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A Theoretical Examination of the Factors Controlling the Catalytic Efficiency of a Transmethylation Enzyme: Catechol *O*-Methyltransferase

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Abstract: The reaction mechanism of the nonenzymatic transmethylation of catechol by *S*-adenosylmethionine (AdoMet, as modeled by sulfonium ion) has been elucidated using *ab initio* and semiempirical quantum mechanical methods. The gas phase reaction between catecholate and sulfonium is extremely fast, involving no overall barrier. The reaction profile to some extent resembles a typical gas phase S_N2 reaction. However, in aqueous solution, this reaction is very slow with a predicted barrier of 37.3 kcal/mol. The calculated $(k_H/k_D)_\alpha$, k^{12}/k^{13}, k^{16}/k^{18}, and k^{32}/k^{34} are 0.80, 1.06, 1.003, and 1.010, respectively. Previously, Schowen and co-workers measured $(k_H/k_D)_\alpha$ and k^{12}/k^{13} to be 0.83 ± 0.05 and 1.09 ± 0.05 for the catechol *O*-methyltransferase (COMT)-catalyzed methylation of 3,4-dihydroxyacetophenone by AdoMet. This good agreement between the calculated kinetic isotope effects for the model reaction and the measured kinetic isotope effects for the enzymatic reaction seems to suggest that the structure of the enzymatic transition state is very similar to that of the nonenzymatic reaction. Factors that modulate the catalytic efficacy of catechol *O*-methyltransferase were discussed in light of the present study on the nonenzymatic reaction and the recently solved X-ray crystal structure.

Introduction

Methyltransferases are enzymes that catalyze the transmethylation reactions involving the transfer of the *S*-methyl group of *S*-adenosylmethionine (AdoMet) to either nitrogen, oxygen, or carbon atoms of a wide variety of nucleophiles. Methyltransferase can modify DNA, RNA, proteins, and small molecules such as catechol. In prokaryotes, DNA methylation protects the host DNA from being degraded by restriction enzymes,[1] while in eukaryotes, DNA methylation appears to have a more diverse role in the control of cellular processes such as differentiation and gene regulation.[2] 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 methylated catechol and *S*-adenosyl-L-homocysteine (AdoHcy).[3] Although, the DNA methyltransferases and catechol *O*-methyltransferase function on very different substrates, recent crystallographic studies have revealed that their three-dimensional structures are strikingly similar.[4−6] They share a

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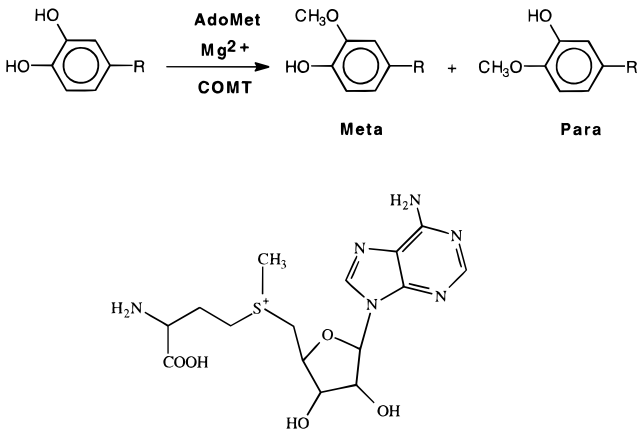
Scheme 1



similar catalytic domain; in the case of DNA methyltransferases, there is also a recognition domain for substrate DNA. A recent structural comparison suggested that many (if not all) AdoMet-dependent methyltransferases have a common catalytic domain structure.[7] Earlier studies demonstrated that the transfer of a methyl group occurs with inversion of configuration at the carbon center, indicating the methyl transfer is a direct S_N2 process.[8] Our interest here is focused on catechol *O*-methyltransferase. Since the AdoMet-dependent methyltransferases have a common catalytic domain structure, their catalytic mechanism should be similar. Thus, our study regarding the catalytic mechanism of catechol *O*-methyltransferase will be of general interest in the study of other AdoMet-utilizing transmethylation enzymes.

Scheme 1 displays a generic reaction catalyzed by COMT. It was suggested earlier that the catalytic mechanism of COMT-catalyzed transmethylation reaction occurs via a general-base-catalyzed proton abstraction from either one or both of the catechol hydroxyl groups (Scheme 2, eq 1).[9] Model reactions were used to examine this proposal. Coward and co-workers investigated COMT model reactions in great detail.[10] Some of the intramolecular model reactions do show large effective molarity (10^6 M) as compared to the intermolecular reactions. General-base catalysis was indeed observed in these model reactions (Scheme 2, eq 2). Later, it was found that COMT is a monomeric enzyme requiring a Mg^{2+} ion for catalysis.[11] Schowen and co-workers measured the α-deuterium and carbon-13 isotope effects for the COMT-catalyzed methylation reactions, and these studies revealed that the rate-determining step is the transfer of the methyl group.[12] They also demonstrated that the S_N2 transition state for the methyl transfer is symmetrical and tight. However, the role of the Mg^{2+} remained unclear until the availability of structural information of a COMT. In 1994, Vidgren *et al.*[6] reported a 2.0 Å resolution crystal structure of rat liver catechol *O*-methyltransferase with bound AdoMet, Mg^{2+}, and an inhibitor (3,5-dinitrocatechol). The rat liver COMT is a monomeric protein having 221 amino acids with a molecular weight of 24.7 kD. According to the crystal structure,[6] Mg^{2+} seems to play a structural role, organizing the active site and bringing together AdoMet and catechol. On the basis of the X-ray crystal structure and recent kinetic studies,[13] it was postulated that AdoMet binds to COMT first, which is then followed by Mg^{2+} and substrate.

In the current study, we examine the reaction mechanism of the nonenzymatic transmethylation reaction between catechol and the sulfonium cation in detail. On the basis of the present study and the recently solved X-ray crystal structure, factors that may affect the catalytic efficiency of COMT will be discussed. A detailed mechanism for COMT-catalyzed methylation will be presented.

