TRANSITION MOMENTS OF RIBOFLAVIN IN THE 300 nm REGION

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Electronic absorption, linear dichroism and polarized excitation spectra of riboflavin (RF) were investigated in anisotropic sheets of polyvinyl alcohol. The absorption spectrum of riboflavin was found to contain a distinct electronic transition, probably of the $\pi \rightarrow \pi^*$ type, responsible for the weak absorption band at about 308 nm. The orientation of the transition moment for the band was also determined.

1. Introduction

In spite of the vast amount of experimental data accumulated [1–3, 6, 8, 10, 11], it is still not clear which and what kind ($\pi \rightarrow \pi^*$ or $n \rightarrow \pi^*$) of electronic transitions are responsible for the absorption by riboflavin (vitamin B_2) in the ultraviolet. Especially controversial and difficult to detect are the transition moments in the range of ca 300 nm, where absorption by riboflavin is relatively weak.

Calculations by quantum chemistry methods fail to yield conclusive results. Fox et al. [11] have pointed to a $\pi \rightarrow \pi^*$ transition of low intensity ($f \simeq 0.056$) at 306 nm. From the calculations of Sun et al. [2], the $\pi \rightarrow \pi^*$ transition lies at 297 nm. Investigations of the quantum yield of riboflavin fluorescence in liquid solutions [1] do not rule out a $n \rightarrow \pi^*$ transition in this spectral region.

In the available literature of RF absorption spectra no experimental evidence is to be found for such transitions as separate bands, or contours of absorption bands.

Electronic absorption transitions of dyes in solution are likely to be influenced by the dispersing phase. The effect of a polymeric matrix on the dye molecules dispersed therein may, among other things, lead to a change in the symmetry of states and a partial "mixing" of states. Consequently, the probability of electronic transitions of the fainter absorption bands and the intensity of certain absorption bands will increase [3]. Therefore, it

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seems useful to investigate electronic transitions of dyes in solutions in which the dispersing phase is a polymeric matrix.

This work is intended to determine the absorption spectra, types of electronic transitions, and orientation of the transition moments responsible for the absorption of riboflavin in the spectral region at about 300 nm. Isotropic and anisotropic PVA sheets were used as a matrix.

2. *Methods of measurements*

Riboflavin ($C_{17}H_{20}N_4O_6$) from BDA Chemical Ltd. (England) and polyvinyl alcohol (PVA) from Loba-Chemie, Wien–Wischamend, were used. Riboflavin was studied in water solutions, PVA-water mixture, and isotropic and anisotropic PVA sheets. The solutions were prepared as described in our previous paper [4]. The RF concentration of the liquid solutions was 10^{-4} M, and that in PVA sheets 6×10^{-4} M. Absorption spectra were measured using a Specort UV–VIS Zeiss spectrophotometer and an „Opton" PMQ II spectrophotometer equipped with a double monochromator MM12Q. An arrangement designed by us was used for measuring slight changes in absorption anisotropy. The absorption of linearly polarized light ($A_{\parallel}$ and $A_{\perp}$) by a Glan prism was measured [6] (the error: $A \pm 0.0003$).

The absorption spectrum of riboflavin in isotropic and anisotropic sheets was obtained on the elimination of matrix absorption by calculations [7]. It is known that the matrix absorption in this spectral region is stronger than the absorption of riboflavin, so that conventional methods of absorption measurement fail. In the calculation method, however, the region 250–180 nm was used. Here two "strong" absorption bands occur, one related to PVA absorption and the other to RF. This allowed us to calculate the extinction of riboflavin with an accuracy of several per cent and to determine the nature of the RF spectrum in the region at about 300 nm.

The emission anisotropy (r) was measured by Price's method [5]. The angle (α) of incidence of the linearly polarized excitation light on the sheets was 55°. The fluorescence anisotropy (r) was calculated for two types of excitation: (*i*) with the electric vector of excited light parallel, and (*ii*) perpendicular to the stretch direction (denoted as $r_{\parallel}$ and $r_{\perp}$). Fluorescence was observed perpendicularly to the excitation beam.

3. *Results and discussion*

The absorption spectrum of riboflavin in the PVA matrix differs from that in aqueous solutions and PVA liquid solutions in the spectral interval from 300 to 320 nm (Fig. 1a). The absorption curve of riboflavin in isotropic and anisotropic PVA sheets reveals the presence of a separate absorption band with λ_{max} at ca 308 nm.

Linear dichroism calculated for anisotropic sheet (Fig. 1, curve *5*), markedly diminishes about ca 310 nm but retains a positive value. This indicates that the transition moment responsible for RF absorption in this spectral range is inclined with respect to the direction

of sheet stretch at an angle δ larger than the angle subtended by the transition moment of the band 366 nm, where $45° > \delta > \delta$ (366).

