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WHAT CAUSES THE VARIATION OF POLARIZATION DEGREE ACROSS THE EMISSION SPECTRUM OF PROTEINS?

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A gradual decrease in fluorescence polarization across the emission spectrum on increase in wavelength has been recorded for a number of proteins and also for tryptophan, *N*-acetyltryptophan and glycyltryptophan. Various factors responsible for this dependence have been analyzed. It is shown that if the emission originates from both the ^1L_a and ^1L_b states, the position and form of the fluorescence spectrum polarization components as well as the slope of the dependence of the degree of polarization upon emission wavelength must always vary with the excitation wavelength. However, this condition, although necessary, is not enough to prove the participation of ^1L_b in emission. The dependence of the form of the emission polarization spectrum upon excitation wavelength obtained for some proteins is explained by tyrosine residues contributing to the emission. Consequently, there are no reasons for assuming that the ^1L_b oscillator participates in emission. It has been observed that for individual emitting centres, the slope of the dependence of the degree of polarization upon emission wavelength is determined by alteration of the vibrational substates, between which the transition with radiation takes place. The heterogeneity in the microenvironment properties of separate tryptophan residues in multitryptophan proteins and the existence, under certain conditions, of a correlation between the radiative lifetime of the emitting centre (determining the degree of the emission polarization) and the completeness of the microenvironment orientational relaxation (determining the emitted quantum of energy) can also affect the slope of this dependence.

1. Introduction

Practically all authors of works on the ultraviolet fluorescence of proteins assume that the emission originates solely from the ^1L_a state. Only in a few papers [1,2] have the contributions of both $^1L_a \rightarrow A$ and $^1L_b \rightarrow A$ transitions to tryptophan fluorescence been indicated. Recently, the question of the dual emission has re-emerged owing to the assumption that the $^1L_b \rightarrow A$ transition contributes to the fluorescence of the proteins with a short-wavelength spectrum position, such as azurin, ribonuclease T_1 and C_2 and L-asparaginase [3]. The solution to the problem of the possible existence of the dual emission in proteins and of the conditions that give rise to it is of great importance for the study of the dynamics of the structure of proteins on the basis of their intrinsic polarizational fluorescence and for understanding of the spectroscopic properties of indole, its derivatives and tryptophan residues in proteins in general. The suggestion that not only the long-wavelength absorption band but also the emission spectra of proteins are due to the two electronic transitions was based on the experimentally obtained variations in polarization across the emission spectrum for proteins and on the existing explanation of such dependences for indole by the contribution of ^1L_b to the emission [4]. However, the results of investigations on such dependences for model compounds are extremely controversial [4–8]. Moreover, the question of what this depen-

dence will be when only one oscillator is responsible for the absorption and emission is also debatable [9–12].

In this work we have examined how convincing the conclusions are concerning the participation of ^1L_b in the emission, based on the study of the dependences of polarization upon emission wavelength; how the $^1L_b \rightarrow A$ radiation is reflected in the spectral and polarizational characteristics; what can serve as a criterion for the participation of ^1L_b in the emission and whether such a situation occurs in proteins. We also attempted to clarify the factors that determine the dependence of the fluorescence polarization across the emission spectrum.

2. Materials and methods

All luminescence measurements were made with a spectrofluorimeter described earlier [13]. Since the existence of a dependence of the fluorescence anisotropy upon the emission wavelength for complex molecules is a problem in itself [9–12], special attention has been paid in this work to the procedure of carrying out fluorescence polarization measurements, the obtained dependences of P upon emission wavelength having been repeatedly rechecked. The degree of emission polarization is determined as follows:

$$P = \frac{I_V^V - FI_V^H}{I_V^V + FI_V^H}, \qquad (1)$$

where $F = I_H^V / I_H^H$ is the coefficient characterizing the contribution of the vertical (V) and horizontal (H) components to the exciting radiation [14]. I_V^V, I_H^V, I_V^H and I_H^H are the components of the emission for different orientations of the electric vector of the plane polarized exciting radiation (the superscripts refer to the state of polarization of the exciting radiation, and the subscripts to the state of polarization of the emitted flux). Eq. 1 can be easily derived from the generally accepted expression for the determination of the degree of polarization:

