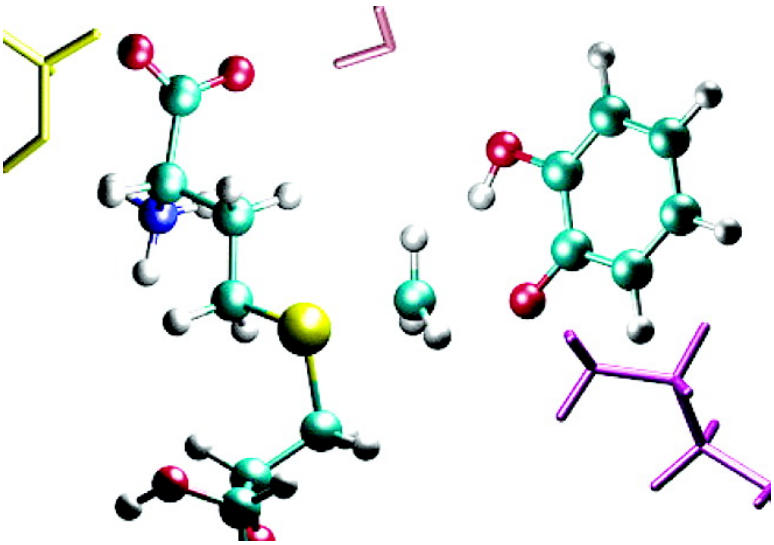
Communication

QM/MM Determination of Kinetic Isotope Effects for COMT-Catalyzed Methyl Transfer Does Not Support Compression Hypothesis

Giuseppe D. Ruggiero, Ian H. Williams, Maite Roca, Vicent Moliner, and Iaki Tun

J. Am. Chem. Soc., **2004**, 126 (28), 8634-8635• DOI: 10.1021/ja048055e • Publication Date (Web): 22 June 2004



J|A|C|S
COMMUNICATIONS

Published on Web 06/22/2004

QM/MM Determination of Kinetic Isotope Effects for COMT-Catalyzed Methyl Transfer Does Not Support Compression Hypothesis

Giuseppe D. Ruggiero and Ian H. Williams*

Department of Chemistry, University of Bath, BA2 7AY Bath, U.K.

Maite Roca and Vicent Moliner

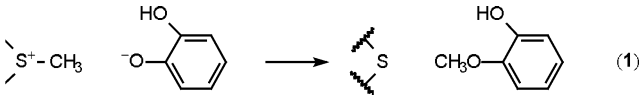
Departament de Ciències Experimentals, Universitat Jaume I, Box 224, 12080 Castellón, Spain

Iñaki Tuñón

Departamento de Química Física, Universidad de Valencia, 46100 Burjasot, Spain

Received April 5, 2004; E-mail: i.h.williams@bath.ac.uk

Experimentally, the secondary α-deuterium kinetic isotope effect (2° α-D KIE) $k(CH_3)/k(CD_3)$ is large and inverse for methyl transfer catalyzed by catechol-*O*-methyltransferase (COMT), much more inverse than for an uncatalyzed reaction in solution.[1] This observation was interpreted in terms of a tighter S_N2 transition state for the COMT-catalyzed reaction than for the nonenzymic reaction. We now report 2° α-D_3 KIEs of 0.94 for the reaction 1 of *S*-adenosylmethionine (AdoMet) with catecholate anion in aqueous solution and 0.85 for the same reaction catalyzed by COMT, both at 25 °C, as computed by a hybrid AM1/TIP3P/CHARMM method. Although these calculated results are in agreement with experiment, they do not support the compression hypothesis.[1]



(1)

