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Reactions of Lumiflavin and Lumiflavin Radicals with $\cdot CO_2^-$ and Alcohol Radicals[†]

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ABSTRACT: The kinetics of reaction of oxidized lumiflavin (F_o) with the radicals $\cdot CO_2^-$, $CH_3\dot{C}HOH$, and $(CH_3)_2\dot{C}OH$ have been investigated at pH 7 and 24 ± 1 °C by the pulse radiolysis technique. The radicals have been shown to react with lumiflavin with second-order rate constants of 36 ± 4, 26 ± 3, and 20 ± 3 in units of 10^8 M^{-1} s^{-1}, respectively. These rate constants are close to the diffusion limit. The main product in each case was the lumiflavin semiquinone radical FH·. By utilization of long pulses (~100 μs), it was shown that the reaction $FH\cdot + \cdot AH(\alpha) \rightarrow FH^- + A(\alpha) + H^+$ [$\cdot AH(\alpha) = \cdot CO_2^-$, $CH_3\dot{C}HOH$, or $(CH_3)_2\dot{C}OH$] proceeded for all three types of $\cdot AH(\alpha)$ radical with second-order rate constants of 17 (+4,−3), 9 (+5,−3), and 9 (+4,−3), respectively, in the above units. The β-carbon radical $\cdot CH_2CH(OH)CH_3$ added to ·FH, forming an alkylated flavin, while the $\cdot CH_2CH_2OH$ radical appeared to be capable of addition or hydrogen atom donation to ·FH.

One-electron reductions of flavin molecules by solvated electrons (e_{aq}) and formate radical ions ($\cdot CO_2^-$) have been used in several laboratories to produce flavin radicals (·FH)[1] and to study their disproportionations and reactions with oxygen and other substances (Land & Swallow, 1969; Anderson, 1976, 1981; Goldberg et al., 1981). Studies in this laboratory (Ahmad & Armstrong, 1982) have demonstrated that these radicals and the α-carbon radicals of simple alcohols [e.g., $CH_3\dot{C}HOH$ and $(CH_3)_2\dot{C}OH$] may also effect *two-electron* reductions of flavins. Writing a general mechanism with AH_2 representing the alcohol, $\cdot AH(\alpha)$ the α-carbon radical, and $A(\alpha)$ the ketone resulting from monodehydrogenation of this radical, one has the following reactions at pH 7:[2]

$$F_o + \cdot AH(\alpha) \rightarrow \cdot FH + A(\alpha) \qquad (1)$$

$$\cdot FH + \cdot AH(\alpha) \rightarrow FH^- + A(\alpha) + H^+ \qquad (2)$$

$$2(\cdot FH) \rightleftharpoons F + FH^-\ H^+ \qquad (3,-3)$$

Reaction 3 is well established from kinetic spectroscopic studies (Land & Swallow, 1969; Vaish & Tollin, 1970; Hemmerich et al., 1980) and the evidence for (1) is unequivocal. However, although (2) can be supported on thermodynamic grounds, it is in competition with the sequence (1) + (3), and its exact contribution cannot be established until the rate constant k_2 is known.

The occurrence of reaction 2 is of general interest in view of its possible role in the reversible reaction between alcohols and flavins. Bruice and co-workers (Bruice & Taulane, 1976; Bruice, 1976) have pointed out that these reactions may proceed through two one-electron transfer steps, involving the $\cdot FH\text{-}\cdot AH(\alpha)$ free radical pair as an intermediate, viz.:

$$F_o + AH_2 \rightleftharpoons \cdot FH + \cdot AH(\alpha) \qquad (4,-4)$$

$$\cdot FH + \cdot AH(\alpha) \rightleftharpoons FH^- + A(\alpha) + H^+ \qquad (2,-2)$$

This pair may possess a relatively short lifetime and never actually separate. Nonetheless, it is of considerable interest to produce ·FH and $\cdot AH(\alpha)$ in a system where free diffusion is occurring and to determine their rate of reaction. The present study was undertaken for that purpose and also to examine the rate of reaction 1 and the spectrum of the ·FH species formed by $\cdot CO_2^-$, $CH_3\dot{C}HOH$, and $(CH_3)_2\dot{C}OH$ radicals. So that reactions 1–4 can also be applied to the formate system, HCO_2^-, $\cdot CO_2^-$, and CO_2 are represented as AH_2, $\cdot AH(\alpha)$, and $A(\alpha)$.

