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# TEMPERATURE EFFECT ON THE LUMINESCENCE OF SYNTHETIZED $Y_t$ BASE IN PVA FILM

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Summary: The mean phosphorescence lifetimes, fluorescence yields and phosphorescence-to-fluorescence quantum yields ratios of $Y_t$ base in PVA films were measured in the temperature range of 100-250°K. The quantum yield of triplet state formation was found to be temperature independent at a wide range of lower temperatures. The radiative and nonradiative rate constants for triplet state were measured.

## Introduction

The physicochemical and photophysical properties of Yeast $tRNA^{phe}$ $Y_t$ base (fig. 1) have been the subject of several theoretical and empirical studies during the last few years [1,2]. The CNDO/S and INDO/S molecular orbital calculation were used for determining the energy of the electronic transitions, the distribution of electronic charge and electric dipole moments of $Y_t$ [1,4]. The high fluorescence efficiency of this base was profitably applied to studies of tRNA tertiary structure by singlet-singlet energy transfer [5] as well as to studies of the interaction between $Y_t$ and adenine - its nearest neighbour base in $tRNA^{phe}$ [6,7]. Photochemical reactions of $Y_t$ have also been investigated [8]. However, the triplet state of $Y_t$, which is responsible for its photochemical properties has received little attention so far. In

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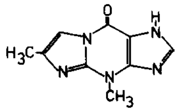

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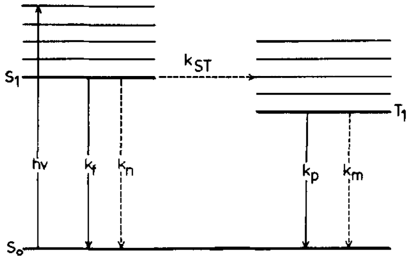

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Fig.1. The chemical structure of $Y_t$ base.

Fig.2. Jablonski diagram. S and T represent singlet and triplet states respectively.

this paper we present our results on studies of the effect of temperature on deactivation of the lowest excited states of $Y_t$ with the special attention given for triplet state.

## Materials and Methods

The $Y_t$ base was synthetized according to Kasai et al. [9] using 3-methylguanine as the key intermediate. Two fold recrystallization from water - methanol mixture yielded a chromatographically pure product.

The techniques used to prepare poly-vinyl alcohol/PVA/ films were similar to those employed by Tanizaki et al. [10] .

The phosphorescence decay measurements were made on the spectrofluorimeter using two coupled shutters. The phosphorescence-to-fluorescence quantum yields ratios $\varphi_p/\varphi_f$ were measured by means of two synchronous choppers supplied by power generator with the frequency of 40 Hz. The real values of $\varphi_p/\varphi_f$ were calculated by applying Fourier analysis of measured signals. The fluorescence quantum yields were measured on the instrument described previously [11]. A special cryostat for optical spectroscopy was employed at temperatures 100 - 300°K. The measuring cell was thermostated with a precision of ± 1°K. The excitation wavelength was 313nm at which optical densities of $Y_t$ in the PVA films did not exceed 0.1. The PVA film, without $Y_t$ base did not show any absorption at this wavelength.

## Results and discussion

All results obtained in this work are listed in Table I. It is noteworthy that the fluorescence quantum yield of $Y_t$ ($\varphi_f$) depends insignificantly on temperature below 160°K while the phosphorescence

**Table I.** $Y_t$ base in PVA film

| No | T [°K] | $\tau_p$ [s] | $\varphi_f$ | $\frac{\varphi_p}{\varphi_f} \times 10^2$ |
|---|---|---|---|---|
| 1 | 100 | 1.71 | 0.632 | 2.87 |
| 2 | 110 | 1.71 | 0.632 | 2.86 |
| 3 | 120 | 1.68 | 0.629 | 2.84 |
| 4 | 130 | 1.65 | 0.628 | 2.80 |
| 5 | 140 | 1.62 | 0.628 | 2.78 |
| 6 | 150 | 1.59 | 0.624 | 2.75 |
| 7 | 160 | 1.56 | 0.619 | 2.71 |
| 8 | 180 | 1.52 | 0.615 | 2.57 |
| 9 | 200 | 1.34 | 0.591 | 2.18 |
| 10 | 220 | 1.09 | 0.534 | 1.57 |
| 11 | 240 | 0.72 | 0.528 | 0.98 |
| 12 | 250 | 0.46 | 0.521 | 0.61 |

lifetime and the ratios of phosphorescence-to-fluorescence quantum yields are steady at much lower temperatures ($T < 120^{\circ}K$) . This means, that deactivation of the lowest excited singlet state $S_1$ (the fluorescent state) has somewhat different character than that of the lowest excited triplet state $T_1$ (the phosphorescent state). Considering the Jablonski diagram (fig. 2) one can write the following expressions for fluorescence $\varphi_f$, phosphorescence $\varphi_p$ and intersystem crossing $\varphi_{ST}$ quantum yields in terms of the rate constants for all radiative and nonradiative processes:

$$\varphi_f = \frac{k_f}{k_f + k_n + k_{ST}} \qquad (1)$$

$$\varphi_p = \frac{k_p}{k_p + k_m} \varphi_{ST} \qquad (2)$$

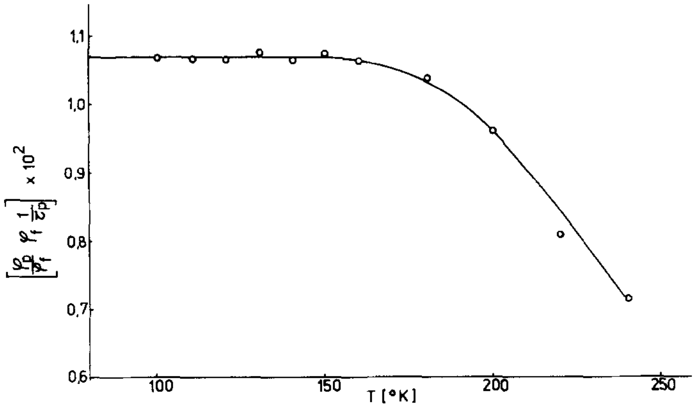

Fig.3. The plot of the left hand of equation /5/ vs temperature.

