Comparison of the Dynamics for Ground-State and Transition-State Structures in the Active Site of Catechol *O*-Methyltransferase

Edmond Y. Lau and Thomas C. Bruice*

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

Received January 24, 2000

Abstract: Three 1-ns molecular dynamics (MD) simulations have been used to study the active-site structure of catechol *O*-methyltransferase when containing catechol, catecholate, or the transition state. It was found that the physical properties of the enzyme active sites are very similar when containing either catecholate or the transition state. The calculated root-mean-squared deviation and positional fluctuations of the active-site residues within 10 Å of the methyl group of *S*-adenosyl-L-methionine (AdoMet) for the catecholate and transition-state simulations are similar, and the average solvent accessible surface areas for these residues are almost identical. It was found in the ground-state simulation with catecholate that interactions between AdoMet and the enzyme are critical in positioning AdoMet into near attack conformers (NACs) which resemble the transition-state geometry. The CB of Tyr68 influences the distance between the methyl carbon of AdoMet and the ionized oxygen of catecholate. Interactions between the backbone amide carbonyls of Met40 and Asp141 control the angular positioning of the AdoMet relative to catecholate. When the interactions dissipated, the angle formed by the sulfur and methyl carbon of AdoMet and ionized oxygen of catecholate decreased and no longer fulfilled the NAC criteria. Comparisons of these 3 MD simulations in combination with results from previous quantum mechanical calculations from this lab suggest that the catalytic power of this enzyme in going from E·S to E·TS is not due to transition-state stabilization but from the ability of the active site of catechol *O*-methyltransferase to arrange the reactants into conformers that closely resemble the transition state. Overall, in E·S going to E·TS one would also consider as a driving force catecholate and AdoMet desolvation.

Introduction

Enzymes have the remarkable ability to accelerate the rate of a reaction by as much as 10^{18} times relative to that of the uncatalyzed reaction.[1] An enzyme is able to lower the activation barrier separating the reactants and products. How an enzyme lowers the activation barrier for a reaction is still under debate.[2] It has been speculated that the active site of enzymes are optimized to bind the transition state, resulting in a lowering of the transition-state energy.[3] Yet, this is not the only rationale advanced to explain the magnitude of rate enhancements in enzyme catalysis. There is growing evidence that supports the early proposal that positioning of the reactants into a conformation that would facilitate entrance to the transition state is an important component of catalysis.[4,5] The importance of the reactant's ground-state conformation to the rate of reaction has been explored in intramolecular reactions using modern computational methods by Lightstone and Bruice.[6] It was found that the number of ground-state conformers that are geometrically similar to the transition-state structure is related to rate enhancement exhibited by the molecule.[7] Although much experimental and computational work has been done on enzymes, a consistent view of enzymatic catalysis still has not been reached, and the subject remains contentious.

A transition state exists for a fleeting amount of time (on the order of femtoseconds). Femtosecond laser spectroscopy has allowed experimentalists to glimpse the transition-state structure for simple gas-phase reactions.[8] Although this method has provided greater understanding of chemical reactions, it has not yet been used commonly to study reactions in biological systems. Experimental characterization of the transition-state structure in biological molecules is usually done indirectly using transition-state analogues. Important interactions between the transition-state analogue and enzyme, that may influence catalysis, are inferred from the structure obtained by X-ray crystallography or NMR. Another method for obtaining the transition-state structure is through computation. Calculations are performed on the reactants to locate the corresponding saddle point on the potential energy surface, which leads to the desired products.[9]

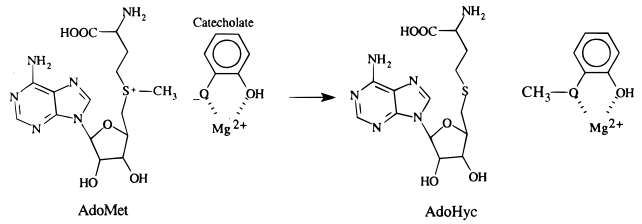
We report a molecular dynamics study to determine if changes occur in the active site of rat liver catechol *O*-methyltransferase (COMT) when binding the reactants or the transition state. Catechol *O*-methyltransferase is a monomeric protein consisting of 221 residues. This enzyme is extremely amenable for theoretical study. The crystal structure of this enzyme has been solved at 2.0 Å resolution containing the inhibitor 3,5-dinitrocatechol.[10] The structure of the enzyme is characterized by an

* To whom correspondence should be addressed. Telephone: 805-893-2044. Fax: 805-893-2229. E-mail: tcbruice@bioorganic.ucsb.edu.

(1) Wolfenden, R.; Lu, X.; Young, G. *J. Am. Chem. Soc.* **1998**, *120*, 6814−6815.
(2) Bruice, T. C.; Benkovic, S. J. *Biochemistry* **2000**, *39*, 6267−6274.
(3) Pauling, L. *Nature* **1948**, *161*, 707−709.
(4) Bruice, T. C.; Pandit, U. K. *Proc. Natl. Acad. Sci. U.S.A.* **1960**, *46*, 402.
(5) Bruice, T. C. *Annu. Rev. Biochem.* **1976**, *45*, 331−373.
(6) Bruice, T. C.; Lightstone, F. C. *Acc. Chem. Res.* **1999**, *32*, 127−136.
(7) Lightstone, F. C.; Bruice, T. C. *J. Am. Chem. Soc.* **1996**, *118*, 2595−2605.
(8) Zewail, A. H. *Faraday Discuss.* **1991**, *91*, 207−237.
(9) Hehre, W. J.; Radom, L.; Schleyer, P. v. R.; Pople, J. A. *Ab Initio Molecular Orbital Theory*; John Wiley and Sons: New York, 1986.

10.1021/ja000265d CCC: $19.00
Published on Web 07/15/2000

Scheme 1



α/β hydrolyase fold, a β-sheet surrounded by α-helices. This enzyme catalyzes the S_N2 displacement of the *S*-adenosyl-L-methionine (AdoMet) methyl group to an oxygen of catecholate (Scheme 1). The reaction mechanism of this enzyme is well characterized from model and enzymatic studies.[11−16] We have reported ab initio quantum mechanical studies on a model of the COMT active site and reactants to determine geometries, activation barriers, and kinetic isotope effects (KIE) for the transmethylation reaction when both oxygens of catecholate are ligated to Mg^{2+}, as in the X-ray structure.[17] The calculated KIE for formation of the transition-state structure, for the model system in the gas phase, was in good agreement with the experimental KIE determined with the enzyme, implying the gas-phase transition state is very similar to the transition-state structure in the enzyme.