Catechol *O*-methyltransferase (COMT, EC 2.1.1.6) is a ubiquitous enzyme that catalyzes the transfer of the activated methyl group of *S*-adenosyl-l-methionine (AdoMet) to an oxygen atom of a catechol.[2] COMT plays a key role in the metabolic inactivation of neurotransmitters and neuroactive xenobiotics, accepting a wide variety of substrates containing a vicinal dihydroxyphenyl moiety. Its inactivation of L-DOPA is particularly significant, since this is currently the most effective drug for the treatment of Parkinson's disease. There is much interest presently in the development of effective COMT inhibitors as potential adjuncts to L-DOPA therapy.[3]

We recently reported extensive molecular dynamics (MD) calculations involving quantum-mechanical/molecular-mechanical (QM/MM) potentials for the COMT-catalyzed reaction 1.[4] The computed AM1[5]/CHARMM[6] free energy barrier was shown to be lower than the AM1/TIP3P[7] free energy barrier for reaction 1 in water.[4] Now we have refined the geometries of representative transition structures (TS) and reactant complexes (RC) for the enzymic and aqueous reactions, both with AdoMet as the methyl donor, by means of GRACE,[8] using the same QM/MM methodology as before. The optimization of structures taken from MD trajectories in the RC and TS regions involved 2040 mobile atoms for the systems in water and 2610 for the enzymic systems. The QM region comprised the 63 atoms of AdoMet and catecholate for reactions in both media. The RCs and TSs are well-characterized minima and first-order saddle points (transition frequencies in Table 2), but they are not unique; other similar stationary points exist with slightly different arrangements of solvent molecules or amino acid residues. Consideration of a small set of 9 TSs for the enzyme suggests the particular structure selected here is representative as compared with the average bond lengths (Table 1). The Hessian computed for the QM atoms was subjected to a projection procedure to ensure that 6 zero frequencies were obtained for the translational and rotational modes and that the 183 frequencies for the vibrational modes of the 63 atoms satisfied the Teller−Redlich product rule.[9] While full ensemble averaging would be desirable, preliminary indications from inspection of Hessians for the 9 individual enzyme TSs suggest that the error in the 2° α-D KIE computed from a single representative TS is likely to be about ±0.03.

The inverse 2° α-D KIEs (Table 2) are dominated by the zero-point energy factor ZPE; the mass/moment-of-inertia factors (MMI) are negligible, and the contributions of excited vibrational frequencies (EXC) are normal. By coincidence, the ZPE factor arising from the frequencies >2200 cm^{-1} (including CH, NH, and OH stretches, of which only the CH are isotopically sensitive) is about the same magnitude as the overall isotope effect for the reaction in water;

Table 1. Representative AM1/MM-Optimized Transition-State Pauling Bond Orders and Bond Lengths for Reaction 1, with Mean Value and Standard Deviation for 9 Enzyme TSs

	bond order	bond length (Å)	average (Å)
C···O (nucleophile)			
water	0.34	2.07	
enzyme	0.36	2.05	2.06 ± 0.001
S···C (leaving group)			
water	0.60	2.06	
enzyme	0.54	2.10	2.09 ± 0.02

Table 2. AM1/MM Transition Frequencies, 2° α-D_3 KIEs for Reaction 1 at 25 °C, with Contributing Factors Including ZPE Contributions from Modes with Frequencies above and below 2200 cm^{-1}, and Average Values of Relaxed Force Constants for Bond Stretching (md $Å^{-1}$) and Angle Bending (md Å rad^{-2}) for Coordinates Involving Isotopically Substituted Atoms

	water		enzyme	
$\nu^{\ddagger}/cm^{-1}$	479*i*		530*i*	
α-D_3 KIE	0.943		0.846	
MMI	1.000		1.000	
EXC	1.057		1.051	
ZPE	0.892		0.805	
>2200	0.936		0.879	
<2200	0.953		0.916	
	RC	TS	RC	TS
<CH stretch>	5.20	5.40	5.13	5.40
<HCH bend>	0.47	0.48	0.47	0.48
<SCH bend>	0.59	0.78	0.60	0.79
<HCO bend>	0.40	0.74	0.31	0.80

