

The Homodimerization of *Thalictrum tuberosum* O-Methyltransferases by Homology-based Modelling

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Received March 6, 2003

Two O-methyltransferases, OMTII-1 and OMTII-4 of meadow rue *Thalictrum tuberosum* showed a high sequence identity. Of 364 amino acids only one residue is not the same, which is Tyr21 or Cys21. Even if the 21st residues in these OMTs are not included in the binding sites of the enzymes, binding affinities of the enzyme homodimers over the same substrate are very different. While the binding affinity of one homodimer over caffeic acid is 100%, that of the other is 25%. Authors tried to predict the three-dimensional structures of *Thalictrum tuberosum* O-methyltransferases using homology-based modelling by a comparison with caffeic acid O-methyltransferase, and explain the reason of the phenomenon mentioned above based on their three dimensional structural studies. In the enzyme homodimer, the better binding affinity may be caused by the shorter distance between the 21st residue and the binding site of the other monomer.

Key Words : Modelling, Molecular dynamics, Homology, Methyltransferase, *Thalictrum tuberosum*

Introduction

Since secondary metabolites of plants play an important role in improving disease resistance of plants against pathogens as well as promoting human health, an increasing number of research groups are studying their biosynthetic pathways.¹ Among many enzymes participated in their biosynthetic pathways, methyltransferases are ubiquitous and create more diverse class of metabolites. Secondary metabolites such as lignin, suberin, flavonoids, antocyanins, and isoflavonoids which are synthesized *via* phenylpropanoid pathway are also modified by methyltransferases.^{2,3} Because they show diversity and play an important role in plant growth and development as well as the interactions of plants with their environment, many studies on methyltransferases have been carried out.⁴ Especially, O-methyltransferases (OMTs) are more common. OMTs can be classified based on their functions: (1) The first group methylates phenylpropanoids in the lignin biosynthesis pathway. (2) The second group methylates flavonoids, including chalcones, flavonols, and flavones. (3) The third group methylates alkaloids and other chemicals such as sugars and scent compounds.⁵ In all three groups, OMTs transfer the methyl group of S-adenosyl-L-methionine (SAM) to the hydroxyl group of phenolic acceptors, and form their methyl ether derivatives and S-adenosyl-L-homocysteine (SAH) as products.⁶ O-methylation not only reduces the chemical reactivity of their phenolic hydroxyl groups but also increases their lipophilicity and their intracellular compartmentation.⁷ Therefore, to know the function of OMTs at a molecular level, many genes encoding OMTs have been isolated and are characterized. In contrast with mammalian

OMTs, plant OMTs show narrow substrate specificities as well as position-specific activities, so that the homology-based comparison such as BLAST could not give enough information about the biological function of each OMTs such as substrate and product.⁸ Molecular modelling method may help us understand the reason why plant OMTs show narrow substrate specificities as well as position-specific activities.^{9,10}

It is known that methyl jasmonate can induce an alkaloid, berberine in cell cultures of meadow rue *Thalictrum tuberosum*.¹¹ Four cDNA encoding O-methyltransferases were isolated. Among these, two cDNAs named OMT II-1 and OMT II-4 showed high sequence homology. Of 364 amino acids only one residue is not the same, which is Tyr21 of OMT II-1 and Cys21 of OMT II-4. Even though both enzymes have the same substrate, caffeic acid, their binding affinities are different. The binding affinity values over caffeic acid of enzyme homodimers were reported by Frick and Kutchan.¹¹ While in the case of OMT II-1 homodimer the binding affinity over caffeic acid is 100%, that of OMT II-4 homodimer is 25%. Of course, if the different amino acid is positioned at the binding site, this phenomenon may be explained. Compared with residues neighboring the binding site of caffeic acid O-methyltransferase (COMT) which shows a high homology over 74% with OMT II, their binding site can be predicted but the 21st residue is not included there.⁸ Authors tried to predict the three-dimensional (3D) structures of *Thalictrum tuberosum* OMTs using homology-based modelling. A template for homology-based modelling was COMT deposited in Protein Data Bank (PDB code: 1KYZ.pdb).¹² Based on their 3D structural studies, we tried to explain the reason why two enzyme homodimers show very different binding affinity over caffeic acid.