Theoretical Procedure

In the current study, both semiempirical and *ab initio* molecular orbital theory methods were used. The PM3[14] Hamiltonian was used in the semiempirical calculations, and these calculations were performed using AMPAC5.0.[15] For the *ab initio* calculations, geometry optimization and a transition state search were performed at the HF/6-31+G-(d,p) level of theory using the GAUSSIAN 94 program.[16] The inclusion of diffuse functions in the basis set is necessary for anionic species. To account for electron correlation effects, the energy of each species was calculated at the MP2/6-31+G(d,p) level of theory. Since the density functional theory method seems to be an attractive alternative to the MP2 method, energy calculations were also performed using a hybrid density functional theory method at the B3LYP/6-31+G(d,p) level of theory.[17] Minima and the transition state were characterized by calculating the harmonic vibrational frequencies.

To examine the effect of hydration on the reaction profile, two approaches were used. First, the solvation effect on the reaction profile was included using Tomasi's polarizable continuum solvation[18] model as implemented in the GAUSSIAN 94 program (the self-consistent isodensity polarized continuum model (SCI-PCM) option).[19] In these solvation calculations, the solute cavity was determined self-consistently from an isodensity surface of the electron density of the solute molecule with an isodensity of 0.0004 au. The dielectric constant of water (78.3) and gas phase geometries were used. It should be pointed out that the *ab initio* SCI-PCM method only accounts for electrostatic contributions to the solvation free energy; specific interactions between solvent and solute such as hydrogen bonding interactions and cavitation terms are not included in the SCI-PCM implementation. Specific interactions between the solvent and solute molecules are very important for small ions, and neglect of these interactions could underestimate the hydration free energies of small ions. Alternatively, the solvation effect can be accounted for by using the PM3-SM3 method, which was developed by Cramer and Truhlar[20] and parametrized to reproduce the experimental hydration free energy of both neutral and charged molecules.

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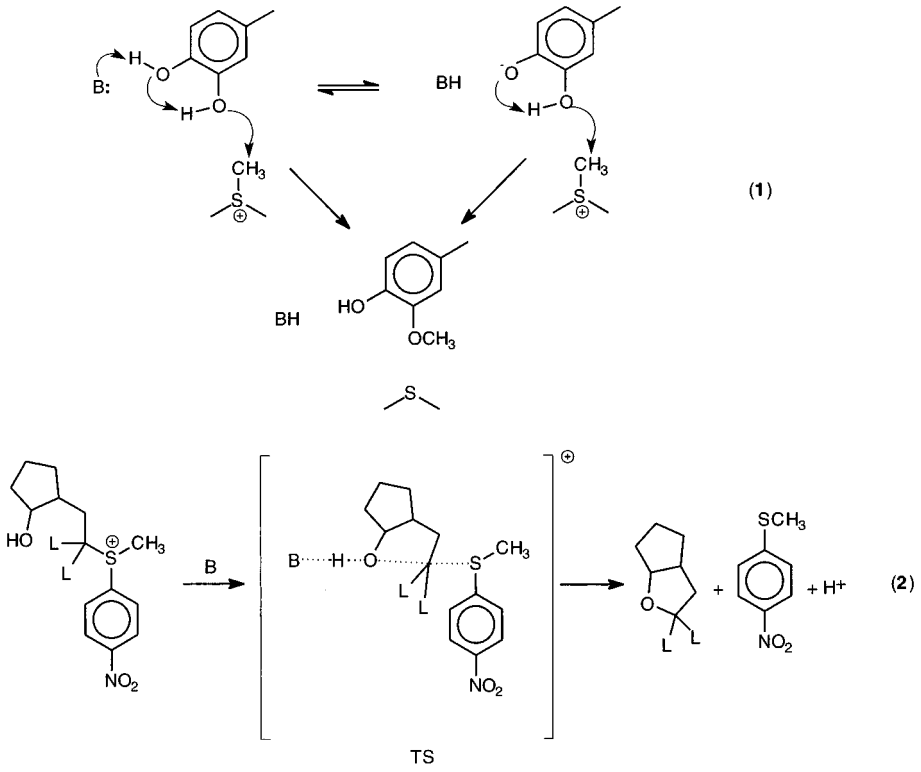
Scheme 2



To examine the effect of the presence of the Mg^{2+} on the acidity of the hydroxyl group of catechol, two models were used: (a) a "naked" Mg^{2+} and (b) Mg^{2+} with bound ligands. The latter model was built from the coordinates of the X-ray crystal structure of rat liver COMT with bound Mg^{2+} and a competitive inhibitor (3,5-dinitrocatechol).[6] In the active site, Mg^{2+} is coordinated to the two hydroxyl groups of catechol, the side chain oxygens of Asp141, Asp169, and Asn170, and a water molecule. In our calculations, the side chain carboxylate groups of Asp141 and Asp169 were replaced by formate and the side chain of Asn170 was replaced by formamide. Due to the large size of the model, calculations were done only at the HF/6-31G(d) level of theory.

Results and Discussion

The *ab initio* and PM3-calculated geometries for the reactants, ion pair complex intermediate, transition state, and products are given in Figures 1 and 2, respectively. The calculated total electronic energies and heats of formation are summarized in Table 1. According to the *ab initio* molecular orbital calculations, catechol, catecholate, and methylcatechol are all planar molecules. The most stable conformation for each compound is the one with an internal hydrogen bond, in agreement with many experimental studies on catechol.[21] The energy difference between the structure with and the one without this hydrogen bonding interaction is about 5.0 kcal/mol for methylcatechol, 13.9 kcal/mol for catecholate, and 4.6 kcal/mol for catechol at the HF/6-31+G(d,p) level of theory, respectively. Thus, in the following discussions, only the most stable substrate species (the ones with the internal hydrogen bond) are considered.

