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Photochemical properties of Y_t base in aqueous solution

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ABSTRACT

Photoreactivity of Y_t base [I] has been studied in aqueous solution [pH~6] saturated with oxygen. Two photoproducts [II,III], resulting from irradiation at $\lambda = 253.7$ nm and $\lambda \geqslant 290$ nm, were isolated and their structures determined. The quantum yield for Y_t base disappearance[φ dis]is 0.002 [λ = 313 nm]. It was shown that dye-sensitized photooxidation of Y_t base in aqueous solution occurs according to a Type I mechanism, as well as with participation of singlet state oxygen. Quantum yields, fluorescence decay times and phosphorescence of Y_t base have been also determined.

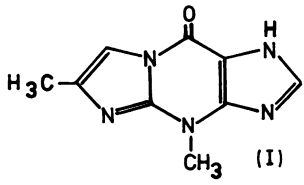
INTRODUCTION

One of distinctive properties of tRNA is its high content of modified bases[1]. The transfer ribonucleic acids specific for phenylalanine contain supermodified Y type bases in addition to the modified bases. Three hydrophobic Y bases have been detected and described in $tRNA^{Phe}$ from different eucaryotic cells:

- Y_{sc} base from baker yeast [Saccharomyces cerevisiae]
- Y peroxy-base from mammalian livers
- Y_t base from yeast[Torulopsis utilis],[I] ; Fig. 1.

Nakanishi et al.[2] determined the structure of Y_{sc} base from bakers' yeast and this was confirmed by Thiebe et al.[3] and by means of chemical synthesis[4]. The fluorescent base peroxy-Y was found in $tRNA^{Phe}$ from mammalian livers[5,6] and in $tRNA^{Phe}$ from Lupinus luteus[7]. Its structure is similar to that of Y_{sc} and the difference consists only in the presence of a peroxide group at carbon-β in side chain. The Y_t base [I]was isolated from Torulopsis utilis[8].

All the Y type bases occupy the position adjacent to



(I)

Fig. 1. Structure of Y_t base.

3'-end of the anticodon and they can be selectively isolated during the acidic hydrolysis of tRNA at pH = 2.9. They possess an intense fluorescence at room temperature [fluorescence maximum at λ= 430 nm, λ_{exc} = 315 nm].

The Y bases are used as fluorescent probes in structural studies of tRNA. Potentially, they could also be suitable photochemical probes, since their absorption in aqueous solution extends to about 350 nm and this fact enables excitation outside the absorption range of other components of nucleic acids. In our laboratory the study of photochemical properties of Y_t base in aqueous solution [pH~6] was undertaken. Studies on photoreactivity of pyrimidine bases and their nucleotides are known[9]. Purines undergo photochemical transformations with greater difficulty compared to pyrimidine bases. Among purine bases, the process of guanosine photooxidation in the presence of dyes as sensitizers is well-known. The sensitized photodynamic oxidation of nucleic acids is usually connected with the degradation of guanine residues[10-13]. It is established that sensitized photooxidation of guanosine may occur with the participation of singlet oxygen, according to the mechanism designated by Gollnick as a Type II, as well as without its participation [Type I mechanism][14-16].

MATERIALS and METHODS

The Y_t base used in this study was prepared by condensation of 3-methylguanine and bromoacetone[17].

The UV spectrum of Y_t base:

λ_{max} pH 1.0: $\varepsilon_{227nm}=41.5\times10^3$; $\varepsilon_{231nm}=40.4\times10^3$; $\varepsilon_{284nm}=9.4\times10^3$

λ_{max} pH 5.9: $\varepsilon_{230nm}=34.5\times10^3$; $\varepsilon_{264nm}=5.9\times10^3$; $\varepsilon_{307nm}=6.5\times10^3$

λ_{max} pH 11 : $\varepsilon_{231nm}=35.0\times10^3$; $\varepsilon_{274nm}=7.2\times10^3$; $\varepsilon_{302nm}=8.9\times10^3$

The Y_t base [H_2O] shows an intense fluorescence at room temperature. Emission maximum λ = 440 nm [corrected spectrum, λ_{exc} = 307 nm]. Relative quantum yield of Y_t base fluorescence in water [pH~6, 20°C.] φ = 0.21. The fluorescence decay time under these conditions is of order of 7.2 ns.