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Table 1. A comparison of α -helices and β -sheets included in OMT II-1, OMT II-4, and COMT (top: α -helices; bottom: β -sheets)

OMT II-1	OMT II-4	COMT
Asn6 - Glu17	Asn6 - Glu17	
Tyr21 - Ser31	Cys21 - Ser31	Glu18 - Ser28
Pro34 - Ala40	Pro34 - Ala40	Ser30 - Leu42
Ile49 - Lys54	Ile49 - Lys54	Asp43 - Ala51
		Ser58 - Ser64
Ile63 - Thr76	Ile63 - Thr76	Asp72 - Tyr87
Asp100 - Arg105	Asp100 - Arg105	Thr110 - Val116
Phe115 - Asp121	Phe115 - Asp121	
Ser124 - Leu130	Ser124 - Leu130	Ile124 - Gln132
Lys135 - Glu139	Lys135 - Glu139	Asp133 - Glu138
		Ser139 - Tyr141
His143 - Val148	His143 - Val148	His142 - Gly150
Gly152 - Phe155	Gly152 - Phe155	Ile152 - Tyr158
Gly160 - His184	Gly160 - His184	Thr161 - His166
		Asp169 - Tyr195
Ser204 - Gly211	Ser204 - Gly211	Gly212 - Thr217
		Ile218 - Tyr222
		Leu232 - Asp238
Ile263 - His276	Ile263 - His276	Ile267 - Trp271
		Ser272 - Leu287
Glu298 - Thr313	Glu298 - Thr313	Ser307 - Asn324
Leu322 - Thr332	Leu322 - Thr332	
		Gln332 Ala342

OMT II-1	OMT II-4	COMT
Leu46 - Glu47	Leu46 - Glu47	
Arg80 - Ala86	Arg80 - Ala86	
Thr92 - Leu98	Thr92 - Leu98	Leu90 - Thr96
		Val102 - Leu108
Leu193 - Lys197	Leu193 - Lys197	Leu204 - Val207
Val216 - Thr221	Val216 - Thr221	
His235 - Asp239	His235 - Asp239	Gly227 - Asp231
Asp252 - Met253	Asp252 - Met253	Val245 - Gly249
Asn283 - Ser287	Asn283 - Ser287	Val262 - Phe263
Pro289 - Ala290	Pro289 - Ala290	Lys292 - Leu300
Ile320 - Met321	Ile320 - Met321	
Lys337 - Leu339	Lys337 - Leu339	Val349 - Ala353
Gly347 - Val351	Gly347 - Val351	Thr356 - Leu362

additional allowed regions, and 1.3% in generously allowed regions. G-factor is 0.11.

OMT II and substrate assembly. The substrate, caffeic

acid and cofactor. SAM binding sites of OMT II-1 and OMT II-4 were determined based on a comparison of the corresponding residues with binding sites of COMT (Table 2). In the case of the substrate binding site, Ile316 of COMT is switched with Leu317 in OMT II-1 and OMT II-4. However, in the case of the SAM binding site, all residues are agreed with each other. Even though OMT IIs form a substrate assembly, a comparison of OMT II-1 with OMT II-4 shows that there is no difference in both the substrate and the cofactor binding sites. Therefore, the different binding affinity over caffeic acid of OMT II-1 and OMT II-4 cannot be explained by their primary sequences composing the binding sites.

The quaternary structure of OMT II-1 and OMT II-4.

In order to explain the binding difference in OMT II-1 and OMT II-4 in other way, we turn our attention to the quaternary structure of these proteins. The homodimer of OMT II-1 was obtained by docking OMT II-1 into another OMT II-1 (Figure 2). The N-terminals from each subunit lie in the opposite direction as found in COMT. It was known that COMT consists of two polymers A and C which are dimerized by contact of the N-terminal domain. To compare OMTII-1 homodimer with COMT homodimer, they are superimposed and its RMSD value is 8.1 Å (Figure 3). A comparison of OMTII-1 monomer with COMT monomer gives the RMSD value of 7.3 Å. That is, the case of dimer shows better RMSD than that of monomer. As shown in Table 1, the N-terminal domain of COMT includes three helices between Glu18 and Ala51. However, OMT II-1

