J. Phys. Chem. A **2006,** *110,* 503−509

Activation Free Energy of Catechol *O*-Methyltransferase. Corrections to the Potential of Mean Force†

Maite Roca and Vicente Moliner*

Departamento de Ciencias Experimentales, Universitat Jaume I, 12080 Castellón, Spain

J. Javier Ruiz-Pernía, Estanislao Silla, and Iñaki Tuñón*

Departamento de Química Física/IcMol, Universitat de València, 46100 Burjasot, Spain

Received: April 22, 2005; In Final Form: July 28, 2005

We use quantum mechanics/molecular mechanics (QM/MM) calculations to estimate the activation free energy for the chemical reaction catalyzed by catechol *O*-methyltransferase. While in many cases the activation free energy of a chemical process is directly determined by the potential of mean force associated with a particular reaction coordinate, here we have included several corrections that have been proposed in the literature. These include the free energy change associated with release of the reaction coordinate motion in the reactant state, consideration of the curvilinear nature of the reaction coordinate, and correction due to the classical treatment of molecular vibrations. In addition, since potentials of mean force are usually obtained from low levels of QM theory to describe the quantum subsystem, we have included an interpolated correction term to improve this description at small additional cost. This last correction term has a dramatic effect, improving the agreement between the theoretical predictions and the experimental value, while the other terms considered make only small contributions to this particular reaction.

1. Introduction

Transition state theory (TST) and its variational extension (VTST) due to the work of Truhlar and co-workers[1] provide a powerful framework for calculating rate constants. In general, the VTST rate constant for a reaction as a function of the temperature (T) is given by[2−5]

$$k(T) = \kappa(T)\frac{k_B T}{h}(C^\circ)^{1-n} \exp\left(-\frac{\Delta G^\ddagger(s)}{RT}\right) \tag{1}$$

where $\kappa(T)$ is the transmission coefficient, k_B is Boltzmann's constant, h is Planck's constant, C° is the standard state concentration, n is the order of the reaction, R is the gas constant, and $\Delta G^\ddagger(s)$ is the standard state molar free energy of activation for a transition state located at a value s of the reaction coordinate (ξ) and is calculated as the difference between the standard state molar free energy of the transition state (TS) and that of the reactant state:

$$\Delta G^\ddagger(s) = G^\ddagger(s) - G^R \tag{2}$$

Usually, $\Delta G^\ddagger$ is the most important factor determining the rate constant, with the effect of the transmission coefficient being significantly smaller. The lowering of the activation free energy is the most important factor responsible for the rate acceleration of chemical reactions in enzymes,[6] and most computational studies of enzymatic processes have been devoted to obtaining and analyzing the changes in the activation free energy when passing from aqueous solution to the enzymatic active sites.[6−11] In many studies, the activation free energy is assimilated to the change in the potential of mean force (PMF) associated with a particular reaction coordinate (ξ):

$$\Delta G^\ddagger(s) \approx \Delta W^\ddagger(s) = W(s) - W(s_0) \tag{3}$$

where $\Delta W^\ddagger$ is evaluated as the difference between the PMF evaluated at the transition state (where $\xi = s$) and the PMF at the reactant value of the reaction coordinate (s_0). However, the precise relationship between the activation free energy and the PMF requires the release of the reaction coordinate motion in the reactant state, an energy term not included in the PMF:

$$G^R_\xi = G^R - W(s_0) \tag{4}$$

Note that free energies and the PMF are not equivalent, since the last one is obtained by integration for particular values of the reaction coordinate (ξ). Then, the free energy associated with the vibrational motion of this coordinate is not included in the PMF. In principle G^R_ξ may contain not only the vibrational free energy of the reactant mode that correlates with the reaction coordinate but also other free energy terms due to the coupling of the reaction coordinate with other coordinates.[12]

Anyway, this assimilation between the activation free energy and the PMF holds exclusively when the reaction coordinate is rectilinear, that is, is a linear combination of atomic Cartesian coordinates.[12] A general reaction coordinate, for example, a bond distance or an antisymmetric combination of bond distances, is curvilinear, and in such a case, the activation free energy should be written as

$$\Delta G^\ddagger = \Delta W^\ddagger(s) - G^R_\xi + W_{curv}(s) \tag{5}$$

where the last term accounts for the curvature of the transition state dividing surface.

---

† Part of the special issue "Donald G. Truhlar Festschrift".

* To whom correspondence should be addressed. E-mail: moliner@exp.uji.es (V.M.); tunon@uv.es (I.T.).

10.1021/jp0520953 CCC: $33.50

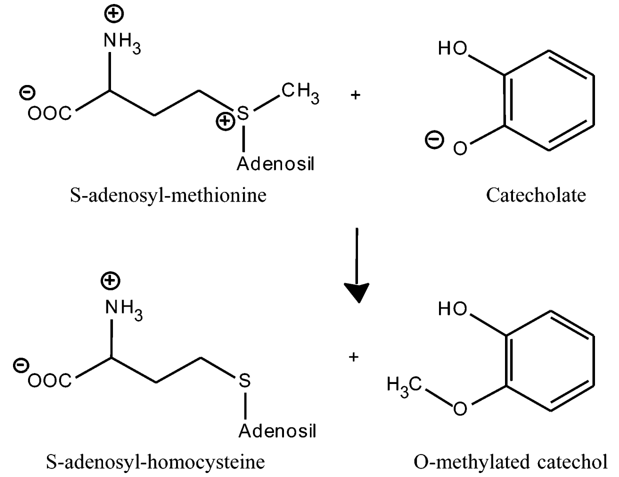

Figure 1. Schematic representation of the reaction catalyzed by COMT. An ionized catechol molecule is methylated by the cofactor (*S*-adenosylmethionine).

