Quenching of the Y_t-Base Fluorescence by Heavy-Atom Induced Intermolecular Singlet-Triplet Energy Transfer *

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Quenching of the Y_t-base fluorescence by a series of halogen derivatives of benzene and naphthalene was measured in dioxane. For the naphthalene derivatives, the presence of the heavy atom in the acceptor molecule was found to affect strongly the fluorescence quenching rate constant, whereas no such effect was observed for the benzene derivatives. The fact is explained with the assumption of a singlet-triplet energy transfer from the Y_t-base molecules to the naphthalene derivatives.

Y_t-base adjacent to adenine in the t-RNA$^{\mathrm{Phe}}$ anticodon has recently become an object of numerous luminescence studies [1–4]. Y_t-base exhibits a high fluorescence quantum yield ($\eta \sim 0.2$) as compared to that observed for the remaining nucleotides (e.g., for adenine $\eta \sim 10^{-6}$ [5]). Thus, the luminescent properties of t-RNA$^{\mathrm{Phe}}$ may be assumed to depend entirely on that former luminophore.

In recent years, the intramolecular electronic excitation energy transfer has been widely investigated in order to determine the distances between chromophores and their mutual orientation. In the case of t-RNA$^{\mathrm{Phe}}$, such studies are possible provided an additional absorption probe is introduced [6]. The energy of the lowest excited singlet state of Y_t-base ($E_{S_1} \approx 27000\ \mathrm{cm}^{-1}$) is lower by several thousand cm^{-1} than the energies of the corresponding states in the remaining nucleotides. Thus, a resonance interaction between the excited Y_t-base molecules and other molecules entering into the composition of t-RNA$^{\mathrm{Phe}}$ is not very probable. No significant effect of adenine upon the fluorescence quantum yield and the lifetime of Y_t-base bounded to adenine in model Y_t-$(\mathrm{CH_2})_n$-adenine systems, has been found [7]. This proves that in the case under investigation, no energy transfer occurs which involves the triplet state of adenine. Singlet-triplet energy transfer in liquid solutions was frequently observed [8, 9]. In each case, however, the efficiency of this process is influenced by the presence of a "heavy atom" in the donor or acceptor molecule, and is also dependent upon the energy difference between the donor singlet and the acceptor triplet levels [10]. Halogen derivatives of benzene (Cl-, Br-, I-benzene), the triplet state energies of which are higher than the energy of the excited singlet state of Y_t-base, and the respective halogen derivatives of naphthalene, with the triplet state energies (approximately equal) lying about 6000 cm^{-1} below the S_1 level of Y_t-base, were used for the quenching of the Y_t-base fluorescence in dioxane.

Experimental

Y_t-base was obtained by the condensation of 3-methylguanine from bromoacetone [11]. Benzene and naphthalene derivatives (Fluka AG and Merck) used as the quenching substances, and dioxane (spectrograde) did not exhibit absorption in the excitation band ($\lambda_{\mathrm{exc}} = 345$ nm). Relative quantum yields were measured on a spectrofluorometer described in [12]. The concentration of Y_t-base was in all cases constant and equal to 1.5×10^{-4} M.

Results and Discussion

Exchange interactions between the excited donor molecules and the quenching molecules may occur in liquid solutions by collision, with the whole process being diffusion controlled. To the simplest approximation, the fluorescence quenching is expressed by the Stern-Volmer equation

$$\eta_0/\eta = 1 + K c_A = 1 + k \tau_0 c_A , \tag{1}$$

where η and η_0 are the donor fluorescence quantum yields in the presence and absence of the quenching substance, respectively, k the rate constant of the excitation energy transfer, and τ_0 the donor mean fluorescence lifetime. The results of the measure-

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ments of the Y_t-base fluorescence quenching by the benzene and naphthalene derivatives are summarized in Figure 1. An essential difference exists in the character of the Y_t-base fluorescence quenching by the benzene derivatives, for which a slight drop in η can be observed for iodobenzene only, and by the naphthalene derivatives. For the latter ones, the quenching rate constant k is markedly affected by the presence of the "heavy atom" in the acceptor molecule. For naphthalene, α-chloro, α-bromo and α-iodonaphthalene, the quenching rate constants amount to 0.0, 0.96, 6.65 and 19.8 l/mole, respectively. This effect, together with the simultaneous lack of quenching by the corresponding benzene derivatives, indicates that in the systems studied, intermolecular singlet-triplet energy transfer is responsible for the deactivation of the Y_t-base excited singlet state. Rate constants, k, for the energy transfer ($\tau_0 = 6.3$ ns [13]) amount to 0.15, 1.06 and 3.14×10^9 l/mole · s for α-chloro, α-bromo and α-iodonaphthalene, respectively. Therefore, the rate constant k increases with the increase of the spin-orbit coupling in the acceptor molecule, for α-iodonaphthalene attaining a value close to that of the diffusion rate constant k_d ($k_d = 8\,RT/3000\,\eta_s$, η_s-solvent viscosity). For dioxane, k_d is equal to 5.5×10^9 l/mole · s at room temperature. This confirms the fact that the singlet-triplet excitation energy transfer in liquid solutions is a diffusion controlled process.

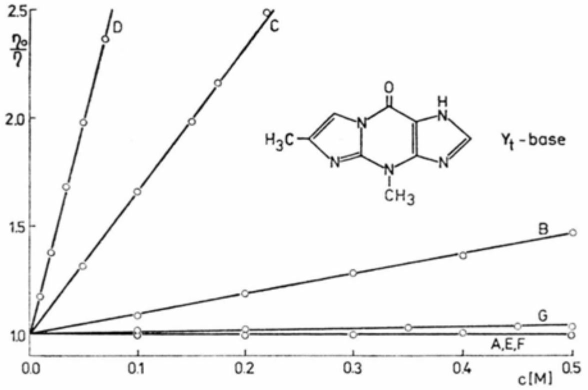

Fig. 1. Stern-Volmer plots for the Y_t-base fluorescence quenching by naphthalene and benzene derivatives. A, B, C, D-naphthalene, α-Cl-naphthalene, α-Br-naphthalene, α-I-naphthalene, respectively; E, F, G-, Cl-, Br-, I-benzene, respectively.

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