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RATE OF INTRAMOLECULAR ELECTRONIC ENERGY TRANSFER IN COUMARIN BICHROMOPHORIC MOLECULES. AN INVESTIGATION BY MULTIFREQUENCY PHASE-MODULATION FLUOROMETRY

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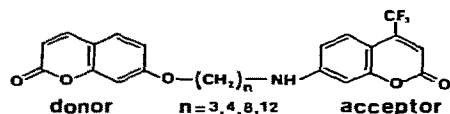
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Dynamics of intramolecular electronic energy transfer in bichromophoric molecules, consisting of two coumarins linked by a variable number of methylene groups is investigated by multifrequency phase-modulation fluorometry. From observation of the acceptor delayed emission, information on the rate of transfer can be obtained.

1. Introduction

Intramolecular electronic energy involving two chromophores separated by a molecular chain or frame is still receiving considerable attention because of its implications in numerous fields: photophysical processes, biological systems, dye lasers, wavelength shifters, etc. The reader is referred to the recent review by Speiser [1].

In a previous paper [2], we have reported a very efficient intramolecular energy transfer in a bichromophoric molecule consisting of two coumarin dyes linked by a short aliphatic chain. Application to frequency conversion of light was suggested. This new type of bichromophore whose formula is



has been further investigated with special attention to the effects of various parameters (temperature, viscosity, nature of the solvent, number of methylene groups separating the donor and acceptor moieties) on the efficiency of energy transfer. The results are being reported separately [3].

An essential feature of energy transfer is the rate of transfer. In most studies, this rate is calculated from measurements of the transfer efficiency and the lifetime of the donor in the absence of transfer. Nevertheless, dynamics of energy transfer is best analyzed by using time-dependent techniques, especially when transfer cannot be characterized by a single rate constant. Pulse fluorometry has been currently used for this purpose but never phase fluorometry. However, thanks to recent improvements based on multifrequency modulation of light and cross-correlation detection [4], phase fluorometry can provide information equivalent to that obtained with pulse fluorometry [5,6]. In the present paper, we report the first investigation of energy-transfer dynamics by phase fluorometry.

Furthermore, in most previous studies, attention

¹ Unité Associée au CNRS No 1103 "Physico-chimie Organique Appliquée"

was paid only to changes in the fluorescence decay of the donor. However, when very efficient energy transfer occurs and/or when the quantum yield of the donor is very low, the donor fluorescence is so weak that detection is difficult because of stray light. Nevertheless, information on transfer rate is contained in time-dependent acceptor fluorescence via donor excitation. For instance, picosecond pulse fluorometry can be used for the determination of the rise of the acceptor fluorescence following pulse excitation of the donor [7]. Alternately, as shown in this paper, phase fluorometry experiments are based on the measurement of the phase shift between the acceptor fluorescence in the presence of transfer with respect to the acceptor fluorescence in the absence of transfer at various frequencies. This kind of experiment is reported in the present work using the above-depicted bichromophoric molecules.

2. Experimental

The synthesis of the coumarin bichromophoric molecules will be reported elsewhere [8]. The experiments were carried out in propylene glycol (Aldrich, gold label) at 25°C. Very dilute solutions (5×10^{-6} M) were used to prevent spurious effects such as intermolecular energy transfer or reabsorption.

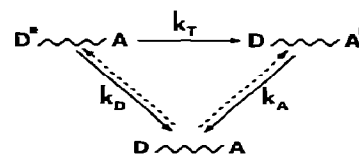
The absorption and emission spectra are given elsewhere [3].

Lifetime measurements were performed with the multifrequency phase fluorometer described by Gratton and Limkeman [4]. The light source used in the experiments reported in this work was an HeCd laser from Liconix Inc., equipped with UV optics for 325 nm. The intensity of the laser was sinusoidally modulated using an electro-optical modulator (model LMA 1 from Lasermetrics Inc.). With this arrangement, continuously variable modulation frequencies can be obtained from 1 to 160 MHz. The detection system is based on the cross-correlation technique introduced by Spencer and Weber [9]. The photomultipliers employed were from Hamamatsu Inc., model R 928. These tubes show negligible color effect in the wavelength range used in the present experiments [4]. Data acquisition was performed using an ISS-ADC interface from ISS Inc. and the lifetime acquisition software provided by ISS. Phase and modulation

data were analyzed for a single and double exponential decay using the ISS lifetime analysis software. This analysis is based on a non-linear least-squares method described by Brandt [10]. The equations used and the attainable resolving power have been reported elsewhere [5,6]. Error analysis is obtained from the computation of the diagonal terms of the covariance matrix of errors as reported by Brandt [10].

