

termination, a value of 1.055 ± 0.001 was obtained (data not shown) and demonstrates the reproducibility of this technique. The open circles are for an uncatalyzed reaction and provide $D(k_u) = 1.044 \pm 0.001$.

$D(k_{\text{obsd}})$ reflects the isotope effects for both the enzyme-catalyzed and uncatalyzed reactions and can be expressed as $D(k_{\text{obsd}}) = D(k_E)C_E + D(k_u)C_u$, where C_E and C_u are fractional contributions to rate limitation of the observed reaction by the enzyme-catalyzed and uncatalyzed reactions.⁷ In the present case, $C_u = k_u/k_{\text{obsd}}$ and, by definition, $C_E = 1 - C_u$.⁷ Rate constants were determined spectrophotometrically⁴ and are $k_{\text{obsd}} = 0.0119 \text{ s}^{-1}$ and $k_u = 0.00421 \text{ s}^{-1}$. Using $D(k_u) = 1.044$ and $D(k_{\text{obsd}}) = 1.081$, we calculate $D(k_E) = 1.106 \pm 0.016$.⁸

The solid line in the lower half of Figure 1 was drawn according to eq 3 with $\alpha = 1.047$ [$D(k_{\text{obsd}}) = 0.955$] and models the situation claimed by Fischer³ where $D(k_u) = 1.05$ and $D(k_E) = 0.91$. This further illustrates that our data cannot be fit to a model incorporating an inverse value for $D(k_E)$.

In summary, the β -deuterium isotope effect of 1.10 that we determined in this study confirms our earlier finding of 1.13⁴ and does not lend support to any mechanism involving nucleophilic catalysis. Mechanisms for this enzyme will likely employ some form of catalysis by distortion.⁴

Acknowledgment. We gratefully acknowledge Dr. Jeff Hermes (Biophysics Department, Merck Research Laboratories) for suggesting this experiment.

(7) Stein, R. L. *J. Org. Chem.* **1981**, *46*, 3328-3330.

(8) The error limit for this isotope effect is the standard deviation of the mean of the individual isotope effects that we calculated at each of seven fractions of reaction.

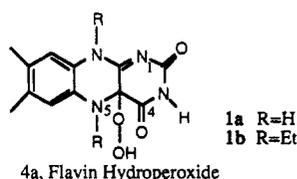
Potential for Water Catalysis in Flavin-Mediated Hydroxylation. A Theoretical Study

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One of the most important biochemical oxygen atom transfer reactions involves catalysis by flavoenzymes.¹⁻⁶ The flavin coenzyme functions as an electron conduit between a variety of substrates. These tricyclic isoalloxazine moieties are among the more versatile of the redox cofactors in biochemistry. When molecular oxygen is the reducible substrate for dihydroflavin reoxidation, the highly cited flavin 4a-hydroperoxide (**1a**) has been



(1) (a) Walsh, C. In *Flavins and Flavoproteins*; Vincent, M., Williams, C. H., Eds.; Elsevier/North Holland: Amsterdam, 1981; pp 121-132. (b) Ballou, D. P. *ibid.*; 1982; pp 301-310. (c) Bruice, T. C. *ibid.*; 1982; pp 265-277. (d) Wierenga, R. K.; Kalk, K. H.; van der Laan, J. M.; Drenth, J.; Hofsteenge, J.; Weijer, W. J.; Jekel, P. A.; Beintema, J. J.; Muller, F.; van Berkel, W. J. *ibid.*; 1982; pp 11-18.

(2) (a) Bruice, T. C. In *Flavins and Flavoproteins*; Bray, R. C., Engel, P. C., Mayhew, S. E., Eds.; Walter DeGruyter and Co.: New York, NY, 1984; pp 45-55. (b) Anderson, R. F. *ibid.*; 1984; pp 57-60.

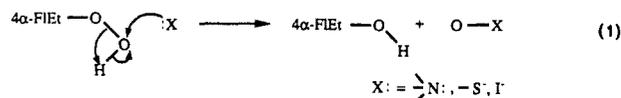
(3) Walsh, C. In *Enzymatic Reaction Mechanisms*; W. H. Freeman and Co.: San Francisco, 1979; pp 406-463.

(4) Visser, C. M. *Eur. J. Biochem.* **1983**, *135*, 543.

(5) (a) Ghisla, S.; Massey, V. *Biochem. J.* **1986**, *239*, 1; (b) *Eur. J. Biochem.* **1989**, *181*, 1.

