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MNDO Calculation of Kinetic Isotope Effects in Model Cytochrome P-450 Oxidations

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Abstract: The semiempirical MNDO SCF-MO method has been used to calculate kinetic isotope effects for the reactions of singlet and triplet oxygen with methylamine, methanol, and ethane. These reactions were chosen as models for cytochrome P-450 catalyzed N-dealkylation, O-dealkylation, and carbon oxidation. The reactant-like transition states were qualitatively similar for each substrate. The singlet reactions led to oxygen insertion via an attachment-rearrangement mechanism, while the triplet reactions proceeded via a hydrogen radical abstraction–recombination mechanism. For the singlet reactions with methylamine, methanol, and ethane, calculated deuterium isotope effects of 1.63, 1.68, and 1.65, respectively, were obtained. For the triplet reactions, the corresponding values were 8.19, 8.35, and 8.75. Comparison with experimental isotope effects suggests that P-450-mediated O-dealkylation and carbon oxidation, but *not* N-dealkylation, proceed via a mechanism involving H· abstraction by a triplet "oxene-like" species.

Cyclochrome P-450's are hemeproteins that catalyze the oxidation of various substrates according to the general equation[2]

$$\mathrm{RH} + \mathrm{O_2} + \mathrm{NADPH} + \mathrm{H^+} \xrightarrow{\text{P-450}} \mathrm{ROH} + \mathrm{H_2O} + \mathrm{NADP^+}$$

The many varied reactions catalyzed by this group of enzymes include aromatic and aliphatic hydroxylation and O- and N-dealkylation.[3] These processes are of interest not only for their importance in the elimination of xenobiotics but also because of their demonstrated ability to transform otherwise innocuous molecules into toxic or carcinogenic species.[4–6]

The mechanism of cytochrome P-450 catalysis has been the subject of a substantial research effort. The current consensus favors an "oxenoid" mechanism, involving the generation of a single oxygen atom bound to the heme iron of the cytochrome, although the spin state of this electron deficient species remains in doubt.[2] Primary deuterium kinetic isotope effects of approximately 11 have been observed for P-450-mediated benzylic[7] and aliphatic hydroxylation[8] as well as O-dealkylation.[9] They have been interpreted as evidence for rate-limiting hydrogen radical abstraction, implicating a triplet oxene mechanism.[2] On the other hand, considerably smaller deuterium isotope effects of 1.2–2.0 have been observed in a number of N-dealkylations,[10–12] leading to speculation that these dealkylations may proceed through a transition state distinct from that of carbon oxidation or alkylation.[11] *N*-Oxide formation,[13] nitrogen-based radical cation formation,[14–16] and the participation of the nitrogen lone pair in stabilizing the transition state have been suggested.[10]

A major problem here concerns both the interpretation and measurement of the kinetic isotope effects themselves. For example, participation of the nitrogen lone pair may well lead to transition-state differences relative to those of the analogous oxygen and carbon cases. However, our present qualitative understanding of the factors determining these effects is too crude to allow even a rough estimate of their probable magnitudes. The second problem concerns the measurement of a kinetic isotope effect in the complex, multistep, enzymatic process in which these oxidations occur. It has been shown that the intrinsic isotope effect on C–H bond breakage can be significantly or even totally masked by other rate factors in the enzymatic process.[17,18]

Encouraged by earlier successful prediction of kinetic isotope effects using the semiempirical MNDO method,[19,20] we have calculated those for the oxidation of various substrate classes by a cytochrome P-450 model system. An oxene model presumed to give a satisfactory qualitative description of cytochrome P-450 oxidations was selected,[21] and the reaction of singlet and triplet oxygen atoms with methylamine, methanol, and ethane were chosen as models of N-dealkylation, O-dealkylation, and aliphatic hydroxylation, respectively. In this way, we hoped to establish the approximate magnitudes of the deuterium and tritium kinetic isotope effects associated with alternative mechanisms.

