Transition-State and Ground-State Structures and Their Interaction with the Active-Site Residues in Catechol *O*-Methyltransferase

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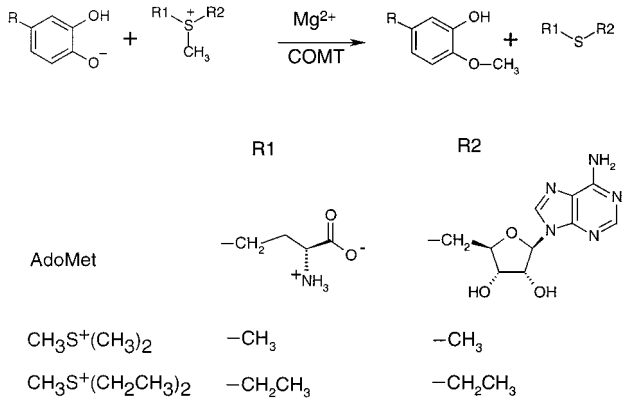
Abstract: The methylation of catecholate, catalyzed by catechol *O*-methyltransferase, has been studied by means of ab initio quantum mechanical calculations. The uncatalyzed reaction proceeds via a strongly interacting reactant complex of catecholate and methyl donor and encounters a significant activation barrier. The enzyme active site dictates an alternative orientation of reactants, which leads to a large reduction of activation energy. The contribution of three active-site groups to catalysis has been evaluated from MP2/6-31+G(d,p) interaction energy profiles and ONIOM MP2:HF energies. The calculations indicate that Tyr68 and peptide carbonyl groups of Met40 and Asp141 interact with the reactant complex more strongly than with the transition state. These results suggest that the enormous rate enhancements brought about by catechol *O*-methyltransferase do not arise from preferential interactions of the transition state with the enzyme. Instead, the catalytic power of this enzyme stems from orienting the reactants into a conformation where little structural rearrangement is needed to form the transition state.

Introduction

Catechol *O*-methyltransferase (COMT, EC 2.1.1.6) is a monomeric enzyme that catalyzes the transfer of a methyl group from *S*-adenosylmethionine (AdoMet) to the hydroxylate oxygen of substituted catechols (Scheme 1).[1] The enzyme is important in the central nervous system where it metabolizes dopamine, adrenaline, noradrenaline, and various xenobiotic catechols. One important substrate for COMT is levodopa, presently the most effective drug for Parkinson's disease.[2] Recently developed COMT inhibitors, tolcapone and entacapone, enhance the availability of levodopa and increase its half-life by 30−50%.[3] However, serious liver toxicity prevents routine use of tolcapone, possibly necessitating the development of new COMT inhibitors.[3,4]

COMT is a member of a large class of AdoMet-dependent methyltransferases, and its catalytic domain is similar to catalytic domains of DNA-(cytosine-5) methyltransferase and DNA-(adenine-N^6) methyltransferase.[1] The enzymatic reaction most likely proceeds by an ordered sequential kinetic mechanism with AdoMet binding first, followed by Mg^{2+} and then by the catechol substrate.[5] The chemical step of the catechol *O*-methyltransferase reaction is a S_N2 transfer of the methyl group from the sulfur of the AdoMet cofactor to the hydroxyl oxygen of the catecholate substrate. The enzyme has been estimated to accelerate this reaction by a factor of 10^{16} relative to the reaction in solution.[6] This large rate enhancement, relatively small size of the enzyme, and its role as the model for other methyltransferases make COMT an interesting system to study. Recent ab initio calculations indicate that the uncatalyzed reaction in solution faces a large activation barrier (21−30 kcal/mol), consistent with the experimentally observed enthalpy barrier of 28.5 kcal/mol for the reaction between trimethylsulfonium ion and phenolate.[7,8] Schowen et al. have measured ^{13}C and α-deuterium kinetic isotope effects in the enzymatic reaction and model systems and proposed that the catalytic power of COMT arises from a specific and strong stabilization of the transition state.[6,9,10] On the basis of molecular dynamics (MD) simulations, Lau and Bruice have argued that bringing together two reactants in a near attack conformation (NAC) is the role that the enzyme plays in the COMT reaction.[11]

Scheme 1



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In this paper we report the energetics of the methyl transfer reaction when the orientation of substrates is fixed to that found in the enzyme. Also, the interactions between active-site residues and the reactant complex and between active-site residues and the transition state have been quantified using ab initio calculations.

