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Importance of Correlated Motions in Forming Highly Reactive Near Attack Conformations in Catechol *O*-Methyltransferase

Edmond Y. Lau and Thomas C. Bruice*

Contribution from the Department of Chemistry, University of California, Santa Barbara, California 93106

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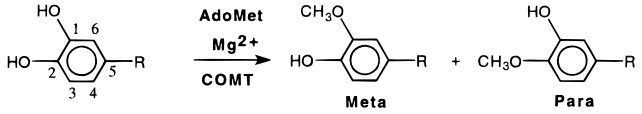
Abstract: The monomeric protein catechol *O*-methyltransferase (COMT) of rat liver is one of a number of methyl, from AdoMet, transferring enzymes that share a common catalytic domain. As a representative enzyme, COMT has been chosen for molecular dynamic (MD) simulation studies in order to ascertain if there are correlated motions in the ES complex that would be useful in decreasing the energy of activation for the S_N2 methyl transfer reaction. Such correlative motions have been found and are discussed. The MD trajectory can also provide insight into the observed preference of *meta*-O-methylation over *para*-O-methylation for ionic substrates such as dopamine and norepinephrine. The catechol ring has a tilt of approximately 30° compared to that of the X-ray structure. This directs any substituent at the 5-position of the catecholate ring, which is *para* to the O-methylation site, into a hydrophobic pocket formed by Trp38 and Tyr200. This pocket accommodates hydrophobic substituents such that *para*-O-methylation is favored. Polar substituents are repelled by this hydrophobic pocket making *meta*-O-methylation favorable.

Introduction

Catechol *O*-methyltransferase (COMT, EC 2.1.1.6) catalyzes the transfer of a methyl group from AdoMet to one hydroxyl oxygen of catechol, forming O-methylated catechol and *S*-adenosyl-L-homocysteine (Scheme 1). COMT is one of a wide variety of AdoMet requiring methyltransferases. Methyltransferases are known which methylate DNA, RNA, proteins, and small molecules. Comparisons between the catalytic domains of the DNA methyltransferases M·*HhaI*, M·*TaqI*, and catechol *O*-methyltransferase suggest that AdoMet-dependent methyltransferases share a universal catalytic domain structure.[1−4] All three enzymes have α/β folds which contain a central mixed β-sheet around which several α-helices are arranged. There are also several residues in the binding site for AdoMet in each methyltransferase which are in the same relative position and form similar contacts with the cofactor.

Scheme 1



The reaction mechanism of COMT has been studied in great detail in model systems by Coward and co-workers.[5] The transmethylation reaction occurs via a general-base-catalyzed proton abstraction from either one or both of the catechol

* To whom correspondence should be addressed. E-mail: tcbruice@bioorganic.ucsb.edu.

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hydroxyl groups concerted with a catecholate-O^- S_N2 displacement of S< from AdoMet. In the enzymatic reaction a Mg^{2+} ion is required for catalytic activity.[6] Schowen and co-workers showed the S_N2 displacement to be rate determining and the S_N2 transition state was found to be symmetric and tight from measured α-deuterium and carbon-13 isotope effects.[7] Zheng and Bruice have determined the geometries, activation barriers, and isotope effects for methyl transfer from trimethyl sulfonium to catecholate in the gas phase and in solution by quantum chemical calculations.[8] Their calculated isotope effects are in excellent agreement with those determined for COMT. This indicates that the transition-state structure in the gas phase is much the same as the one within COMT. Due to the similarity of the catalytic domain and the relative positioning of the active site residues of the methyltransferases, we believe that a detailed understanding of the reaction mechanism of COMT may provide valuable insights into the other two methyltransferases and to AdoMet-dependent transmethylating proteins in general.

There is interest in the role of dynamic motions in the enzyme structure as a possible feature in their catalytic mechanisms. Atomic displacements from nuclear motions[9] to domain motions[10] have been studied in proteins. We report here our studies of the molecular dynamics (MD) of a catechol *O*-methyltransferase·catecholate complex. The COMT of rat liver was chosen for our study because it is monomeric, it consists of only 221 residues, and a crystal structure of the enzyme containing AdoMet and the competitive inhibitor 3,5-dinitrocatechol has been solved at 2.0 Å.[3] Our study reveals that the observed correlated motions between catechol, AdoMet, and the side chain of Tyr68 are important in the formation of near attack conformations in this methyltransferase.[11]