Method

All calculations were carried out by using the MNDO semiempirical molecular orbital procedure described by Dewar and Thiel.[22] Singlet-state calculations were based on standard RHF wave functions, while triplet states were treated within the UHF formalism.[23] Approximate transition-state geometries were located via coarse two-dimensional searches of the energy surface and subsequently refined by minimizing the square of the gradient vector[24] with respect to all geometrical vari-

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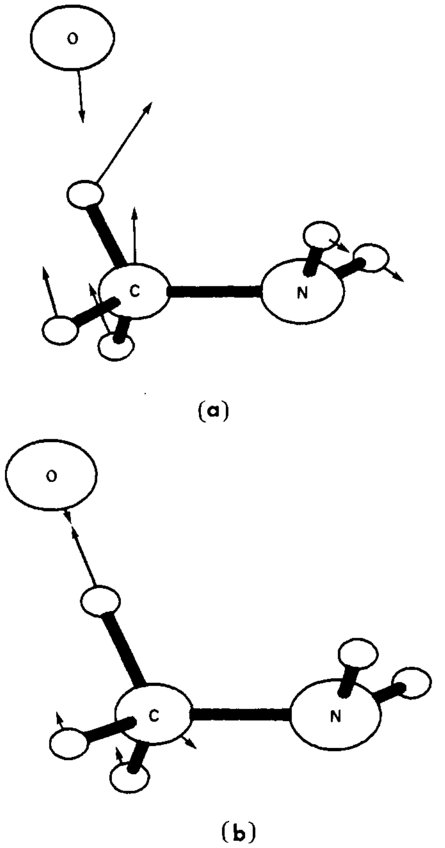

Figure 1. (a) Geometry of transition state and reaction coordinate displacements for the reaction of methylamine with O(^{1}D). (b) Geometry of transition state and reaction coordinate displacements for the reaction of methylamine with O(^{3}P).

ables. The resulting geometries were shown to be genuine transition states (saddle points) on the MNDO surface by calculating and diagonalizing the Cartesian force constant matrices, which revealed a single negative eigenvalue in every case. Kinetic isotope effects, k_H/k_D and k_H/k_T, were calculated via the Bigeleisen equation[25] as before[19] by using the MNDO vibrational frequencies for the appropriate isotopically substituted species.

$$CH_3NH_2 + O \rightarrow HOCH_2NH_2 \qquad (1)$$

$$CH_3OH + O \rightarrow HOCH_2OH \qquad (2)$$

$$CH_3CH_3 + O \rightarrow HOCH_2CH_3 \qquad (3)$$

For reactions 1–3, the relevant expressions are given by eq 4–7. Superscripts A and B refer to the substrate and transition state, respectively. Subscripts 1 and 2 refer to the light and heavy isotopes, ν refers top the frequency of the imaginary mode of the transition state and $u = h\nu/(kT)$.

$$\frac{k_1}{k_2} = \frac{s_2{}^A s_1{}^B}{s_1{}^A s_2{}^B} = \text{HRR} = \frac{\nu_1}{\nu_2}(\text{VP})(\text{EXC})(\text{ZPE}) \qquad (4)$$

$$\text{VP} = \prod^{3n-6}{}^{A}\frac{u_{2i}}{u_{1i}} \prod^{3n-7}{}^{B}\frac{u_{1j}}{u_{2j}} \qquad (5)$$

$$\text{EXC} = \prod^{3n-6}{}^{A}\frac{1-e^{-u_{1i}}}{1-e^{-u_{2i}}} \prod^{3n-7}{}^{B}\frac{1-e^{-u_{2i}}}{1-e^{-u_{1i}}} \qquad (6)$$

$$\text{ZPE} = \exp[\sum^{3n-6}{}^{A}(u_{1i} - l_{2i})/2]/\exp[\sum^{3n-7}{}^{B}(u_{1i} - u_{2i})/2] \qquad (7)$$