The phosphorescence spectrum of synthetic Y_t base in ethyl alcohol [T = 77°K] shows fine structure [corrected spectrum λ_{exc} = 313 nm]. The quantum yield of Y_t base phosphorescence determined under the same conditions φ_{ph} = 0.01 and lifetime τ_{ph} = 2.6 s; Fig. 2.

The irradiation of aqueous solutions of Y_t base [c = 3.0×10^{-4}M, pH~6] was carried out in a cylindrical quartz reactor using low pressure mercury immersion lamp TNN 32 Original Hanau [λ = 253.7 nm] and high pressure mercury lamp TQ 150 provided with a cylindrical Pyrex filter with a wall thickness of about 1.5 mm [the system cuts off the radiation of $\lambda < 290$ nm]. The temperature of the irradiated solutions was maintained in the

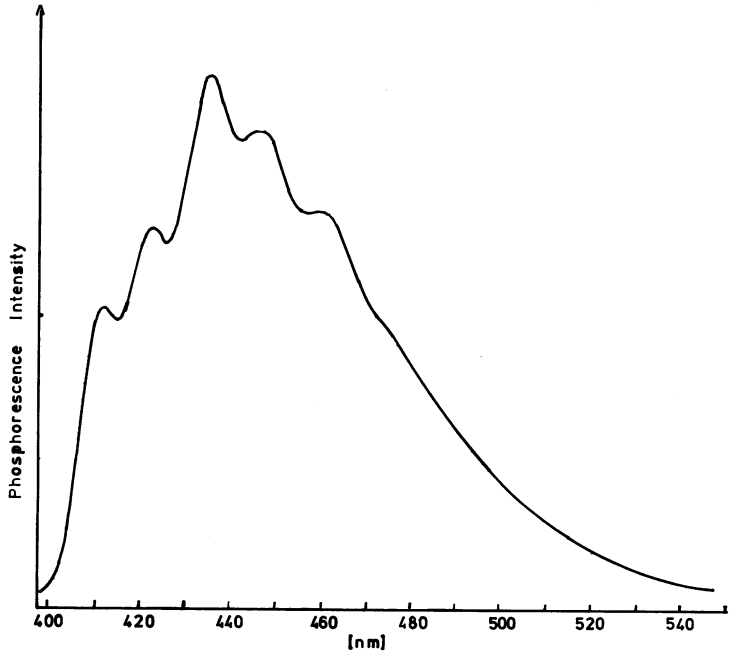


Fig. 2. Phosphorescence spectrum of Y_t base in EtOH at 77°K.

range of 20 ± 1°C. The point source light [I_o = 0.013x10^{-3} E/h ml] HBO 200 NARVA was used to determine the quantum yield for photooxidation of Y_t base. The above light source was provided with a suitable interference filter to obtain monochromatic light λ = 313 nm [bandwidth is 9 nm] . Uranyl oxalate was employed as an actinometer.

The photoproducts and unreacted Y_t base were separated by column chromatography [silica gel] in the solvent system consisting of 86% n-butanol and 14% water. The R_f values [silica gel HF_{254}] of the obtained photoproducts [II,III]:
a. $CHCl_3$: [MeOH 4 : 1 v/v]
b. 86% n-butanol - 14% water

	solvent a	solvent b
II	0.15	0.22
III	0.67	0.46

The mass spectra of the photoproducts were recorded on JEOL JMSD-100 mass spectrometer and nuclear magnetic resonance spectra on Perkin-Elmer R-32 spectrometer [90 MHz].

