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Formation of Radical Cations of Dihydropteridine and Dihydroflavin in the Photoreduction of Pteridine and Flavin Analogues by Benzyl Alcohol Derivatives

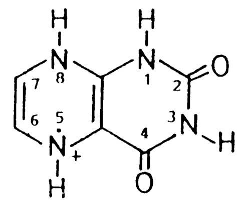
Shunichi FUKUZUMI,* Kumiko TANII, and Toshio TANAKA
Department of Applied Chemistry, Faculty of Engineering,
Osaka University, Suita, Osaka 565

Radical cations of dihydropteridine and dihydroflavin are formed in the photoreduction of pteridine and flavin analogues, respectively, by benzyl alcohol derivatives in the presence of perchloric acid in acetonitrile *via* photoinduced electron transfer from benzyl alcohol derivatives to the triplet excited states of protonated pteridine and flavin analogues. Their ESR spectra and the stabilities are compared.

The reduced form of folic acid which contains a dicyclic pteridine ring, is known as a highly versatile carrier of activated one-carbon units.[1)] On the other hand, the versatile roles of flavins, which contain a tricyclic isoalloxazine ring, in biological redox reactions are also well established.[2,3)] In contrast with flavins, however, little is known of the redox properties of pteridine derivatives, especially of their one-electron redox properties, in spite of the apparent similarity between pteridine and isoalloxazine rings. We wish to report herein the formation of the radical cations in the photoreduction of a pteridine derivative (lumazine) and a flavin analogue (tetraacetylriboflavin) with benzyl alcohol derivatives in the presence of $HClO_4$ in acetonitrile (MeCN). The spin densities and the stabilities of radical cations of dihydropteridine and dihydroflavin are compared.

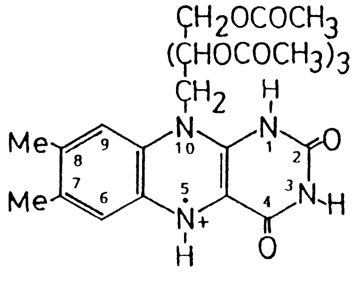
Irradiation of an deaerated MeCN solution containing lumazine (Lu),[4)] benzyl alcohol, and $HClO_4$ with light (290 nm $< \lambda <$ 420 nm) at 298 K resulted in the decrease in the absorbance due to a protonated lumazine (LuH^+: λ_{max} 350 nm), accompanied by the appearance of a new absorption band at 326 and 416 nm, which disappeared gradually when the irradiation was stopped. In order to assign the unstable photoreduced product, the ESR measurements[4)] were carried out under irradiation with a Xenon lamp. The ESR spectrum observed is shown in Fig. 1 (part a), together with the computer simulation spectrum, from which the paramagnetic species can be assigned as the dihydrolumazine radical cation ($LuH_2^{+\bullet}$). Upon cutting off the irradiation light, the ESR spectrum disappeared within 20 min, obeying the second-order kinetics, *i.e.*, $d[LuH_2^{+\bullet}]/dt = -k_d[LuH_2^{+\bullet}]^2$. Thus, $LuH_2^{+\bullet}$ is formed by the photoreduction of LuH^+ by a benzyl alcohol derivative (Eq. 1),[5)] and the $LuH_2^{+\bullet}$ radicals decay by the disproportionation reaction (Eq. 2).

$$2LuH^+ + X\text{-}C_6H_4CH_2OH \xrightarrow{h\nu} 2LuH_2^{+\bullet} + X\text{-}C_6H_4CHO \qquad (1)$$



$LuH_2^{+\bullet}$

hfc	/ mT
a(5-N)	0.801
a(5-H)	1.402
a(8-N)	0.530
a(7-H)	0.106
ΔH_{msl}	0.040



$FlH_2^{+\bullet}$

hfc	/ mT
a(5-N)	0.630
a(5-H)	1.411
a(8-Me)	0.305
a(10-N)	0.477
a(10-CH_2)	0.477
ΔH_{msl}	0.080

