1 shows the geometries and heats of formation (ΔH_f) calculated for **1** and **2**. Both are planar, except for the methyl hydrogen atoms in **2**. The indicated (**2**) conformation, with C_{2h} symmetry, was found to be the global energy minimum. While no experimental ΔH_f values are available for comparison, a reasonably reliable estimate can be made for **1** by using the bond-energy scheme of Dewar and de Llano.[13] This (60.5 kcal/mol) is quite close to the MNDO value. **4** was found to be nonplanar (Figure 2), the central ring being bent into a boat geometry as a result of steric interactions. Planar **4** was less stable by 11 kcal/mol. Table 2 lists orbital types and energies calculated for the three molecules, together with the observed[1,2] ionization energies. Values for **4** are given both for a planar geometry (D_{2h}) and for the optimum one (C_{2v}), symmetry designations being for the former. Calculations for **1**$^+$ and **5** were carried out by the spin-unrestricted version (UMNDO[16]) of MNDO, which has been found[17] to give good estimates (Koopmans' theorem) of ionization energies of radicals. The ΔH_f calculated for **1**$^+$ (227.7 kcal/mol) corresponds to an adiabatic ionization energy for **1** of 7.44 eV, the observed (vertical) value[1,2] being 7.87 eV. Cartesian coordinates are available as Supplementary Material.

According to MNDO, the first two bands in the UV-PES of **1** correspond to π ionizations (B_{1u} and B_{2g}). The third π ionization (B_{3g}) corresponds to the low-energy end of the third band, with a tail at higher energies due to the first σ ionization. The next two bands are each assigned to three superimposed ionizations, an assignment consistent with their position and appearance. The position of the sixth band is also correctly predicted. The published spectrum indicates that, as usual, the sensitivity of the spectrometer fell off at higher ionization energies. The appearance of the sixth band shows that it must also involve, as predicted, several superimposed ionizations.

The agreement between the MNDO calculations for **2** and its observed UV-PES would be equally satisfactory[14] were it not for the band (B_x) at 9.8 eV. For reasons indicated above, this cannot correspond to a simple ionization because its intensity is too small. The only way it could arise from **2** would be if it were due to an I + E process and were thus analogous to a double excitation. The excitation probability for such a process might well be low. This, however, seems to be ruled out by the small difference in energy (2 eV) between B_1 and B_x since, as noted above, this is unlikely to be much less than the minimum excitation energy of **2**. Support for this argument is provided by the HOMO/LUMO separations calculated here for **1** (7.6 eV) and **1**$^+$ (7.1 eV). On this basis the first I + E ionization of **1** should appear at ca. 12.2 eV.

We are therefore forced back to the inherently reasonable suggestion that B_x corresponds to some species other than **2**. While there is no direct evidence concerning the compound responsible, one obvious candidate seems to be the 2,4,5-trimethylbenzyl radical (**5**), which might very well have been formed during the pyrolytic synthesis of **2**. As Table I shows, **5** is indeed predicted to have three ionizations in the range 9.6–10.2 eV. A small amount of **5** could then lead to a broad weak band at 9.8 eV, due to coalescence of bands due to these ionizations.

As Table I shows, the MNDO calculations also reproduce the observed[4] UV-PES of **4** in a satisfactory manner. The first two peaks in this correspond to single ionizations and the third to two overlapping ionizations, as predicted.

There is therefore no reason to suppose that the UV-PES of **1**, **2**, or **4** are in any way abnormal or that any unusual effect operates in the corresponding ions. It would indeed be very surprising if **1**, an apparently normal polyene, showed unique behavior, of a kind that has never been encountered in any of the very many hydrocarbons that have been studied by UV-PES. Very strong evidence would be needed to refute the interpretations given here, which indeed seem to illustrate once again the usefulness of MNDO as an aid in assigning ultraviolet photoelectron spectra.

Acknowledgment. This work was supported by the National Science Foundation (Grant CHE78-03213).

Registry No. **1**, 502-86-3; **2**, 63238-49-3; **4**, 3302-51-0; 2,4,5-trimethylbenzyl radical, 15220-27-6.