The fluorescence measurements for Y_t base were carried out using Perkin-Elmer MPF-3 spectrofluorometer and the measurements of the phosphorescence spectrum and the phosphorescence decay time were taken with a spectrofluorometer provided with an accessory for phosphorescence measurements, made by IChF PAN, Warsaw. The measurements of fluorescence decay time were carried out at the Institute of Physics, University of Giessen by single photon counting.

The pK_a values of photoproducts were determined by means of spectrophotometric titration. The sensitizing dyes methylene blue and rose bengal were crystallized twice from ethyl alcohol and dried in a vacuum desiccator at room temperature. A standard system was used to irradiate 2 ml of aqueous solution of Y_t base [c = 3.0x10^{-4} M] in 10 mm quartz cells in the presence of sensitizers [c = 2.7x10^{-6} M]. A 1000W halogen lamp [POLAM OR-3m] was used as irradiation source. A glass filter [W/O Mashpriborintorg, U.S.S.R.] cutting off at $\lambda < 380$ nm was placed between the cell and the light source. Sodium azide was used as a quencher of singlet oxygen.

RESULTS and DISCUSSION

A decrease in absorption at λ = 307 nm and an increase at λ = 268 nm were observed during irradiation of aqueous solutions of Y_t base in the presence of air at λ = 253.7 and $\lambda \geqslant 290$ nm; Fig. 3a and Fig. 3b.

Irradiation with low pressure mercury lamp causes a significant decrease in absorption after 45 minutes exposure [37% of Y_t base conversion]. Extention of the irradiation time beyond 90 minutes leads to a progressive degradation of the products formed. Contrary to the short-wave exposure, irradiation of Y_t base with high pressure mercury lamp causes an appreciable reduction in absorption value only after 90 minutes [37% of reacted Y_t base]. The existence of an isosbestic point at λ = 287 nm proves that the photoproducts II/III ratio does not change during the reaction.

Thin layer chromatography of photolyzed solutions showed the presence of two products and trace amount of substrate. Two chromatographically identical photoproducts: II and III

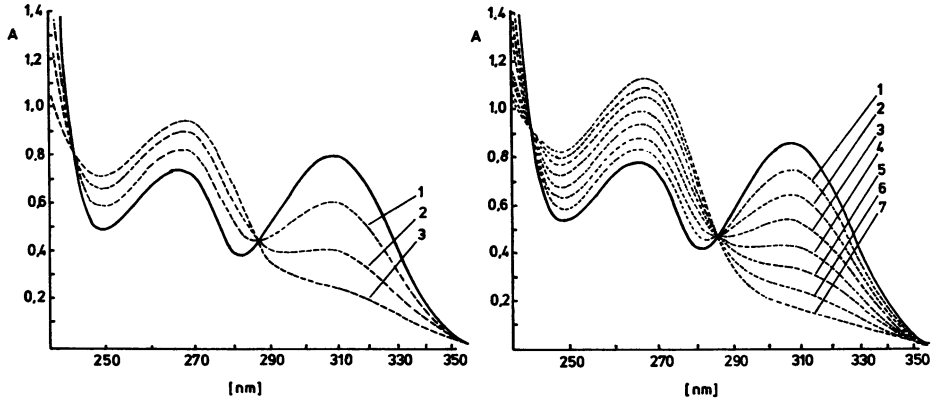
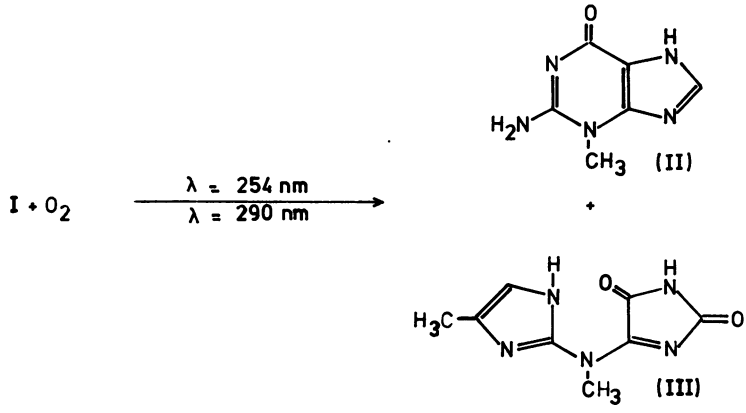


Fig. 3a. UV spectra of Y_t base in aqueous solution [pH~6]
a. before irradiation [—]
b. after irradiation [---] at λ = 253.7 nm ; (1) 30 min.; (2) 60 min.; (3) 90 min.