Results

Details of the calculated transition states for reactions 1–3, involving both singlet (^{1}D) and triplet (^{3}P) oxygen atoms are summarized in Table I. Tables I also includes the calculated activation energies and the frequencies of the imaginary modes. The transition-state geometries for reaction 1 are shown in Figure 1 and illustrate the displacement vectors corresponding to the atomic motions along the reaction coordinate. For reaction with both singlet and triplet oxygen, the transition-state geometries were similar for each substrate and corresponded to reactant-like species. This is apparent from the relatively small elongation of the breaking CH bonds (1.14–1.31 Å) and by the lack of significant electronic reorganization in the transition states. Moreover, for the triplet reactions, the net spin density was almost entirely localized on the oxygen atom. Calculated primary deuterium and tritium KIEs are presented in Table II. The components of the deuterium KIE for reaction 1 as a function of temperature are given in Table III.

Table I. Details of the Calculated Transition States for Reactions 1–3[a]

reaction	d_{CH}	$\delta_{CH}{}^b$	d_{CO}	$\delta_{CO}{}^c$	$\Delta H^{\ddagger\ d}$	imaginary mode, cm^{-1}
CH_3NH_2 + O(^{1}D)	1.141	0.024	2.861	1.470	6.9	361.6
CH_3OH + O(^{1}D)	1.143	0.028	2.820	1.427	7.4	373.2
CH_3CH_3 + O(^{1}D)	1.136	0.045	2.785	1.394	8.5	338.1
CH_3NH_2 + O(^{3}P)	1.231	0.114	2.586	*e*	23.5	2243.7
CH_3OH + O(^{3}P)	1.233	0.118	2.584	*e*	23.8	2294.4
CH_3CH_3 + O(^{3}P)	1.314	0.223	2.545	*e*	26.5	2458.1

[a] Distances in angstroms; energies in kcal mol^{-1}. [b] Extension of the CH bond length relative to that of the isolated reactants. [c] Extension of the CO bond length relative to that of the isolated products. [d] Calculated relative to O(^{1}D) = 112.8 kcal mol^{-1} and O(^{3}P) = 59.6. [e] The triplet product corresponds to the separated OH and CH_2X radicals.

Table II. Calculated Deuterium and Tritium Kinetic Isotope Effects[a]

reaction	singlet reaction		triplet reaction	
	R = D	R = T	R = D	R = T
CR_3NH_2/CH_3NH_2 + O	1.63	2.04	8.20	20.18
CRH_2NH_2/CH_3NH_2 + O[b]		1.91	8.11	19.57
CR_3OH/CH_3OH + O	1.68	2.12	8.35	20.73
CRH_2OH/CH_3OH + O[b]		1.96	8.21	19.93
CR_3CH_3/CH_3CH_3 + O[c]	1.65	2.08	8.75	22.26
CRH_2CH_3/CH_3CH_3 + O[b,d]		1.88	8.58	21.28

[a] Calculated harmonic rate ratios. Cf. footnotes *c* and *d*. [b] Primary isotope effects. [c] Does not include a symmetry factor of 2. [d] Does not include a symmetry factor of 6.

Table III. Deuterium Kinetic Isotope Effects for Reaction 1 as a Function of Temperature

state	*T*, K	$\nu_1{}^*/\nu_2{}^*$	VP	EXC	ZPE	HRR
triplet	200.0	1.36	1.01	0.94	16.86	21.80
	300.0	1.36	1.01	0.90	6.66	8.20
	400.0	1.36	1.01	0.86	4.11	4.85
	500.0	1.36	1.01	0.84	3.10	3.54
	600.0	1.36	1.01	0.82	2.56	2.87
singlet	200.0	1.10	1.25	0.85	1.71	1.99
	300.0	1.10	1.25	0.83	1.43	1.62
	400.0	1.10	1.25	0.82	1.31	1.46
	500.0	1.10	1.25	0.81	1.24	1.37
	600.0	1.10	1.25	0.80	1.20	1.31