Theoretical Procedure

A 1.05 ns simulation of COMT with catecholate in the active site was performed using the program CHARMM (version 25b2).[12] The starting structure used for COMT was the crystal structure with 3,5-dinitrocatechol at the active site (Brookhaven Protein Data Bank entry name 1VID). A catechol monoanion was placed into the active site by overlaying the structure on the inhibitor 3,5-dinitrocatechol, once coordinates for the catechol monoanion were generated the inhibitor was removed. The hydroxyl group on the catechol closest to the methyl group of the AdoMet was ionized. Both oxygens of the catechol and the closest oxygen from Asp141, Asp169, Asn170, and Wat400 were explicitly bonded to the Mg^{2+} ion to complete the octahedral coordination (Figure 1). Partial atomic charges were assigned to the Mg^{2+} ion, the coordinating residues, and the AdoMet from a PM3 calculation.[13] CHARMM was used to add the appropriate number of hydrogens to the heavy atoms of the enzyme−substrate (ES) complex and the crystallographic waters. This ES complex was solvated in a cube of TIP3P water 62.2 Å along each axis.[14] If the oxygen of a water molecule was within 2.3 Å of any atom of the ES complex, the water molecule was deleted. The water pool and ES consisted of 24 209 atoms. The potential energy of the system was minimized using a combination of the steepest descents and adopted-basis Newton−Raphson methods.[12] A MD simulation was performed on the energy minimized system. The system was heated to 300 K in 5 ps and equilibrated for 45 ps. Production dynamics were performed for 1 ns. The system was treated as a microcanonical (NVE) ensemble. The equations of motion were integrated using the Verlet leapfrog algorithm with a time step of 2 fs.[15] The nonbonded list was updated every 20 time steps and nonbonded interactions were cut off using a force-shifting function for the Coulombic term at 13 Å and a switching function for the van der Waals term between 11 and 13 Å.[12] Periodic boundary conditions were used to simulate a continuous system and the SHAKE algorithm was used to constrain bonds containing hydrogens to their equilibrium length.[16] Coordinates were saved every 100 time steps. The 1 ns of production dynamics was used for all analysis.

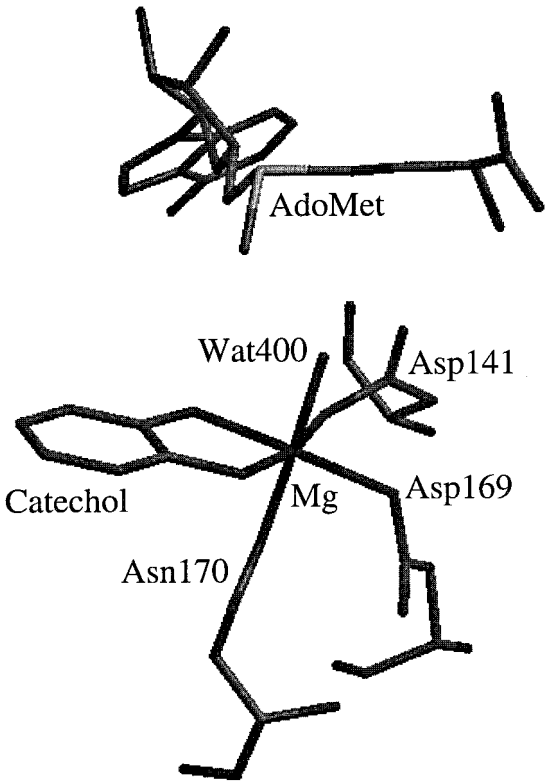

Figure 1. Active site of catechol *O*-methyltransferase showing the coordination of the Mg^{2+} ion from the minimized starting structure.

Results and Discussion

The calculated root-mean-squared deviation (RMSD) of the catechol *O*-methyltransferase backbone atoms (N, C, and Cα) from the trajectory relative to the crystal structure rose quickly initially in the simulation but plateaued after 200 ps; it then fluctuated between 1.7 and 2.1 Å during the remainder of the trajectory (Figure 2). The positional fluctuations of the Cα in the protein backbone were calculated from the MD trajectory and compared to those obtained from the Debye−Waller *B*-factors of the crystal structure (Figure 3) using the equation $\langle \Delta r^2 \rangle^{1/2} = (3B/8\pi^2)^{1/2}$.[17] The positional fluctuations from the simulation are in good qualitative agreement with those obtained by crystallography. Regions which show flexibility in the crystal structure are well-reproduced in the trajectory although the magnitude of the fluctuations for the Cα in the simulation tends

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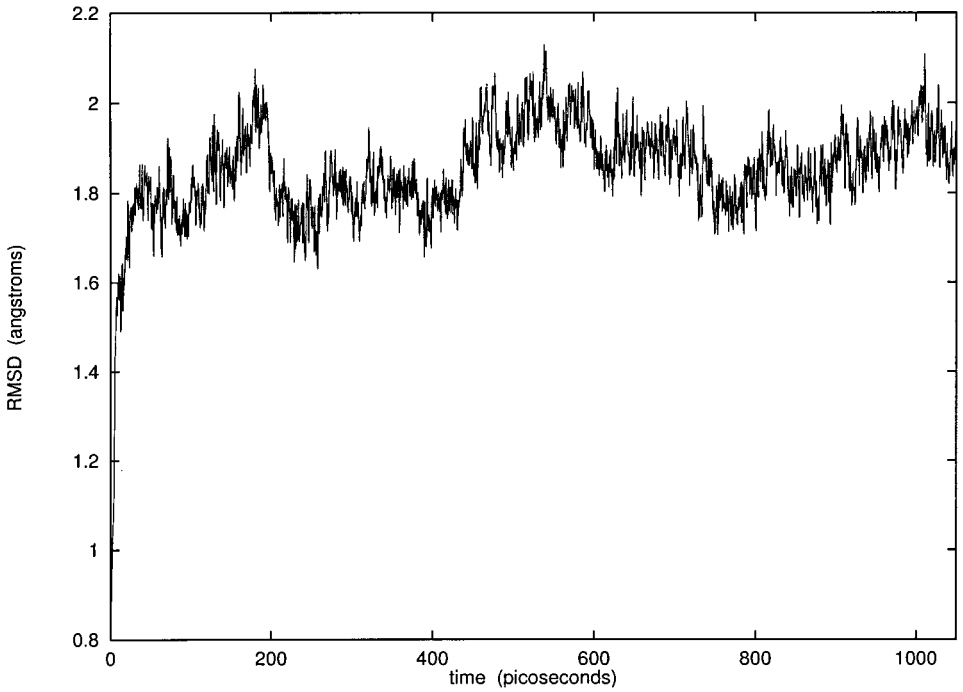

Figure 2. Root-mean-squared deviation (RMSD) of the heavy atoms (N, C, and Cα) in the backbone of COMT relative to that of the crystal structure.