Acidity of the Substrate. Although general-base catalysis was observed for model reactions using cyclopentanols and phenols,[10] the possibility of specific-base catalysis for reactions involving catechols is very high due to their acidity. In the case of specific-base catalysis, catechol is deprotonated first. This is followed by the attack of the oxyanion on the sulfonium ion. The function of the hydroxyl group in the catecholate ion is to stabilize the oxyanion (acting like a surrogate solvent). Since it is very likely that the catechol is deprotonated before the S_N2 reaction, we examined the acidity of the catechol hydroxyl group using the *ab initio* molecular orbital method. The calculated deprotonation energies are shown in Figure 3. The deprotonation energy for catechol in the gas phase is 355.9 and 360.6 kcal/mol at the HF/6-31+G(d,p) and HF/6-31G(d) levels of theory, respectively. The presence of Mg^{2+} greatly increases the acidity of catechol in the gas phase, and the deprotonation energy in the presence of Mg^{2+} is 132.1 kcal/mol at the HF/6-31+G(d,p) level. However, when all of the coordination numbers of Mg^{2+} are satisfied and the positive charge on Mg^{2+} is neutralized by the ligands, the effect of Mg^{2+} on the acidity of catechol hydroxyl is greatly reduced. As shown in Figure 3, the deprotonation energy is now 335.5 kcal/mol at the HF/6-31G(d) level of theory. Although Vidgren *et al.*[6] speculated that the Mg^{2+} probably lowers the pK_a of the hydroxyl group of catechol, surprisingly, as revealed by the experimentally measured pK_a values,[22] the presence of Mg^{2+} in COMT does not seem to change the pK_a values of catechols compared to their pK_a values in aqueous solution. This is unexpected since even in aqueous solution, the Mg^{2+} bound water has a pK_a of 11.4,[23] which is several pK_a units lower than the pK_a of pure water (15.7). Further experimental investigations are needed to resolve this issue.

Reaction in the Gas Phase. The reaction we investigated is shown in Scheme 3. The first step of the reaction is deprotonation of catechol. The resulting catecholate anion attacks the trimethylsulfonium ion in an S_N2 reaction. Since the reactants are oppositely charged and the products are neutral, the reaction is extremely exothermic in the gas phase. When the catecholate and trimethyl sulfonium ions approach each

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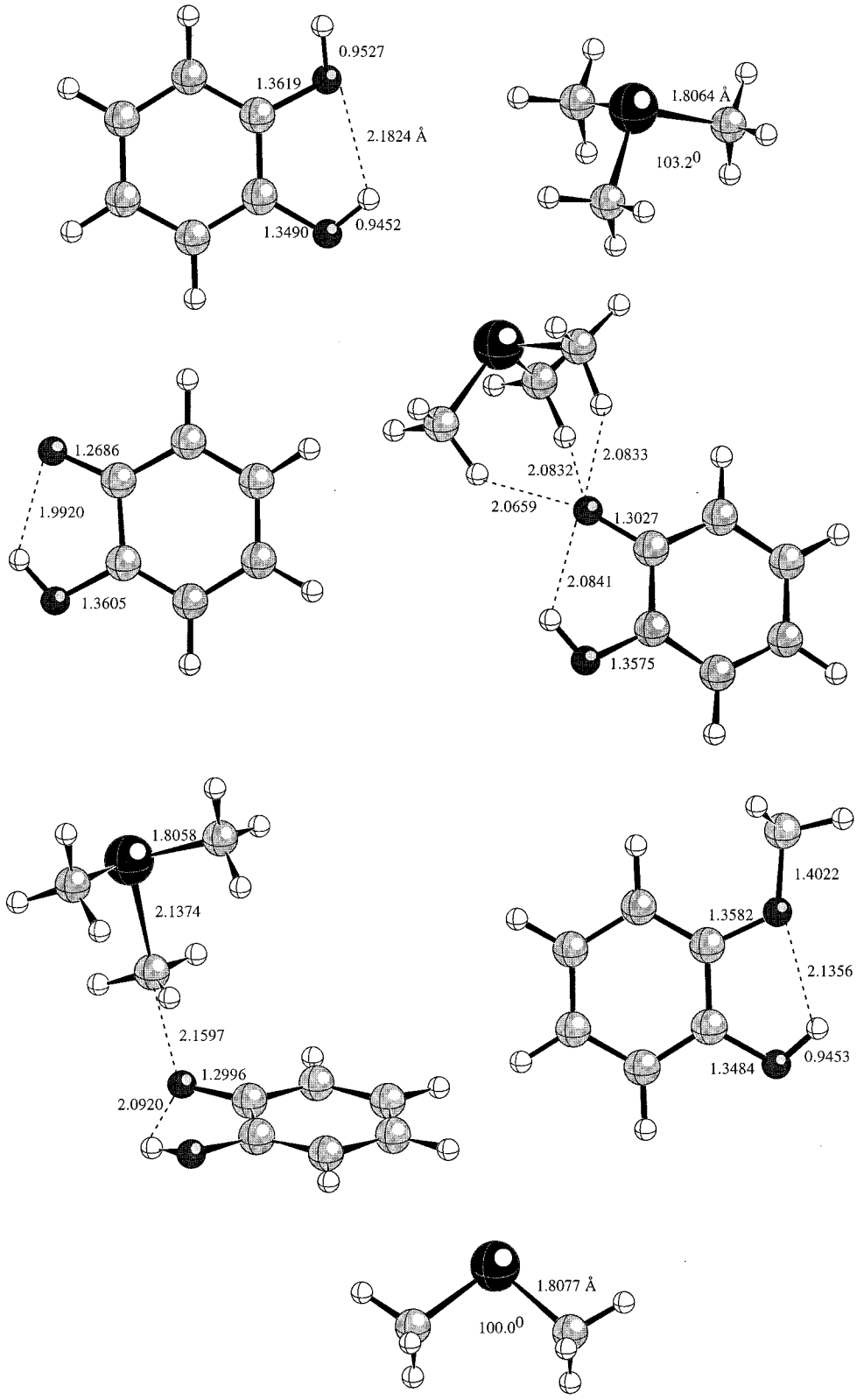

Figure 1. Optimized geometries at the HF/6-31+G(d,p) level of theory.

