The Influence of Reverse Nonradiative Excitation Energy Transfer on the Fluorescence Spectra of Two-component Fluorescent Solutions

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The reverse non-radiative excitation energy transfer (RNEET) in the system of rhodamine 6G (R6G, donor) and rhodamine B (RB, acceptor) is investigated. The presence of RNEET from the energy acceptors to the energy donors is demonstrated using steady-state fluorescence and fluorescence anisotropy techniques. The spectra are deconvoluted and obtained results are analysed.

1. Introduction

In the last decade progress has been made in understanding the mechanism of nonradiative energy transport in solutions by taking into account the reverse energy transport from the excited acceptors to the energy donors ($A^* + D \rightarrow A + D^*$) [1–9]. The precondition for this effect is the spectral overlapping between the acceptor emission and the donor absorption bands (overlap integral $I_{AD} > 0$). This phenomenon manifests itself in many natural and artificial systems of biophysical interest, i.e. chlorophyll a and chlorophyll b in a photosynthetic unit [10] as well as in different ionic forms of the same dye [11–12]. It is inseparably connected with the systems consisting of monomers and fluorescent dimers [13–17] and can affect the intramolecular distance determination between the unlike fluorophores [18].

Very recently, coherent statistical theories based on the hopping [19–21] and diagrammatic models [22–23] have been elaborated to describe the behavior of luminescent observables in the presence of reverse non-radiative excitation energy transfer (RNEET) [1–5, 7–9]. Still, however, not very much experimental evidence has been reported. The only results concern the concentration changes in the emission anisotropy and the quantum yield [24, 25] as well as some Monte-Carlo simulations [9, 16]. The reasons are not only the novelty of the problem but also serious experimental difficulties in the selection and preparation of the samples as well as the demand for a very sensitive equipment. Besides, complex numerical analysis is often indispensable.

The aim of this study is to fill partially the experimental gap be demonstrating the influence of RNEET on the fluorescence spectra of two-component systems. Additionally, high donor fluorescence depolarization due to RNEET supports the results presented. Experiments dedicated to the time-resolved observables and the comparison to analytical theories, in view of the complexity of numerical computations, will be reported elsewhere.

2. Experimental Results and Discussion

The experiment consists in the recording of the fluorescence spectra of the R6G–RB mixture in glycerol. The idea of the experiment looks as follows: the two-component system is excited at 590 nm. For this wavelength practically only acceptors are excited (Figure 1). If, apart from the acceptor fluorescence, the contribution of the donor to the total measured spectrum is observed, it will evidence the non-radiative transfer from acceptors to the donor ensemble (of course, radiative transfer must be eliminated).

The experiment was prepared in the following manner: measurements were carried out in especially designed micrometer cuvettes thin enough to meet the relation

$$2.3\,\varepsilon_{\max} C d \leq 0.1, \qquad (1)$$

where $\varepsilon_{\max}$ is the maximum extinction coefficient, d is the thickness of the cuvette and C denotes the concen-

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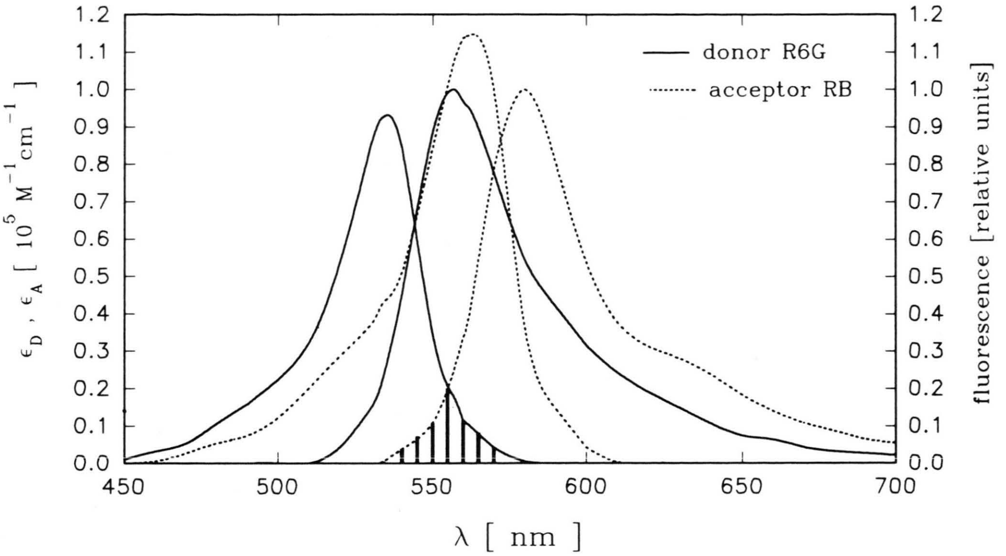

