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Neutral versus Charged Species in Enzyme Catalysis. Classical and Free Energy Barriers for Oxygen Atom Transfer from C4a-Hydroperoxyflavin to Dimethyl Sulfide

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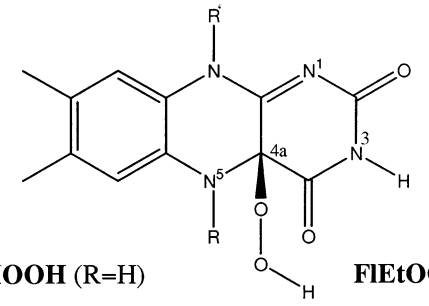
Theoretical calculations at the B3LYP/6-31+G(*d*,*p*) level have been used to study the oxidation of dimethyl sulfide by a series of bicyclic and tricyclic model C4a-flavin hydroperoxides. The intrinsic gas-phase reactivity of tricyclic C4a-hydroperoxyflavin **4** is ca. 10^9 greater than *t*-BuOOH but is ca. 10^7 less reactive toward the oxidation of dimethyl sulfide than peroxyformic acid. The S_N2-like attack of the nucleophile on the distal oxygen of the hydroperoxide and the relative reactivity of the peracid are in excellent agreement with the earlier experimental data of Bruice. The effect of N^1 or N^5 hydrogen-bonding interactions on the activation barriers for oxygen atom transfer have been examined. Classical energy barriers for oxygen atom transfer from neutral and ion-paired forms of C4a-hydroperoxyflavin to dimethyl sulfide are predicted to differ by a small margin, suggesting that proton distribution exerts a relatively small influence on the reactivity of alkyl hydroperoxides. Isolated N^1- and N^5-protonated cations exhibit artificially low barriers as a consequence of their location in a high energy region of the potential energy surface domain.

1. Introduction

One of the most important oxygen atom transfer reactions in biochemistry involves catalysis by flavoenzymes.[1] These tricyclic isoalloxazine moieties are among the more versatile of the redox cofactors in biochemistry. Molecular oxygen is the reducible substrate for dihydroflavin reoxidation, and C4a-hydroperoxyflavin (4a-FlHOOH) has been implicated as the key intermediate that serves as the oxygen donor. Native FADHOOH is a highly reactive oxygen donor with a half-life of the order of 2.5 ms.[2] Considerable effort has been expended in the past to determine the mechanistic details of flavin-dependent oxygenases. These enzymes are able to oxidize a number of hydroxy-substituted phenols.[3] *p*-Hydroxybenzoate hydroxylase (PHBH) has become the paradigm aromatic hydroxylase because of extensive kinetic and X-ray structural studies.[4] When the N^5 nitrogen in C4a-hydroperoxyflavin is alkylated with an ethyl group, the N^5-ethyl group prevents the elimination of H_2O_2, and this model C4a-hydroperoxyflavin (4a-FlEtOOH) can be prepared and isolated in the laboratory.[5] The intrinsic reactivity of the N^5-ethyl derivative has been extensively studied by Bruice in a series of papers where it has been reacted with amines, sulfides, alkenes, and I^-, and its reactivity has been compared to common oxidants such as hydroperoxides and peracids.



FlHOOH (R=H) **FlEtOOH** (R=Et)

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The reactivity of the enzymes toward phenolic substrates has also been recently investigated both by experimental[3] and theoretical methods.[6] In an earlier report on the reactivity of substituted methyl hydroperoxides, we suggested that the equivalent of hydrogen bonding to the N^5 hydrogen of native FADHOOH could afford a stabilized transition structure for oxygen transfer, providing a novel explanation for the unusual monooxygenase reactivity of flavins.[7] That study suggested that Coulombic effects can make a substantial contribution to the oxygen-donor capacity of C4a-hydroperoxyflavin. Many species of interest in biochemistry are reported to be in the form of ions at the active site. In particular, nitrogen atoms are sometimes represented in their protonated form at physiological pH, based on typical pK_a values, and theoretical studies have been based upon such positively charged ammonium ion species. The classical barrier for oxygen transfer to a substrate is typically very sensitive to the proton distribution both on the oxidizing and the substrate fragments. For example, the MP2/6-31G(*d*) classical barrier for oxygen transfer from H_2O_2 dimer to dimethyl sulfide (DMS) is 40.8 kcal mol^{-1}.[6] The corresponding barrier for oxygen transfer from protonated H_2O_2 is lowered to 33.2 kcal mol^{-1}.[6] In a recent QM/MM study of the enzymatic oxidation of phenol, Ridder[3] observed a proton transfer from the substrate to the carboxylate group of Asp54 in a system with $-1e$ net charge. Given the sensitivity of calculated reaction barriers on the total charge of the system under investigation, we extend our model to include the proton donor/acceptor responsible for the charge on the substrate/cofactor. The net charge on the whole system is zero in the case of the hydrogen bonded models. The protonated species are represented as both naked cations and as ion pairs and the effect of the net charge on the hydroperoxide on the barrier for oxygen atom transfer has been examined. For this series of model flavin hydroperoxides, including a realistic tricyclic model C4a-hydroperoxyflavin (**4**), we have also compared the relative activation barriers for oxygen atom transfer to several more classical oxygen transfer reagents.

2. Computational Details

Quantum chemistry calculations were carried out using the Gaussian98 program[8] system utilizing gradient geometry optimization.[9] All geometries were fully optimized using the B3LYP functional[10] with 6-31G(*d*) and 6-31+G(*d*,*p*) basis sets. Vibrational frequency calculations at the same level of the geometry optimization were performed to characterize the stationary points as either minima or first-order saddle points. Frequency calculations for the larger tricyclic systems were at 6-31G(*d*). The modified Perdew−Wang 1-parameter functional for kinetics (MPW1K) optimized by Truhlar[11] for reaction barriers was used for selected molecules. The reactants and transition structure at the CCSD(T)//CCSD/6-31G(*d*) and CCSD(T)/6-311G(*d*,*p*) levels for the oxygen transfer reaction from methyl hydroperoxide to dimethyl sulfide and from methyl hydroperoxide to ammonia were computed using the program ACES II.[12] Molecular structures in Figures 1−10 have been drawn with the program Moldraw.[13] Free energies (*G*) in the gas phase have been computed at 298 K within the harmonic oscillator-rigid rotor approximation.[14] Proton affinities were calculated as differences in the potential energy (including the zero point energy) between the protonated and the corresponding neutral species. It should be emphasized that all the barrier heights reported herein are computed relative to isolated reactants, i.e., dimethyl sulfide (DMS) and the oxygen donor.

