Academician of the Academy of Sciences of the Belorussian SSR A. N. SEVCHENKO, S. V. KONEV, M. A. KATYSHENKOV

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Figure 1

Figure 1: Figure 1

Abstract

Full Text

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ON THE OSCILLATOR NATURE OF THE LONG-WAVELENGTH ABSORPTION BAND OF TRYPTOPHAN

Weber (1), on the basis of the discrepancy between the polarization spectra of fluorescence and the absorption spectra of tryptophan, put forward the suggestion that two oscillators, corresponding to two different electronic transitions and oriented relative to one another at an angle close to a right angle, are responsible for the long-wavelength absorption band of tryptophan at 280 mμ. In this case the absorption spectrum of the "negative" oscillator, i.e., of the oscillator perpendicular to the fluorescence oscillator, lies within the broader absorption spectrum of the "positive" oscillator. This circumstance, in Weber' s opinion, leads to a dip in the polarization spectrum of fluorescence precisely at the place where the partial contribution of the negative oscillator to the total absorption is maximal (289 mμ).

In the present work, in order to resolve the question of the number and mutual orientation of the absorption oscillators, data from measurements of the polarization of fluorescence of anisotropic films, in which tryptophan is oriented along the direction of stretching, have been used. The films were prepared on the basis of polyvinyl alcohol in the following manner: aqueous solutions of tryptophan and polyvinyl alcohol were mixed, the mixture was poured onto a glass substrate, and the water was removed by drying in air. Then the optically transparent film was removed from the substrate and its volume was measured in order to estimate the final concentration of tryptophan in the film. To orient the tryptophan molecules, the film was stretched lengthwise on a special frame by approximately a factor of three.

Fig. 1. Absorption spectrum of tryptophan in polyvinyl alcohol. 2, 7—dependences $P = f(\lambda_{\text{excit.}})$ for tryptophan in an isotropic film of polyvinyl alcohol, respectively upon excitation with polarized light in the vertical and horizontal directions. 3, 4—the same as 2 and 7, but for tryptophan in an anisotropic film. 5, 6—spontaneous polarization of the fluorescence of tryptophan, respectively

for the vertical and horizontal positions of the stretched film.

Tryptophan in the film has the usual absorption spectrum (Fig. 1), shifted somewhat into the short-wavelength region, and a fluorescence spectrum with a maximum at 325 mμ, with the tryptophan triplet typical of low-temperature luminescence spectra. Already at tryptophan concentrations of $5 \cdot 10^{-3}$ M, in the spectral region of interest to us, the intensity of luminescence of the film material is approximately two orders of magnitude lower than the luminescence intensity of the activator.

The polarization spectra of fluorescence were recorded on an apparatus described by us earlier (2).

Excitation and observation of the luminescence were carried out from the side of the front face of the film, the film being positioned in such a way that its stretching axis coincided with the vertical principal direction.

Measurement of the polarization of luminescence was carried out at an angle of 90° to the direction of the exciting beam. When measuring the spontaneous polarization, a stack of quartz plane-parallel plates was placed at the exit of the spectrophotometer in order to eliminate the partially polarized light introduced by the monochromator.

Figure 1 shows the changes in the degree of polarization as a function of the wavelength of the exciting light, $P = f(\lambda_{\text{exc}})$—the polarization spectra for tryptophan in isotropic and anisotropic films, obtained upon excitation of fluorescence by polarized light in the vertical and horizontal directions, as well as the spontaneous polarization. For clarity, in addition to the dependence $P = f(\lambda_{\text{exc}})$, the figure also shows the spectrum of the long-wavelength absorption band of tryptophan in polyvinyl alcohol (curve 1).

From the data presented it follows first of all that the polarization spectrum of tryptophan in an isotropic film upon excitation by polarized light in the vertical plane, in the form and position of the maxima (curve 2), coincides with the polarization spectrum obtained earlier for viscous solutions of tryptophan (1). Upon excitation by polarized light in the horizontal direction, complete depolarization of the fluorescence is observed throughout the spectrum, in accordance with theory (curve 7) ([3]).