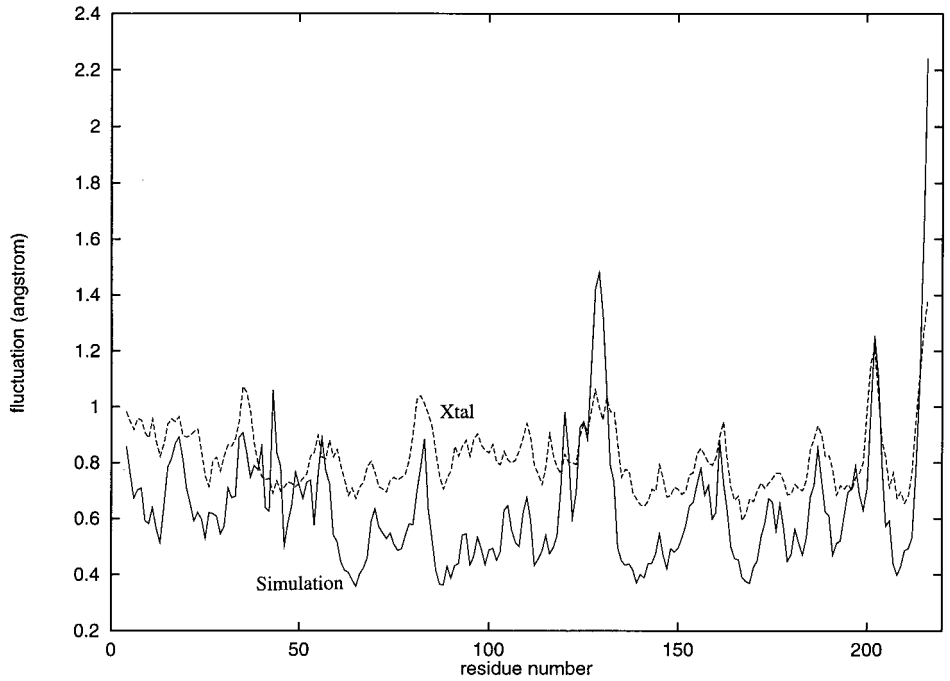

Figure 3. Positional fluctuation of each Cα in COMT determined by X-ray crystallography (Xtal, dashed line) and MD simulation (solid line).

to be less than those from crystallography. This discrepancy could be due either to insufficient sampling of all possible conformations of COMT within the time scale of the simulation or to static (lattice) disorder within the crystal.[18,19] The crystal structure was solved at 2.0 Å and could contain lattice defects which would increase the *B*-factors. This increased *B*-factor when converted to positional fluctuations will have an enhanced value in which only a portion of the value is actually from thermal motions. Only residues 127−131 in the simulation showed motions much greater than those from crystallography. These residues form a turn on the surface of the enzyme. In the simulation, the turn is solvent-exposed and is expected to undergo large scale motions. It is likely that the turn would have its motions attenuated by packing forces when in a crystal.

With these caveats, we believe that the local motions sampled by the COMT−catecholate complex in this MD trajectory are reasonable. Catechol *O*-methyltransferase accelerates the transfer of the methyl group of AdoMet to catechol by 10^{16} relative to the reaction in solution.[7b] The catalytic efficacy of the enzyme is governed by how often the nucleophile and electrophile are

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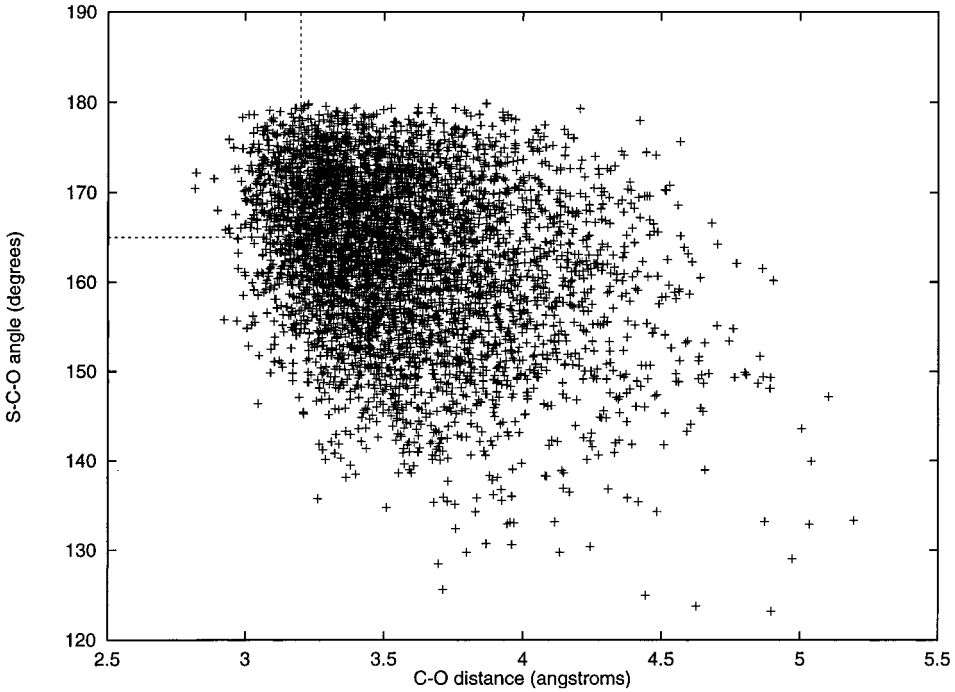

