

EXCITED ELECTRONIC STATES OF FLAVIN-CONTAINING COENZYME MODELS

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Introduction

In order to gain further insight into the physical basis of flavin-catalyzed reactions, the interactions of a flavin with a second flavin and with other aromatic ring systems were studied. For this purpose compounds 2 through 5 (Fig.1) were synthesized (1,2). These compounds contain the interacting units in defined geometric orientation. A monomeric flavin (1) and glutathione reductase (6), a flavoenzyme of known active-site chemistry (3), were included as reference molecules. The present report deals with excited triplet states of the flavin compounds as studied by optical spectroscopy and optically detected magnetic resonances (ODMR) at 1.3K.

Results and Discussion

The flavin-containing compounds shown in Figure 1 were studied in polycrystalline form as well as at low concentrations in a 1:1 mixture of ethylene glycol/water which forms a glass matrix at 1.3K. Singulet and triplet states (via intersystem

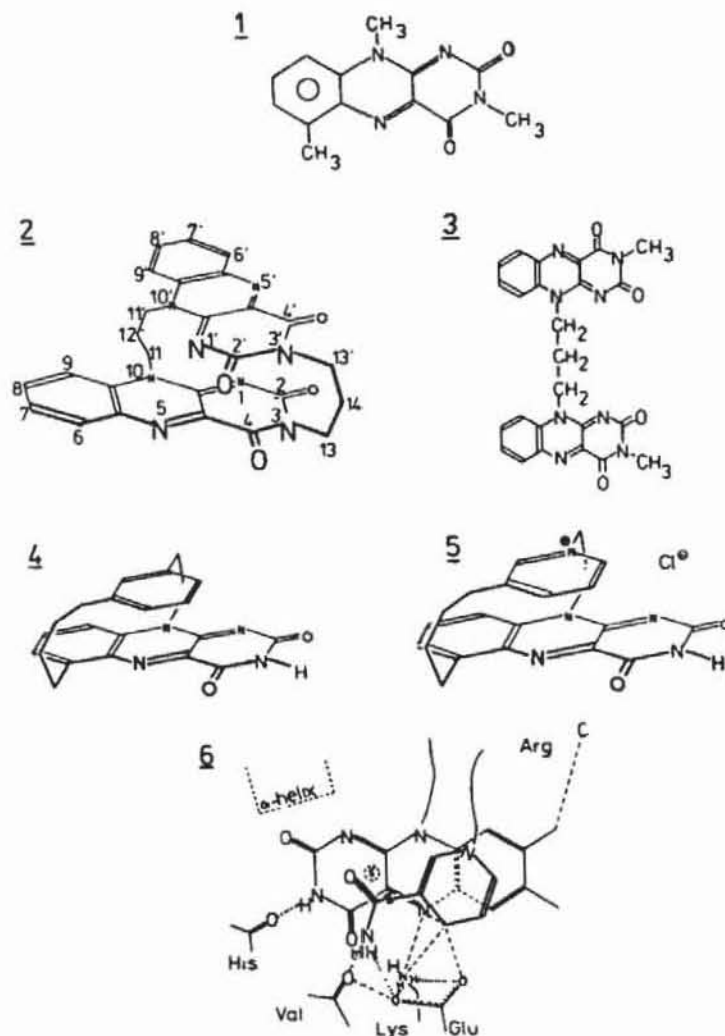


Figure 1. The Structures of the Flavin-containing Compounds 1-6.

1 = 3,6,10-Trimethylisoalloxazine,
2 = (3,3')(3,10')-Isoalloxazinophane,
3 = (10,10')-Trimethylenebis-(3,3'-dimethylisoalloxazine),
4 = (4)Metacyclo-(3)(10,6)-isoalloxazinophane,
5 = (4)(3,1)Pyridinio-(3)(10,6)-isoalloxazinophane chloride,
6 = sketch of one active site of glutathione reductase. The ring systems of flavin and nicotinamide are stacked. A number of amino acid residues located at the catalytic site are indicated.

crossing) were excited by using the 476.5 nm line of an argon ion laser. The characteristic emission spectra of flavins were obtained (Figs.2a and 2b, lower part) (4). In order to identify emissions with longer lifetimes than 1 ms - phosphorescence (Ph) and delayed fluorescence (DF) - the fluorescence of the spectra was eliminated by using crossed rotors in the excitation and detection pathways. All compounds showed a typical flavin phosphorescence; in addition, the flavin dimers 2 and 3 exhibited a relatively strong delayed fluorescence (Ref.4 and Fig.2a, upper part). While other flavin compounds (1,4-6) did not show DF, in the case of the dimers 2 and 3 a weak delayed fluorescence was observed even at a concentration of $<10 \mu\text{mol}$ per liter glass matrix (Fig.2b).

