QM/MM Studies Show Substantial Tunneling for the Hydrogen-Transfer Reaction in Methylamine Dehydrogenase

Paul F. Faulder,[†] Gary Tresadern,[†] Kamaldeep K. Chohan,[‡] Nigel S. Scrutton,[§] Michael J. Sutcliffe,[‡] Ian H. Hillier,[†] and Neil A. Burton*,[†]

Department of Chemistry, University of Manchester Manchester, M13 9PL, U.K.
Departments of Chemistry and Biochemistry University of Leicester, Leicester, LE1 7RH, U.K.

Received May 17, 2001

Methylamine dehydrogenase (MADH) (EC 1.4.99.3) catalyses the oxidative demethylation of methylamine to formaldehyde and ammonia.[1−2] Recent stopped-flow kinetic studies[2] of this enzyme have shown an unusually high, temperature-independent primary deuterium kinetic isotope effect (KIE) of 16.8 ± 0.5, but strongly temperature dependent reaction rates that were interpreted as indicating a thermally induced vibrationally driven extreme tunneling mechanism for the rate-limiting hydrogen abstraction step (Figure 1). Here we present hybrid quantum mechanical and molecular mechanical (QM/MM) computational studies of this reaction step, with canonical variational transition-state theory incorporating multidimensional tunneling effects to predict reaction rates. We find an unusual reaction free energy profile which indicates that a combination of quantum tunneling (>90%) and classical protein motion is responsible for the large KIE.

There is currently great interest in the possibility of enhanced hydrogen tunneling arising from protein flexibility, particularly in the case of the alcohol dehydrogenase family of enzymes.[3−6] Perhaps the most studied enzyme in this series, both experimentally[7,8] and theoretically,[9,10] is liver alcohol dehydrogenase (LADH) where it has been shown that protein flexibility correlates with hydride tunneling for thermophilic and mesophilic enzymes, although the measured KIEs are quite small (2−3), in contrast to the value for the proton shift in MADH studied here. Hybrid QM/MM methods[11,12] are well suited to the study of enzyme reaction paths since they include an accurate quantum mechanical description of the active site, where the electronic structure is important and bonds are broken and formed, while approximating the surrounding effects of the enzyme framework with a molecular mechanical potential. Such hybrid QM/MM methods have recently been combined with semiclassical variational transition-state theory methods[9,12] to study hydride shift reactions and their associated KIEs.

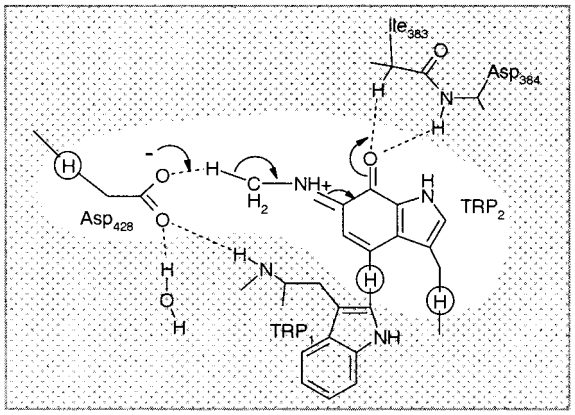

Figure 1. Active site of MADH. QM region is shown unshaded with link atoms circled.

An initial iminoquinone reactant structure was constructed from the crystal coordinates of MADH taken from *Methylophilus methylotrophus*[13] by replacing the carbonyl oxygen of the tryptophan tryptophylquinone (TTQ) cofactor with methylamine as shown in Figure 1 (the two tryptophan residues of TTQ are labeled separately as TRP_1 and TRP_2). The entire dimer was then protonated, solvated, and then minimized using the AMBER molecular mechanics force field.[14] For the hybrid calculations, the QM region was limited to 31 atoms (see Figure 1) which includes the catalytic base, Asp_{428}, and the catalytically active rings of one tryptophan (TRP_2) of the TTQ cofactor after reduction with methylamine. This QM region was subsequently minimized to find an optimized reactant complex, using our QM/MM program which utilizes the Gaussian94[15] and AMBER[16] codes and keeping the link atoms and MM regions fixed.[17] In this study we have used the PM3 semiempirical Hamiltonian which has proved reliable to study other enzyme mechanisms,[11,12] and enables the large number of second derivatives required for the direct dynamics calculation to be evaluated along the reaction path.