$$P = \frac{I_V^V - GI_H^V}{I_V^V + GI_H^V} \qquad (2)$$

by substituting in eq. 2 the expression of the correction coefficient $G = I_V^H / I_H^H$ that characterizes the difference in the sensitivity of the recording system to the vertical (V) and horizontal (H) components of the emission. As expected, the value of the correction coefficient G depends upon the wavelength of observation, which leads to an additional and quite significant error in determining the dependence of P upon emission wavelength. The advantage of the procedure used in these measurements lies in that F does not depend upon the emission wavelength. At the same time, it should be pointed out that for our apparatus F differs considerably from unity and depends essentially upon the excitation wavelength. This means that significant errors may occur in determination of the absolute degree of polarization when made without a polarizer in the excitation beam (since 'natural' radiation is always partially polarized) and that the polarization spectra may be distorted (since the value of F can change with λ_{ex}).

The dependences of P upon λ_{em} have been measured in the wavelength range from 305–315 to 380 nm. The upper limit of wavelengths was determined by the technical characteristics of the polarizing prism (analyzer) we used. The lower limit of emission wavelengths depends upon the quantum yield, the fluorescence spectrum position of the object under test and excitation wavelength. The longer the wavelength region of excitation, the lower the fluorescence quantum yield and the longer the wavelength range of the fluorescence spectrum the narrower is the region of reliable measurements of emission polarization. It should be noted that emission polarization measurements in the short-wavelength region of the fluorescence spectrum always present a difficult problem owing to the possible influence of scattered exciting light and to the fluorescence of solvent impurities. For these reasons we doubt the reliability of the curves P (λ_{em}) presented in ref. 15 for some proteins in the short-wavelength region of their spectra.

The study was conducted with DL-tryptophan (chemically pure, Soviet product), *N*-acetyl-DL-tryptophan (Chemapol, Czechoslovakia), glycyl-DL-tryptophan (Reanal, Hungary), bovine serum albumin (Koch-Light, England) and trypsin

(Spofa, Czechoslovakia; additionally purified by chromatography on CM-cellulose). We used L-asparaginase preparations produced by the Institute of Organic Synthesis, Academy of Sciences of the Latvian S.S.R. (Riga). Rabbit actin preparations were kindly provided by S.Yu. Khaitlina, basic myelin protein by Ya.T. Terletskaya (Institute of Molecular Biology and Genetics, Academy of Sciences of the Ukrainian S.S.R., Kiev) and human ceruloplasmin by K.A. Moshkov (Institute of Experimental Medicine, Academy of Medical Sciences of the U.S.S.R., Leningrad). Measurements were made in buffer solutions: at pH 3 for trypsin and at neutral pH for other proteins. Glycerol (Fluka, Switzerland) with low absorptivity and low impurity fluorescence in the working region of the spectrum was used as the viscous medium. Glycerol concentration in the solutions, i.e., their viscosity, was determined according to tabulated data [16] by means of the refractive index obtained with an Abbé refractometer (Zeiss, Jena, G.D.R.). All measurements, unless otherwise mentioned, were performed at 25.0 ± 0.5°C. The accuracy of measurement of the degree of polarization was ± 0.002.