Discussion

The transition-state geometries for the reactions on the singlet energy surfaces were calculated to be somewhat earlier than their triplet counterparts, which is presumably connected with their significantly greater exothermicity (Table IV).[26] Nevertheless, the calculated geometries for both singlet and triplet reactions

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Table IV. Calculated (Observed)[a] Heats of Reaction for Gas-Phase Oxygen Insertion and H· Abstraction

substrate	CH insertion[b] O(^{1}D)	H· abstraction[c] O(^{3}P)
CH_3NH_2	−160.1 (−137[d])	−34.5 (−7.7[e])
CH_3OH	−160.5 (−148[f])	−33.7 (−8.3[g])
CH_3CH_3	−156.5 (−140.9)	−29.2 (−4.1[g])

[a] From data in ref 32 and 27 with additional data noted. [b] Calculations based on a single determinantal RHF wave function. See the text. [c] Heats of formation of radicals obtained from UHF calculations. [d] Estimated value. $\Delta H_f[CH_2(OH)_2] \approx \Delta H_f[CH_2(OMe)_2] + 2\Delta H_f[MeOH] - 2\Delta H_f[Me_2O] = -91.4$.[32] [e] Colussi, A. J.; Benson, S. W. *Int. J. Chem. Kinet.* 1977, *9*, 307–316. [f] Estimated value. $\Delta H_f[CH_2(OH)NH_2] \approx \Delta H_f[CH_2(OH)NEt_2] + \Delta H_f[EtNH_2] - \Delta H_f[NEt_3] = -37.8$.[32] [g] Golden, D. M.; Benson, S. W. *Chem. Rev.* 1969, *69*, 125–134.

were qualitatively similar. However, the motions of the atoms along the reaction coordinates were quite different. On the singlet surface, the eigenvectors of the force constant matrices corresponding to the reaction coordinate clearly indicated the motion of the hydrogen atom to be roughly perpendicular to the line joining the carbon atom of the substrate and the incoming oxygen atom in each case. Thus, according to our calculations, reactions with the singlet oxygen atom proceed via a concerted attachment–rearrangement mechanism resulting in oxygen insertion while the triplet reactions proceed via a hydrogen radical abstraction–recombination mechanism.[21] Here the motion of the abstracted hydrogen coincides with the axis of the linear C–H–O arrangement. This difference is reflected in the calculated frequencies of the imaginary vibrational mode interconverting reactants and products. In the singlet case, this was calculated to be ~350 cm^{-1}, corresponding to a low-frequency bending vibration. The triplet abstractions were associated with very much higher frequencies, ~2300 cm^{-1}, associated with a weakened CH stretching vibration. As can be seen in Table III, the difference between isotope effects in the singlet and triplet reactions can be attributed almost totally to the ZPE contribution. This is the result of the loss of the higher energy vibrational mode in the triplet transition state.