Supplementary Material Available: Listing of Cartesian coordinates for **1**, **2**, and **4** (1 page). Ordering information is given on any current masthead page.

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(13) Dewar, M. J. S.; de Llano, C. *J. Am. Chem. Soc.* **1969**, *91*, 789.

(14) Changes in ionization energy due to methyl substitution are systematically underestimated by MNDO[15]. The errors for **2** are just what would be expected from analogy with other compounds[15] and the results for **1**.

(15) Compare the following[10] calculated (observed) differences (eV) in I_1 between methyl derivatives and parent compounds: CH_3NH_2/NH_3, 0.64 (1.04); $PhCH_3/PhH$, 0.11 (0.43); C_3H_6/C_2H_4, 0.21 (0.63); CH_3OH/H_2O, 0.78 (1.66); CH_3CN/HCN, 0.62 (1.39).

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One- and Two-Electron Reduction of Oxygen by 1,5-Dihydroflavins

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Considerable attention has been paid to the mechanism by which molecular oxygen reacts with 1,5-dihydroisoalloxazine molecules.[1] This concern stems from the importance of the reaction in the mechanism of action of the flavoxygenase enzymes. The flavomono- and flavodioxygenase reactions are the only non-metal-ion-requiring oxygen activation reactions in biochemistry. Notable advances in the understanding of the mechanisms of oxygen transfer from intermediate enzyme-bound[2] and model[3] flavin 4a-hydroperoxides to substrate have been made, yet the mechanism of reaction of 1,5-dihydroflavin with $^3O_2$ has not been clarified. Hamilton[1c] considered the problem of spin forbiddeness in the formation of a flavin hydroperoxide by direct reaction of triplet oxygen with singlet dihydroflavin. Spin inversion is not required if the initial step involves a one-electron transfer.[4] The central 1,4-dihydropyrazine ring of the 1,5-dihydroisoalloxazine

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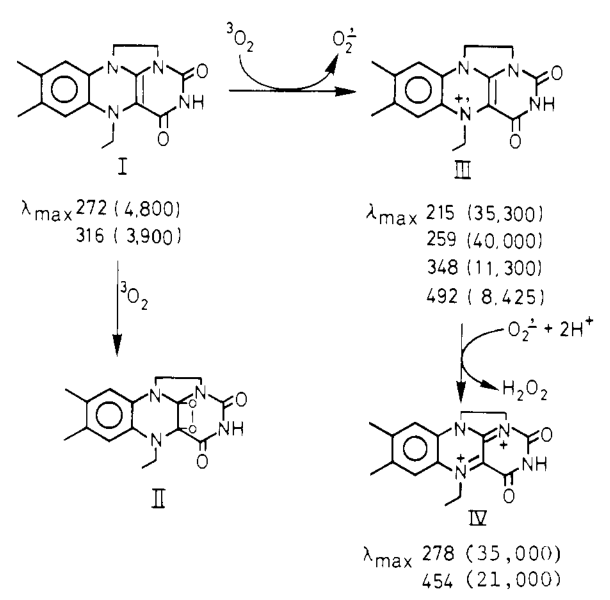
Scheme I



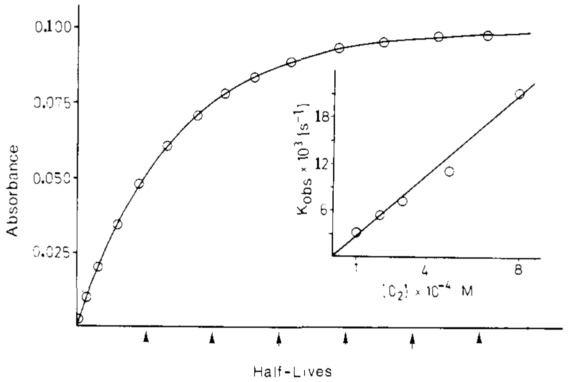


Figure 1. First-order appearance of 1,10-ethano-5-ethylflavinium radical (III) on oxidation of 1,10-ethano-5-ethyl-1,5-dihydrolumiflavin (I) (at 1.1×10^{-5} M, 30 °C, pH 1.8 with $[O_2] = 3 \times 10^{-4}$ M). Points are experimental and line theoretical for a first-order rate constant (k_{obsd}) of 5.66×10^{-3} s^{-1}. The inset is a plot of k_{obsd} vs. $[O_2]$ with a slope of 25 M^{-1} s^{-1}.