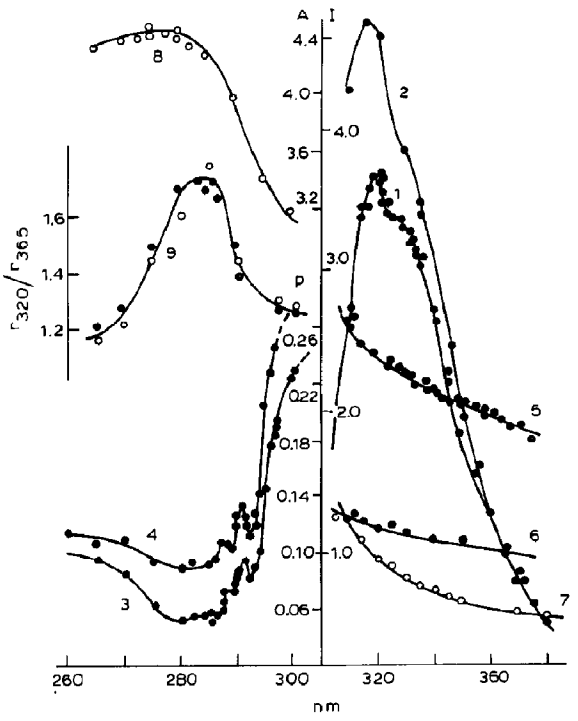

Fig. 1. Spectral and polarizational characteristics of L-asparaginase. (1, 2) Fluorescence spectra excited at 296.8 and 280 nm; (3, 4) polarizational spectra monitored at 365 and 320 nm; (5, 6, 7) dependences of P upon emission wavelength, excited at 296.8, 265 and 280 nm; (8, 9) dependences of the parameter A and the ratio r_{320}/r_{365} on excitation wavelength. On curve 9 experimental values are indicated by open circles and calculated values by closed circles.

3. Results and Discussion

A gradual decrease in fluorescence polarization across the emission spectrum with increasing wavelength was observed for all the proteins studied (figs. 1 and 2). The curves are similar to those registered for some other proteins in refs. 1 and 2. The gradient of the dependences of P upon λ_{em} was characterized quantitatively by using the ratio of the emission anisotropies at 320 and 365 nm: $A_r = r_{320}/r_{365}$. The parameter A_r was chosen by analogy with the parameter $A = I_{320}/I_{365}$ [17], that fits well for characterizing the form and position of the ultraviolet fluorescence spectra of proteins. It has been found that the value of this parameter is always greater than unity; it varies somewhat for different proteins and in certain cases depends upon the excitation wavelength. For the majority of proteins A_r is greater than that for tryptophan, *N*-acetyltryptophan and glycyltryptophan in glycerol/water solutions. Both for these model components with low molecular weight and for proteins, the value of A_r depends, though very slightly, upon the solution viscosity (see table 1).

3.1. How the dual emission is reflected in the spectral and polarizational characteristics

While studying the question of how the existence of the dual emission should be reflected in the spectral and polarizational characteristics, two cases have been analysed: (1) the rate constant of the interconversion $^1L_b \rightsquigarrow {}^1L_a$ is similar to the rate of deactivation of the excited state ^1L_b ($k_{ba} \approx k_b$); (2) the process of interconversion between

Table 1

Gradients of the dependences of P upon λ_{em} for proteins and model compounds

Values of r_{320}/r_{365}, obtained by extrapolation to conditions of infinite viscosity, are given in parentheses.

Object	r_{320}/r_{365}	
	λ_{ex} = 280 nm	λ_{ex} = 296.8 nm
Basic myelin protein	2.55	1.85
Trypsin (pH 3.0)	1.26	1.25
L-Asparaginase	1.60	1.30
Human ceruloplasmin	1.65	1.15
Rabbit G-actin		1.55
Rabbit F-actin		1.23
Bovine serum albumin	1.35	1.24
		(1.18)
Human serum albumin	1.42	1.20
		(1.22)
Indole in 99% glycerol	1.10	
DL-Tryptophan in		
88% glycerol	1.35	
98% glycerol	1.10	
N-Acetyl-DL-tryptophan in		
87% glycerol	1.16	
98% glycerol	1.12	
Glycyl-DL-tryptophan in		
89% glycerol	1.17	
99% glycerol	1.11	
	(1.08)	