3. Results and Discussion

To better understand the origin of the reactivity of 4a-FlHOOH, the inductive and hydrogen bonding effects of the various functionalities in flavin hydroperoxide have been examined (sections 3.1−3.3). Three independent models have been examined for the reaction: C4a-hydroperoxyflavin + dimethyl sulfide, systems (a) without intrinsic *H*-bonds (isolated reactants), (b) N^1-hydrogen-bonded or -protonated, and (c) N^5-hydrogen-bonded. Formic acid was used as a model carboxylic acid to examine the effect of hydrogen bonding interactions with N^1 and N^5 on the oxidation barriers. Due to the considerable size of the tricyclic 4a-FlHOOH molecule, the aromatic ring was excluded from some systems and a bicyclic model was used. Self-consistent reaction field (SCRF) calculations on transition structures involving comparable oxidations of NH_3 and H_2S by hydrogen peroxide suggest that these relatively high barrier heights are reduced very little by inclusion of solvation effects.[6] Hence, we have not included solvation effects since our interest is mainly concerned with the intrinsic barrier differences. Since the oxygen transfer from C4a-hydroperoxyflavin to 4-hydroxybenzoate is computationally very demanding, we chose initially to perform a preliminary investigation on the intrinsic reactivity of C4a-hydroperoxyflavin as an oxygen atom donor with a simple model substrate such as dimethyl sulfide (DMS, **1**). The FlEtOOH model flavin has also been used for the oxidation of sulfur compounds[15] and has also been generated in situ by the addition of HOOH to 4a-hydroperoxyflavins.[16]

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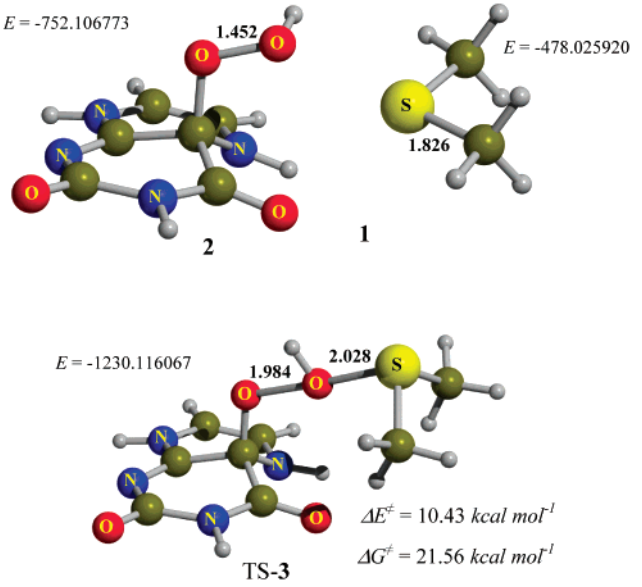
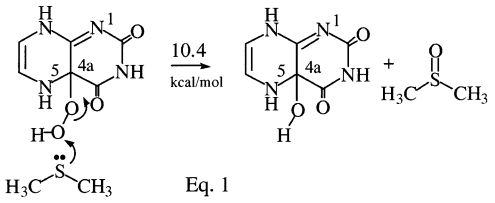
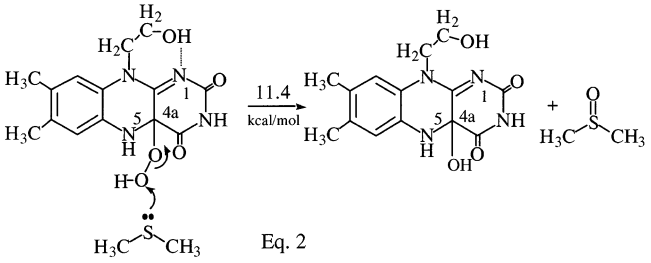


FIGURE 1. Geometry of reactants for the oxygen atom transfer reaction to DMS (**1**) from the neutral bicyclic model C4a-hydroperoxyflavin (**2**). The relative potential energy ($\Delta E^{\ddagger}$) and free energy ($\Delta G^{\ddagger}$) of the transition structure (TS-**3**) are reported. Geometries are optimized at the B3LYP/6-31+G(*d*,*p*) level; distances are in Å.

3.1. Hydroperoxyflavin Models (2 and 4) + Dimethyl Sulfide (TS-3 and TS-5). We report in Figure 1 a bicyclic model of hydroperoxyflavin **2** (eq 1) where the aromatic ring has been omitted and its corresponding transition structure for the oxidation of DMS (TS-**3**). The classical reaction barrier for dimethyl sulfoxide formation ($\Delta E^{\ddagger}$ = 10.4 kcal mol^{-1}) is rather low at the B3LYP/6-31+G(*d*,*p*) level (Table 1).



Eq. 1



Eq. 2

To examine the possible role of the ribityl side chain of the flavin, a β-hydroxyethyl group was included to model the 2′-OH group. We also included the third ring in an effort to examine the effect of the dimethylbenzene ring on the activation barrier (minimum **4**). Oxygen atom transfer from tricyclic model C4a-hydroperoxyflavin **4** to DMS (TS-**5**, Figure 2, eq 2) actually exhibits a slightly higher activation barrier at the B3LYP/6-31+G(*d*,*p*) level ($\Delta E^{\ddagger}$ = 11.4 kcal mol^{-1}).

Thus, extension of model hydroperoxide **2** to include the dimethylbenzene ring and the intramolecular hydrogen bonded "ribityl side chain" has only a modest impact upon the activation barrier for oxygen atom transfer. One would predict a priori that the aromatic ring should help to stabilize the developing alkoxide leaving group (FlO^{-}) in the TS for O−O bond cleavage and lower the activation barrier. For this rather simple model system, in the absence of intermolecular H-bonding of local residues at an active site, the electronic effect of the conjugated benzene ring on the reaction barrier actually increases the barrier by 1.0 kcal mol^{-1}. This suggests that the role of this hydrophobic aromatic fragment of C4a-hydroper-

