Phosphorescence Lifetime Measured with a SPEX® Phosphorimeter

Introduction

A phosphorimeter integral to the SPEX® FLUOROMAX®-P or as an accessory on the SPEX® FLUOROLOG® system can uncover important information on various systems of chemical and biochemical interest, by revealing data normally masked by intense but rapid fluorescence.

This *Application Note* describes an example, using the SPEX® phosphorimeter, of energy-transfer from a peptide-terbium-complex donor to a fluorescein acceptor. The peptide in the complex absorbs light at 280 nm, the terbium phosphoresces at 485 nm, where fluorescein dye absorbs. The fluorescence of fluorescein can be observed when the sample is excited at 280 nm, using a phosphorimeter accessory.

Experiment

Samples of peptide-terbium complex were dissolved in aqueous solution, along with fluorescein in some samples. The measurements were taken on a FLUOROLOG®-322 spectrofluorometer: fluorescence with a 450-W CW xenon lamp; phosphorescence with a xenon flash lamp; and an R928 photomultiplier operated at 950 V in the photon-counting mode. The SPEX® FL-1042 phosphorimeter accessory includes a dual lamp housing (with both CW and pulsed Xe lamps), and all control electronics. A light pulse excites the sample, and variable delays control when the detection window opens, and for how long. Sample excitation was 280 nm, with 100 flashes measured. For luminescence spectra, the integration time was 0.2 s, except as noted. The scans were taken under ambient room conditions.

Results and Discussion

Figure 1 compares luminescence from the peptide-Tb complex with no delay and a 50-µs delay following the excitation light pulse. With the delay, the fluorescence at 363 nm from the peptide disappears, isolating the Tb phosphorescence at 486 and 540 nm. Figure 2 shows fluorescence from a solution of peptide + fluorescein dye, also with and without delay. With a 50-µs delay, all fluorescence

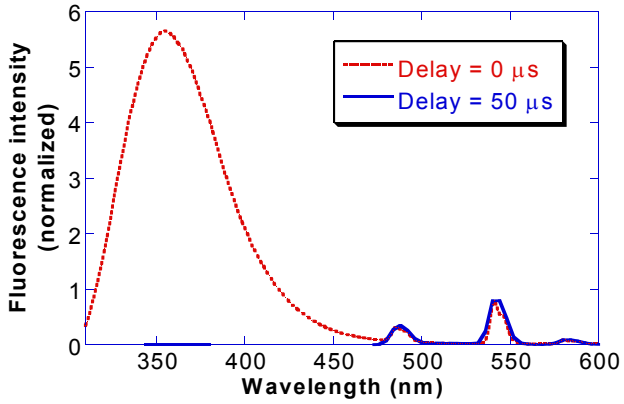

Figure 1. Luminescence spectra of peptide + terbium with (solid line) and without (dotted line) a 50-µs delay. Excitation was at 285 nm, and bandpass was 2 nm for both excitation and emission.

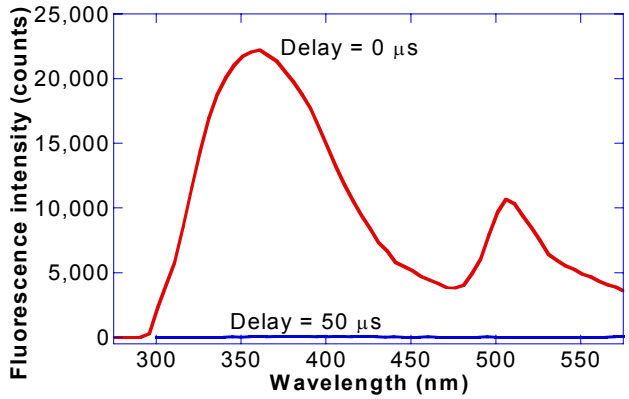

Figure 2. Luminescence spectra of peptide + fluorescein with and without a 50-µs delay. Bandpass was 5 nm for both excitation and emission.

from the peptide + fluorescein vanishes. Taken together, Figures 1 and 2 show that the phosphorescent species is Tb.