^1L_a and ^1L_b states is much faster than that of deactivation of the excited states (k_{ba}, $k_{ab} \gg k_a$, k_b), i.e., a Bolzmann distribution between the excited states ^1L_a and ^1L_b is attained before emission. It is evident that in the first case the form and position of the fluorescence spectrum are dependent on, and in the second case independent of the initial populations of the ^1L_a and ^1L_b states, i.e., upon the wavelength of the exciting light (see, e.g., ref. 17). Nonetheless (as will be shown later), even if fast interconversion is ensured (k_{ab}, $k_{ba} \gg k_a$, k_b), the polarizational components of the emission spectrum and the ratio r_{320}/r_{365} will still depend upon the initial populations of ^1L_a and ^1L_b. This occurs because the emission anisotropy retains 'the memory' of its origin: whether it arose directly from absorption of a quantum exciting radiation or from energy interconversion from the other, previously excited, state. The latter is due to the fact that $r_a \neq r_{b \rightsquigarrow a}$ and $r_b \neq r_{a \rightsquigarrow b}$, while the emission anisotropy is determined as follows:

$$r = r_a f_a + r_b f_b + r_{b \rightsquigarrow a} f_{b \rightsquigarrow a} + r_{a \rightsquigarrow b} f_{a \rightsquigarrow b}. \quad (3)$$

The relationship between r_a, r_b and $r_{a \rightsquigarrow b}$, $r_{b \rightsquigarrow a}$ is

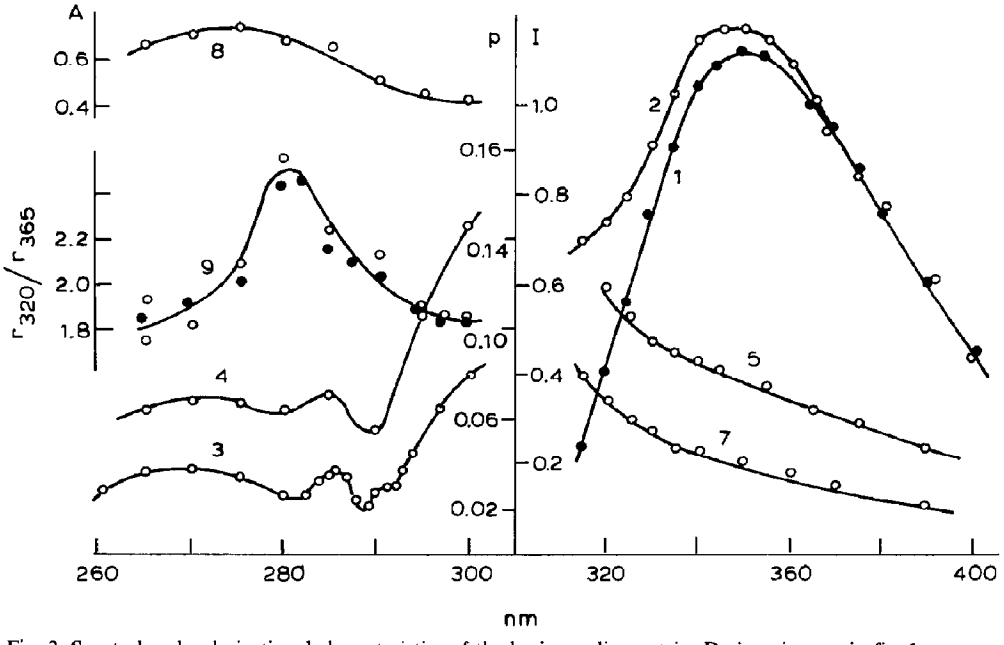

Fig. 2. Spectral and polarizational characteristics of the basic myelin protein. Designations as in fig. 1.