As in earlier studies (Land & Swallow, 1969; Anderson, 1976; Bielski & Gebicke, 1977; Ahmad & Armstrong, 1982), the radicals were produced in situ by the action of ionizing radiation on nitrous oxide purged formate or alcohol solutions containing flavin. Solute concentrations were adjusted so that the following chemical reactions occurred within ~10^{-8} s of absorption of the radiation energy, leaving the α-radicals to react with flavin on a 10^{-6} s or longer time scale:

$$4.2H_2O \overset{\text{radiation}}{\rightsquigarrow} 2.7e_{aq} + 0.6H\cdot + 2.8\dot{O}H + 2.7H^+_{aq} + 0.5H_2 + 0.7H_2O_2 \qquad (5)$$

$$e_{aq} + N_2O + H_2O \rightarrow N_2 + OH^-_{aq} + \cdot OH \qquad (6)$$

$$\cdot OH\ (\text{or}\ \cdot H) + A(H_2) \rightarrow H_2O\ (\text{or}\ H_2) + \cdot AH(\alpha) \qquad (7)$$

All experiments were performed with lumiflavin.

One complicating factor is the fact that about 15% of the ·OH attacks on ethanol or 2-propanol produce the β-radicals $\cdot CH_2CH_2OH$ and $\cdot CH_2CH(CH_3)OH$, respectively (Asmus et al., 1973). These radicals are not as reductive and tend to *add* to ·FH radicals, giving rise to alkylated flavin (Ahmad & Armstrong, 1982). The amounts of these products can be estimated from changes in ultraviolet absorption on addition of atmospheric oxygen.

Experimental Procedures

Materials. Triply distilled water (alkaline $KMnO_4$, acid $K_2Cr_2O_7$) was used in all solution preparations, and research-grade N_2O was passed through a Ridox column (Fisher Scientific Co.) before being used to purge the solutions. Lu-

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[1] Abbreviations: F_o, oxidized flavin; FH·, flavin radical; FH_2 or FH^-, dihydroflavin; AH_2, alcohol or formate; $\cdot AH(\alpha)$, α-carbon radical of alcohol or $\cdot CO_2^-$; $\cdot AH(\beta)$, β-carbon radical of alcohol; $A(\alpha)$, ketone formed by 2-electron oxidation of alcohol; e_{aq}, solvated electron; EDTA, ethylenediaminetetraacetic acid.

[2] pK of FH· = 8.5; pK of FH_2 = 6.5; and at pH 7, the dihydro form is mainly FH^-.

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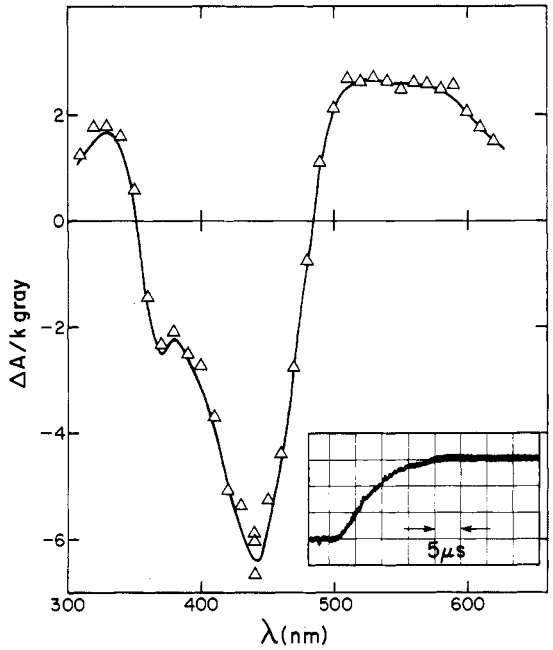

FIGURE 1: Transient spectra after pulse irradiation of a N_2O-saturated aqueous solution of 51.4 μM F_o + 20 mM EtOH at pH 7.0: (solid line) at 30 μs; (Δ) at the half-life (5 μs), normalized to the solid line at 550 nm. (Inset) Oscillograph trace of transient at 550 nm produced under above conditions. Dose of 3.4 grays per pulse.

miflavin was obtained from Sigma Chemical Co.

