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CHEMILUMINESCENT REACTIONS OF "UNMODIFIED" FLAVINS

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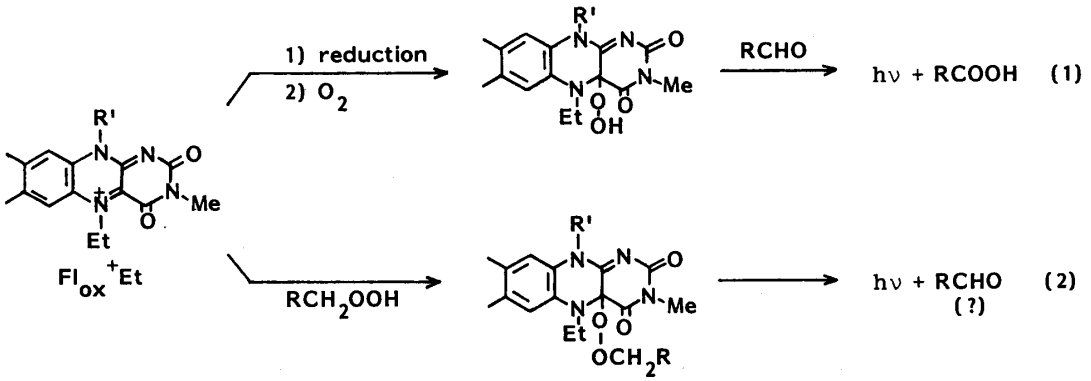
We have found that 3-methyl-10-arylisoalloxazines emit visible light when treated with Triton B and H_2O_2 in aqueous DMF. This is the first example of chemiluminescence in "unmodified" flavins.

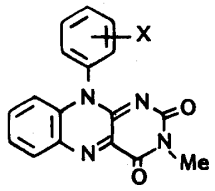
KEYWORDS — chemiluminescence; unmodified flavin; visible light emission; Triton B; hydrogen peroxide; fluorescence; excited species

Chemical reactions that result in light emission have been intensively investigated during the past two decades, and attention to these intriguing reactions is still increasing. Bacterial luciferase chemiluminescence is thought to arise from enzyme-bound flavin-4a-hydroperoxide which enters into the chemiluminescent oxidation of long-chain aldehyde substrates at the active site of the enzymes.[1] A similar chemiluminescent reaction can be imitated in a non-enzymatic system by using N(5)-alkyl flavinium cations ($Fl_{ox}{}^{+}R$): chemiluminescence accompanies the decomposition of the adduct of a flavin-4a-hydroperoxide with RCHO (Eq. 1) or the adduct of an alkyl hydroperoxide with $Fl_{ox}{}^{+}R$ (Eq. 2).[2-5] However, the structure of the excited-state species is not yet established unequivocally.[3-7]



Here, a question arises as to the role of the N(5)-alkyl group: it may stabilize the flavin-4a-hydroperoxide or it may enhance the electrophilicity of C(4a). If the latter role contributes significantly to the subsequent chemiluminescent reaction, it follows that "unmodified" flavins could produce the excited-state species when strong nucleophiles attack the less electrophilic flavin skeleton. It is known that OH^- and OOH^- are very highly nucleophilic in dipolar aprotic solvents. In the course of our studies of flavin chemistry,[8] we unexpectedly found that "unmodified" flavins emit visible light when treated with Triton B (benzyltrimethylammonium hydroxide) and H_2O_2 in aqueous N,N-dimethylformamide (DMF).

Table I. Cheminuminescent Properties of 3-Methyl-10-arylisoalloxazines[a)]

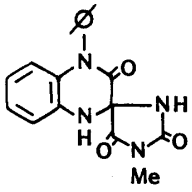


	Time to maximum[b)] s	k[c)] s^{-1}	Emission maximum nm	Φ
X = p-Cl	2	0.050	527	1.1×10^{-7}
X = H	7	0.051	550	4.6×10^{-7}
X = o-Et	9	0.043	550	2.5×10^{-7}

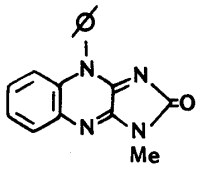
a) 60 °C, DMF:H_2O=56:44 (v/v), [Triton B]=0.844 M, [H_2O_2]=1.09 M, [isoalloxazine]=(6-35)x10^{-3} M.

b) The time to reach the emission maximum.

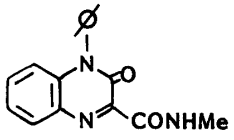
c) First-order rate constant for the emission decay.



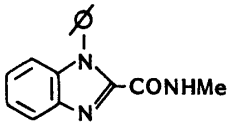
(I)



(II)



(III)



(IV)

3-Methyl-10-arylisoalloxazine (Fl-X: see Table I) in DMF was mixed with Triton B and H_2O_2 at 60 °C in an anaerobic (N_2) Thunberg cuvette and chemiluminescence was followed by a fluorescence spectrophotometer (Hitachi 650-10S) at 60 °C. The concentrations of these reactants in the final reaction solution are recorded in a footnote to Table I. Broad emission spectra spreading at 450-650 nm appeared immediately after the reactants were mixed. The emission maxima were observed at 527-550 nm (Table I), which is comparable with those for $Fl_{ox}{}^{+}R$ (ca. 530 nm).[2-5] The "white" light emission was visible to the dark-adapted eye. This is the first example of chemiluminescence from "unmodified" flavins.