defined by the ratio:

$$r_{a\to b(b\to a)} = \tfrac{1}{2}(3\cos^2\alpha - 1)r_{b(a)}, \quad (4)$$

where α is the angle between the direction of the donor absorption and acceptor emission transition moments (it is assumed that α does not depend upon which oscillator is the donor and which the acceptor). If we admit that the angle between ^1L_a and ^1L_b transition moments equals 90° [19], then the molecules in which interconversion precedes emission will contribute negatively to the emission anisotropy ($r_{a\to b} = -1/2\ r_b$; $r_{b\to a} = -1/2\ r_a$). Using the known expressions for determination of the populations of closely spaced excited states (see, e.g., refs. 18 and 20), we can obtained their contributions to the emission f_a, f_b, $f_{a\to b}$ and $f_{b\to a}$. Eq. 3 will then be as follows:

$$r = r_0\big[(p_a q_a \kappa_{ba} + p_b q_b \kappa_{ab}) + (3\cos^2\alpha - 1)(q_a p_b \kappa_{ba} + q_b p_a \kappa_{ab})\big] \times \big[p_a q_a \kappa_{ba} + p_b q_b \kappa_{ab} + p_b q_a \kappa_{ba} + p_a q_b \kappa_{ab}\big]^{-1}, \quad (5)$$

where p_i and q_i are the fractions of the i-th state in absorption and emission, respectively; it is assumed that $r_a = r_b = r_0$ (r_0, limiting anisotropy). By virtue of eq. 5, we obtain:

$$\left(\frac{r_{320}}{r_{365}}\right)_{\lambda_1} - \left(\frac{r_{320}}{r_{365}}\right)_{\lambda_2} = f(\lambda_1, \lambda_2)\big(p_{b,\lambda_1} p_{a,\lambda_2} - P_{b,\lambda_2} P_{a,\lambda_1}\big) \times \big(q_{a,365} q_{b,320} - q_{a,320} q_{b,365}\big), \quad (6)$$

where $f(\lambda_1, \lambda_2) > 0$. Thus, even if dual emission results from two closely spaced electronic states in thermal equilibrium, it will affect the dependence of the polarization upon the emission wavelength. The gradient of the dependence of P upon λ_{em} will not change with excitation wavelength only if the absorption or emission spectra of the ^1L_a and ^1L_b transitions coincide. Since it does not occur in the case of dual emission, the gradient of the curve $P(\lambda_{em})$ must always depend upon the excitation wavelength. The position and form of the fluorescence spectrum (parameter A) will also depend upon the excitation wavelength if the emission from the ^1L_a and ^1L_b states initiates before the Bolzmann distribution is attained. However, one should bear in mind that there are some other factors responsible for the dependence of the ratio r_{320}/r_{365} and parameter A upon the excitation wavelength. This is why the existence of such dependences is a necessary but insufficient condition for the participation of ^1L_b in emission.

3.2. Spectral and polarizational characteristics of proteins and model compounds

For all the investigated proteins, except trypsin, the dependences of r_{320}/r_{365} and A upon λ_{ex} are of complex character (figs. 1–4). With regard to the participation of the ^1L_b transition moment in emission, the possibility of which has been assumed in the literature (see, e.g., ref. 3), we can presume that the dependences found are caused by the changes in the contributions of the ^1L_a and ^1L_b transition moments across the absorption and emission bands. However, such dependences have been obtained not only for proteins with a short-wavelength fluorescence spectrum position (L-asparaginase), in which a significant emission resulting from the ^1L_b state was assumed [3], but

