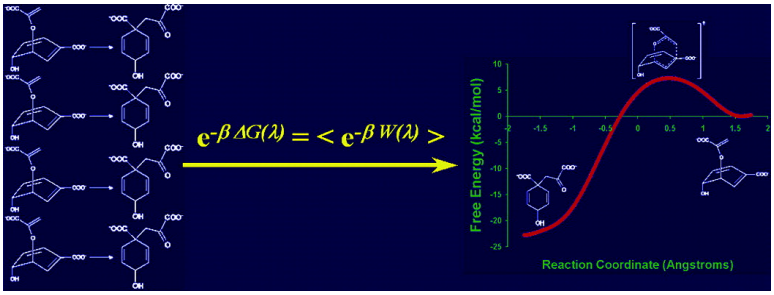
Communication

Multiple-Steering QM–MM Calculation of the Free Energy Profile in Chorismate Mutase

Alejandro Crespo, Marcelo A. Mart, Daro A. Estrin, and Adrian E. Roitberg

J. Am. Chem. Soc., **2005**, 127 (19), 6940-6941• DOI: 10.1021/ja0452830 • Publication Date (Web): 23 April 2005



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 10 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
COMMUNICATIONS

Published on Web 04/23/2005

Multiple-Steering QM−MM Calculation of the Free Energy Profile in Chorismate Mutase

Alejandro Crespo,[†,‡] Marcelo A. Martí,[†] Darío A. Estrin,*[,†] and Adrian E. Roitberg*[,‡]

Departamento de Química Inorgánica, Analítica y Química-Física and INQUIMAE-CONICET, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Ciudad Universitaria - Pab II, C1428EHA, Buenos Aires, Argentina, and Quantum Theory Project and Department of Chemistry, University of Florida, Gainesville, Florida 32611-8435

Received August 4, 2004; E-mail: roitberg@qtp.ufl.edu; dario@qi.fcen.uba.ar

Investigation of chemical events that take place in a small region of a large system is a demanding goal because modeling of chemical phenomena requires computationally expensive quantum mechanically based models and large configurational sampling is needed in most biological applications. The potential energy of the entire system has been used as a tool to compute reaction enthalpies in biomolecules. However, in complex systems, predictions based on the total potential energy may present flaws because motions in regions distant to the active site during pathway minimizations may significantly affect the energy profile. Predictions based on the computation of free energies will yield much more meaningful results. We will illustrate in this work a novel technique for computing free energy profiles, employing the multiple steering molecular dynamics (MSMD) approach, originally proposed by Jarzynski,[1] in the context of an efficient quantum mechanical−molecular mechanical (QM−MM) density functional (DFT) based scheme optimized for biomolecules.

We have chosen for illustrating our scheme the conversion reaction of chorismate to prephenate, catalyzed by the *Bacillus subtilis* enzyme chorismate mutase, a problem for which there is an important body of theoretical and experimental data.[2−8] This reaction is involved in the biosynthesis of aromatic amino acids in bacteria, fungi, and plants. The experimental activation parameters for the enzyme-catalyzed reaction of chorismate to prephenate reported by Kast et al.[5] are $\Delta G^{\ddagger} = 15.4$ kcal/mol, $\Delta H^{\ddagger} = 12.7$ kcal/mol and $\Delta S^{\ddagger} = -9.1$ eu. There have been several theoretical works that provided estimates for the activation parameters of chorismate mutase. Mulholland and co-workers studied the energetic profile of the reaction at the RHF/6-31G(d) QM−MM using CHARMM and corrected it by ab initio calculations in the gas phase (DFT and MP2).[3] Lee et al.[4] assessed the role of different residues in the stabilization of the transition state by performing QM−MM calculations at the HF level. We have studied this system within the same setup as in the present communication, using DFT and MM to compute the activation energies.[2] Brooks and co-workers applied a QM−MM replica path method at the HF and DFT levels for computing potentials of mean force, calculating the activation enthalpy.[6]