Computational Methods

The ab initio molecular orbital calculations were performed with the Gaussian 98 program.[12] The enzymatic reaction was modeled as methyl transfer from a methyl donor to the catecholate anion. Two methyl donors, trimethylsulfonium cation and diethylmethylsulfonium cation were used as the models of AdoMet (Scheme 1). For the gas-phase reaction between the trimethylsulfonium ion and catecholate anion, the structures of the reactant complex (RC_g) and the transition state (TS_g) were taken from a previous study[7] and correspond to the stationary points at the HF/6-31+G(d,p) surface. The structures for RC_g and TS_g for the reaction between diethylmethylsulfonium ion and catecholate were optimized at the HF/6-31+G(d,p) level. Subsequent frequency calculations indicated that RC_g is a minimum and TS_g is a first-order saddle point.

The structure selected for the enzyme-bound reactant complex (RC_e) was based upon analysis of twelve randomly picked near attack conformations (NAC) from a previously published MD simulation.[11] The AdoMet from MD structures was superimposed with diethylmethylsulfonium cation, AdoMet was removed, and the energy of the resulting bimolecular complex was calculated at the HF/6-31+G(d,p) level. The lowest energy NAC (S−C−O angle 177°; C−O−C angle 96°; $CO-CC_{OH}$ torsion −114.8°) was used as an initial structure for the subsequent partial optimization of the reactant complex at the HF/6-31+G(d,p) level. During the optimization of RC_e, two dihedral angles defining the mutual orientation of the reactants ($CO-CC_{OH}$ and $C_{R1}S-OC$) and the S−C−O angle were constrained to the values found in the lowest energy NAC. Control calculations, where the values of the two torsional angles were varied within 10°, showed that the potential energy is rather insensitive function of these dihedrals. To obtain the structure of the enzyme-bound transition state (TS_e), an initial guess was made based on the structures of RC_e and TS_g. Subsequent partial optimization was performed using analytically calculated force constants, and two dihedral angles between the methyl donor and catecholate were fixed to the same values as in the RC_e. To test if any artificial strain has been introduced by restricting the optimization, the transition state with diethylmethylsulfonium ion as a methyl donor was further optimized with only one torsional angle ($C_{R2}S-OC = -133°$) frozen. During this optimization, $CO-CC_{OH}$ torsion increased from −114.8° to −96.4°, but the overall effect on the energetics of methyl transfer was negligible. The final energy evaluations were performed at the MP2/6-31+G(d,p)//HF/6-31+G(d,p) level. Throughout, all electrons were included in the correlation treatment. Basis set superposition error (BSSE) upon formation of reactant complex and transition state was estimated using a seven-point counterpoise correction.[13] It was found that 4−5 kcal/mol of apparent interaction energy is due to BSSE, but this contribution was approximately the same for reactant complex and transition state, and the BSSE effect largely cancels out in calculation of activation barriers.[14]