$$\varphi_{ST} = \frac{k_{ST}}{k_{ST} + k_f + k_n} \qquad (3)$$

While the fluorescence and phosphorescence quantum yields can be calculated directly from the absorption and emission measurements, the value of intersystem crossing yield can only obtained from a combination of luminescence decay and quantum yields measurements. The mean phosphorescence lifetime is given by the following equation:

$$\frac{1}{\tau_p} = k_p + k_m \qquad (4)$$

Combination of (1) - (4) gives the following relation:

$$\frac{\varphi_p}{\varphi_f}\,\varphi_f\,\frac{1}{\tau_p} = k_p\,\varphi_{ST} \qquad (5)$$

It is still not possible to calculate $\varphi_{ST}$ from equation (5) . However the dependence of $\varphi_{ST}$ upon the temperature can be examined (the phosphorescence rate constant $k_p$ does not depend on temperature). The plot of the left hand of equation (5) against temperature is presented in fig. 3. As can be seen in this figure no temperature dependence is observed below 170°K. This indicates that the inter-

Table II

| Compound | $\varphi_f^o$ | $\varphi_{ST}^o$ | $\varphi_p^o$ | $k_p[s^{-1}]$ $\times 10^2$ | $\tau_p^o[s]$ | $k_m^o[s^{-1}]$ | A | $\Delta E$ $[cm^{-1}]$ |
|---|---|---|---|---|---|---|---|---|
| $Y_t$-base in PVA film | 0.632 | 0.368 | 0.018 | 2.86 | 1.71 | 0.56 | 4.53 | 4.28 |

system crossing quantum yield becomes constant below this temperature.

In a solid matrix as PVA film, the temperature dependent nonradiative rate constant $k_n$ becomes negligible with the lowering of temperature and the only nonradiative process of importance in competition with fluorescence is an intersystem crossing. This means that, at the temperatures below 170°K the fluorescence and intersystem crossing quantum yields of $Y_t$ in PVA film sum to unity:

$$\varphi_f + \varphi_{ST} \approx 1 \qquad (6)$$

Thus, by the simple combination of quantum yield and lifetime measurement radiative rate constant $k_p$ can be calculated. This value, as well as the other parameters are listed in Table II. Relatively small phosphorescence quantum yield $\varphi_p$ compared to the intersystem crossing quantum yield $\varphi_{ST}$ indicates that the nonradiative transition $k_m$ is the main deactivating factor of the triplet state.

Dependence of $k_m$ on temperature can be expressed by:

$$k_m = k_m^o \left[1 + A_{exp} \left\{\frac{-\Delta E}{kT}\right\}\right] \qquad (7)$$

and, from the dependence of mean phosphorence lifetime upon the temperature, the following expression can be derived:

$$\frac{1}{\tau} - \frac{1}{\tau_0} = k_m^o \, A \exp \left\{\frac{-\Delta E}{kT}\right\} \qquad (8)$$

or

$$\ln\left(\frac{\tau^{o}}{\tau} - 1\right) = k_m^o\ \tau_o\ A - \frac{\Delta E}{kT} \qquad (9)$$

where $\tau_o \approx \tau_{100^oK} = 1.71$ s.

A and $\Delta$ E parameters were calculated using the least squares method. The calculated value of $\Delta E \approx 430$ $cm^{-1}$ is similar to those obtained for a number of aromatic hydrocarbons [12] and indicates that intramolecular out of plane bonding vibrations can be responsible for nonradiative deactivation of the triplet state of $Y_t$ base.

## References

[1] E.M. Evleth and D.A. Lerner, Photochem. Photobiol. 26, 103 (1977) there may be found earlier refer.
[2] M. Rafalska Ph.D. Thesis, University of Poznań, Poland, Poland (1977)
[3] G. Nygjerd, J.Mc Alister and M. Sundaralingam, Acta Cryst. B 31, 413 (1975) .
[4] I. Gryczyński, Ch. Jung, A. Kawski, S. Paszyc and B. Skalski, Z. Naturforsch. 34a, 172 (1979) .
[5] J. Eisinger, B. Feuer and T. Yamane, Proc. Nat. Acad. Sci. USA, vol. 65, No 3, 638 (1980) .
[6] I. Gryczyński, A. Kawski, S. Paszyc, M. Rafalska and B. Skalski Bull. Acad. Sci. Ser. Sci. Polon. Phys. Astronom. in press.
[7] I. Gryczyński, A. Kawski, S. Paszyc and B. Skalski Z. Naturforsch. in press.
[8] S. Paszyc and M. Rafalska, Nucleic Acid Res. 6, 385 (1979) .
[9] H. Kasai, M. Goto, S. Takemura, T. Goto and S. Matsuura, Tetrahedron Lett. 2725 (1971) .
[10] Y. Tanizaki, T. Kobayashi and N. Ando, Bull. Chem. Soc. Jpn., 32, 119 (1959) .
[11] A. Kawski, J. Kamiński and E. Kuteń, J. Phys. B. Atom. Molec. Phys. 4, 609 (1971) .
[12] P.F. Jones and S. Siegel, J. Chem. Phys. vol. 50, No 3, 1134 (1969)