We describe herein three 1-ns molecular dynamics simulations of fully solvated COMT in which the active site is occupied by AdoMet with catechol or catecholate or by the transition state. Comparisons of properties of COMT from these simulations show that there is little difference between the active-site structure when occupied by catecholate or the transition state.

Methods

The program CHARMM (version 25b2) was used for all molecular dynamics simulations.[18] All starting structures for the simulations were based on the crystal structure of COMT complex with 3,5-dinitrocatechol (PDB entry 1VID).[10] In the simulation of COMT containing the gas-phase transition state, the transition-state structure calculated by Zheng and Bruice was modeled into the active site by overlaying the gas-phase structure with the analogous residues in the enzyme.[17] Both oxygens of catecholate, Asp141, Asp169, Asn170, and Wat400 were explicitly bonded to the essential Mg^{2+} (Figure 1). Partial atomic charges for this moiety were obtained from a PM3 Mulliken population analysis calculation.[19] High force constants were used to maintain the geometry of the transition-state structure throughout the simulation (500 kcal·mol^{-1}·Å^{-2}). CHARMM was used to add the appropriate number of hydrogens to the heavy atoms of the enzyme and substrates. The structure was energy minimized by using a combination of steepest descents and adopted basis Newton−Raphson methods.[18] High-force constants, which were removed during dynamics, were placed on the heavy backbone atoms of COMT during energy minimization. This allowed side chains of residues to change position to accommodate the transition-state structure, but left the backbone of COMT unperturbed. The energy-minimized structure was solvated in a 62.2 × 62.2 × 62.2 Å^3 cube of TIP3P water.[20] If the oxygen of a water molecule was within 2.3 Å of any atom of the enzyme·transition-state complex, the water molecule was deleted. The final system contained 24245 atoms (6939 water molecules). The solvated system was further energy-minimized. Molecular dynamics was performed on the energy-minimized system, and periodic boundary conditions were used to simulate a continuous system. The system was heated to 300 K in 5 ps and equilibrated for 45 ps. Production dynamics was performed for 1018 ps. The SHAKE algorithm was used to constrain bonds containing hydrogens to their equilibrium length.[21] The Verlet leapfrog algorithm

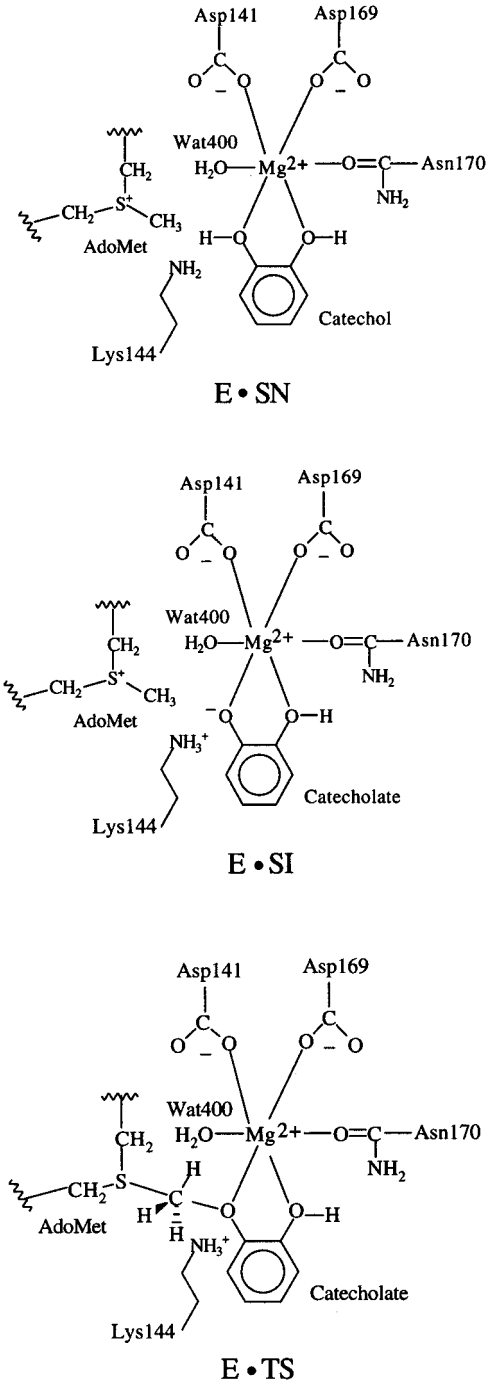


Figure 1. Active-site structures of catechol *O*-methyltransferase used in the simulations.

(10) Vidgren, J.; Svensson, L. A.; Liljas, A. *Nature* **1994**, *368*, 354−358.
(11) Knipe, J. O.; Coward, J. K. *J. Am. Chem. Soc.* **1979**, *101*, 4339−4348.
(12) Mihel, I.; Knipe, J. O.; Coward, J. K.; Schowen, R. L. *J. Am. Chem. Soc.* **1979**, *101*, 4349−4351.
(13) Knipe, J. O.; Vasquez, P. J.; Coward, J. K. *J. Am. Chem. Soc.* **1982**, *104*, 3202−3209.
(14) Hegazi, M. F.; Borchardt, R. T.; Schowen, R. L. *J. Am. Chem. Soc.* **1979**, *101*, 4359−4365.
(15) Gray, C. H.; Coward, J. K.; Schowen, K. B.; Schowen, R. L. *J. Am. Chem. Soc.* **1979**, *101*, 4351−4358.
(16) Rogers, J.; Femec, D. A.; Schowen, R. L. *J. Am. Chem. Soc.* **1982**, *104*, 3263−3268.
(17) Zheng, Y.-J.; Bruice, T. C. *J. Am. Chem. Soc.* **1997**, *119*, 8137−8145.
(18) Brooks, B. R.; Bruccoleri, R. E.; Olafson, B. D.; States, D. J.; Swaminathan, S.; Karplus, M. *J. Comput. Chem.* **1983**, *4*, 187−217.
(19) Stewart, J. J. P. *J. Comput. Chem.* **1989**, *10*, 209−220.
(20) Jorgensen, W. L.; Chandrasekhar, J.; Madura, J. D.; Impey, R. W.; Klein, M. L. *J. Chem. Phys.* **1983**, *79*, 926−935.

