Influence of Equatorial CH···O Interactions on Secondary Kinetic Isotope Effects for Methyl Transfer

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Abstract: DFT calculations for methyl cation complexed within a constrained cage of water molecules permit the controlled manipulation of the “axial” donor/acceptor distance and the “equatorial” distance to hydrogen-bond acceptors. The kinetic isotope effect \( k(\text{CH}_3)/k(\text{CD}_3) \) for methyl transfer within a cage with a short axial distance becomes less inverse for shorter equatorial C···O distances: a decrease of 0.5 Å results in a 3% increase at 298 K. Kinetic isotope effects in AdoMet-dependent methyltransferases may be modulated by CH···O hydrogen bonding, and factors other than axial compression may contribute, at least partially, to recently reported isotope-effect variations for catechol-O-methyltransferase and its mutant structures.

Quantum-mechanical (QM) calculations for a model methyl-transfer reaction occurring inside a constrained cage of water molecules have revealed that secondary kinetic isotope effects \((2^\alpha \text{KIEs}), k(\text{CH}_3)/k(\text{CD}_3)\) and \(k(\text{CH}_3)/k(\text{CT}_3)\), vary significantly in response to controlled changes in CH···O interactions in the equatorial plane of the transition state (TS) for a fixed donor–acceptor distance along the methyl-transfer axis. This finding indicates that CH···O hydrogen bonding in AdoMet-dependent methyltransferases as noted by Trievel and co-workers\(^5\) may also modulate 2\(^\alpha\) KIEs and that factors other than axial compression might contribute, at least partially, to the intriguing KIE variations for catechol-O-methyltransferase (COMT) and its mutant structures reported recently by Klinman and co-workers\(^1\)\(^,\)\(^2\).

Besides providing the prototypical example of an \( S_N 2\) mechanism, methyl transfer is an important component of many biological processes, not least in reactions mediated by AdoMet.\(^7\) In view of the small size of the methyl group, it is not obvious how an enzyme might preferentially stabilize the TS for methyl transfer relative to the reactant state (RS). The observation of an inverse \( D_3\) KIE of unusually large magnitude in the COMT-catalyzed reaction of AdoMet with catecholate led to a hypothesis that catalysis might be facilitated by mechanical compression along the nucleophile/nucleofuge axis.\(^3\) However, hybrid QM/molecular-mechanical (MM) computational simulations of this KIE for methyl transfer in solution and in the active site of COMT did not support the compression hypothesis: the trend in the KIEs was reproduced but without any significant difference in the average distance between the methyl donor and acceptor atoms in the corresponding TSs.\(^6\)\(^,\)\(^7\) Nonetheless, an apparent trend in recent experimental \( T_3\) KIEs for wild-type and mutant COMTs has been interpreted as new evidence for compression.\(^2\)\(^,\)\(^3\) Meanwhile, the functional importance of unconventional CH···O hydrogen bonding in AdoMet-dependent methyltransferases has been noted from a survey of high-resolution crystal structures,\(^2\) but the possible influence of these interactions on KIEs for methyl transfer is unknown.

Herein we present results for computational investigations of the isotopic sensitivity of the methyl cation trapped inside a constrained cage (Figure 1) which permits the controlled manipulation of both the “axial” distance between donor/acceptor atoms and the “equatorial” distance to hydrogen-bond acceptors. This model system offers access to structures not amenable to experiment but which help to provide a framework for the interpretation of KIEs that might be observed for reactions in highly structured environments, such as enzyme active sites or, potentially, within the cavities of nanoporous materials.

The cage comprises five water molecules arranged at the vertices of a trigonal bipyramid. Quasi-\( D_{3h}\)-symmetric structures are obtained by placing a methyl cation at its center, coplanar with the three equatorial water molecules and with collinear CH···O\(_{eq}\) interactions, and perpendicular to the plane of the two axial water molecules. Each water molecule is frozen at particular fixed values of the C···O\(_{ax}(r_{ax})\) and C···O\(_{eq}(r_{eq})\) distances in the symmetric structures, and the rigid cage structure is maintained even when the methyl position is allowed to relax axially to form a tetrahedral CH\(_2\)OH\(_2^+\) RS adduct with one of the axial water molecules. All calculations were performed with the B3LYP/aug-cc-PVDZ density-functional method. Isotope effects were...
The D₃ isotope effect for the transfer of methyl cation from vacuum to water, evaluated as an average over 40 solvent configurations (each a locally relaxed snapshot from a hybrid AM1/TIP3P molecular-dynamics simulation at 298 K), is 0.85;[9] the closest water molecules in both the axial and equatorial directions in these QM/MM structures are located at C···O distances ranging from 2.95 to 3.20 Å (i.e. close to the sum of the van der Waals radii). The transfer of methyl cation (alone) from vacuum to the center of the water cage (in which the complex is a TS with respect to methyl transfer along the axial direction with an imaginary frequency for antisymmetric C···O ax stretching) similarly yields a D₃ isotope effect of 0.86 for rₑq = 3.0 Å at 298 K, thus showing the reasonableness of the calculation procedures used with the cage model. However, the magnitude of this IE increases (in an inverse sense) to 0.90 for rₑq = 2 Å, rₑq = 3.0 Å because loss of methyl-group translational and rotational motions is inadequately compensated by vibrational gains within the smaller cage.

