ABSORPTION AND FLUORESCENCE SPECTRA OF Y_t-BASE IN BINARY SOLVENTS

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Shifts of absorption and fluorescence spectra were investigated in dioxane/N, N-dimethyl-formamide (nonpolar + polar solvent) mixtures. The values of dipole moments were determined in the ground state μ_g, and the lowest excited singlet electronic state μ_e, based on the measurements carried out. Good agreement was found with the dipole moments computed by the INDO/S method.

Introduction

Y_t-base (Fig. 1) is a modified nucleotide occurring in the t-RNA^{Phe} anticodon in the immediate vicinity of adenine. As opposed to other nucleotides (purine and pyrimidine bases), Y_t-base displays fluorescence of a marked quantum yield ($\eta \approx 0.5$) even at room temperature. This enables the Y_t-base to be employed as a natural fluorescence probe for the investigations of interactions and electronic excitation energy transfer in t-RNA. Intermolecular interactions depend strongly on the electric properties of a molecule, first of all on the magnitude and direction of the electric dipole moment. The aim of this paper is to determine dipole moments μ_g and μ_e of Y_t-base in the ground state (very low solubility of Y_t-base makes the dielectric measurements impossible) and in the fluorescent excited state S_1, respectively, by means of a spectroscopic method.
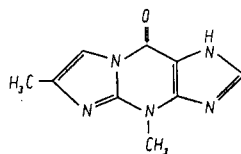

Fig. 1. Y_t-base

Experimental

The Y_t-base was synthetized by direct condensation of 3-methylaquanin with brom-acetone following the method of KASAI ET AL. [1]. Twofold recrystallization from water or methanol yielded a chromatographically pure product.

Absorption spectra were measured on a Beckman Model 25 spectrophotometer. The arrangement for the fluorescence measurements had been described in a previous paper [2]. The solvents used were of a spectroscopical grade.

Results and discussion

The use of mixtures as solvents (polar + nonpolar) when investigating absorption and fluorescence offers the possibility of determining electric dipole moments in the ground and singlet excited states, respectively [3]. The shift of the absorption (fluorescence) spectrum is, among other things, a function of the dipole moment in the ground (excited) electronic state. The relation between the relative band shift and parameters characterizing the solute molecule and the solvent can be written as:

$$\ln\left(\frac{1}{\Delta v_{a,f}^{rel}}-1\right) = \ln\left(\frac{1}{x}-1\right) - \frac{\mu^2}{a^3}\left(\frac{C_{II}-C_I}{kT}\right) + \frac{\langle\mu_s^{II}\rangle^2}{a_s^3}\cdot\frac{C_{II}}{kT} - \frac{\langle\mu_s^I\rangle^2}{a_s^3}\frac{C_I}{kT} \qquad (1)$$

where:

$$\Delta v_{a,F}^{rel} = \frac{\tilde{v}_{a,F}^I - \tilde{v}_{a,F}}{\tilde{v}_{a,F}^I - \tilde{v}_{a,F}^{II}}$$

x — is the mole fraction of the polar solvent,
μ — is the dipole moment of the solute molecule
   (μ_g — stands for absorption, μ_e — for fluorescence)
a — is the Onsager radius

$$C_{I,II} = \frac{\varepsilon_{II,I}-1}{\varepsilon_{II,I}+2}$$

$\langle\mu_s^{I,II}\rangle$ — is the mean value of the dipole moment of the polar (II) and nonpolar (I) solvents in a solvation shell,
a_s — is the Onsager radius of the solvent molecules.

Polar and nonpolar solvents should be selected so that the refractive indices be similar, their Onsager radii not differing markedly. A system of N, N-dimethylformamide (DMF) and dioxane (D), $n_I \approx n_{II}$ and $a_s^I \approx a_s^{II} \approx 3.4$ Å is suitable. Fig. 2 shows the absorption and fluorescence spectra of Y_t-base in DMF and D. The fluorescence spectrum has been found to display bathochromic shift with increasing solvent polarity, whereas the absorption spectrum is shifted in an opposite direction (hypsochromic effect). Such behaviour is characteristic of a case when the directions of the
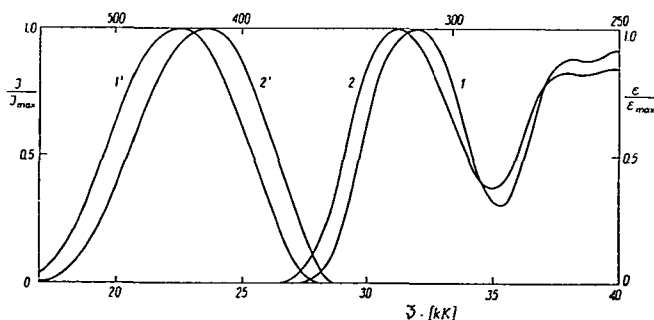

