chemically diverse inhibitors relate to each other. To get insight into the inhibitor binding domain in complex I, we synthesized a photoreactive acetogenin [[125I](trifluoromethyl)phenyldiazirinylacetogenin, [125I]TDA], in which an aryldiazirine group serves as both a photoreactive group and a substitute for the -lactone ring that is a common toxophore of numerous natural acetogenins, and carried out photoaffinity labeling to identify the labeled subunit using bovine heart submitochondrial particles (SMP). When SMP were UV-irradiated in the presence of [125I]TDA, radioactivity was predominantly incorporated into an ~30 kDa band on a SDS gel. Blue native gel electrophoresis of the [125I]TDA-labeled SMP revealed that the majority of radioactivity was observed in complex I. Analysis of complex I on a SDS gel showed a predominant peak of radioactivity at ~30 kDa. Immnoprecipitation of the [125I]TDA-labeled complex I with anti-bovine ND1 antibody indicated that the labeled protein is the ND1 subunit. A variety of complex I inhibitors such as piericidin A and rotenone efficiently suppressed the specific binding of [125I]TDA to ND1, indicating that they share a common binding domain. However, the suppression efficiency of lac-acetogenin, a new type of complex I inhibitor synthesized in our laboratory, was much lower than that of the traditional inhibitors. Our results unequivocally reveal that the ND1 subunit constructs the inhibitor binding domain, though the contribution of this subunit has been challenged. Further, the present study corroborates our previous proposition that the inhibition site of lac-acetogenins differs from that of traditional inhibitors.

5) **Okazaki, Y., Ishizuka, A., Ishihara, A., Nishioka, T. and Iwamura, H. :New Dimeric Compounds of Avenanthramide Phytoalexin in Oats. Journal of Organic Chemistry, 72 (10): 3830 -3839, 2007.**

Abstract: Avenanthramide B is an oat phytoalexin produced in response to pathogen attack and elicitation. We found the formation of new dimers (1-5) of avenanthramide B in elicited oat leaves. The dimers were synthesized by a reaction of peroxidase and avenanthramide B in the presence of hydrogen peroxide. The structures of 1-5 were determined by spectroscopic analyses, chemical derivatization, and 15N labeling. Compound 1 was a dehydrodimer of avenanthramide B with a bisbutane lactam skeleton, while 2-4 were monohydrated dehydrodimers with butane lactam structures. Compound 5 was also a monohydrated dehydrodimer but with a tetrahydrofuran structure. All the compounds were classified into lignanamides that were formed by an 8'-8' coupling reaction between two avenanthramide B units.

6) **Yoshida, T., Murai, M., Abe, M., Ichimaru, N., Harada, T., Nishioka, T. and Miyoshi, H. : Crucial Structural Factors and Mode of Action of Polyene Amides as Inhibitors for Mitochondrial NADH-Ubiquinone Oxidoreductase (Complex I). Biochemistry, 46: 10365-10372, 2007.**

Abstract: Natural antibiotic polyene amides such as myxalamides are potent inhibitors of mitochondrial complex I. Because of the significant instability of this series of compounds due to an extended pi-conjugation skeleton, a detailed characterization of their inhibitory action has not been performed. To elucidate the action mechanism as well as binding manner of polyene amides with complex I, identification of the roles of each functional group in the inhibitory action is needed. We here synthesized a series of amide analogues and carried out structure-activity studies with bovine heart mitochondrial complex I. With respect to the left-hand portion, the natural pi-conjugation skeleton common to many

natural products is not required for the inhibition and can be substituted with a simpler substructure such as a conjugated diene. The geometry and shape of the left-hand portion were shown to be important for the inhibition, suggesting that this portion may bind to a narrow hydrophobic pocket in the enzyme rather than merely partitioning into the lipid membrane phase. Concerning the right-hand portion of the inhibitor, the presence of the 2-methyl, amide NH, and (S)-1'-methyl groups was crucial for the activity, suggesting that both methyl groups neighboring the amide group finely adjust the hydrogen-bonding ability of the amide group. In contrast, modifications of the 2'-OH group did not significantly influence the activity, suggesting that the role of this functional group is not to serve as a hydrogen bond donor to the enzyme but to act as a hydrophilic anchor directing the right-hand portion at or near the membrane surface. Detailed characterization of the action mechanism indicated that the polyene amides share a common binding domain with other complex I inhibitors, though their binding position (or manner) within the domain may differ considerably from that of other inhibitors.

7) **Horiuchi, J., Muroi, A., Takabayashi, J. and Nishioka, T. :Exposing Arabidopsis seedlings to borneol and bornyl acetate affects root growth: Specificity due to the chemical and optical structures of the compounds. Journal of Plant Interactions, 2 (2): 101 - 104, 2007.**

Abstract: Root growth of Arabidopsis seedlings on the surface of agar plates was measured after the seedlings were exposed to volatile organic compounds. Similar to the roots of unexposed seedlings, the roots of seedlings exposed to volatile methanol (control) grew straight down. On the other hand, seedlings exposed to volatile bornyl acetate produced wavy roots. Interestingly, the wavy roots from seedlings exposed to (+)-bornyl acetate were significantly longer than those from seedlings exposed to (-)-bornyl acetate. Exposure to either (+)- or (-)-borneol resulted in thick root tips and reduced root growth. The roots from seedlings treated with (+)-borneol were significantly longer than those from seedlings exposed to (-)-borneol. The interactions between root length and the concentrations of (+)- or (-)-borneol were significantly different, showing that the Arabidopsis seedlings specifically responded to the molecular configuration of the borneol.

◇総　説

1) 西岡孝明：メタボローム：細胞の化学分析がゲノムと環境の相互作用を明らかにする．文部科学省科学研究費特定領域研究「ゲノム」4領域編，：ゲノムは何をどのように決めているのか？：生命システムの理解へ向けて．クバプロ，43-55, 2007.
2) 光野秀文，櫻井健志，西岡孝明：昆虫の匂い受容体に関する研究．杉山産業化学研究所年報，平成18年度：33-51, 2007.

◇学会報告　（*：特別講演，シンポジウム，ワークショップ等）

1) 西岡孝明：MassBank：マススペクトルデータベースの現状．第55回質量分析総合討論会，2007, 5.15 -17，広島市.
2) 西岡孝明，蓬莱尚幸，有田正規："MassBank：メタボローム－マススペクトルデータベースの現状"，第2回メタボロームシンポジウム，2007, 11.5-6，東京都.

◇その他

1) 西岡孝明：メタボローム解析の現状と将来．ゲノムテクノロジー第164委員会第24回研究会，日本学術振興会，2007, 9.7，福岡市.