Free energy profiles have also been reported. Jorgensen and co-workers studied the environment effects of the reaction by using a combined (AM1) QM−MM Monte Carlo/free energy perturbation (MC/FEP) method.[7] Marti et al.[8] calculated the free energy profile for the reaction in aqueous solution and in the enzyme, using an umbrella sampling AM1 scheme. Most of these studies agree that the enzyme reduces the activation free energy of the reaction by providing an environment which preferentially stabilizes the transition state. We present, for the first time, predictions of free energy profiles in an enzymatic system using the MSMD ideas proposed by Jarzynski, employing a QM−MM DFT Hamiltonian.

The MSMD[1] approach establishes a relation between the nonequilibrium dynamics and equilibrium properties.[9] For a more detailed description of the ideas, we refer to ref 10. We present the relevant equations which allow for efficient calculation of free energy profiles. Let $H(\mathbf{r},\lambda)$ be the Hamiltonian of a system that is subject to an external time-dependent perturbation ($\lambda = \lambda(t)$) and respectively let $\Delta G(\lambda)$ and $W(\lambda)$ be the change in free energy and the external work performed on the system as it evolves from $\lambda = \lambda_0$ to λ. Here $\mathbf{r}$ indicates a configuration of the whole system, while λ is a reaction coordinate. Then, $\Delta G(\lambda)$ and $W(\lambda)$ are related to each other by the following identity

$$e^{-\beta \Delta G(\lambda)} = \langle e^{-\beta W(\lambda)} \rangle \qquad (1)$$

where the brackets represent an average taken over an ensemble of molecular dynamics trajectories, provided the initial ensemble is equilibrated. Equation 1 is valid under the assumption of a converged average, formally needing an infinite number of realizations of the process. The Hamiltonian $H(\mathbf{r},\lambda)$ can be written as the sum of the time independent Hamiltonian of the unperturbed system, $H_0(\mathbf{r})$, plus a time-dependent external potential. As usual, the perturbation has been chosen to be a harmonic potential, whose minimum position moves at constant velocity υ according to

$$H(\mathbf{r},\lambda) = H_0(\mathbf{r}) + {}^1/_2 k\,[\lambda(r) - \lambda_0 - \upsilon t\,]^2 \qquad (2)$$

where $\lambda(\mathbf{r})$ represents a chosen reaction path.

Thus, the free energy of a process along a reaction coordinate can be computed performing a number of finite time transformations, collecting the work done at each time step, and then properly averaging it as in eq 1.

The simulation details are presented in the Supporting Information. The system consists of the enzyme chorismate mutase solvated with a sphere of 496 water molecules centered on one of the active sites. Our QM−MM scheme[2] uses, for the description of the QM region, a very efficient implementation of density functional theory (DFT) based on numerical basis sets called SIESTA (Spanish Initiative for Electronic Simulation of Thousands of Atoms).[11]

A purely MM dynamics run was first done to equilibrate the system at a value of the reaction coordinate corresponding to the reactant (product). From the last 2 ns of the classical simulation of chorismate and prephenate, we collected 20 starting structures of each (40 total). Only atoms within a sphere of 15 Å from the active site were allowed to move. Each structure was equilibrated at the QM−MM level for 0.5 ps at 300 K with a time step of 0.5 fs. The substrate moiety was treated with QM at the DFT level, and the

[†] Universidad de Buenos Aires.
[‡] University of Florida.