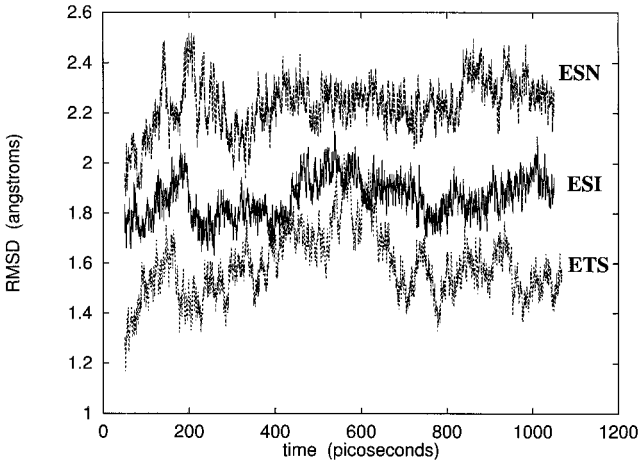


Figure 2. Root-mean-squared deviations (rmsd) of the α-carbons (CA) of COMT from the crystal structure during dynamics.

was used to integrate the equations of motion.[22] A time step of 1.5 fs was used to prevent the total energy from drifting, and the nonbonded interactions were updated every 20 time steps. The nonbonded interactions were cut off using smoothing functions.[18] The Coulombic term was cut off at 13 Å by means of a force-shifting function, and the Lennard-Jones term was cut off between 11 and 12 Å by means of a switching function.

The procedures for performing the simulation of COMT with catecholate was described previously by Lau and Bruice.[23] The setup for the COMT simulation containing catechol is exactly the same as that described for COMT with catecholate. The final system contained 24284 atoms (6952 water molecules). Lysine 144 was modeled as a neutral residue in this simulation. In the crystal structure, this lysine is hydrogen-bonded to an oxygen of 3,5-dintrocatechol and has been speculated to be the base that abstracts the hydrogen from catechol.[17] The pK_a for catechol is ∼9.4, thus initially bound catechol is neutral.[24] The partial charges for neutral lysine was obtained from the residue topology file supplied with the program QUANTA.[25] Partial atomic charges for Asp141, Asp169, Asn170, Wat400, catechol (neutral), and Mg^{2+} were obtained from a Mulliken population analysis from a PM3 calculation. The same procedures for cutting off the nonbonded interactions discussed above were used for this simulation. The heating and equilibration times used above were used for this simulation. Production dynamics was performed for 1000 ps. A time step of 2.0 fs was used for this simulation.

Solvent accessible surface areas (SASA) for the enzyme were calculated, using the program NACCESS, from the coordinates generated every 1 ps of production dynamics.[26] Standard atomic radii and a slice thickness of 0.05 Å were used for the calculations.

Results and Discussion

To avoid confusion when discussing the three different simulations, they will be identified as follows (see Figure 1): E·SN (ground-state simulation containing AdoMet, catechol, and neutral Lys144), E·SI (ground-state simulation containing AdoMet, catecholate, and protonated Lys144), and E·TS (transition-state simulation).

Root-Mean-Squared Deviations. The root-mean-squared deviations (rmsd) between the protein backbone α-carbons (CA) during all three simulations relative to the crystal structure were calculated (Figure 2). The E·TS simulation had the smallest deviation from the crystal structure, the rmsd reached 2.0 Å but for the majority of the trajectory the rmsd was ∼1.6 Å. The simulation of COMT containing neutral catechol had the largest rmsd that ranged between 2.2 and 2.3 Å for most of the simulation. The rmsd of COMT in the E·SI simulation fluctuated between 1.7 and 2.1 Å. Comparisons of the average structure of COMT from each simulation show that E·SI and E·TS are the most similar (Table 1). The RSMD of the heavy backbone atoms (C, CA, and N for residues 6−213) of E·SI to E·TS is 0.89 Å. Only the rmsd between the crystal structure and E·SI was lower, 0.85 Å.

Table 1. Comparison of the Root-Mean-Squared Deviations Between the Average Structures of COMT[a]

	ESN	ESI	ETS
Xtal	1.37	0.85	1.00
ESN		1.12	0.97
ESI			0.89

[a] Values are in Å.

Positional Fluctuations. The positional fluctuations of the CA in the three different COMT simulations were calculated and can be compared to those estimated from the crystallographic B-factors (Figure 3).[27] All three simulations exhibit positional fluctuations that are in reasonable agreement with those from crystallography. In all three simulations, most of the calculated fluctuations are less than those estimated from crystallography. The only region in COMT that consistently exhibit fluctuations greater than those from crystallography was the C-terminus.

Active Site. In this study, both oxygens of catecholate are coordinated to the essential Mg^{2+} which is also coordinated to residues Asp141, Asp169, Asn170, and a water to complete the octahedral coordination (Figure 1). The geometry of residues within 10 Å of the methyl group of AdoMet show only minor differences between simulations. There are 27 residues within 10 Å of the methyl group of AdoMet that were monitored throughout the three simulations. The dynamic properties of these active-site residues are similar for the E·SI and E·TS simulations. The rmsd for all of the heavy atoms for these active-site residues are comparable in the 3 simulations (Table 2). The largest differences observed for the rmsd between simulations are observed for Tyr68 and Tyr71; both have rmsd's that are ∼1 Å greater in the E·TS simulation than in the E·SN or E·SI simulations. The large rmsd values from the E·TS simulation are deceiving since there is no large-scale displacements in either tyrosine. Instead, the great change is due to a ∼180° dihedral transition around the angle formed by CD1−CG−CB−CA in both residues. The only atoms that have changed positions are the CD and CE atoms in the phenyl ring. The initial positions of atoms CD1 and CE1 are almost superimposable with those of CD2 and CE2 in the final structures. If the rmsd's are calculated for these two residues without the CD and CE atoms, the values are reduced to 0.17 and 0.28 Å for Tyr68 and Tyr71, respectively. This is in good agreement with the E·SN and E·SI simulations. Another discrepancy between rmsd is between Met40 in the E·SI and E·TS simulations. The deviation in the E·SI simulation is more than twice that from the E·TS simulation. This difference in value is due to a dihedral transition in the Met40 side chain in the E·SI simulation. The dihedral transition causes the last three atoms in the side chain to change positions. The positional fluctuations for the active-site residues are relatively small in the CA backbone atoms, in agreement