Figure 4. Conformations sampled by the methyl carbon of AdoMet and ionized hydroxyl oxygen of catechol during the simulation. Structures within the box formed by the dashed lines are NACs.

present in *n*ear *a*ttack *c*onformations, (NACs).[11] A NAC is an energy minimum structure that geometrically must be formed prior to reaching a transition state in a reaction pathway. The criterion for forming a NAC in COMT was a separation of less than 3.2 Å between the catecolate-O^- nucleophile and the carbon of the $CH_3-S^+<$ moiety of AdoMet in combination with an angle greater than 165° formed by the sulfur and carbon of $CH_3-S^+<$ in AdoMet and the ionized oxygen of catechol (S−C···O). A similar criterion was used for NACs in the formation of cyclic anhydrides.[11] Of the 5000 structures collected during production dynamics, 378 contained NACs. The majority of the NACs are generated between 400 and 900 ps of the simulation. Figure 4 graphically shows that there are many other structures which are just beyond the criterion for a NAC. Indeed, the average catecholate-O^- to methyl C distance and S−C···O angle for the MD simulation were 3.55 ± 0.33 Å and 163.3 ± 8.9°, respectively.[20] The average structure sampled during the nanosecond is almost a NAC. Although only ∼7.6% of the conformations sampled within this trajectory will lead to transmethylation, the production of NACs within COMT is likely not rate limiting since the measured turnover for the enzyme (k_{cat}) is 24 min^{-1}.[21] Salient features of a NAC structure in COMT are provided in Figure 5 and discussed below.

When removed from solvent and bound within COMT, the geometry of catechol is restricted by its coordination to the Mg^{2+} ion, and the motions of AdoMet are hindered by the protein. The positional fluctuations for the heavy atoms of AdoMet ranged from 0.18 to 0.57 Å in the simulation and are in good agreement with crystallography (Table 1). COMT is able to

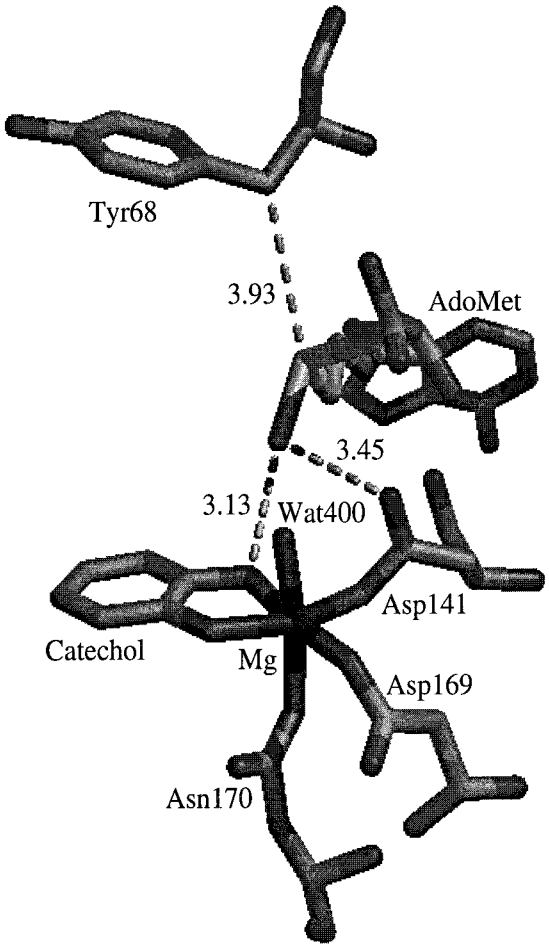

Figure 5. Near attack conformation formed in COMT at 591.2 ps in the trajectory. The numerical values correspond to the distances, in angstroms, for C···O (3.13), S···CB (3.93), and AdoMet methyl carbon to Asp141 OD1 (3.45). The atoms C2*, C3*, and the attached oxygens have been removed from AdoMet for ease of viewing.

(20) Interestingly, the C···O distance and S−C···O angle formed by AdoMet and the inhibitor 3,5-dinitrocatechol (DNC) in the crystal structure are 2.63 Å and 173.2°, respectively. The C···O separation of 2.63 Å is only 0.47 Å greater than the separation found in the calculated transition-state structure for catecholate and trimethyl sulfonium by Zheng and Bruice (ref 8). The difference between C···O separations of the crystal structure and that of the simulation is likely due to the charge state of the substrate. We deal with a catechol monoanion COMT complex, whereas the X-ray structure is of a COMT dinitrocatecholate dianion.