Fig. 1. Absorption and emission spectra of R6G and RB in anhydrous glycol at 293 K. Shaded area is the spectral overlap of the acceptor fluorescence and donor absorption, and represents the precondiction for the RNEET.

tration of the solution. The fulfilment of this condition enabled the secondary effects to be neglected [26], and particularly to eliminate the radiative transfer between D and A molecules. Each sample contained donors and acceptors with relatively low acceptor to donor concentration ratio, C_A/C_D (see Table 1 for the exact values and Table 2 for more physical information on the systems investigated). Donor concentrations were chosen from the range $10^{-3} \div 5 \cdot 10^{-3}$ M. Within this range aggregate-formation in the system investigated can be fully neglected [24]. Acceptor concentrations were smaller than those of donors: $5 \cdot 10^{-5} \div 2 \cdot 10^{-3}$ M. On the one hand, such a choice ensured a strong enough fluorescence signal, and on the other, it created a situation in which acceptors are surrounded by numerous donors (for most of the systems investigated). This enabled, in turn, a relatively high efficiency for the reverse transfer. The absorptions of R6G and RB were measured separately at low concentrations (10^{-5} M each) in the long-wavelength tails of the spectra, using 5 cm thick cuvettes. The purpose was to check in practice, whether the donors (R6G) absorb the light responsible for the acceptor excitation (RB), and to determine the exact extinction coefficients corresponding to the excitation wavelength. If this absorption had been significant compared to that of donors it could have complicated the interpretation of the results. Figure 2 shows long-

Table 1. Basic physical data for some of the systems investigated.

System	Subsystem	Solvent	C_A [M]	C_A/C_D [M]	Viscosity [Pa s]	λ_{exc} [nm]
R6G	Ia	an-	$5 \cdot 10^{-5}$	1/20		
+RB	Ib	hydrous	$5 \cdot 10^{-5}$	1/40	1.5	590
	Ic	glycerol	$5 \cdot 10^{-5}$	1/100		
	IIa		$2 \cdot 10^{-4}$	1/5		
	IIb		$2 \cdot 10^{-4}$	1/10		
	IIc		$2 \cdot 10^{-4}$	1/25		
	IIIa		$5 \cdot 10^{-4}$	1/2		
	IIIb		$5 \cdot 10^{-4}$	1/5		
	IIIc		$5 \cdot 10^{-4}$	1/10		
	IVa		10^{-3}	1/1		
	IVb		10^{-3}	1/2		
	IVc		10^{-3}	1/5		
	Va		$2 \cdot 10^{-3}$	2/1		
	Vb		$2 \cdot 10^{-3}$	1/1		
	Vc		$2 \cdot 10^{-3}$	1/2.5		

Table 2. Energy transfer parameters for the R6G/RB system in glycerol.

