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Computational studies on bergaptol *O*-methyltransferase from *Ammi majus* L.: The substrate specificity

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Abstract

In order to understand the mechanisms of substrate specificity and the interaction between bergaptol and bergaptol *O*-methyltransferase (BMT), a 3D model of BMT is generated based on the crystal structure of caffeic acid 3-*O*-methyltransferase (COMT EC 2.1.1.68, PDB code 1KYZ) by using the InsightII/Homology module. With the aid of the molecular mechanics and molecular dynamics methods, the final refined model is obtained and its reliability is further assessed by PROCHECK and ProSa2003. With this model, a flexible docking study is performed and the results indicate that BMT has narrow substrate specificity. Although the homology between both proteins is higher than 65% and all amino acids surrounding the binding site, except four residues, are similar in their sequences, the two proteins exhibit different substrate preferences. The differences in substrate specificity can be explained on the basis of the structures of the protein and the substrate. Our results indicate that His259 may be the catalytic base for the reaction, and Glu320, Glu287 bracket the catalytic His259. Especially, Glu320 forms a weak hydrogen bond with His259 and promotes transfer of an H ion.

Keywords: BMT; COMT; Homology modeling

1. Introduction

Plants produce over 100,000 secondary metabolites which are not considered essential for their basic metabolic processes, most of which belongs to the isoprenoids, phenylpropanoids, alkaloids, and polyketides [1–14]. Among these the flavonoid compounds play an important role in plant growth and development as well as in the interactions of plants with their environment. These are catalyzed by substrate-specific, position-oriented enzymes [13,14]. Enzymatic methylation is mediated by a family of methyltransferases which involve the transfer of the group of *S*-adenosyl-L-methionine (AdoMet) to the oxygen, nitrogen, or carbon atoms of various acceptor molecules with the formation of the methylated derivative and *S*-adenosyl-L-homocysteine (AdoHcy) [13]. In contrast with mammalian enzymes, however, plant OMTs show narrow substrate specificities as well as position-specific activities [1,13,14,18]. Recently, three OMTs, chalcone *O*-methyltransferase (ChOMT), isoflavone *O*-methyltransferase (IOMT), and caffeic acid *O*-methyltransferase (COMT) [15–18], have been determined using X-ray crystallography. The crystal structures of these OMTs can exhibit the complex of enzymes and their substrates as well as the structures of their binding sites, and can help us to predict the function of other OMTs.

The primary sequence of bergaptol *O*-methyltransferase (BMT) from *Ammi majus* L. shows 65% homology with COMT [1]. COMT recognizes caffeic acid as well as caffeoyl alcohol, caffeoyl aldehyde, 5-hydroxyferulic acid, 5-hydroxyconiferyl alcohol, and 5-hydroxyconiferaldehyde [14,15]. Because of the high homology, the substrate of BMT is expected to be one of the caffeic acid derivatives. However, only bergaptol is accepted as substrate by BMT [1]. Therefore, in order to reveal why the two proteins with the high homology

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exhibit different substrate preferences, it is necessary to determine the 3D structure of BMT. To the best of our knowledge, the 3D structure of BMT is not known until now. In the present investigation, the 3D model of BMT was built by a homology modeling procedure based on the crystal structure of caffeic acid 3-*O*-methyltransferase (COMT EC 2.1.1.68, PDB code 1KYZ) [18] and further used to search the binding site of substrate. The mechanism of substrate specificity for BMT was also discussed.

2. Computational methodology

2.1. Target and template proteins

The amino acid sequence of the target protein, BMT, was obtained from GenBank (Accession No. AAR24096) with 354 residues involved. The template protein used was a caffeic acid/5-hydroxyferulic acid OMT, COMT deposited in Protein Data Bank (PDB code 1KYZ.pdb) [18]. 1KYZ includes three dimer chains: A, C, E. While A and E chains form a dimer, the polymer chain C is separated from the others. Because BMT functions as a monomer [14], the chain C is chosen as a template.

2.2. Molecular modeling

The molecular modeling was carried out on the SGI O3800 workstation with the software InsightII [19]. The forcefield CVFF provided by Accelrys [20,21] was used for molecular dynamics (MD) and energy minimization, and BLAST search algorithm was used for the online search (http://www.ncbi.nlm.nih.gov) [22]. Program Modeler, which is an implementation of an automated approach to comparative modeling by satisfaction of spatial restraints [23], was employed to build the 3D structure of BMT. All sequences were imported into ClustalW program (version 1.83) (Fig. 1). Ribbon illustration was made using Swiss-Pdb Viewer [24].

The initial model was improved by energy minimization. After 200 steps of conjugate gradient (CG) minimization, the MD simulation was carried out to examine the quality of the model structures by checking their stability via performing 400 ps simulations at a constant temperature 298 K. An explicit solvent model TIP3P water was used with a 10 Å water cap from the center of mass of BMT. Finally, a conjugate gradient energy minimization of full protein was carried out until the root mean-square (rms) gradient energy was lower than 0.001 kcal mol^{-1} $Å^{-1}$. All the calculations mentioned above were accomplished by using Discover3 software package [25]. In this step, the quality of the initial model was improved. The structure with the lowest energy was checked using PROCHECK [26] and ProSa2003. In our studies, the binding site module [27] and CASTp (http://cast.engr.uic.edu/cast/) [28] were used to identify all the cavities associated with the model and the template as well as to measure the volume they contained. The results were further used for the protein–ligand docking investigations.