The purpose of this paper is to evaluate the last two terms of eq 5 for a particular enzymatic reaction in order to gain knowledge about the relative magnitude of these terms. To have a realistic evaluation of their importance in the framework of standard PMF calculations, we will compare them with other corrections that are not always included in the evaluation of the activation free energy. In particular, PMFs are usually obtained by means of molecular dynamic simulations where the atomic motions are described classically. This classical description of the vibrational motion can introduce a non-negligible error in the calculation of the activation free energies, as zero-point energies are not evaluated. Thus, we will also take into account a correction term due to the discrete nature of quantum mechanical vibrational energy[13−15] which is not properly accounted in classical simulations:

$$\Delta W^{\ddagger} - G^{\mathrm{R}}_{\xi} = \Delta W^{\ddagger}_{\mathrm{clas}}(s) - G^{\mathrm{R}}_{clas,\xi} + \Delta\Delta G_{\mathrm{vib}} \qquad (6)$$

where $\Delta\Delta G_{\mathrm{vib}}$ is the difference between the classical and quantum vibrational contributions to the activation free energy, as detailed in section 3.3. Our evaluation of the activation free energy will be done according to

$$\Delta G^{\ddagger}(s) = \Delta W^{\ddagger}_{\mathrm{clas}}(s) - G^{\mathrm{R}}_{\mathrm{clas},\xi} + \Delta\Delta G_{\mathrm{vib}} + W_{\mathrm{curv}}(s) \qquad (7)$$

In this work, we use eq 7 to evaluate the activation free energy of the enzymatic reaction catalyzed by catechol *O*-methyltransferase (COMT, EC 2.1.1.6).[16] COMT is an important enzyme in the central nervous system where it metabolizes dopamine, adrenaline, noradrenaline, and various xenobiotic catechols. The reaction is a methyl transfer from *S*-adenosylmethionine (SAM) to the hydroxylate oxygen of a substituted catechol (see Figure 1). One important substrate for COMT is levodopa, presently the most effective drug for Parkinson's disease.[17] Other contributions to the rate constant, due to recrossings and tunneling,[6] are not considered in this work.

2. Methodology

The AM1/MM PMF of the reaction was already presented in ref 18: The initial coordinates for the enzyme calculations were taken from the X-ray crystal structure of a COMT-inhibitor complex including 3,5-dinitrocatechol and the cofactor SAM;[19] the nitro groups were removed and one of the hydroxyl groups of catechol was ionized by proton transfer to Lys144. Then, the quantum mechanical (QM) subsystem consisted of the cofactor SAM and the substrate catecholate (63 atoms), while the molecular mechanical (MM) subsystem contained the reminder of the enzyme, a magnesium cation present in the active site, and water molecules of crystallization. The system was then placed and equilibrated inside a cubic box of 55.8 Å side of TIP3P[20] water molecules. The PMF for the methyl transfer was obtained along the antisymmetric combination of the distances describing the breaking and forming bonds ($\xi = r_{\mathrm{SC}} - r_{\mathrm{CO}}$) using the DYNAMO[21] program. Molecular dynamic simulations were carried out in the NVT ensemble at a reference temperature of 300 K and using periodic boundary conditions. The umbrella-sampling approach[22,23] was used to restrain the system by means of a parabolic energy penalty (with a force constant of 2500 kJ mol^{-1} Å^{-2}) centered at the desired value of the reaction coordinate. The probability distributions were put together by means of the weighted histogram analysis method (WHAM).[24] The free energy barrier directly obtained from this AM1/MM PMF was 10.4 kcal/mol, clearly below the experimental estimation (18 kcal/mol).[25] An MP2 single-point correction to the internal energy based on the averaged geometries of the reactant and transition structures led to a much better agreement with experiment.[18] The reactant and the transition states appeared at reaction coordinate values of −1.09 and 0.07 Å, respectively. These equilibrated configurations have been considered as the starting points for the additional simulations and optimizations carried out in this work, as explained below.

3. Results

3.1. Evaluation of $G^{\mathrm{R}}_{\mathrm{clas},\xi}$. First of all, the free energy change associated with release of the reaction coordinate motion in the reactant state has to be evaluated. To do this, we have assimilated the PMF defined at a frozen value of the reaction coordinate as the free energy of a restrained reactant state ($G^{\mathrm{R}}_{\mathrm{clas}}(\xi_{\mathrm{res}})$) where the (classical) vibrational free energy of the reaction coordinate is removed ($G_{\mathrm{clas,vib}}(\xi_{\mathrm{res}})$) Then, the free energy of releasing the reaction coordinate can be expressed as

$$G^{\mathrm{R}}_{\mathrm{clas},\xi} = G^{\mathrm{R}}_{\mathrm{clas}} - W_{\mathrm{clas}}(s_0) \approx G^{\mathrm{R}}_{\mathrm{clas}} - [G^{\mathrm{R}}_{\mathrm{clas}}(\xi_{\mathrm{res}}) - G_{\mathrm{clas,vib}}(\xi_{\mathrm{res}})] \qquad (8)$$

The motion of the reaction coordinate was restrained by imposing a harmonic umbrella potential to keep it at the value ($\xi = s_0$) corresponding to the minimum of the PMF. To evaluate the free energy difference between the free and restrained reactants, we used a thermodynamic integration technique through the introduction of a perturbation parameter λ applied to an umbrella potential, defining thus a restraining Hamiltonian as

$$H_{\lambda} = \lambda U_{\mathrm{umb}} = \frac{1}{2} k_{\mathrm{umb}} \lambda (\xi - s_0)^2 \qquad (9)$$

In this equation, $\lambda = 1$ defines the restrained reactant state, while $\lambda = 0$ defines the free reactant state. The free energy change is consequently obtained as

$$G^{\mathrm{R}}_{\mathrm{clas}} - G^{\mathrm{R}}_{\mathrm{clas}}(\xi_{\mathrm{res}}) = \int_0^1 \mathrm{d}\lambda \left\langle \frac{\partial H_{\lambda}}{\partial \lambda} \right\rangle \qquad (10)$$