R_{0DD} [Å]	R_{0DA} [Å]	R_{0AA} [Å]	R_{0AD} [Å]	$C_{0DD}\ 10^{-3}$ [M]	$C_{0DA}\ 10^{-3}$ [M]	$C_{0AA}\ 10^{-3}$ [M]	$C_{0AD}\ 10^{-3}$ [M]
48.92	62.55	51.83	35.48	3.387	1.620	2.848	8.889

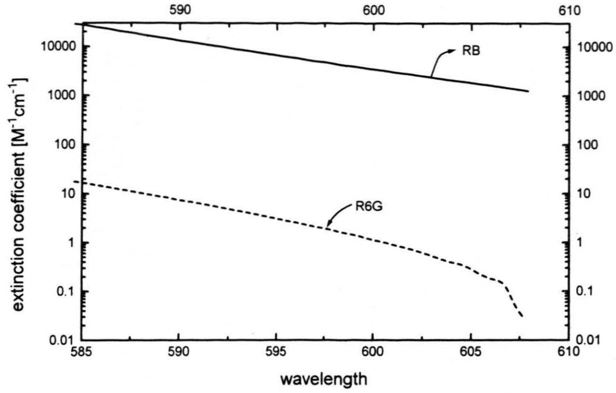

Fig. 2. Logarithms of long-wavelength extinction coefficients of R6G and RB.

Table 3. Donor emission anisotropy, r_D/r_{0D}, and peak-to-peak fluorescence intensity ratio (PPR) for the R6G/RB system. Particular subsystems are described in Table 1.

System	Ia	Ib	Ic	IIa	IIb	IIc	IIIa	IIIb	IIIc
r_D/r_{0D}	0.019	0.016	0.011	0.018	0.017	0.009	0.015	0.010	0.01
PPR	0.075	0.124	0.179	0.072	0.125	0.174	0.052	0.089	0.13

System	IVa	IVb	IVc	Va	Vb	Vc
r_D/r_{0D}	0.013	0.012	0.006	0.01	0.007	0.007
PPR	0.03	0.072	0.093	0*	0.061	0.073

* Not measurable in terms of the convergence of the numerical procedure.

wavelength tails of absorptions for both compounds. It is clearly seen that upon the experimental conditions (590 nm) the absorption of R6G is insignificant (approximately 0.01% of the maximal extinction). Additionally, the emissive signals coming from the single components were measured at the excitation 590 nm. No emission of rhodamine 6G was found within the sensitivity of our equipment. The fluorescence of rhodamine B was easily measured.

The fluorescence measurements were carried out with a fully computerized non-commercial apparatus, based on a phase and intensity modulation method, built in our laboratory and described previously in detail [27]. The absorption was measured by a fully computerized Specord M-40 spectrofluorometer. Emission anisotropy measurements were carried out using a two-channel single photon-counting technique [28, 29]. All measurements were performed at $T = 293$ K.

Figure 3 shows the experimental results for a given acceptor and variable donor concentrations. Figure 3a shows the summaric spectra of RB and R6G, measured for the excitation 590 nm (excitation line eliminated). It is clearly seen that with the increase in the donor concentration the short-wavelength contribution to the total fluorescence becomes higher. Simultaneously, a certain decrease in the emission peak intensity was found with rise in the donor concentration, for which also the reverse transfer is responsible. In the same figure also the spectrum of pure RB is presented for reference. Figure 3b shows an example of spectra deconvolution. The separation was performed using the standard nonlinear least-squares method assuming the real shapes of the single spectra (no arbitrary distribution function is introduced). It was supposed that the component spectra hidden in the summaric spectrum have the same shape as those measured individually. The spectra could, however, differ in amplitudes and peaks location. As references served the specta of pure RB measured for the excitation 590 nm, and R6G for 557 nm, which corresponds to the center of the spectral overlapping region (cf. Figure 1). Table 3 shows the emission anisotropy of R6G measured at 525 nm upon the excitation of RB at 590 nm. Almost complete depolarization of R6G fluorescence was observed, which is consistent with the well known theoretical prediction that the molecules excited by the act of nonradiative transfer emit essentially depolarized light even in a viscous solution [30, 31]. Moreover, the emission anisotropy did not change much with the acceptor concentration for the system with moderate donor concentration (see columns Ic ÷ Vc in Table 3) which may indicate that indeed even one act of non-radiative transfer is enough to depolarize the fluorescence light almost completely. This proof is, in our opinion, more straightforward than that based on the direct energy transfer measurement in which, apart from the acceptor emission, also highly polarized donor fluorescence is usually unavoidable. Similar systems, but with higher C_A concentration and lower C_A/C_D ratio were also investigated. Figure 4 shows the results for $C_A = 2 \cdot 10^{-3}$ M and $C_A/C_D = 1/2.5$ (system Vc) presented in the same form as in Figure 3. For this system the energy migration within the acceptor set becomes significant, which lowers the probability of the excitation to be transferred to the donor ensemble. This is the reason why in this system the effect observed is weaker. A certain measure of the intuitive "strength" of RNEET may be the peak-to-peak fluorescence intensity ratio, $I^{\max}_{R6G}/I^{\max}_{RB}$, obtained from the deconvolu-