Scheme II

$$\text{I} + O_2 \underset{\substack{10^{11}\ s^{-1}\\ k_{-1}}}{\overset{\substack{k_1\\ 17\ M^{-1}\ s^{-1}}}{\rightleftharpoons}} \text{III} + O_2^{\cdot -} \underset{\substack{5 \times 10^2\ M^{-1}\ s^{-1}\\ k_{-2}}}{\overset{\substack{k_2\\ \geqslant 10^{11}\ s^{-1}}}{\rightleftharpoons}} \text{IV} + H_2O_2$$

$$\text{I} + \text{IV} \underset{\substack{\sim 2 \times 10^5\ M^{-1}\ s^{-1}\\ k_{-3}}}{\overset{\substack{k_3\\ \sim 10^8\ M^{-1}\ s^{-1}}}{\rightleftharpoons}} 2\text{III}$$

molecule should confer to the latter a propensity to act as a one-electron donor.[5] Thus, when in the more stable bent conformation, Hoffmann[6] orbital splitting of the lone-pair electrons on the N(10) and N(5) positions may be expected to place one pair of electrons in a higher energy orbital, the splitting being relieved on radical formation. When in the planar conformation, the 1,4-dihydropyrazine ring may be recognized as being antiaromatic. This destablizing feature is also relieved on one-electron abstraction. Arguments for one-electron transfer from dihydroflavin to triplet oxygen have been presented,[1f] and models for this process that involve one-electron reduction of nitroxides by dihydroflavins have been investigated.[5] By molecular orbital calculations,[7,8] the energy of the HOMO of 1,5-reduced flavin is nonbonding to slightly antibonding and $^3O_2$ attack at the 4a and 10a positions is favorable. Orf and Dolphin[8] proposed the first intermediate of $^3O_2$ attack upon 1,5-dihydroflavin to be a 4a,10a-dioxetane. Hemmerich and collaborators[9] also favored a reaction between $^3O_2$ and dihydroflavin that involves a two-electron transfer process.

The oxidation of 1,5-dihydroflavins by $^3O_2$ in water are autocatalytic (eq 1)[1d,f] due to the facts that oxidized flavin reacts with

$$Fl_{red} + O_2 \xrightarrow{2H^+} Fl_{oxid} + H_2O_2 \tag{1a}$$

$$Fl_{red} + Fl_{oxidized} \rightarrow 2Fl_{radical} \tag{1b}$$

$$Fl_{radical} + O_2 \rightarrow Fl_{oxid} + O_2^{-\cdot} \tag{1c}$$

$$Fl_{radical} + O_2^{-\cdot} \xrightarrow{2H^+} Fl_{oxid} + H_2O_2 \tag{1d}$$

reduced flavin much more rapidly than does O_2 and that flavin radical is rapidly oxidized by oxygen to yield oxidized flavin. Thus, after but a few percent oxidation of 1,5-dihydroflavin by O_2 (eq 1a), the remainder of the reduced flavin is consumed by reactions 1b-d. For this reason, direct investigation of the kinetics of reaction 1a are not possible.

In order to investigate the mechanism of the direct reaction of O_2 with 1,5-dihydroflavin, it is required that the rate-limiting step becomes that of eq 1a and that flavin radical is not reactive with oxygen. We have chosen to study the reaction of O_2 with 1,5-dihydro-1,10-ethano-5-ethyllumiflavin (I).[10] The reaction of I with $^3O_2$ can, in principal, yield the 4a,10a-dioxethane (II) or the radical (III) (Scheme I). However, the formation of IV from I by $^3O_2$ oxidation is not thermodynamically favored due to the two positive charges at the termini of the 1,5-quinoid structure of IV. Also, the radical III is rather stable in the presence of $^3O_2$. These features have allowed the direct investigation of the mechanism of eq 1a with I.