TABLE 1. Reaction Potential Energy Barriers ($\Delta E^{\ddagger}$, kcal mol^{-1}) Based upon B3LYP/6-31+G(*d*,*p*) and MPW1K/6-31+G(*d*,*p*) Calculations for the Reaction of Oxygen Atom Transfer to DMS from Various Model Flavin Hydroperoxides[a]

process	protonation	charge	B3LYP	MPW1K
1 + **2** → TS-**3**	none	0	10.43 (21.56)	21.64 (32.63)
1 + **4** → TS-**5**	none	0	11.41	
1 + **6** → TS-**7**	N^1	1	−4.74 (5.82)	4.20 (15.79)
1 + **8** → TS-**9**	N^1	1	−1.20	
1 + **10a** → TS-**11a**	N^1 + HCOO$^-$	0	18.28	15.96
1 + **10a** → TS-**11b**	N^1 + HCOO$^-$	0	7.22	
1 + **10b** → TS-**11b**	N^1 + HCOO$^-$	0	−5.95	
1 + **6** → TS-**13**	N^5	1	15.61 (26.38)	24.13 (19.84)
1 + **12** → TS-**13**	N^5	1	−7.96 (2.57)	−3.52 (11.87)
1 + **10a** → TS-**15**	N^5 + HCOO$^-$	0	10.14	19.95
1 + **14** → TS-**15**	N^5 + HCOO$^-$	0	−15.52	−4.73

[a] Activation Gibbs free energies at 298 K ($\Delta G^{\ddagger}$, kcal mol^{-1}) for selected processes are given in parentheses.

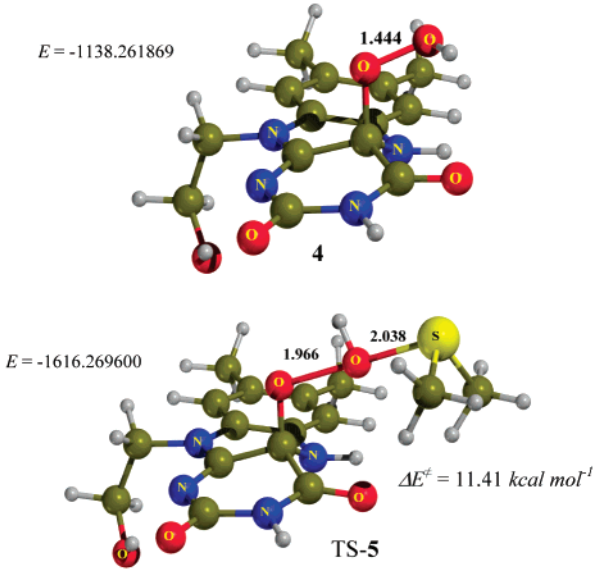


FIGURE 2. Relative energy ($\Delta E^{\ddagger}$) of reactant model tricyclic C4a-hydroperoxyflavin (**4**) and the transition structure for oxygen atom transfer to DMS (TS-**5**). Geometries are optimized at the B3LYP/6-31+G(*d*,*p*) level; distances are in Å.

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TABLE 2. Reaction Barriers[a] from the Isolated Oxidizing Agent and Substrate

substrate	oxidizing agent	barrier, kcal mol^{-1}	imaginary freq, cm^{-1}
C_2H_4	peroxyformic acid	14.1; **14.9**	−448.0
C_2H_4	bicyclic flavin model **2**	24.0; **24.1**	−392.3
cyclohexene	peroxyformic acid	9.7; **10.1**	−401.5
(*E*)-2-butene	peroxyformic acid	10.5; **11.0** [20.1][c]	−411.1
(*E*)-2-butene	bicyclic flavin model **2**	19.9; **19.8**	−408.6
DMS	peroxyformic acid	5.6; **3.7**	−303.0
DMS	CH_3OOH	27.1 (**32.4**) [39.7][c]	**-697.9**
DMS	CH_3OOH dimer	10.8; **10.5** (22.5; **19.4**)	−332.5
DMS	*t*-BuOOH	30.0; **27.2** (36.5; **32.2**)	−672.5
DMS	bicyclic flavin model **2**	13.5 [21.4][c]	−258.5
DMS	tricyclic flavin model **4**	14.3	−267.6

[a] Reaction barriers are based upon total energies (without ZPE corrections). [b] Numbers in parentheses are barriers calculated with respect to the pre-reaction complex. Plain numbers are at the B3LYP/6-31G(*d*) level, bold numbers correspond to the B3LYP/6-31+G(*d*,*p*) calculations. [c] Barriers in brackets are calculated at the MPW1K/6-31+G(*d*,*p*) level of theory.

oxyflavin **4** could be simply one of internal solvation to facilitate binding at the active site. The transition structure for oxygen-atom transfer from model C4a-hydroperoxyflavin **4** to DMS (TS-**5**) corroborates the earlier suggestion by Bruice[5] that the *N*-oxidation of amines by 4a-FlEtOOH involved nucleophilic displacement by sp^3-hybridized nitrogen on the terminal oxygen of the hydroperoxide (eq 2). It was further suggested that the efficiency of 4a-FlEtOOH in N-oxidation was more than 4 orders of magnitude greater than that of H_2O_2 or *t*-BuOOH. On the contrary, *m*-chloroperbenzoic acid was found to be ca. 10^5 times more reactive than 4a-FlEtOOH.[5] We now provide theoretical data that agree well with this assessment of the reactivity of C4a-hydroperoxyflavin as an oxygen atom donor.

Another objective of the current study is to compare the intrinsic gas-phase reactivity of bicyclic and tricyclic C4a-hydroperoxyflavin models **2** and **4** to that of other more general oxidizing agents. This type of rate data provides a useful measure of the role that must be played by activation of the hydroperoxide by the local environment in an enzymatic oxidation. Using the paradigm model theoretical oxygen atom donor peroxyformic acid (H(C═O)OOH) as a measure, the barriers for the epoxidation of ethylene, (*E*)-2-butene and DMS with bicyclic model **2** are an average of 9 kcal mol^{-1} greater than that for peroxyformic acid (Table 2). Peroxyformic acid is also much more reactive toward DMS than methyl hydroperoxide ($\Delta\Delta E^{\ddagger}$ = 23 kcal mol^{-1}). It is also evident that DMS is a much better gas-phase nucleophile than ethylene, as evidenced by their difference in activation barriers ($\Delta\Delta E^{\ddagger}$ = 11 kcal mol^{-1}) for reaction with peroxyformic acid. While CH_3OOH and *t*-BuOOH have comparable barriers for the oxidation of DMS (27.2 and 27.1 kcal mol^{-1}), bicyclic model flavin **2** exhibits a much lower activation barrier of only 10.4 kcal mol^{-1} ($\Delta\Delta E^{\ddagger}$ = 16.8 kcal mol^{-1}). Thus, one may consider bicyclic hydroperoxide **2** to be a "tertiary alkyl hydroperoxide" with electronegative substituents that is a much better oxygen donor than *t*-BuOOH. The correlation of pK_a of the departing "alcohol" for a series of ROOH oxygen donors led to the suggestion[5] that the increased reactivity of C4a-hydroperoxyflavin can be attributed to the inductive stabilization of the alcoholate leaving group by the electronegative elements surrounding C4. The pK_a of 4a-FlEtOOH is 9.4 while the pK_a of *t*-BuOH is approximately twice that. We estimate the proton affinity (PA) of the C4a-hydroxyflavin (Fl-OH) derived from oxygen transfer from **4** to be 339 kcal mol^{-1} (B3LYP/6-31+G(*d*,*p*)) while that of *t*-BuOH is much greater at 381 kcal mol^{-1}. Thus, in the oxygen transfer step, the developing oxyanion of **4** (Fl-O$^-$) is much better stabilized as the O−O bond is cleaved than *t*-butoxy anion and this is clearly reflected in their difference in activation energies for the oxidation of DMS ($\Delta\Delta E^{\ddagger}$ = 15.7 kcal mol^{-1}, Table 2).