The free energy $G^{\mathrm{R}}_{\mathrm{clas},\xi}$ is finally obtained by adding the (classical) vibrational free energy of the restrained reaction coordinate. The vibration of this coordinate is a function of the umbrella force constant (k_{umb}) and the force constant of the normal mode associated with the reaction coordinate in the reactant state (k_ξ):

$$G^{\mathrm{R}}_{\mathrm{clas},\xi} = \int_0^1 \mathrm{d}\lambda \left\langle \frac{\partial H_\lambda}{\partial \lambda} \right\rangle + RT \ln \frac{h}{2\pi k_{\mathrm{B}} T} \left(\frac{k_{\mathrm{umb}} + k_\xi}{\mu_\xi} \right)^{1/2} \qquad (11)$$

with μ_ξ being the reduced mass associated with the reaction coordinate, which is straightforwardly obtained from the Wilson matrix.[26] The transformation from $\lambda = 1$ to $\lambda = 0$ was carried out in 50 windows. In each one of these windows, the value of $\partial H/\partial\lambda$ or $\partial H_\lambda/\partial\lambda$ was averaged for 10 ps of the AM1/MM molecular dynamics simulation under the same conditions as those employed to obtain the PMF. The value of the umbrella force constant was 2500 kJ mol^{-1} Å^{-2}.

It is interesting to note that this term may contain not only the vibrational free energy due to the mode corresponding to this coordinate (the antysimmetric combination of the sulfur−carbon and carbon−oxygen distances) but also other contributions associated with setting the reaction coordinate to its value in the reactant state. The origin of these contributions is the possible coupling between the reaction coordinates and other coordinates of the system. The estimation obtained for this term through eq 11 is very close to zero, resulting from the cancellation between the removal of the umbrella restraint (−1.25 kcal/mol) and the consideration of the vibrational free energy of the reactant under the effect of the umbrella force constant (1.30 kcal/mol). However, an estimation of this contribution based uniquely on the vibrational contribution of the reaction coordinate could be obtained from its averaged frequency (see below):

$$G^{\mathrm{R}}_{\mathrm{clas,vib}}(\xi) = RT \ln \frac{h\langle \nu^{\mathrm{R}}_\xi \rangle}{k_{\mathrm{B}} T} \qquad (12)$$

Using eq 12, the obtained value is about 0.7 kcal/mol. It is then probable that other contributions were included in $G^{\mathrm{R}}_{\mathrm{clas},\xi}$. The origin of these contributions can be clarified if we analyze the reactant structure, shown in Figure 2. COMT binds catechol in its neutral form, and once it is placed in the active site, one of the hydroxyl groups is ionized by proton transfer to Lys144.[27] The negatively charged oxygen atom is now prepared to be methylated by SAM. However, during the first stages of the reaction, the approach of the methyl group must be accompanied by a lengthening of the distance between the nucleophilic oxygen and the protonated Lys144. The oxygen atom must be *desolvated* in order to be prepared to receive the positive methyl group. In other words, the selected reaction coordinate is strongly coupled to other coordinates of the environment in the reactant state. Thus, setting the reaction coordinate to its particular value in the reactant state is accompanied by changes in other coordinates. This is illustrated by comparing the averaged distances obtained from the first and last simulation windows obtained during the removal of the umbrella potential added to the reaction coordinate. When the reaction coordinate is restrained, the averaged carbon−oxygen distance is 2.96 Å and the distance from this nucleophilic oxygen to the closest proton of Lys144 is 2.28 Å. When the umbrella force constant is removed, the averaged distance between the carbon atom of the methyl group and the nucleophilic oxygen is slightly longer (3.06 Å) and the distance between the oxygen and the proton of Lys144 is somewhat shorter (2.03 Å).

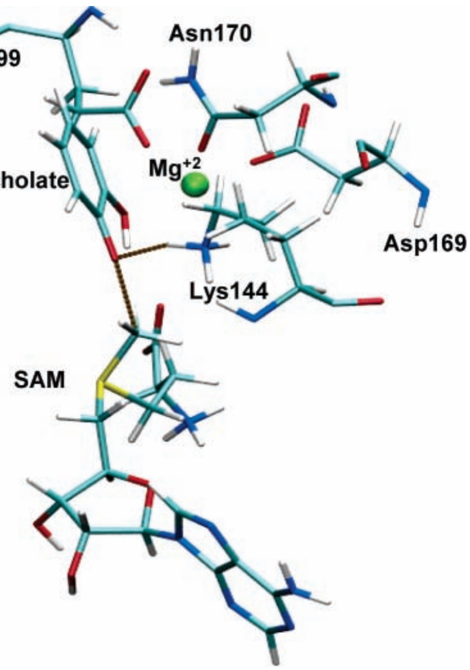

Figure 2. Snapshot of the reactant state showing the catecholate substrate, the cofactor (SAM), the Mg^{2+} cation, and some residues close to the active site.