All kinetic and electrochemical measurements have been made at pH 4.6 and 30 °C, in aqueous solution. Under the pseudo-first-order conditions $[^3O_2] >> [I] = 8.7 \times 10^{-5}$ to 10^{-6} M, the formation of III (492 nm) from I follows the first-order rate law to completion of reaction between pH 1 and 4.6 (Figure 1).[11] The reaction is not acid catalyzed. (The reaction of I with $^3O_2$ to

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(10) For I: mp 240 °C (un); IR (KBr disk) ν(N^3-H) 3020, ν(C^4=O) 1740, ν(C^2=O) 1680, ν(Ar) 1510 cm^{-1}; ^{1}H NMR (CF$_3$COOH, Me$_4$Si) δ 1.00 (3 H, t, N^{5}CH$_2$CH$_3$), 2.13 (6 H, s, two aryl-CH$_3$), 3.60 (2 H, q, N^5-CH$_2$CH$_3$), 4.20 (4 H, N^{10}-CH$_2$-CH$_2$-^{1}N), 4.77 (1 H, s, N^{3}H), 6.72 (1 H, s, aryl-H), 7.19 (1 H, s, aryl-H). Anal. Calcd for $C_{16}H_{18}N_4O_2 \cdot {}^1/_3H_2O$: C, 63.14; H, 6.18; N, 18.41. Found: C, 63.30; H, 6.20; N, 18.19.

(11) Kinetic studies were conducted at 30 °C in solution of μ = 0.1 (KCl) employing as buffers sulfate-bisulfate (pH 2-3), acetate-acetic acid (pH 3.5-5.5), and carbonate-bicarbonate (pH 9-11). In the reaction of I with oxygen, the desired volumes of O_2-saturated and N_2-saturated buffers (pH 4.6) were placed into a cuvette by injection through a rubber septum cap. After thermal equilibration in the cell compartment of the spectrophotometer, there was then added 0.1 mL of an anaerobic methanolic solution 2.7 mM in I. The solution was mixed, and the appearance of III was followed with time at 492 nm. The reaction of Va was carried out in a similar manner, and the formation of the oxidation product was followed at 484 nm (appearance of the radical). The reaction of $^3O_2$ with dihydroflavins Vb-Vf were studied with a Durrum stopped-flow spectrophotometer (D-110) with deadtime ~2 ms and mixing time ~4.6 ms. The spectrophotometer was sealed in a glovebox under nitrogen ($[O_2]$ ~5×10^{-8} M). To elucidate the initial rate constant of the reaction between dihydroflavins and oxygen, equal volumes of oxygen-saturated acetate buffer (μ = 1.0, C = 2.0 M L^{-1}, $[O_2] = 8 \times 10^{-4}$ M L^{-1}) and dihydroflavin/buffer solution (μ = 1.0; C = 0.2 M L^{-1}, [I] = 8 $\times 10^{-5}$ M L^{-1} were mixed together on the stopped-flow bench. The reactions were followed at 426 (Vb), 585 (Vc), 444 (Vd), 430 (Ve), 423 (Vf), and 365 nm (Vg). The tangential slopes for the first 3-5% of reaction were employed to calculate the initial rate constants.

provide III becomes difficult to follow at pH values greater than 6 due to a further reaction of III not involving $^3O_2$.)[12] From the slopes of plots of $[^3O_2]$ vs. the pseudo-first-order rate constants for conversion of I $\rightarrow$ III (inset to Figure 1) there is obtained the apparent second-order rate constants for reaction of I with $^3O_2$. Thus, the formation of III occurs in a reaction first order in I and first order in $^3O_2$. The spectrophotometrically determined yields of III based on the initial concentrations of I, are 100%. At completion of the oxidation of I, the reaction solutions were analyzed for H_2O_2.[13] In five separate experiments, the percentage yield of H_2O_2, based on the initial concentration of I, was found to be 45%, 52%, 47%, 47%, and 47%.