Our theoretical data are quite consistent with the experimental[5] rate ratios for oxygen atom transfer. Thus, native FADHOOH as modeled in this work by **4**, while intermediate in reactivity between a peracid and ROOH, is still capable of enzymatically oxidizing the benzene ring in *p*-hydroxybenzoic acid.[1] It should also be noted that, at the B3LYP/6-31+G(*d*,*p*) level, oxygen transfer from C4a-hydroperoxyflavin to DMS ($\Delta E^{\ddagger}$ = 10.4 kcal mol^{-1}) is considerably faster, with respect to the corresponding oxygen transfer step, than oxidation of neutral *p*-hydroxybenzoic acid ($\Delta E^{\ddagger}$ = 18.2 kcal mol^{-1}).[17] Thus, it is very likely that enzymatic hydroxylation of *p*-hydroxybenzoic acid occurs as its carboxylate.

3.2. N^1-Protonated and Hydrogen-Bonded Model C4a-Hydroperoxyflavins (6 and 8) + Dimethyl Sulfide (TS-7 and TS-9). It is generally thought that the potential H-bonding interactions at N^1 and N^5 with residues at an active site should lower the barrier for oxygen transfer. Proton transfer to these basic sites could be used, in the limit, to model the effects of H-bonding on the reaction barrier. The two basic nitrogen sites in bicyclic model C4a-hydroperoxyflavin **2** are N^1 and N^5, and the proton affinities of these sites are estimated to be 218.4 and 194.6 kcal mol^{-1} (B3LYP/6-31+G(*d*,*p*) + ZPE).[18] Consequently, we first examined the relative reactivity of the more basic N^1-protonated hydroperoxide **6** (Figure 3).

Significantly, the activation barrier for oxygen atom transfer to DMS is reduced from 10.4 kcal mol^{-1} in neutral TS-**3** to −4.7 kcal mol^{-1} in cationic TS-**7**.[19] While we do not specifically identify the source of the proton in TS-**7**, it is emphasized that the molecular architecture in flavin hydroperoxide 4a-FlHOOH would not permit proton transfer from N^1 to the developing FlO$^-$ as the O−O bond is cleaved. In the transition structure for the formal transfer of OH$^+$, the distance is simply too big for a concerted proton transfer. The marked reduction in the activation barrier ($\Delta\Delta E^{\ddagger}$ = 15.2 kcal mol^{-1}) is a consequence of the fact that naked gas-phase cations such as **6** often exhibit artificially low barriers, relative to their corresponding neutral counterparts such as **2** because charged species (including anions) are elevated in energy and hence are inherently more reactive.

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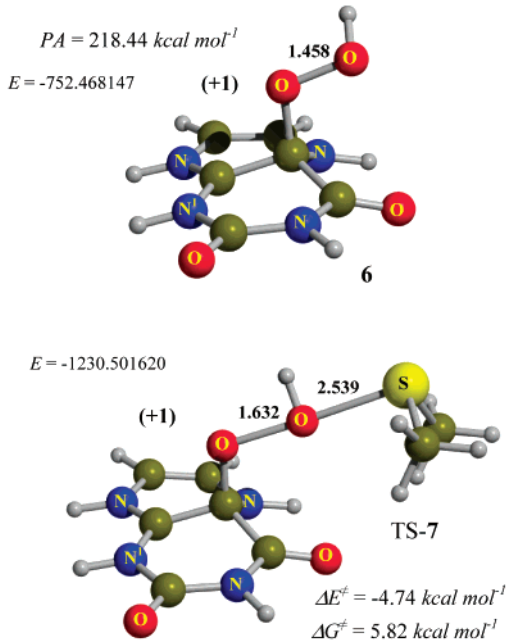

FIGURE 3. Relative energy ($\Delta E^{\ddagger}$) and free energy ($\Delta G^{\ddagger}$) of reactant model bicyclic N^1-protonated C4a-hydroperoxyflavin (**6**) and the transition structure for oxygen atom transfer to DMS (TS-**7**). Geometries are optimized at the B3LYP/6-31+G(*d*,*p*) level; distances are in Å. The proton affinity (PA) at N^1 is also reported.

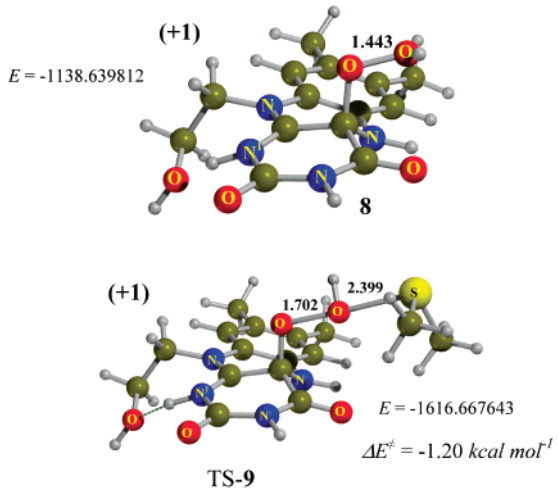

FIGURE 4. Reactant model tricyclic-ring N^1-protonated C4a-hydroperoxyflavin (**8**) and the transition structure for oxygen atom transfer to DMS (TS-**9**). Geometries are optimized at the B3LYP/6-31+G(*d*,*p*) level; distances are in Å.