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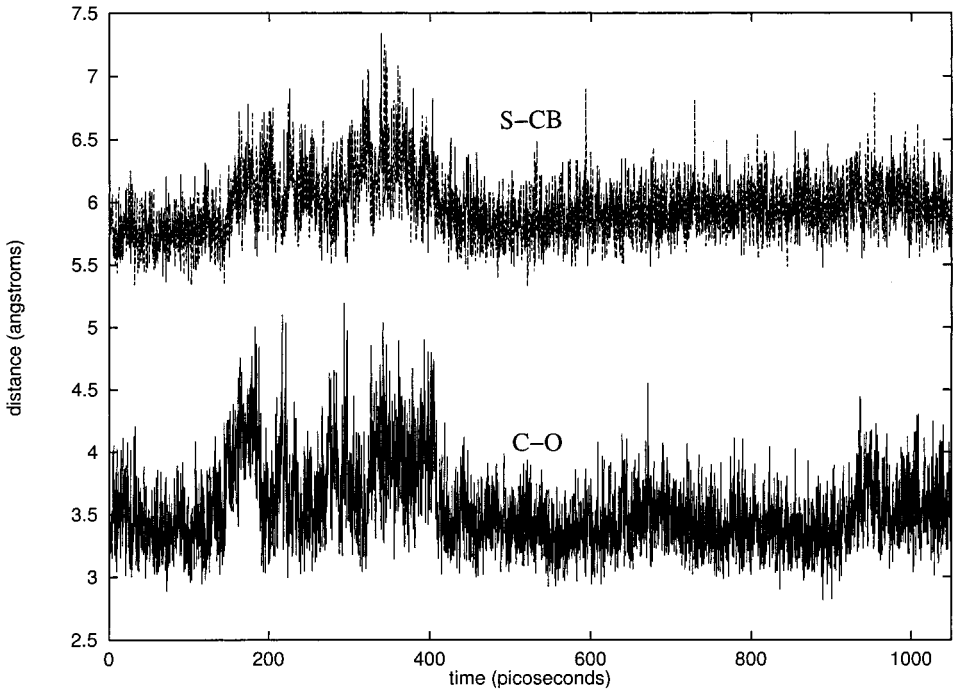

Figure 6. Separation between the methyl carbon of AdoMet and charged hydroxyl oxygen of catechol (C···O) and sulfur of AdoMet and CB of Tyr68 (S···CB) during the simulation. The S···CB separation has been offset by 2 Å for ease of viewing.

Table 1. Positional Fluctuations for Non-Hydrogen Atoms of AdoMet[a]

	MD	Xtal
N	0.38	0.65
CA	0.34	0.66
C	0.44	0.70
O	0.52	0.73
OXT	0.57	0.73
CB	0.33	0.71
CG	0.29	0.70
SD	0.32	0.72
CE	0.43	0.64
C5*	0.28	0.75
C4*	0.22	0.74
O4*	0.22	0.77
C3*	0.24	0.75
O3*	0.32	0.74
C2*	0.23	0.75
O2*	0.31	0.82
C1*	0.19	0.77
N9	0.19	0.75
C8	0.31	0.68
N7	0.33	0.75
C5	0.21	0.76
C6	0.25	0.76
N6	0.36	0.78
N1	0.34	0.75
C2	0.39	0.74
N3	0.33	0.75
C4	0.18	0.73

[a] Values are in angstroms.

accommodate the charge of the catecholate not only by coordination to the Mg^{2+} ion but also by formation of a salt bridge between the catecholate-O^- and protonated NZ of Lys144. This interaction is stable throughout the simulation (3.03 ± 0.23 Å). The other hydroxyl group of catechol forms a hydrogen bond with OE1 of Glu199 which is stable throughout the simulation. The charge on the carboxylate of Glu199 is neutralized by formation of a salt bridge with Lys46. Two interactions appear to be very important in orienting the methyl group of AdoMet to the ionized oxygen of catechol. The carboxylate oxygen of Asp141, not coordinated to the Mg^{2+} ion, forms close contacts with both the carbon and sulfur in $CH_3-S^+<$ of AdoMet, 3.46 ± 0.26 and 4.12 ± 0.31 Å, respectively during the simulation. The opposing charges of the AdoMet and Asp141 form a stabilizing interaction which serves to orient the cofactor into a NAC. A similar arrangement is seen in the crystal structure of glycine-*N*-methyltransferase (GNMT) from rat liver. The OE1 of Glu15B points to the positively charged sulfur of AdoMet. The separation between these two atoms in GNMT is 4.0 Å, which is much the same as that observed in COMT.[22]

There are close contacts between the catecholate-O^- to C of $CH_3-S^+<$ (C···O) and AdoMet sulfur to the methylene carbon (CB) of Tyr68 (S···CB). The latter is positioned directly above both the AdoMet and catechol in COMT. The amount of conformational space sampled by these four atoms is intertwined. The S···CB distance is correlated with the C···O distance and appears to restrict the C···O distance (eq 1 and

$$\begin{array}{l} R-O^- \overleftarrow{} CH3-S^+< \overrightarrow{} CB \\ \qquad\qquad R-O^- \overrightarrow{} CH_3-S^+< \overleftarrow{} CB \end{array} \quad (1)$$

Figure 6). Equation 1 illustrates the change in directions for the CB of Tyr68 and the catecholate-O^- relative to the $CH_3-S^+<$ moiety when large scale motions occur. In the time frame of 50 to 150 ps, both the C···O and S···CB distances are short, 3.44 ± 0.26 and 3.78 ± 0.16 Å, respectively. Although the sampling is limited, it can be seen from Figure 7A that the conformation space sampled is in a confined region. A total of 63 NACs are formed in this span of 100 ps. Between 150 and 400 ps, there is an increase in the distances for C···O and S···CB, 3.84 ± 0.38 and 4.18 ± 0.27 Å, respectively which leads to many nonreactive conformations. A much broader region of