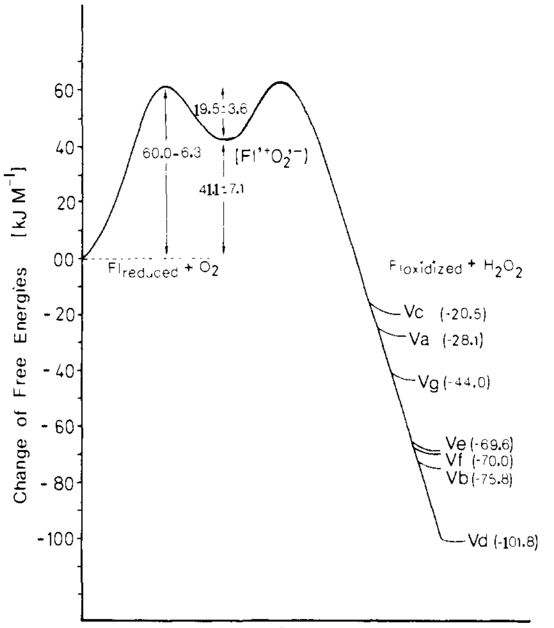


Figure 2. Averaged cartoon of the reaction coordinant for the reactions of a series of 1,5-dihydroisoalloxazines with O_2 at 30 °C, pH 4.6.

The mechanism for formation of III as the product of the reaction of $^3O_2$ with I is shown, along with *calculated rate constants* at pH 4.6, in Scheme II. Individual proton-transfer steps are not shown (vide infra). The individual rate constants were obtained in the following manner. The free energy (pH 4.6) for $Fl_{red} + O_2 \rightarrow Fl_{radical} + O_2^{-\cdot}$ (ΔG^1) and $Fl_{radical} + O_2^{-\cdot} \rightarrow Fl_{oxid} + H_2O_2$ (ΔG^2) were determined from the electrochemical half-reactions of eq 2. The standard potentials for eq 2c and 2d were

$$Fl_{oxid} + 1e^- \xrightarrow{E^1} Fl_{radical} \qquad (2a)$$

$$Fl_{radical} + 1e^- \xrightarrow{E^2} Fl_{red} \qquad (2b)$$

$$^3O_2 + 1e^- \xrightarrow{-310\ \mathrm{mV}} \text{superoxide} \qquad (2c)$$

$$\text{superoxide} + 1e^- \xrightarrow{1203\ \mathrm{mV}} \text{hydrogen peroxide} \qquad (2d)$$

obtained from literature sources[14] and corrected to pH 4.6 by use of the Nernst equation. The potentials for eq 2a and 2b were determined by thin-layer cyclic voltametry[15] at pH 4.6, and each potential was established to be associated with the transfer of one electron by Coulometry. The redox potentials E^1 and E^2 (eq 2a and 2b) for the 1,10-ethano-5-ethyllumiflavin system are provided in eq 3. The values of ΔG^1 and ΔG^2 were determined to be 57.3

$$\text{IV} \xrightarrow[687\ \mathrm{mV}]{1e^-} \text{III} \xrightarrow[284\ \mathrm{mV}]{1e^-} \text{I at pH 4.6} \qquad (3)$$

and −49.8 kJ m^{-1}, respectively. The free energy (pH 4.6) for eq 4 was calculated from ΔG^1 and ΔG^2. It follows from eq 4 that

$$\text{I} + {}^3O_2 \underset{\text{pH 4.6}}{\overset{\Delta G = 7.5\ \mathrm{kJ\ M^{-1}}}{\rightleftarrows}} \text{IV} + H_2O_2 \qquad (4)$$

the oxidation I $\rightarrow$ IV is endothermic. For this reason IV must be at steady state and k_1 (Scheme II) must be rate determining (eq 5) so that k_1 is the experimentally determined second-order

$$\frac{d[\mathrm{III}]}{dt} = \frac{k_1k_3[\mathrm{I}]^2[O_2]}{k_{-2}[H_2O_2] + k_3[\mathrm{I}]} = k_1[\mathrm{I}][O_2] \qquad (5)$$

rate constant for the reaction of $^3O_2$ with I, and therefore, $\Delta G^\ddagger$ for the overall reaction pertains to k_1. The value of k_{-1} was then calculated from ΔG^1 and $\Delta G^\ddagger$. Since k_2 cannot be rate determining, $k_2 \geq k_{-1}$. The rate constant k_{-2} was determined from eq 6 (30 °C) where ΔG is from eq 4. The value of k_3 exceeds 10^7