(6) Jones, K. C.; Ballou, D. P. *J. Biol. Chem.* **1968**, *261*, 2553.

implicated as the key intermediate that serves as either the oxygen donor or its immediate precursor. The generally accepted mechanism for oxygen transfer is attack by the substrate on the distal oxygen with a direct nucleophilic displacement of the β -peroxy oxygen (eq 1).^{1c} Model enzyme **1b** is intermediate in



reactivity between a peracid and an alkyl hydrogen peroxide.⁷ In discussions of this mechanism to date, no provision has been made for the energy requirements of the 1,2-hydrogen shift. In the following paper in this issue⁸ we established the potential energy surface for oxygen donation from H_2O_2 to amines and alkenes in the gas phase to be almost entirely dominated by the energetic requirements (56.0 kcal/mol)⁹ for the 1,2-hydrogen migration to afford water oxide. We now describe the remarkable catalytic effect of one and two molecules of water on the transition state for the oxidation of ammonia.^{9,10}

Although the barriers (Table I) for a concerted $\text{S}_{\text{N}}2$ displacement by ammonia on hydrogen peroxide are surprisingly high (51.4 kcal/mol), a direct displacement of OH^- from H_2O_2 without a 1,2-hydrogen shift would afford OH^- and H_3NOH^+ that is 198.2 kcal/mol (HF/6-31G*) above the energy of the reactants in the gas phase. Solvation of a hydroxide ion by one water molecule affording bihydroxide ion (H_3O_2^-) is accompanied by only 35.2 kcal/mol (HF/6-31G*) of stabilization. Consequently, the oxidation of NH_3 must be accompanied by a formal 1,2-hydrogen migration that is complete prior to N-O bond making in the transition state. The overall oxygen transfer is therefore very likely a two-step process involving $\text{S}_{\text{N}}2$ attack by ammonia on water oxide.⁸ By analogy, a comparable reaction occurring in a flavin-mediated oxygen atom donation in the absence of solvent participation must also involve a similar hydrogen shift to the α -oxygen prior to displacement of the pseudobase (4a-FIOH). Secondly, the transition structure for this type of concerted reaction also has a complete octet of electrons around the so-called "electrophilic" oxygen. Given the observed half-life for native **1a** (~ 2.5 ms),^{2b} such a process involving a simple 1,2-hydrogen migration (eq 1) would exhibit an energy barrier that is far in excess of that of an oxygen donor that is capable of achieving aromatic hydroxylation.

The origin of the barrier for a 1,2-hydrogen shift across an O-O bond may be attributed in part to the fact that the rearrangement is a four-electron process involving a filled-in-plane σ -type O-O bond at the migration terminus that has pseudo- π^* symmetry¹¹ and the concerted process is formally forbidden on the basis of symmetry arguments. The barrier for formation of water oxide is 56.0 kcal/mol⁸ while a concerted rearrangement of H_2O_2 hydrogen bonded to one molecule of water (TS-1) still exhibits a barrier of 44.1 kcal/mol (MP4SDTQ/6-31G**/HF/6-31G*) from the $\text{H}_2\text{O}_2 \cdot \text{H}_2\text{O}$ complex. In principle, a water molecule serving as a catalyst can circumvent this problem in an alkyl hydrogen peroxide like **1a** by accepting a proton from the distal peroxide oxygen and transferring one of its hydrogens to the

(7) The N^5 -ethylflavin **1b** is about a 10^3 -fold better oxygen donor than *m*-chloroperbenzoic acid but 10^3 - 10^6 -fold less reactive than ROOH .^{2a}

(8) Bach, R. D.; McDouall, J. J. W.; Owensby, A. L.; Schlegel, H. B. *J. Am. Chem. Soc.*, following paper in this issue.

(9) Unless otherwise specified, all energies refer to structures optimized at the MP2/6-31G* level with Møller-Plesset electron correlation to full fourth order (MP4SDTQ/6-31G**/MP2/6-31G*) without zero point energy. The three transition structures are first-order saddle points and water oxide solvated with one and two water molecules gives stationary points as established by frequency calculations at the HF/6-31G* level.