Fig. 3b. UV spectra of Y_t base in aqueous solutions [pH~6]
a. before irradiation [—]
b. after irradiation [---] at $\lambda \geqslant 290$ nm ; (1) 30 min.; (2) 60 min.; (3) 90 min.; (4) 120 min.; (5) 150 min.; (6) 180 min.; (7) 210 min.



Scheme 1.

are formed, using both irradiation sources; Scheme 1.

No photochemical changes were observed when pure nitrogen was bubbled through the irradiated solution, indicating that the photochemical reaction of Y_t base is photooxidation. The above described reaction occurs much faster in the solution saturated with oxygen, leading to the same products; Fig. 4.

Photoproduct II

The values as follows were obtained:

mass spectrum: m/e 165.0626 [M^+, $C_6H_7N_5O$] relative intensity 100% ; 123.0440 [$C_5H_5N_3O$] 28% ; 95.0438 [$C_4H_5N_3$] 25% ; 68.0080 [$C_3H_4N_5$] 37% ; 58.0112 [C_2HN_2] 17%.

NMR spectrum [CF_3COOH]: δ 4.05 [3H, s, N_3-CH_3] ; 8.08 [broad, N-H] ; 8.63 [1H, s, C_8H].

UV spectrum; Fig. 5.

λ_{max} pH 1.0: ε_{244nm}[sh]= 8.1×10^3 ; $\varepsilon_{264nm} = 11.0 \times 10^3$

λ_{max} pH 6.2: $\varepsilon_{235nm} = 6.2 \times 10^3$; $\varepsilon_{269nm} = 9.8 \times 10^3$

λ_{max} pH 11 : $\varepsilon_{273nm} = 13.3 \times 10^3$

IR spectrum [KBr]: 3410, 3340, 3150, 1650, 1520, 1440, 1220, 1170, 1120, 1100, 920, 760 cm^{-1}.

pK_a values [in water]; 4.35 ± 0.05, 8.15 ± 0.05, 9.65 ± 0.05.