Methods. All experiments were conducted at 24 ± 1 °C in solutions buffered at pH 7 with 5 mM phosphate buffer. The 1.5-MeV accelerator and optical system, which were used to follow reactions 1 and 2 on a microsecond time scale, have already been described (Elliot et al., 1980; Wu et al., 1982). The accelerator was adjusted to produce either pulses of 1.5 μs or much longer pulses of 80–100 μs duration. These are subsequently referred to as short- or long-pulse experiments. In some experiments of the latter type, the 1-cm optical cuvette was removed from the Van de Graaff cell holder after pulsing, and the spectrum was taken on a Cary 219 spectrophotometer. A few reference spectra were also taken after irradiation at a low dose rate with a ^{60}Co γ cell as previously described (Ahmad & Armstrong, 1982). Dosimetry for γ radiolysis was performed with the Fricke dosimeter (Draganić & Draganić, 1971), while 10^{-2} M ferrocyanide (N_2O saturated) with $G\epsilon$ for ferricyanide = 6.3×10^3 (±0.09) (Schuler et al., 1980) was used for the pulse radiolysis experiments. Here, G was the yield of radicals and ϵ the difference in the extinction coefficients of ferricyanide and ferrocyanide. Doses of radiation energy absorbed in the solutions have been reported in grays (1 gray = 1 J/kg of solution).

Results and Discussion

(I) Experiments with Short Pulses—Spectrum of FH· and Rate of Reaction 1. The change in absorbance (ΔA), which occurred as the free radicals reacted with lumiflavin in reaction 1, was followed as a function of time at wavelengths in the range 320–620 nm. For 40 μM F solutions, the absorbance changes at 550 nm, where only FH· absorbs (see Figure 2 below), reached maxima at about 30 μs, and the spectra produced by the formate, ethanol, and 2-propanol radicals at that point were identical within our precision of ΔA = ±0.0002. However, one may note that the magnitudes of ΔA per unit dose were somewhat lower for the alcohol radicals than for formate. This is to be expected because of the presence of the ~15% nonreducing β-radicals, which probably react more slowly than the α-radicals (Ahmad & Armstrong, 1982).

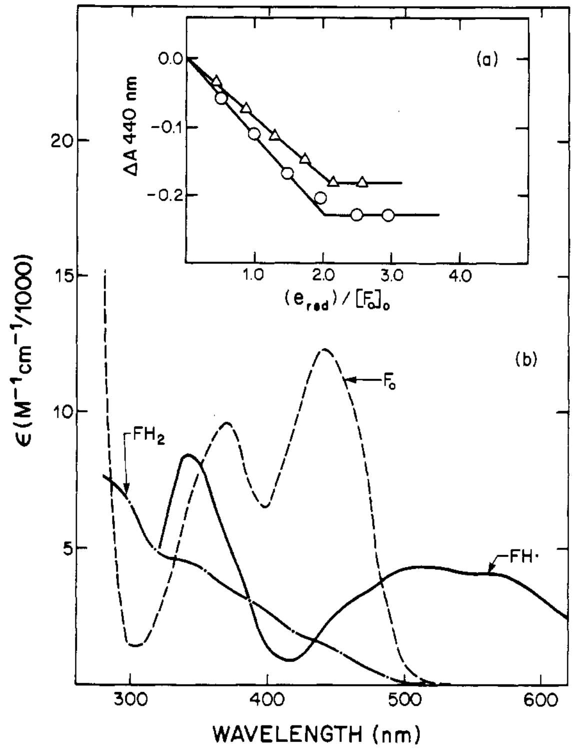

FIGURE 2: (a) Change in absorbance at 440 nm vs. $e_{red}/[F_o]_o$, moles of radical species reacted per mole of flavin present initially, for F_o + $\cdot CO_2^-$ (O) and F_o + 2-propanol radicals (Δ). (b) Spectra of lumiflavin species at pH 7.0: F_o (- -); FH_2 (-·-); FH· (—).