Product and transition-state structures, corresponding to proton transfer from the methylamine to Asp_{428}, were also found, starting with the reactant enzyme structure but only optimizing the QM region. The potential energy barrier to reaction was found to be 9.1 kcal/mol which is lower than the experimentally determined enthalpic barrier of reaction (10.7 kcal/mol) determined from the temperature dependence of the rates.[2] A reaction path profile was generated using the Page and McIver intrinsic reaction coordinate (IRC) algorithm[18] as implemented within the POLYRATE[19] code and interfaced to our QM/MM program. This reaction profile (see Figure 2a) shows a narrow potential energy (PE) barrier (the transition state having an imaginary frequency of 1997*i* cm^{-1})

* To whom correspondence should be addressed.
[†] Department of Chemistry, University of Manchester.
[‡] Department of Chemistry, University of Leicester.
[§] Department of Biochemistry, University of Leicester.

(1) Chen, L.; Doi, M.; Durley, R. C.; Christoserdov, A. Y.; Lidstrom, M. E.; Davidson, V. L.; Mathews, F. S. *J. Mol. Biol.* **1998**, *276*, 131−149.
(2) Basran, J.; Sutcliffe, M. J.; Scrutton, N. S. *Biochemistry* **1999**, *38*, 3218−3222.
(3) Bruno, W. J.; Bialek, W. *Biophys. J.* **1992**, *63*, 689−699.
(4) Kohen, A.; Klinman, J. P. *Acc. Chem. Res.* **1998**, *31*, 397−404.
(5) Basran, J.; Patel, S.; Sutcliffe, M. J.; Scrutton, N. S. *J. Biol. Chem.* **2001**, *276*, 6234−6242.
(6) Northrop, D. B.; Cho, Y.-K. *Biochemistry* **2000**, *39*, 2406−2412.
(7) Bahnson, B. J.; Colby, T. D.; Chin, J. K.; Goldstein, B. M.; Klinman, J. P. *Proc. Natl. Acad. Sci. U.S.A.*, **1997**, *94*, 12797−12802.
(8) Kohen, A.; Klinman, J. P. *J. Am. Chem. Soc.* **2000**, *122*, 10738−10739.
(9) Alhambra, C.; Corchado, J. C.; Sanchez, M. L., Gao, J.; Truhlar, D. G. *J. Am. Chem. Soc.* **2000**, *122*, 8197−8203.
(10) Webb, S. P.; Agarwal, P. K.; Hammes-Schiffer, S. *J. Phys. Chem.* **2000**, *104*, 8884−8894.
(11) Burton, N. A.; Harrison, M. J.; Hart, J. C.; Hillier, I. H.; Sheppard, D. W. *Faraday Discuss.* **1998**, *110*, 463−475.
(12) Nicoll, R. M.; Hindle, S. A.; MacKenzie, G.; Hillier, I. H.; Burton, N. A. *Theor. Chem. Acc.* **2001**, *106*, 105−112.
(13) X-ray crystal coordinates from Mathews, F. S.; personal communication.
(14) Cornell, W. D.; Cieplak, P.; Bayly, C. I.; Gould, I. R.; Merz, K. M.; Ferguson, D. M.; Spellmeyer, D. C.; Fox, T.; Caldwell, J. W.; Kollman, P. A. *J. Am. Chem. Soc.* **1995**, *117*, 5179−5197.
(15) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Gill, P. M. W.; Johnson, B. G.; Robb, M. A.; Cheeseman, J. R.; Keith T. A.; Petersson, G. A.; Montgomery, J. A.; Raghavachari, K.; Al-Laham, M. A.; Zakrzewski, V. G.; Ortiz, J. V.; Foresman, J. B.; Cioslowski, J.; Stefanov, B. B.; Nanayakkara, A.; Challacombe, M.; Peng, C. Y.; Ayala, P. Y.; Chen, W.; Wong, M. W.; Andres, J. L.; Replogle, E. S.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Binkley, J. S.; Defrees, D. J.; Baker, J.; Stewart, J. P.; Head-Gordon, M.; Gonzales, C.; Pople, J. A. *GAUSSIAN94*; Gaussian Inc.: Pittsburgh, PA, 1995.
(16) Pearlman, D. A.; Case, D. A.; Caldwell, J. W.; Ross, W. S.; Cheatham, T. E.; Ferguson, D. M.; Seibel, G. L.; Singh, U. C.; Weiner, P. K.; Kollman, P. A. *AMBER 4.1*; University of California: San Francisco, 1995.
(17) Hall, R. J.; Hindle, S. A.; Burton, N. A.; Hillier, I. H. *J. Comput. Chem.* **2000**, *21*, 1433−1441.
(18) Page, M.; McIver, J. W. *J. Phys. Chem.* **1988**, *88*, 922−935.