(21) Ryckaert, J. P.; Ciccotti, G.; Berendsen, H. J. C. *J. Comput. Phys.* **1977**, *23*, 327−341.
(22) Verlet, L. *Phys. Rev.* **1967**, *159*, 98−113.
(23) Lau, E. Y.; Bruice, T. C. *J. Am. Chem. Soc.* **1998**, *120*, 12387−12394.
(24) Serjeant, E. P.; Dempsey, B. *Ionisation Constants of Organic Acids in Aqueous Solution*; Pergamon Press: Oxford, 1979.
(25) *QUANTA*; Molecular Simulations Inc.: San Diego, CA.
(26) Hubbard, S. J.; Thornton, J. M. *NACCESS*; Department of Biochemistry and Molecular Biology, University College: London, 1993.
(27) McCammon, J. A.; Harvey, S. C. *Dynamics of Proteins and Nucleic Acids*; Cambridge University Press: Cambridge, 1987.

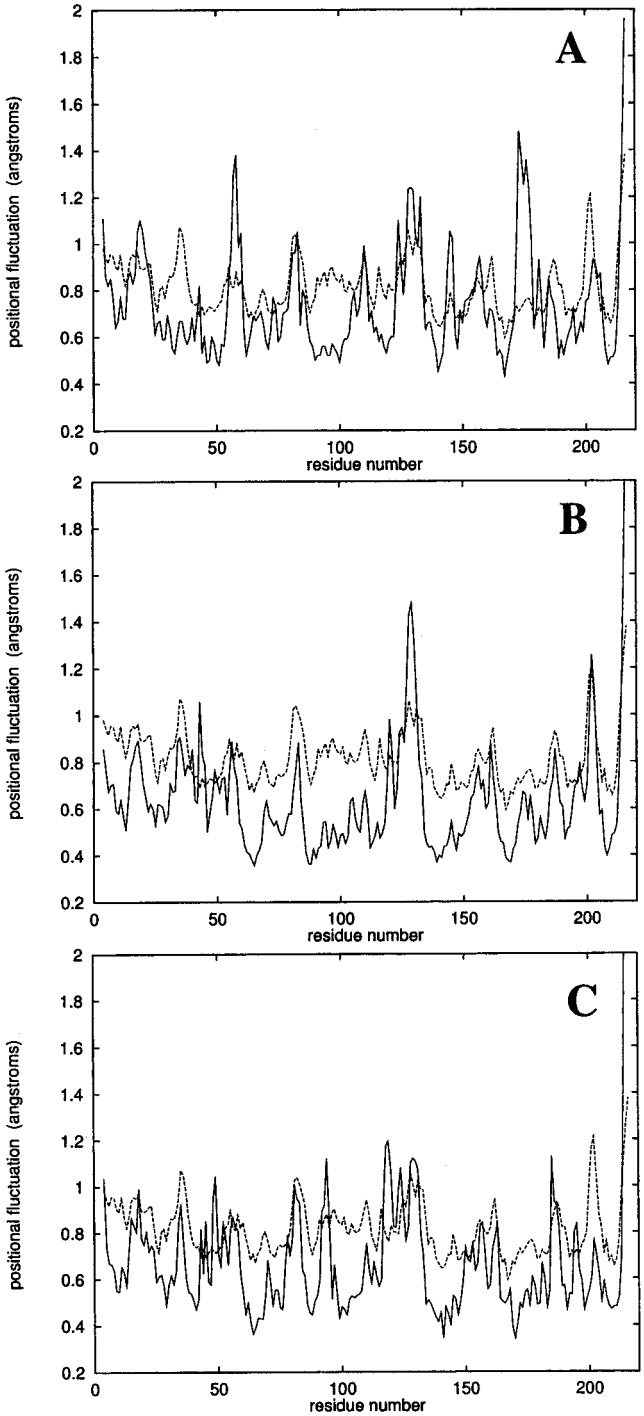


Figure 3. Positional fluctuations of the α-carbons (CA) in COMT from the E·SN (A), E·SI (B), and E·TS (C) simulations. Dashed lines in each panel is for the crystal structure and solid line is for the simulation.

with the observation from crystallography (Table 3). The greatest discrepancy is in the E·SN simulation where Lys144 and Pro174 exhibit large-scale fluctuations. It is not surprising that Lys144 is more mobile in this simulation. The side chain for this residue was modeled as a neutral species and does not form a salt bridge with the oxygen of catechol (Figure 1). Pro174, which is in van der Waals contact with the phenyl ring of 3,5-dinitrocatechol in the crystal structure, moves away from the phenyl ring in all three simulations.

Solvent Accessible Surface Area. The packing of heavy atoms in the active site of COMT in the E·SI and E·TS simulations differ little as evidenced by the solvent accessible surface area (SASA) of 27 active-site residues (residues within 10 Å of the methyl group of AdoMet). The average SASAs for the active-site residues in the E·SI and E·TS simulations only differ by 1.0 Å^2 (Table 4). There is a slight (13 to 14 Å^2) increase in the SASA for the active-site residues in the E·SN simulation, relative to the other two simulations. The difference is due to greater solvent exposure of the hydrophilic atoms. The similarity in properties for the active-site residues in the E·SI and E·TS simulations can be further assessed between different components of the SASA for the two simulations. Figure 4 shows that not only are the total SASA in correspondence, but the SASA of main chain, side chain, hydrophilic, and hydrophobic atoms of the active-site residues are in good agreement between the two simulations. There is a slight deviation in the highly solvent-exposed atoms, but agreement between the values is clearly shown. These individual properties for the active site of COMT lead to the conclusion that there is little change in residue position when the active site contains the catecholate or transition-state structures.

