

FLUORESCENCE QUENCHING OF Y-4,9-DIHYDRO-4,6-DIMETHYL-9-OXO-1H-IMIDAZO-1,2-a PURINE (Y_t BASE) AND THE MODEL COMPOUNDS Y_t-(CH₂)_n-ADENINE BY α-BROMONAPHTHALENE

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Summary

The fluorescence quenching of Y-4,9-dihydro-4,6-dimethyl-9-oxo-1H-imidazo-1,2-a purine (Y_t base) and of some model compounds Y_t-(CH₂)_n-adenine ($n = 2, 3, 5$) by α-bromonaphthalene was studied in various solvents. The dependence of the ratio k/k_d (where k is the rate constant for energy transfer from Y_t to α-bromonaphthalene and k_d is the diffusion rate constant) on the integrated overlap of the Y_t fluorescence and the singlet-triplet absorption of α-bromonaphthalene was linear. The constant k for the model compounds varied with the length of the polymethylene chain and attained its lowest value for $n = 3$. This is probably due to an increased degree of base stacking interaction in the trimethylene-bridged compound.

1. Introduction

The high fluorescence efficiency and the ease with which Y-4,9-dihydro-4,6-dimethyl-9-oxo-1H-imidazo-1,2-a purine (Y_t base) can be excited in transfer ribonucleic acid (t-RNA) has led to the use of Y_t emission for probing the structure and conformation of t-RNA and has stimulated much interest in its luminescence and photochemical properties [1 - 6]. The luminescence properties of some model compounds in which Y_t is linked to adenine (Ade) by a polymethylene chain (Fig. 1) have also been investigated [7, 8]. We have recently reported [9] that the fluorescence of the Y_t base in dioxane is strongly quenched by α-halonaphthalenes (the triplet state energies of α-halonaphthalenes are approximately equal and lie about 6000 cm⁻¹ below the S₁ level of the Y_t base). No such effect is observed for halogen derivatives of benzene in which the triplet states have higher energies than the excited singlet state of the Y_t base. Furthermore the presence of the heavy atom in the quencher molecule has a marked effect on the fluorescence quenching rate constant k ($k_I > k_{Br} > k_{Cl}$).

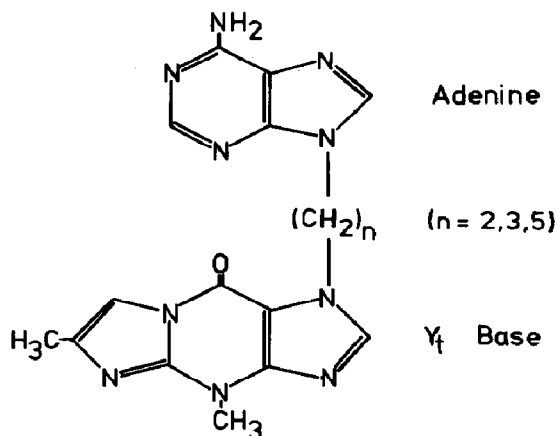


Fig. 1. The structure of the model compounds $Y_t-(CH_2)_n-Ade$.

These observations have led to the conclusion that intermolecular singlet-triplet energy transfer from Y_t to α -halonaphthalene is responsible for the fluorescence quenching of the Y_t base. In the present work we have studied this process for the Y_t base and the model compounds $Y_t-(CH_2)_n-Ade$ in various solvents.

2. Materials and methods

The Y_t base was prepared by reacting 3-methylguanine with bromoacetone [10]. The $Y_t-(CH_2)_n-Ade$ compounds were synthesized and purified as described previously [7]. Neither the solvents nor the α -bromonaphthalene quencher showed any absorption in the excitation band ($\lambda_{exc} = 345$ nm). The viscosities of the solvents were determined using a Höppler viscosimeter. The fluorescence was measured using the spectrofluorometer described in ref. 11. The Y_t concentrations were the same in all the solutions and were equal to 1.5×10^{-4} M. The lifetimes were measured using a pulse fluorometer similar to that described in ref. 12 and assuming a monoexponential decay. The singlet-triplet absorption spectra of α -bromonaphthalene were measured using a Beckmann model 25 spectrometer in the presence of 50% methyl iodide. The Stern-Volmer quenching constants K were calculated using the least-squares method.