2.3. Docking of substrate and co-factor into protein

Affinity was chosen as docking program [29]. The 3D structure of the substrate (bergaptol) was constructed by InsightII/builder and the geometry of the substrate was further optimized using AM1 method. The substrates of COMT, 5-hydroxyferulate and caffeate, were also optimized using AM1 method. After determining the binding site, the co-factor (AdoMet) was docked into the protein. The potential function of the complex was assigned by using the CVFF forcefield and the cell multipole approach was also used for non-bonding interactions. As mentioned above, the substrate was docked into the binding site which is the most similar site to that of template protein (COMT). After the substrate, bergaptol, the co-factor, AdoMet, and the protein were created, the assemblies were embedded in a 5 Å layer of water to imitate solvent conditions. Finally, the docked complex of BMT with bergaptol was selected by the criteria of interacting energy combined with the geometrical matching quality. The global structure with the lowest energy was chosen for computing intermolecular binding energies. In general a higher Ludi score represents a higher affinity and a stronger binding of a ligand to the receptor, thus, the Ludi program was used to characterize the affinity and the binding preference of a ligand to the receptor.

3. Results and discussion

3.1. Sequence alignment and homology models validation

It should be noted that the sequences of amino acids determine the tertiary structure of a protein, as proposed by Anfinsen et al. [30]. High level of sequence can guarantee more accurate alignment between the target sequence and template structure. So BLAST search algorithm is used and the sequence identity between the BMT and the reference protein COMT (PDB code 1KYZc) is 65%, which allows straightforward sequence alignment (Fig. 1). All the side chains of model protein are set by rotamers. With this procedure, the initial model is completed. This model is refined by MM optimization and MD simulation, and then the final stable structure of BMT is obtained as displayed in Fig. 2. The root mean sequence deviation of the Cα atoms (Cα RMSD) between BMT and COMT (PDB code 1KYZc) is 0.69 Å, and this indicates a good overall structural alignment with COMT. We select, through the PROCHECK assessment, the best as the final 3D structure in terms of average stereochemical properties, which shows more than 75% of residues in most favored regions of the Ramachandran plot, 25% of residues in additional allowed regions (Table 1). For the X-ray structure of 1KYZc, it shows 85.4% residues in most favored regions, 12.9% of residues in additional allowed regions, 1.3% of residues in generously allowed regions, and 0.3% of residues in disallowed regions (Table 1). This result is comparable with high-quality crystallographic structure determined at a resolution of more than 2 Å (COMT) [18]. Moreover, the analysis with ProSa2003 program was used to perform on the template and the final model shows that their