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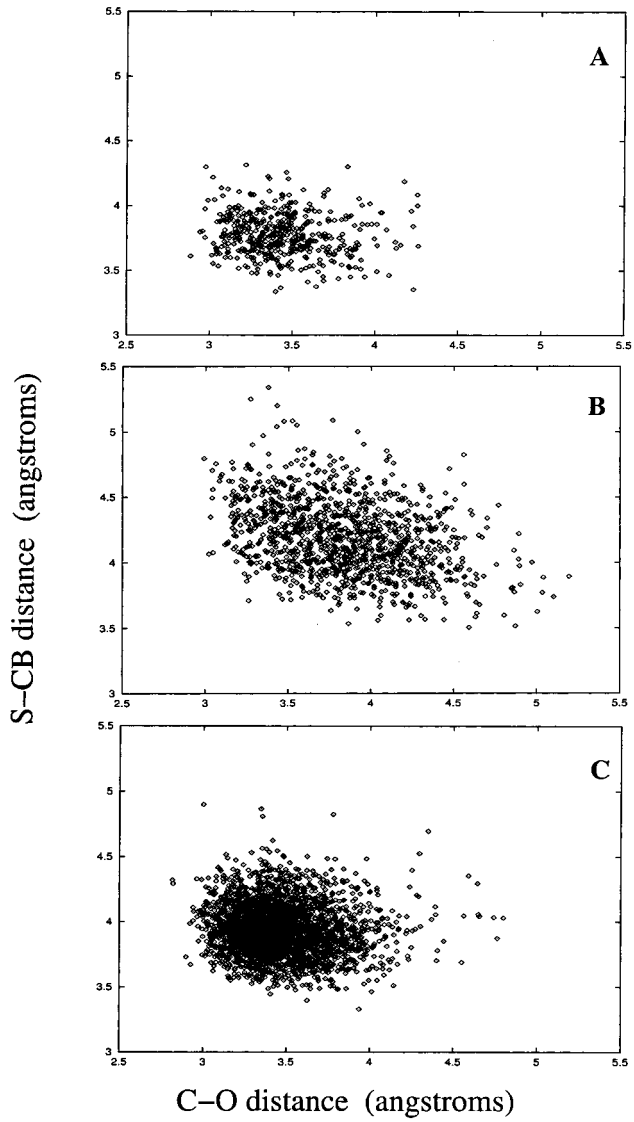

Figure 7. Plot of the correlation between the methyl carbon of AdoMet and charged hydroxyl oxygen of catechol (C···O) and sulfur of AdoMet and CB of Tyr68 (S···CB) distances during the simulation from 50 to 150 ps (A), 150 to 400 ps (B), and 400 to 1050 ps (C).

conformation space is sampled during this time period (Figure 7B), but this only leads to 30 NACs. After 400 ps, both the C···O and S···CB distances decreased to 3.46 ± 0.25 and 3.94 ± 0.19 Å, respectively, for the remainder of the simulation which again allows formation of numerous NACs. It can be seen in Figure 7C that the C···O and S···CB distances have returned to the confined region of conformation space and are correlated. A total of 285 NACs are formed after 400 ps. The correlation of C···O and S···CB distances can be seen in Figure 6, which shows the correlated and reactive conformations to be formed by compressing motions decreasing the sum of the two distances by ~0.5 Å. The position of the side chain of Tyr68 is important in the formation of NACs; ~80% of the NACs have S···CB distances below 4.2 Å (the sum of the van der Waals radii for the two atoms). Interestingly, in the time frame 150 to 400 ps, all of the NACs formed have S···CB distances greater than 4.0 Å. This leads to two distinct populations of NACs sampled during the MD simulation, those formed when Tyr68 is distant from AdoMet (150−400 ps) and those formed from compressing motions by the CB of Tyr68 (50−150 and 400−1050 ps). NACs formed from the compressing motions occur 5 times more often than NACs formed when Tyr68 is distant from AdoMet. When the motions of the AdoMet are hindered within the active site, the methyl group can only sample a limited portion of conformation space, and changes in conformation are minimal. The limited sampling will allow the AdoMet and catechol to rapidly form a NAC which then leads to the transfer of the methyl group to the nucleophilic catechol. Greater conformational freedom, as seen when Tyr68 is distant from AdoMet, leads to lower NAC formation and rapid changes in conformations which may affect the rate of reaction. This difference in the populations of the two NAC species corresponds, kinetically, to approximately 0.5 kcal/mol should the two NAC species have equal rates of methyl transfer. Although this energy difference is small, the reactivity of these two different NACs could be quite different. Arguments can be advanced on the reactivity of the compressed NAC, but a definitive answer will not be found until accurate calculations are performed to determine the energetics of these two different conformations.