The difference spectrum produced by the reaction of $CH_3\dot{C}HOH$ radicals with 51.4 μM F at 30 μs is shown by the solid line in Figure 1. The points represent the spectrum at 5 μs (the half-life for these conditions), normalized to the solid line in the 520–580 nm region. It is evident that on that time scale the spectrum grows homogeneously. The same result was obtained with $\cdot CO_2^-$.

The absorbance changes per unit dose obtained with $\cdot CO_2^-$ at 30 μs were converted to absolute values of $\epsilon(FH\cdot) - \epsilon(F_o)$ at each wavelength by dividing ΔA by the concentration of $\cdot CO_2^-$ produced per kilogray (6.7×10^{-4} M). Addition of $\epsilon(F_o)$ then generated the spectrum of FH· in Figure 2b. Also shown are the spectrum of F_o used in the above calculation and that of FH_2. Both were taken on the Cary 219 with 15–50 μM flavin solutions. The FH_2 was prepared by two-electron reduction of F_o with $\cdot CO_2^-$ formed by γ radiolysis (Ahmad & Armstrong, 1982). The titration plot has been presented in Figure 2a.

The spectrum of FH· in Figure 2b is similar to those of the flavin radicals from flavin adenine dinucleotide (Anderson, 1976; A. J. Elliot, L. McIntosh, R. Ahmad, K. J. Stevenson, and D. A. Armstrong, unpublished results) and riboflavin (Land & Swallow, 1969). The main features, including the minimum at 415 nm, maxima at 340 and 510, and broad shoulder at 570 nm, occur at the same wavelengths as those of the neutral radical of riboflavin. Our extinction coefficients are about 25% lower. However, Land and Swallow used a smaller yield of radicals (5.8×10^{-4} M/kilogray). If their data are normalized to the above concentration of 6.7×10^{-4} M/kilogray, the extinction coefficients agree within 10%.

The inset in Figure 1 shows the growth of the absorbance of FH· at 550 nm. Due to the large concentration of F_o relative to $\cdot AH(\alpha)$ (~2 μM), it was psuedo first order. The slopes of plots of the pseudo-first-order rate constants vs. $[F_o]$ for

Table I: Summary of Rate Constants in Units of 10^8 M^{-1} s^{-1} [a]

major radical	k_1	$k_2 + k_{-4}$
$\cdot CO_2^-$	36	18
$CH_3\dot{C}(OH)H$	26	12
$(CH_3)_2\dot{C}OH$	20	8.8

[a] Uncertainties: ±4 for k_1 of $\cdot CO_2^-$; ±3 otherwise.

three concentrations in the range 15–55 μM gave the values of k_1 summarized in Table I. The magnitudes are similar to those for reactions of $\cdot CO_2^-$, $CH_3\dot{C}HOH$, and $(CH_3)_2\dot{C}OH$ with nitromethane and nitrosobenzene, which are all in the range (3–5) × 10^9 M^{-1} s^{-1} (Rabani et al., 1965; Asmus et al., 1966; Fojtik et al., 1970). These reactions are considered to be diffusion controlled. Since the larger flavin molecules would have smaller diffusion coefficients than the above molecules, the slightly lower rate constants for the present reactions would suggest that they are also diffusion controlled. The present value of $k_1 = 3.6 \times 10^8$ M^{-1} s^{-1} for $\cdot CO_2^-$ reacting with lumiflavin may also be compared with 3.6×10^9 M^{-1} s^{-1} for $\cdot CO_2^-$ reacting with riboflavin (Land & Swallow, 1969).

(*II*) *Experiments with Long Pulses—Products of Reaction 2.* The magnitudes of k_1 found above are such that at γ radiolysis dose rates, where the radical formation rate was only 1.4×10^{-7} M s^{-1}, all α-radicals would have undergone that reaction as long as a significant concentration of F_0 remained. Reactions 3 and −3 would therefore have dominated over (2) in controlling the concentration of FH·. The species formed under these conditions are discussed first.