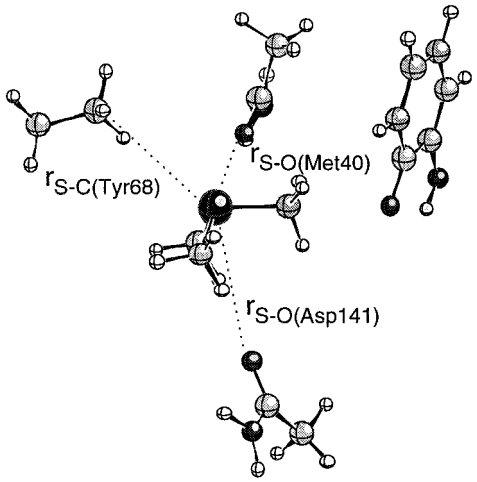

Figure 1. Positions of three active-site residue models relative to the HF/6-31+G(d,p)-optimized ground state in catechol *O*-methyltransferase with the trimethylsulfonium ion as a model for the methyl donor. The positions of residues were obtained from the lowest-energy NACs as determined in the ground-state MD simulation.[11] Energy profiles shown in Figures 4−6 were obtained by varying the distances indicated in this figure.

Enzyme residues observed to interact with the reactants and the transition state are the $-CH_2-$ moiety of Tyr68,[11] and peptide units of Met40 and Asp141 (Figure 1).[15] The interaction energy between RC_e and these three active-site groups and the interaction energy between TS_e and the same enzymatic groups were calculated by two methods: (i) supermolecule approach and (ii) ONIOM energy subtraction method. In the supermolecule approach, the MP2(full)/6-31+G(d,p) energies of the system consisting of an enzyme residue and RC_e or TS_e were calculated, and energies of the isolated enzyme residue and RC_e (or TS_e) were subtracted. To study the effect of the methylene carbon of Tyr68, which comes into close contact with AdoMet during the MD simulation, the interaction energies were calculated as a function of the distance between the methylene carbon and the AdoMet sulfur. The methylene moiety of Tyr68 was modeled as MP2/6-31+G(d,p) optimized ethane, diethylmethylsulfonium ion was employed as a model of AdoMet. The effect of peptide groups of Met40 and Asp141, which were represented as MP2/6-31+G(d,p) optimized acetamide molecules, was studied similarly, but now trimethylsulfonium ion was used to model AdoMet. To position the enzymatic groups relative to the reacting system, a steepest descents molecular mechanics minimization of catechol *O*-methyltransferase was performed using the lowest energy NAC structure as a starting point. The same CHARMM22 force field[16] as in the original MD simulation[11] was employed in this minimization. The resulting structure was characterized by distances of 3.98, 3.70, and 4.10 Å for $S-C_{Tyr68}$, $S-O_{Met40}$, and $S-O_{Asp141}$ respectively. Each of these distances was systematically varied during three series of energy evaluations while all angles and torsional degrees of freedom were frozen by using appropriately designed *z*-matrix input. Interaction energies calculated by subtracting the energy of isolated molecules from the energy of the complex are subject to a BSSE.[17] The correction for both Met40-RC_e and Met40-TS_e interactions was made as follows. The

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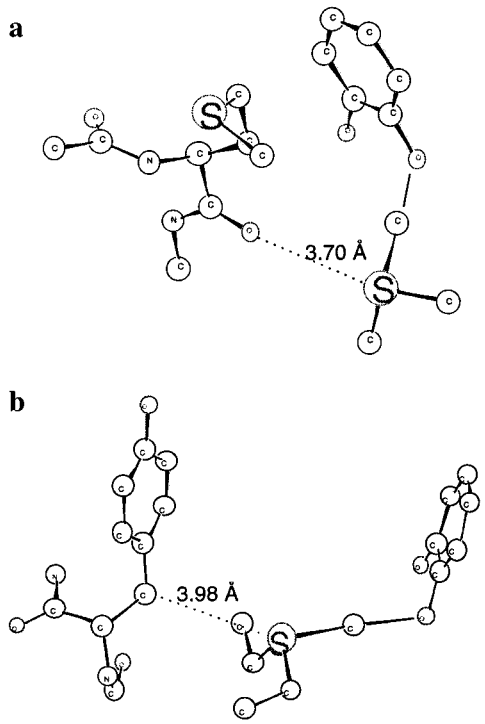

Figure 2. Larger models of the active site residues Met40 (a) and Tyr68 (b) which were employed in the ONIOM MP2:HF calculation.