When the N^1-protonated model hydroperoxide is extended to include the effect of the conjugated aromatic third ring, as shown in Figure 4, tricyclic cation **8** and TS-**9** exhibit a barrier for the oxygen transfer to DMS of -1.2 kcal mol^{-1},[20] a decrease of 12.6 kcal mol^{-1} relative to neutral tricyclic TS-**5** (Table 1). This decrease in activation energy, due largely to the fact that the substrate is positively charged, is one of the issues that we specifically address in this study. While localized charges at the active site of an enzyme can vary due to very rapid proton relays, when using gas-phase calculations to model enzymatic reactions one should always remain cognizant of the fact that charged substrates often exhibit reduced activation barriers. The substrate, in the absence of a counterion, has an artificially high energy *relative* to its neutral counterpart and this is reflected in a reduced barrier. Thus, caution should be exercised in drawing mechanistic conclusions from such reduced activation barriers.

We next addressed the effect of the proximity of a counterion to the protonated N^1 site of **6**, on the barrier for the oxygen transfer to DMS. One of the most acidic moieties in any protein environment is the carboxylic group of aspartic acid ($pK_a = 3.86$). While we do not model a specific interaction, we used a formate anion and the protonated C4a-hydroperoxyflavin to illustrate this point. Geometry optimization of bicyclic model **2** hydrogen bonded to formic acid leads to intermediate **10a** (Figure 5). The PA of formic acid (339.4 kcal mol^{-1}, B3LYP/6-31+G(*d*,*p*) + ZPE) is much greater than that of **2**, so the proton moves back to the formate ion.[21] The activation barrier for the oxidation of DMS is 7.2 kcal mol^{-1} (TS-**11a**). When the N^1-H distance is constrained to its value in the isolated cation **6**, zwitterionic structure **10b** results, but this ammonium salt is not a critical point on the potential energy surface and it is 13.2 kcal mol^{-1} higher in energy than **10a**. The activation barrier for oxygen transfer from *constrained cation* **10b** is reduced to 5.1 kcal mol^{-1}. The strong hydrogen bond between the proton donor and acceptor does not change significantly along the reaction coordinate. Structure **10b** would be a true minimum in an environment providing enough stabilization energy through hydrogen bonding. However, this stabilization involves coordinates not represented in the reaction path for oxygen atom transfer and would equally apply to the reactant and the transition structure, with only a modest effect on the reaction barrier. This behavior has previously been observed in the decarboxylation of 2-aminoformylacetic acid in water[22] and in a model pyridoxal 5′-phosphate-bound glycine,[23] where a proton transfer from formic acid to the weakly basic pyridine nitrogen of PLP requires only 3.9 kcal mol^{-1} at the MP2/6-31G(*d*)//HF/6-31G(*d*) level of theory. Thus, this apparently lower barrier is a consequence of the geometry constraints imposed on both structures, **10b** and TS-**11b**. When the activation energy for TS-**11a** is measured relative to unconstrained minimum **10a**, the barrier is 18.3 kcal/mol.

3.3. N^5-Hydrogen-Bonded C4a-Hydroperoxyflavin (12 and 14) + Dimethyl Sulfide (TS-13 and TS-15). In both model hydroperoxides **2** and **4** the imine nitrogen at N^1 is considerably more basic than the trisubstituted conjugated nitrogen at N^5. The difference in basicity of

(20) Negative barriers can arise when the barrier height is calculated relative to isolated reactants. This reaction would exhibit a positive activation barrier if calculated relative to a stabilized pre-reaction complex.

(21) (a) For an excellent discussion of the energetics of salt-bridges in a nonaqueous environment, see: Zheng, Ya-J.; Ornstein, R. L. *J. Am. Chem. Soc.* **1996**, *118*, 11237. (b) For a discussion of the charge distribution and hydrogen bonding in ammonium carboxylates, see: Bach, R. D.; Dmitrenko, O.; Glukhovtsev, M. N. *J. Am. Chem. Soc.* **2001**, *123*, 7134.

(22) Bach, R. D.; Canepa, C. *J. Am. Chem. Soc.* **1997**, *119*, 11725−11733.

(23) Bach, R. D.; Canepa, C.; Glukhovtsev, M. N. *J. Am. Chem. Soc.* **1999**, *121*, 6542−6555.

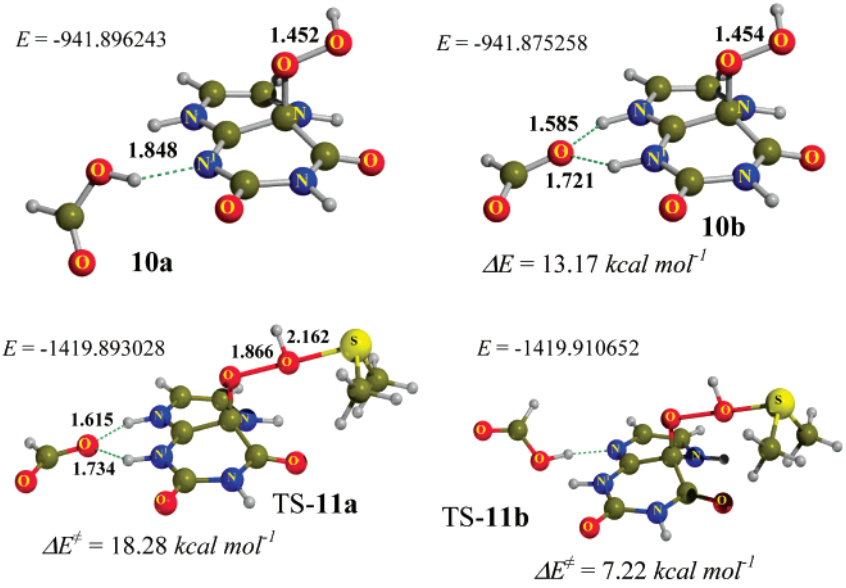

FIGURE 5. Relative energy ($\Delta E^{\ddagger}$) of neutral (**10a**), ion-paired (**10b**) bicyclic N^1-protonated C4a-hydroperoxyflavin, and the corresponding transition structures for oxygen atom transfer to DMS (TS-**11a,** TS-**11b**). Geometries are optimized at the B3LYP/6-31+G(*d*,*p*) level; distances are in Å. The N^1−H distance was constrained in minimum **10b** and TS-**11a** to 1.0089 Å.