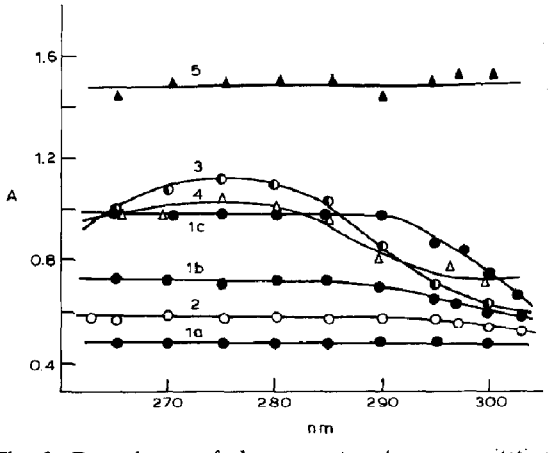

Fig. 3. Dependences of the parameter A upon excitation wavelength. (1) Tryptophan in aqueous solution (a), 98% glycerol (b) and 98% glycerol at 10°C (c); (2) *N*-acetyltryptophan in 98% glycerol; (3) mixture of tryptophan (3×10^{-5} M) and tyrosine (5×10^{-5} M) in 98% glycerol; (4) bovine serum albumin; (5) trypsin.

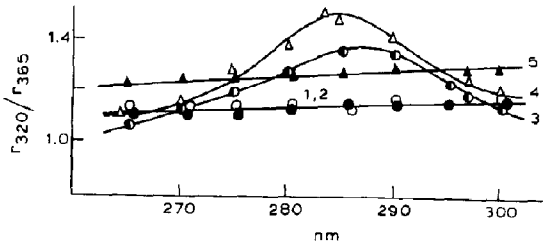

Fig. 4. Dependences of the ratio r_{320}/r_{365} upon excitation wavelength. Designations as in fig. 3.

also for those with a long-wavelength spectrum position (e.g., myelin basic protein).

Comparison of the fluorescence spectra, measured at different excitation wavelengths, and construction of the differential spectra bear witness to the fact that the dependence of the parameter A upon excitation wavelength is due to alteration in the contribution of tyrosine residues to the emission, whose relative value at 320 nm is determined as follows:

$$f_{\mathrm{Tyr},320}(\lambda) = \left(\frac{I_{\mathrm{Tyr}}}{I}\right)_{320} = \frac{A_\lambda - A_{300}}{A_\lambda}, \qquad (7)$$

where $A_\lambda = (I_{320}/I_{365})_\lambda$ is the parameter that characterizes the form and position of the fluorescence spectrum at the excitation wavelength λ. The relative number of tyrosine and tryptophan residues in the studied proteins varies greatly, viz., 12 : 1 for L-asparaginase, 4 : 1 for basic myelin protein, 18 : 2 for bovine serum albumin, 18 : 1 for human serum albumin, 64 : 22 for ceruloplasmin and 10 : 4 for trypsin. However, in accordance with existing literature, the contribution of tyrosine residues to emission does not correlate with their relative contents in protein. Although nearly 35% of the exciting light is absorbed by tyrosine residues in trypsin upon excitation at 280 nm, they practically do not contribute to the emission (see, e.g., ref. 21).

The change in the ratio r_{320}/r_{365} across the absorption spectrum appears to be greatest for proteins with a considerable contribution from tyrosine to the emission spectrum. It has been assumed that the bell-like character of the dependence $r_{320}/r_{365} = f(\lambda_{ex})$ is determined by the increased contribution of tyrosine residues to absorption and their simultaneously decreased emission polarization with a decrease in excitation wavelength. In confirmation of this hypothesis a similar dependence has been obtained for a mixture of tryptophan and tyrosine in water/glycerol solution (fig. 4). Although the cause of the change in r_{320}/r_{365} with excitation wavelength is the dependence of the relative contribution of tyrosine residues to the absorption, the value of this effect, i.e., the amplitude of the changes in r_{320}/r_{365}, is determined by the relative contribution of tyrosine residues to the emission and by the values of the emission polarization of tyrosine and tryptophan residues in proteins. The ratio r_{320}/r_{365} is related to the above-mentioned values as follows:

$$\frac{r_{320}(\lambda)}{r_{365}(\lambda)} = \frac{r_{320,\mathrm{Tyr}}(\lambda) f_{\mathrm{Tyr}}(\lambda)}{r_{365}(\lambda)} + \frac{r_{320,\mathrm{Trp}}(\lambda)(1 - f_{\mathrm{Tyr}}(\lambda))}{r_{365}(\lambda)}, \qquad (8)$$