(10) (a) Molecular orbital calculations have been carried out using the GAUSSIAN 88 program system^{10b} utilizing gradient geometry optimization.^{10c} (b) Frisch, M. J.; Head-Gordon, M.; Schlegel, H. B.; Raghavachari, K.; Binkley, J. S.; Gonzales, C.; DeFrees, D. J.; Fox, D. J.; Whiteside, R. A.; Seeger, R.; Melius, C. F.; Baker, J.; Martin, R. L.; Kahn, R. L.; Stewart, J. J. P.; Fluder, E. M.; Topiol, S.; Pople, J. A. Gaussian, Inc., Pittsburgh, PA, 1988; GAUSSIAN 88 is a trademark of Gaussian, Inc. (c) Schlegel, H. B. *J. Comput. Chem.* **1982**, *3*, 214.

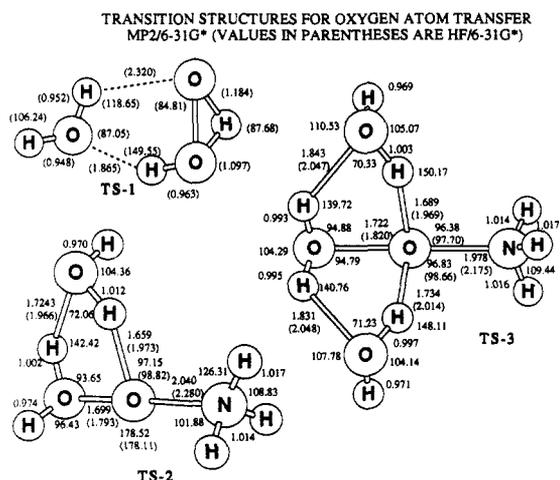
(11) Bach, R. D.; Wolber, G. J. *J. Am. Chem. Soc.* **1984**, *106*, 1410.

Table I. Barriers^a Relative to Isolated Reactants for NH₃ + H₂O₂ + n(H₂O) [n = 0, 1, 2]

	HF/6-31G*// HF/6-31G*	MP4/6-31G*// HF/6-31G* ^b	MP2/6-31G*// MP2/6-31G* ^c	MP4/6-31G*// MP2/6-31G* ^{b,c}
(n = 0) ^d	41.7	52.1 ^e	57.5	51.4 ^f
TS-2 (n = 1)	28.8	31.8	34.6	29.4 (39.1) ^g
TS-3 (n = 2)	18.5	14.9	16.0	11.2 (33.1) ^h

^a In kilocalories per mole. ^b All MP4 calculations used a frozen core and include single, double, triple, and quadruple excitations. ^c All MP2 calculations used full orbital space. ^d See ref 8 for transition structure. ^e The barrier relative to the H₂O₂-NH₃ linear complex is 62.9 kcal/mol.⁸ ^f The barrier relative to the water oxide + NH₃ cyclic complex is 24.3 kcal/mol.⁸ ^g The barrier relative to the H₂O₂-H₂O complex and isolated NH₃. ^h The barrier relative to the H₂O₂-2H₂O complex and isolated NH₃.

proximal oxygen (α -oxygen of the ROOH) effecting a formal 1,4-hydrogen shift as depicted in TS-2. The reaction path for



oxygen transfer to NH₃ was followed in internal coordinates¹² and led from TS-2 back to solvated water oxide and ammonia, supporting a simple protonation-deprotonation pathway. Although a remarkable decrease in the ΔE^\ddagger results from this water catalysis (TS-2),¹³ the barrier of 29.4 kcal/mol relative to the isolated reactants and 39.1 from hydrogen-bonded H₂O₂ would still be much too high to be commensurate with the oxygen donation potential of native 1a. We therefore employed a second water molecule (TS-3), and the barrier for oxygen atom transfer from hydrogen peroxide and two isolated water molecules dropped to 11.2 kcal/mol for an overall decrease in activation energy of 40.2 kcal/mol.¹³ A decrease in barrier of 18.3 kcal/mol for H₂O₂ relative to H₂O₂ + 2H₂O reflects the energy difference of 28.9 kcal/mol for the H₂O₂-2H₂O complex and water oxide solvated by two water molecules. More significantly, the barrier for oxygen transfer (TS-3) from solvated water oxide is only 4.2 kcal/mol, which falls in an energy range that would be consistent with the extremely high oxidizing capacity exhibited by native 1a. Such a dramatic energy decrease is far in excess of that which would be anticipated on the basis of simple hydrogen bonding.¹⁴