3.2. Evaluation of $W_{\mathrm{curv}}(s)$. According to the work of Truhlar and co-workers,[12] the free energy correction due to the use of a nonlinear reaction coordinate to obtain the PMF can be conveniently expressed as

$$W_{\mathrm{curv}}(s) = -k_{\mathrm{B}} T \ln \frac{\Phi(s)}{\Phi(s_0)} \qquad (13)$$

where for an arbitrary generalized reaction coordinate ξ

$$\Phi(s) = \left\langle \left(\frac{h^2 \beta Z_\xi}{2\pi} \right)^{1/2} \right\rangle_\xi \qquad (14)$$

h is Planck's constant, $\beta = 1/k_{\mathrm{B}}T$, and Z_ξ is defined by

$$Z_\xi = \sum_{k=1}^{3N} \frac{1}{M_k} \left(\frac{\partial \xi}{\partial x_k} \right)^2 \qquad (15)$$

where x denotes the set of 3N atomic Cartesian coordinates of those atoms involved in the definition of the reaction coordinate and M_k the atomic mass of each atom. This $W_{\mathrm{curv}}(s)$ contains correction terms from both the transition state ($\Phi^{\mathrm{TS}}(\xi)$) and the reactant state ($\Phi^{\mathrm{R}}(\xi)$). Considering the case of the system studied, the reaction coordinate chosen for the methyl transfer is

$$\xi = r_{\mathrm{SC}} - r_{\mathrm{CO}} \qquad (16)$$

where S, C, and O are the atoms involved in the reaction; S is the donor atom, C is the carbon atom of the methyl group which is transferred, and O is the acceptor atom. Equation 15 yields

$$Z_\xi = \frac{1}{m_{\mathrm{S}}} + \frac{1}{m_{\mathrm{O}}} + \frac{2}{m_{\mathrm{C}}}(1 - \cos\theta_{\mathrm{SCO}}) \qquad (17)$$

where θ_{SCO} is the S−C−O bond angle. Thus, the correction is

$$W_{\mathrm{curv}}(s) = -k_{\mathrm{B}}T \ln \frac{\left\langle \left(\frac{1}{m_{\mathrm{S}}} + \frac{1}{m_{\mathrm{O}}} + \frac{2(1-\cos\theta_{\mathrm{SCO}})}{m_{\mathrm{C}}} \right)^{1/2} \right\rangle_{s}}{\left\langle \left(\frac{1}{m_{\mathrm{S}}} \frac{1}{m_{\mathrm{O}}} + \frac{2(1-\cos\theta_{\mathrm{SCO}})}{m_{\mathrm{C}}} \right)^{1/2} \right\rangle_{s_0}} \quad (18)$$

To calculate this correction, we ran 600 ps of AM1/MM molecular dynamics simulation in the TS and reactant region, under the same conditions as those used for the computation of the PMF. We obtained the values of θ_{SCO} from these trajectories, and thus, we computed the averaged values appearing in the preceding equation for both the transition and reactant states. As advanced by Truhlar and co-workers,[12] the free energy term that accounts for the curvature of the selected reaction coordinate (the antisymmetric combination of the bond forming and bond breaking distances) is very small. In fact, for this particular reaction, we have concluded that this free energy term is negligible (−0.03 kcal/mol).

3.3. Evaluation of $\Delta\Delta G_{\mathrm{vib}}$. The contribution of the vibrations of a single molecular structure to its free energy can be easily obtained, considering the quantum nature of its motions, under the harmonic approximation using the following expression:

$$G_{\mathrm{QM,vib}} = \sum_{i=1}^{m} \frac{1}{2} h\nu_i + \sum_{i=1}^{m} RT \ln(1 - \mathrm{e}^{-h\nu_i/k_{\mathrm{B}}T}) = \mathrm{ZPE} + G'_{\mathrm{QM,vib}}(T) \quad (19)$$

where m is the number of vibrational modes to be considered and ν_i the fundamental frequency associated with each one of these modes. The first term is the well-known zero-point energy, while the second one contains the thermal contribution of the vibrations to the molecular free energy.

If the vibrations are being described classically, then the contribution of the vibrational motion to the free energy is given by

$$G_{\mathrm{clas,vib}} = \sum_{i=1}^{m} RT \ln \frac{h\nu_i}{k_{\mathrm{B}}T} = G_{\mathrm{clas,vib}}(T) \quad (20)$$

Then, the molecular free energy estimated from a classical molecular dynamics simulation needs to be corrected by adding the term corresponding to the quantum description of the vibrations and subtracting the term corresponding to the classical description:

$$\Delta G_{\mathrm{vib}} = G_{\mathrm{QM,vib}} - G_{\mathrm{clas,vib}} = \mathrm{ZPE} + G'_{\mathrm{QM,vib}}(T) - G_{\mathrm{clas,vib}}(T) \quad (21)$$

If we are estimating the correction to the activation free energy, we must take into account that this is obtained as the difference between the free energy of the transition state and the reactant states:

$$\Delta\Delta G_{\mathrm{vib}} = \Delta G_{\mathrm{vib}}^{\mathrm{TS}} - \Delta G_{\mathrm{vib}}^{\mathrm{R}} = \Delta\mathrm{ZPE} + \Delta G'_{\mathrm{QM,vib}}(T) - \Delta G_{\mathrm{clas,vib}}(T) \quad (22)$$

