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PHOTO-EXCITED REACTIVITY OF FLAVIN STUDIED BY CIDNP TECHNIQUE

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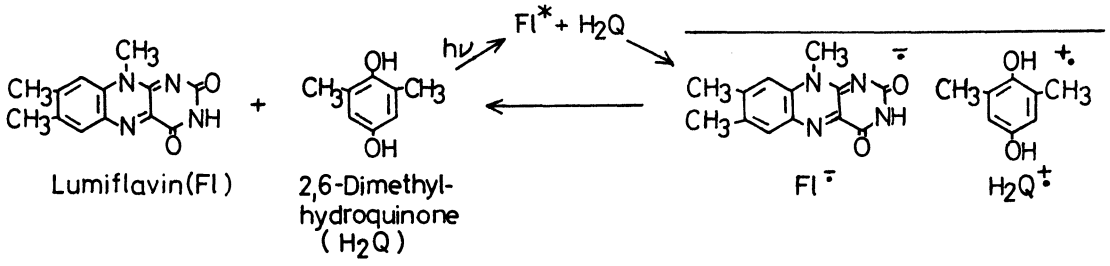
The photo-excited flavin was found to undergo the electron-transfer reaction with a variety of electron donors. The relative importance of the different spin states of flavin concerned is governed by the nature and the concentration of electron donors.

Photochemical behaviors of flavin have attracted much attention because flavin in its excited state was suggested to show the similar reactivity to that found in the biological system.[1] By applying CIDNP technique the photo-excited flavin was proposed to undergo the hydrogen-abstraction reaction from the hydroxyl group of phenols such as tyrosine in its triplet excited state.[2]

On the basis of the detailed study by CIDNP technique we wish to report here that the photochemical reactivity of flavin is delineated by the electron-transfer mechanism in nature — not by the hydrogen-abstraction mechanism as previously proposed[2] — and that the spin multiplicity concerned is dependent upon the nature and the concentration of the electron donor. In Fig.1 illustrated are the CIDNP signals observed upon irradiation of lumiflavin in the presence of 2,6-dimethylhydroquinone as an electron donor.[3] Two methyl-H's of lumiflavin (at the 8- and 10-position) showed enhanced emissions and the ring-H (at the 6-position) showed an enhanced absorption, respectively. Of 2,6-dimethylhydroquinone, on the other hand, an enhanced absorption of the methyl-H and an enhanced emission of the ring-H were detected. The similar polarization due to lumiflavin can be observed when other hydroquinones as well as phenols as the electron donor are subjected to the photo-induced reaction of flavin (see Fig.2). Hydroquinone dimethyl ether, which has no hydroxyl groups, was also found to give rise to the similar CIDNP signals in its reaction with the photo-excited lumiflavin. These results strongly suggest the contribution of electron-transfer process in the reaction of the photo-excited flavin (Eq.1). Based upon Kaptein's rule, the triplet excited state is suggested to make the major contribution in the photo-induced reaction of flavin with phenols and/or hydroquinones.[4]



(Eq.1)