10.1021/ja048055e CCC: $27.50

this contribution from CH stretching modes has been noted previously.[12,14] However, for the enzymic reaction this fortuitous cancellation of the other factors does not occur; although the largest contribution to the inverse KIE comes from CH stretching, the frequencies $<2200\ cm^{-1}$ (including in particular the *bending* modes involving the isotopically substituted atoms) are significant in determining the overall KIE. Inspection of the relaxed force constants[15] (averaged over the three isotopic positions) shows not only the expected increase for CH stretching but also very significant increases for SCH and HCO angle bending about the transferring methyl group as between RC and TS. We have previously noted large ZPE factors arising from corresponding bending modes in 2° α-D KIEs for symmetrical methyl transfer reactions in a vacuum.[16]

Schowen and co-workers determined 2° α-D KIEs of $V^{CH_3}/V^{CD_3} = 0.83 \pm 0.05$ for methylation of 3,4-dihydroxyacetophenone with AdoMet at 37 °C catalyzed by COMT and $k^{CH_3}/k^{CD_3} = 0.97 \pm 0.02$ for methylation of methoxide ion by *S*-methyldibenzothiophenium ion at 25 °C in methanol.[1] Model vibrational analysis (BEBOVIB) calculations suggested Pauling bond orders for the making and breaking bonds to be ∼0.1 greater in the enzymic TS than in the nonenzymic TS, corresponding to a shortening of ∼0.06 Å for each of these bonds in the enzymic TS.[10] It was suggested that, as a consequence of this compression, the enzyme might be able to distinguish the S_N2 TS structurally from the preceding reactant state and the succeeding product state in order to stabilize it specifically.[11] QM calculations have indeed demonstrated that this hypothesis is reasonable for carefully constructed model systems.[12] However, the present AM1/MM optimizations predict bonds lengths C···O (from the transferring methyl group to the nucleophile) and S···C (the distance to the leaving group) for TSs in water and enzyme (Table 1), which suggests (a) that the Pauling bond order[13] to the nucleophile in each TS is significantly less than that to the leaving group and (b) that the sum of the making and breaking bond orders is about the same for the enzymic and aqueous TSs.

The TS bending force constants employed in the original BEBOVIB modeling study[10] for the SCH and HCO angles were estimated by multiplying the TS bond order (for S···C or C···O) by the value of the corresponding force constant in the reactant or product. This had the effect of underestimating these critical force constants. To reproduce the observed 2° α-D KIEs, Schowen and co-workers inevitably used higher bond orders for the enzymic reaction.[10] The present results do not support the compression hypothesis, and the previous study of electrostatic factors in COMT catalysis has suggested that it is unnecessary for reaction 1: the TS is distinguished from the RC by its less unfavorable electrostatic interaction with the environment, relative to the same reaction in water.[7]

The terms "tight" and "loose" as applied to TSs are potentially ambiguous. Often, these terms are taken as geometrical descriptors: tight TSs would have relatively shorter bonds or higher bond orders for the making and breaking bonds than loose TSs. However, a tight TS could also be one that is *stiffer* than a loose one, even though it is geometrically similar. The TS for the COMT-catalyzed reaction is stiffer than that for the uncatalyzed reaction in water because the force constants for out-of-plane angle bending (and CH bond stretching) of the transferring methyl group are significantly higher; in this sense, the enzymic TS is "tighter". This distinction between geometrical structure and stiffness implies that there is no simple linear relationship between bond orders and force constants. Consequently, care should be exercised in regard to the interpretation of KIEs as measures of transition-state structure, particularly if this information would be used for the design of TS analogues as inhibitors.