Close Contacts. The average position of AdoMet in the ground state relative to that of catechol differs depending if catechol is neutral or ionized (Table 5). AdoMet backs away from the neutral catechol. The average distance, in the E·SN

Table 2. Root-Mean-Squared Deviations for the Heavy Atoms of active-site Residues[a]

	ESN	ESI	ETS		ESN	ESI	ETS
Trp38	0.43	0.27	0.20	Glu90	0.14	0.21	0.23
Ala39	0.12	0.12	0.12	Tyr95	0.18	0.15	0.16
Met40	0.56	0.76	0.37	Phe139	0.28	0.16	0.28
Asn41	0.55	0.82	0.31	Leu140	0.58	0.91	0.64
Val42	0.53	0.53	0.58	Asp141	0.62	0.30	0.19
Glu64	0.73	0.26	0.31	His142	0.29	1.14	0.69
Leu65	0.75	1.05	1.07	Trp143	0.26	0.23	0.48
Gly66	0.36	0.09	0.07	Lys144	0.65	0.37	0.56
Ala67	0.50	0.13	0.09	Tyr147	0.24	0.20	0.32
Tyr68	0.35	0.26	1.31	Asp169	0.11	0.14	0.15
Cys69	0.18	0.29	0.23	Asn170	0.17	0.17	0.21
Gly70	0.15	0.35	0.23	Pro174	0.22	0.31	0.36
Tyr71	0.22	0.28	1.33	Glu199	0.38	1.14	0.76
Ser72	0.39	0.16	0.48				

[a] Values are in Å.

Table 3. Positional Fluctuations of the CA of Active-Site Residues[a,b]

	ESN	ESI	ETS		ESN	ESI	ETS
Trp38	0.57	0.79	0.54	Glu90	0.50	0.39	0.53
Ala39	0.60	0.77	0.54	Tyr95	0.52	0.43	0.85
Met40	0.68	0.86	0.51	Phe139	0.55	0.37	0.41
Asn41	0.59	0.64	0.47	Leu140	0.45	0.40	0.46
Val42	0.69	0.63	0.51	Asp141	0.49	0.39	0.35
Glu64	0.63	0.38	0.36	His142	0.53	0.44	0.48
Leu65	0.69	0.36	0.39	Trp143	0.69	0.44	0.45
Gly66	0.67	0.40	0.43	Lys144	0.90	0.47	0.40
Ala67	0.69	0.42	0.43	Tyr147	0.61	0.42	0.44
Tyr68	0.71	0.46	0.43	Asp169	0.56	0.37	0.40
Cys69	0.66	0.56	0.52	Asn170	0.62	0.43	0.34
Gly70	0.58	0.63	0.68	Pro174	1.37	0.67	0.54
Tyr71	0.55	0.57	0.59	Glu199	0.62	0.63	0.51
Ser72	0.63	0.55	0.49				

[a] The value reported for a residue is the sum of the positional fluctuation for all heavy atoms in the residue. [b] Values are in Å.

Table 4. Average Solvent-Accessible Surface Areas (SASAs) for Active-Site Residues[a]

	total	sidechain	mainchain	hydrophobic	hydrophilic
ESN	579.3 (41.5)	524.9 (39.2)	54.4 (7.1)	434.3 (34.2)	145.0 (15.9)
ESI	566.1 (41.1)	513.9 (37.7)	52.2 (8.1)	437.6 (32.6)	128.4 (13.1)
ETS	565.1 (37.7)	520.7 (36.2)	44.4 (4.4)	448.0 (36.3)	117.1 (9.3)

[a] Values are in Å^3.

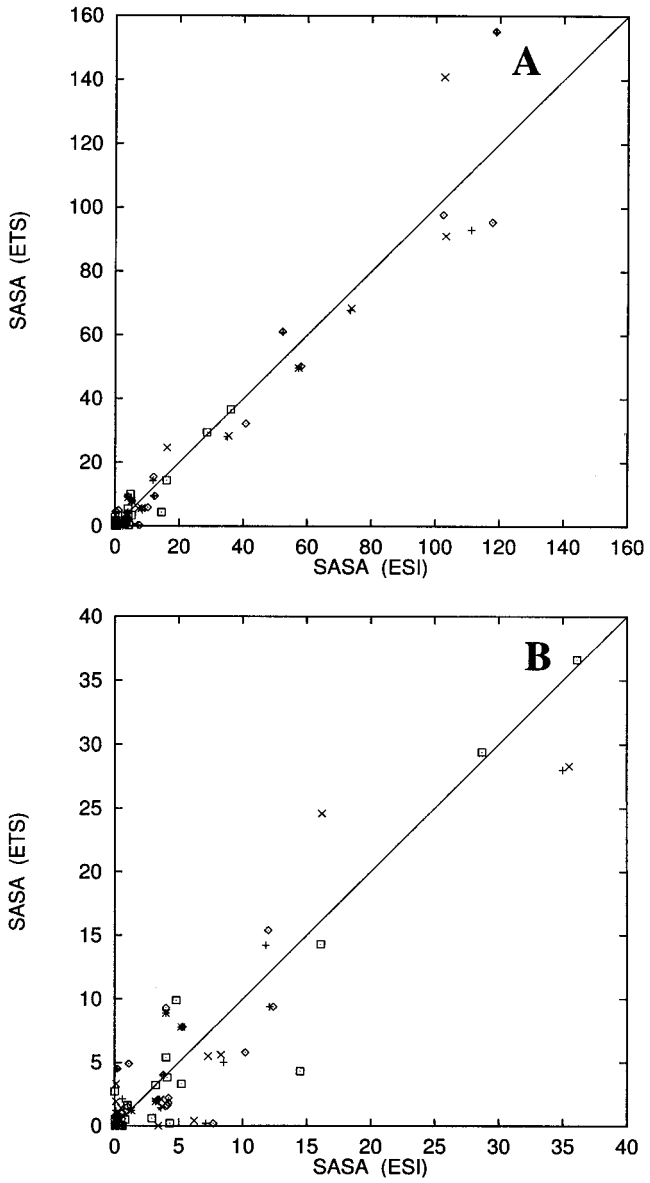


Figure 4. Plot of the correlation between the average solvent accessible surface area (SASA) for main chain (◇), side chain (+) hydrophilic (□), and hydrophobic (×) atoms of the 27 active-site residues from the E·SI and E·TS simulations. Panel B provides a view of the lower SASA values from Panel A. The line in both panels shows a perfect correlation. Values are in Å^2.