Protonation of the ring N-9 [N-7] in the imidazole moiety of

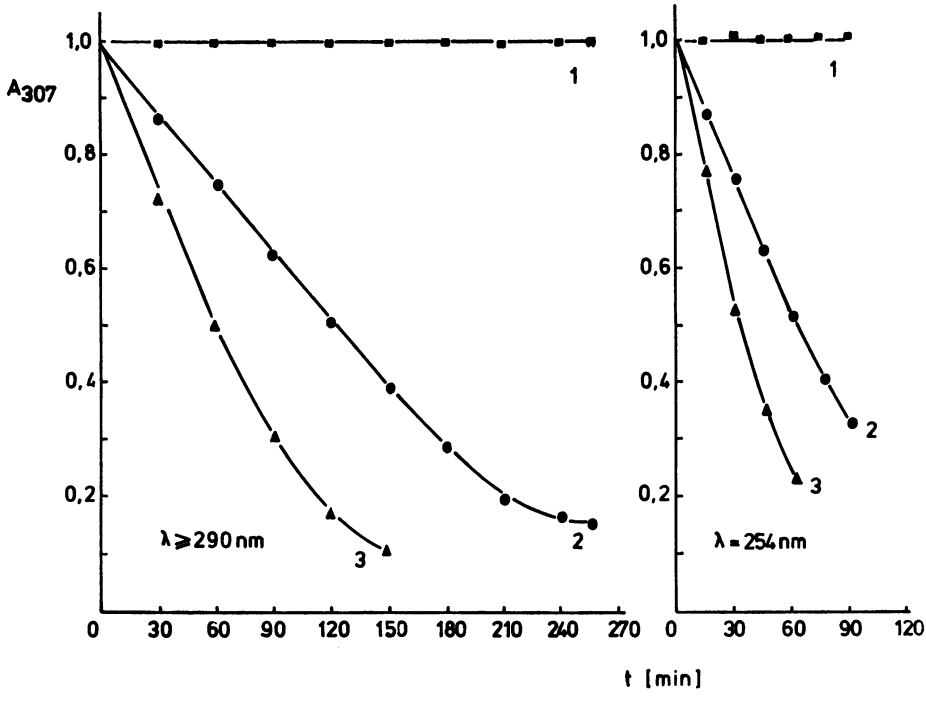


Fig. 4. Changes in absorption of aqueous solution of Y_t base [pH~6] during irradiation; 1 - in oxygen free solution [N_2], 2 - in air, 3 - in oxygen atmosphere.

3MeGua leads to the formation of a monocation , pK_a = 4.35. Deprotonation of the hydrogen at N-1 in the pyrimidine ring and at N-7 in the imidazole ring leads to the formation of a monoanion , pK_a = 8.15 and a dianion , pK_a = 9.65.

Photoproduct III

mass spectrum: m/e 207.0718 [M^+ , $C_8H_9N_5O_2$] relative intensity 39% ; 192.0497 [$C_7H_6N_5O_2$], 100% ; 149.0446 [$C_6H_5N_4O$] 23% ; 123.0431 [$C_5H_5N_3O$] 8% ; 95.0443 [$C_4H_5N_3$] 9%.
The suggested structure was confirmed also by high-resolution mass spectrum of deuterated derivative of this photoproduct.

IR spectrum [KBr]: 3100, 3040, 2850, 1715, 1575, 1545, 1390, 1385, 1160, 1130, 970, 820, 760 cm^{-1}.

UV spectrum of the photoproduct pointed to destruction of a part of Y_t base chromophoric system; Fig. 6.

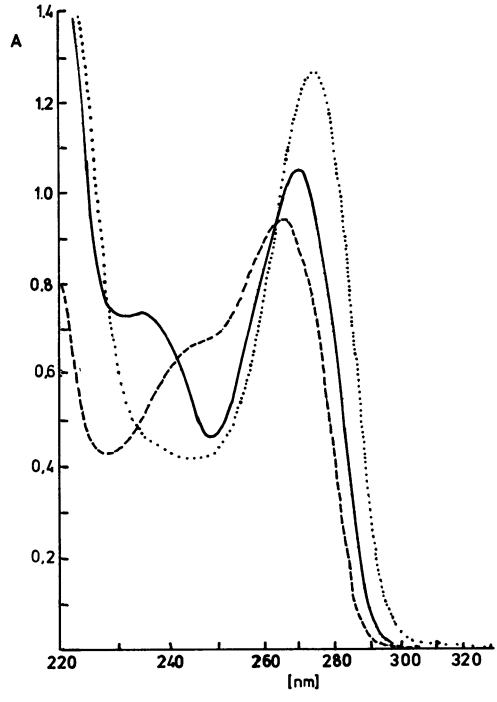


Fig. 5. UV spectra of photoproduct II :[---] pH = 1.0 ; [—] pH = 6.2 ; [...] pH = 11 .