To apply this correction to the activation free energy of an enzymatic reaction, we face at least two problems associated with the complexity of our system. The first one is the very large number of vibrational modes. However, as we are interested in a relative free energy, only those modes whose frequencies change from the reactant state to the transition state will make a contribution to the vibrational correction. Then, we restrict eq 22 to the vibrational modes of the QM subsystem. Moreover, the six lowest frequency modes of the transition state and the reactant structures correspond to very low frequency librational motions[28] and the quantum correction is expected to be small. Thus, we omit these modes and the correction is calculated over the $3N - 6$ (in the reactant state) or $3N - 7$ (in the transition state) frequency modes, with N being the number of atoms in the QM subsystems. The second problem is due to the fact that in complex systems the thermodynamic states (reactant and transition states) are not properly represented by a single structure. Then, thermodynamical statistics cannot be simply applied to the energy levels corresponding to a unique energy well (or saddle point). To circumvent this problem, averaged frequencies have been computed as a representative of the corresponding thermodynamic states.[14] It has also been proposed to directly evaluate the ratio of the classical and quantum vibrational partition functions.[29] Here, we use an equivalent strategy in which the average is carried out over the thermodynamic corrections calculated over stationary structures. Note that the difference among these strategies designed to obtain averaged vibrational free energy corrections is just in the quantity that is averaged: frequencies, partition functions, or free energies. In our scheme, we selected 12 different configurations from the molecular dynamic simulations of the reactant and transition states: These configurations were separated by 50 ps each, ensuring then that they were independent. Each of these structures was then refined and characterized as saddle points and minima by means of QM/MM methods using a micro/macroiteration approach implemented in a modified DYNAMO program.[30] Following the idea of the GRACE algorithm,[31] we defined a control space containing the coordinates of all of the QM atoms. The Hessian matrix is obtained for this subspace, and then, it is used to guide the search algorithm. At each step in the control space, the rest of the coordinates of the system (the complementary space) are minimized using the gradients. Once the stationary-point structures (12 minima and 12 saddle points) are located and characterized (through inspection of the frequencies obtained for the control space), the vibrational contributions to the free energy are obtained and averaged to define the correction to be applied to the activation free energy:

$$\begin{aligned}\Delta\Delta G_{\mathrm{vib}} = {} & [\langle \mathrm{ZPE}\rangle_{\mathrm{TS}} - \langle \mathrm{ZPE}\rangle_{\mathrm{R}}] + \left[\left\langle \sum_{i=1}^{3N-7} RT \ln(1 - \mathrm{e}^{-h\nu_i/k_{\mathrm{B}}T}) \right\rangle_{\mathrm{TS}} - \right. \\ & \left. \left\langle \sum_{i=1}^{3N-6} RT \ln(1 - \mathrm{e}^{-h\nu_i/k_{\mathrm{B}}T}) \right\rangle_{\mathrm{R}} \right] - \left[\left\langle \sum_{i=1}^{3N-7} RT \ln \frac{h\nu_i}{k_{\mathrm{B}}T} \right\rangle_{\mathrm{TS}} - \right. \\ & \left. \left\langle \sum_{i=1}^{3N-6} RT \ln \frac{h\nu_i}{k_{\mathrm{B}}T} \right\rangle_{\mathrm{R}} \right] = \langle \Delta\mathrm{ZPE}\rangle + \langle \Delta G'_{\mathrm{QM,vib}}(T)\rangle - \\ & \langle \Delta G_{\mathrm{clas,vib}}(T)\rangle \end{aligned} \quad (23)$$

The total value of this correction is small but, surprisingly, is positive and thus contributes to slightly increase the activation free energy (about 0.2 kcal/mol). Table 1 shows the decomposition of this contribution into the zero-point energy (ZPE) contribution and the quantum correction to the thermal vibrational term. In hydrogen transfer reactions, the ZPE usually diminishes the activation free energy by about 2 or 3 kcal/mol.[32−34] However, in this methyl transfer reaction, the ZPE makes a negligible contribution to the activation free energy.

TABLE 1: Vibrational Correction to the PMF in COMT and Its Zero-Point and Thermal Contributions (See Text for Details)[a]

$\Delta\Delta G_{\mathrm{vib}}$	0.2
$\langle\Delta \mathrm{ZPE}\rangle$	−0.1 (±0.7)
$\langle\Delta G'_{\mathrm{QM,vib}}(T) - \Delta G_{\mathrm{clas,vib}}(T)\rangle$	0.3 (±0.8)

[a] All values are listed in kilocalories per mole.

Although the transition structure has one vibrational mode less, its ZPE is very close to that of the reactant state (324.2 ± 0.4 kcal/mol for the TS and 324.3 ± 0.6 kcal/mol for the reactant state). This similarity in the ZPE of the reactant and transition structures in this particular reaction is partly due to the fact that the vibration associated with the transferred methyl group has a fundamental frequency that is smaller than in the case of hydrogen atoms. The vibration corresponding to the sulfur−carbon bond displays an averaged frequency of 630 cm^{-1} in the reactant state, significantly smaller than that for hydrogen transfers, where the corresponding stretching can take values around 3000 cm^{-1}. In the transition state, the symmetric sulfur−carbon−oxygen vibration has a frequency of 290 cm^{-1}, while differences of more than 1000 cm^{-1} have been observed in the case of hydrogen atom transfer and thus large contributions of the ZPE are obtained in these cases.[14] Moreover, the loss of ZPE in this particular mode in the transition state is compensated by the fact that the averaged frequency of the remaining modes in the transition state is slightly larger than that in the reactant state (1246 cm^{-1} versus 1239 cm^{-1}). This larger averaged frequency reflects that in the transition structure the two reacting fragments are more tightly bound than in the reactant state and thus the vibrations display larger associated force constants.

The correction of the thermal vibrational contribution to the activation free energy due to its quantum nature is also modest, amounting to 0.3 kcal/mol. This value results from the difference between the quantum thermal contribution ($\langle\Delta G'_{\mathrm{QM,vib}}(T)\rangle$ = 0.5 kcal/mol) and the classical thermal contribution ($\langle\Delta G_{\mathrm{clas,vib}}(T)\rangle$ = 0.2 kcal/mol.). We can also directly compare the quantum contribution of vibrations to the activation free energy ($\langle\Delta G_{\mathrm{QM,vib}}\rangle$ = 0.4 kcal/mol) and the classical contribution ($\langle\Delta G_{\mathrm{clas,vib}}\rangle$ = 0.2 kcal/mol) by adding the ZPE. For this particular reaction, where a methyl group is being transferred, the quantum correction to the vibrational contribution to the activation free energy obtained from classical simulations seems to be quite modest.