3. Theory

In a first approach, we consider a simple kinetic scheme involving a single rate constant k_T for transfer from donor to acceptor. If the emission spectrum of the acceptor does not overlap the absorption spectrum of the donor, there is no back transfer.



k_D and k_A are the emission rate constants of the donor and the acceptor, respectively they are equal to the reciprocal of the lifetimes τ_D^0 and τ_A^0 , respectively, in the absence of transfer.

Following an infinitely short pulse of light, the concentrations in excited donor and acceptor obey the following system of differential equations:

$$d[D^*]/dt = -(k_D + k_T)[D^*], \quad (1)$$

$$d[A^*]/dt = k_T[D^*] - k_A[A^*]. \quad (2)$$

Assuming that k_T is not time dependent (Stern-Volmer kinetics), the solutions of this system are the impulse response functions [11]:

$$[D^*] = \alpha_{0D} \exp(-m_0 t), \quad (3)$$

$$[A^*] = \alpha_{0A} \exp(-m_0 t) + \alpha_{1A} \exp(-m_1 t), \quad (4)$$

where

$$m_0 = k_D + k_T, \quad (5)$$

$$m_1 = k_A, \quad (6)$$

$$\alpha_{0D} = [D^*]_0, \quad (7)$$

$$\alpha_{0A} = -\frac{k_T [D^*]_0}{k_D + k_T - k_A}, \quad (8)$$

$$\alpha_{1A} = \frac{k_T}{k_D + k_T - k_A} [D^*]_0 + [A^*]_0. \quad (9)$$

At the excitation wavelength, both donor and acceptor absorb and their initial concentrations are $[D^*]_0$ and $[A^*]_0$, respectively.

In phase fluorometry experiments, the exciting light is sinusoidally modulated and the harmonic responses that are observed, are the Fourier transforms of the impulse responses. The quantities that can be measured are ϕ , the phase shift of emission with respect to excitation, and M , the modulation ratio (ac/dc ratio) [12]

$$\phi = \tan^{-1}(S/G), \quad (10)$$

$$M = (S^2 + G^2)^{-1/2}, \quad (11)$$

where S and G are the sine and cosine transforms of the impulse response $I(t)$:

$$S = \int_0^{\infty} I(t) \sin(\omega t) dt / \int_0^{\infty} I(t) dt, \quad (12)$$

$$G = \int_0^{\infty} I(t) \cos(\omega t) dt / \int_0^{\infty} I(t) dt, \quad (13)$$

ω is the angular frequency of the exciting light.

In the present case, $I(t)$ is given by eq. (3) for the donor and eq. (4) for the acceptor. Eqs. (12) and (13) thus yield:

$$S_D = \frac{m_0 \omega}{\omega^2 + m_0^2}, \quad (14)$$

$$G_D = \frac{m_0^2}{\omega^2 + m_0^2}, \quad (15)$$

$$S_A = \frac{m_0 m_1}{m_1 \alpha_{0A} + m_0 \alpha_{1A}} \left(\frac{\omega \alpha_{0A}}{m_0^2 + \omega^2} + \frac{\omega \alpha_{1A}}{m_1^2 + \omega^2} \right), \quad (16)$$

$$G_A = \frac{m_0 m_1}{m_1 \alpha_{0A} + m_0 \alpha_{1A}} \left(\frac{m_0 \alpha_{0A}}{m_0^2 + \omega^2} + \frac{m_1 \alpha_{1A}}{m_1^2 + \omega^2} \right). \quad (17)$$

3.1. Calculation of ϕ

Let us assume first that we can find an exciting wavelength λ_1 at which only the donor absorbs ($[A^*]_0 = 0$ in eq. (9)). Eqs. (4) can be now written in the following form:

$$[A^*] = \frac{[D^*]_0 k_T}{k_D + k_T - k_A} \times \{ \exp(-k_A t) - \exp[-(k_D + k_T)t] \}. \quad (18)$$