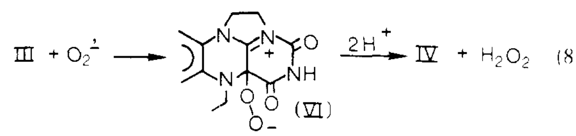
$$\frac{\Delta G^\ddagger - \Delta G}{1.386} = \log \frac{k_{-2}}{k_1} \qquad (6)$$

and is reasonably assumed to be $\sim 10^8$ M^{-1} s^{-1}. The equilibrium constant of eq 7 was determined from the potentials of eq 3. From

$$2\text{III} \underset{\text{pH 4.6}}{\overset{K_e = 1.6 \times 10^{-3}}{\rightleftarrows}} \text{IV} + \text{I} \qquad (7)$$

k_3 and K_e was calculated the rate constant k_{-3}.

Examination of Scheme II shows that III + $O_2^{-\cdot}$ would reside in a shallow free-energy well and that the rate constants for partitioning of $\{\mathrm{III}, O_2^{-\cdot}\}$ to starting state and products approach the limit for diffusion apart from the III and $O_2^{-\cdot}$ moieties.[21] One is led, therefore, to the conclusion that the transition-state structure for O_2 oxidation of I to IV plus H_2O_2 closely resembles or is identical with the structure of the radical pair $\{\mathrm{III}, O_2^{-\cdot}\}$.[17] That is to say, the overall oxidation involves the transfer of two electrons, but the transition state is reached upon transfer of the first electron. Because neither specific nor general acid catalysis is involved in the reaction of $^3O_2$ with I (yet H_2O_2 must be a product), a covalent intermediate must be formed on collapse of the transition state. The intermediate may be either II or the 4a-hydroperoxylflavin zwitterion VI, which hydrolyzes to H_2O_2 and IV in a non-rate-determining step. Previous studies in nonaqueous solution have shown that $O_2^{-\cdot}$ combines with flavin radical to yield 4a-hydroperoxylflavin,[18] which, in the presence of protic solvent, dissociates to oxidized flavin and hydrogen peroxide.[1f,3b]



(8)

The formation of III as the final product (Scheme II) involves the trapping of the steady-state intermediate IV by I. Since the pseudobase pK_R for IV is quite low (eq 9) one might, a priori,

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(12) Kemal, C.; Bruice, T. C. *J. Am. Chem. Soc.* **1976**, *98*, 3955.

(13) Hydrogen peroxide product was determined by withdrawing 0.4 mL of the reaction solution and adding this to 3 mL of aqueous and oxygen-free 0.1 M sodium iodide solution. The appearance of I_3^- was monitored with time at 358 nm. The time course for I_3^- appearance was found to be biphasic. The rapid first-order production of I_3^- due to the presence of H_2O_2 is followed by a much slower production of I_3^- due to the O_2 present in the reaction solution as a reagent. The time course for I_3^- formation due to H_2O_2 was isolated by use of a computer program designed by Dr. Patrick Shannon for this purpose. Thus, the $[I_3^-]$ formed from reaction with H_2O_2 could be calculated, as could the second-order rate constant for the reaction of I^- with H_2O_2. The rate constants so determined were found to be identical with the second-order rate constant for reaction of I^- with authentic H_2O_2 at the pH employed ($k_2' =$ 156 M^{-1} s^{-1}).

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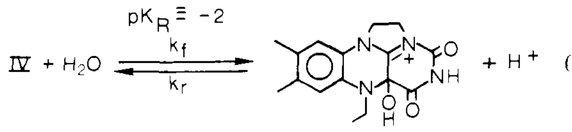

(9)

anticipate that IV might also be trapped by H_2O solvent. If this should have occurred, then pseudobase rather than III would have resulted as product. From the pH of the experiment (4.6) and pK_R, it can be calculated[19] that $k_f = 10^7 k_r$. Since pseudobase was not observed, it must be assumed that $k_3[I] >> k_{H_2O}[H_2O] < 10^2$ s^{-1}.