The results from this MD study support the proposition of Zheng and Bruice that factors such as desolvation and bringing together the nucleophile and electrophile in the correct juxtaposition play an important role in the catalytic efficacy of catechol *O*-methyltransferase. Much the same has been reported for the S_N2 displacement of Cl^- from 1,2-dichloroethane by Asp120-CO_2^- in the mechanism of the *Xanthobactor autotrophicus* haloalkane dehalogenase.[23]

It has been shown experimentally that catechol *O*-methyltransferase tends to favor one hydroxyl group over the other in the substrate for methylation. Creveling et al. showed that the ratio of *meta*-O-methylated to *para*-O-methylated products from COMT can be severely affected by the length of the R group attached to the phenyl ring and whether R contains a charged group (Scheme 1).[24] The *meta* product is highly favored over the *para* product when the R group contains an ionized atom, but the *meta:para* ratio of products becomes closer to unity when the ionized atom is neutralized. Based on the experimental data, it has been proposed that a hydrophobic pocket exist in COMT near the 5-position of catechol.[24] From inspection of the crystal structure, Trp38 and Pro174 are seen to be the only residues in COMT that can provide the hydrophobic pocket without large-scale changes to the protein's structure. This MD trajectory provides some insight to the observed preference for one methylation site over the other. In the crystal structure, Trp38 forms an edge-to-face interaction with the phenyl ring of the catechol.[3] During the simulation, the ring of the catechol begins to tilt toward Trp38 relative to its position in the crystal structure. There is an approximately 30° change in the angle formed by C2 and C5 of the phenyl ring of catechol and CZ2 of Trp38 relative to that of the starting structure (Figure 8). This tilting of the phenyl ring of catechol appears to be due to changing the substrate from 3,5-dinitrocatechol to catechol. In the crystal structure, Trp38 is in close contact with the nitro group at the 5-position of catechol. The CH2 and CZ2 of Trp38 are 3.72 and 3.57 Å from the nitrogen and an oxygen of the nitro group, respectively. The conformation of Trp38 around the catechol is likely not as stable without the interactions with the nitro group, and in the simulation the indole group moves away from the phenyl ring which allows the catechol to adopt

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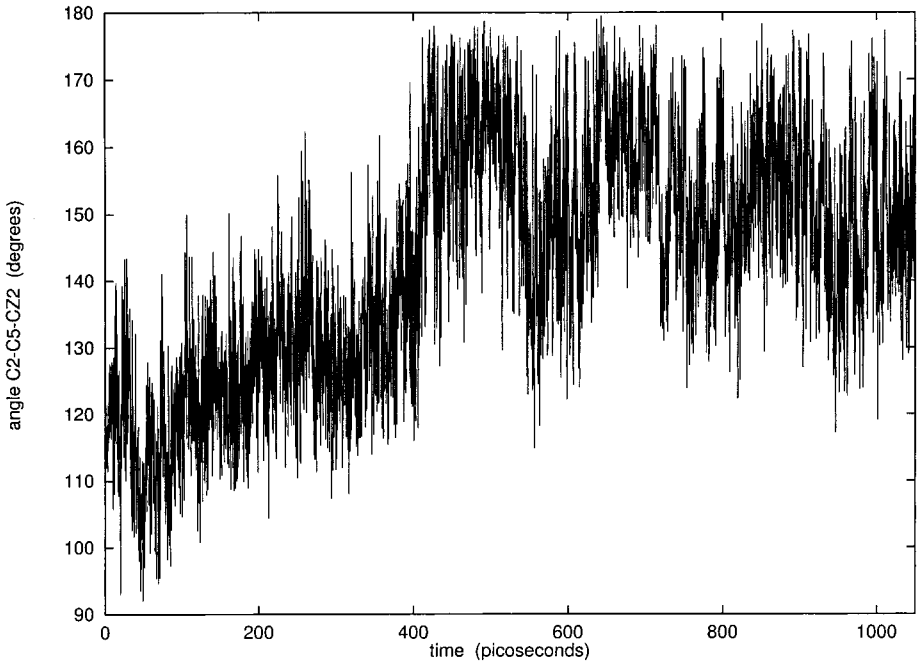

Figure 8. Change in the angle formed by the C2 and C5 of catechol and CZ2 of Trp38 during the MD simulation.

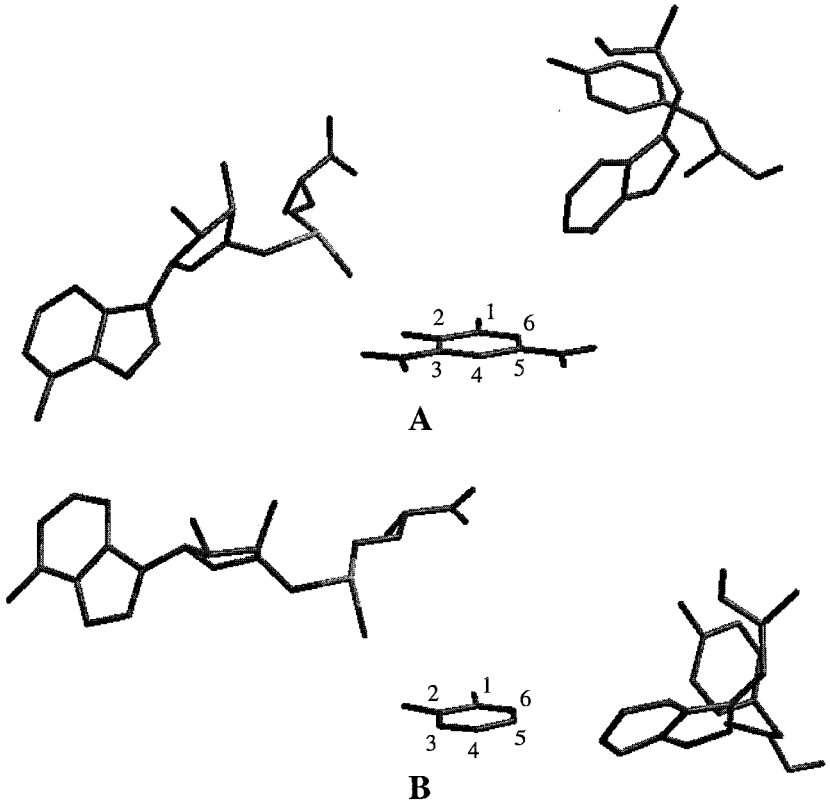

Figure 9. Diagram of the positions of AdoMet, Trp38, and Tyr200 relative to those of 3,5-dinitrocatechol in the crystal structure (A) and catechol after 1050 ps of dynamics (B).