3. Results and discussion

3.1. Quenching of the Y_t base fluorescence by heavy-atom-induced intermolecular singlet-triplet energy transfer

The singlet-triplet energy transfer can only take place by the exchange mechanism at normal encounter distances (about 10 Å) and in liquid solu-

tions this process is diffusion controlled. As a first approximation the fluorescence quenching can be described by the Stern–Volmer equation

$$\begin{aligned}\phi_0/\phi &= 1 + KC_A \\ &= 1 + k\tau_D^0 C_A\end{aligned}\quad (1)$$

where ϕ and ϕ_0 are the fluorescence quantum yields of the donor in the presence and the absence of the acceptor respectively, k is the excitation energy transfer rate constant, τ_D^0 is the fluorescence mean lifetime of the donor and C_A is the acceptor concentration.

The results for the quenching of the Y_t base fluorescence by α -bromonaphthalene in various solvents are shown in Fig. 2. As can be seen the quenching constant K and the energy transfer rate constant k depend strongly on the viscosity of the solvent. However, it is not a linear relation and the constant k is not directly proportional to the diffusion rate constant k_d . For example, in ethyl acetate the fluorescence of the Y_t base is quenched more strongly than in acetonitrile which has a lower viscosity. Thus another factor must be involved in the quenching process. When the solvent polarity changes, the emission of the Y_t base exhibits a pronounced red shift whereas the position of the singlet–triplet absorption of α -bromonaphthalene remains almost unchanged. Therefore the spectral overlap of the donor emission and the acceptor absorption and the probability of excitation energy transfer will depend on the solvent polarity. In liquid solutions the probability of fluorescence quenching by singlet–triplet energy transfer is proportional to

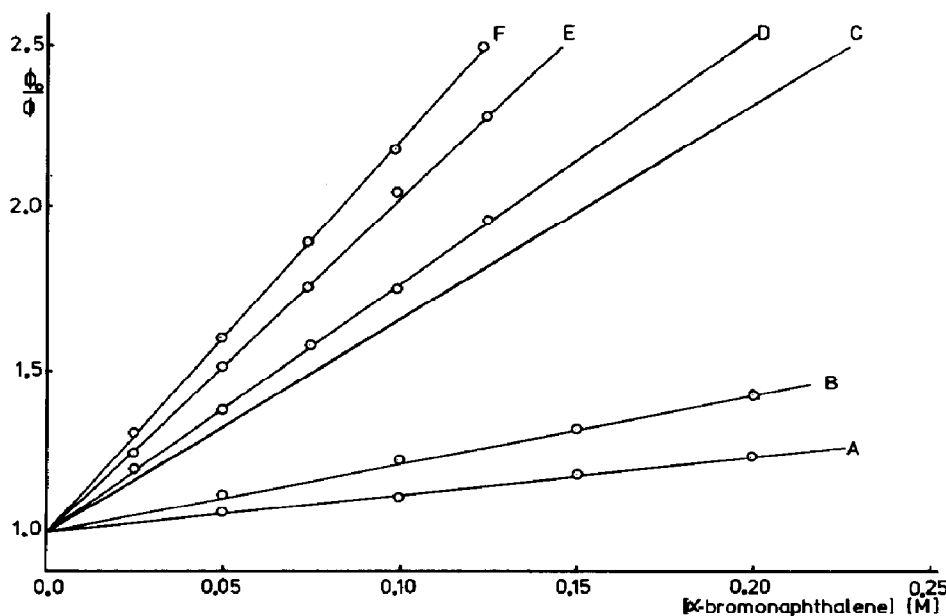


Fig. 2. Stern–Volmer plots of the quenching of the Y_t base by α -bromonaphthalene in various solvents: A, hexanol; B, *n*-butanol; C, dioxane (taken from ref. 9); D, methanol; E, acetonitrile; F, ethyl acetate.

the product of the probability of encounter and the probability of singlet-triplet energy transfer. This means that the rate constant for energy transfer is directly proportional to the product of the diffusion constant k_d and the probability $n(R)$ of energy transfer by exchange interaction:

$$k \approx k_d n(R) \quad (2)$$

The following equation has been derived [13 - 15] for the probability of energy transfer by an exchange mechanism:

$$n(R) = \frac{2\pi}{\hbar} z^2 \int f_D(\tilde{\nu}) f_A(\tilde{\nu}) d\tilde{\nu} \quad (3)$$

$$z^2 = A^2 \exp\left(-\frac{2R}{L}\right) \quad (4)$$

where $f_D(\tilde{\nu})$ and $f_A(\tilde{\nu})$ represent the donor emission spectrum and the acceptor absorption spectrum respectively, normalized so that $\int f_D(\tilde{\nu}) d\tilde{\nu}$ and $\int f_A(\tilde{\nu}) d\tilde{\nu}$ are both unity. A is a constant with the dimensions of energy, L is the effective Bohr radius and R is the donor-acceptor distance. Substitution of $n(R)$ in eqn. (2) gives

$$\frac{k}{k_d} \approx \frac{2\pi}{\hbar} z^2 \int f_D(\tilde{\nu}) f_A(\tilde{\nu}) d\tilde{\nu} \quad (5)$$