3.4. Refining the PMF. To reduce the errors associated with the low level (LL) of theory employed in describing the QM region to obtain the PMF, we recalculated it applying a correction method recently implemented in our group.[35] The method is based on the original work of Truhlar and co-workers[36−38] and considers the inclusion of an interpolated correction (IC) energy term obtained as a function of the reaction coordinate:

$$E = E_{\mathrm{QM}}^{\mathrm{LL}} + E_{\mathrm{QM/MM}}^{\mathrm{LL}} + E_{\mathrm{MM}} + \Delta E(\xi) \qquad (24)$$

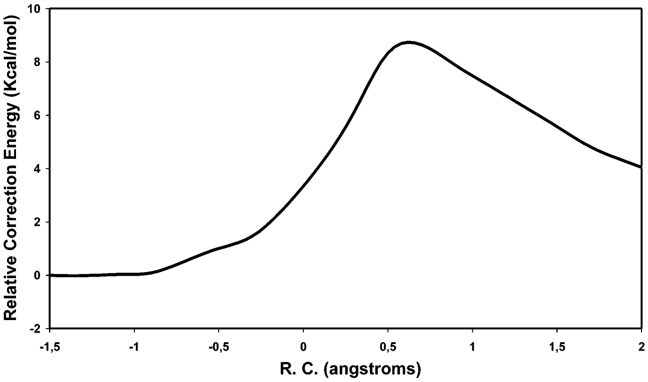

Figure 3. Correction energy relative to the reactants as a function of the reaction coordinate. The correction energy function is obtained as the averaged difference between gas phase MP2/6-31+G* and AM1 calculations on QM structures appearing along four reaction paths.

This correction term $\Delta E(\xi)$ is obtained as the difference between the energy provided by the low level (LL) method and a single-point energy gas phase calculation at a high level (HL) of theory method for particular configurations of the QM subsystem obtained along the chosen reaction coordinate. In this case, the LL single-point gas phase energies were obtained using the AM1 Hamiltonian,[39] while the HL energies were obtained at the MP2/6-31+G*[40−42] level of theory. This level is a good compromise between accuracy and computational effort considering the size of the QM subsystem (63 atoms). The selected configurations were obtained along several AM1/MM minimum energy paths traced down from their transition structures to the products and reactant valleys. An initial transition structure was localized and characterized using the GRACE algorithm[31] (see details in ref 18) with a control space containing the coordinates of all of the QM atoms. From the set of transition structures described in the previous point, we selected three additional structures obtained from initial configurations separated by at least 100 ps during the molecular dynamic simulation of the transition state. A different correction energy term was then obtained for each one of the energy paths traced down from the different transition structures:

$$\Delta E_i(\xi) = E_{\mathrm{QM},i}^{\mathrm{HL}} - E_{\mathrm{QM},i}^{\mathrm{LL}} \qquad (25)$$

We finally obtained the correction energy applied to obtain the new PMF as the average over the energy paths:

$$\Delta E(\xi) = \frac{1}{L}\sum_{i=1}^{L}\Delta E_i(\xi) \qquad (26)$$

where L is the number of considered reaction paths ($L = 4$ in this case). We have checked that, in this case, four paths are enough to reduce the statistical deviation of the correction term contribution to the reaction barrier to less than 0.5 kcal/mol. This value is very similar to that obtained for other systems and gives an idea of the sensitivity of the correction term to changes in other coordinates.[35]

A representation of this correction term as a function of the reaction coordinate is given in Figure 3. The correction energy destabilizes the transition state with respect to the reactant state, and thus, larger activation energies are expected when this term is included. The most important changes in these correction terms take place during the bond breaking and bond forming processes; this is in the surroundings of the TS. For the products, AM1 calculations predict an exothermicity that is too large which is corrected at the MP2 level. Splines under tension[43,44] are used to interpolate these correction energy terms at any value of the reaction coordinate. In this way, we obtain a continuous function in ξ, with continuous first and second derivatives, which are necessary to perform molecular dynamics simulations. Technical details are provided elsewhere.[35−38]

The corrected PMF was obtained on the new potential energy surface defined by eq 24. As described above, we used the umbrella-sampling approach[22,23] to restrain the system in a particular value of the reaction coordinate by means of a parabolic energy penalty (with a force constant of 2500 kJ mol^{-1}

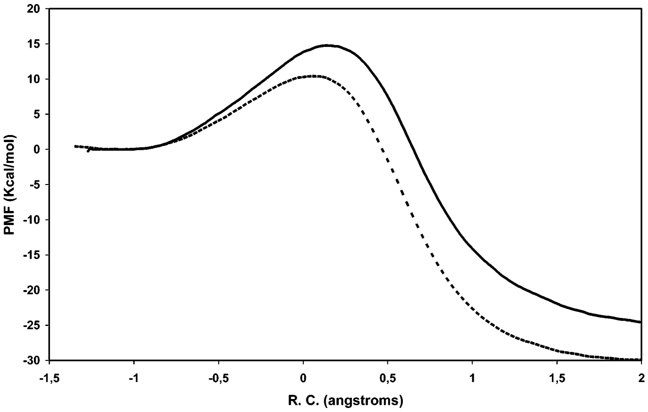

Figure 4. Potentials of mean force as a function of the reaction coordinate obtained at the AM1/MM level (dashed line) and including interpolated corrections (solid line).

TABLE 2: PMF Barriers and Activation Free Energies for the Reaction Catalyzed by COMT Including Different Corrections, as Explained in the Text[a]

$\Delta W^{\ddagger}_{\text{clas}}$(AM1)	10.4[18]
$\Delta W^{\ddagger}_{\text{clas}}$(IC/MP2)	14.8
$\Delta G^{\ddagger}$(AM1)	10.6
$\Delta G^{\ddagger}$(IC/MP2)	15.0
$\Delta G^{\ddagger}$(exp)	18[25]

[a] All values are listed in kilocalories per mole.