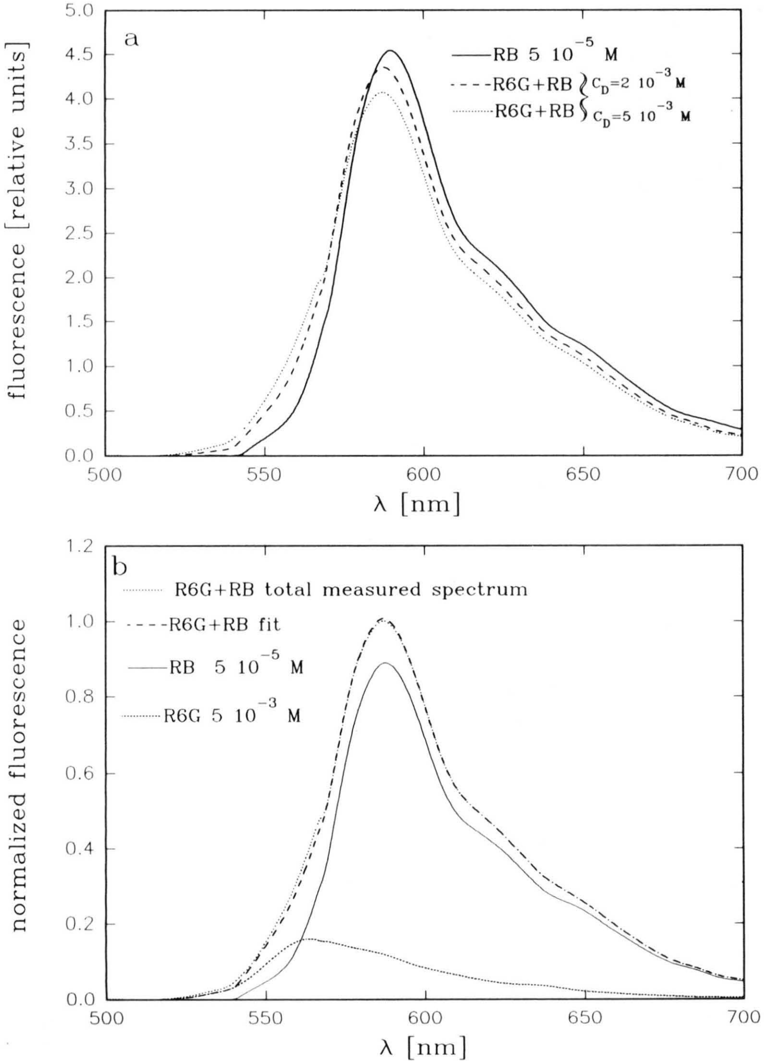

Fig. 3. An example of the experimental results of R6G and RB emission spectra measurements ($C_A = 5 \cdot 10^{-5}$ M): a) spectra of two-component systems and spectrum of pure RB as a reference, b) separated spectra.