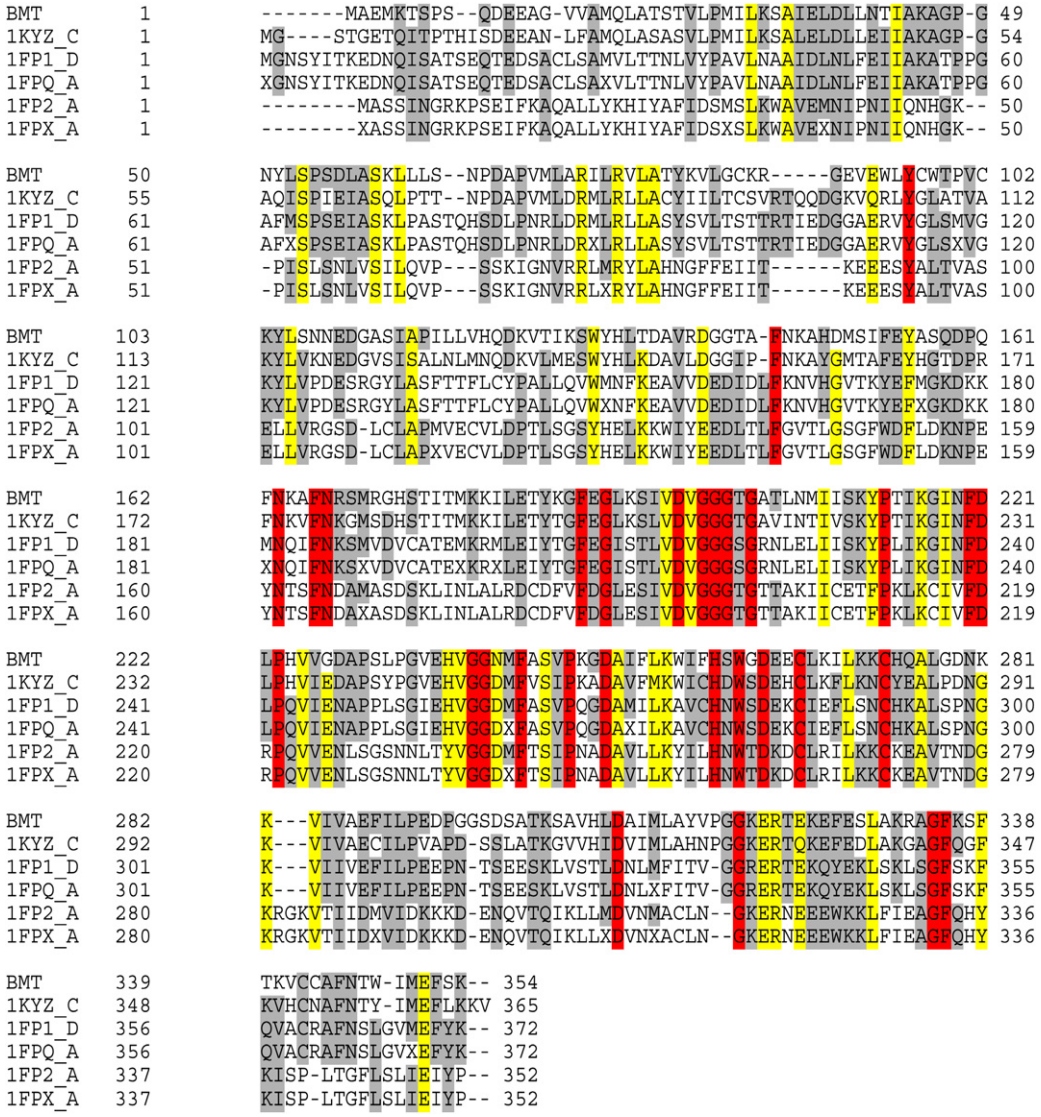

Fig. 1. Sequence alignment of amino acids of seven enzymes on secondary structure and sequence. BMT: bergaptol *O*-methyltransferase, 1KYZc: caffeic acid/5-hydroxyferulic acid 3/5-*O*-methyltransferase, 1FP1d: chalcone *O*-methyltransferase, 1FPQa: selenomethionine substituted chalcone *O*-methyltransferase, 1FP2a: isoflavone *O*-methyltransferase, 1FPXa: selenomethionine substituted isoflavone *O*-methyltransferase, 1TW3b: carminomycin-4-*O*-methyltransferase. Red boxes show identical residues, yellow boxes show chemically similar residues, and gray boxes show semiconserved substitutions. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Z-scores are comparable (from Table 1, BMT is −8.28, and COMT is −9.49), and these results mean that the model structure is consistent with their fold. From Fig. 3, with the same program to calculate the pseudoenergy profiles based on the knowledge-based mean field for the template and the model, we can find that they are similar. By checking with two different criteria mentioned above, we believe that the homology model is reliable.

3.2. Comparison of the homology model with the X-ray structure of COMT

By comparing the homology model with the X-ray structure of 1KYZ, the structure of BMT is found to be similar to those of previously characterized plant phenolic *O*-methyltransferases [12,16,17] (Fig. 2). The catalytic C-terminal domain consists of a core α/β Rossmann fold found in nucleotide binding proteins. This extensive β-sheet motif is shared by all structurally AdoMet-dependent methyltransferases [18]. The *N*-terminal domain contributes to the formation of the active site by providing residues that line the back wall of the substrate-binding cavity and help to enclose the recognition surface [14,18].

Since the superimposition of backbone of the two proteins gives the RMSD value of 0.69 Å, it can be considered that they have a high similarity. Comparison of the model of the 3D structure of BMT with the crystal structure of COMT shows similar distributions of secondary structures. In the secondary structure of BMT, there are 17-α-helixes, labeled H1−H17, a six-stranded mixed β-sheet, labeled βA1−βA6, and a two-stranded antiparallel β-sheet. Whereas COMT (PDB code 1KYZc) includes twenty α-helixes and nine β-sheets.

