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Influence of spectral heterogeneity of prodan and laurdan solutions on the transfer of electronic energy to octadecyl rhodamine B

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Abstract

Fluorescence quenching and resonance energy transfer have been studied by steady-state fluorescence spectroscopy. The experimental and theoretical values for the rate constants of the electronic energy transfer (k_{ET}) and critical radius (R_0) were determined for prodan and laurdan as donors and octadecyl rhodamine B as acceptor. The spectroscopic data show, that prodan and laurdan in solution create an inhomogeneous spectroscopic medium in which multi-channel luminescence phenomena take place. This finding indicated that the modified form of the Stern–Volmer relation should be used for analyzing fluorescence quenching data. Results of performed studies point out, that dipole–dipole interaction is responsible for the resonance energy transfer from prodan and laurdan to octadecyl rhodamine B. The relative quenching efficiencies of both dyes depend on polarity of the medium and are higher for more polar solvent (AcN).

Keywords: Prodan; Laurdan; Fluorescence quenching; Heterogeneously emitting systems; Energy transfer

1. Introduction

The quenching of fluorescence caused by the resonance energy transfer from electronically excited donor molecule, D^*, to an acceptor molecule, A, which becomes excited to a higher electronic state, is a widely studied phenomenon [1–4]. The studies of energy transfer have both theoretical as well as practical importance. This phenomenon has found applications in many fields including the photochemistry for elucidating mechanisms of photochemical reactions [5] and has been effectively used as a spectroscopic ruler in a wide variety of biological macromolecules and assemblies [2,3,6]. Furthermore, energy transfer is a key step in biologically important processes such as the photosynthetic process in plants [7].

The quenching of fluorescence requires a close approach of fluorophore and quencher, and usually is interpreted according to theories assuming either dynamic or static quenching, or a combination of both [6]. The Stern–Volmer theory has been applied successfully to a wide variety of quenching studies in isotropic and low-viscosity solutions. When a system contains a fluorophore in different environments (e.g. a fluorophore embedded in microheterogeneous materials as sol–gel matrices, polymers, etc.) or more than one fluorophore (e.g. different tryptophanyl residues of a protein), the classical Stern–Volmer theory cannot be applied. A proper treatment of quenching processes in heterogeneously emitting systems requires additional considerations [1,2,10].

In this paper we focus besides the spectral properties of prodan (6-propionyl-2-dimethylaminonaphtalene) and laurdan (6-dodecanoyl-2-dimethylaminonaphtalene), the quenching of their excited states by octadecyl rhodamine B (ORB) (shown on Scheme 1). A particular attention has been paid to these organic molecules since they are capable of simultaneous creation of locally excited S_1(LE) and charge transfer S_1(CT) excited states [11–16]. As it has been shown in a series of our previous papers [13–16], the solutions of prodan and laurdan in neat solvents and phospholipid vesicles are spectrally an inhomogeneous system in which multi-channel luminescence phenomena take place. The spectroscopic heterogeneity is due to distribution of space conformational forms possessing different twisting angles φ between the mutual orientation

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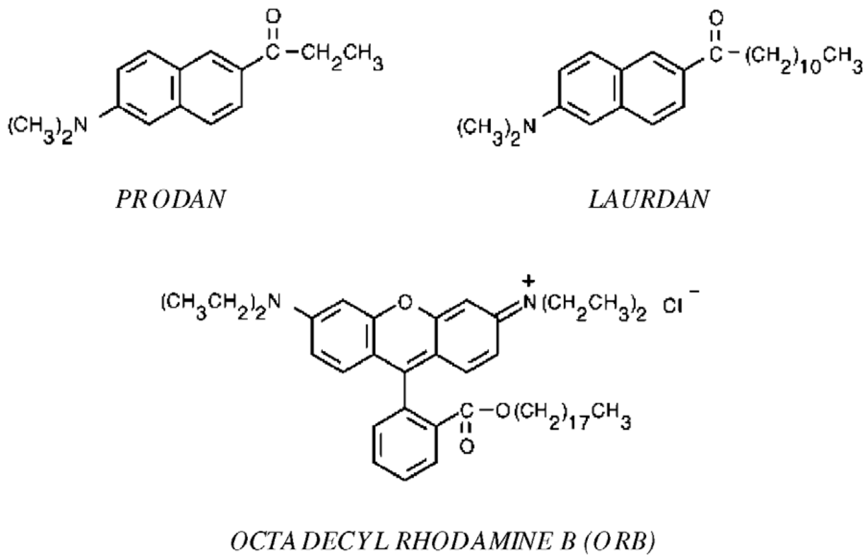