Furthermore, at a higher exciting wavelength λ_2 at which only the acceptor absorbs, we have

$$[A^*] = [A^*]_0 \exp(-k_A t). \quad (19)$$

Denoting by ϕ_A and ϕ_A^0 the phase shifts corresponding to eqs. (18) and (19) respectively, it is of interest to calculate $\tan(\phi_A - \phi_A^0)$. This calculation can be done by means of eqs. (12) and (13) and leads to a very simple expression:

$$\tan(\phi_A - \phi_A^0) = \omega / (k_D + k_T). \quad (20)$$

It is remarkable that this expression is identical to the tangent of the phase shift of the donor in the presence transfer:

$$\tan \phi_D = \omega / (k_D + k_T). \quad (21)$$

Therefore, equivalent information is obtained from observation of the donor and the acceptor provided that the phase of reference is appropriately chosen.

In practice, there is no wavelength at which the donor can be excited with negligible absorption of the acceptor. When both donor and acceptor absorb ($[A^*]_0 \neq 0$ in eq. (9)), the same procedure of calculation can be used and leads to:

$$\Delta = \tan(\phi_A - \phi_A^0) = \frac{\omega}{k_D + k_T - ([A^*]_0 / [D^*]_0) [(k_T + k_D)^2 + \omega^2] / k_T}. \quad (22)$$

Once k_D and the ratio $[A^*]_0 / [D^*]_0$ are evaluated, the measurement of Δ provides a straightforward way to determine k_T .

It is now worth examining what kind of information can be drawn from the measurement of the modulation ratio.

3.2. Calculation of M

Using eq (11) together with eqs. (16) and (17), we obtain:

$$M_A^2 = \left(\frac{m_0 m_1}{m_1 \alpha_{0A} + m_0 \alpha_{1A}} \right)^2 \times \frac{(m_1 \alpha_{0A} + m_0 \alpha_{1A})^2 + \omega^2 (\alpha_{0A} + \alpha_{1A})^2}{(m_0^2 + \omega^2)(m_1^2 + \omega^2)} \quad (23)$$

Substituting the values of m_0 , m_1 , α_{0A} , α_{1A} in this equation, the following expression for M_A is obtained:

$$M_A = M_A^0 \frac{k_D + k_T}{[(k_D + k_T)^2 + \omega^2]^{1/2}} \times \left(1 + \frac{\omega^2 [A^*]_0^2 / [D^*]_0^2}{[k_T + (k_T + k_D)[A^*]_0 / [D^*]_0]^2} \right)^{1/2}, \quad (24)$$

where M_A^0 is the modulation ratio of the acceptor in the absence of transfer,

$$M_A^0 = k_A / (k_A^2 + \omega^2)^{1/2}. \quad (25)$$

In the present investigation, the modulation frequency ranges from 1 to 160 MHz. Therefore $\omega^2 \leq 10^{18}$. In addition, the lifetime of the donor τ_D^0 is found to be 0.76 ns ($k_D = 1.31 \times 10^9 \text{ s}^{-1}$). Furthermore, steady-state experiments have shown that the transfer efficiencies are about 0.93 [3]; k_T is thus expected to be of the order of 10^{10} s^{-1} . Therefore,

$$(k_D + k_T)^2 \gg \omega^2. \quad (26)$$

Moreover, if the exciting wavelength is chosen to be the wavelength of maximum absorption of the donor, we have, $[A^*]_0 / [D^*]_0 < 0.1$. Therefore,

$$\frac{\omega^2 [A^*]_0^2 / [D^*]_0^2}{[k_T + (k_T + k_D)[A^*]_0 / [D^*]_0]^2} \ll 1 \quad (27)$$

Taking into account the inequalities (26) and (27), eqs. (22) and (23) reduce to

$$\Delta = \tan(\phi_A - \phi_A^0) \approx \frac{\omega}{k_D + k_T - ([A^*]_0^2 / [D^*]_0^2)(k_T + k_D)^2 / k_T}, \quad (28)$$

$$M_A \approx M_A^0. \quad (29)$$

Eq. (28) shows that a linear dependence of Δ as a function of ω is expected. The slope permits determination of k_T . Any departure from a linear dependence is evidence that there is not a single rate constant, or that this rate constant is time dependent.