BSSE was calculated by the counterpoise method[18,19] at intermolecular O−S distances of 2.5, 2.8, 3.2, 4.0, and 5.0 Å. The calculated BSSE was found to be an exponential function of intermolecular distance, and BSSE at other distances were estimated from a single-exponential fit. The BSSE correction was 3.6 kcal/mol for Met40-RC_e, and 3.13 kcal/mol for Met40-TS_e at 3.2 Å, indicating that effects due to BSSE largely cancel out when interactions with reactant complex and transition state are compared. For this reason, BSSE was not estimated for interactions of Asp141 and Tyr68 with the reacting system.

The inclusion of electron correlation is necessary to describe short-range dispersion interaction,[20] but it limits the size of the enzymatic group that can be modeled. To alleviate this size limitation, an energy subtraction scheme, ONIOM MP2/6-31+G(d,p):HF/6-31+G(d,p), was employed to calculate single point energies between larger models of active site residues and the reacting system.[21−23] The ONIOM MP2:HF calculation yields energy of the enzyme residue−reacting system complex where electron correlation is included only in the reacting system. Polarization of the reacting system by enzymatic residues is well-described with the use of 6-31+G(d,p) basis set.[24] The residues considered for the ONIOM calculation were Met40 and Tyr68 (Figure 2 a and b). ONIOM calculation with Asp141 was not performed because this residue interacts with the active site Mg ion, which in turn is coordinated to catecholate, Asp 169, Asn170, and water molecule. Bond lengths and bond angles in Met40 and Tyr68 were optimized at the HF/6-31G(d,p) level in the absence of catecholate and the methyl donor prior to the ONIOM calculation. During the optimization, dihedral angles for the heavy atoms were frozen to values determined by minimization of the complete enzyme using the CHARMM22 force field[16] (vide supra).

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Results

The energies of the isolated reactants and products and reaction energies for a gas-phase methylation of catechol (Scheme 1, R = H) with two methyl donors (R1 = R2 = $-CH_3$, and R1 = R2 = $-CH_2CH_3$) are reported in Table 1. The overall reaction is slightly less exothermic with $CH_3S^+(CH_2CH_3)_2$ compared to $CH_3S^+(CH_3)_2$, but it appears that the trimethylsulfonium ion is an adequate model for AdoMet. Electron correlation effects are significant and decrease exothermicity of the reaction by at least 10 kcal/mol.

The analysis of the crystal structure of COMT,[25] and of the gas-phase reactant complex between trimethylsulfonium ion and catecholate[7] indicated that the structure of the reactant complex differs in the gas phase and in the enzyme. In the gas phase a strongly interacting complex with C_S symmetry (Figure 1 in ref 7) forms. However, the mutual orientation of two reactants is not favorable for methyl transfer via the in-line S_N2 pathway. A sample of near attack conformations from a previous MD study[11] was analyzed, and it was found that the reactant complex in the enzyme, RC_e, poses a conformation that is different from that observed in the gas phase. The orientation of the trimethylsulfonium ion and catecholate in RC_e and the positions of adjacent enzyme residues are illustrated in Figure 1. Energy evaluations showed that the two substrates interact much more weakly in the orientation allowed by the enzyme (Table 2) than they do in the gas-phase reactant complex. The intrinsic energy of the trimethylsulfonium ion−catecholate complex in the enzymatic orientation was 20.7 kcal/mol above the energy of RC_g. Similarly, RC_e was 18.8 kcal/mol above RC_g with the $CH_3S^+(CH_2CH_3)_2$ model.

