

Fluorescence Resonance Energy Transfer Using Spiropyran and Diarylethene Photochromic Acceptors

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Abstract: We describe the preparation and photophysical characterization of two model compounds designed to test a new approach for the quantitative determination of Fluorescence Resonance Energy Transfer (FRET) in biological systems. The method enables modulation of FRET by exploiting the unique reversible spectral properties of photochromic diarylethenes and spiropyrans to create switchable energy acceptors.

Introduction

Fluorescence resonance energy transfer [1] (FRET) is a physical process by which energy is transferred in a non-radiative manner through a dipole-dipole interaction. Previously, we have proposed a method based on the modulation of fluorescence emission of a donor by a photochromic acceptor [2,3], in which only the absorption of one of its photochromic-isomeric forms overlaps with donor emission. A light-induced structural transition of the acceptor results in changes of its excitation properties, which in turn can "switch on" and "off" donor fluorescence in a reversible fashion. The method is especially suited for microscopy because it operates over a range ($< 100 \text{ \AA}$) that surpasses optical resolution of most light microscopes ($\sim 0.3 \text{ \mu m}$) and it generates the necessary reference state required for quantitative FRET determinations without the need of photochemical destruction of donor or acceptor [4]. Additionally, continuous observations within living cells are feasible.

In this work we perform a comparative analysis of photochromic spiropyrans and diarylethenes as potential acceptors for FRET.

Experimental

Synthesis. Lucifer yellow cadaverine-6-nitroBIPS (LYC-BIPS) (1) or Lucifer yellow cadaverinediarylethene (2)

Lucifer yellow cadaverine was coupled with the succinimidyl ester of 1',3'-dihydro-3',3'-dimethyl-

1'-(2-carboxy)-6-nitrospiro [2H-1-benzopyran-2',2'-(2H) indole] (6-nitroBIPS) or carboxyethyldiarylethene in dry acetonitrile to yield compounds **1** or **2**. The products were HPLC purified and identified by $^1\text{H-NMR}$ and mass spectrometry.

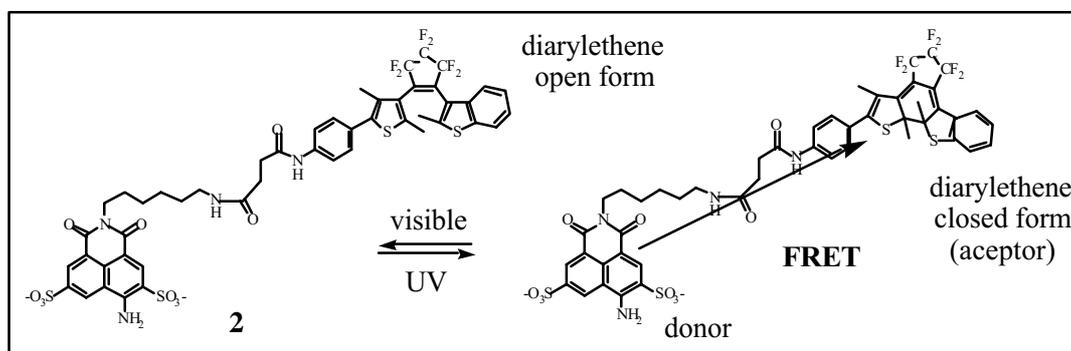
Photophysical studies

Absorption spectra of compounds **1** and **2** were performed at different stages of acceptor photoisomerization. The kinetics of thermal reversion was studied for the spiropyran derivative. FRET was determined by evaluating the donor emission steady state spectra (ex. 420 nm) in each of the acceptor forms and donor lifetime emission, measured by the phase and modulation method.

Results and Discussion

Light induced photo-conversion at 254 nm of the spiropyran form of 6-nitroBIPS to the merocyanine form (FRET acceptor) resulted in a 35% decrease in donor emission quantum yield. Irradiation at 546 nm yielded the initial spiropyran form with the original donor emission intensity. The irradiation could be repeated for at least 8 cycles without any apparent fatigue. In addition to this light driven process, the merocyanine reverts to the spiropyran form by a thermal mechanism ($k_T = 0.039 \text{ min}^{-1}$). The light-independent transition from spiropyrans to merocyanine in polar solvents limits the use of this compound in aqueous solutions.

Thermally stable diarylethenes were used as acceptors for FRET in model compound **2**. Photoisomerization from the open to the closed form induced by irradiation at 313 nm switched "on" and "off" the FRET process ($E = 0.25$). The modulation of the absorption and fluorescence could be cycled at least 20 times without any noticeable degradation. The degree of conversion between open and closed form was wavelength dependent. In contrast to the merocyanine acceptors, thermal stability of both open and closed diarylethene forms allowed repeated absorption and fluorescence determinations. The excellent performance of diarylethene acceptors encourages their use for future studies in FRET microscopy.



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References and Notes

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