S 8-3

Functional Analysis of *O*-methyltransferases and Glycosyltransferase from Bacteria

Bong-Gyu Kim , Jae hyung Ko, Yoon Jung Lee, Yoongho Lim and Joong-Hoon Ahn

Bio/Molecular Informatics Center, Department of Molecular Biotechnology, KonKuk University, Seoul, 1 Hwayang-dong, Gwangin-gu, Seoul 143-701, Korea

Introduction

Biological modification of natural compound with enzymes is one of attractive area because it provides compounds with structural diversity and regioselectivity. The sources of enzymes could come from various organisms whose genome sequences are completed. With the aid of genome sequence database, it is easy to clone and characterize a certain gene with defined functions. In this study, flavonoids, one of common natural products found in nature, are used as model compounds for structural modifications since they contain several active hydroxyl groups. These groups could be acceptors for methyl group or glucose groups by enzymatic reaction mediated by methyltransferases and glycosyltransferases.

Methyltransferases are classified into three groups depending on the methylated atom; C-methylatransferase (CMT), N-methyltransferase (NMT) and O-methyltransferse (OMT). *O*-Methyltransferases (OMTs) catalyze the transfer of a methyl group from *S*-adenosine-L-methionine (AdoMet) to a hydroxyl group of an acceptor molecule to form methyl ether derivatives. Plant OMTs can be divided into two groups based on their molecular weight. Both groups with molecular weights from 26 to 28-kDa and from 38 to 43 kDa are represented by caffeoyl coenzyme A OMTs (CCoAOMTs and caffeic acid OMT (COMT), respectively. Biological function of *O*-methylation in plant is diverse. For an example, ferulic acid and sinapic acid, which are both methylated compounds, are precursors of the monolignols that serve as building blocks for lignin biosynthesis. *O*-methylation of flavonoids results in lowering the chemical activity and enhancing their lipophilicity and antimicrobial activity.

Glycosylation is one of the major modification reactions and occurrs often in the last step of the biosynthesis of natural compounds. The enzymes leading to glycoside formation, the glycosyltransferases (GTs), transfer nucleotide-diphosphate-activated sugars to low molecular weight substrates. Usually, the activated sugar form is UDP-glucose, but UDP-galactose and UDP-rhamnose are also found. Sugar acceptors include all major classes of secondary metabolites, including phenolics, terpenoids, cyanohydrins and alkaloids. Mammalian UDP-glucuronosyl-transferases have been extensively studied because they are important in drug and xenobiotic metabolism. Whereas plant GTs are important in the stabilization of pigments, the regulation of plant growth regulators and in the enhancement of aglycone solubility. Also,

GTs take part in cell wall biosynthesis, and glycoprotein synthesis.

Here, we report the characterization of an OMT and GT, BcOMT1, BcOMT2, and BcGT1 from *Bacullus cereus* and SaOMT-2 from *Streptomyces avermilitilis* and the combinational modification of flavonoids with them.

Results and Discussions

O-methyltransferases (BcOMT-1 and BcOMT-2) from *Bacillus cereus*

BcOMT-1 and BcOMT-2, methyltransferase from *Bacillus cereus* was cloned to study the biological modification reaction of flavonoids. It consisted of 635 bp and 668 bp ORF, respective ly. BcOMT-1 and BcOMT-2 were subcloned into *E. coli* expression vector pET-15b with His-tag expression system. *E.coli* transformant containing BcOMT-1 and BcOMT-2 were used for biotransformation of flavonoids such as quercetin, taxifolin, eriodictyol, luteolin, naringenin, and keampferol. The reaction products were analyzed by thin layer chromatography and HPLC. BcOMT-1 and BcOMT-2 produced two reaction products with flavonoids containing ortho hydroxy group, which were methylated at either 3' or 4' hydroxyl group.