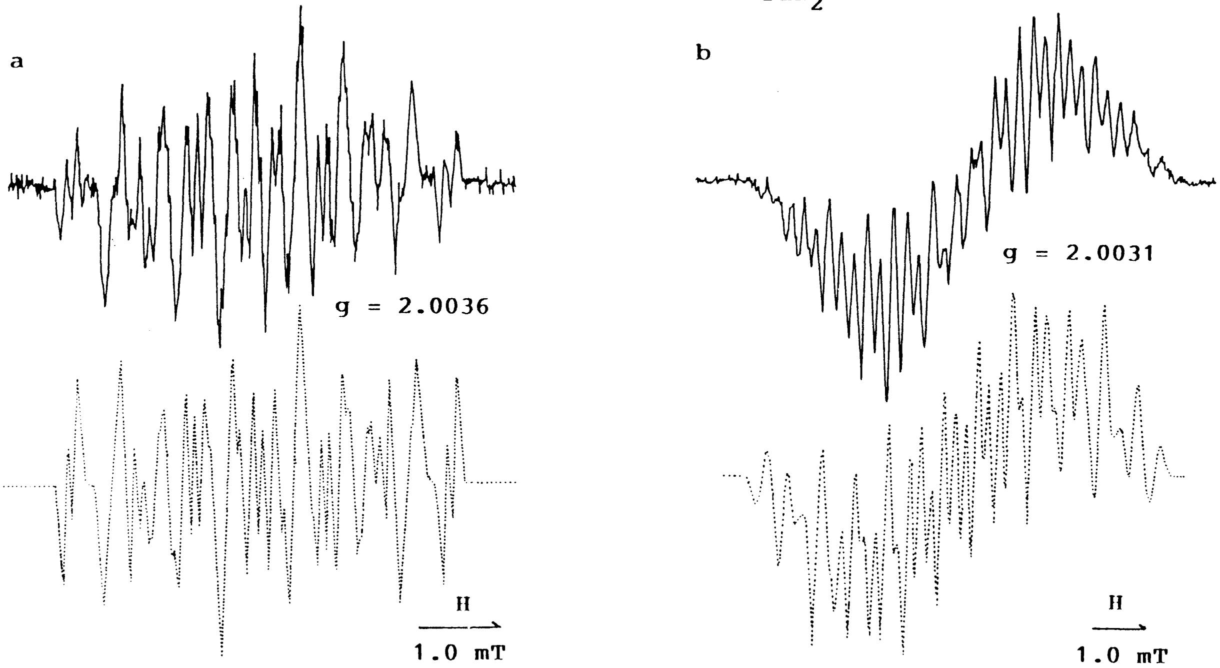


Fig. 1. The ESR spectra of $LuH_2^{+\bullet}$ (a) and $FlH_2^{+\bullet}$ (b) formed by the photoreduction of lumazine (8.1×10^{-3} mol dm^{-3}) and tetraacetylriboflavin (3.0×10^{-3} mol dm^{-3}) by $4\text{-}ClC_6H_4CH_2OH$ (5.0×10^{-2} mol dm^{-3}) and $4\text{-}MeC_6H_4CH_2OH$ (5.0×10^{-2} mol dm^{-3}), respectively, in the presence of $HClO_4$ (0.17 mol dm^{-3}) in MeCN under degassed conditions. The dotted line spectra show the corresponding computer simulation spectra using the hfc values listed above.