Table 5. Distances of Important Interactions Between Substrate and Enzyme[a]

	ESN	ESI	ETS
AdoMet CH3−catechol O	4.49 ± 0.66	3.55 ± 0.33	2.16 ± 0.02
AdoMet S−Tyr68 CB	4.53 ± 0.41	3.99 ± 0.25	4.19 ± 0.21
AdoMet S−Met40 O	4.78 ± 0.58	4.28 ± 0.95	4.44 ± 0.73
AdoMet S−Asp141	6.07 ± 0.29	4.38 ± 0.54	3.88 ± 0.20
catechol O−Lys144 NZ	4.73 ± 0.62	3.03 ± 0.23	3.14 ± 0.18
AdoMet CH3−Met40 SD	5.62 ± 0.96	5.23 ± 1.05	4.14 ± 0.50
AdoMet CH3−Asp141 OD1	3.55 ± 0.35	3.47 ± 0.26	3.52 ± 0.21

[a] Values are in Å.

simulation, between the methyl carbon of AdoMet to oxygen in catechol ($CH_3\cdots O$) is 4.49 ± 0.66 Å. In comparison, the average $CH_3\cdots O^-$ distance is 3.55 ± 0.33 Å in the E·SI simulation. Not surprisingly, the negative charge on the catecholate oxygen is important in orienting cationic AdoMet. The strong attraction between the opposing charges keeps these two species into close proximity, increasing the probability of methyl

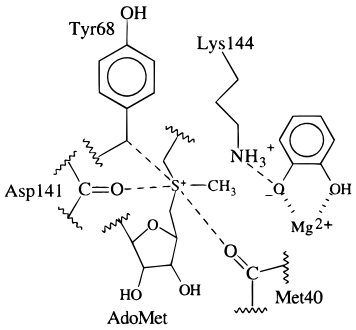


Figure 5. Picture of important interactions between the sulfur of AdoMet to CB of Tyr68, and the carbonyl oxygens of Met40 and Asp141.

transfer. It has been previously shown that the methylene of Tyr68 is in van der Waals contact with the sulfur of AdoMet (CB···S) in the E·SI simulation (Figure 5). It was speculated that this interaction could influence catalysis.

A study utilizing ab initio quantum mechanical calculations has been performed by Kahn and Bruice to determine the effect of having the Tyr68 methylene group in close proximity with the sulfur of AdoMet in models of the E·SI and E·TS complexes.[28] In that study, the methyl group of ethane was used as the model for the CB of Tyr68, and diethylmethylsulfonium and catecholate were used as models for the reactants in COMT. The interaction energy (the difference in ab initio energy between the reactant complex and the sum of the isolated molecules) was calculated as a function of the CH_3 from ethane to the sulfur of diethylmethylsulfonium separation for the ground-state and transition-state structures. It was found that the minimums in interaction energy for the ground-state and transition-state structures are centered at approximately the same separation with the ground-state being lower in energy by ∼0.5 kcal/mol. The maximum interaction energy supplied by this methyl group to sulfur interaction is small (−2 kcal/mol) for both the ground state and the transition state. It was concluded that this interaction did not greatly influence the energetics of the S_N2 methylation reaction in COMT.

The average distances between the methyl carbon of AdoMet and the OD1 of Asp141 (Figure 1) are very similar in all three simulations and range from 3.47 to 3.55 Å (Table 5). In the E·SN and E·SI simulations, SD of Met40 moves away from the methyl group of AdoMet. The side chain of Met40 remains in van der Waals contact to the methyl group in the E·TS simulation. There are two amide carbonyl oxygens (Met40 and Asp141) in close contact with the sulfur of AdoMet in the crystal structure (Figure 5). These two interactions are stabile for the majority of the E·SI simulation. Only during the last 150 ps did both interactions break. When the interactions ceased, the angle formed by the sulfur and methyl carbon of AdoMet and ionized oxygen of catecholate ($\angle S^+-CH_3\cdots O^-$) changed significantly. Initially the average $\angle S^+-CH_3\cdots O^-$ is near the criteria for a NAC, 165° or greater, and persisted until the interactions between the carbonyl oxygens and sulfur dissipated (Figure 6).

During the E·SI simulation, 378 out of 5000 conformers saved (7.6%) fulfilled the NAC criteria (methyl carbon of AdoMet to oxygen of catechol, $CH_3\cdots O^-$ distance is ≤3.2 Å and the angle formed by the sulfur and methyl carbon of AdoMet and ionized oxygen of catecholate, $\angle S^+-CH_3\cdots O^-$, is ≥165°). Figure 7 shows the number of NACs during the E·SI simulation in 100 ps intervals. The lower production of NACs between 200 and

(28) Kahn, K.; Bruice, T. C. *J. Am. Chem. Soc.* **2000**, *122*, 46−51.