10.1021/ja0452830 CCC: $30.25

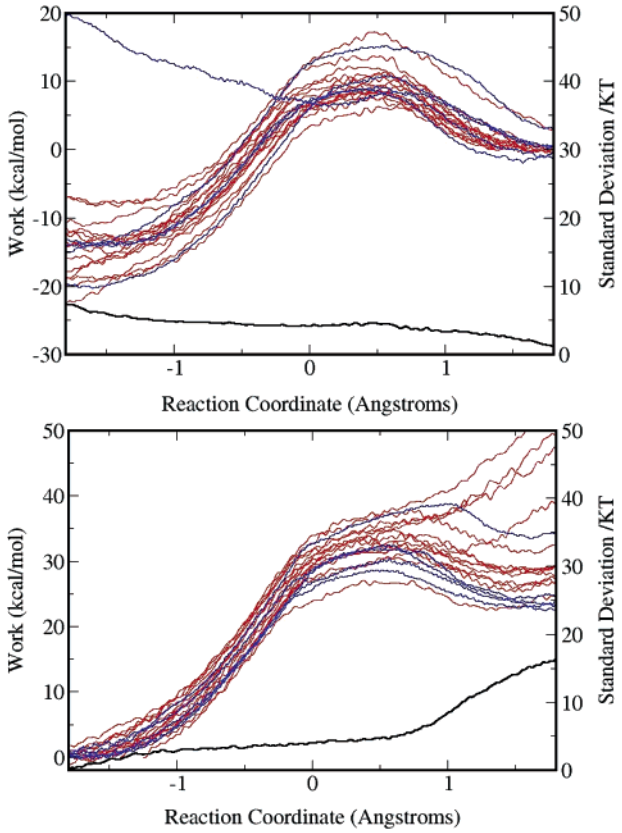
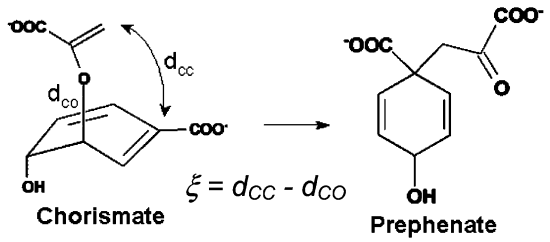


Figure 1. (top) Chorismate to prephenate work for the 20 runs (set 1: red, set 2: blue) and the standard deviation (thick black line). (bottom) Prephenate to chorismate work for the 20 runs (set 1: red, set 2: blue) and the standard deviation (thick black line).

Scheme 1. Chorismate to Prephenate Conversion Reaction



rest of the system was treated at the MM level using the Wang et al. force field parametrization.[2,12]

The reaction coordinate of this reaction (Scheme 1), $\xi = d_{cc} - d_{co}$ has been shown to adequately represent the process.[2−4,8] The 20+20 QM−MM structures were used as starting points for the multiple steering molecular dynamics (MSMD) runs. The reaction coordinate was changed from $\xi = -1.8$ to 1.8 Å for a set of 15+15 QM−MM structures (set 1) at a constant speed of 2 Å/ps, and for another set of 5+5 QM−MM structures (set 2) at a constant speed of 1 Å/ps, to asses the role of the pulling velocity. A force constant of 200 kcal/(molÅ) was used in all cases.

In Figure 1 (top) we show the values of accumulated work vs ξ for chorismate to prephenate conversion for the 20 repetitions. Also shown is the standard deviation of the work values. These data should be trusted from $\xi = 1.8$ to -0.5 Å, at which point $\sigma_W > 3k_BT$. Figure 1 (bottom) has the same data starting at the prephenate side of the reaction. These data are good only from $\xi = -1.8$ to $+0.5$ Å.

Figure 2, in red, shows the Jarzynski estimator for the free energy of set 1 (15+15 structures, pulling speed of 2 Å/ps) according to eq 1. In green, we present the same results for set 2 (5+5 structures,

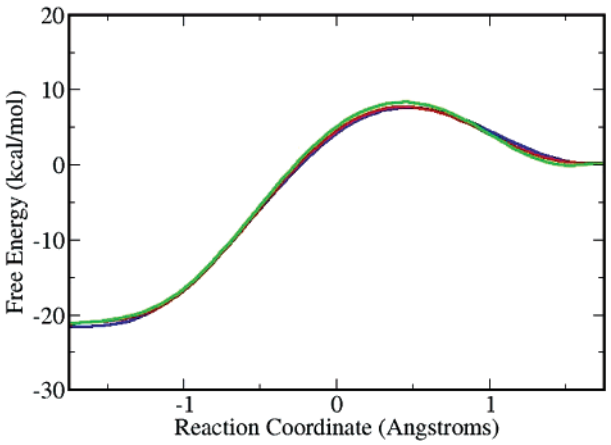


Figure 2. Free energy profile from chorismate ($\xi \approx 1.75$ Å) to prephenate ($\xi \approx -1.75$ Å), calculated using Jarzynski's equality (both forward and reverse data are used) for set 1 (red), for set 2 (green), and for umbrella sampling scheme (blue).