10.1021/ja016219a CCC: $20.00
Published on Web 08/10/2001

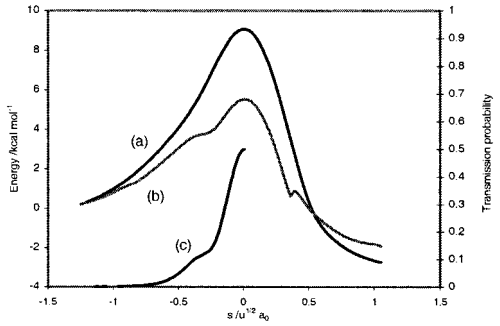

Figure 2. Energetics and tunneling probabilities along the reaction coordinate. (a) Potential energy relative to reactant. (b) Vibrationally adiabatic potential energy relative to reactant. (c) Tunneling transmission probability.

with a rapid drop in potential on the product side (corresponding to positive mass-weighted reaction coordinate, *s*) as the proton bonds to the aspartate oxygen.

The vibrationally adiabatic potential energy surface (Figure 2b) was generated by inclusion of the zero-point energies (ZPEs) and has two definite shoulders. The reactant side feature, which has been previously suggested for aromatic alcohol dehydrogenase,[5] is the result of coupling between the C−H stretch and the reaction coordinate causing the ZPE to change rapidly between the reaction coordinate value, $s = -0.40$ a_0 and $s = -0.23$ a_0. This region of the PE surface corresponds to C−H lengths of 1.15 to 1.22 Å, and the reaction coordinate from here through the transition state (1.33 Å) corresponds to mostly hydrogen motion. In this region, the distance between the oxygen of Asp_{428} and the methylamine group (O−C) is effectively constant at 2.64 Å compared to values of 2.75 and 2.73 Å in the reactant and product, respectively. Such conformational compression through concerted motion of the H-bond donors has been noted in previous dynamical studies of other hydrogen-transfer reactions.[20]

The predicted primary deuterium KIEs for this hydrogen shift are summarized in Table 1. Transition-state theory (TST) and canonical variational TST (CVT) predict relatively large KIEs but we see the importance of inclusion of quantum mechanical tunneling from the large transmission coefficients, κ: For hydrogen and deuterium, the optimized multidimensional tunneling (OMT) transmission coefficients are predicted to be 23.1 and 12.6 respectively and are considerably larger than the simpler Wigner (W) tunneling corrections[21] ($\kappa = 4.9$ and 3.1, respectively). The CVT/OMT method predicts a KIE of 11.1 at 298 K which is in good agreement with experimental observations. Here the OMT method is the small curvature tunneling (SCT) approximation[22] which incorporates the curvature of the adiabatic potential curve, and we can see from the variation in the transmission probability along the reaction path coordinate (Figure 2c) a sharp increase at the strong coupling region of closest C−O approach. This region also corresponds to a sharp reduction in the effective reduced mass of the proton, resulting in the large KIE.

Table 1. Calculated KIE for Proton Transfer in MADH

T/K	TST	CVT	TST/W	CVT/OMT
273	7.2	7.2	11.5	13.2
298	6.1	6.1	9.6	11.1
323	5.3	5.4	8.2	9.5

To bring the proton to a position where it can tunnel significantly, approximately 3.7 kcal/mol below the classical barrier, there is a region of classical atomic motion within the active site. We would suggest that these changes in structure may be achieved through thermal vibrations within the system and protein scaffold, although in this static model the groups must approach by some relaxation within the active site. In contrast to proton transfer in MADH, the reaction coordinate for hydride transfer in horse LADH, using the same methodology, involves heavy atom motion to within approximately 0.2−0.4 kcal/mol of the transition state and thus promotes a more classical hydrogen-transfer mechanism and results in a smaller KIE. This is consistent with ∼40% of the reaction predicted to occur classically in LADH[9] compared with only ∼4% in MADH.

These results provide a consistent and interesting interpretation of experimental observations that C−H bond breakage in methylamine by MADH involves extensive proton tunneling.

Acknowledgment. We thank D. G. Truhlar for use of the POLYRATE code, EPSRC and BBSRC for financial support.

JA016219A

(19) Steckler, R.; Chuang, Y.-Y.; Fast, P. L.; Coitiño, E. L.; Corchado, J. C.; Hu, W.-P.; Corchado, J. C.; Liu, Y.-P.; Lynch, G. C.; Nguyen, K. A.; Jackels, C. F.; Gu, M. Z.; Rossi, I.; Clayton, S.; Melissas, V. S.; Garrett, B. C.; Isaacson, A. D.; Truhlar, D. G. *POLYRATE*, version 7.4; University of Minnesota: Minneapolis, 1997.

(20) Pantono, S.; Alber, F.; Carloni, P. *J. Mol. Struct. (THEOCHEM)* **2000**, *530*, 177−181.

(21) Bell, R. P. *The Tunnel Effect in Chemistry*; Chapman & Hall: London, 1980.

(22) Liu, Y.-P.; Lynch, G. C.; Truong, T. N.; Lu, D.-H.; Truhlar, D. G.; Garrett, B. C. *J. Am. Chem. Soc.* **1993**, *115*, 2408.