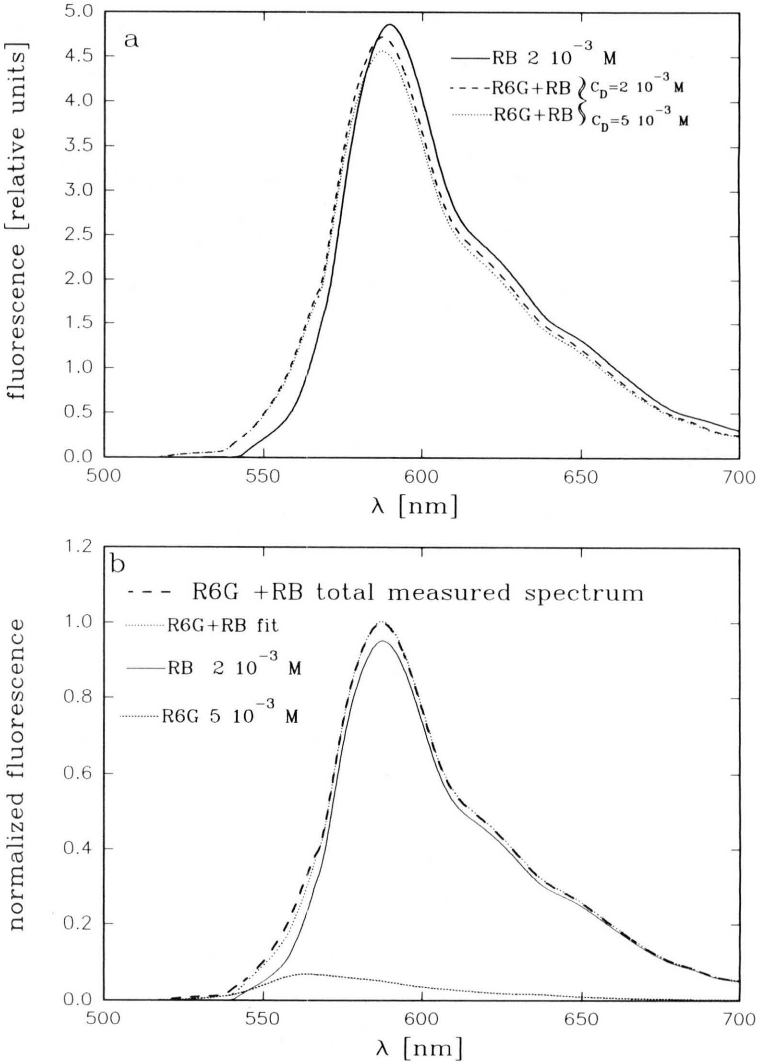

Fig. 4. An example of the experimental results of R6G and RB emission spectra measurements ($C_A = 2 \cdot 10^{-3}$ M): a) spectra of two-component systems and spectrum of pure RB as a reference, b) separated spectra.

tion. The values of this ratio for several systems are listed in Table 3. If C_D and C_A are moderate (order of 10^{-3} M) and comparable in concentration, it becomes difficult to separate the spectra and conclude about RNEET. However, it does not form a general rule concerning the detection of RNEET using other fluorescence observables, i.e. quantum yield or emission anisotropy, which are sensitive to RNEET in a somewhat different manner [24, 25]. Of course, RNEET almost certainly takes place in systems with high C_A and C_D concentrations. However, the results obtained for these systems are much more difficult to discuss in view of aggregate-formation. Fluorescent dimers do not act only as traps for the excitation, but also unavoidably contribute to the reverse transfer to monomers. Another problem would be the elimination of secondary effects. This would demand either a cuvette thicknes much smaller than 1 micrometer or a good microscope and image intensifier to be employed.

3. Conclusions

It was found that the effect of RNEET affects the fluorescence spectra of two-component systems with closely located S_1 levels. This result is strongly supported by the emission anisotropy measurements in which high donor depolarization due to RNEET was found. The effect of reverse transfer is strongly dependent on the acceptor concentration, C_A, and the C_A/C_D ratio. It is especially evident in systems with weak acceptor concentration and low C_A/C_D ratio. Energy migration within the set of acceptors diminishes the effectiveness of RNEET. This is because the multistep energy migration opens a new path for the excitation deactivation. Further experimental investigations concerning the steady-state and time-resolved observables in the presence of RNEET seem useful and advisable.

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