other, an ion pair complex is formed. This complex is separated from the products by a transition state. The calculated potential energy surface is given in Figure 4. The gas phase reaction profile to some extent resembles a typical S_N2 reaction of an anion with a neutral molecule;[24] however, there are some obvious differences. Normally, the stabilization for the formation of an ion−molecule complex is less than 15 kcal/mol. In the current study, since the two reactants are oppositely charged, there is an extremely large attraction in the gas phase. This results in a very large stabilization for the formation of the initial ion pair complex (e.g., 89.9 kcal/mol at the HF/6-31+G(d,p)

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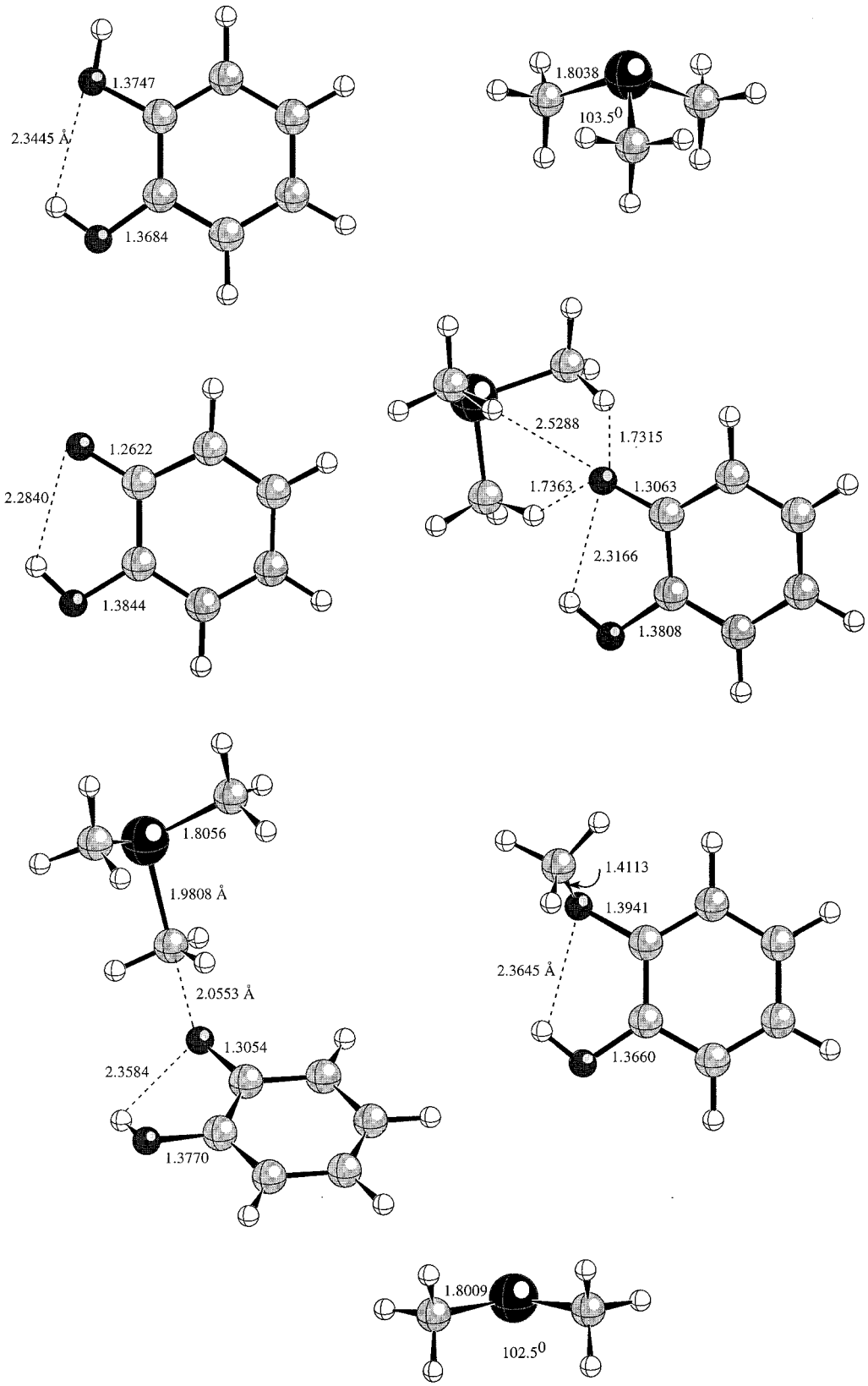


Figure 2. Optimized geometries using the PM3 method.

level of theory). After passing the transition state, the charges are essentially annihilated, so no significant stabilization is observed when the two products are close together. Since the transition state has a lower energy than the sum of the two separate reactants, the overall reaction has no barrier. The initial ion pair complex is separated from the products by a large barrier.

As revealed in Figure 4, all four methods [HF/6-31+G(d,p), PM3, MP2/6-31+G(d,p), and B3LYP/6-31+G(d,p)] predict a large stabilization for the formation of the initial ion pair complex. The variation among these four methods is rather small; i.e., the maximum difference is 6.4 kcal/mol. It should be pointed out that since the PM3 method was parametrized to reproduce experimental data such as heats of formation, the thermal effects are built-in through parametrization. However, the *ab initio* and density functional theory values (corresponding

Table 1. Calculated Electronic Energies (hartrees, 1 hartree = 627.5 kcal/mol) and Heats of Formation (PM3 in kcal/mol) for Each Species

compound	HF/ 6-31+G(d,p)	B3LYP/ 6-31+G(d,p)	MP2/ 6-31+G(d,p)	PM3
catechol (**1**)	−380.446 92			−65.0
catecholate (**2**)	−379.879 83	−382.167 91	−381.054 44	−92.3
$S(CH_3)_3^+$ (**3**)	−516.126 87	−517.683 56	−516.698 05	149.9
complex (**CP**)	−896.149 99	−899.996 42	−897.905 90	−35.6
TS	−896.114 50	−899.972 00	−897.872 27	−8.9
$S(CH_3)_2$ (**5**)	−476.746 08	−478.025 23	−477.173 35	−11.0
methylcatechol (**4**)	−419.470 49	−422.021 91	−420.773 53	−58.1