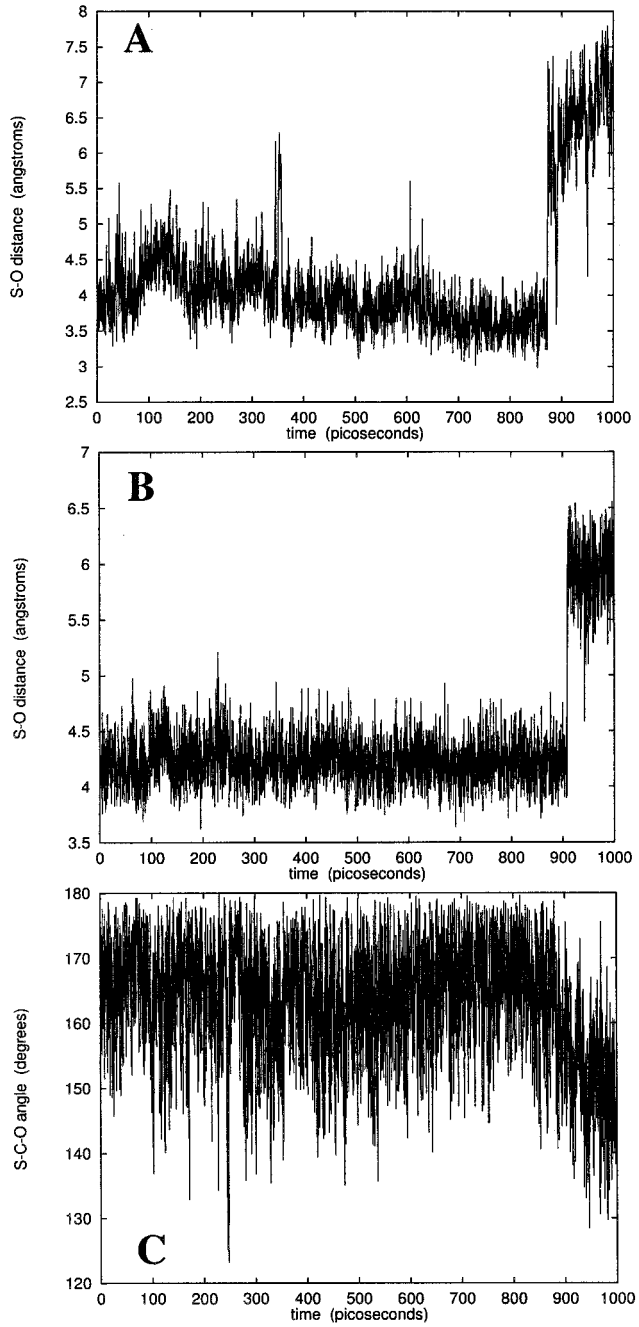


Figure 6. Plots of the distance between sulfur of AdoMet to carbonyl oxygen of Met40 (A) and amide carbonyl oxygen of Asp141 (B). Panel C shows the angle formed by the sulfur and methyl carbon of AdoMet and oxygen of catecholate. All data is from the production dynamics portion of the E·SI simulation.

400 ps has been previously shown to be due to the CB of Tyr68 backing away from the sulfur of AdoMet, causing a lengthening of the $CH_3\cdots O^-$ distance (Figure 5).[23] Three NACs were formed during the last 100 ps of dynamics. The decrease in NACs was not due to an increase in the $CH_3\cdots O^-$ distance since the average was 3.58 ± 0.24 Å during this time interval which is close to the average for the entire simulation (3.55 ± 0.33 Å). The substantial drop in NACs is due to a change in the $\angle S^+-CH_3\cdot\cdot\cdot O^-$ which shifts from ≥165° to 151.8 ± 8.0° during the last 100 ps of the E·SI trajectory (Figure 6C). It appears that a function of the amide carbonyl groups of Met40 and Asp141 is to position the AdoMet into conformers that can easily react with the catecholate. The cationic sulfur of AdoMet should have a significant stabilizing influence with the polar carbonyls.

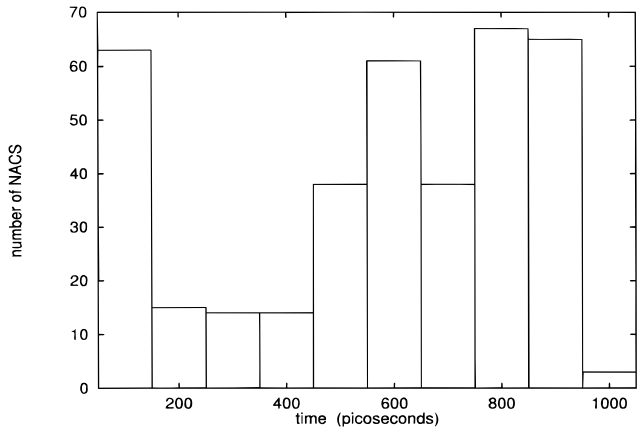


Figure 7. Number of near attack conformers (NACs) during 100 ps intervals from the E·SI simulation.

Ab initio calculations by Kahn and Bruice have determined the interaction energy as a function of distance between the amide carbonyl oxygen (Met40 and Asp141) and AdoMet sulfur using model compounds to assess the importance of these interactions to catalysis.[28] In their model, the carbonyl of an acetamide interacts with the sulfur of trimethylsulfonium. The acetamide was used to represent the peptide backbone, and trimethylsulfonium and catecholate were used as models of the reactants in COMT. The acetamide interacted with trimethylsulfonium at positions corresponding to Asp141 or Met40 initially. The interaction energy as a function of the carbonyl oxygen-to-sulfur of trimethylsulfonium separation was calculated. The interaction energy is the difference in ab initio energy between the complex and the sum of the isolated molecules. The minimum interaction energy for the ground-state structure is 2 kcal/mol lower than the minimum interaction energy for the transition-state structure. It is not surprising that the ground-state structure has a lower interaction energy, charge annihilation is occurring in the TS lowering the charge on the sulfur of trimethylsulfonium and weakening the dipolar interaction. In the ab initio calculations by Kahn and Bruice, the difference in interaction energies of the carbonyl oxygen with the sulfur of trimethylsulfonium at the minimum (centered at 3.8 Å, S···O distance) is 2 kcal/mol for the carbonyl of acetamide at the analogous position of Asp141 and 4 kcal/mol for the acetamide carbonyl at the Met40 position (minimum centered at 3.0 Å, S···O distance).

Comparison of the distances, from the MD simulations, between the amide carbonyl oxygens of Asp141 and Met40 with the sulfur of AdoMet (S···O) shows that the separations differ between the structures (Table 5). In the E·TS simulation, the S···O interaction for Asp141 is very stable and persisted throughout the simulation. The average distance is 3.88 ± 0.20 Å. Interestingly, the average distance in the E·SI simulation from 50 to 900 ps is 4.23 ± 0.19 Å. Although ab initio calculations show the minimum in the interaction energy for the ground state is lower than the one for the transition state, the carbonyl oxygen of Asp141 comes in closer contact with the sulfur of AdoMet in E·TS than E·SI. The shorter distance for Asp141 carbonyl oxygen to the sulfur of AdoMet in the E·TS simulation is due to having a shorter methyl carbon of AdoMet to oxygen of catechol distance, 2.16 and 3.55 Å for E·TS and E·SI, respectively (see Figure 1). The shorter methyl carbon of AdoMet to oxygen of catechol puts the sulfur of AdoMet in closer contact with the backbone carbonyl oxygen of Asp141. The interaction energy of these two different interactions can be estimated by utilizing the average separations of S···O from the E·SI and E·TS simulations and the quantum

mechanical energy profiles for the ground-state and transition-state structures of Kahn and Bruice. There is almost no difference in the interaction energy for the two species. The interaction energy for both interactions are approximately −10 kcal/mol.