λ_{max} pH 1.0: $\varepsilon_{263nm} = 1x10^4$

λ_{max} pH 5.9: $\varepsilon_{225nm} = 14.6x10^3$; $\varepsilon_{268nm} = 11.8x10^3$

λ_{max} pH 10 : ε_{230nm}[sh]= $12.4x10^3$; $\varepsilon_{275nm} = 11.8x10^3$

The signals: δ = 2.27 [3H, d] , 3.83 [3H, s] , 8.21 [1H, q] , appeared in the NMR spectrum taken in D_2O. They can be ascribed to aromatic methyl, N-methyl and aromatic protons respectively.

pK_a values [in water] ; 2.55 ± 0.05, 8.15 ± 0.05, 9.82 ± 0.05. The pK_a value of 2.55 [monocation] may correspond to the protonation at a amine nitrogen atom. Deprotonation at nitrogens in the imidazole rings leads to formation of a monocation , pK_a = 8.15 and a dianion, pK_a = 9.82 .

We suggest that both products of Y_t base photooxidation in aqueous solution are formed as a result of decomposition

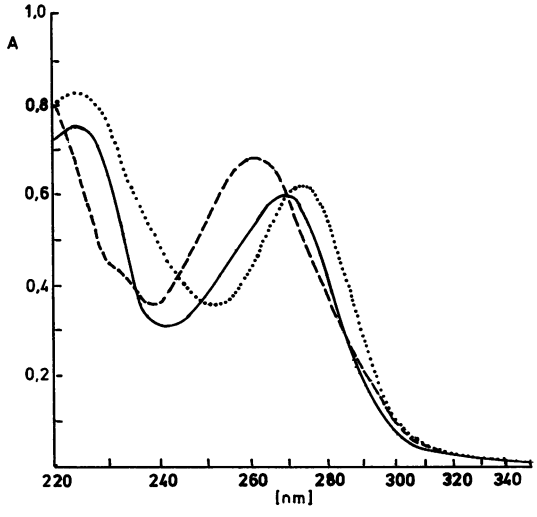
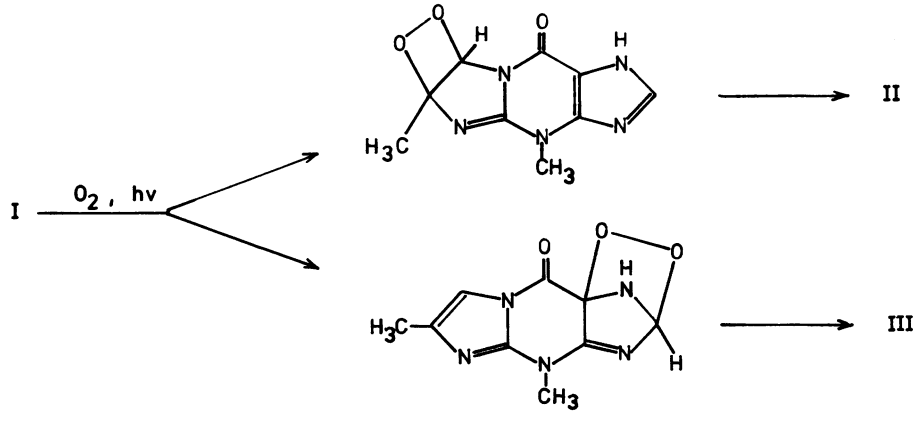


Fig. 6. UV spectra of photoproduct III :[---] pH = 1.0 ; [—] pH = 5.9 ; [...] pH = 10 .

of respective unstable intermediates. We did not succeed in isolating the intermediates formed during the reaction; Scheme 2. The quantum yield [φ dis] of Y_t base disappearance process in aqueous solution [pH~6] λ = 313 nm is 0.002.

The photooxidation of Y_t base in aqueous solution



Scheme 2.