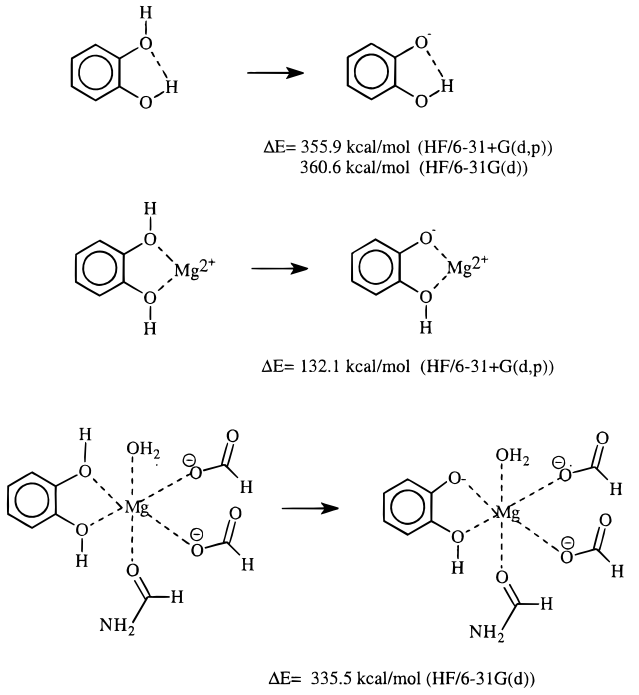
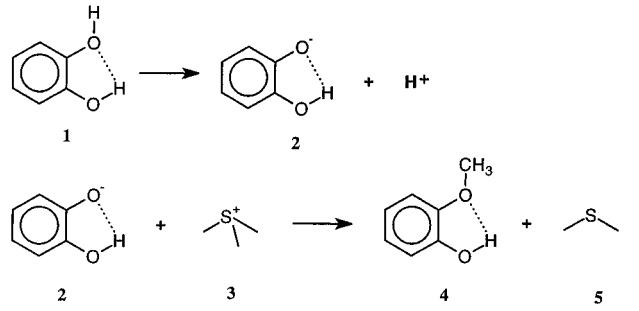


Figure 3. Calculated deprotonation energies for catechol with and without the presence of Mg^{2+} at the HF/6-31G(d) and HF/6-31+G-(d,p) levels.

Scheme 3



to 0 K) do not include thermal corrections and zero-point energy corrections. The calculated barriers from the ion pair to the transition state are also similar among the four methods. The B3LYP barrier is about 5.8 kcal/mol lower than the MP2 value. On the other hand, the PM3 barrier is about 5.6 kcal/mol higher than the MP2 barrier. By comparison, the variation among the calculated reverse barriers is larger. The HF/6-31+G(d,p) and PM3 barriers are similar and are the MP2 and B3LYP barriers. However, the HF/6-31+G(d,p) and PM3 barriers are uniformly larger than the MP2 and B3LYP barriers.

Recently, Radom and co-workers examined the performance of B3LYP density functional theory on S_N2 reactions of Cl^- with CH_3Cl and CH_3Br in detail.[25] According to their study, the complexation energy for the formation of the ion−molecule complex is not sensitive to the method used, but the central barrier varies considerably. For instance, the MP2/6-31+G(d) and B3LYP/6-31+G(d) barriers differ by 34.0 kcal/mol for the reaction between Cl^- and CH_3Cl. A similar trend was also found for the reaction between Cl^- and CH_3Br.[25] The B3LYP density functional theory greatly underestimates the central barrier for S_N2 reactions of Cl^- with CH_3Cl and CH_3Br.[25] However, as shown in Figure 4, we did not observe a similar phenomenon in the reaction between the catecholate ion and trimethylsulfonium ion. Although the B3LYP density functional theory method has become a popular alternative to the correlated *ab initio* molecular orbital method such as MP2, care must be exercised when the density functional theory method is used.

As shown in Figure 1, there is an internal hydrogen bond in the catecholate anion. The O···H distance is 1.992 Å in the HF/6-31+G(d,p) geometry. In the initial complex formed between the positively charged sulfonium ion and the negatively charged catecholate ion, the oxygen anion of catechol interacts with the sulfonium ion through three hydrogen bonds of 2.083, 2.083, and 2.066 Å. As reflected by the hydrogen bonding distance (2.084 Å in the initial complex vs 1.992 Å in the free catecholate anion), the internal hydrogen bond between the oxygen anion and the hydroxyl group of catechol becomes weaker when the negative charge on the oxygen anion is being stabilized by the sulfonium cation. However, the orientation of the methyl groups of the sulfonium ion is such that a direct S_N2 reaction is not possible without further structural rearrangement. In this sense, the formation of such a complex is not productive for an enzymatic reaction, and the enzyme has devised an elegant way to solve this problem (*vide infra*). The calculated transition state structure is also given in Figure 1. In the transition state, the forming C···O distance is 2.160 Å and the breaking C···S distance is 2.137 Å. The attacking oxygen, the transferring methyl carbon, and the leaving sulfur atom are in an almost linear arrangement, the angle being 177°. The transferring methyl group is close to being planar. Again, the internal hydrogen bond of catechol is weaker (2.092 Å) than in the anion. Earlier isotope labeling studies by Schowen and co-workers indicated that the S_N2 transition state for COMT-catalyzed reaction is tight and symmetrical.[12] Our results are consistent with their observation. Although, our calculated results refer to the gas phase reaction and their studies were for the enzyme, as shown recently, the S_N2 transition state appears to be implastic and the structure changes insignificantly in a different environment.[26] In the methylcatechol, the internal hydrogen bond distance is 2.136 Å.