It was observed that the previously reported transition state for the catecholate methylation by trimethylsulfonium ion, obtained by quantum mechanical calculations,[7] is sterically prohibited in the enzyme. This becomes even more obvious when bulkier diethylmethylsulfonium ion is used as the model of AdoMet. Thus, the transition state in the enzymatic reaction (Figure 3a) differs somewhat from that in the gas phase (Figure 3b). The two transition states have different dihedral angles between the methyl donor and acceptor, but other structural features, such as the bond lengths and angles are almost identical (Figure 3 and Table 2). The total energy of the transition state is rather insensitive to this torsional degree of freedom. MP2/6-31+G(d,p)//HF calculations show that TS_e is 2.6 kcal/mol above TS_g with trimethylsulfonium ion as the model for AdoMet. The correction for BSSE had negligible effect on this energy difference. The optimization of the transition state at the MP2/6-31+G(d,p) level leads to contraction of C−O (2.159 to 2.104 Å) and C−S (2.119 to 2.047 Å) bonds; the addition of a second set of polarization functions at the HF level did not result in significant changes in TS geometry (data not shown). Current ab initio transition-state geometry appears to be consistent with known experimental isotope effects for transmethylation reaction in model systems and in catechol *O*-methyltransferase.[6,7,9,10,26]

The results in Table 2 indicate that the activation barrier for methyl transfer is strongly dependent on the mutual orientation of the reactants. In accord with previous work,[7] the energy difference between the transition state and the reactant complex is large, 21−23 kcal/mol, when the reactant complex adopts the minimum energy configuration in the gas phase. Unfavorable mutual orientation of reactants may also explain the high activation barriers, 25−28.5 kcal/mol, measured in homoge-

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Table 1. (a) Calculated Electronic Energies (a.u.), HF/6-31+G(d,p) ZPVEs (kcal/mol) for Reactants and Products for the COMT Reaction, and (b) ZPVE-Corrected Reaction Enthalpies for the Reaction between the Catecholate Anion and the Methyl Donor at 0 K

	HF/6-31+G(d,p)// HF/6-31+G(d,p)	MP2/6-31+G(d,p)// HF/6-31+G(d,p)	MP2/AUG-cc-pVDZ// MP2/6-31+G(d,p)	ZPVE
		(**a**)		
catecholate anion	−379.879835	−381.091441	−381.159266	64.55
O-methylcatechol	−419.470489	−420.815287	−420.877723	92.28
$CH_3S^+(CH_3)_2$	−516.126873	−516.725885	−516.758664	77.17
$CH_3S^+(CH_2CH_3)_2$	−594.210120	−595.112741	−595.151479	115.38
dimethyl sulfide	−476.746082	−477.195358	−477.230241	50.69
diethyl sulfide	−554.822249	−555.575579	−555.615356	88.93
		(**b**) Methyl Donor		
$CH_3S^+(CH_3)_2$	−130.54	−120.16	−118.10	
$CH_3S^+(CH_2CH_3)_2$	−126.08	−115.97	−113.25	

Table 2. Structural and Energetic Characteristics of Ground-State Reactant Complexes (RC) and Transition States (TS) in the Gas Phase (g) and Enzymatic (e) Orientation at the HF/6-31+G(d,p) Optimized Level[a]

system	r_{C-O}	r_{C-S}	$\theta_{C(R1)S-OC}$	$\theta_{CO-CC(OH)}$	HF/6-31+G(d,p)	MP2/6-31+G(d,p)
			Trimethylsulfonium as the Methyl Donor			
RC(g)	2.996	1.807	180.0	180.0	−896.149990	−897.972174
RC(e)	2.568	1.829	131.4	−114.8	−896.119627	−897.939216
TS(g)	2.160	2.137	5.5	−88.1	−896.114497	−897.938151
TS(e)	2.159	2.119	131.4	−114.8	−896.112481	−897.934000
			Diethylmethylsulfonium as the Methyl Donor			
RC(g)	3.014	1.808	180.0	180.0	−974.228294	−976.355901
RC(e)	2.646	1.822	119.2	−96.4	−974.200522	−976.325862
TS(g)	2.148	2.156	5.1	−90.0	−974.192484	−976.321132
TS(e)	2.151	2.141	121.4	−96.4	−974.190852	−976.317780

[a] θ Is the dihedral angle that determines the orientation of catecholate relative to the methyl donor.