Eq. (29) is of practical interest. It shows that the modulation ratio is not affected by transfer. Therefore, it is not necessary to use a second excitation wavelength at which the donor does not absorb; this would require, for the measurement of very small phase shifts, to take into account a possible slight "color effect" of the photomultiplier. As a matter of fact, the modulation lifetime $(\tau_A)_M$ of the acceptor in the presence of transfer is expected to be constant versus frequency and equal to the modulation lifetime $(\tau_A^0)_M$ in the absence of transfer. Besides, the lifetime $(\tau_A^0)_\phi$ measured by phase is given by

$$(\tau_A^0)_\phi = \omega^{-1} \tan \phi_A^0. \quad (30)$$

Since in the absence of transfer, the decay of the acceptor is assumed to be single exponential, these two lifetimes are identical and therefore ϕ_A^0 can be calculated by means of the following relation:

$$\phi_A^0 = \tan^{-1} [\omega(\tau_A)_M]. \quad (31)$$

4. Results and discussion

The lifetimes of the models for the donor and the acceptor described in ref. [3] were measured in propylene glycol at 25°C. The donor model was excited at 325 nm and the acceptor model at 442 nm. A multifrequency phase and modulation analysis leads to a single decay time. 0.759 ± 0.003 ns for the donor and 4.620 ± 0.040 ns for the acceptor.

The bichromophores were excited at 325 nm. At this wavelength, the ratio $[A^*]_0 / [D^*]_0$ is close to the minimum and equal to 0.083. The acceptor fluorescence was selected by using a Corning 3.71 filter. The modulation lifetime of the acceptor is constant versus

frequency; the average values are 4.77 ± 0.04 ns for $n = 3$, 4.66 ± 0.04 ns for $n = 4$, 4.71 ± 0.04 ns for $n = 8$ and 4.63 ± 0.05 ns for $n = 12$. These results show that the approximations used in section 3 are valid. For frequencies less than 10 MHz, the accuracy of the modulation lifetime is poor because the modulation ratio is very close to 1. Therefore, the above

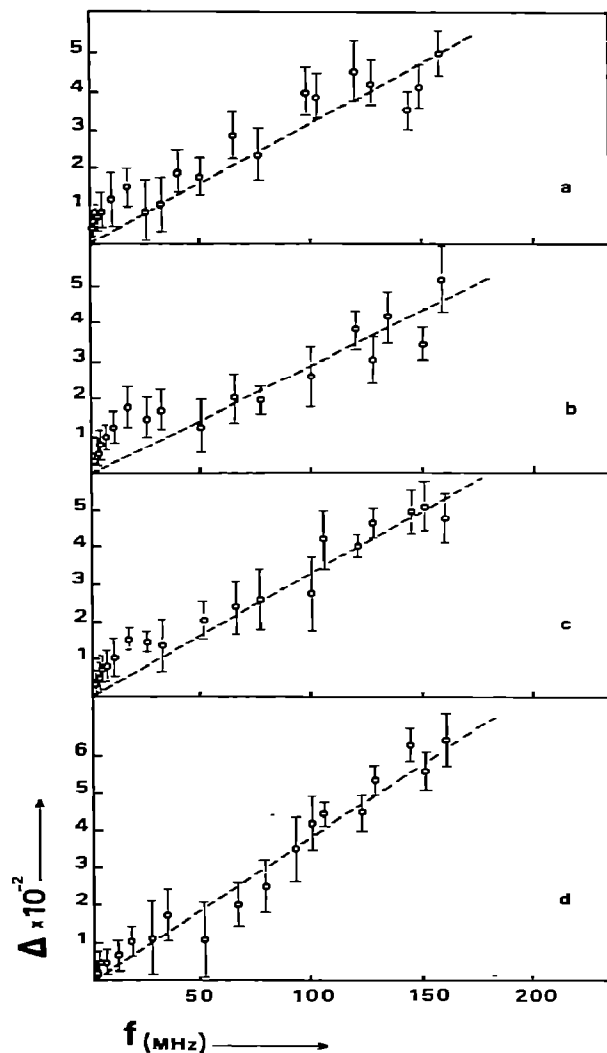


Fig. 1 Variations in $\Delta = \tan(\phi_A - \phi_A^0)$ versus frequency for $n = 3$ (a), $n = 4$ (b), $n = 8$ (c), $n = 12$ (d). Solvent, propylene glycol, temperature, 25°C.