As in the earlier study (Ahmad & Armstrong, 1982), $\cdot CO_2^-$ was unique in that FH^- was the only significant product and addition of atmospheric oxygen completely restored the initial lumiflavin. However, in the case of the alcohol radicals, oxygen-stable products were also present. That for the 2-propanol system could not be photolytically converted to F even after 30-min illumination with a 250-W tungsten lamp. They are taken to be C4a-alkylated, 4a,5-dihydro derivatives of lumiflavin, produced by addition of the $\cdot CH_2CH_2OH$ and $\cdot CH_2CH(OH)CH_3$ β-alcohol radicals to FH·.

The proportions of lumiflavin converted to these products (0.30 and 0.13 for 2-propanol and ethanol radicals, respectively) and their absorption spectra were determined as previously described (Ahmad & Armstrong, 1982). As illustrated for the 2-propanol case in Figure 3b, they exhibited maxima near 360 nm. It may be noted that methylated C4a-alkylated flavins have properties similar to those described above, while N5-alkylated flavins possess absorption maxima at shorter wavelengths and on reaction with O_2 give rise to a radical with strong absorption above 500 nm (Ghisla et al., 1973; Walker et al., 1970).

Ahmad and Armstrong found evidence that the β-radicals of alcohols reacted relatively slowly with F_0 but added efficiently to FH· formed in reaction 1 or −3. The titration plot for 2-propanol in Figure 2a confirms a high efficiency for that type of reaction, since only 2.1 alcohol radicals reacted per lumiflavin molecule consumed. This feature also applied to the ethanol system. For both alcohols the fraction of β-radicals formed by ·OH attack is 0.15 (Asmus et al., 1973) which means that at the end points 0.3 ·AH(β) had reacted per mol of flavin. The fact that the proportion of flavin converted to oxygen-stable product in the 2-propanol system was also 0.3 could be explained if every $\cdot CH_2CH(OH)CH_3$ underwent reaction

$$FH\cdot + \cdot AH(\beta) \rightarrow FH\text{–}C4a\text{–}AH(\beta) \qquad (8a)$$

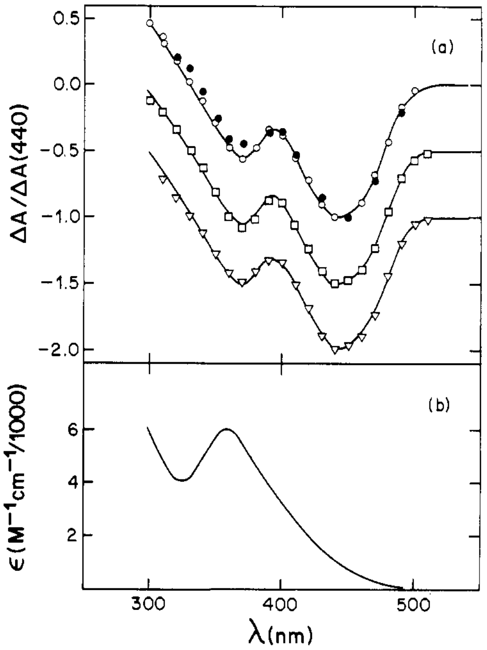

FIGURE 3: (a) Difference spectra for the γ (—) and long-pulse Van de Graaff (symbols) radiolyses of N_2O-saturated lumiflavin solutions at pH 7, $[F_0] = 20$ μM for γ and 5 μM for pulse radiolysis. Radicals were from 0.02–0.1 M formate (○,●), ethanol (□) (ordinate displaced by −0.5), and 2-propanol (▽) (ordinate displaced by −1.0). Filled symbols were taken on pulse radiolysis spectrometer, all others were on Cary 219. (b) Absorption spectrum of oxygen-stable product formed in 2-propanol solutions at pH 7.