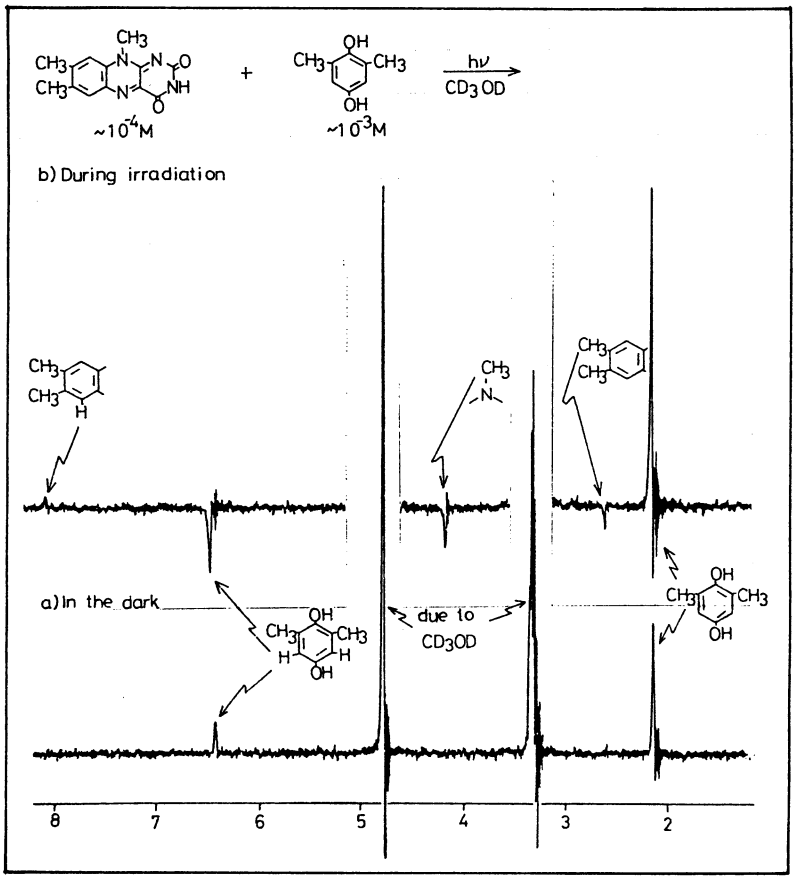


Fig.1. CIDNP Signals observed upon irradiation of lumiflavin in the presence of 2,6-dimethylhydroquinone.
(a) In the dark.
(b) During irradiation.
Solvent: CD_3OD (at room temperature).

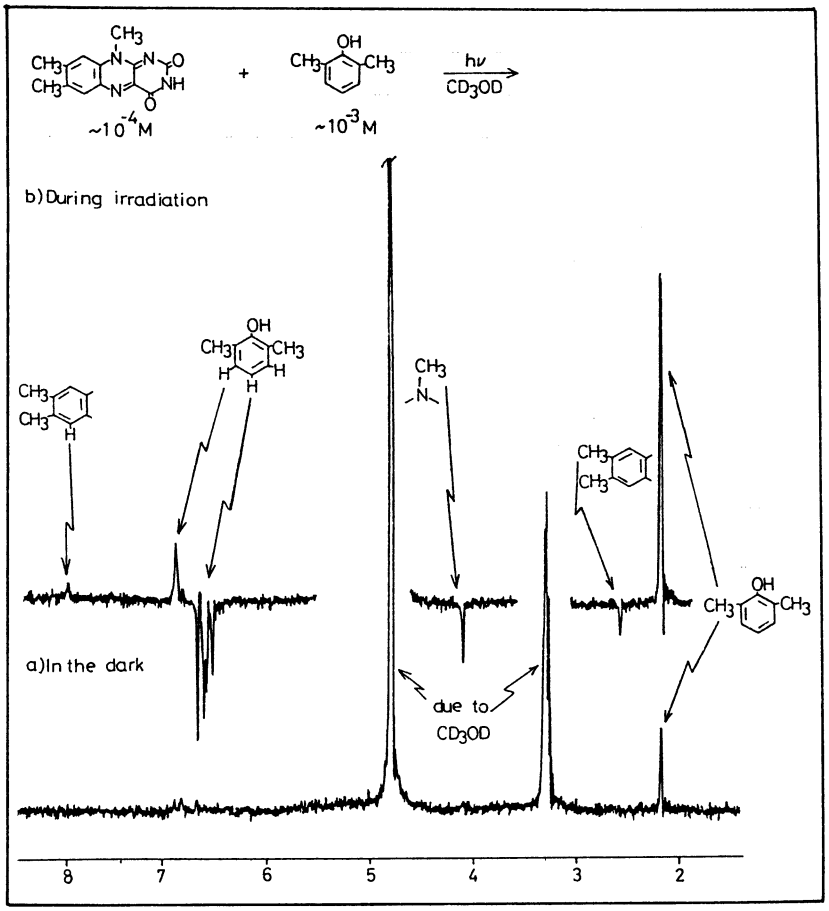


Fig.2. CIDNP Signals observed upon irradiation of lumiflavin in the presence of 2,6-dimethylphenol.
(a) In the dark.
(b) During irradiation.
Solvent: CD_3OD (at room temperature).