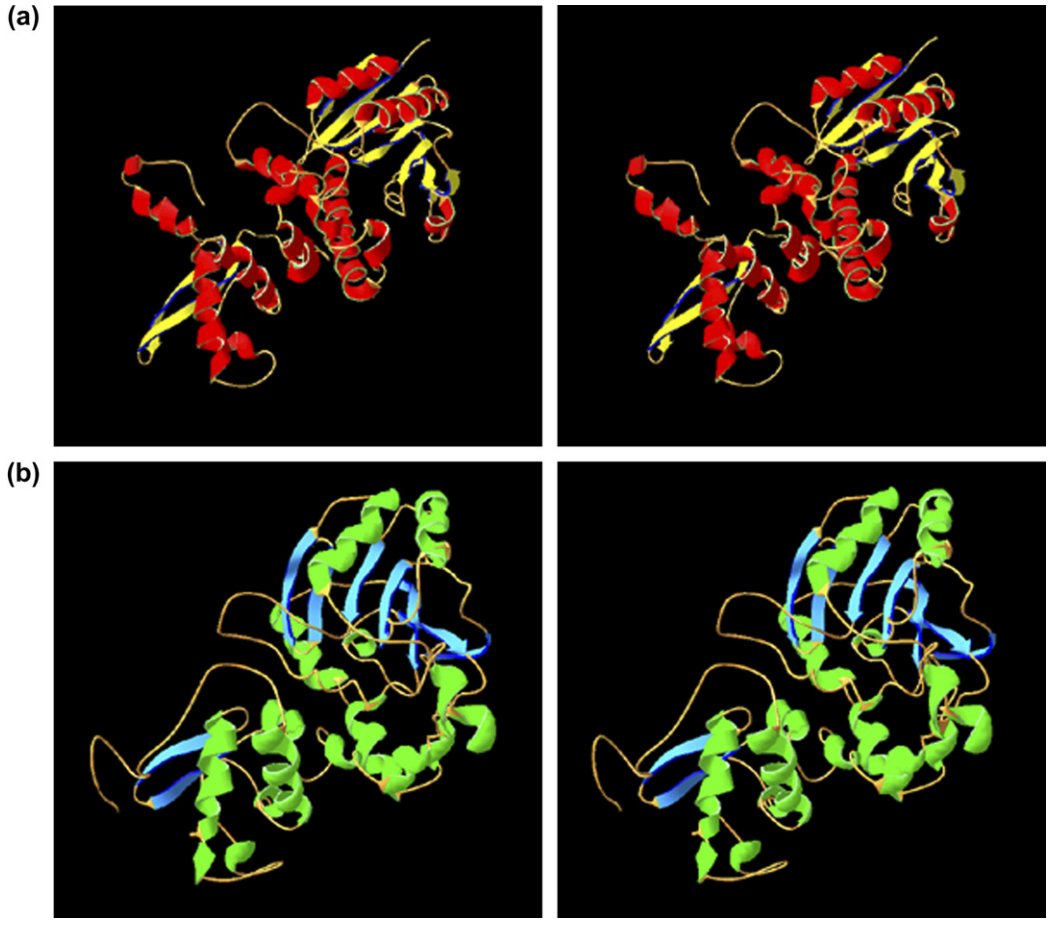

Fig. 2. (a) The 3D structure of COMT obtained by X-ray crystallography (1KYZc.pdb) and (b) the predicted structure of BMT based on homology-based modeling.

A topology diagram is shown in Fig. 4, which also shows the arrangement and labeling of the secondary structure of BMT. Among these, Helix 17, for COMT, includes one residue near the AdoMet binding site (Trp271). However, for BMT, the corresponding residue is Trp261 which is not positioned in the helix. As a result, Trp261 in BMT may be flexible. In order to investigate its flexibility, phi and psi dihedral angles are plotted against MD time, shown in Fig. 5a, in which the variations of phi and psi dihedral angles are placed within 170°. But for Glu265, phi and psi dihedral angles position in helix16 placed with 60° (Fig. 5b) do not show variation. Likewise, for Trp271 included in the helix of COMT, the dihedral angles are placed within 60° (Fig. 5c). As a result, the difference between the residues Trp271 in COMT included in helix 17 and Trp261 in BMT which are not included in a helix can make a difference in stability of AdoMet, and this can result in different binding affinities in COMT and BMT. This situation was also appeared in AtOMT [14].

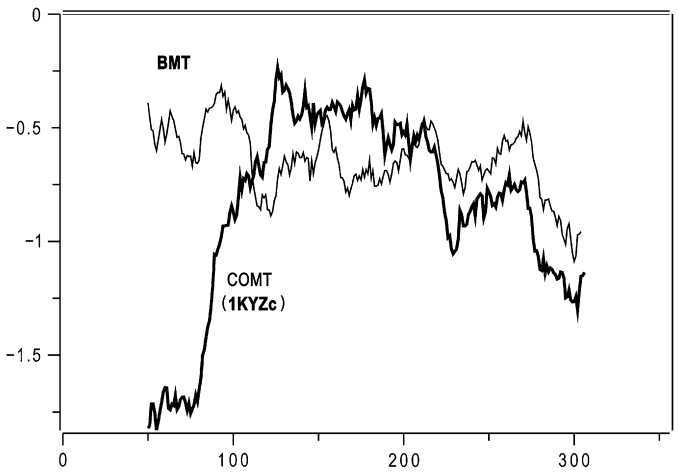

Fig. 3. Pair energy graph of COMT (PDB code 1KYZc) and BMT. It shows that they are strictly similar.

Table 1
Distribution of residues of the model in the Ramachandran plot after PROCHECK calculation and *Z*-scores evaluated with ProSa2003

Models	% Residues in the most favored regions (*A*,*B*,*L*)	% Residues in additional allowed regions (*a*,*b*,*l*,*ρ*)	% Residues in generously allowed regions (∼*a*,∼*b*,∼1,∼*ρ*)	% Residues in disallowed regions	Total	ProSa2003 *Z*-score
COMT	85.4	12.9	1.3	0.3	356	−9.49
BMT	75	25	0	0	354	−8.28

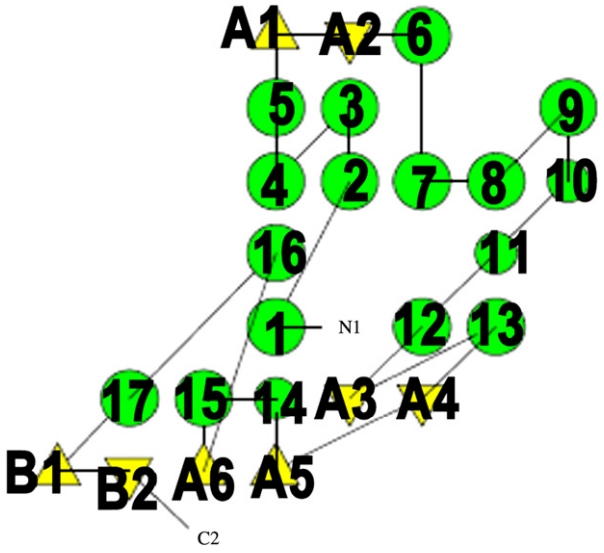

Fig. 4. Topology diagram showing the arrangement of secondary structural elements in BMT. Helixes are represented as circles and β-strands as triangles.