On the other hand, only 0.13 mol of that product/mol of F_0 was observed from ethanol radicals. Thus only about half of the $\cdot CH_2CH_2OH$ radicals could have undergone (8a). The bond dissociation energies $FH_2 \rightarrow FH\cdot + H\cdot$ and $\cdot CH_2CHOH\text{–}H \rightarrow CH_2CHOH + H$ have been estimated to be 59[3] and 40 kcal/mol (Kerr, 1966). It is therefore likely that the remaining ethanol β-radicals formed dihydroflavin via the exothermic reaction 8b:

$$FH\cdot + \cdot CH_2CH_2OH \rightarrow FH_2 + CH_2CHOH \qquad (8b)$$

The fact that steric and statistical factors make the analogue of reaction 8b with $\cdot CH_2CH(OH)CH_3$ radicals less likely is in line with the above findings for 2-propanol. However, one cannot overlook the possibility that some of these radicals do form FH_2 and a fraction of the $CH_3\dot{C}(OH)CH_3$ α-radicals added to FH· to produce the same net yield of alkylated flavin. If this were so, the proportion of that product should rise substantially when reaction 3 is suppressed and all FH· molecules are forced to react with alcohol radicals. The very high radical formation rates in the long-pulse experiments (~0.6 M s^{-1} vs. ~1.4×10^{-7} M s^{-1} in the γ radiolyses) were explicitly designed to establish that condition, and the kinetic analysis in section III demonstrates that they did.

When the optical cuvette was subjected to long-pulse irradiations from the Van de Graaff and the spectra of the irradiated solutions were taken within a few minutes on the Cary 219 spectrophotometer, the difference spectra for the products were identical with those observed at the γ radiolysis dose rate. This is demonstrated by the open symbols and solid lines in Figure 3a. In addition, the proportions of oxygen-stable products for the three solute systems did not increase. Thus the products formed when reaction 2 dominated were quantitatively similar to those discussed above for γ radiolysis conditions, and there was no evidence for alkylated flavin formation by addition of either α-radical to FH·.

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[3] D. A. Armstrong, unpublished calculation.

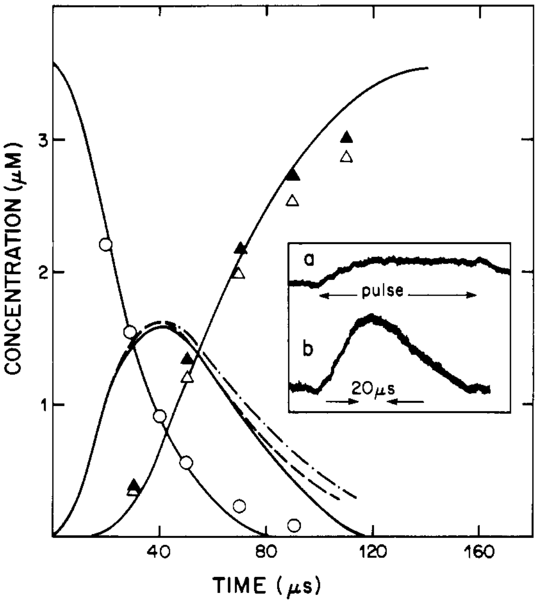

FIGURE 4: Concentration–time profiles for typical long-pulse experiments at pH 7. (Inset) Oscilloscope traces: (a) $\cdot CO_2^-$ absorption at 352 nm in the absence of F_o; (b) FH· absorption at 550 nm. Solid curves are experimental concentrations calculated from oscilloscope traces (see text) for $\cdot CO_2^- + F_o$ reactions. Symbols and dashed lines are computed with rate constants in units of 10^8 M^{-1} s^{-1}: $k_1 = 36$, $k_2 = 18$, $2k_3 = 15$, $k_{-4} = 0$ (○, ▲, – –); effect of changing k_2 to 16 and k_{-4} to 2 (△, –·–). $[F_o]_0 = 3.6$ μM; $[HCO_2^-] = 20$ mM; 0.01 M buffer (N_2O saturated). Dose rate of 100 grays s^{-1}.