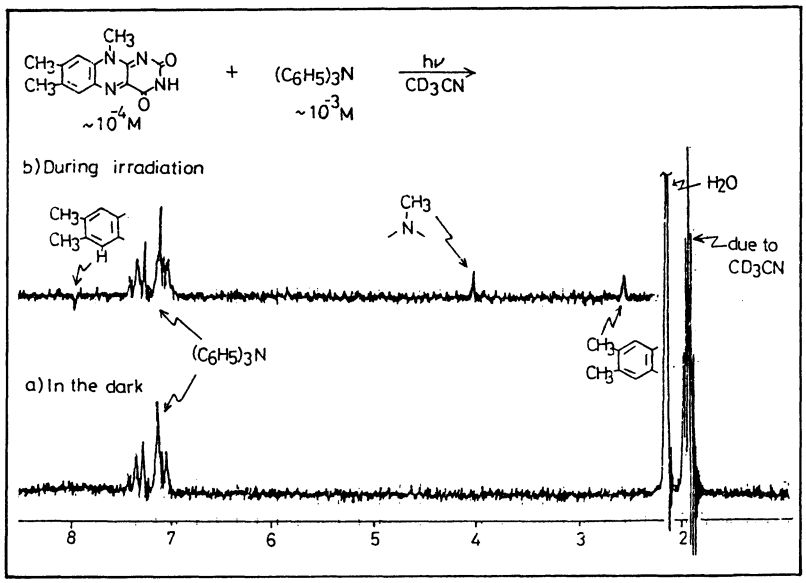


Fig.3. CIDNP Signals observed upon irradiation of lumiflavin in the presence of triphenylamine.
(a) In the dark.
(b) During irradiation.
Solvent: CD_3CN (at room temperature).

On the other hand, when amines such as triphenylamine were used as the electron donor, the observed CIDNP signals due to lumiflavin were completely inverted in their directions as shown in Fig.3. This result not only supports further the electron-transfer character of the flavin-sensitized reaction but also indicates the major contibution of the singlet excited flavin in its reaction with amines as the electron donor. Thus, the contributing spin state of the excited flavin in the electron-transfer reaction is dependent upon the nature of the electron donor.

The fluorescence of flavin, which indicates the intervention of the singlet excited state, is quenched linearly by the addition of the electron donor such as phenols, hydroquinones and/or amines. By analyzing the Stern-Volmer plot of the fluorescence quenching of lumiflavin, $k_q\tau$'s were estimated for typical donors as listed in Table 1. The fluorescence of lumiflavin was more efficiently quenched by amines as the electron donor than by phenols and/or hydroquinones. The efficient quenching by amines results in the overwhelming contribution of the singlet state to the photo-induced electron-transfer over that of the triplet state. Although the quenching efficiency by phenols and/or hydroquinones is rather poor, the relative contribution of the singlet state is expected to increase with the concentration of the electron donor such as hydroquinones. Actually at the higher concentration of hydroquinones,[5] the overwhelming contribution of the singlet radical pair was confirmed by the CIDNP signals, where the methyl-H's of lumiflavin (at the 8- and the 10-position) showed enhanced absorptions (similar to those in Fig.3). The more efficient quenching of the singlet excited state of flavin results in the more important contribution of the singlet radical pair in the flavin-sensitized reaction. Thus, it is concluded that the spin multiplicity of the major contributing excited flavin in the electron-transfer process is dependent upon the nature and the concentration of the electron donor.

Table 1. Fluorescence Quenching of Lumiflavin by Electron Donor (at 23°C)

Electron Donor	$k_q\tau$	Solvent
Triphenylamine	133 M^{-1}	CH_3CN
3-Methoxy-N,N-dimethylaniline	130	CH_3OH
Hydroquinone	81	CH_3CN
2,6-Dimethylhydroquinone	65	CH_3OH

References

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2) R. Kaptein, K. Dijksta, F. Müller, C. G. van Schagen, and A. J. W. G. Visser, J. Mag. Res., 31, 171 (1978). R. Kaptein, "Nuclear Magnetic Resonance Spectroscopy in Molecular Biology," ed by B. Pullman, D.Reidel, Holland (1978), p. 211.

3) Methanol-d_4, acetonitrile-d_3 and deuterium oxide as solvent gave the similar results of CIDNP study.

4) For the analysis of CIDNP signals, the g-value of flavin anion radical (g=2.0032)[a] is assumed to be smaller than those of hydroquinone cation radical (g=2.0034),[b] phenol cation radical and/or amine cation radical (g=2.0044).[c] The hyperfine coupling constant of the methyl-H's (at the 8- and the 10-position) of lumiflavin anion radical and the methyl-H of 2,6-dimethylhydroquinone cation radical are positive, and the ring-H (at the 6-position) of lumiflavin anion radical and the ring-H of 2,6-dimethylhydroquinone cation radical are negative, respectively.[a,b]
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b) P. D. Sullivan and J. R. Bolton, J. Am. Chem. Soc., 90, 5366 (1968).
c) A. Tench, J. Chem. Phys., 38, 593 (1963). W. C. Danen, C. T. West, and T. T. Kensler, J. Am. Chem. Soc., 95, 5716 (1973).

5) When the concentration of 2,6-dimethylhydroquinone was increased up to $\sim 10^{-2}$ M, the contribution of the singlet radical pair could be unequivocally indicated by the CIDNP signals due to lumiflavin, although the CIDNP signals due to 2,6-dimethylhydroquinone could hardly be observed because of its too high concentration.

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