It is reasonable to suppose that changes in the substituents on the 1,5-dihydroisoalloxazine ring system might decrease ΔG^1 relative to $\Delta G^{\ddagger}$ and thereby result in the formation of $Fl_{radical}$ and $O_2^{-\cdot}$ as discrete intermediates. Apparent $\Delta G^{\ddagger}$ values for the bimolecular reaction of $^3O_2$ with seven assorted 1,5-dihydroflavins (Va–Vg) at pH 4.6 were obtained from initial rate constants (eq 1a).[11] The potentials E_1 and E_2 (eq 2a and 2b) were determined for each flavin and corrected to pH 4.6 (as in the case of the 1,10-ethano-5-ethyllumiflavin system loc. cit.).[20] From the calculated values of $\Delta G^{\ddagger}$, ΔG^1, and ΔG^2,[14] there may be constructed the averaged cartoon of Figure 2. Inspection of Figure 2 reveals that $Fl_{radical} + O_2^{-\cdot}$ exists in a potential well. Return to the starting state is associated with a potential barrier of 19.7 ± 3 kJ m^{-1}. The barrier for formation of products from $Fl_{radical}$ + $O_2^{-\cdot}$ cannot be greater than that for return to the starting state and in the cartoon has been set equal to this value. The free-energy difference of 19.7 kJ M^{-1} corresponds to a diffusion-controlled rate constant of 2.6×10^9 s^{-1}.[21] Thus, the moieties $Fl_{radical}$ and $O_2^{-\cdot}$ may diffuse from the $\{Fl_{radical}, O_2^{-\cdot}\}$ radical pair, and the former are true intermediates. One may conclude that the $^3O_2$ oxidation of the 1,5-dihydroflavins Va–Vg[15] occurs by two consecutive one-electron transfer steps. The driving force for the reaction(s), overall, is provided by ΔG^2 (product stability). Changes in the potentials of eq 2a, 2b, and 2c and the rate constant for the reaction of eq 1a with pH will change the reaction-coordinant cartoon. Other data[1f] indicate, however, that the values of ΔG^1 remain less than $\Delta G^{\ddagger}$ so that the one-electron transfer reaction from 1,5-reduced flavin to $^3O_2$ is allowed at other pH values.

We have shown in this investigation that: (i) dependent upon 1,5-dihydroflavin structure, the reaction of these compounds with $^3O_2$ involves a transition state closely resembling a flavin radical plus $O_2^{-\cdot}$ or a flavin radical and $O_2^{-\cdot}$ as discrete intermediates; (ii) a covalent intermediate is required in the $^3O_2$ oxidation of 1,5-dihydroflavin to flavin plus hydrogen peroxide in water.

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Registry No. I, 80720-87-2; **III**, 80720-88-3; **Va**, 76030-63-2; **Vb**, 64910-62-9; **Vc**, 50387-36-5; **Vd**, 50635-52-4; **Ve**, 64910-61-8; **Vf**, 80720-89-4; **Vg**, 79075-88-0; O_2, 7782-44-7.