(III) Experiments with Long Pulses—Reaction Rates. The magnitude of $2k_9$ in units of 10^9 M^{-1} s^{-1} has been shown to

$$2[\cdot AH(\alpha)] \rightarrow A + AH_2 \text{ or } HA\text{–}AH \qquad (9)$$

be 1.5 at ionic strength 0.03 for $\cdot CO_2^-$ (Buxton & Sellers, 1973) and 2.3 and 1.4 for ethanol and 2-propanol radicals, respectively (Simic et al., 1969). On irradiation of solutions of these solutes at dose rates producing 0.64 M s^{-1} $\cdot AH(\alpha)$, stationary radical concentrations should therefore be reached within a few tens of microseconds, giving $[\cdot AH(\alpha)] = [0.64/(2k_9)]^{1/2}$. This has been illustrated for the case of formate by trace a in the inset of Figure 4, which shows the absorption due to $\cdot CO_2^-$ at 352 nm during a 100-μs pulse. In this case, the stationary radical concentration can be calculated from the known value of ϵ (at 352 nm, 216 M^{-1} cm^{-1}; Buxton & Sellers, 1973) or the above formula. The average was 20 ± 1 μM for the two methods.

Irradiation of 3–5 μM flavin solutions at the above dose rate with long (~100 μs) pulses led to an initial rapid rise in [FH·], followed by a decay (trace b, inset to Figure 4). The latter was not due to reaction 3, because $t_{1/2}$ for that process would be expected to be $(2k_3[FH\cdot]_{max})^{-1}$ or typically $(\simeq 2 \times 10^9 \times 3 \times 10^{-6})^{-1} \simeq 160$ μs from reported values of k_3 (A. J. Elliot, L. McIntosh, R. Ahmad, K. J. Stevenson, and D. A. Armstrong, unpublished results; Hemmerich et al., 1980; Land & Swallow, 1969), and we observed half-lives in that region using short pulses here. The time at which $[FH\cdot]_{max}$ occurred decreased with increasing dose rate, which is consistent with reaction 2 as the major cause of decay.

The concentrations of FH·, F_o, and FH_2 during the course of a long pulse can be calculated from the absorbance changes at 550 nm and at the isosbestic points at 434 and 484 nm, respectively (see Figure 2). An example has been given in Figure 4 for $\cdot CO_2^-$.

Ultimately, when all flavin has been converted to FH_2, the observed difference spectrum should be given by $[\epsilon(\text{product}) - \epsilon(F_o)][F_o]_0$, where $[F_o]_0$ is the initial flavin concentration. This has been illustrated by the filled symbols on the uppermost plot in Figure 3a. These were taken with the pulse radiolysis optical system near the end of a 120-μs pulse. The absorptions below 380 nm are high due to the presence of residual $\cdot CO_2^-$, but apart from this, the spectrum agrees with that taken on the Cary instrument (see above). Similar results were obtained with radicals produced from ethanol and 2-propanol.

If one neglects reaction 3, which will in fact be justified later, and the presence of β-radicals, the main processes controlling the concentration of FH· near its maximum (see traces in Figure 4) should be reactions 1 and 2 and possible also –4 and 10. The rate of 10 was checked by prepulsing a solution

$$FH\cdot + \cdot AH(\alpha) \rightarrow F_o + AH_2 \qquad (-4)$$

$$FH_2 + \cdot AH(\alpha) \rightarrow FH\cdot + AH_2 \qquad (10)$$

several times, which converted all F_o to FH_2, and then following the increase in absorption at 550 nm in a further pulse. A steady state was quickly reached, due to $k_{10}[FH_2][\cdot AH(\alpha)] = k_2[FH\cdot][\cdot AH(\alpha)]$. Since [FH·] could be calculated from ΔA at 550 nm and $[FH_2]$ was equal to $[FH_2]_0 - [FH\cdot]$, one could evaluate k_{10}/k_2. For all three radicals, $k_{10}/k_2 \lesssim 0.04$, which for present purposes is negligible. At the maximum in the radical concentration, one therefore has $k_1[F_o][\cdot AH(\alpha)] = (k_2 + k_{-4})[FH\cdot][\cdot AH(\alpha)]$ or $(k_2 + k_{-4}) = k_1[F_o]/[FH\cdot]$. Since both $[F_o]$ and [FH·] are known from plots such as those in Figure 4, the quantities $k_2 + k_{-4}$ can be calculated from the values of k_1 in Table I. The results obtained from the mean of three experiments for each solute system are given in column three of Table I. In each case, $k_2 + k_{-4}$ is about half of k_1, a feature that may reflect more stringent steric requirements for reaction of $\cdot AH(\alpha)$ with FH· than F_o.