Å^{-2}). The corrected PMF was calculated along 80 windows. Simulations were run for 10 ps for each window. The obtained PMF, together with the AM1/MM one, is presented in Figure 4. The free energy difference between the transition state and the reactant state obtained from the AM1/MM potential of mean force is about 10.4 kcal/mol, a value clearly below the experimental estimation of the activation free energy (18 kcal/mol).[25] The use of the interpolated correction scheme leads to a free energy barrier that is much closer to the experimental data. Adding an interpolated correction term based on the difference between MP2/6-31+G* and AM1 calculations to the energetic description of the system leads to a new potential of mean force with a barrier of 14.8 kcal/mol. This means that the error, defined as the difference between the experimental result and the theoretical prediction, is reduced to less than half at a very similar computational effort (the additional cost of the evaluation of the derivatives of the correction energy term is negligible). PMF barriers and estimated activation free energies are summarized in Table 2. To further reduce the difference from the experimental value, further improvements of the theoretical treatment would need to consider corrections not only along the semiempirical reaction paths but also on other regions of the PES. Work is in progress for this.

4. Conclusions

In this work, we have considered three different contributions or corrections that should be added to the PMF in order to estimate the activation free energy of a given chemical process: the free energy associated with the release of the reaction coordinate in the reactant state ($G^{\text{R}}_{\text{clas},\xi}$), a correction due to the use of curvilinear reaction coordinates ($W_{\text{curv}}(s)$), and finally a correction of the classical free energy due to the quantum nature of these degrees of freedom ($\Delta\Delta G_{\text{vib}}$). The magnitude of each of these contributions depends on the particular selected reaction and also on the nature of the distinguished reaction coordinate used to trace the PMF. In addition, we have also considered the dependence of the potential of mean force on the computational level chosen to describe the quantum subsystem. For the particular example analyzed in this paper, the methylation of catecholate catalyzed by catechol *O*-methyltransferase, consideration of an adequate quantum treatment in the PMF, even through simple correction schemes, is essential in order to have a good quantitative description of the process. The corrections introduced at the MP2 level, using the 6-31+G* basis set, considerably reduce the error in predicting the activation free energy (as compared to the AM1/MM value).

None of the rest of the contributions or corrections to the activation free energy are quantitatively significant. The selection of an antysimmetric combination of the bond breaking and bond forming distances due to its curvilinear nature gives an almost zero correction (−0.03 kcal/mol). This result was not unexpected, but it should be taken into account that the magnitude of this contribution depends on the particular form selected for the reaction coordinate and that it could be a significant contribution for particular cases.[12] The release of the reaction coordinate motion in the reactant state, here evaluated using a thermodynamic integration technique, is also zero as a result of the compensation between the vibrational free energy and the work spent setting the reaction coordinate to a particular value. This process can be accompanied by changes in other coupled coordinates, for example, those reflecting the *solvation* of the nucleophilic oxygen. Finally, the correction of the vibrational free energy term is also very small (0.2 kcal/mol). This is not obviously a general conclusion, since in the case of hydrogen transfer the zero-point energy can lower the activation free energy by as much as 2−3 kcal/mol. In this particular reaction, the loss of one vibrational degree of freedom in the zero-point energy of the transition state is not so important because the stretching mode corresponding to the sulfur−carbon bond has an associated frequency that is significantly smaller than in the case of hydrogen transfers. Moreover, this effect is compensated by the rest of the normal modes that have slightly larger frequencies in the transition state than in the reactant state, resulting thus in a final positive contribution to the activation free energy.

Acknowledgment. The authors acknowledge Prof. D. G. Truhlar and Dr. M. García-Viloca for reading and commenting on the manuscript. We thank the DGI for project DGI BQU2003-04168-C03, BANCAIXA for project P1-1B2002-02, and Generalitat Valenciana for projects GV04B-21, GV04B-131, and GRUPOS04/28. E.S. acknowledges the hospitality of Prof. O. Tapia at the University of Uppsala. M.R. and J.J.R.-P. acknowledge a doctoral fellowship of the Ministerio de Educación y Ciencia.

References and Notes

(1) Truhlar, D. G.; Garret, B. C.; Klippenstein, S. J. *J. Phys. Chem.* **1996**, *100*, 12771−12800.

(2) Truhlar, D. G., Isaacson, A. D., Garret, B. C. In *Theory of Chemical Reaction Dynamics*; Baer, M., Ed.; CRC: Boca Raton, FL, 1985; Vol. 4, pp 65−138.

(3) Truhlar, D. G.; Garret, B. C. *Acc. Chem. Res.* **1980**, *13*, 440−448.

(4) Truhlar, D. G.; Isaacson, A. D.; Skodje, R. T.; Garret, B. C. *J. Phys. Chem.* **1982**, *86*, 2252−2261.

(5) Tucker, S. C.; Truhlar, D. G. In *New Theoretical Concepts for Understanding Organic Reactions*; Bertrán, J., Csizamadia, I. G., Eds. Kluwer: Dordrecht, The Netherlands, 1989; p 291.

(6) García-Viloca, M.; Gao, J.; Karplus, M.; Truhlar, D. G. *Science* **2004**, *303*, 186−195.

(7) Kollman, P. A.; Kuhn, B.; Donini, O.; Peräkylä, M.; Stanton, R.; Bakowies, D. *Acc. Chem. Res.* **2001**, *34,* 72.

(8) Villà, J.; Warshel, A. *J. Phys. Chem. B* **2001**, *105*, 7887−7907.

(9) Bruice, T. C. *Acc. Chem. Res.* **2002**, *35,* 139−148.

(10) Martí, S.; Roca, M.; Andrés, J.; Moliner, V.; Silla, E.; Tuñón, I.; Bertrán, J. *Chem. Soc. Rev.* **2004**, *33*, 98−107.