Symmetric axial structures [H₂O···CH₃⁺−OH₃] without the three equatorial water molecules (rₑq = ∞) possess an imaginary frequency for methyl transfer. Figure 2 shows D₃ equilibrium isotope effects (EIEs) for the transfer of these structures from the vacuum into the center of the three-water equatorial ring of the cage as a function of rₑq for different rₐx distances. A decrease in rₑq from 4 to 3 Å for rₑq = 3 Å has very little effect on the EIE (ca. 1), but the same change in rₑq for rₑq = 2 Å (corresponding very closely to the optimized C···O distance in the gas-phase Sₙ2 TS) inversely increases the EIE from 0.99 to 0.84. Equatorial CH···O interactions affect the EIE significantly.

For each rₑq distance, variation in the axial nucleophile–nucleofuge distance changes the D₃ KIE from RS to TS within the cage dramatically from about 0.1 at rₑq = 2 Å to about 3 at rₑq = 4 Å. This increase in the D₃ KIE corresponds to a change from a relatively tight Sₙ2 TS to a very loose “exploded” Sₙ2 TS.[8,11] On a per deuterium basis, these KIEs are equivalent to about 1.03 for rₑq = 2 Å and about 1.4 for rₑq = 4 Å, which are plausible values for 2° α-D KIEs.[9] The Sₙ2-like behavior from RS to TS.[13] However, the respective factors (D₃ and T₃ CH str) diminish in magnitude (i.e. become less inverse) as rₑq decreases, because ΔFCH also decreases as the CH···O interactions strengthen (Table 1). The CH bond-stretching factor (which itself is dominated by changes in zero-point energy) is responsible for the trend in the KIEs with changing rₑq, whereas the overall normal direction of these isotope effects is due to all the other modes (especially bending and vibration of the methyl group within the constrained cage). The CH bonds are shorter and stiffer in TS than in RS.

The strengthened CH···O hydrogen-bonding interactions also cause the energy-barrier height for methyl transfer within the cage to decrease from 23.2 kJ mol⁻¹ at rₑq = 4 Å to 17.5 kJ mol⁻¹ at rₑq = 3 Å (Table 1), equivalent to an order-
of-magnitude increase in catalysis. Wolfe et al. reported calculations of $D_3$ KIEs for intramolecular methyl transfer between O atoms confined within a rigid template, but the effect that they called transverse compression appears to have been purely repulsive in nature: there were no stabilizing interactions with hydrogen-bond acceptors, and the transverse force distorted the TSs away from collinearity along the methyl-transfer axis. Moliner and Williams considered $D_3$ KIEs for intra-bridgehead methyl transfer inside a symmetrical cryptand containing CH···O interactions, but this system could not be manipulated independently of the donor–acceptor distance.

Although these results have been obtained for a model system, nonetheless they clearly suggest a possible role for CH···O interactions in modulating the magnitude of $D_3$ and $T_3$ KIEs in methyl-transfer reactions. Use of an anionic hydrogen-bond acceptor (e.g. carboxylate) instead of neutral water might be expected to enhance these effects. The functional importance of CH···O hydrogen bonding in AdoMet-dependent methyltransferases has been argued but a link to KIEs has not been previously proposed. It is known, however, that CD bond stretching frequencies are sensitive to the local electric field within a protein environment. Klinman and co-workers have reported $T_3$ KIEs on $r_{eq}/K_m$ in human COMT: 0.791 ± 0.012 for the wild-type enzyme and 0.822 ± 0.021 and 0.850 ± 0.012 for its Y68F and Y68A mutants, respectively. The trend in these KIEs has been interpreted in terms of mediation of the distance between the methyl donor and acceptor groups, or “active-site compaction”. In terms of the cage model presented herein, the variations in KIE for COMT mutants would be attributed to changes in $r_{eq}$, whereas we now suggest a significant role for changes in $r_{eq}$ which may lead to variations in KIE of similar magnitudes to those reported by Klinman and co-workers. It is possible that factors other than compression along the methyl donor–acceptor axis may contribute, at least partially, to the intriguing KIE variations for COMT and its mutant structures. It has already been shown that $D_3$ KIEs are very sensitive to the local dielectric constant. Certainly, the present results for a model methyl transfer strongly indicate the necessity for explicit inclusion of CH···O interactions in the QM region and Hessian in any new QM/MM simulations of these KIEs for COMT-catalyzed methyl transfer. Earlier calculations included these interactions only across the QM/MM boundary and did not include in the Hessian the Met40 and Asp141 residues, which make close contact with the methyl-group H atoms. An electrostatic origin for catalysis in COMT and its mutants has been demonstrated and should also serve to explain trends in KIEs.

The practical utility of KIEs as part of a multidisciplinary approach to determining TS structure, and thence to designing potential drugs as TS-analogue enzyme inhibitors, has been demonstrated. Computational modeling plays an important role within this approach, and the quality of the information it provides about TS structures for enzymatic reactions depends upon the reliability of the method used for KIE calculations in protein environments. The insight provided by the present study of the influence of CH···O interactions on $D_3$ KIEs in methyl transfer may be of value for computational modeling not only of methyltransferases (e.g. COMT as a potential target for pancreatic-cancer therapy) but of many other enzymes for which KIE data are available.

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Der kinetische Isotopeneffekt $k(CH_3)/-k(CT_3)$ für den Methyl-Transfer in einem Käfig aus Wassermolekülen hängt von den CH···O-Wasserstoffbrücken ab: Er steigt um 3 %, wenn bei konstantem axialen Abstand $r_{ax} = 2.0 \text{ Å}$ der äquatoriale Abstand $r_{eq}$ von 3.5 auf 3.0 Å abnimmt (siehe Diagramm), was vermuten lässt, dass andere Faktoren als die axiale Kompression die unterschiedlichen Isotopenwirkungen bei Methyltransferase und ihren mutierten Strukturen verursachen könnten.