If one utilizes the steady-state concentrations of $\cdot AH(\alpha)$ from the relation $[\cdot AH(\alpha)] = [0.64/(2k_9)]^{1/2}$, independent values of k_{-4} and k_2 can be estimated from the instantaneous slopes $d[F_o]/dt$ and $d[FH_2]/dt$ and the relationships

$$d[F_o]/dt = k_{-4}[\cdot AH(\alpha)][FH\cdot] - k_1[\cdot AH(\alpha)][F_o]$$

and

$$d[FH_2]/dt = k_2[FH\cdot][\cdot AH(\alpha)]$$

respectively. These calculations led to the conclusion that k_{-4} was relatively small, viz., $\lesssim 1$, $\lesssim 3$, and $\lesssim 1$ in units of 10^8 M^{-1} s^{-1} for formate, ethanol, and 2-propanol radicals, respectively. Thus the magnitudes of k_2 are 17 (+4,–3), 9 (+5,–3), and 9 (+4,–3) in units of 10^8 M^{-1} s^{-1}, respectively. For the alcohol radicals these rate constants should strictly be taken as averages for reactions 2 and 8. However, since the fraction of β-radicals is only 0.15, the true values of k_2 should be well within the overall uncertainties given.

In the case of the formate system, the correctness of the foregoing analysis was checked by a computer program for the calculation of concentrations of reactants and products in complex system driven by ionizing radiation.[4] The program included reactions 1–4 and –4. The dashed lines and points in Figure 4 show the computer-calculated concentrations for $k_1 = 36$, $2k_3 = 15$, $k_2 = 16$, $k_{-4} = 2$, $k_2 = 18$, and $k_{-4} = 0$ in units of 10^8 M^{-1} s^{-1}. It is evident that k_{-4} must be within the limit given above, since larger values cause too slow a decay of [FH·]. The computer program also gave the concentrations of FH^- produced via reactions 2 and 3 as 3.1 and 0.1 μM, respectively, which confirms that the former process dominates strongly in the long-pulse experiments.

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[4] We are indebted to Atomic Energy of Canada, Chalk River Nuclear Laboratories, and Dr. A. J. Elliot for running the program for us.

Before closing this section, it should be recognized that reaction −4 is similar to the "reverse hydrogen transfer" proposed (Vaish & Tollin, 1970; Heelis et al., 1979, and earlier references cited therein) to explain the decay of FH·, when $^3F_0$ is quenched by phenols and indoles:

$$^3F_0 + RH_2 \rightarrow FH\cdot + RH\cdot \qquad (11)$$

$$FH\cdot + RH\cdot \rightarrow F_0 + RH_2 \qquad (12)$$

This type of reaction was shown here to be unimportant for the $\cdot AH(\alpha)$ radicals of ethanol and 2-propranol and for $\cdot CO_2^-$, which rather resemble the radical species produced in reaction 11 from EDTA and methionine in that they donate hydrogen atoms (or electrons) to FH·. At the same time, our studies indicate that $\cdot AH(\beta)$ radicals of the higher alcohols add to FH· as do the radicals formed by photodecarboxylation of certain acids (Walker et al., 1970; Bowen & Yang, 1975; Novak et al., 1980; Hemmerich et al., 1980). The $\cdot CH_2CH_2OH$ species appears to undergo both reactions, the proportions being roughly 1:1 for the FH· of lumiflavin. Clearly, the nature of the radical has an important bearing on the type of reaction it undergoes with FH·. Comparison of the present data with our earlier results also indicates a dependence on the flavin type. This point will be explored in more detail.

Acknowledgments

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Registry No. F_0, 84709-10-4; FH·, 64135-79-1; $\cdot CO_2^-$, 14485-07-5; $CH_3\dot{C}HOH$, 2348-46-1; $(CH_3)_2\dot{C}OH$, 5131-95-3; $\cdot CH_2CH(OH)CH_3$, 7277-18-1; $\cdot CH_2CH_2OH$, 4422-54-2.

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