Table 1

Rate constants and efficiencies of transfer determined by phase fluorometry and steady-state methods in propylene glycol at 25°C

	Phase fluorometry		Steady-state method ^{a)}
	k_T (10^{10} s^{-1})	η_T	η_T
$n = 3$	1.7 ± 0.4	0.93 ± 0.02	0.96 ± 0.04
$n = 4$	1.9 ± 0.2	0.935 ± 0.01	0.95 ± 0.04
$n = 8$	1.5 ± 0.2	0.92 ± 0.01	0.93 ± 0.04
$n = 12$	1.4 ± 0.2	0.91 ± 0.01	0.91 ± 0.04

^{a)} From ref. [3].

average values were used to calculate ϕ_A^0 by means of eq. (31). At frequencies higher than 10 MHz, the calculation was performed by using the actual value of $(\tau_A)_M$ at each frequency instead of using the average value, in order to minimize the effect of slight systematic deviations from the average value

The values of $\Delta = \tan(\phi_A - \phi_A^0)$ are reported in fig. 1. Apart from small deviations at low frequencies, the variations of Δ versus frequency are approximately linear, as expected from eq. (28). The calculation of the average slopes leads to the values of the rate constant which are reported in table 1. These values are in the range predicted by Förster; dipole-dipole interaction is in fact highly probable as a consequence of the large spectral overlap between donor emission and acceptor absorption [3]. The transfer rate constants are slightly sensitive to the number of methylene groups. This fact is in agreement with the small changes in transfer efficiencies determined by steady-state methods (whereas larger changes were observed when dimethylformamide is used as a solvent) [3]. The consistency between dynamic and steady-state methods is further confirmed by the values of transfer efficiencies η_T calculated from the rate constants (table 1) by means of the following relation:

$$\eta_T = k_T \tau_D^0 / (1 + k_T \tau_D^0). \quad (32)$$

The agreement between steady-state results and time-resolved results indicates that the kinetics are simple; the small deviations at low frequencies are more likely accounted for by experimental error than by complex kinetics (the experiment is in fact more difficult to perform at the lowest frequencies). Several phenomena, such as distribution of interchromo-

phoric distance, intramolecular motions, etc., could lead to complex kinetics but the effects are expected to be small in the present case since the transfer efficiency is high

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References

- [1] S. Speiser, *J. Photochem.* 22 (1983) 195, and references therein
- [2] J. Bourson, J. Mugnier and B. Valeur, *Chem. Phys. Letters* 92 (1982) 430.
- [3] J. Mugnier, J. Pouget, J. Bourson and B. Valeur, *J. Luminescence*, to be published.
- [4] E. Gratton and M. Limkeman, *Biophys. J.* 44 (1983) 315.
- [5] J.R. Lakowicz, G. Laczko, H. Cherek, E. Gratton and M. Limkeman, *Biophys. J.* 46 (1984) 463.
- [6] E. Gratton, M. Limkeman, J.R. Lakowicz, B.P. Maliwal, H. Cherek and G. Laczko, *Biophys. J.* 46 (1984) 479.
- [7] B. Kopynsky, W. Kaiser and F.R. Schäfer, *Chem. Phys. Letters* 56 (1978) 458.
- [8] J. Bourson, to be published.
- [9] R.D. Spencer and G. Weber, *Ann. NY Acad. Sci.* 158 (1969) 361
- [10] S. Brandt, *Statistical and computational methods in data analysis*, 2nd Ed. (North-Holland, Amsterdam, 1976)
- [11] J.B. Birks, S. Georghiou and I. Munro, *J. Phys. B1* (1968) 266
- [12] G. Weber, *J. Phys. Chem.* 85 (1981) 949.