Fig. 2. Absorption and fluorescence spectra of Y_t-base:
1 and 1' — absorption and fluorescence in DMF,
2 and 2' — absorption and fluorescence in D

dipole moments in the ground and excited states are opposite, according to the theory of the effect of the solvent on the spectra of dissolved molecules [4]. Quantummechanical INDO/S computations for Y_t-base yield an angle 168° between μ_g and μ_e [5]. The magnitudes of absorption and fluorescence shifts are presented in Fig. 3. As can be seen, about 80% of the shift occurs even if the polar solvent content in the solution is not higher then 20%. Plots of relation (1) are shown in Fig. 4. The values of μ_g and μ_e are given in tabulated form.

Table I

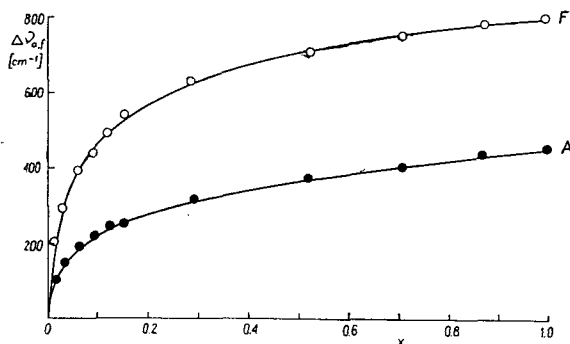| Substance	a[Å][5]	μ_g[D]	μ_e[D]	INDO/S computations		
				μ_g[D]	μ_e[D]	$\sphericalangle^\varphi_{\mu_g,\mu_e}$
Y_t-base	5.8	3.9	5.8	3.73	5.67	168°

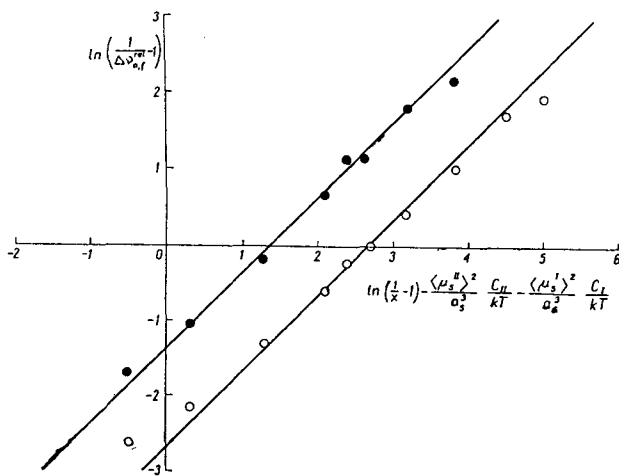*Fig. 3.* Absorption and fluorescence shifts

Fig. 4. Plots of relation (1)

Conclusions

— Y_t-base exhibits bathochromic fluorescence shift with simultaneous hypochsromic shift of the absorption spectrum, which is due to the antiparallel position of the μ_g and μ_e directions.

— The shift of the absorption and fluorescence spectra is almost complete even if the polar solvent content is slight.

— The values of μ_g and μ_e obtained from the absorption and fluorescence shifts are in good agreement with the theoretical computations.

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[5] *Gryczynski, I., C. Jung, A. Kawski, S. Paszyc, S. Skalski:* Z. Naturforsch. **34a**, 172 (1979).

СПЕКТРЫ ПОГЛОЩЕНИЯ И ФЛУОРЕСЦЕНЦИИ Y_t-ОСНОВЫ В БИНАРНЫХ РАСТВОРИТЕЛЯХ

И. Гричиньски, Й. Чайко, С. Пашыц и Б. Скалски

Исследованы сдвиги спектров погложения и люминесценции в смесях диоксан/диметил-формамид (неполярный и полярный растворитель). На основе выполнении измерений опре-делены значения дипольных моментов в основном μ_g и низком возбужденном синглетном электронном состоянии μ_e. Существует хорошее соответствие с величинами диполных момен-тов вычисленным методом INDO/S.