these two nitrogen sites is slightly larger in hydroperoxide **4**. The ΔPA of 30.3 kcal mol^{-1} for tricyclic C4a-hydroperoxyflavin **4** [evaluated with the B3LYP/6-31+G(*d*,*p*) potential energy + the B3LYP/6-31G(*d*) ZPE] is ∼7 kcal mol^{-1} larger than the ΔPA of the bicyclic model. Protonation of bicyclic model **2** at position 5 affords the naked N^5-cation **12**, which is 23.6 kcal mol^{-1} higher in energy with respect to the N^1-protonated cation **6**. The activation barrier for oxygen transfer to DMS (TS-**13**, −8.0 kcal/mol)[18] is 3.3 kcal mol^{-1} lower in energy than the corresponding N^1-protonated transition state (TS-**7**). However, TS-**13** (Figure 6) has a relative energy of 20.3 kcal mol^{-1} with respect to N^1-protonated TS-**7** involving the more basic imine N^1 nitrogen. Hydrogen bonding of formic acid to the N^5 position of bicyclic C4a-hydroperoxyflavin **2** affords hydrogen-bonded hydroperoxide **14**. The barrier for oxygen atom transfer to DMS, with respect to oxygen donor **14** is −15.5 kcal mol^{-1} (TS-**15**, Figure 7), and 9.6 kcal mol^{-1} lower than the barrier in the comparable system with H-bonding to N^1 (**1** + **10b** → TS-**11b**, Figure 5) despite its much weaker basicity.

If free motion of the local residues were possible, as it is in solution, then obviously only the lower energy N^1-protonated TS would be involved ($\Delta\Delta E^{\ddagger}$ = 20.3 kcal mol^{-1}). However, once the substrate is bound at an active site both basic nitrogen sites could potentially interact with local residues. The proton affinity data, however, tend to suggest that hydrogen bonding interactions could be much more effective at the N^1 position that is also more remote from the C4-OOH moiety. However, in this model study both reaction barriers were modestly decreased as a consequence of hydrogen bonding interactions with model formic acid. Also, as noted above, the fully protonated transition structures (TS-**7** and TS-**13**) afford the lowest barrier at the N^5-protonated site. This suggests that a modest Coulombic stabilization is also at work and the developing alcoholate leaving group (FlO^-) may be stabilized by the adjacent partially protonated N^5 (see below).

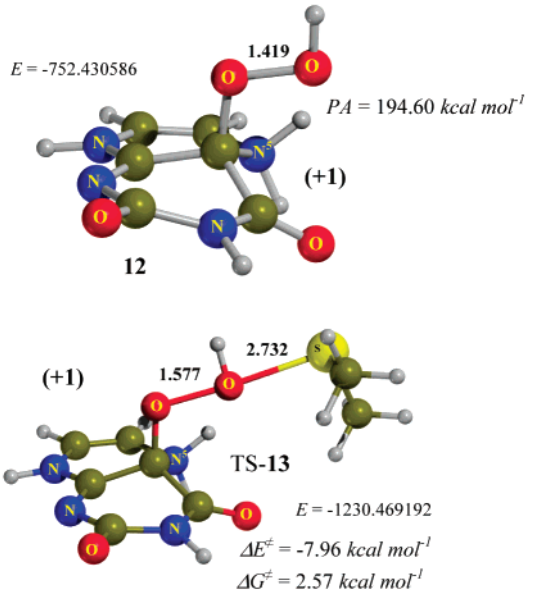

FIGURE 6. Relative energy ($\Delta E^{\ddagger}$) and free energy ($\Delta G^{\ddagger}$) of reactant model bicyclic N^5-protonated C4a-hydroperoxyflavin (**12**) and the transition structure for oxygen atom transfer to DMS (TS-**13**). Geometries are optimized at the B3LYP/6-31+G(*d*,*p*) level; distances are in Å. The proton affinity (PA) at N^5 is also reported.

The classical and free energy barriers discussed above, summarized in Tables 1 and 2, suggest a remarkably different behavior between neutral and charged cationic systems. Typically, a neutral system exhibits an electronic barrier for oxygen atom transfer from C4a-hydroperoxyflavin to DMS of ∼10 kcal mol^{-1}. Gas-phase protonation of the oxidant reduces the reaction barrier to approximately −5 to −8 kcal mol^{-1}. However, the high binding energies of cations **6** and **12** indicate that a naked cation should be considered as a species in the high-energy region of the potential energy surface. The binding energy of the formate anion to cation **6**, uncorrected for

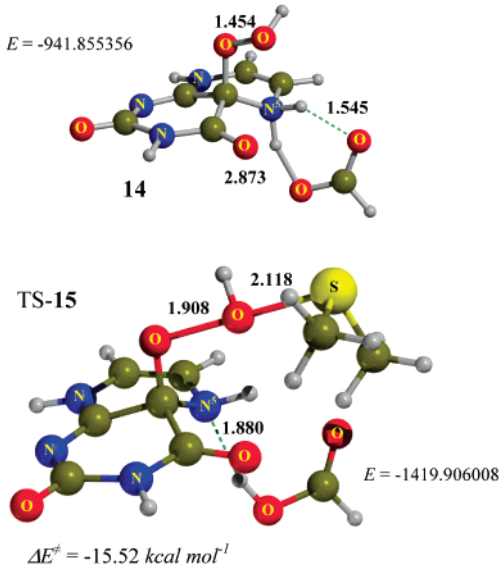

FIGURE 7. Relative energy ($\Delta E^{\ddagger}$) of ion-paired bicyclic N^5-protonated C4a-hydroperoxyflavin (**14**) and the corresponding transition structure for oxygen atom transfer to DMS (TS-**15**). Geometries are optimized at the B3LYP/6-31+G(*d*,*p*) level. The N^5−H distance is constrained to 1.0123 Å in structure **14**.

BSSE, is 120.3 kcal mol^{-1}. In fact, we can ideally form the protonated charged species **6** and **12** by a proton transfer from an acidic source, followed by separation of the resulting ion pair at infinite distance. Separating the ion pair requires energies of the order of 100 kcal mol^{-1}, vastly in excess of what would be available from any coupled biochemical process. We conclude that reaction barriers calculated from the isolated ions as reactants are artificially low compared to what is obtained from an extended model including the proton donor. The above data suggest that the high reactivity of flavin hydroperoxides can be attributed to a combination of inductive effects of the substituents at C4[5] and partly to potential hydrogen bonding interactions at *both* basic nitrogen sites. Thus, C4a-hydroperoxyflavin does not exhibit remarkable oxidant capabilities, its reaction barrier for oxygen transfer to dimethyl sulfide is considerably higher than the corresponding oxygen atom transfer from typical oxidants such as peracids. The ability of C4a-hydroperoxyflavin to act in the enzyme as an oxidant toward aromatic systems could be also due to nonlocal cooperative effects of the protein environment[24] including hydrogen-bonding interactions.