The fluorescence of tryptophan in stretched films upon excitation by natural light and observation along the exciting beam is polarized. The spontaneous polarization of tryptophan, caused by partial orientation of the molecules, as in the case of dyes, does not depend on the wavelength of the exciting light. Moreover, when the azimuth of the film is changed, i.e., the angle between the stretching axis of the film and the selected principal vertical direction ([3]), maximum positive values of the spontaneous polarization are obtained at $\psi = 0°$ and 180° (curve 5), and maximum negative values at $\psi = 90°$ (curve 6). At $\psi = 45°$ the degree of polarization is equal to zero. Such a dependence of the spontaneous polarization on the azimuth of the stretched film is very remarkable and may indicate that the emission oscillator and the long-wavelength absorption oscillator are oriented along the stretching axis of the film. This proposition has already been checked more than once on dye molecules (5). Upon excitation of the fluorescence of tryptophan by polarized light in the vertical direction, the polarization depends on λ_{exc} (curve 3), the course of the polarization spectrum coinciding with the course of the polarization spectrum in the isotropic film, but with a higher value of the degree of polarization. The latter circumstance, as is known, is due to the partial orientation of the luminescing molecules along the stretching axis. Upon excitation of the fluorescence of tryptophan by light polarized in the horizontal direction, the polarization also depends on λ_{exc}, but the shape of this polarization spectrum differs sharply from the polarization spectrum obtained with vertical excitation (curve 4). Thus, at the positions of the former maxima in this spectrum, minima are observed, and vice versa.

Let us consider in more detail the nature of the spectrum obtained. In this case all chaotically arranged molecules, according to theory, will give depolarized fluorescence throughout the entire absorption spectrum.

Therefore, the entire polarization spectrum can be formed only at the expense of oriented tryptophan molecules. However, a positive oscillator, located vertically along the stretching direction, cannot absorb light polarized in the horizontal direction. Therefore, in the case of a single oscillator, the polarization of the fluorescence should be low over the entire absorption band, as is the case for most dyes ([3]).

Consequently, the existence of a polarization spectrum of fluorescence directly indicates that we are dealing not with one, but with two oscillators. The second, negative oscillator, located at an angle close to

toward the direct positive one; as a result of the orientation of the film at an angle of 45° to the vector of the horizontally polarized exciting light, it is successfully excited by this component.

In this case the resulting polarization spectrum in fact reflects changes in the percentage contribution of the absorption of the negative oscillator relative to the total absorption of chaotically arranged molecules.

Judging from the form of 4, it may be concluded that the negative oscillator does indeed have an absorption maximum at 289 mμ, practically does not absorb at 300 mμ, has an absorption minimum at 270 mμ (the maximum of the polarization spectrum of chaotically arranged tryptophan), and an increase in absorption toward the short-wavelength side, which accounts for the fall of the polarization spectrum of tryptophan in solution in the region 255—240 mμ.

Thus, the complex character of the polarization spectra of tryptophan in viscous media can be interpreted in the following way. At least two oscillators are responsible for the long-wavelength absorption band, corresponding to two electronic transitions and oriented with respect to one another at an angle close to a right angle. In this case the maximum values of the degree of polarization at

$\lambda = 272$ and 302 mμ evidently correspond to electronic transitions for which the absorption and emission oscillators coincide in direction. The minimum values of the degree of polarization at $\lambda = 289$ mμ correspond to electronic transitions for which the absorption and emission oscillators do not coincide in direction. This conclusion is in good agreement with Weber' s previously proposed hypothetical scheme (^{1}) and may serve as its qualitative substantiation. At the same time, a similar dependence in the polarization spectrum may also be observed in the case when not two but three electronic transitions are responsible for the long-wavelength absorption band of tryptophan. Investigations in this direction are continuing.

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Note: Figure translations are in progress. See original paper for figures.