where $r_{365}(\lambda)$ is the experimental value of the fluorescence anisotropy registered at 365 nm; $r_{320,\mathrm{Trp}}(\lambda)$ and $r_{320,\mathrm{Tyr}}(\lambda)$ are the fluorescence anisotropies of tryptophan and tyrosine residues at $\lambda_{\mathrm{reg}} = 320$ nm. The value of $r_{320,\mathrm{Trp}}(\lambda)$ can be calculated on the assumption that the form of the polarization spectrum of tryptophan residues does not depend upon the emission wavelength:

$$r_{320,\mathrm{Trp}}(\lambda) = r_{365}(\lambda)\frac{r_{320}(300)}{r_{365}(300)}. \qquad (9)$$

The value of $r_{320,\mathrm{Tyr}}(\lambda)$ can be determined as follows:

$$r_{320,\mathrm{Tyr}}(\lambda) = \kappa r_0(\lambda), \qquad (10)$$

where $r_0(\lambda)$ is the polarization spectrum of tyrosine fluorescence in a solution of infinite viscosity (see, e.g., ref. 19) and κ the coefficient characterising the degree of depolarization for tyrosine residues in protein governed by the change in their orientation during the lifetime of the excited state (intramolecular mobility and rotational motion with macromolecule as a whole) and by nonradiative Tyr-Tyr energy transfer. The value of κ can be

determined as follows:

$$\kappa = \langle \kappa(\lambda) \rangle = \left\langle \frac{r_{320}(\lambda) - \left(1 - f_{Tyr}(\lambda)\right) r_{320,Trp}(\lambda)}{f_{Tyr}(\lambda) r_0(\lambda)} \right\rangle. \quad (11)$$

The value of $f_{Tyr}(\lambda)$, determined from eq. 7, depends upon the absorption of tyrosine (ε_{Tyr}) and tryptophan (ε_{Trp}) residues and their quantum yields (q_{Tyr} and q_{Trp}) in protein:

$$f_{Tyr}(\lambda) = \frac{1}{1 + \frac{\varepsilon_{Trp}}{\varepsilon_{Tyr}} \cdot \frac{q_{Trp}}{q_{Tyr}}}. \quad (12)$$

This equation is valid for solutions of low absorbance ($A \to 0$). Figs. 1 and 2 show that the experimental and calculated curves $r_{320}(\lambda)/r_{365}(\lambda) = f(\lambda)$ for basic myelin protein and L-asparaginase coincide fairly well. This proves the validity of the proposed interpretation of these dependences. In the case of trypsin, for which the value of the parameter A remains fairly constant across the emission band, i.e., the contribution of tyrosine fluorescence in emission is small, the value of the ratio r_{320}/r_{365} practically does not depend upon the excitation wavelength (fig. 4). As expected, the ratio r_{320}/r_{365} remains fairly constant across the emission spectrum for tryptophan, *N*-acetyltryptophan and glycyltryptophan in viscous solutions. For these compounds in liquid solution the parameter A also remains constant across the emission band. The red shift of the fluorescence spectra of these compounds (decrease in the parameter A) with increasing excitation wavelength in the long-wavelength absorption edge in viscous solutions (fig. 3) can be easily explained by the so-called heterogeneous spectrum broadening theory (see, for instance, refs. 22–24). The same effect may be the cause of the decrease in A at the long-wave excitation in the case of proteins, but this is difficult to distinguish from that of the change in A due to the decrease in the contribution of tyrosine residues to the emission in this spectral region of excitation.

As follows from this analysis of the spectral and polarizational characteristics, there are no reasons to assume the participation of the ^1L_b oscillator in emission (even for proteins with a short-wavelength spectrum position, such as L-asparaginase).