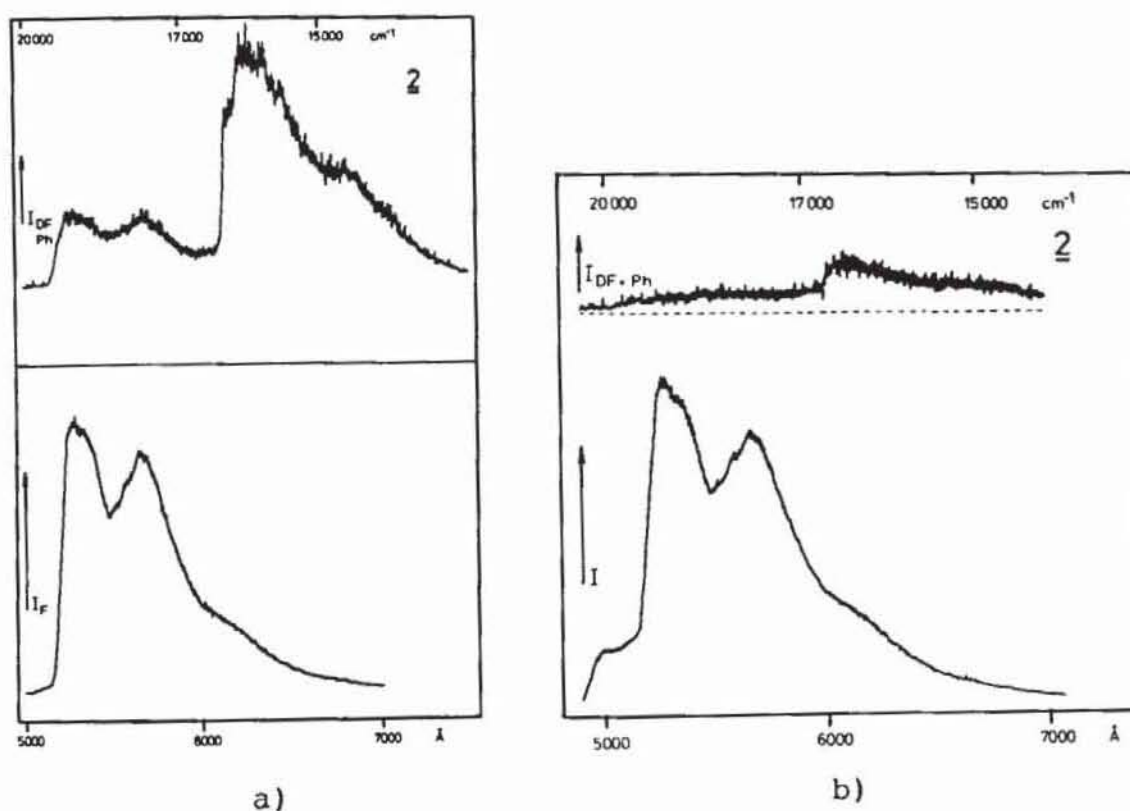
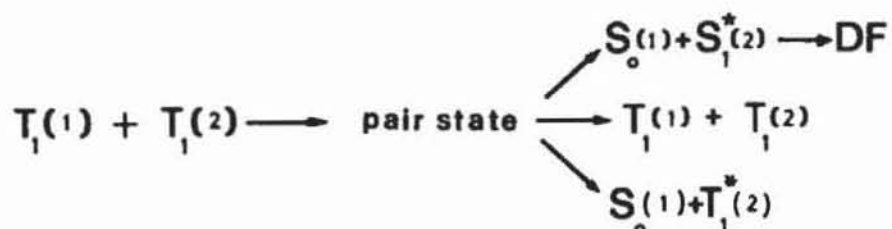


Figure 2. Emission Spectra of Compound 2 in Polycrystalline Form (a) and in 'Dissolved' Form (b). In each panel, the lower spectrum represents the total emission whereas the upper spectrum shows only the long-living emission contributed by delayed fluorescence (DF) and phosphorescence (Ph).

DF which was described so far only in crystals can be explained in the following way (5). The lowest excited triplet state is populated via intersystem crossing from the singlet manifold. In crystals, there are singlet and triplet exciton bands in which excitation energy can be transported over many lattice sites. A detailed description of such Frenkel excitons is given in ref. (6). The collision of two excitonic triplet states or one excitonic triplet state and a localized triplet trap state results in the formation of a pair state as shown in the following scheme.



Scheme of a Triplet-Triplet Annihilation

The two interacting triplet states are able to add their energies and spins and then to decay according to group-theoretical selection rules. One of the possible pathways leads to an excited singlet state. The deactivation of this state can be observed as a delayed fluorescence with a lifetime in the ms range. According to theory, this lifetime corresponds to the half-lifetime of a triplet exciton state (7). Within experimental error, our values of 23.5 ms and 8.5 ms meet this expectation (Table 1, lines 5 and 6).

As emphasized above, DF is based on an effective intersystem crossing process resulting in a high density of excitonic triplet states within crystals. The really surprising property of the flavin dimers is therefore the moderate DF observed in the case of isolated dimer molecules.