3.3. Identification of substrate-binding region and co-factor-binding region in BMT

OMTs have a common co-factor, AdoMet. BMT receives methyl group from AdoMet and catalyzes the methylation of bergaptol. The methylation results in the formation of AdoHcy and bergapten, respectively [1,15]. To find the binding sites between the protein and the substrate, InsightII/Binding site module is used. Among several binding sites given by InsightII/Binding site module, the most similar one to the binding site of COMT determined from the X-ray crystal structure is chosen. The cavity volume estimated by CASTp [28] is dependent on the radius of the probe sphere; a probe radius of 1.4 Å outlines a cavity of 197.1 Å^3 for BMT, while a probe radius of 1.4 Å outlines a cavity of 5327.0 Å^3 for COMT. The cavity volume measures by Binding site is dependent on the maximum distance between grid points exposed at the aperture to the cavity; the default value of 7 Å outlines a cavity of 87 Å^3 for BMT, whereas the default value of 7 Å outlines a cavity of 2517 Å^3 for COMT. From these results, we can conjecture that the active site of BMT is less spacious than that of COMT, and it can lead to substrate specificity between BMT and COMT.

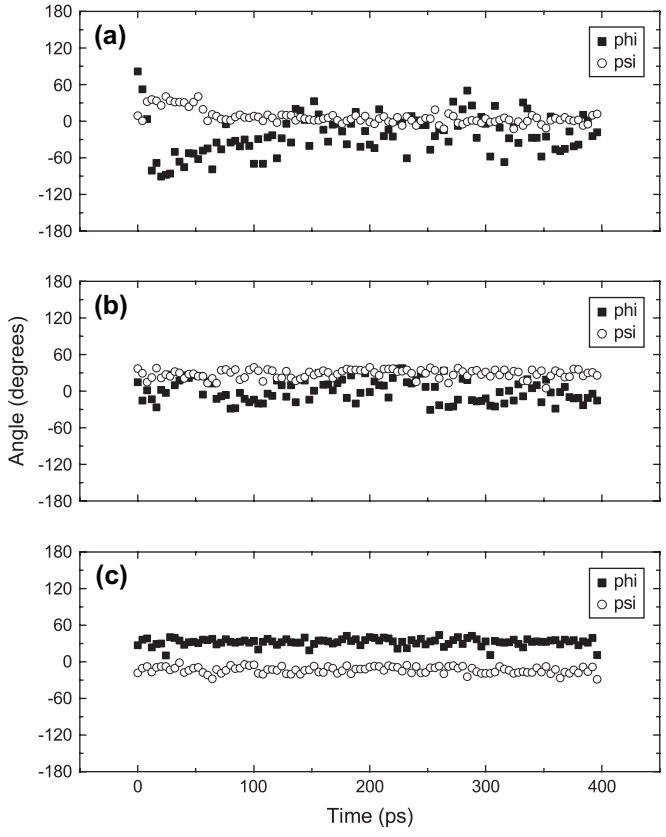

Fig. 5. Phi and psi dihedral angles of (a) Trp261, (b) Glu265 in BMT, and (c) Trp271 in COMT during MD.

Compared with the residues near the binding site of the COMT, the residues of BMT participating in the docking are selected as follows: Val120, His121, Phe166, Met170, His259, Phe288, Lys302, Leu307, Ile310, Met311, Val315, and Glu320. However, in the case of BMT, four residues (Val120, His121, Leu307, and Val315) surrounding the binding site of bergaptol are different from that in COMT as listed in Table 2. In order to confirm whether the binding site determination for bergaptol is correct, the residues surrounding the substrate-binding site of COMT as well as those of ChOMT and IOMT (they are also two AdoMet-dependent methyltransferases) [15–18] are listed in Table 2 for comparison. From Table 2, we can find that all the residues surrounding the binding site of four enzymes are similar, so we believe that the binding site of bergaptol in BMT is reasonable.

On the other hand, the residues acted as a gate for the entrance of the substrate are important for substrate specificity [14]. It is known that the active site of BMT is buried and the enzyme must undergo a conformational change in order to go through a long channel for the substrate to reach the active site. The characteristics of the entrance surface, such as the shape, the size, or the static potential etc., are important factors for the enzymatic activity of methyltransferases. In COMT, Gly324 and Asp268 are the corresponding residues to the gate of the cleft. Likewise, two residues, Ser260 and Gly317, corresponding to the gate of the cleft are observed in BMT. Fig. 6 shows the shape and the hydrophobic quality of the entrance for substrate accessing on the surface of BMT. In BMT, serine is substituted by Asp and switching of this single residue causes a change of the volume within the entrance site. Asp268 in COMT has more methylene group than Ser260 in BMT. The difference between their molar volumes can change the entrance size and can further influence the substrate specificity. In addition, Ser260 is near the catalytic residue His259. When the bergaptol, which is larger than the caffeic acids, enters into the cleft via serine, it may encounter less sterically block during the catalytic process compared to Asp residue in the gate.