Although the reduction in the barrier must reflect both the amelioration of the 1,2-hydrogen shift barrier and a decrease in electron density at the "electrophilic" oxygen, the greatest effect comes from stabilization of water oxide itself by the water molecules.¹³ Since alkyl hydrogen peroxide 1b has been established to be 10⁴ times more reactive than H₂O₂ as an oxygen donor,⁷ comparable inductive effects should further lower the energy of TS-3. The present study strongly suggests that a simple S_N2 displacement (eq 1) cannot account for the oxygen donation potential of 1a. *The oxygen transfer process requires an oxenoid*

oxygen atom and a neutral leaving group. The hydrogen transfer can also be achieved by simple protonation-deprotonation circumventing the more energetically demanding 1,2-hydrogen shift. We therefore suggest that hydrogen bonding by the N₃ hydrogen on one side¹⁵ and one (or two) water molecule(s) on the other would afford a transition structure in native flavin 1a that resembles TS-3, providing a novel explanation for the unusual monooxygenase reactivity of flavins. Hence, the potentially ex-orbitant barrier for this overall process may be abated by the stabilizing influence of one or more water molecules.¹⁶ The data in the present study also strongly suggests that a structure resembling TS-3 is involved in a great many reactions of hydrogen peroxide in protic solvents.

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Registry No. NH₃, 7664-41-7; H₂O₂, 7722-84-1; H₂O, 7732-18-5.

(15) The importance of five-membered intramolecular hydrogen bonding in α -azo hydroperoxides has been firmly established to play a major role in oxygen atom transfer: Baumstark, A. L.; Vasquez, P. C. *J. Org. Chem.* **1987**, *52*, 1939.

(16) For a discussion of solvent-assisted proton transfer in alkyl hydrogen peroxides, see: (a) Dankleff, M. A. P.; Ruggero, C.; Edwards, J. O.; Pyun, H. Y. *J. Am. Chem. Soc.* **1968**, *90*, 3209. (b) Swern, D. *Organic Peroxides*; Wiley Interscience: New York, 1971; Vol. II, pp 73-74.

Theoretical Study of Oxygen Atom Transfer. The Role of Electron Correlation

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The transfer of an oxygen atom involving cleavage of an oxygen-oxygen σ bond is one of the most significant biological transformations known,¹ and this type of oxidative insertion also enjoys a unique status in synthetic organic chemistry.² Despite the generality of such reactions, the literature is essentially void of high level ab initio calculations involving oxygen transfer where an O-O bond is involved.³ Pople et al. reported⁴ that a 1,2-

(1) (a) Walsh, C. In *Flavins and Flavoproteins*; Vincent, M., Williams, C. H., Eds.; Elsevier/North Holland: Amsterdam, 1981; pp 121-132. (b) Walsh, C. In *Enzymatic Reaction Mechanisms*; W. H. Freeman and Co.: San Francisco, 1979; pp 406-463. (c) Bruce, T. C. In *Flavins and Flavoproteins*; Bray, R. C., Engel, P. C., Mayhew, S. E., Eds.; Walter DeGruyter and Co.: New York, 1984; pp 45-55.

(2) (a) Finn, M. G.; Sharpless, K. B. *Asymmetric Synth.* **1985**, *5*, 247. (b) Sheldon, R. A.; Kochi, J. *Metal-Catalyzed Oxidations of Organic Compounds*; Academic Press: New York, 1981; Chapter 9. (c) Mimoun, H.; Chaumette, P.; Mignard, M.; Saussine, L. *Nouv. J. Chim.* **1983**, *7*, 467.

(3) For a highly detailed theoretical study of the reaction of ozone with alkenes, see: Cremer, D.; Bock, C. W. *J. Am. Chem. Soc.* **1986**, *108*, 3375 and references therein.

(12) Gonzalez, G.; Schlegel, H. B. *J. Chem. Phys.* **1989**, *90*, 2154.

(13) Water oxide complexed by one water molecule in a cyclic array resembling that in TS-2 exists at an energy minimum that is stabilized by 21.6 kcal/mol. The hydrogen-bonded bicyclic array, involving two water molecules, is 42.7 kcal/mol lower in energy than its separated entities and only 7.0 kcal/mol above the energy of isolated H₂O₂ plus two water molecules.

(14) For example, the enthalpy values for the formation of water dimer at the MP4SDTQ/6-31G*//HF/6-31G* and MP4/6-31+G(2d,2p) levels are -7.4 and -3.6 kcal/mol, respectively: Del Bene, J. E. *J. Phys. Chem.* **1988**, *92*, 2874. At the former level the hydrogen-bonding stabilization of H₂O₂ and H₂O is 9.3 kcal/mol.