The pair formation of triplet states in an isolated dimer can occur only if both monomeric moieties are excited. Since the lifetime of triplet states is much longer than of the singlet state simultaneous excitation of triplet states of neighbouring units by singlet absorption seems possible provided that effective intersystem crossing takes place. In addition, the relatively short average distance between the two units allows an excitonic interaction leading to pair formation. In this context it should be emphasized that the interaction of the two flavin moieties in the excited triplet state must be very weak because otherwise symmetric charge transfer terms would prevent the two monomers from being excited simultaneously (8,9).

Table 1. Lifetimes of the Triplet States. Ph, phosphorescence; DF, delayed fluorescence

Compound	Emmission Wavelength		Lifetime at 1.3K
		nm	
<u>1</u>	Ph,	630	53
<u>2</u>	Ph,	630	< 3
<u>2</u>	Ph,	700	< 3
<u>2</u>	DF,	550	< 3
<u>3</u>	Ph,	610	23.5
<u>3</u>	DF,	550	8.7
<u>4</u>	Ph,	630	62
<u>4</u>	Ph,	650	62
<u>5</u>	Ph,	650	27
<u>6</u>	Ph,	630	41.6
Riboflavin-	Ph,	605	170
tetrabutyrate (15)	Ph,	640	100

An alternative explanation, namely that the observed DF is due to an effective absorption of two quanta by the same monomer, is unsatisfactory for two reasons: (i) the relatively long lifetime of the DF (Table 1) cannot be explained sufficiently by a 2-quanta absorption; (ii) we were unable to obtain a DF by direct singlet-triplet excitation ($S_0 \rightarrow T_1$) using a narrow banded dye laser. This indicates that in spite of the comparatively high quantum yield of the intersystem crossing process ($S_1 \rightarrow T_1$) (10) the $S_0 \rightarrow T_1$ transition is strongly forbidden. In consequence, an effective 2-quanta absorption seems unfavourable in this case. In contrast, the process of pair formation is not limited by intercombination selection rules and can - especially under these conditions - be most effective. Thus the indirect decay of the triplet state via pair formation and delayed fluorescence is able to compete with the direct phosphorescence decay. On the other hand the occurrence of DF in the case of the dimeric flavins 2 and 3 is not associated with a fundamental change of the electronic properties with respect to monomeric flavins.

Evidence for this are other spectroscopic data, such as the energy levels of the singlet and triplet states as well as the zero field splitting parameters and the triplet lifetimes. These data are shown in Tables 1 - 3 together with literature values for other flavins (11 - 15). In particular, the comparison of the $|D|$ and $|E|$ parameters of compounds 2 to 6 with those of monomeric flavins indicates that no appreciable charge transfer occurs in the triplet state T_1 ; otherwise one would expect a strong reduction of the $|D|$ parameters in 2 to 6 (16).

Table 2. Energies of the Singlet and Triplet Levels of Compounds 1 to 6 Measured as Polycrystalline Material. Protein crystals contain approximately 50% solvent; consequently the spectrum of 6 represents a mixture of crystalline and solvated molecules.

Compound	S_1 (cm^{-1})	T_1 (cm^{-1})
<u>1</u>	19840	16750
<u>2</u>	19460	16370
<u>3</u>	20200	17390
<u>4</u>	20450	17290
<u>5</u>	20120	17180
<u>6</u>	20600	17250
N3-Undecylflavin (15)	20956	-
8-Methylalloxazine (14)	20410	17545
Lumiflavin (14)	21275	-

Table 3. Fine Structure Parameters. Dashes (-) referring to 1 to 6 indicate that these parameters could not be determined experimentally.

Compound	$ D+E $ (MHz)	$ D-E $ (MHz)	$ 2E $ (MHz)	$ D _{-1}$ (cm^{-1})	$ E _{-1}$ (cm^{-1})
<u>1</u>	2164	1086,3	-	0,0542	0,0179
<u>2</u>	1985	1008	-	0,0499	0,0163
<u>3</u>	-	-	-	-	-
<u>4</u>	2071	1040	-	0,05188	0,0172
<u>5</u>	-	-	-	-	-
<u>6</u>	-	-	-	-	-
FAD (12,13)	-	-	-	0,059	0,019
Riboflavin (13)	-	-	-	0,059	0,019
Riboflavin (11)	-	-	-	0,061	0,015
Alloxazine (13)	-	-	-	0,079	0,019
FMN (13)	-	-	-	0,058	0,019

Conclusion

For the two compounds 2 and 3 containing two flavin units we detected delayed fluorescence (DF) associated with a most efficient excitonic interaction. In this process, two low-energetic quanta are transformed into one high-energetic quantum with an unusually high quantum yield. An activation energy as high as $34000 \text{ cm}^{-1} \approx 4.2 \text{ eV} \approx 96.8 \text{ kcal per mol}$ can be provided in this process. Therefore analogous molecular arrangements might be relevant for flavoenzyme-catalyzed reactions.

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