In conclusion, it should be pointed out that the contribution of tyrosine residues to the emission in the short-wavelength region of the fluorescence spectrum determines not only the 'steepness' of the dependences of P upon λ_{em}, but also the dependence of the excitation polarization spectrum form upon the registration wavelength. It has thus been shown that the contribution of tyrosine residues to the emission is an essential factor determining the form of the excitation polarization spectrum, registered at the short-wavelength region.

3.3. Reasons for the appearance of the dependence of P upon λ_{em} across the emission band

It has been shown in section 3.2 that the emission of tyrosine residues can affect the dependence of P upon λ_{em}. However, the form of the curves remains the same for the model compounds (tryptophan, *N*-acetyltryptophan and glycyltryptophan), trypsin, for which the contribution of tyrosine residues in emission is small, and all other proteins at the excitation in the long-wavelength absorption band, where the contribution of the tyrosine residues is negligible. Therefore, the contribution of tyrosine residues to the emission cannot be the initial cause of the dependence of the fluorescence anisotropy across the emission band.

We shall now analyse some other factors that can determine or influence the dependence in question. It is well known (see ref. 25) that the fluorescence spectra of separate tryptophan residues may differ one from another. If, at the same time, some other characteristics, such as the lifetime of the excited state and the participation of the indole rings of tryptophan residues in intramolecular mobility, also differ, then this may well lead to the dependence of the fluorescence anisotropy upon the registration wavelength. In the case of tryptophan, *N*-acetyltryptophan and glycyltryptophan, the heterogeneity of the fluorescence centres may be due to the existence of equilibrium populations of conformers that differ

in spectral position and other fluorescence characteristics [26]. We cannot exclude the heterogeneity of emission centres even in the case of proteins with a single tryptophan residue, since some of them exhibit decay curves of a multiexponential character and the contributions from different components vary across the emission spectrum (see, e.g., refs. 27–32). Without denying the possible contribution of heterogeneity of the emission centres to the registered curves, we may also claim that this factor is not the initial cause of the dependence under discussion because alteration of the fluorescence anisotropy across the emission spectrum proceeds in the same way for indole in 98% glycerol.

One more factor that determines the form of the dependences of P upon λ_{em} may be the spectral dependence of the lifetime of the emitting molecule [33]. The latter can be explained in the following way: molecules with a longer lifetime radiate a light quantum of lower energy (i.e., their contribution to the emission is greater in the long-wavelength region of the fluorescence spectrum) and at the same time, due to the longer lifetime their emission is more strongly subjected to rotational depolarization (see, e.g., ref. 9). Evidently, this factor can occur only in the case when the time of rotational relaxation of the fluorescent group, the time of the relaxation of its microenvironment and the lifetime of the excited state are of a comparable magnitude. In order to evaluate the role of this factor, the $P(\lambda_{em})$ curves have been measured for tryptophan, *N*-acetyltryptophan, glycyltryptophan and some proteins in glycerol/water mixtures of different viscosity (table 1). It has been found that the slope of the curves decreases somewhat with increasing viscosity. Nevertheless, the curves retain their form even on extrapolation to infinity viscosity.

It remains to be concluded that the initial cause of the dependence of P upon λ_{em} is the change in the limiting polarization P_0. This occurs due to the change in angle between the absorption and emission transition moments, that is caused by alteration of the vibrational states between which the transition with radiation takes place. Such an interpretation of the dependences of P upon λ_{em} for dyes was proposed in [9]. However, it remains a controversial point [10–12]. In the opinion of the authors in refs. 10 and 12, the value of the emitting anisotropy must always remain constant across the emission band in solid solutions for spectroscopically complex molecules. The authors in refs. 10 and 12 express the view that in all cases when the dependence of P upon λ_{em} was recorded, it was caused by the inherent heterogeneity of the emitting centres, that was not considered by Gurinovich et al. [11]. It has been noted [10,12] that the emergence of heterogeneity of the emitting centres is most probable when polymer matrices are used as a solvent. However, our results prove the existence of the dependence of P upon λ_{em} for individual emitting centres and confirm the concepts generalized in the work [9].

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