The results presented in Fig. 1b show that in the region of ca 305 nm fluorescence anisotropy decreases. The decrease in fluorescence anisotropy is particularly marked in anisotropic sheets. A similar shape of the polarized excitation spectra of riboflavin has been reported in [8]. With regard to the nature of the absorption spectrum, it seems un-

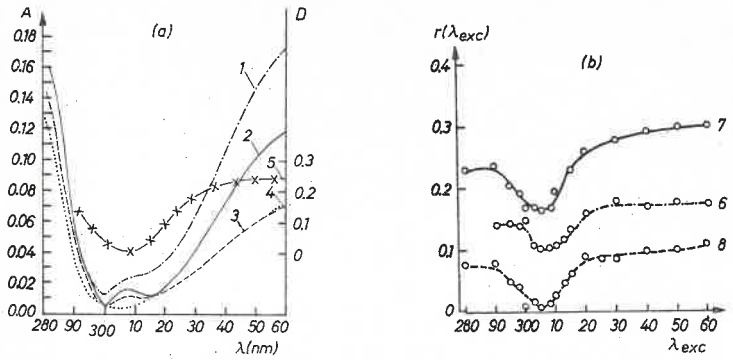

Fig. 1. Absorption spectra and polarized excitation spectra of riboflavin: (a) — absorption spectra of riboflavin: curve *1* — in isotropic sheets, curve *2* — parallel component of anisotropic sheets ($A_{||}$), curve *3* — perpendicular components of anisotropic sheets ($A_{\perp}$), curve *4* — in aqueous solution and liquid PVA solution, curve *5* — dichroism D of riboflavin in anisotropic sheets. $D = \frac{A_{||} - A_{\perp}}{A_{||} + A_{\perp}}$; (b) — polarized excitation spectra: curve *6* — in isotropic sheets, curve *7* — $r_{||}$ in anisotropic sheets, curve *8* — $r_{\perp}$ in anisotropic sheets. A — optical density, r — emission anisotropy

justified to assign a lower emission anisotropy to a group of vibrational transitions of different polarization than the other transitions within one of the two adjacent electronic bands [9]. Hence the change in emission anisotropy in this interval of excitation may be due to excitation within a distinct electric transition.

TABLE I

Anisotropy of emission of the riboflavin in isotropic sheets and relative orientation of the transition moments

r_{I}	r_{II}	$r_{II'}$	β_{I}	β_{II}	$\beta_{II'}$	$\theta_{I\,II}$	$\theta_{I\,II'}$
0.293	0.175	0.115	$25° \pm 2°$	$38° \pm 2°$	$>44°$	13°	$>19°$

where $\theta = \Delta\beta$

To determine the alignment configuration, within the molecule, of the transition moment responsible for the 308 nm band (II') with respect to the transition moments of the 366 nm and 446 nm bands (II and I, respectively), the polarized excitation spectrum of riboflavin in the PVA isotropic matrix in the region between 280 nm and 490 nm

was measured. The mean values of emission anisotropy in the maximum ranges of the bands I and II were used to calculate according to Perrin [5] the angles β between the emission oscillator and the corresponding absorption oscillators (β_{I} and β_{II}), (Table I).

The minimum value of the emission anisotropy of the isotropic sheet at 300 nm to 320 nm (Fig. 1(b), curve *6*) was used to estimate the angle $\beta_{\mathrm{II}'}$. The estimate is rough due to the fact that the value of the emission anisotropy r used for the calculations is the sum of the anisotropy of the emission excited at 308 nm and that of the two neighbouring strong bands and more strongly anisotropic. Hence, the minimum emission anisotropy value $r = 0.115$ obtained from these measurements is overestimated and the calculated value of the angle $\beta_{\mathrm{II}'} = 44°$ lowered.

On this basis (Table I), one can determine the approximate position of the absorption oscillator of the band II′ in the molecule (Fig. 2). It was assumed after Song [10] that the

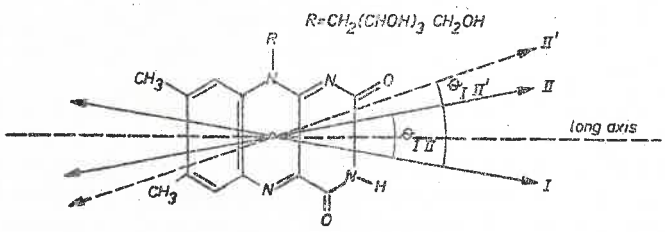

Fig. 2. Relative orientation of the transition moments of riboflavin

absorption oscillators of the bands I and II are oriented almost symmetrically with respect to the long axis of the riboflavin molecule.

The results reported here prove the existence of a distinct electronic transition, responsible for riboflavin absorption at ca 308 nm. The absorption oscillator of the transition is oriented at a larger angle to the emission oscillator and direction of sheet stretching than the oscillator of band II. It should be noted that vibrations out of the plane of the molecule can be induced by the matrix for vibronic states $\pi\pi^*$ [3]. These vibrations give similar changes in the emission anisotropy in the form of activated $n \rightarrow \pi^*$ transitions. Since, however, the angle $\theta_{\mathrm{I\,II}'}$ is much less than 90° it may be assumed that band II′ is associated with a $\pi \rightarrow \pi^*$ transition. This does not exclude the possibility that the $n \rightarrow \pi^*$ transition is also in this spectral region as suggested in a previous paper[1] [1].

The appearance of a weak absorption band with $\lambda_{\max}$ at ca 308 nm in the RF absorption spectrum in PVA sheets may be attributed to the active role of the polymeric matrix in perturbating the vibronic states of the dye as a result of which the probability of transitions in this excitation region increases.

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[1] The latest results of our study show that the lowest electronic state of riboflavin is rather a $\pi\pi^*$ than a $n\pi^*$ state, as had been suggested in our previous paper [1].

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