The PM3-calculated structures are shown in Figure 2. Overall, the PM3 structures are very close to the *ab initio* structures. However, there are some noticeable differences. In the PM3 structures, the internal hydrogen bonding is weaker in catechol, catecholate, and methylcatechol as indicated by the C···O distances. Even though, like the *ab initio* calculations, PM3 predicts that the breaking C···S distance is shorter than the forming C···O distance, the calculated distances are both significantly smaller than the *ab initio* ones. The C···O distance is about 0.10 Å shorter, while the C···S distance is 0.16 Å shorter. The angle around the transferring methyl (O−C−S) is smaller (163.9°) than that in the *ab initio* structure (177°). Thus, the PM3 transition state is tighter than the *ab initio* one.

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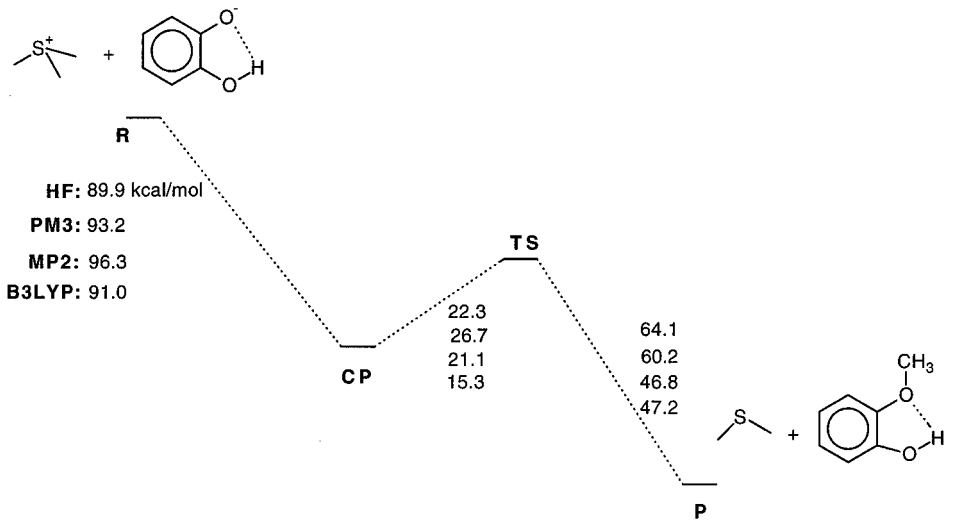

Figure 4. Calculated schematic potential energy profiles in the gas phase for the reaction between catecholate and trimethylsulfonium.

Table 2. Calculated Energies in the Presence of a Self-Consistent Reaction Field (SCI-PCM) with the Dielectric Constant of Water (78.3)

compound	HF/6-31+G(d,p)	B3LYP/6-31+G(d,p)
catecholate (**2**)	−379.954 88	−382.237 52
$S(CH_3)_3^+$ (**3**)	−516.217 62	−517.774 48
complex (**CP**)	−896.179 69	−900.024 06
TS	−896.145 75	−899.997 39
$S(CH_3)_2$ (**5**)	−476.749 35	−478.028 18
methylcatechol (**4**)	−419.477 96	−422.027 88

In the PM3-optimized structure of methylcatechol, the methyl group is twisted out of the catechol aromatic plane, and this structure is about 0.5 kcal/mol lower in energy than the one with the methyl group in the catechol plane.

Solution Reaction. Since both reactants are charged, solvation by solvent molecules will have a dramatic effect on the energetics of this reaction. To estimate the solvation effect, we used a self-consistent reaction field to mimic the solvent. Energy calculations were performed for each species using the SCI-PCM model in GAUSSIAN 94 and the PM3-SM3 method developed by Cramer and Truhlar.[20] Table 2 lists the calculated energies using the SCI-PCM solvation model at the HF/6-31+G(d,p) and B3LYP/6-31+G(d,p) levels. The resulting reaction profiles are given in Figure 5.

As expected, inclusion of a solvent has a dramatic effect on the calculated reaction profile. The gas phase reaction has no overall barrier, but according to the calculations, there is a large barrier in solution for the nucleophilic reaction between the catecholate anion and the trimethylsulfonium ion. The SCI-PCM calculations at the HF/6-31+G(d,p) level give a barrier of 21.3 kcal/mol, with the ion pair complex still being a minimum on the reaction surface. Since the SCI-PCM model only calculates electrostatic contributions to the free energy of solvation, it probably underestimates the solvation free energy of ions. This could have a large effect on reactions involving charge annihilation. Therefore, the SCI-PCM results are likely to be inaccurate for such reactions. On the other hand, since the PM3-SM3 method was parametrized to reproduce experimental hydration free energies, it is suited for reactions involving charged species. On the PM3-SM3 reaction surface, the ion pair complex is no longer a minimum. The overall reaction is nearly thermoneutral with a forward barrier of 37.3 kcal/mol. The experimental barrier has not been determined for the reaction of catecholate ion with trimethylsulfonium ion, but the enthalpy barrier for the reaction between phenolate ion and

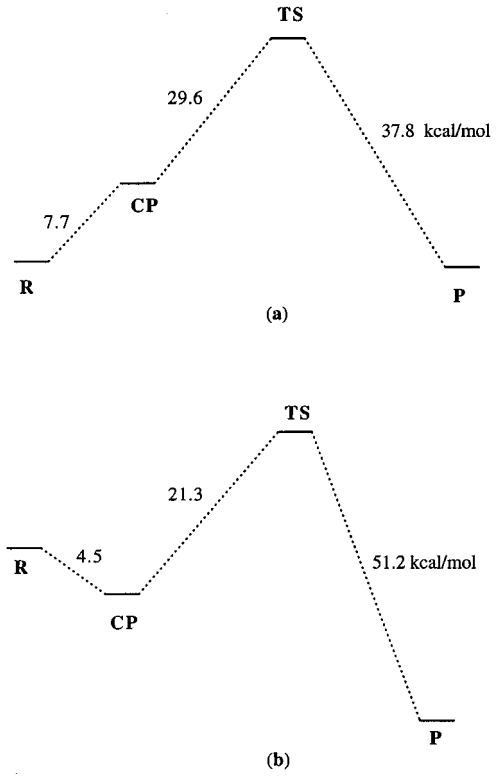

Figure 5. Calculated reaction profiles for the reaction of catecholate with trimethylsulfonium in solution using the PM3-SM3 (a) and the SCI-PCM (b) method at the HF/6-31+G(d,p) level.

trimethyl sulfonium ion has been determined to be 28.5 kcal/mol at 80 °C.[27] It is known that phenolate ion is more basic in aqueous solution than catecholate ion. Because of the positive Brønsted constant for nucleophilic attack ($\beta_{nuc} = 0.3$),[28] the barrier for the reaction between catecholate ion and trimethylsulfonium ion is expected to be higher than that for the reaction between phenolate and trimethylsulfonium ion. Thus, the PM3-SM3 barrier for the nucleophilic substitution reaction between catecholate ion and trimethylsulfonium ion may be a good estimate of the actual barrier in solution. However, in view of the performance of PM3 in the gas phase reaction, the calculated PM3-SM3 barrier is likely an upper limit.