$$2LuH_2^{+\bullet} \longrightarrow LuH^+ + LuH_3^+ \qquad (2)$$

When lumazine is replaced by tetraacetylriboflavin (Fl) in the photoreduction by benzyl alcohol (Eq. 1), the dihydroflavin radical cation ($FlH_2^{+\bullet}$) is formed as shown in Fig. 1 (part b). In contrast with $LuH_2^{+\bullet}$, $FlH_2^{+\bullet}$ in the presence of $HClO_4$ in MeCN are extremely stable, and it took about 10 h for $FlH_2^{+\bullet}$ to disappear even in the presence of oxygen. The hyperfine coupling constants (hfc) of $FlH_2^{+\bullet}$ agree well with the reported values of other dihydroflavin radical cations.[6] In both cases, no appreciable hfc on the pyrimidine ring has been observed. The hfc values due to N(5) and N(8) of $LuH_2^{+\bullet}$ are larger than those due to N(5) and N(10) of $FlH_2^{+\bullet}$. The presence of hfc due to C(8)-Me protons of $FlH_2^{+\bullet}$ indicates the unpaired electron of $FlH_2^{+\bullet}$ is more delocalized than that of $LuH_2^{+\bullet}$. This

Table 1. Limiting quantum yields (Φ_∞) and the quenching constants (K_{obsd}) of the excited state of FlH^+ (or LuH^+) in the photoreduction of FlH^+ (or LuH^+) by $X-C_6H_4CH_2OH$ in the presence of $HClO_4$ (1.4×10^{-2} mol dm^{-3}), and the fluorescence quenching constants (K_q) of $^1FlH^{+*}$ by $X-C_6H_4CH_2OH$ in MeCN at 298 K

$X-C_6H_4CH_2OH$	Φ_∞ a,b)	K_{obsd} a) dm^3 mol^{-1}	K_q a) dm^3 mol^{-1}
X = $4-NO_2$	1.8×10^{-1}	1.3	
2-Br	1.7×10^{-1}	6.8×10	
H	2.8×10^{-1}	2.8×10^2	2.5×10
4-Cl	2.5×10^{-1} (4.0×10^{-1})	4.5×10^2	2.7×10
4-Me	3.0×10^{-1}	4.7×10^2	2.8×10
$4-Pr^i$	3.1×10^{-1} (4.6×10^{-1})	2.5×10^2	2.6×10
4-OH	1.3×10^{-3}	$> 10^3$	
4-MeO	1.6×10^{-3}	$> 10^3$	3.6×10

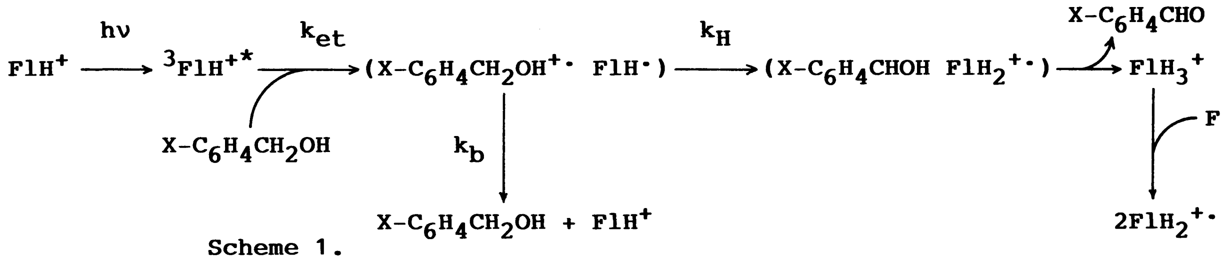
a)The experimental errors are within ±10%. b)The values in parentheses are those of LuH^+ (the experimental values are within ±20%).7)

difference in the spin delocalization, albeit it is small, may cause the drastic difference in the stabilities between $LuH_2^{+\cdot}$ and $FlH_2^{+\cdot}$.