Although the calculated activation energies in Table I are all lower for the reaction with the singlet oxygen atom, O(^{1}D) is some 50 kcal mol^{-1} higher in energy than the ^{3}P ground state.[27] Thus, the singlet transition states are, in fact, higher energy species than their triplet counterparts. Unfortunately, in neither case are the calculated activation energies expected to be quantitatively reliable. MNDO, like minimum basis set ab initio procedures, systematically overestimates the energies of hydrogen abstraction reactions.[28] Thus, the calculated activation energies for the gas-phase reactions of O(^{3}P) with methanol and ethane exceed those determined experimentally by 20.8[29] and 20.1[30] kcal mol^{-1}, respectively. The corresponding error for methylamine is not known since the available data are complicated by competing *N*-oxide formation.[31] Experimental data are not available for the reactions of O(^{1}D), although the MNDO activation energies are likely to again be overestimated. A further complication arises for the singlet reactions, since the closed shell RHF formalism we have used leads to a description of the lowest singlet state of the oxygen atom which corresponds not to the ^{1}D state but to a weighted average of the ^{1}D and ^{1}S states.[33] Thus, the predicted heat of formation of the lowest singlet state (112.8 kcal mol^{-1}) is somewhat higher than that determined experimentally (104.9 kcal mol^{-1}).[27] To some extent, this problem also applied to the transition states, although not to the oxygenated products. This, therefore, raises the possibility that the calculated transition-state geometries may be too reactant-like. However, relocating the transition state for the singlet oxygenation of methylamine (reaction 1), including CI sufficient to properly trace the five microstates of O(^{1}D) along the reaction path, revealed this effect to be rather slight.[34] The calculated exothermicities of the triplet reactions are also exaggerated but for a different reason. This is due to the tendency of the MNDO UHF procedure to lead to heats of formation of radicals that are too negative. The situation is improved slightly by using the "half-electron"[36] in place of the UHF formalism but not sufficiently to justify its significantly greater cost.[37] Thus, again, the calculated transition-state geometries may be somewhat too early. Similar calculations were carried out at the MNDO level by Pudzianowski and Loew[38-40] for the reaction between O(^{3}P) and several hydrocarbons, including methane. The latter reaction has also been the subject of a rather detailed ab initio study by Walch and Dunning.[41] Comparison of the transition-state geometries calculated by the two methods revealed very similar CO distances (MNDO, 2.53 Å; ab initio, 2.56 Å), while the hydrogen transfer was calculated to be rather more advanced in the ab initio study (MNDO, CH = 1.23 Å; ab initio; CH = 1.36 Å). In support of their calculated transition-state geometry, Pudzianoski and Loew[38] noted the excellent agreement between the calculated and observed entropies of activation. This does indeed suggest that the relative positions of the heavy atoms are calculated correctly. Unfortunately, in the present case, the inertial product, and hence the rotational entropy (by far the largest geometry-dependent contributor), is rather insensitive to the precise location of the transferring hydrogen. In all likelihood, the ab initio geometry is closer to the truth, the slightly earlier MNDO one probably being an artifact of the excessive exothermicity calculated for the reaction.

Previous experience[19] suggests that isotope effects calculated under these conditions may be somewhat lower than would otherwise be the case. Nevertheless, we expect our qualitative conclusions to be correct. These are that, in the absence of medium and other proximity effects, (1) oxygenation by O(^{1}D) leads to deuterium KIE's that only modestly exceed unity, while oxygenation by O(^{3}P) leads to deuterium KIE's that are approximately 6 times larger, and (2) in neither case are the magnitudes of the isotope effects greatly influenced by the adjacent substituents.

Observed deuterium isotope effects for P-450-mediated reactions have ranged from 1.5 to 11. However, intramolecular competition experiments, designed to reduce the masking effect of enzymatic rate constants not associated with the catalytic step,[42] have consistently revealed high (10–11) deuterium isotope effects for O-alkylations, benzylic hydroxylations, and carbon oxidations.[7-9] Our calculations show that, for the model system adopted here,

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(33) When represented by the appropriate combination of configurations, based on the half-electron wave function for $2s^22p^22p^12p^1$, the heats of formation of the ^{1}D and ^{1}S states of oxygen in the MNDO approximation are 95.1 and 148.3 kcal mol^{-1}, respectively.

(34) Relocating the transition state for oxygenation of methylamine using a 7 × 7 half-electron CI treatment (cf. ref 35) gave CH = 1.16 Å and CO = 2.78 Å with $\Delta H^{\ddagger}$ = 12.1 kcal mol^{-1}.

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(37) The calculated heats of reaction for O(^{3}P) in Table IV are less negative by 2.3, 1.4, and 2.6 kcal mol^{-1} at this level.

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triplet hydrogen radical abstractions lead to deuterium isotope effects of approximately that magnitude. Since the calculations also demonstrate that the corresponding reactions of singlet oxygen atoms have significantly lower deuterium KIE's, we feel that these data support the hypothesis of triplet, radical abstraction mechanisms for P-450-mediated carbon oxidation, benzylic hydroxylation, and O-dealkylations where high deuterium KIE's have been observed.