Kinetic Isotope Effects. Kinetic isotope effects have been used widely to probe the structure of the transition state of

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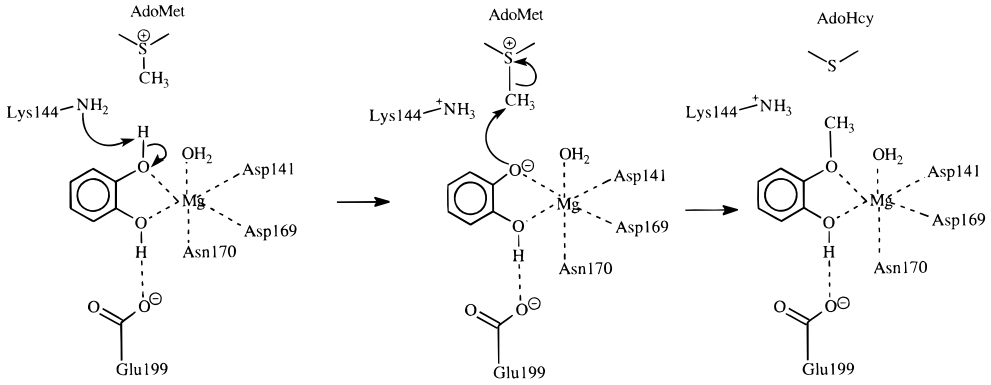
Scheme 4



chemical and enzymatic reactions.[29] Since Showen and co-workers have measured kinetic isotope effects for COMT-catalyzed transmethylation reaction between 3,4-dihydroxyacetophenone and AdoMet,[12] it would be of interest to compare the experimentally measured values with the calculated ones based on the gas phase *ab initio* transition state structure of the model reaction. The kinetic isotope effects were calculated according to the following formula at 298.15 K.[30] The free energies were calculated using standard techniques.[31]

$$k_L/k_H = \exp((\Delta G^{\ddagger}_L - \Delta G^{\ddagger}_H)/RT)$$

$$\Delta G^{\ddagger} = G_{TS} - G_{reactant}$$

The calculated $(k_H/k_D)_\alpha$, k^{12}/k^{13}, k^{16}/k^{18}, and k^{32}/k^{34} are 0.80, 1.06, 1.003, and 1.010, respectively. Previously, Schowen and co-workers found $(k_H/k_D)_\alpha$ and k^{12}/k^{13} to be 0.83 ± 0.05 and 1.09 ± 0.05 for the COMT-catalyzed methylation of 3,4-dihydroxyacetophenone by AdoMet.[12] Clearly, our calculated values are in reasonable agreement with the experimental data. It should be noted that the calculated values are based on the gas phase geometries. This suggests that the transition state structure for the methyl transfer in the enzymatic reaction is probably very similar to the gas phase transition state geometry. This is not surprising because recent studies on the S_N2 displacement of a chloride anion from 1,2-dichloroethane by a carboxylate ion, showed that the transition state structure for the S_N2 reaction remains essentially the same regardless of the environment even when the enzyme is included in the calculations.[26b,c]

Enzymatic Reaction. It was estimated that the enzymatic methylation reaction is a factor of 10^{16} times faster than the nonenzymatic reaction of catechol with sulfonium ion.[10b] It would be beneficial to examine how COMT could achieve such efficacy. Coward *et al.* proposed in 1973 that the COMT-catalyzed methylation of catechol by AdoMet is via a general-base catalysis.[9] Subsequent studies on model reactions provided further support for this proposal.[10] However, it was not clear at that time how Mg^{2+} fits into the proposed mechanism. The availability of the crystal structure[6] of rat liver COMT with a bound inhibitor, 3,5-dinitrocatechol, revealed that the Mg^{2+} ion is coordinated to the two hydroxyl oxygen atoms of 3,5-dinitrocatechol, the side chain oxygens of Asp141, Asp169, and Asn170, and a crystallographic water (Figure 6).

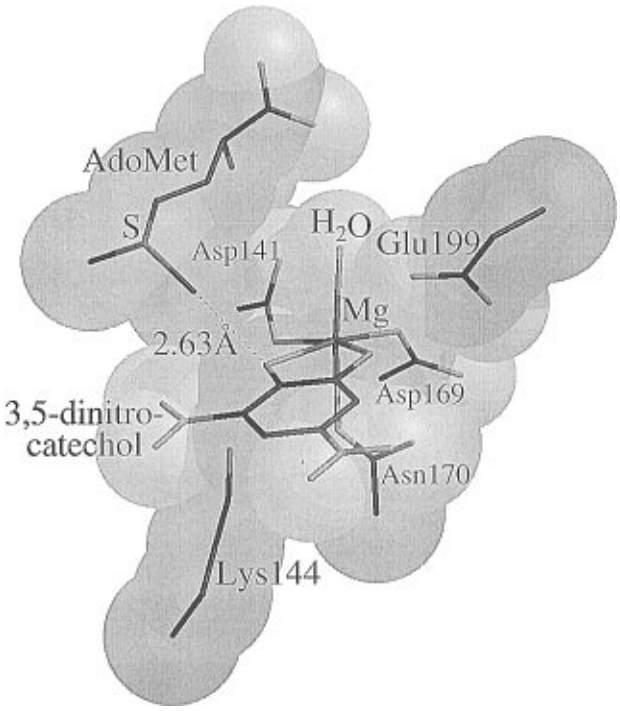


Figure 6. Active site structure of rat liver COMT with bound AdoMet, Mg^{2+}, and inhibitor (3,5-dinitrocatechol) as revealed by the X-ray crystallographic study. In the crystal structure, the side chain carboxylate of Glu199 is hydrogen bonded to one of the hydroxyl groups of 3,5-dinitrocatechol. The other hydroxyl of 3,5-dinitrocatechol appeared to be deprotonated, and the oxyanion is 2.63 Å away from the methyl group of AdoMet.