The opposite result occurs for the carbonyl oxygen of Met40. In this case, the E·SI has a shorter distance than E·TS, 4.28 ± 0.95 and 4.44 ± 0.73 Å, respectively. At these two distances, the interaction energy for E·SI is ∼2 kcal/mol lower than for E·TS. In the E·TS simulation this interaction is highly unstable and exists in two conformers. One conformer has the carbonyl directed toward the sulfur of AdoMet; in the other conformer a dihedral transition occurs (∼90°) and directs the carbonyl away from the sulfur. The relative differences between the interaction energies for the ground state and the transition state are small and likely cannot explain the large rate enhancement of 10^{16} for the methylation reaction with this enzyme.[15]

These simulations have shown the amide carbonyls' interactions are important in positioning the AdoMet into conformers that are catalytically productive. It cannot be determined from these simulations if interactions of both carbonyls are needed to position the AdoMet or if only one of these carbonyls is necessary for catalysis. In the E·SN simulation both carbonyls are not in close contact with the AdoMet sulfur which allows larger angular changes in the relative orientation between the AdoMet and catechol. The average $\angle S^+-CH_3\cdots O^-$ for the E·SN simulation is 156.1 ± 10.8° relative to 163.2 ± 9.0° for the E·SI simulation.

Individually the minor differences in values for the rmsd, positional fluctuations, and SASA imply that only small changes in the active site occur upon binding the substrates in the E·SI and the transition state in E·TS. Taken together, this shows that for this enzyme the transition-state conformers do not differ greatly from those of the ground state containing charged reactants on the nanosecond time scale. The active site of catechol *O*-methyltransferase appears to be optimized to position the substrates into conformation in which little structural rearrangement needs to occur for the reaction to proceed. Recently, Kuhn and Kollman have calculated the cratic free energy contribution for bringing together the reactants (trimethylsulfonium and catecholate) in water.[29] The free energy needed to desolvate and bring the reactants into a reactive conformation is 9−13 kcal/mol. COMT is able to overcome this high free energy cost for bringing the reactants together to form a NAC and has a favorable binding free energy.

Conclusions

There is a long held belief that transition-state stabilization was the underlying cause for the catalytic power for all enzymes. Yet there is growing evidence that transition-state stabilization is not the only explanation for enzymatic catalytic efficiency. Cho and Northrop have interpreted their high-pressure experiments to indicate that the transition state is not as tightly bound as the ground state in yeast alcohol dehydrogenase.[30] Crystal structures of carbamoyl phosphate synthetase and methylmalonyl-CoA mutase show a minimum amount of change when binding analogues of ground and intermediate substrates.[31,32] Computational studies on the strain-free ring-closure reactions of dicarboxylic monoesters to form five- and six-membered cyclic anhydrides and the Claisen rearrangement of chorismate to prephenate have been able to show that the observed rate enhancements are due to positioning the reactants as conformers which closely resemble the transition state.[7,33] An example of how much positioning of the reactants can affect a reaction is illustrated from the calculations of Kahn and Bruice for the S_N2 methylation reaction of catechol by diethylmethylsulfonium.[28] The energy difference between the optimized ab initio minimum-energy structure and the transition-state structure is 22 kcal/mol. If the geometries of the reactants (diethylmethylsulfonium and catecholate) are restricted to that observed in the NACs formed in the COMT E·S complex, the energy difference between ground state and the associated transition state is 5 kcal/mol. The energy difference is due to the orientation of the diethylmethylsulfonium; in the ab initio minimum-energy structure the two ethyl groups reside directly above the phenyl ring of catechol. In the enzyme NAC, the two ethyl groups are directed away from the phenyl group which removes unfavorable interactions when the transition state is formed (Figure 8). Transition-state stabilization has almost universally been invoked for the rate enhancement observed in enzymes. It has now become apparent that preferential transition state binding is not in effect for a number of enzymes.[6,34] Thus, this feature is not a general requirement for the large rate enhancements characteristic of enzyme reactions. A general feature of enzymatic reactions is the formation of reactant ground-state conformers which closely resemble the transition-state structure (NACs) and desolvation. NACs are the ground-state turnstiles through which the E·S ground state must pass to become a transition state.

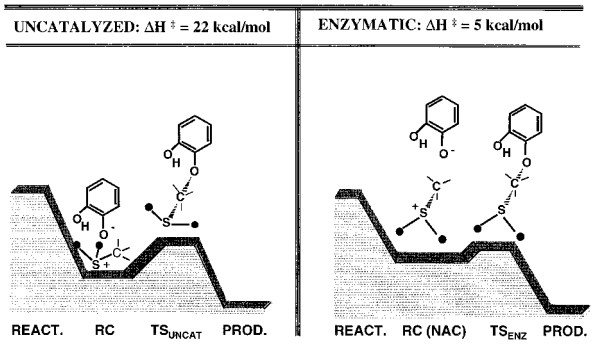


Figure 8. Plot of the relative geometries of trimethylsulfonium to catecholate and their associated energies for the ab initio optimized structure (uncatalyzed) and near attack conformer (enzymatic) in the gas phase. The reported energy differences ($\Delta H^{\ddagger}$) are between the reactant complex (RC) and the transition state (TS).

Acknowledgment. This work was funded by a grant from the National Science Foundation (MCB-9727937). The authors gratefully acknowledge computer time at the National Center for Supercomputer Applications, Maui High Performance Computing Center, and UCSB's Origin2000 (funded by NSF grant CDA96-01954 and Silicon Graphics Inc.). The authors thank Dr. Kalju Kahn for preparing Figure 8.

Supporting Information Available: Partial atomic charges used for the active-site residues and AdoMet; numbering system used for catechol and AdoMet (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA000265D

(29) Kuhn, B.; Kollman, P. A. *J. Am. Chem. Soc.* **2000**, *122*, 2586−2596.
(30) Cho, Y.-K.; Northrop, D. B. *Biochemistry* **1999**, *38*, 7470−7475.
(31) Thoden, J. B.; Huang, X.; Raushel, F. M.; Holden, H. M. *Biochemistry* **1999**, *38*, 16158−16166.
(32) Mancia, F.; Smith, G. A.; Evans, P. R. *Biochemistry* **1999**, *38*, 7999−8005.
(33) Khanjin, N. A.; Snyder, J. P.; Menger, F. M. *J. Am. Chem. Soc.* **1999**, *121*, 11831−11846.
(34) Torres, R. A.; Schiott, B.; Bruice, T. C. *J. Am. Chem. Soc.* **1999**, *121*, 8164−8173.