Finally, we have computed 1° ^{13}C KIEs using the same AM1/MM Hessians and obtain values of 1.061 and 1.059 for reaction 1 in water and in the enzyme, respectively. These agree reasonably well with the experimental determinations of 1.080 ± 0.010 for methylation of methoxide ion by *S*-methyldibenzothiophenium ion at 25 °C in methanol[1,10] and 1.09 ± 0.02 (or 0.05) for methylation of 3,4-dihydroxyacetophenone with AdoMet at 37 °C catalyzed by COMT.[1] The present AM1/MM computed value of 1.010 for the ^{18}O KIE differs from the range of about 0.96−0.98 consistent with the preferred TS in the earlier BEBOVIB study, although the present value of 1.007 for the ^{34}S KIE accords with the previously predicted range of about 1.007−1.019;[10] however, there are no experimental values with which to compare these values.

Acknowledgment. We thank the EPSRC for a ROPA Grant (GR/94060) and the Royal Society for a Joint Project Grant.

References

(1) (a) Hegazi, M. F.; Borchardt, R. T.; Schowen, R. L. *J. Am. Chem. Soc.* **1979**, *101*, 4359−4365. (b) Gray, C. H.; Coward, J. K.; Schowen, K. B.; Schowen, R. L. *J. Am. Chem. Soc.* **1979**, *101*, 4351.
(2) Takusagawa, F.; Fujioka, M.; Spies, A.; Schowen, R. L. In *Comprehensive Biological Catalysis*; Sinnott, M., Ed.; Academic Press: San Diego, 1998; Vol. 1, pp 1−30.
(3) Männistö, P. T.; Kaakkola, S. *Pharmacol. Rev.* **1999**, *51*, 593−628.
(4) Roca, M.; Martí, S.; Andrés, J.; Moliner, V.; Tuñón, I.; Bertrán, J.; Williams, I. H. *J. Am. Chem. Soc.* **2003**, *125*, 7726−7737.
(5) Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Stewart, J. J. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902−3909.
(6) Brooks, B. R.; Bruccoleri, R. E.; Olafson, B. D.; States, D. J.; Swaminathan, S.; Karplus, M. *J. Comput. Chem.* **1983**, *4*, 187−217.
(7) Jorgensen, W. L.; Chandrasekhar, J.; Madura, J. D.; Impey, R. W.; Klein, M. L. *J. Chem. Phys.* **1983**, *79*, 926−935. Note that CHARMM contains a modified TIP3P potential that includes intramolecular terms.
(8) (a) Moliner, V.; Turner, A. J.; Williams, I. H. *J. Chem. Soc., Chem. Commun.* **1997**, 1271. (b) Turner, A. J.; Moliner, V.; Williams, I. H. *J. Phys. Chem. Chem. Phys.* **1999**, *1*, 1323−1331.
(9) Ruggiero, G. D.; Guy, S. J.; Martí, S.; Moliner, V.; Williams, I. H. *J. Phys. Org. Chem.* **2004**, *in press*.
(10) Rodgers, J.; Femec, D. A.; Schowen, R. L. *J. Am. Chem. Soc.* **1982**, *104*, 3263−3268.
(11) Olsen, J.; Wu, Y.-S.; Borchardt, R. T.; Schowen, R. L. In *Transmethylation*; Usdin, E., Borchardt, R. T., Creveling, C. R., Eds.; Elsevier: New York, 1979.
(12) (a) Williams, I. H. *J. Am. Chem. Soc.* **1984**, *106*, 7206−7212. (b) Moliner, V.; Williams, I. H. *J. Am. Chem. Soc.* **2000**, *122*, 10895−10902.
(13) Bond order $n = \exp[(R_1 - R_n)/c]$, where $c = 0.6$; note that $c = 0.3$ in ref 10.
(14) (a) Zhao, X. G.; Tucker, S. C.; Truhlar, D. G. *J. Am. Chem. Soc.* **1991**, *113*, 826. (b) Glad, S. S.; Jensen, F. *J. Am. Chem. Soc.* **1997**, *119*, 227.
(15) Williams, I. H. *Chem. Phys. Lett.* **1982**, *88*, 462.
(16) Ruggiero, G. D.; Williams, I. H. *J. Chem. Soc., Perkin Trans. 2* **2002**, 591−597.

JA048055E