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(20) (a) 5-Ethyl-10-(2′,6′-dimethylphenyl)-3-methyl-1,5-dihydroisoalloxazine (Va) ($\Delta G^{\ddagger}$ (kJ/M) = 70.6, ΔG^1 = 52.4, ΔG = −28.5; E^1 (mV) [pH 4.6, NHE] = 365 mV, E^2 = 233 mV). 10-(2′,6′-Dimethylphenyl)-3-methyl-1,5-dihydroisoalloxaxine (Vb) ($\Delta G^{\ddagger}$ = 54.0, ΔG^1 = 37.2, ΔG = −76.5; E^1 = 25, E^2 = 75). 5-Ethyl-3-methyl-1,5-dihydrolumiflavin (Vc) ($\Delta G^{\ddagger}$ = 64.4; ΔG^1 = 49.1, ΔG = −21.2; E^1 = 474, E^2 = 199).[14b] 3-Carboxymethyllumiflavin (Vd) ($\Delta G^{\ddagger}$ = 59.1, ΔG^1 = 25.6, ΔG = −102.6; E^1 = −125, E^2 = −45).[14c] 7-Cyano-3,10-dimethyl-1,5-dihydroisoalloxazine (Ve) ($\Delta G^{\ddagger}$ = 54.9, ΔG^1 = 39.1, ΔG = −71.7; E^1 = 55, E^2 = 95). 7-Chloro-3,10-dimethyl-1,5-dihydroisoalloxazine (Vf) ($\Delta G^{\ddagger}$ = 55.7, ΔG^1 = 40.5, ΔG = −68.9; E^1 = 70, E^2 = 110). 5-Ethyl-3-methyl-1,5-dihydro-1-deaza-1-carbaflavin (Vg) ($\Delta G^{\ddagger}$ = 65.4, ΔG^1 = 42.9, ΔG = −44.7; E^1 = 295, E^2 = 135). (b) Calculated from values obtained by: Draper, R. D.; Ingram, L. L. *Arch. Biochem. Biophys.* **1968**, *125*, 802. (c) E_m obtained by Schug are about 70 mV lower: Schug, C. "Diplomarbeit"; Konstanz, Germany, 1978.

(21) For the individual 1,5-dihydroisoalloxazines investigated (compounds Va–Vg[10]), the calculated rate constants for partitioning of the $Fl_{radical} + O_2^{-\cdot}$ to initial state vary from 4×10^7 to 2.6×10^{10} s^{-1}. Dissociation rate constants have been shown to range from $\sim 10^{12}$ to $\sim 10^5$ s^{-1} (see ref 16 and literature citations therein).

Use of Large Magnetic Fields To Probe Photoinduced Electron-Transfer Reactions: An Example from Photosynthetic Reaction Centers

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We will outline an experimental approach that uses large magnetic fields and simple optical methods, combined with a method of data analysis, that is ideally suited for studying the mechanism of rapid photoinduced electron-transfer (ET) reactions over a very wide range of conditions. Results are presented from experiments on the primary photochemistry in photosynthetic reaction centers (RCs), along with predictions for a variety of chemically interesting ET reactions.

Many investigators are attempting to prepare photosynthetic analogues, including covalently connected donor–acceptor pairs,[1] organized assemblies (micelles, bilayers, surfaces),[2] and solid-state materials such as amorphous silicon.[3] Time-resolved optical data are widely used to study charge separation in these systems; however, the absorption spectrum of the primary photoproduct, a radical-ion pair (RIP), is frequently difficult to distinguish from that of excited singlet and triplet intermediates. In order to resolve this ambiguity and prove the intermediacy of RIPs, it is important to demonstrate that the intermediates possess spin angular momentum. For long-lived radicals, ESR is the method of choice, but this fails when the lifetime of the RIP is less than about 200 ns. Indirect magnetic resonance methods, such as reaction yield detected magnetic resonance (RYDMR) can also be applied when the conditions are favorable.[3,4]

A fairly general description of the kinetics in a singlet ET reaction is shown in Scheme I. Donor D is excited to its singlet state and forms a RIP with acceptor A. This pair recombines to the ground state with the magnetic-field-independent rate constant k_S. If k_S is sufficiently slow, the phase of the unpaired spins in the correlated RIP may evolve to the triplet RIP. Spin-allowed recombination in the latter can produce ^3D (or ^3A), providing the reaction is sufficiently exothermic. Such long-lived triplet products are a useful probe of the short-lived RIP.

The effects of *small* magnetic fields on the yields of reactions of this type have been widely discussed.[5,6] As illustrated in Figure 1, if the exchange interaction (J) in the RIP is small relative to the interactions that mix radical-pair singlet and triplet states, then application of a small field (typically less than 500 G) will *decrease* the yield of ^3D (ϕ_T), because the field removes the T_+ and T_- levels from the vicinity of S. However, certain systems suspected to undergo ET show negligible triplet formation at low

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