The chemiluminescence intensity at the emission maximum increased rapidly and then decayed according to the first-order kinetics. In Table I, we record the time to reach the maximum intensity and the first-order rate constants (k) for the emission decay. In the initial burst reaction, Fl-Cl reached the maximum faster than Fl-H and Fl-Et. Probably this is accounted for by the fact that the electron-withdrawing Cl group facilitates the nucleophilic attack of OOH^-. On the other hand, a significant substituent effect was not found for the k values. The quantum yields of light emission were determined by comparing them with the chemiluminescence from the standardized luminol-hemin-H_2O_2 reaction run in exactly the same geometry.[9] The quantum yields calculated on the basis of the total flavin concentration were in the order of 10^{-7} (Table I), which is smaller by about three orders of magnitude than the yields for $Fl_{ox}{}^{+}R$ ($\Phi \approx 10^{-4}$).[2-5]

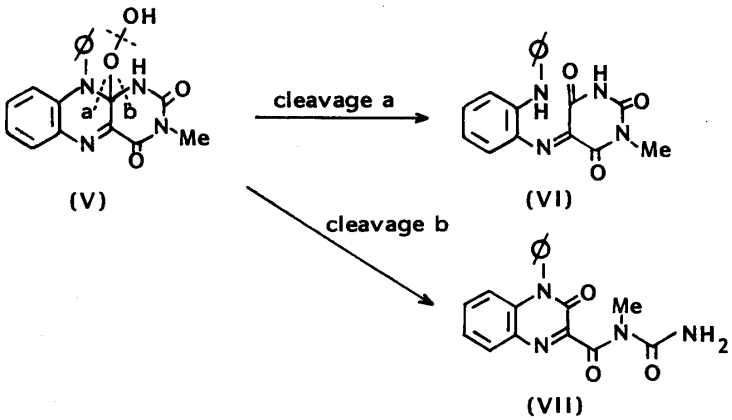
It is known that the alkaline hydrolysis of 3-methyl-10-arylisoalloxazines with Triton B in DMF-methanol (60:40 v/v) at room temperature gives (I) (major) and (II) (minor).[8] Further treatment

of (I) with Triton B in DMF at 130 °C gives (III) (major) and (IV) (minor). (I) and (II) resulted from the nucleophilic attack of OH^- at C(10a) and C(4) of Fl-X, respectively.

Treatment of Fl-X with Triton B plus H_2O_2 at 60 °C afforded a similar product distribution. We therefore tried to find an excited-state species which might have the fluorescence maximum in the visible region. However, none of them emitted visible light at λ_{max} >500 nm: the fluorescence maxima at 60 °C in DMF-H_2O (56:44, v/v) were 374 nm for (I), 420 nm for (II), 370 nm for (III), and 371 nm for (IV).[10] This suggests that the excited species may exist along a reaction route from 10-arylisoalloxazines to (I)-(IV). Although we can not exclude other excited species such as ones derived from an OOH^- adduct to C(4), we consider at present that the excited species can be assigned to either (VI) or (VII). Thus, OOH^- would attack mainly the C(10a) position to yield (V) and then (V) would decompose thermally according to two different routes: N(10)-C(10a) cleavage (cleavage a) to afford (VI) and C(10a)-N(1) cleavage (cleavage b) to afford (VII). Particularly, the structure of (VI) is similar to that of the intermediate generated from flavin-4a-hydroperoxide by p-hydroxybenzoate hydroxylase.[6,11]

In conclusion, the present paper demonstrates for the first time that "unmodified" flavins can emit visible light in the presence of a strong alkaline reagent and H_2O_2.

We thank Miss Y. Tanaka for technical assistance.



(V) (VI) (VII)

REFERENCES AND NOTES

1) For a general review see J.W.Hastings, CRC Crit. Rev. Biochem., 1978, 163.
2) C.Kemal, T.W.Chan, and T.C.Bruice, Proc. Natl. Acad. Sci. U.S.A., 74, 405 (1977).
3) C.Kemal and T.C.Bruice, J. Am. Chem. Soc., 99, 7064 (1977).
4) P.T.Shepherd and T.C.Bruice, J. Am. Chem. Soc., 102, 7774 (1980).
5) T.C.Bruice, Acc. Chem. Res., 13, 256 (1980).
6) A.Wissiak and T.C.Bruice, J. Am. Chem. Soc., 105, 4809 (1983).
7) T.Matsuura, Kagaku, 34, 72 (1979).
8) T.Harayama, Y.Tezuka, T.Taga, and F.Yoneda, Tetrahedron Lett. 25, 4015 (1984).
9) J.Lee and H.H.Selinger, Photochem. Photobiol., 4, 1015 (1965).
10) 10-Ethyl-3-methylisoalloxazine 5-oxide showed a fluorescence maximum at 546 nm very close to that of 3-methyl-10-arylisoalloxazine (see Table I). At present stage, it is difficult to determine whether the 5-oxide is the excited-state species for chemiluminescence or not, because we have no evidence supporting the formation of the 5-oxide from isoalloxazine under the reaction conditions (Triton B-H_2O_2 in DMF at 60 °C).
11) B.Entsch, D.P.Ballou, and V.Massey, "Flavins and Flavoproteins," Elsevier, Amsterdam, 1976, p. 111.

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