pulling speed of 1 Å/ps). They both have been obtained by joining the forward and reverse free energies curves obtained by exponential averaging of work from Figure 1, respectively. We can conclude that the different pulling velocities do not change quantitatively the $\Delta G^{\ddagger}$ values obtained. Although our $\Delta G^{\ddagger}$ values are lower compared to the experimental ones, (8 kcal/mol approximately vs 15 kcal/mol) due to flaws in our DFT description, the calculated entropic effect is negative, in agreement with the experimental value (−9.1 eu).[5] Previous calculation of the entropic effect for this reaction in ref 8 computes the wrong sign. In addition, the potential of mean force has been computed using an umbrella sampling scheme, for comparison. A total of 12 windows simulations of 5 ps each have been employed, using as starting structures the snapshots of the constrained energy minimizations performed previously.[2] The blue curve in Figure 2 presents the umbrella sampling data, in perfect agreement with the MSMD estimator, but computed in a much less straightforward way.

Acknowledgment. A.E.R. thanks Chris Jarzynski for insightful discussions and acknowledges partial support for this work by DOE Contract DE-FG02-02ER45995. This work was partially suported by grants from Fundacion Antorchas, Universidad de Buenos Aires, and ANPCYT.

Supporting Information Available: This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Jarzynski, C. *Phys. Rev. Lett.* **1997**, *78*, 2690.
(2) Crespo, A.; Scherlis, D. A.; Martí, M. A.; Ordejón, P.; Roitberg, A. E.; Estrin, D. *J. Phys. Chem. B* **2003**, *107*, 13728.
(3) Ranaghan, K. E.; Ridder, L.; Szefczyk, B.; Sokalski, A. W.; Hermann, J. C.; Mulholland, A. J. *Org. Biomol. Chem.* **2004**, *7*, 968.
(4) Lee, Y. S.; Worthington, S. E.; Krauss, M.; Brooks, B. R. *J. Phys. Chem. B* **2002**, *106*, 12059.
(5) Kast, P.; Tewari, Y. B.; Wiest, O.; Hilvert, D.; Houk, K. N.; Goldberg, R. N. *J. Phys. Chem. B* **1997**, *101*, 10976.
(6) Woodcock, H. L.; Hodoscek, M.; Sherwood, P.; Lee, Y. S.; Schaefer, H. F., III; Brooks, B. R. *Theor. Chem. Acc.* **2003**, *109*, 140.
(7) Guimarães, C. R. W.; Repasky, M. P.; Chandrasekhar, J.; Tirado-Rives, J.; Jorgensen, W. L. *J. Am. Chem. Soc.* **2003**, *125*, 6892.
(8) Martí, S.; Andrés, J.; Moliner, V.; Silla, E.; Tuñon, I.; Bertrán, J.; Field, M. J. *J. Am. Chem. Soc.* **2001**, *123*, 1709.
(9) Vandevondele, J.; Rothlisberger, U. *J. Am. Chem. Soc.* **2002**, *124*, 8163 and references therein.
(10) Hummer, G.; Szabo, A. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 3658.
(11) Soler, J. M.; Artacho, E.; Gale, J. D.; García, A.; Junquera, J.; Ordejón, P.; Sánchez-Portal, D. *J. Phys.: Condens. Matter* **2002**, *14*, 2745.
(12) Wang, J.; Cieplak, P.; Kollman, P. A. *J. Comput. Chem.* **2000**, *21*, 1049.

JA0452830