For N-dealkylations, however, the picture is much less clear. To date, no deuterium KIE above 2.00 has been observed in N-dealkylation, even with intramolecular competition experiments.[11,12] The lack of a large observed deuterium KIE for N-dealkylations has led to speculation that these reactions proceed via a transition state distinct from that of other P-450 oxidations, and alternative mechanisms have been proposed. One such mechanism receiving current experimental attention is the initial formation of a nitrogen-based radical cation, which can lose a proton to generate a carbon-based radical (eq 8) or undergo an additional one electron oxidation to form an iminium ion.[14-16]

$$CH_3NH_2 \xrightarrow{P\text{-}450} CH_3\dot{N}H_2^+ \xrightarrow{-H^+} \dot{C}H_2NH_2 \tag{8}$$

The corresponding gas-phase reaction of $O(^3P)$ is endothermic by about 180 kcal mol^{-1}, which, if it occurs in the enzymatic reaction, must be offset by powerful energy lowering proximity effects. The absence of even qualitative estimates of these, either here or for the H· abstraction reaction, obviously precludes any meaningful discussion of their relative energetics under enzymatic conditions. However, we can make a reasonable estimate of the equilibrium isotope effect for the first step in reaction 8, since this is independent of the oxidizing species. If K_H is the equilibrium constant for the first step in eq 8, then K_H/K_D corresponds to the equilibrium constant for the reaction.

$$CH_3NH_2 + CD_3\dot{N}H_2^+ \rightleftharpoons CD_3NH_2 + CH_3\dot{N}H_2^+$$

For a general isotopic exchange process

$$A + B' \rightleftharpoons A' + B$$

the equilibrium constant may be computed from the harmonic frequencies of the light (subscript 1) and heavy (subscript 2) isotopes of A and B via eq 9.[43]

$$K = \frac{\prod\limits^{3n-6}{}_{A} \dfrac{u_{2i}}{u_{1i}} \dfrac{1 - e^{-u_{1i}}}{1 - e^{-u_{2i}}} \exp[(u_{1i} - u_{2i})/2]}{\prod\limits^{3n-6}{}_{B} \dfrac{u_{2j}}{u_{1j}} \dfrac{1 - e^{-u_{1j}}}{1 - e^{-u_{2j}}} \exp[(u_{1j} - u_{2j})/2]} \tag{9}$$

From the MNDO frequencies of the species involved, we obtained $K_H/K_D = 1.38$. Intermolecular isotope effects of approximately this magnitude have indeed been measured for both chemical and electrochemical oxidation of aliphatic amines.[44,45] However, very recently, Shono et al.[46] have discovered examples of both electrochemical and microsomal N-dealkylation leading to low (1.6–1.9) intramolecular deuterium isotope effects. In these cases, the measured isotope effects arise from competitive loss of CH_3 and CD_3 from the *same* radical cation. In particular, there can be no isotope effect on the initial oxidation step. The low measured isotope effects cannot, therefore, be explained in terms of a rate-limiting nitrogen oxidation. The close similarity between the electrochemical and enzymatic isotope effects suggest that the latter are close to the intrinsic isotope values. However, our calculations require a much larger isotope effect for radical abstraction by a triplet oxene species. It appears, therefore, that P-450-mediated N-dealkylation does *not* proceed via a pathway analogous to that for O-dealkylation and carbon oxidation. The proposed mechanistic alternative (eq 8) would also appear to require modification. In its present form, this mechanism requires a CH bond fission associated with an unnaturally low deuterium isotope effect.

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Registry No. Methylamine, 74-89-5; methanol, 67-56-1; ethane, 74-84-0; atomic oxygen, 17778-80-2; cytochrome P-450, 9035-51-2; deuterium, 7782-39-0.

Supplementary Material Available: Calculated Cartesian coordinates and vibrational frequencies for all transition states (3 pages). Ordering information is given on any current masthead page.

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