It was reported that recognition and sequestration of AdoMet/AdoHcy were highly conserved in all AdoMet-dependent methyltransferases and the involved residues form a readily recognizable signature motif [15]. From Table 2, we can

Table 2
A comparison of residues' neighboring binding sites

Enzyme								
	Residues neighboring the binding site of AdoHcy							
COMT	Asp206	Gly208	Asp231	Leu232	*Asp251*	Met252	Lys265	Trp271
BMT	Asp196	Gly198	Asp221	Leu222	*Asn241*	Met242	Lys255	Trp261
IOMT	Asp194	Gly196	Asp219	Arg220	Asp239	Met240	Lys253	Trp259
ChOMT	Asp215	Gly217	Asp240	Leu241	Asp260	Met261	Lys274	Trp280
	Residues neighboring the binding site of substrate							
COMT	*Met130*	*Asn131*	Phe176	Met180	*Ile316*	Ile319	Met320	*Asn324*
BMT	*Val120*	*His121*	Phe166	Met170	*Leu307*	Ile310	Met311	*Val315*
IOMT	Cys117	Val118	Phe164	Met168	Met307	Asn310	Met311	Leu314
ChOMT	Phe318	Leu319	Phe185	Met189	Leu325	Leu328	Met329	Thr332

also see that Asp251 participating in the AdoMet binding site of COMT is switched with Asn241 in BMT. Although the hydrogen bonding of AdoMet is highly conserved, the hydrophobic and aromatic residues interacting with AdoMet can vary among methyltransferases, similar to the results of several energetically favorable sets of van der Waals interaction with the adenine ring [15]. Therefore, the substitution of Asn241 in BMT which has neutral group for Asp251 in COMT which has negative charged group may cause the different binding affinities and BMT.

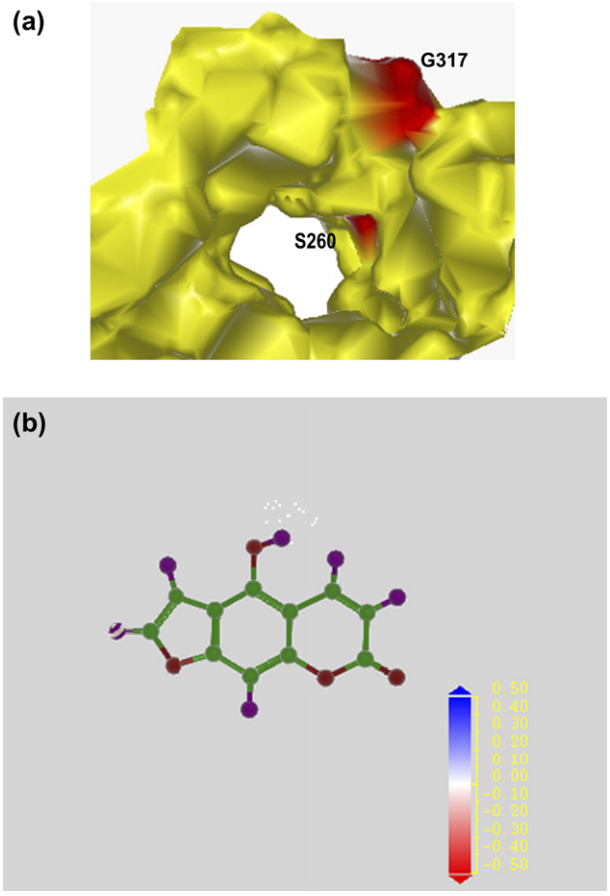

Fig. 6. (a) The solvent accessible surfaces of the entrance for substrate accessing on the enzymes' surface and (b) the structure of the bergaptol optimized by AM1 method, colored by surface static potential. Dots point to the site which can be hydroxylated by the enzyme.

3.4. Substrate specificity of BMT

As mentioned before, COMT recognizes caffeic acid as well as caffeoyl alcohol, caffeoyl aldehyde, 5-hydroxyferulic acid, 5-hydroxyconiferyl alcohol, and 5-hydroxyconiferaldehyde [18,19,31]. However, only bergaptol is accepted as a substrate by BMT [1]. This substrate specificity for BMT can be explained by the substrate affinity of BMT to AdoMet and bergaptol based on its 3D structure and docking experiment, and by comparative docking of 5-hydroxyferulate and caffeate with BMT. The protein–ligand structures are analyzed by using Ludi method, and the results show that the global structure with the lowest energy which is chosen to compute intermolecular binding energies has higher Ludi score.

It was reported that the substrate hydroxyl group residues 7–9 Å away from the donor methyl moiety of AdoMet can be helpful to the transmethylation process of COMT [14,15]. The transportation of the methyl group from sulfur of AdoMet to the hydroxyl groups of bergaptol, 5-hydroxyferulate, and caffeate is also studied. While the distance between sulfur of AdoMet and hydroxyl group of bergaptol is 6.61 Å, the distance between sulfur of AdoMet and 5-methoxy group of 5-hydroxyferulate in BMT is 17.65 Å, and the distance between sulfur of AdoMet and 3-methoxy group of caffeate in BMT is 18.56 Å. These results indicate that the transportation of the methyl group from sulfur of AdoMet to hydroxyl group of the bergaptol is easier than that of 5-hydroxyferulate or caffeate.

Compared with BMT and COMT, four residues in active site are changed as shown by Table 2 (Val120, His121, Leu307 and Val315 in BMT; Met130, Asn131, Ile316 and Asn324 in COMT). For the four mutation residues of BMT, Val120 is a small aliphatic residue and situated near the end of psoralen ring of substrate, where it can stabilize the hydrophobic moieties of potential substrates. So this side chain will not interfere with the small phenolic ring group, such as 5-hydroxyferulate and caffeate. Specifically, the Asn324 in COMT which can interact with hydroxyl groups, most likely assisted in the repositioning of the phenolic ring closer to AdoMet molecule and in the positioning of the hydroxyl moiety for transmethylation, changes to Val315 in BMT. Val315 is a hydrophobic residue. So in BMT, the necessity of accommodating the substrate, bergaptol, in a more hydrophobic environment can help to prevent repositioning of the substrate and thus can proceed

