Rotational Motions of Synthetized Y_t-Base, Free or Bounded to Adenine, in Solvents of Various Viscosities *

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Mean lifetimes in the singlet excited state and emission anisotropies of Y_t base and Y_t-$(CH_2)_5$-adenine were measured as a function of solvent viscosity.

Effective volumes of the molecules studied and the corresponding radii were determined. The fluorescence depolarization of Y_t-base in solutions was found to be due to the rotational motion, for the most part about the main x-axis.

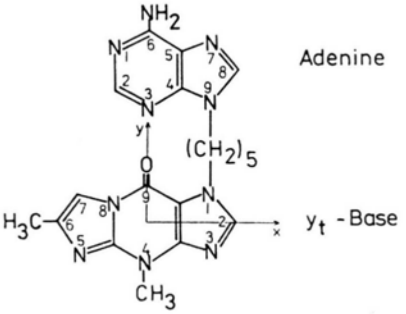
1. Introduction

In recent years, several papers dealt the physical-chemical properties of the Y_t-base, which is the main luminophore in t-RNA^{Phe} [1 – 3]. High fluorescence quantum yield enables to use this compound as a fluorescent probe or as a donor of electronic excitation energy in investigations of the energy transfer in biological systems [4]. The occurrence of the Y_t-base in anticodon of t-RNA^{Phe} in close vicinity of adenine inspired investigations of the luminescence of synthetized Y_t-(CH_2)n-adenine [Y_t-$(CH_2)_n$-Ade] systems [5]. Adenine and the polymethylene chain do not affect significantly the luminescence of the system, nevertheless free motions of the Y_t-base may be hindered.

The theory of rotational fluorescence depolarization [6 – 8] permits the investigation of rotational motions of molecules during electronic excitation. It was found that for prolate molecules, particularly for those having short lifetimes in the excited state, the emission anisotropy ** can attain considerably high values even in solvents with low viscosities [9]. Advancement in the measuring techniques in recent years, particularly emission anisotropy measurements, permits the above-mentioned effects to be investigated also for more spherical molecules.

2. Experimental

The Y_t-base was prepared by condensation of 3-methylguanine and bromoacetone [10]. Synthesis and purification of Y_t-$(CH_2)_n$-Ade were carried out as described previously [5]. The viscosities of the solvents were determined by means of a Höppler viscosimeter, the lifetimes on a pulse fluorimeter similar to that reported in [11], assuming a mono-exponential decay. The emission anisotropy was measured on an apparatus adjusted to low degree of polarization (emission anisotropy) [12].



3. Results and Discussion

Table 1 gives the measured mean lifetimes and emission anisotropies for the Y_t-base and Y_t-$(CH_2)_5$-Ade in solvents of different viscosities. While the mean lifetimes remain essentially unchanged, the emission anisotropy depends strongly on the viscosity of the solvents. In solvents with high viscosity (ethylene glycol, cyclohexanol) the emission anisotropy is much greater than that in solvents with low

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** For plane polarized exciting light

$$r = \frac{J_{\parallel} - J_{\perp}}{J_{\parallel} + 2\,J_{\perp}} = \frac{2\,P}{3 - P}\,,$$

where $J_{\parallel}$ and $J_{\perp}$ are the components of the emitted intensity parallel and perpendicular to the direction of vibration of the electric vector of the exciting light; P is the degree of Polarization.

Table 1.

		η	Y_t		Y_t-$(CH_2)_5$-Ade	
Nr.	Solvent	(cP)	τ (ns)	r	τ (ns)	r
1	Water	0.94	4.5	0.0005	5.9	0.0036
2	Ethanol	1.42	5.2	0.0010	6.2	0.0042
3	Dioxane	1.45	6.3	0.0005	7.2	0.0038
4	*n*-Butanol	2.6	5.1	0.0021	5.8	0.0060
5	Hexanol	3.4	4.9	0.0024	5.6	0.0094
6	Heptanol	4.5	4.9	0.0034	5.6	0.0114
7	Decanol	9.6	5.6	0.0055	5.9	0.0215
8	Ethylene glycol	17	5.0	0.0093	5.8	0.0348
9	Cyklohexanol	62	5.4	0.0241	5.9	0.0880
10	PMMA	—	6.1	0.14	5.4	0.18

viscosity (water). The mean lifetime of free Y_t-base is in each solvent shorter than that of Y_t bounded to adenine by a pentamethylene bridge. None the less, the emission anisotropy of Y_t-$(CH_2)_5$-Ade is markedly higher than that of Y_t-base, thus indicating that the rotational motions of the former are strongly hindered by adenine and the polymethylene chain. The fluorescence depolarization of molecules is conditioned by their rotations (occurring during the lifetime of an excited state), which result in a change in the direction of the electric transition moment. For the Y_t-base, the electric transition moment computed by the CNDO/S method [13] is almost perpendicular to the main x-axis of the molecule. Therefore, free rotations about the single N-C bond should not influence the change in anisotropy of the emitted radiation. The change in the emission anisotropy of the Y_t molecule is caused by rotational motions about the x- and z-axis, which are hindered by the polymethylene chain and adenine. The dependence of the emission anisotropy upon the solvent viscosity and the mean lifetime of a molecule in the excited state is given by the Perrin formula

$$\frac{1}{r} = \frac{1}{r_0} + \frac{1}{r_0} \frac{kT}{V} \frac{\tau}{\eta}, \tag{1}$$

where r_0 is the limiting emission anisotropy, τ the mean lifetime in the excited state, η the solvent viscosity and V the effective volume of a dissolved molecule. The plot of $1/r$ vs. τ/η is presented in Figure 1. The effective volumes determined amount to 107.7 Å^3 and 350.3 Å^3 for free Y_t-base and Y_t-base bounded to adenine, respectively. The radii corresponding to the above volumes, assuming spherical shape, are 3.0 Å and 4.4 Å. As was observed by several authors [14 – 17], these values are considerably lower than those of the geometrical radius of the Y_t-base, which implies that the fluorescence depolarization of the Y_t-base in solutions is caused by the rotational motion occurring about the x-axis mainly. Rotations about the x-axis, which require less space than those about the remaining axes, are also the reason of low ratios of the emission anisotropies in rigid solutions, e. g., in polymethyl methacrylate PMMA.

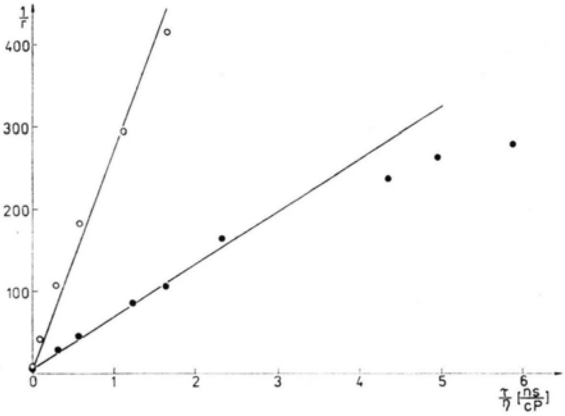

Fig. 1. Dependence of $1/r$ (r is the emission anisotropy) on τ/η (τ the mean lifetime, η the viscosity): ○ — free Y_t-base ● — $Y_t(CH_2)_5$-adenine.

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