3.4. The Effect of Basis Set upon the Activation Barriers. We also briefly examined which DFT functional gives the most accurate barrier for this class of reactions. While the B3LYP functional has been widely used in the literature, the novel MPW1K[11] does not enjoy the benefit of extensive testing. Moreover, it has been calibrated against experimental barriers for hydrogen transfer reactions. We chose this particular reaction (oxygen atom transfer reaction from methyl hydroperoxide to ammonia, see Figure 8) since the reaction vector was comprised largely of lighter atom hydrogen transfer, as evidenced by the relatively large imaginary frequency

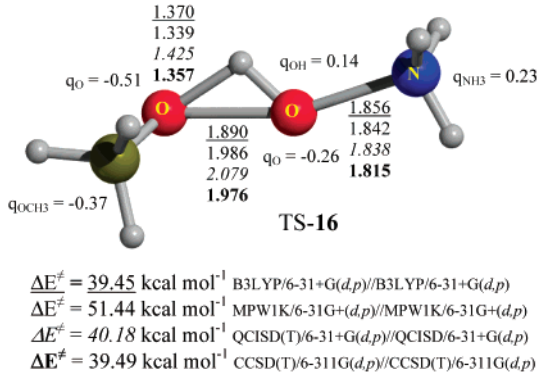

FIGURE 8. Transition structure (TS-**16**) for the reaction of oxygen atom transfer from methyl hydroperoxide to ammonia. The barriers are calculated relative to isolated reactants at the B3LYP/6-31+G(*d*,*p*)//B3LYP/6-31+G(*d*,*p*) (underlined numbers), MPW1K/6-31G+(*d*,*p*)//MPW1K/6-31G+(*d*,*p*) (plain numbers), QCISD(T)/6-31+G(*d*,*p*)//QCISD/6-31+G(*d*,*p*) (italic numbers), and CCSD(T)/6-311G(*d*,*p*)//CCSD(T)/6-311G(*d*,*p*) (bold numbers). O−O, O−S, and O−H distances are in Å. Mulliken charges (*q*), for this overall neutral system, are calculated at the B3LYP/6-31+G(*d*,*p*) level.

[ν_i = −1013.9 cm^{-1}, B3LYP/6-31+G(*d*,*p*)]. Activation barriers for oxygen atom transfer from ROOH are dramatically influenced by the position of the migrating hydrogen in the TS. Since a 1,2-hydrogen shift across the O−O bond has an exceptionally high barrier, those transition structures with little involvement of the peroxide hydrogen tend to have lower barriers. To this effect the oxygen atom transfer reaction from methyl hydroperoxide to ammonia (TS-**16**) has been studied at the B3LYP/6-31+G(*d*,*p*)//B3LYP/6-31+G(*d*,*p*), MPW1K/6-31+G(*d*,*p*)//MPW1K/6-31+G(*d*,*p*), QCISD(T)/6-31+G(*d*,*p*)//QCISD/6-31+G(*d*,*p*), and CCSD(T)/6-311G(*d*,*p*)//CCSD(T)/6-311G(*d*,*p*) levels. The classical barriers (reported in Figure 8) show that the MPW1K functional gives a somewhat higher barrier than the ab initio methods. When oxygen transfer dominated the reaction vector, the MPW1K functional gave activation barriers that were 30−50% higher than B3LYP barriers (Table 1). To place this in perspective, for the peracid epoxidation of a series of substituted alkenes the B3LYP functional gave classical activation barriers that were an average of 3 kcal mol^{-1} lower than QCISD(T)//QCISD barriers.[25] We, therefore, advocate the continued use of the B3LYP functional for calculations involving ROOH but with the caveat that it sometimes provides lower activation barriers.

The comparative studies on the MeOOH/DMS reaction (Figure 9) suggest that the MPW1K functional may overestimate reaction barriers for oxygen transfer by about the same amount as the B3LYP method underestimates them.

3.5. Coulombic versus Inductive Effects on the Reaction Barriers. We briefly revisit our earlier study on the potential role of Coulombic stabilization on the barriers to oxygen atom transfer.[7] Using a rather simplistic series of model hydroperoxides, we presented data that showed a remarkably large reduction in the barriers

(24) Canepa, C.; Bach, R. D. *Phys. Chem. Chem. Phys.* **2001**, *3*, 4072−4081.

(25) Bach, R. D., Dmitrenko, O., Adam, W.; Schambony, S. *J. Am. Chem. Soc.*, accepted.

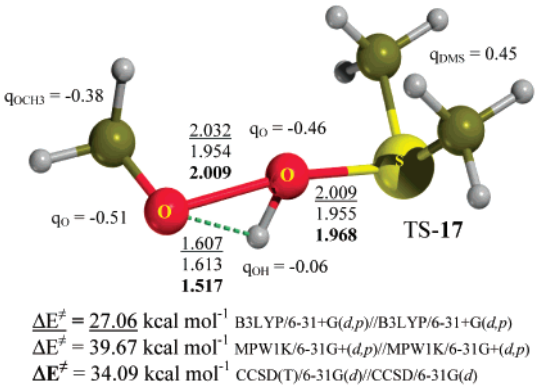

FIGURE 9. Transition structure (TS-**17**) for the reaction of oxygen atom transfer from methyl hydroperoxide to DMS. The reaction barriers ($\Delta E^{\ddagger}$, kcal mol^{-1}) are calculated at the B3LYP/6-31+G(*d*,*p*)//B3LYP/6-31+G(*d*,*p*) (underlined numbers), MPW1K/6-31G+(*d*,*p*)//MPW1K/6-31G+(*d*,*p*) (plain numbers), and CCSD(T)/6-31G(*d*)//CCSD/6-31G(*d*) (bold numbers) levels of theory and are relative to isolated reactants. O−O, O−S, and O−H distances are in Å. Mulliken charges (*q*) are calculated within the B3LYP/6-31+G(*d*,*p*) level.