to the surrounding environment. The tilt in the ring causes any substituent located at the 5-position of the ring to be directed into the indole ring of Trp38 (Figure 9). In addition, Tyr200 shifts in position after 700 ps and moves approximately 1.5 Å toward Trp38, forming a hydrophobic pocket in the vicinity of the 5-position of catechol (Figure 10). Both Trp38 and Tyr200 reside in loops which allow structural changes without greatly perturbing the enzyme's tertiary structure. The indole group of Trp38 and the phenyl ring of Tyr200 are not in van der Waals contact with the catechol. Inspection of the coordinates shows that both residues are solvent-exposed. The C5 carbon of catechol is on average 5.32 Å from the CE2 of Trp38. This spacing would allow catechol to have short substituents attached to the ring and not greatly affect the *meta:para* product ratio. This result is seen in the *meta:para* ratios which are close to unity when the side chain is a methyl or ethyl group or alkyl

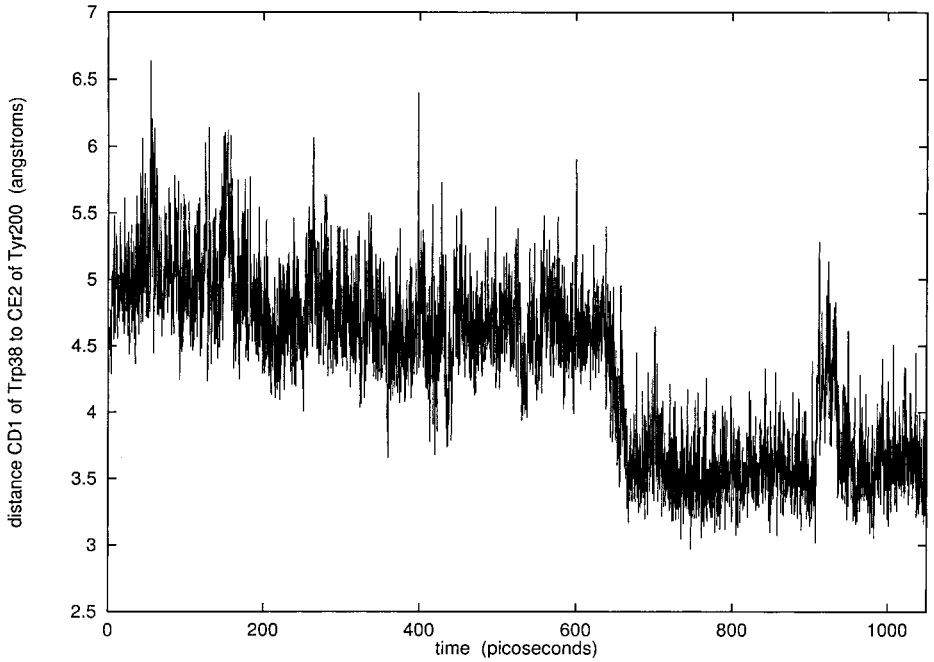

Figure 10. Separation between the CD1 of Trp38 and CE2 of Tyr200 during the MD simulation.

ethers. The very high *meta:para* product ratio can be further rationalized for the amines and carboxylic acids by this change in the orientation of the catechol ring. When the amine or acid is ionized and attached to a side chain of 2 carbons or more (such as dopamine or norepinephrine), having the side chain oriented toward the hydrophobic indole ring would be an unfavorable interaction making *para*-O-methylation unlikely. When catechol is oriented for *meta*-O-methylation, the ionic side chain will be directed away from the hydrophobic environment of the protein and into solvent. This is a much more favorable situation and apparently makes the conformation for *meta*-O-methylation the dominant arrangement.

In conclusion, this MD simulation has been able to show a correlation between the distances of CB of Tyr68 to S of $CH_3-S^+<$ (S$\cdots$CB) and catecholate-O^- to C of $CH_3-S^+<$ (C$\cdots$O). This correlation is important in forming reactive near attack conformations. When the motions are compressive, NACs are formed in abundance in COMT. Formation of NACs can also be formed without the compressing motion, but the frequency for generating a reactive conformation is 5 times lower. It is currently not known which of the two NACs are more reactive, but QM/MM studies are underway to determine the energetics of these two different conformations and their role in the catalysis of catechol in COMT.

This simulation also showed that a hydrophobic pocket is formed by Trp38 and Tyr200 in the vicinity of the 5-position of catechol. The tilting of the catechol ring during the MD trajectory will direct any substituent attached at the 5-position toward the indole ring of Trp38, providing an explanation for the observed preference for *meta*-O-methylated products over *para*-O-methylated products.

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