SaOMT-2

A variety of bacterial and fungal methyltransferases are involved in antibiotic and aflatoxin biosynthesis, as well as in the methylation of other compounds. O-methyltransferases isolated and characterized from streptomyces avermitilis. The SaOMT-2 had an apparent molecular mass of 37.5 kDa for denatured protein, with a pI of 5.23. The blast result showed that it showed high homology with Carminomycin 4-O-methyltrans. In order to determine the substrate of ROMT9, SaOMT-2 was expressed in *E. coli* as a His-tag fusion protein. Several substrates including naringenin, quercetin, daidzein, genisteinm, esuletin, kaempferol, catechol, caffeic acid and apigenin were tested. Reaction product was analyzed by TLC and HPLC. It converts quercetin , daidzein, genistein, esculetin, kaempferol, quercetin, naringenin. The methylation position is determined to be at 7 position by TLC and HPLC with sakuranetin (7 methylated Naringenin) as a standard. We concluded that *in vivo* substrate of SaOMT-2 would be naringenin and SaOMT-2 mediates methylation of naringenin at the 7 position.

Glycosyltransfease(BcGT1) from *Bacillus cereus*

One of the UDP-glycosyltransferase, BcGT1 from *Bacillus cereus* was cloned by polymerase chain reaction and sequenced. It showed the homology with UDP-glycosyltransferase (UDPGT). BcGT1 was expressed in *Escherichia coli* BL21 DE3 strain with his-tag and purified by using His-tag affinity column. To determine substrate specificity, apigenin, daidzein, genistein, kaempferol, luteolin, naringenin and quercetin were used as tentative substrates and reactions products were analyzed with thin layer chromatography (TLC) and high performance liquid chromatography (HPLC). All substrates tested were converted into the corresponding glucosides. Also, glycosylations at different hydroxyl groups were observed in some flavonoids, indicating that BcGT1 has a broad substrate range.

Multiple expression

Flavonoids are typical plant secondary metabolites belonging to a group of polyphenols. Over 6,000 natural flavonoids have been reported, and many of them are common in higher plants. Flavonoids constitute a relatively diverse family of phenolic molecules. These compounds are modified by genes such as *O*-methyltransferase(OMT) and glycosyltransferase(GT). We investigated the multiple expressions of ROMT-9, SOMT-2, and BcGT-1. ROMT-9 and SOMT-2 are 3' and 4'-*O*-methyltransferse of quercetin and was cloned from Rice and Soybean, respectively. Also BcGT-1 is 7-glycosytranferase and cloned from *Bacillus cereus*. Three genes were cloned to pET-21 vector system, respectively. In addition, ROMT-9 and SOMT-2 was independently cloned into one pET-21 vector and the resulting plasmid is named as RSOMT. ROMT-9, SOMT-2 and BcGT-1 conjugated vector is named as RSBK. Five constructs were expressed in *E. coli* BL21 to study multiple expression of RSOMT and RSBK for quercetin as substrate. HPLC analysis of reaction product from three construct having each gene showed one apparent product peak: 3'-*O*-methylated quercetin for ROMT-9, 4'-*O*-methylated quercetin for SOMT-2, and 7-*O*-glycosylated quercetin for BcGT-1. When RSOMT was expressed in BL21, three reaction products were detected, containing 3', 4'-*O*-dimethylated quercetin. In case of RSBK reaction products showed six product peaks, which will be further identified through mass spectrometry and NMR analysis.

References

1. Ibrahim, R. K., Bruneau A., Bantignies, B., 1998. Plant *O*-methyltransferase: molecular analysis, common signature and classification. Plant Mol. Biol. 36, 1-10.
2. Mwafaq Ibdah, Xing-Hai Zhang, Jurgen Schmidt, and Thomas Bogt. (2003) A Novel $Mg2^{+}$-dependent *O* -Methyltransferase in the Phenylpropanoid Metabolism of Mesembryanthemum crystallinum. J. of Biol. Chem., 278 : 43961-43972.
3. Whetten, R.W., MacKay, J.J., and Sederoff, R.R. (1998). Recent advances in understanding lignin biosynthesis. Annu. Rev. Plant Physiol. Plant Mol. Biol. 49, 585-609.
4. Vogt T, Jones P (2000) Glycosyltransferases in plant natural product synthesis: characterization of a supergene family. Trends Plant Sci 5:380-386.
5. Willits MG, Giovanni M, Prata RTN, Kramer CM, Luca VD, Steffens JC, Grase G (2004) Bio-fermentation of modified flavonoids: an example of in vivo diversification of secondary metabolites. Phytochemistry 65:31-41