TABLE 3. Coulombic versus Inductive Effects on the Activation Barriers (kcal mol^{-1}) to the Oxidation of Ammonia (MP4//MP2/6-31G(*d*))[a]

hydroperoxide	barrier
CH_3OOH	46.6
H_2NCH_2OOH	44.4
FCH_2OOH	35.0
$HOCF_2OOH$	28.9
$^+H_3NCH_2OOH$	10.3
$^+H_3NCH(CH{=}O)OOH$	2.9

[a] Data taken from ref 7.

for the oxidation of ammonia when the substrate had a positive charge adjacent to the departing alkoxide (Table 3). Comparison of the activation energies for the formation of ammonia oxide shows that inductive effects due to adjacent electronegative elements have only a modest affect upon the activation barrier. While a $HOCF_2$ substitutent does lower the barrier by 17.7 kcal mol^{-1}, the cationic hydroperoxide $^+H_3NCH_2OOH$ induces a dramatic decrease in the barrier to 10.3 kcal mol^{-1}. However, the additional inductive effect of an adjacent carbonyl group does result in a further reduction in activation energy to 2.9 kcal mol^{-1}. We concluded, at that time, that through-space Coulombic effects are more effective than through-bond inductive effects in activating alkyl hydroperoxides toward oxygen atom donation. We did not specifically address the question of the "cation effect" on activation barriers.

In the present study, we have compared several classical activation barriers for the oxidation of DMS (Figure 10). The barrier for oxygen atom transfer from CH_3OOH ($\Delta E^{\ddagger}$ = 27.1 kcal mol^{-1}, B3LYP/6-31+G(*d*,*p*)) is only reduced to 26.6 kcal mol^{-1} by the inductive effect of an α-amino substitutent (H_2NCH_2OOH, TS-**18**). However, as noted above, cationic hydroperoxide $^+H_3NCH_2$-OOH exhibits a negative barrier[18] ($\Delta E^{\ddagger} = -5.0$ kcal mol^{-1}, TS-**19**) with a substantial overall reduction in activation energy. A large fraction of this decrease in barrier is obviously due simply to the fact that the transition structure is cationic. However, Coulombic effects can also

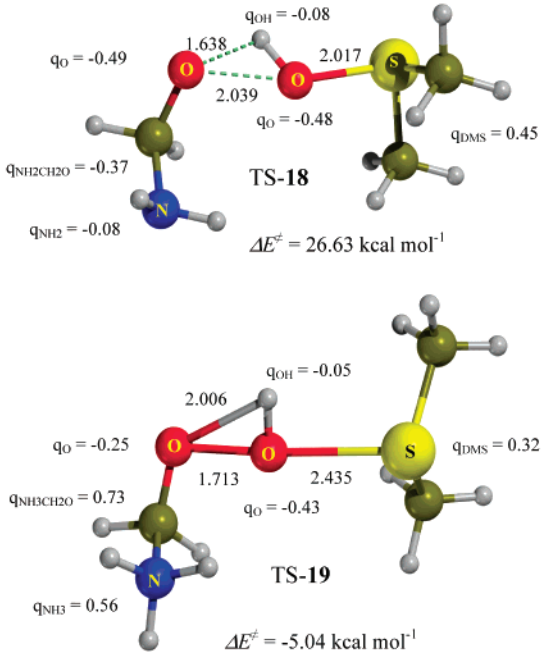

FIGURE 10. Transition structures (TS-**18** and TS-**19**) for the reaction of oxygen atom transfer from H_2NCH_2OOH and cationic $^+H_3NCH_2OOH$ to DMS. The reaction barriers ($\Delta E^{\ddagger}$, kcal mol^{-1}) are calculated at the B3LYP/6-31+G(*d*,*p*)//B3LYP/6-31+G(*d*,*p*) level of theory and are relative to isolated reactants. O−O, O−S, and O−H distances are in Å. Mulliken charges (*q*) are calculated within the B3LYP/6-31+G(*d*,*p*) level.

play a role since a large portion of the positive charge is localized on the H_3N^+ fragment in TS-**19** (q_{NH3} = 0.56) and the charge on the alcoholate oxygen is reduced to −0.25 *e* (Figure 10). Interestingly, in cationic TS-**19**, the negative charge on the distal oxygen is reduced by 50% in comparison with TS-**18**, thus, it is not as good a hydrogen acceptor as it was in the neutral case. This may explain the markedly reduced value of the imaginary frequency [−183.2 cm^{-1} in TS-**19** vs −691.1 cm^{-1} in TS-**18**, B3LYP/6-31G(*d*)], which, in the case of the cationic system, represents pure oxygen atom transfer without contamination by hydrogen migration. As noted above with the much larger systems, the introduction of an adjacent positive charge at N^5 does result in a modest reduction in activation barrier relative to the more remote N^1 position.

4. Conclusion

C4a-hydroperoxyflavin **4** is intermediate in reactivity as an oxygen atom donor between a peracid and an alkyl hydroperoxide. The rate ratio for the oxidation of dimethyl sulfide by C4a-hydroperoxyflavin **4** versus *tert*-butyl hydroperoxide is ca. 10^9. However, **4** is about 10^7 less reactive toward DMS than peroxyformic acid. These theoretical rate ratios are in good accord with those based upon the earlier experimental work of Bruice.[5a−d] Bicyclic hydroperoxide **2** is a slightly more effective oxidizing agent than tricyclic hydroperoxide **4**, suggesting that the function of the dimethyl benzene moiety in **4** is to play a role in increasing the binding capacity at a hydrophobic active site. The proton affinity of the C4a-hydroxyflavin (Fl-OH) derived from oxygen transfer from **4** is about 40

kcal mol^{-1} lower than that of *t*-BuOH, making it a much better leaving group (FlO^-) and hence a more effective oxidizing agent. Relative to neutral C4a-hydroperoxyflavin **2**, N^1-protonated hydroperoxide **6** exhibits a much reduced activation barrier for the oxidation of DMS ($\Delta\Delta E^{\ddagger}$ = 15.1 kcal mol^{-1}) pointing out problems associated with the use of naked cations (or anions) as model substrates for enzymatic reactions. Hydrogen bonding of the model carboxylic acid, formic acid, to N^1 or N^5 of C4a-hydroperoxyflavin results in a reduction in the activation barrier for the oxidation of DMS of 3.2 and 5.1 kcal mol^{-1}.

The proton distribution of the cofactor in oxygen transfer reactions catalyzed by flavoenzymes does not significantly affect the reaction barrier. Counterions should be included in the quantum chemistry model to avoid underestimation of the reaction barriers due to gas-phase isolated ions.

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Supporting Information Available: Geometries and energies for all structures. This material is available free of charge via the Internet at http://pubs.acs.org.

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