In order to check this relation the spectral overlap integrals were calculated from the singlet-triplet absorption of α -bromonaphthalene and the fluorescence emission spectra of the Y_t base in various solvents (Table 1) and were plotted against k/k_d ratios (Fig. 3). A reasonably straight line was obtained. This result proves that the singlet-triplet energy transfer in liquid solution occurs by a short-range exchange interaction.

TABLE 1

The fluorescence data for the quenching of the Y_t base by α -bromonaphthalene in various solvents

Solvent	η_s (cp)	τ_D^0 (ns)	k ($\times 10^{-8} \text{ l mol}^{-1} \text{ s}^{-1}$)	$\bar{\nu}_D^F$ (cm^{-1})	$\int f_D(\tilde{\nu}) f_A(\tilde{\nu}) d\tilde{\nu}$ ($\times 10^{-4} \text{ cm}^{-1}$)
Acetonitrile	0.35	5.6 ± 0.1	18.3 ± 0.2	22.200	2.06
Ethyl acetate	0.46	5.9 ± 0.1	19.8 ± 0.2	22.900	2.41
Methanol	0.58	5.7 ± 0.1^a	13.0 ± 0.2	22.100	1.94
Dioxane	1.25	6.3 ± 0.1^a	10.6 ± 0.3^b	22.700	3.37
<i>n</i> -butanol	2.6	5.1 ± 0.1^c	3.7 ± 0.3	22.400	2.20
Hexanol	3.4	4.9 ± 0.1^c	2.3 ± 0.5	23.000	2.66

^aData from ref. 7.

^bData from ref. 9.

^cData from ref. 8.

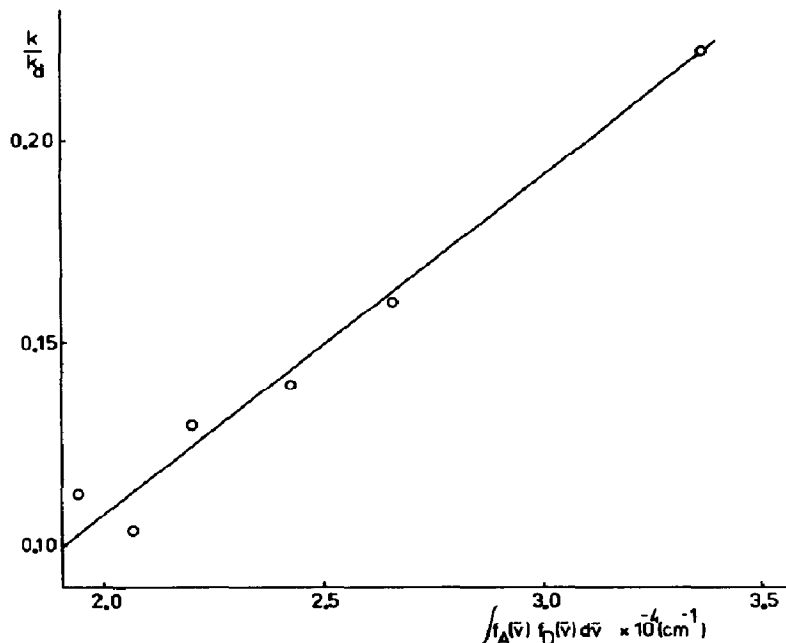


Fig. 3. The dependence of the ratio k/k_d on the integrated overlap of the donor (Y_t base) emission spectrum and the acceptor (α -bromonaphthalene) absorption spectrum.

3.2. The fluorescence quenching of the model compounds $Y_t-(CH_2)_n-Ade$ by α -bromonaphthalene

The fluorescence quenching of the dinucleotide analogues $Y_t-(CH_2)_n-Ade$ ($n = 2, 3, 5$) by α -bromonaphthalene was examined in dioxane and methanol solutions.