Table 3
The calculated energies (kcal mol^{-1}) of the ligand tested for BMT binding

Ligand	E_{vdw} (kcal mol^{-1})	E_{ele} (kcal mol^{-1})	E_{total} (kcal mol^{-1})	Ludi score	No. of H-bonds	Relative activity[a] (%)
Bergaptol	−47.39	−5.61	−53.00	516	1	100
5-Hydroxyferulate	−34.31	−6.90	−35.21	210	0	<1
Caffeate	−38.68	−1.14	−39.82	223	0	<1

[a] Proposed by Hehmann et al.

the transportation of the methyl group to hydroxyl group of the bergaptol. To sum up, the changes of four residues in active site increase the hydrophobic region of the active pocket of BMT, and this can make the neutral bergaptol easily to slide into the active pocket.

In order to compare with the substrate-binding affinity of BMT, the Ludi program is used and the results are listed in Table 3. From Table 3, we can see that the total interaction energy between bergaptol–BMT (−53.00 kcal mol^{-1}) is lower than those of 5-hydroxyferulate–BMT (−35.21 kcal mol^{-1}) and caffeate–BMT (−39.82 kcal mol^{-1}). It is reported that the phenolic substrates, not the AdoMet, undergo dynamic shifts as transmethylation proceeds [15]. So the largely van der Waals substrate–protein interactions allow a high degree of substrate sliding and conform to the general shape of phenolic skeleton [14,15,18]. The total interaction energy between 5-hydroxyferulate–BMT and caffeate–BMT is high, and the high energy indicates that these complexes are not stable and thus cannot undergo this process. So only bergaptol is accepted as substrate by BMT. In addition, Ludi score for bergaptol–BMT (516) is higher than 5-hydroxyferulate–BMT (210) and caffeate–BMT (223). This indicates that bergaptol has higher affinity and stronger binding than those of 5-hydroxyferulate and caffeate. This result is consistent with the experimental facts [1].

In order to explore the effect of the site mutation in which Val120, His121, Leu307, and Val315 are replaced by Met, Asn, Ile, and Asn (V120M, H121N, L307I, and V315N), the complexes of mbergaptol–BMT, m5-hydroxyferulate–BMT, and mcaffeate–BMT are developed by affinity module. The Ludi program is used and the results are listed in Table 4. From Table 4, we can see that the total interaction energy between mbergaptol–BMT (−37.59 kcal mol^{-1}) is higher than those of m5-hydroxyferulate–BMT (−54.10 kcal mol^{-1}) and mcaffeate–BMT (−64.52 kcal mol^{-1}). In addition, Ludi score for mbergaptol–BMT (203) is lower than m5-hydroxyferulate–BMT (326) and mcaffeate–BMT (387). So these site mutations may influence the substrate specificity of BMT, which agrees with the result that was previously inferred by experimental study [18].

Table 4
The calculated energies (kcal mol^{-1}) of the ligand tested for BMT (V120M, H121N, L307I, V315N) binding

Ligand	E_{vdw} (kcal mol^{-1})	E_{ele} (kcal mol^{-1})	E_{total} (kcal mol^{-1})	Ludi score
Bergaptol	−32.50	−5.09	−37.59	203
5-Hydroxyferulate	−48.75	−5.35	−54.10	326
Caffeate	−39.81	−24.71	−64.52	387

From the docking studies, we observe a network of hydrogen bonding interaction with BMT, which, in turn, stabilized the docked structure. There is one hydrogen bond between BMT and bergaptol (Fig. 7a). The hydroxyl group of the substrate, bergaptol, is involved in potential hydrogen bond