(11) Nam, K.; Prat-Resina, X.; García-Viloca, M.; Devi-Kesavan, L. S.; Gao, J. *J. Am. Chem. Soc.* **2004**, *126*, 1369−1376.

(12) Schenter, G. K.; Garret, B. C.; Truhlar, D. G. *J. Chem. Phys.* **2003**, *119*, 5828−5833.

(13) Alhambra, C.; Corchado, J. C.; Sanchez, M. L.; García-Viloca, M.; Gao, J.; Truhlar, D. G. *J. Phys. Chem. B* **2001**, *105*, 11326−11340.

(14) García-Viloca, M.; Alhambra, C.; Truhlar, D. G.; Gao, J. *J. Chem. Phys.* **2001**, *114*, 9953−9958.

(15) Truhlar, D. G.; Gao, J.; Alhambra, C.; García-Viloca, M.; Corchado, J.; Sanchez, M. L.; Villà, J. *Acc. Chem. Res.* **2002**, *35*, 341−349.

(16) Takusagawa, F.; Fujioka, M.; Spies, A.; Schowen, R. L. In *Comprehensive Biological Catalysis*; Sinnott, M., Ed.; Academic Press: San Diego, CA, 1998; Vol. 1, pp 1−30.

(17) Gulberg, H. C.; Marsden, C. A. *Pharmacol. Rev.* **1975**, *27*, 135−206.

(18) Roca, M.; Martí, S.; Andrés, J.; Moliner, V.; Tuñón, I.; Bertrán, J.; Williams, I. H. *J. Am. Chem. Soc.* **2003**, *125*, 7726−7737.

(19) Vidgren, J.; Svensson, L. A.; Liljas, A. *Nature* **1994**, *368*, 354−358.

(20) Jorgensen, W. L.; Chandrasekhar, J.; Madura, J. D.; Impey, R. W.; Klein, M. L. *J. Chem. Phys.* **1983**, *79*, 926−935.

(21) Field, M. J.; Albe, M.; Bret, C.; Proust-De Martin, F.; Thomas, A. *J. Comput. Chem.* **2000**, *21*, 1088−1100.

(22) Torrie, G. M.; Valleau, J. P. *J. Comput. Phys.* **1977**, *23*, 187−199.

(23) Valleau, J. P.; Torrie, G. M. In *Statistical Mechanics A. Modern Theoretical Chemistry*; Berne, B. J., Ed.; Plenum Press: New York, 1977; Vol. 5, p 137.

(24) Kumar, S.; Bouzida, D.; Swendsen, R. H.; Kollman, P. A.; Rosenberg, J. M. *J. Comput. Chem.* **1992**, *13*, 1011−1021.

(25) Schultz, E.; Nissinem, E. *Biochem. Pharmacol.* **1989**, *38*, 3953−3956.

(26) Wilson, E. B.; Decius, J. C.; Cross, P. C. *Molecular Vibrations*; Dover: New York, 1955.

(27) Zheng, Y. J.; Bruice, T. C. *J. Am. Chem. Soc.* **1997**, *119*, 8137−8145.

(28) Stern, M. J.; van Hook, W. A.; Wolfsberg, M. *J. Chem. Phys.* **1963**, *39*, 3179−3196.

(29) García-Viloca, M.; Truhlar, D. G.; Gao, J. *Biochemistry* **2003**, *42*, 13558−13575.

(30) A modified DYNAMO code[21] was written to implement the same methodology as that employed by GRACE[31] to locate first-order saddle points (corresponding to transition structures of chemical reactions).

(31) (a) Moliner, V.; Turner, A. J.; Williams, I. H. *J. Chem. Soc., Chem. Commun.* **1997**, *14*, 1271. (b) Turner, A. J.; Moliner, V.; Williams, I. H. *Phys. Chem. Chem. Phys.* **1999**, *1*, 1323−1331.

(32) Alhambra, C.; Gao, J.; Corchado, J. C.; Villà, J.; Truhlar, D. G. *J. Am. Chem. Soc.* **1999**, *121*, 2253−2258.

(33) Alhambra, C.; Corchado, J. C.; Sanchez, M. L.; Gao, J.; Truhlar, D. G. *J. Am. Chem. Soc.* **2000**, *122*, 8197−8203.

(34) Webb, S. P.; Agarwal, P. K.; Hammes-Schiffer, S. *J. Phys. Chem. B* **2000**, *104*, 8884−8894.

(35) Ruiz-Pernía, J. J.; Silla, E.; Tuñón, I.; Martí, S.; Moliner, V. *J. Phys. Chem. B* **2004**, *108*, 8427−8433.

(36) Corchado, J. C.; Coitiño, E. L.; Chuang, Y.; Fast, P. L.; Truhlar, D. G. *J. Phys. Chem. A* **1998**, *102*, 2424−2438.

(37) Chuang, Y.; Corchado, J. C.; Truhlar, D. G. *J. Phys. Chem. A* **1999**, *103*, 1140−1144.

(38) Nguyen, K. A.; Rossi, I.; Truhlar, D. G. *J. Chem. Phys.* **1995**, *103*, 5522−5530.

(39) Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902−3909.

(40) Head-Gordon, M.; Pople, J. A.; Frisch, M. J. *Chem. Phys. Lett.* **1988**, *153*, 503−506.

(41) Frisch, M. J.; Head-Gordon, M.; Pople, J. A. *Chem. Phys. Lett.* **1990**, *166*, 275−280.

(42) Frisch, M. J.; Head-Gordon, M.; Pople, J. A. *Chem. Phys. Lett.* **1990**, *166*, 281−289.

(43) Renka, R. J. *SIAM J. Sci. Stat. Comput.* **1987**, *8*, 393−415.

(44) Renka, R. J. *ACM Trans. Math. Software* **1993**, *19*, 81−94.