Figure 3 (on back) combines all three species, as a plot of peptide-terbium complex with and without 0.67-µ*M* fluorescein, without and with a 50-µs delay between excitation with a xenon flash lamp. Again, the spurious fluorescence at 363 nm is removed by delaying the sample window for 50 µs. Curves 3-b and 3-c directly compare fluorescein-containing and fluorescein-lacking solutions, showing fluorescein phosphorescence at 511 nm caused

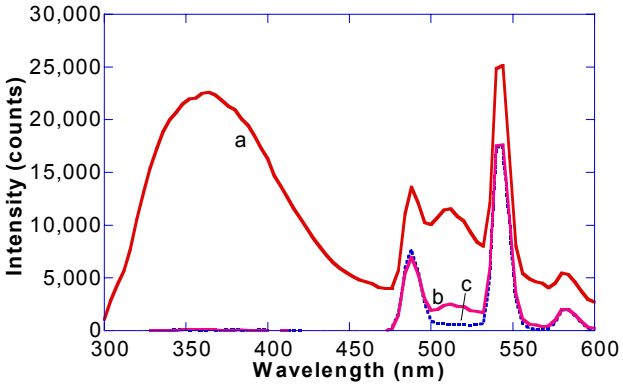

Figure 3. Luminescence spectra of complex, with (a) 0.67-µ*M* fluorescein and no delay after excitation, (b) 0.67-µ*M* fluorescein and a 50-µs delay after excitation, and (c, dotted line) no fluorescein and a 50-µs delay after excitation. Bandpass = 5 nm for both excitation and emission.

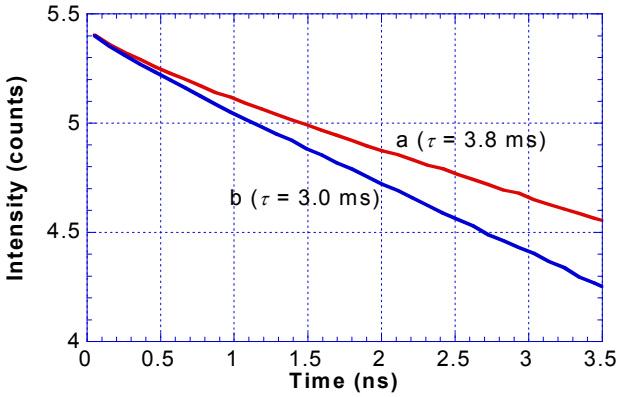

Figure 4. Phosphorescence decay from an aqueous solution of: (a) complex alone, and (b) complex + fluorescein, (integration time = 0.4 s). Bandpass is 5 nm on excitation (280 nm) and emission (540 nm).

by energy transfer from the complex to the fluorescein.

Figure 4 shows phosphorescence-decay curves of peptide with terbium ion alone (τ = 3.8 ms), and in the presence of fluorescein (τ = 3.0 ms). The faster phosphorescence decay in the presence of fluorescein (curve 4-b) confirms energy-transfer from the peptide-Tb donor to the fluorescein acceptor.

Similar experiments can be performed with the FLUOROMAX-P® spectrofluorometer, containing an integrated phosphorimeter along with CW lamp.

Conclusions

Use of a SPEX® phosphorimeter, including gated delay of signal-acquisition, can reveal extra information about the physical and chemical properties of materials.

In the USA:
Jobin Yvon Inc.
3880 Park Avenue, Edison, NJ 08820
Tel: 1-732-494-8660
Fax: 1-732-549-5157
E-Mail: fluorescence@jyhoriba.com
1-800-533-5946

In France:
16-18, rue du Canal
91165 Longjumeau cedex
Tel: (33) 1/64.54.13.00
Fax: (33) 1/69.09.93.19

Japan: (81) 3/5823.0140
China: (86) 10/6836.6542
Germany: (49) 89/46.23.17-0
Italy: (39) 2/57.60.47.62
U.K.: (44) 208/2048142