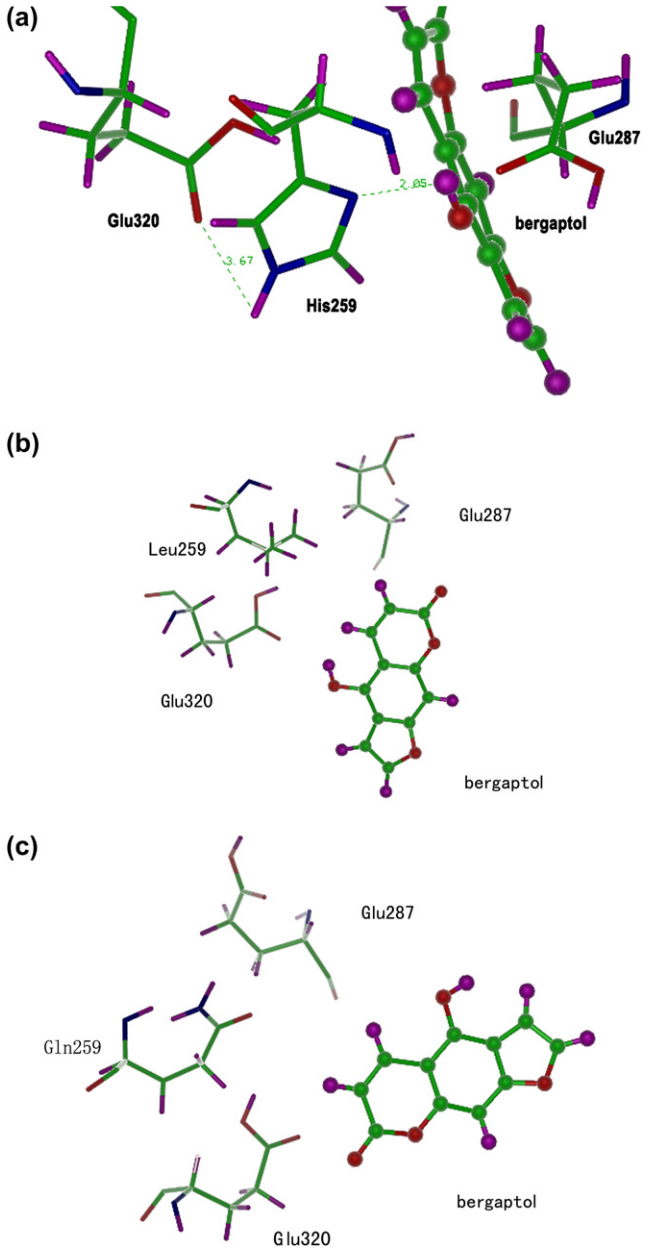

Fig. 7. (a) Schematic drawing of interaction between BMT and bergaptol, (b) BMT(H259L) and bergaptol, and (c) BMT(H259Q) and bergaptol hydrogen bonds is shown as green dashed line. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

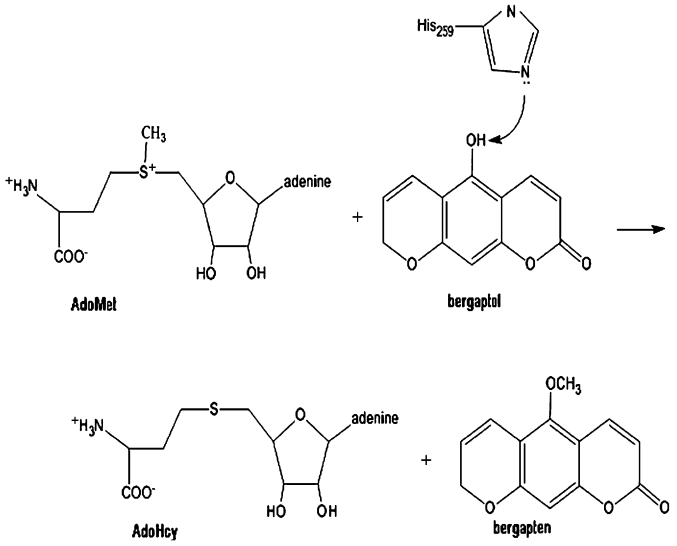

Fig. 8. Reaction catalyzed by BMT.

interactions with His259 (His259—N···HO, 2.05 Å, −1.66 kcal mol^{-1}). This interaction can be helpful to the optimal orientation of the imidazole group for deprotonation of the hydroxyl group of the substrate by Nε in His259. Based upon both the structures of BMT and COMT and the sequence alignment with the large family of plant OMTs, methylation most likely proceeds via base-assisted deprotonation of the hydroxyl group followed by a nucleophilic attack on the reactive methyl group of AdoMet (Fig. 8). In BMT, deprotonation of the bergaptol by His259 set up the subsequent attack by the hydroxyl anion on the methyl group of AdoMet. Because the sulfur of AdoMet is positively charged, the transmethylation process is easily facilitated by the deprotonation step. Such linear arrangement of the nucleophile, the methyl group and the leaving thioester group in transition state is required for the classic S_N2 reaction mechanism used by most other OMTs [14,15,18]. Glu287 and Glu320 bracket the catalytic His259, and the Nδ nitrogen of His259 makes a weak hydrogen bonding interaction with carboxylate group of Glu320 (Fig. 7a). The putative role of His as a catalytic base has only been seen in another structurally characterized methyltransferase, PRMT3 (protein arginine *N*-methyltransferase) [32]. The role of His in BMT is similar to that of His in the reaction mechanism proposed for PRMT3, which uses a His-Asp proton relay system. Mutation of His259 to Leu and Gln will completely eliminate methyltransferase activity. From Fig. 7b and c, we concluded that either Leu or Gln cannot form a hydrogen bond with the substrate, and the substrate cannot deprotonate, so mutation of the active site His259 to Leu or Gln completely eliminates methyltransferase activity, implicates that His259 is an important catalytic residue. This result is consistent with the experimental facts [18].

4. Conclusion

The 3D structure of BMT is obtained by homology modeling by taking COMT as a template. Then the model structure is refined by the energy minimization and molecular dynamics simulation. The 3D structure of BMT is stable after the molecular dynamics simulation and the reliability is assessed by PROCHECK and ProSa2003 module. The putative binding pocket of BMT is determined by binding site search module, which is helpful for the realization of the experiment results. The docking studies show that bergaptol has higher affinity and stronger binding than those of 5-hydroxyferulate and caffeate, and only bergaptol is accepted as substrate by BMT. This result is consistent with the experimental facts. Even though there are only four amino acid differences in the residues near the substrate-binding sites of COMT and BMT, MET130, Asn131, Ile316, and Asn324 instead of Val120, His121, Leu307, and Val315, respectively, both enzymes exhibit distinct substrate specificities. We also docked the substrates into the mutation enzymes (V120M, H121N, L307I, V315N) and found that the substrates specificity is changed. The reason conjectures that four amino acid differences in the binding site are sufficient to determine substrate specificity. Our results show that His259 is the catalytic base for the reaction, and Glu320, Glu287 bracketed the catalytic His259. Especially, Glu320 can form a weak hydrogen bond with His259 and promote transfer of an H ion.

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