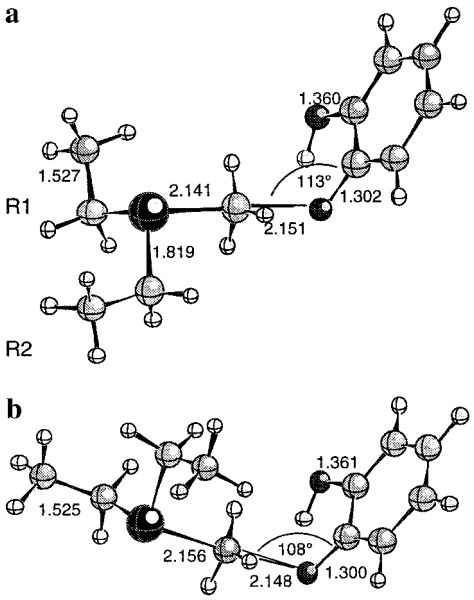

Figure 3. Optimized geometries of the transition states TS_e (a) and TS_g (b) with diethylmethylsulfonium ion as model for the methyl donor.

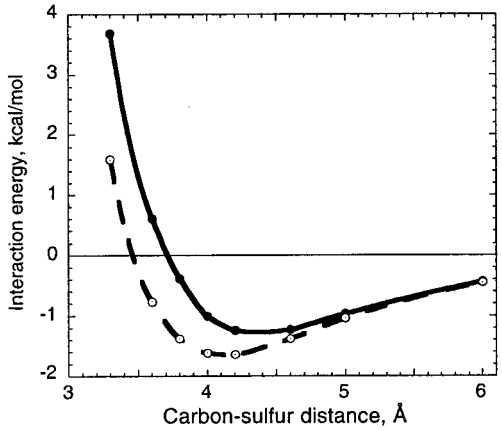

Figure 4. Interaction energy between the transition state and model of Tyr68 methylene (solid line), and between the reactant complex and model of Tyr68 methylene (dashed line) at the MP2(Full)/6-31+G-(d,p) level. $CH_3S^+(CH_2CH_3)_2$ was used as the methyl donor. (See Figure 1)

neous solution for analogous reactions.[8] A much smaller energy difference between the transition state and the reactant complex was found when the reacting system was constrained to the orientation present in the enzyme. The activation energy was only 3.27 kcal/mol with the trimethylsulfonium ion and 5.08 kcal/mol with the diethylmethylsulfonium ion. This result strongly suggests that a large part of the catalytic efficiency of COMT arises from the proper alignment of substrates in the active site.

The MP2/6-31+G(d,p) interaction energies of three enzymatic groups with the reactant complex and the transition state as functions of intermolecular distances are shown in Figures 4−6. Figure 4 shows that there is a very weak attraction between the methylene of Tyr68 and substrates, and slightly weaker attraction occurs between the methylene of Tyr68 and the transition state. In contrast, the HF/6-31+G(d,p) interaction profiles, which neglect electron correlation and thus do not describe dispersion forces, do not predict any attraction between the methylene unit of Tyr68 and the reacting system (data not shown). The ONIOM MP2:HF calculation with the model shown in Figure 2b suggests that the presence of Tyr68 increases the activation enthalpy by 0.6 kcal/mol, in good agreement with data in Figure 4. Figures 5 and 6 indicate that the polar peptide groups of Met40 and Asp141 interact strongly with both ground state and transition state, but the interactions are stronger in the ground state. This interaction arises mainly due to the dipolar nature of the carbonyl groups and at short distances is balanced by repulsion between electron clouds of the carbonyl oxygen and the sulfur atom. Qualitatively similar results were obtained at the HF/6-31+G-