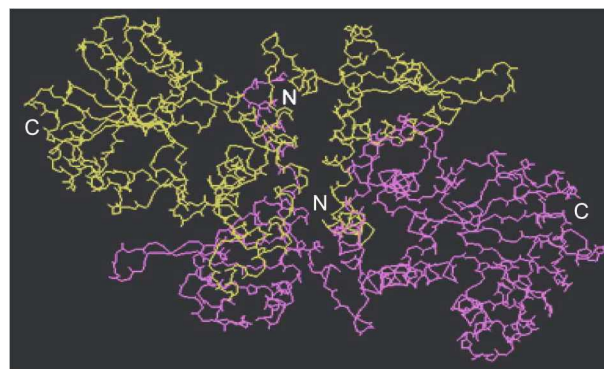


Figure 2. The structure of OMT II-1 homodimer. (white, a monomer; grey, another monomer)

Table 2. A comparison of residues neighboring binding sites of OMT II-1, OMT II-4, and COMT

Enzyme	substrate binding site												
COMT	Met130	Asn131	Leu136	Ala162	His166	Phe172	Phe176	Met180	His183	Ile316	Ile319	Met320	Asn324
OMTII-1	Met131	Asn132	Leu137	Ala163	His167	Phe173	Phe177	Met181	His184	Leu317	Ile320	Met321	Asn325
OMTII-4	Met131	Asn132	Leu137	Ala163	His167	Phe173	Phe177	Met181	His184	Leu317	Ile320	Met321	Asn325

Enzyme	SAM binding site													
COMT	Asp206	Val207	Gly208	Gly209	Gly210	Thr211	Gly212	Asp231	Leu232	Asp251	Met252	Phe253	Lys265	Trp271
OMTII-1	Asp207	Val208	Gly209	Gly210	Gly211	Thr212	Gly213	Asp232	Leu233	Asp252	Met253	Phe254	Lys266	Trp272
OMTII-4	Asp207	Val208	Gly209	Gly210	Gly211	Thr212	Gly213	Asp232	Leu233	Asp252	Met253	Phe254	Lys266	Trp272

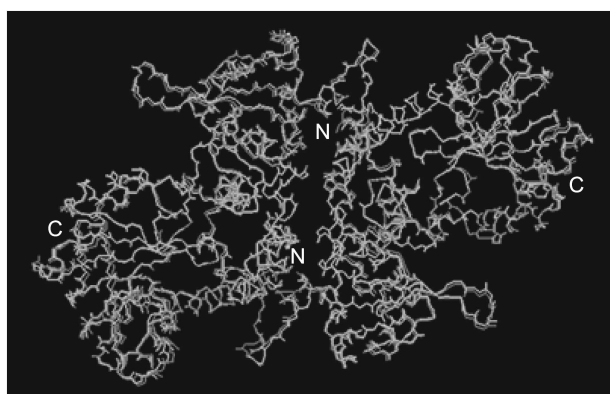


Figure 3. A superimposition of OMTII-1 homodimer (white) on COMT homodimer (grey).

appears to have one more helix that is composed of twelve amino acids between Asn6 and Glu17. Since the helix structure is not flexible, the presence of one more helix in OMT II-1 can make wider contact area and it could facilitate dimer formation. The driving forces for dimerization are electrostatic interactions and van der Waals interactions. As listed in Table 1, having one more helix in OMT II-1 results in more van der Waals force for dimer formation. Comparing the residues showing van der Waals interaction, included in opposing α -helices of each N-terminal, OMT II-1 has nine more residues than COMT. It would confer more van der Waals force when OMT II-1 forms dimer than when COMT does. In addition, while there are six continuing Glu residues in the first helix of OMT II-1, there are only two Glu residues in COMT. As a result, it may cause stronger electrostatic interactions. Taken together, OMT II-1 is predicted to form dimer.¹¹ Two OMT cDNA clones were isolated from freshwater weed *Chrysosplenium americanum*.¹⁵ They have only three residue difference but they show a large substrate specificity. While one OMT (NCBI Accession No.: P59049) shows 100% binding affinity for quercetin, another OMT (NCBI Accession No.: Q42653) does 38%. Of course, three residues are included in the binding sites. Therefore, this phenomenon can be explained based on the dimer interface interaction.