Scheme 1. Chemical structure of donor (prodan, laurdan) and acceptor (octadecyl rhodamine B) molecules.

planes of the dimethyl group ($-N(CH_3)_2$) and the naphthalene moiety, and by accompanying intra- and intermolecular charge transfer phenomena.

In the following, we report results of performed intermolecular electronic energy transfer studies in isotropic solutions of the heterogeneously emitting systems. A special attention has been paid to the description of the fluorescence quenching occurring in the heterogeneously emitting systems.

2. Experimental details

Prodan, laurdan and ORB were purchased from Molecular Probes (Eugene, OR) and used without further purification. The solvents tetrahydrofuran (THF) and acetonitrile (AcN) were of spectroscopic grade and provided by Merck (Darmstadt, Germany).

The absorption spectra of the dyes were measured on Shimadzu UV-2401 PC spectrophotometer. The fluorescence spectra of the compounds and their mixtures were carried out with a Shimadzu RF-5301 spectrofluorometer. Fluorescence quenching measurements were performed using triangular cuvette with frontal excitation to minimize the effect of reabsorption of donor emission by the acceptor. The emission was observed perpendicular to the direction of the exciting beam. Thus, the errors due to fluorescence reabsorption were reduced in a way that mathematical corrections were superfluous.

The fluorescence lifetime measurements were performed by means of the Time Correlated Single Photon Counting Technique (TCSPC). The experimental set up for the performed time resolved measurements has been described elsewhere [16,17].

The quantum yield, Φ_F, was determined by comparison with a standard solution using β-carboline in 1 N sulphuric acid as a reference compound [6,18]. The value of quantum yield of the used standard solution is equal $\Phi_F{}^S = 0.6$ [6].

3. Results and discussion

3.1. Spectral properties of one compound solution

In Fig. 1 the emission spectra of prodan and laurdan in THF and AcN are reported. As expected for a dye molecule containing a donor and acceptor groups, its emission is dependent on the polarity of the environment. As can be seen on Fig. 1, the emission maxima of prodan and laurdan in THF are 431 and 432 nm. While those values are considerably red shifted in AcN, and equal 456 and 452 nm, respectively. The preceding of experimental observation [14–16] agree with the results of the quantum mechanical calculations [12,13] which predict the existence of S_1(LE) and S_1(CT) states. The fluorescence spectra of prodan and laurdan obtained in our studies (see Fig. 1), as well as by other authors [11–16] show that the emission of both molecules arises from two different excited states. As it is seen on Fig. 1, the emission spectra of both dyes can be decomposed into two separated bands (Fig. 2) which correspond to the emission arising from either S_1(LE) or S_1(CT) states. From the decomposition of the fluorescence spectrum into the separated Gaussian bands, we establish the λ_{max} value of the LE and CT bands and their half width. We found that the two bands participate in the fluorescence spectrum at the ratio: 1 : 4.8 (THF) and 1 : 1.4 (AcN) for prodan, and 1 : 2 (THF) and 1 : 2.2 (AcN) for laurdan. In conclusion, the number of molecules with changed structural conformation emitting from the S_1(CT) state depends on the solvent polarity.

As can be seen from Fig. 2, the absorption spectra of prodan in THF and AcN at room temperature are similar, indicating that the ground states of this compound in the two solvents are identical. The same results were obtained for laurdan molecules (data not shown). Furthermore, the overlap between the donor (prodan and laurdan) emission and acceptor (ORB) absorption spectra is noted. It points to the fact that the donor–acceptor