Catechol is expected to bind in the same way as 3,5-dinitrocatechol in the active site. In view of the crystal structure, the proposed catalytic reaction mechanism needs to be revised. Scheme 4 depicts a modified mechanism. As revealed by the crystal structure, the role of the Mg^{2+} is mainly to organize the binding site for catechol and bring together the electrophile and nucleophile. Since the pK_a of catechol is 9.48 in aqueous solution,[33] under physiological conditions only a small percent of catechol is ionized and the majority of catechol is in the neutral form. As a result, catechol probably binds to COMT in the neutral form. However, in order for the methylation to proceed, the catechol has to be deprotonated first. We propose that the side chain NH_2 group of Lys144 acts as the base to abstract the proton from the enzyme-bound catechol. A similar role for lysine has been invoked in a number of enzymatic

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reactions.[33] Certainly, if the substrate is already ionized in solution, e.g., at high pH or using acidic catechol derivatives as the substrate, this deprotonation step will not be necessary. The resulting catecholate ion then reacts with AdoMet, forming methylcatechol and AdoHcy. Methylation of the catecholate ion reduces the interaction between the catechol moiety and Mg^{2+}, making the release of the product occur readily. It was suggested that a conformational change is probably required for the release of AdoHcy from the active site.[6] Although the crystal structure of COMT with bound AdoHcy is not available, a recent study on a DNA methyltransferase has shown that AdoMet and AdoHcy bind differently to the methyltransferase, indicating a conformational change accompanies the methyl transfer.[34] The methyl transfer step is the rate determining step of the COMT-catalyzed reaction.[12]

The present investigation of the nonenzymatic reaction in the gas phase and in aqueous solution has clearly demonstrated (a) the methylation reaction is extremely fast in the gas phase and (b) the solution reaction is very slow due to the large barrier imposed by solvation. The obvious means to achieve high efficiency for the enzyme is to provide an environment where solvent molecules are excluded. Previous experimental studies on model reactions showed that bringing the electrophile and nucleophile together, e.g., in an intramolecular reaction, could provide a rate enhancement of 10^6 M. In the X-ray crystal structure[6] of rat liver COMT with bound inhibitor, the attacking oxygen of the catecholate was only about 2.63 Å away from the transferring methyl group and the O···C···S angle was 173.2°. The relative orientation of the nucleophile (catecholate ion) to electrophile (AdoMet) in the crystal structure resembles the transition state of the model reaction very closely. Clearly, the enzyme orients the nucleophile and electrophile in a near attack conformation (NAC)[35] such that the S_N2 reaction can occur without much structural reorganization. Further, the catalytic efficacy depends on how close the NAC matches the transition state, and catalytic efficacy reaches a maximum when the NAC and transition state become the same.

Since the catalytic efficacy of the methyltransferases depends on the correct juxtaposition of the nucleophile and the electrophile, it is very surprising to note that, in the model of a DNA−N^6-adenine methyltransferase M·*Tag*I with AdoMet and a DNA substrate, the transferring methyl group points in the wrong direction.[7d] This led Schluckebier *et al*. to propose that a rearrangement of the cofactor AdoMet is required to bring the cofactor and substrate close enough to allow methyl transfer to occur. This is clearly in conflict with what is discussed above concerning the NAC arrangement of the nucleophile and the electrophile. Indeed, recently, after a careful reevaluation, AdoMet is indeed in a catalytically competent orientation with respect to the base of the DNA, and the previously proposed conformational rearrangement of AdoMet becomes unnecessary.[34]

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Conclusions

In the present study, *ab initio* and semiempirical molecular orbital theory methods are used to examine the methylation of catechol by AdoMet. The gas phase reaction profile for the nucleophilic substitution reaction of catecholate ion with sulfonium ion is similar to a typical gas phase S_N2 reaction of an anion with a neutral molecule, but there are some significant differences. It was found that when the negatively charged catecholate ion and the positively charged sulfonium ion approach each other, a very stable ion pair complex is formed. All four methods [HF/6-31+G(d,p), MP2/6-31+G(d,p), B3LYP/6-31+G(d,p), and PM3] gave similar stabilization energies for the formation of the ion pair complex. The ion pair complex is separated from the products by a large barrier. The barrier calculated by the four methods varies by several kilocalories per mole, and the reverse barrier varies even more. In contrast to the fast reaction in the gas phase, the reaction in solution is very slow. The calculated reaction barrier in solution is about 37.4 kcal/mol.

Kinetic isotope effects were calculated for the structures of the transition state and reactants in the gas phase. The calculated values for $(k_H/k_D)_\alpha$ and k^{12}/k^{13} are in good agreement with the measured values for the COMT-catalyzed methylation of 3,4-dihydroxyacetophenone by AdoMet, indicating that the transition state structure of the enzymatic reaction is probably very similar to that of the nonenzymatic reaction.[26]

On the basis of the calculations on the nonenzymatic reaction and the recent X-ray crystal structure of rat liver COMT with bound AdoMet and a competitive inhibitor (3,5-dinitrocatechol),[6] it is clear that the catalytic efficacy of COMT comes from factors such as desolvation and bringing together the electrophile and nucleophile in the correct juxtaposition. Presently, it is not clear whether Mg^{2+} plays any role in lowering the pK_a of the bound catechol. Now, with a better understanding of the nonenzymatic reaction, one could start to examine the enzymatic reaction directly in order to get a more quantitative description of the catalytic power of COMT.

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