occurs with the participation of singlet oxygen. We carried out a test to detect the presence of such a kind of species. To perform this test, an aqueous solution of Y_t base was irradiated at λ = 253.7 nm in the presence of sodium azide. Two methods are usually used to demonstrate the participation of singlet oxygen in photochemical reaction: comparison of reaction rates in presence and absence of singlet oxygen quenchers[16] or in normal and deuterated solvents[15]. We have chosen sodium azide [c = $4x10^{-4}$ M] as a quencher of singlet oxygen[19]. This led to the inhibition of reaction. After 90 minutes irradiation in the absence of sodium azide, 68% of Y_t base reacted, whereas in the presence of sodium azide the conversion was only 40%, R_q/R_o = 0.58 [R_q - the amount of Y_t base which reacted in the presence of sodium azide ; R_o - the respective value in the case of the absence of sodium azide] . The obtained value of R_q/R_o = 0.58 proves that ground state oxygen also takes part in the reaction according to a Type I mechanism. This result was confirmed by comparing photooxidation of Y_t base in oxygen saturated H_2O and D_2O. The photooxidation rate was ca. 2x higher in D_2O. Since the increase was much less than usually expected for a pure singlet oxygen reaction, these results suggest that photooxidation proceeds by both singlet oxygen and Type I mechanism.

In the case of sensitized photooxidation of Y_t base in water, methylene blue and rose bengal were used as sensitizers. We found that the same products are formed as with non-sensitized photooxidation of Y_t base. The experiments were carried out using solutions saturated with oxygen, as well as in air, and the results are presented in Fig.7.

Sodium azide influences the dye-sensitized reaction of Y_t base photooxidation only to a small extent. We suggest that under normal conditions, in the presence of oxygen from air, while using rose bengal, the photooxidation reaction of Y_t base occurs, in practice, without participation of singlet oxygen. On the other hand, some increase in the participation of singlet oxygen was observed during the photooxidation of Y_t base in oxygen-saturated solutions in the presence of methylene blue. We suggest that the sensitized photooxidation of Y_t base

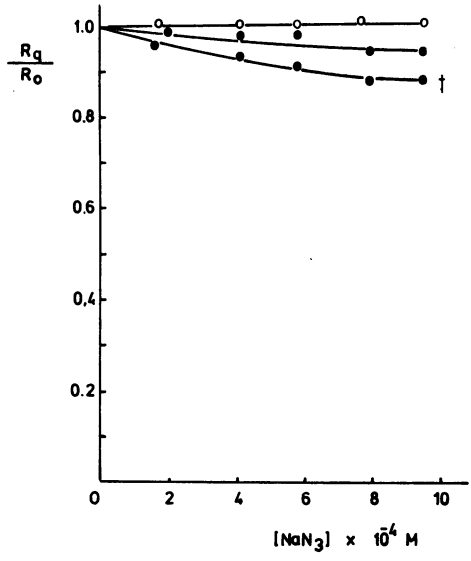


Fig. 7. Inhibiting effect of NaN_3 on the reaction of Y_t base photooxidation, sensitized by rose bengal [o] and methylene blue [•]; R_o - reacted Y_t base in the absence of NaN_3; R_q - reacted Y_t base in the presence of NaN_3; † - in oxygen-saturated solution.

in aqueous solution occurs, first and foremost, according to Type I mechanism. It is worth-while to mention that a few mechanisms of sensitized photooxidation of purines in the absence of singlet oxygen are known in the literature[20].

$$^1S \xrightarrow{h\nu} {}^1S^* \xrightarrow{ISC} {}^3S^* \qquad (1)$$

$$^3S^* + {}^1Y_t \longrightarrow {}^3(S\text{-}Y_t)^* \qquad (2)$$

$$^3(S\text{-}Y_t)^* + {}^3O_2 \longrightarrow {}^1S + Y_t\text{-}O_2 \qquad (3)$$

A sensitizer molecule in a ground electronic state, while absorbing a light quantum, converts into an electronic excited triplet state via the lowest singlet state (1). The quenching of the dye triplet state under the influence of Y_t base is caused by the formation of excited $^3(S\text{-}Y_t)^*$ complex. This intermediate can react with molecular oxygen in the solution and endoperoxide is formed as a result of such a reaction (3). In addition to these reactions, competitive reactions of exci-

ted complex deactivation can occur as well.

Having in view the future use of Y_t base as a native fluorescence probe in tRNA, we have determined for the first time the fluorescence and phosphorescence quantum yields and decay time of this compound see[MATERIALS and METHODS].

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