The Stern-Volmer quenching constants K and the energy transfer rate constants k obtained from these measurements are listed in Table 2. No significant change in the emission of the model compounds compared with that of the free Y_t base was observed. This implies that the spectral overlaps of

TABLE 2

Fluorescence parameters for the quenching of the model compounds in dioxane and methanol

Compound	Dioxane			Methanol		
	τ_D^a (ns)	K (l mol ⁻¹)	k ($\times 10^{-8}$ l mol ⁻¹ s ⁻¹)	τ_D^a (ns)	K (l mol ⁻¹)	k ($\times 10^{-8}$ l mol ⁻¹ s ⁻¹)
$Y_t-(CH_2)_2-Ade$	6.4 ± 0.1	4.3 ± 0.05	6.9 ± 0.2	5.4 ± 0.1	5.0 ± 0.08	9.3 ± 0.3
$Y_t-(CH_2)_3-Ade$	6.7 ± 0.1	4.3 ± 0.04	6.4 ± 0.2	5.6 ± 0.1	4.6 ± 0.1	8.2 ± 0.3
$Y_t-(CH_2)_5-Ade$	7.2 ± 0.2	6.0 ± 0.18	8.3 ± 0.5	5.8 ± 0.1	5.3 ± 0.15	9.1 ± 0.4

^aData from ref. 7.

the Y_t fluorescence and the singlet-triplet absorption of α -bromonaphthalene are the same for the same solvent, and thus no variation in the k values for these compounds is expected.

As can be seen from Table 2, however, the energy transfer rate constants k for the model compounds are lower than those for the Y_t base and vary with the length of the polymethylene chain. The reduction in k values for the model compounds compared with those for the free Y_t base can easily be explained by a screening effect of Ade which makes the Y_t base in these compounds less accessible to the quencher molecules. It is obvious that the screening effect must be strongly dependent on the length of the polymethylene chain because of the different degree of base stacking interaction.

The investigation of the effect of the synthetic spacer length on base-base interactions for many dinucleotide analogues $B-(CH_2)_n-B'$ ($n = 2, 3, 5, 6$) in water showed that the strongest interaction occurred in trimethylene-bridged compounds with folded or stacked conformations [16]. Therefore it is quite possible that the variation in the energy transfer rate constant k for the $Y_t-(CH_2)_n$ -Ade model compounds reflects the differences in the base-base interactions, and the occurrence of the minimum value of k for $n = 3$ may be due to an increase in the degree of the Y_t -Ade stacking conformation in the trimethylene-bridged compound.

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References

- 1 E. M. Evleth and D. A. Lerner, *Photochem. Photobiol.*, **26** (1977) 103.
- 2 R. J. Hoover, K. F. S. Luk and A. H. Maki, *J. Mol. Biol.*, **89** (1974) 363.
- 3 D. R. Kearns and K. L. Wong, *Proc. Natl. Acad. Sci. U.S.A.*, **70** (1973) 3843.
- 4 S. Paszyc and M. Rafalska, *Nucleic Acids Res.*, **6** (1979) 385.
- 5 I. Gryczyński, Ch. Jung, A. Kawski, S. Paszyc and B. Skalski, *Z. Naturforsch.*, **34a** (1979) 179.
- 6 J. Eisinger, B. Feuer and T. Yamane, *Proc. Natl. Acad. Sci. U.S.A.*, **65** (1970) 638.
- 7 I. Gryczyński, A. Kawski, S. Paszyc, M. Rafalska and B. Skalski, *Bull. Acad. Pol. Sci., Sér. Sci. Phys., Astron.*, **27** (1979) 271.
- 8 I. Gryczyński, A. Kawski, S. Paszyc and B. Skalski, *Z. Naturforsch.*, **35a** (1980) 1265.
- 9 I. Gryczyński, A. Kawski, S. Paszyc and T. Skowryra, *Z. Naturforsch.*, **36a** (1981) 76.
- 10 H. Kasai, M. Goto, K. Ikeda, M. Zama, Y. Mizuno, S. Takemura, T. Sugimoto and T. Goto, *Biochemistry*, **15** (1976) 838.
- 11 A. Kawski, J. Kamiński and E. Kuteń, *J. Phys. B*, **4** (1971) 609.
- 12 W. Becker, S. Dähne, K. Teuchner and K. Selige, *Exp. Tech. Phys.*, **23** (1975) 297.
- 13 D. L. Dexter, *J. Chem. Phys.*, **21** (1953) 836.
- 14 R. F. Vassilev, *Nature (London)*, **200** (1963) 773.
- 15 M. Inokuti and F. Mirayama, *J. Chem. Phys.*, **43** (1965) 1978.
- 16 N. J. Leonard, *Acc. Chem. Res.*, **12** (1979) 423, and references cited therein.