Delayed Light Emission from Flavins in Solid Matrices

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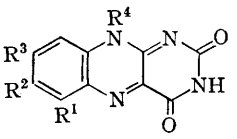
THE photochemistry of riboflavin and related isoalloxazine derivatives has been studied by several groups.[1] Flavin triplets have been implicated in some reactions.[2,3] Because of the possible role of flavin nucleotides in photobiological processes[1] it was argued that the inclusion of such molecules in solid matrices might give rise to properties which are more relevant to their biological function and are not directly observable for solutions.[4]

We now report our observations on the nature of the delayed light emission obtained when flavins trapped in solution in an acrylamide gel are illuminated. The preparations give transparent films. This delayed emission was measured with a spectrofluorimeter equipped with two 200 c./sec. choppers at right angles to each other in between the two monochromators. When the synchronised chopper disks are put out of phase (by a mechanical method) no direct fluorescence is detectable. Excitation is brought about by a 250 w stabilised xenon arc and emission from the front surface of polyacrylamide films is detected at an angled position with the aid of suitable mirrors, lenses, and a detector system.

At room temperature the delayed light emission can be identified as E-type fluorescence[5] which is brought about by thermal re-excitation of the triplet to the first singlet excited state. This is shown by the following observations: (a) the emitted light has the wavelength distribution of fluorescence (Table) and its excitation spectrum corresponds to the flavin absorption spectra; (b) the intensity of the emitted light is linearly dependent on the intensity of the exciting light; (c) no phosphorescence is observed at room temperature but at liquid nitrogen temperature the emission maximum is shifted to 605 mμ, the

TABLE

Delayed fluorescence decay times for flavin derivatives



Substituent				Trivial name	Half decay time at room temp. (msec.)
R^1	R^2	R^3	R^4		
H	Me	Me	Me	lumiflavin	38
H	Me	Me	ribityl	riboflavin	37
H	Me	Me	ribitylphosphate	FMN	41
H	Me	Me	ribitylphosphate-AMP	FAD	38
H	Me	H	$CH_2{\cdot}CH_2{\cdot}NEt_2$		29
H	Cl	H	$CH_2{\cdot}CH_2{\text{-}}NEt_2$		20
H	Cl	OMe	$CH_2{\cdot}CH_2{\cdot}N{\cdot}[CH_2]_3{\cdot}CH_2$		59
Me	Me	H	ribityl	isoriboflavin	22

The emission maxima at room temperature lie between 520—530 mμ and at liquid nitrogen temperature ~605 mμ.

wavelength of phosphorescence of FMN,[6] under these conditions no significant contribution at 530 mμ can be observed; (d) the lifetime of the emitted fluorescence is long (see the Table). These lifetimes are measured using a mechanical shutter and following the exponential decay curves on a Telequipment type S43 oscilloscope equipped with a polaroid camera. They are reproducible to within 10% and do not depend significantly on the concentration of the flavin in the acrylamide films, or on the concentration of the initiator employed in making the cross-linked polymer. They depend on the degree of drying of the films but reach a maximal value after about 48 hr. in a desiccator. Similar delayed emission is observed when FMN is trapped in a gelatin or a polyvinylpyrrolidone film, but the results are less satisfactory, because the films are opaque and the emission intensities are low.

The decay times for the various flavins cover the same relative range as their photochemical reactivities,[2,7] and in many cases follow the same relative orders. FAD is a notable exception in that its decay time is the same as that for FMN yet its photochemical reactivity is considerably lower.[2] This suggests that just like fluorescence[8] the triplet state is only observed in the unfolded form of FAD.

Our measurements oppose the view that the flavin triplet does not correspond to the measured phosphorescence at 605 mμ, for if phosphorescence emission occurred at half this energy (as predicted)[9] the thermal energy required for the regeneration of the singlet excited state from it would be too large (26 kcal./mole) in contrast to the 5—7 kcal./mole required if phosphorescence were at 605 mμ.

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