The quantum yields (Φ) of formation of $FlH_2^{+\cdot}$ were determined using a ferroxalate actinometer.7) The Φ value at a fixed concentration of a benzyl alcohol derivative increased with an increase in the $HClO_4$ concentration to reach a constant value in the region $[HClO_4] > 3 \times 10^{-3}$ mol dm^{-3} when Fl is protonated to form FlH^+. The quantum yield increased also with an increase in the concentration of each benzyl alcohol derivative to approach a limiting value (Φ_∞) in accordance with Eq. 3. From the linear plots between Φ^{-1} and $[X-C_6H_4CH_2OH]^{-1}$ are obtained the

$$\Phi^{-1} = \Phi_\infty^{-1}[1 + (K_{obsd}[X-C_6H_4CH_2OH])^{-1}] \qquad (3)$$

Φ_∞ and K_{obsd} values, which are listed in Table 1. The K_{obsd} value increases generally with increasing the donor ability of the substituent X. On the other hand, the quenching constants K_q determined by the fluorescence quenching of the singlet excited state $^1FlH^+$ (the values are listed also in Table 1) are more than 10-fold smaller than the K_{obsd} values. Thus, the photoreduction of FlH^+ by $X-C_6H_4-CH_2OH$ may proceed via the triplet excited state of FlH^+ rather than the singlet excited state. When $4-MeC_6H_4CH_2OH$ is replaced by the dideuterated analogue ($4-Me-C_6H_4CD_2OH$),8) the small primary kinetic isotope effect was observed ($\Phi_H/\Phi_D = 1.2 \pm 0.1$). Based on the above results, the reaction mechanism of the photoreduction FlH^+ by $X-C_6H_4CH_2OH$ may be given as shown in Scheme 1, where photoinduced electron transfer from $X-C_6H_4CH_2OH$ to the triplet excited state $^3FlH^{+*}$ generates the radical pair ($X-C_6H_4CH_2OH^{+\cdot}$ $FlH^\cdot$) which disappears either by the back electron transfer to regenerate the reactant pair or by the proton transfer from $X-C_6H_4CH_2OH^{+\cdot}$ to $FlH^\cdot$, followed by the hydrogen transfer to yield $X-C_6H_4CHO$ and FlH_3^+, the latter of which is converted to $FlH_2^{+\cdot}$ by the comproportionation reaction with FlH^+. According to



Scheme 1.

Scheme 1, the limiting quantum yield may be determined by the competition between the back electron transfer (k_b) and the proton transfer (k_H). In such a case, the primary kinetic isotope effect due to the proton transfer step may not be observed appreciably, since the photoinduced electron transfer becomes the rate-determining step when the proton transfer is faster than the back electron transfer. This may be the reason why only the small primary kinetic isotope effect is observed ($\Phi_H/\Phi_D = 1.2 \pm 0.1$). The strongly electron-donating substituents favor the electron-transfer process, but disfavor the proton-transfer process from $X-C_6H_4CH_2OH^{+\cdot}$ to $FlH^\cdot$, since it is known that an increase in the electron-donor ability of substituents leads to a decrease in the proton-donor ability of the oxidized species.[9] This may be the reason why the limiting quantum yield with the strongly electron donating substituent X = 4-OH or 4-MeO becomes significantly smaller compared with the other substituents (Table 1).

The photoreduction of LuH^+ may proceed also via the photoinduced electron transfer from $X-C_6H_4CH_2OH$ to $^3LuH^{+*}$. The similar Φ_∞ values between FlH^+ and LuH^+ (Table 1) indicate the similarity of their one-electron redox properties. Despite of such similarity, the third ring of Fl may be essential for the much greater stability of $FlH_2^{+\cdot}$ than $LuH_2^{+\cdot}$

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4) Lumazine (Lu) is hardly soluble in MeCN, but the protonated species (LuH^+) becomes soluble in the presence of $HClO_4$ in MeCN.
5) The oxidized products $X-C_6H_4CHO$ were identified by the ^1H NMR spectra.
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7) The experimental errors in determining the quantum yields of $LuH_2^{+\cdot}$ are larger than those of $FlH_2^{+\cdot}$ (Table 1).
8) The dideuterio-4-methylbenzyl alcohol ($4-MeC_6H_4CD_2OH$) was prepared according to the literature; L. Roecker and T. J. Meyer, J. Am. Chem. Soc., 109, 746 (1987).
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