OMT II-4 homodimer was also obtained by the same method as applied on OMT II-1. As shown in Figure 1, Tyr21 of OMT II-1 is switched with Cys21 in OMT II-4. However, the comparison of helices of OMT II-1 with those of OMT II-4 shows that there is no difference (Table 1). While OMT II-1 homodimer showed the binding affinity of 100% over caffeic acid, OMT II-4 homodimer did 25%.¹¹ Since OMT IIs are predicted to form dimer, the quaternary structures of OMT IIs cause the substrate binding differences.

The characteristic in the dimer formation of COMT is that Ser28 sticks out into the binding site of the other monomer.¹⁶ At the same time, the residues being close to Ser 28 move closer to the binding site of the other monomer. It helps to exclude solvent in the substrate binding pocket. In the cases of OMT IIs, Ser29 matches Ser28 of COMT. The second helix of OMT IIs is composed of twelve residues between

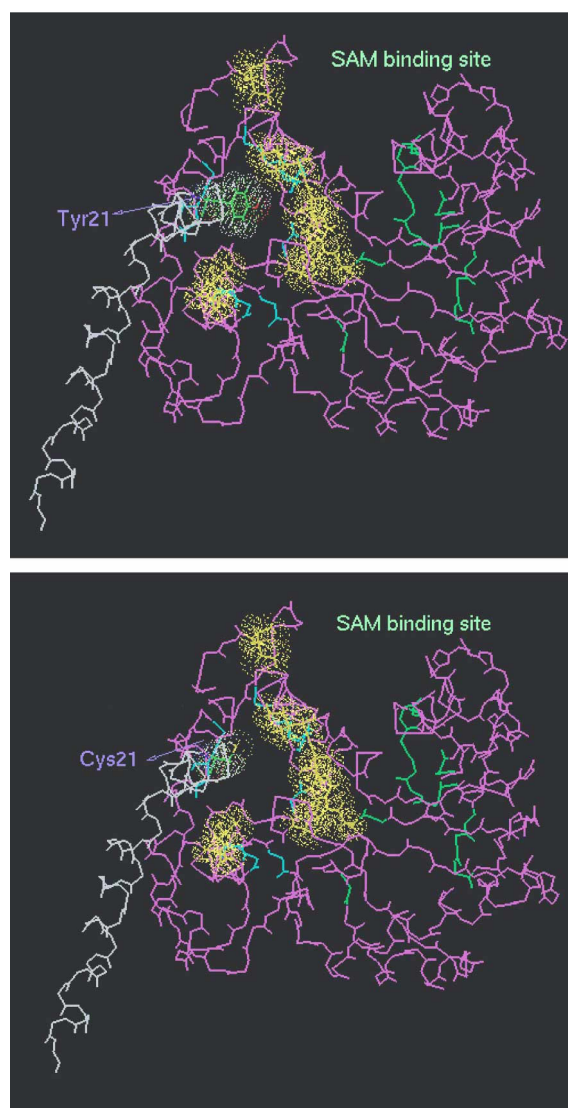


Figure 4. A comparison of the N-terminal domain of OMT II-1 homodimer backbone (top) with that of OMT II-4 homodimer backbone (bottom). (Dots denote the residues showing van der Waals interaction with Tyr21 or Cys21.)

the 21st residue and the 31st. Tyr21 and Cys21 are the starting residue of the helix. Therefore, the residues being close to Ser29 might move closer to the substrate binding pocket as those of COMT. To clarify this conjecture, the residues showing van der Waals interaction with Tyr21 of OMT II-1 were investigated in the other monomer. Eight residues of the other monomer were found, which were Phe115, Met125, His184, Ile187, Thr188, Thr313, Phe355, and Gly356. Unlike OMT II-1, only three residues were observed in Cys21 of OMT II-4, which were Phe115, Met125, and Phe355. This phenomenon can be explained by the reason why Tyr21 being closer to the binding site of the other monomer can help to keep out solvent molecules from the binding site and make the substrate access the binding site easier. As a result, the shorter distance between Tyr21 and the binding site of the other monomer probably causes the better binding affinity.

Acknowledgement. This work was supported by Frontier 21 Crop Functional Genomics Center, Korea Ministry of Science and Technology (CG2214).

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