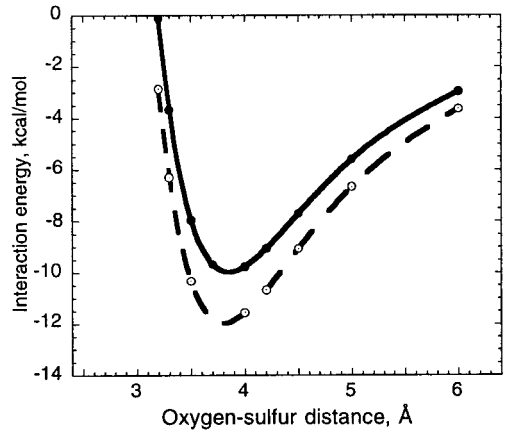

Figure 5. Interaction energy between the transition state and peptide group of Asp141 (solid line), and between the reactant complex and the peptide group of Asp141 (dashed line) at the MP2(Full)/6-31+G(d,p) level. $CH_3S^+(CH_3)_2$ was used as the methyl donor. (See Figure 1)

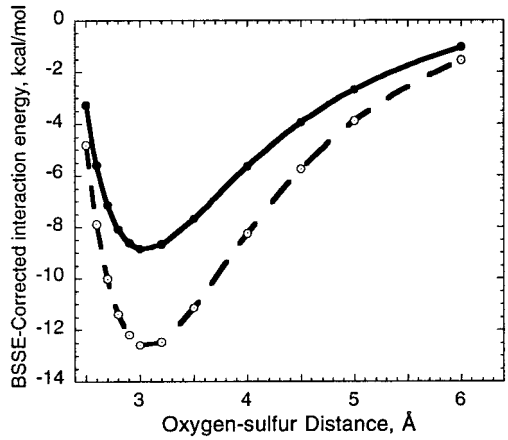

Figure 6. Interaction energy between the transition state and the peptide group of Met40 (solid line), and between the reactant complex and the peptide group of Met40 (dashed line) at the MP2(Full)/6-31+G(d,p) level. $CH_3S^+(CH_3)_2$ was used as the methyl donor. (See Figure 1)

(d,p) level as well. Stronger interaction with the ground state is also observed in the ONIOM MP2:HF calculation where inclusion of complete Met40 residue and adjacent backbone atoms (Figure 2a) increases the activation barrier from 3.27 to 5.87 kcal/mol.

Discussion

The methyl transfer reaction catalyzed by COMT (Scheme 1) is an interesting and important example of an enzymatic S_N2 reaction mechanism. The solution reaction is very slow and the enzyme accelerates the process enormously. The origin of this rate acceleration has not been clearly established, but two hypotheses, transition-state stabilization[6,9,10] and formation of highly reactive near attack ground-state conformations,[7,11] have been advanced. In addition, desolvation likely plays a role in the COMT catalysis because the transition state for the methyl transfer reaction is less strongly solvated than the ionic reactant state.[26]

It is usually believed that enzymes interact more strongly with transition states than with ground states. A popular view suggest that this preferential interaction is achieved due to the presence of preoriented dipoles.[27,28] We have analyzed here the effect of two such strong dipoles (peptide groups of Met40 and Asp141) and found that, while the interaction of these groups with the transition state is strong, it is even stronger in the ground state. This finding should not be surprising, considering that the reactant ground state consists of an ion pair while partial charge transfer has occurred in the transition state. It can be argued that charges or dipoles far from the active site might interact preferentially with the transition state,[29] but it is not clear how this can be achieved because the transition state for the methyl transfer is less polar than the ground state. Overall, it appears that the transition state of the COMT reaction interacts more weakly with enzyme dipoles than does the ground state.

The orientation of reactants is of crucial importance, in agreement with model studies of the transmethylation reactions where intramolecular accelerations as large as 10^6 M have been observed.[1,6] The reaction in the gas phase and in the enzyme active site follow slightly different reaction coordinates. Specifically, steric requirements of the enzyme active site prevent formation of the strong ion−ion complex which is favored in the gas phase. Instead, a weaker complex with alternative orientation of reactants is formed in the active site. This effectively amounts to the destabilization of ground state, and it can be described as a strain effect, deformation of a contact pair into a conformation with high internal energy but favorable orientation for the methyl transfer. Such near attack conformations (NACs) have been proposed to be important also for intermolecular reactions, and in catalytic cycles of other enzymes, such as haloalkane dehalogenase and formate dehydrogenase.[30,31]

The present results also suggest that the role of the desolvation of reactants and the transition state by the enzyme is limited. The transition state for this transmethylation reaction is less polar than the reactant complex, and a polar environment will lead to decreased rates of reaction when compared to the gas phase.[1,32] The increase in the activation energy with the increasing solvent polarity has been reported in a previous SCRF study of the COMT reaction.[7] Also, it has been shown that the activation barrier for the reaction between $(CH_3)_3S^+$ and Cl^- increases from 29.2 kcal/mol to 32.4 kcal/mol when the dielectric constant increases from 25 to 79.[33] It thus follows that the gas-phase activation barrier we now find for the COMT reaction, 21−23 kcal/mol, is the lowest limit for a reaction which follows the gas-phase reaction coordinate. The reaction in the aqueous solution most likely follows a similar reaction coordinate and faces an activation barrier of about 28 kcal/mol.[8] If the reaction in the COMT active site follows the gas-phase reaction coordinate, the enzymatic reaction would be faster than reaction in water, but it would still face an activation barrier larger than 21 kcal/mol. The only way efficient catalysis can be achieved would be to rearrange the reactants in the ground state so that they resemble the transition state. We have

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previously reported that the NAC structures can be found to form in the ES complex by use of MD simulation.[11] Binding of the two substrates in the NAC orientation is a critical prerequisite for the efficient transfer of the methyl group from AdoMet to catecholate.

The calculations reported here illustrate some difficulties associated with ab initio estimation of activation energies for enzymatic reactions. The calculated activation barrier for two isolated reactants in enzymatic alignment is 3−5 kcal/mol. The interaction of the rigid reacting system with three active-site residue models (Figures 4−6) appears to increases the activation energy by about 6 kcal/mol, assuming pairwise additivity of interactions. The relaxation of reactant state or transition-state geometries in the active site probably balances these effects to a certain extent. Ideally, optimization of the reactant complex and the transition state in the presence of active-site residues is needed to obtain a better estimate of the activation barrier in the enzyme. However, such optimizations are challenging not only because the system is large but also due to the effect of basis set superposition error on intermolecular energies and energy gradients. Recent advances in combined quantum mechanical−molecular mechanical (QM/MM) methods[34,35] hold great promise for making such calculations both feasible and accurate. The QM/MM treatment of the active site often requires calibration of nonbonded parameters for active-site residues to accurately describe interactions between the enzyme and the reacting system.[36] The methodology described in the present paper is suitable for obtaining accurate and BSSE-free interaction energies which serve as calibration data for QM/MM calculations.

Conclusions

Analysis of the catechol *O*-methyltransferase reaction suggests that the lowering of activation enthalpy relative to the uncatalyzed reaction is mainly achieved by orienting the nucleophile and electrophile into a conformation that closely resembles the structure of the transition state. The interaction of transition state with three active site residues—Met40, Tyr68, and Asp141—is weaker than interaction of the more polar ground state with the same residues. Thus, interaction between these three enzymatic groups and the reacting system does not lower the activation barrier for this transmethylation reaction. The role played by Met40, Tyr68, and Asp141 is to assist in creation of a ground-state conformation that closely resembles that of the transition state.

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