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FLUORESCENCE LIFETIMES OF α- AND β-CAROTENES*

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SUMMARY

The fluorescence lifetimes of α- and β-carotenes are both measured by a streak camera technique to be 55 ± 10 ps at a concentration of $6 \cdot 10^{-2}$ M in chloroform. Carotenes are present in chloroplasts at approximately this concentration.

Accurate lifetime measurements for fluorescence in the sub-nanosecond range have not been possible until recently. With the advent of picosecond laser pulse techniques measurement of sub-nanosecond processes in biological systems [1] are now possible. In this communication, we report measurements of the decay time of the fluorescence from both α- and β-carotene. The samples are pumped with picosecond pulses at 0.53 μm, and the fluorescence is detected with an ultrafast streak camera [2, 3] with a temporal resolution of 10 ps.

The experimental arrangement, to be discussed in detail in a forthcoming paper, consists of a mode-locked Nd/glass laser, a KDP second harmonic generation crystal, the sample, a lens to collect the fluorescence, and the streak camera. The pulses at 0.53 μm (5 ps duration) excite the samples, and the fluorescence at wavelengths longer than 5800 Å is detected by appropriate filtering. The temporal resolution of the streak camera, a home-made Los Alamos Scientific Laboratory device with S-20 response, is 10 ps. Fluorescence produced by a single picosecond pulse is photographed on Eastman Kodak No. 2484 film. The response of the streak camera and the film were accurately calibrated so that a densitometer trace of the film was sufficient to directly determine the lifetime on a single shot.

The samples were prepared in the following way. A crude pigment extract was obtained from *Chlorella pyrenoidosa* [4] (Sorokin's high temperature strain ICC No. 1230) which had been osmotically shocked by repeated freeze-thaw procedures [5], followed by lyophilization. Wet-packed cells weighing 100 g were extracted three times each with 500 ml of methanol and 125 ml of petroleum ether which was maintained at 4 °C during the extraction. Each extract was subsequently diluted with one l of saturated NaCl solution which resulted in a transfer of the pigments to the petroleum ether layer. The aqueous layer was successively extracted with 150-ml portions of petroleum ether until most of the pigment transfer was effected.

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The extract was concentrated in vacuo at 15 °C, dried over anhydrous sodium sulfate, and lyophilized. It was then dissolved in petroleum ether containing 0.5 % *n*-propanol and chromatographed on a 10 cm × 50 cm column of powdered sugar [6] which had been equilibrated with petroleum ether.

The carotene band from the column was collected and concentrated in vacuo at 15 °C. The α- and β-carotenes were separated chromatographically on a 2 cm × 25 cm column of activated magnesia and heat-treated siliceous earth (Celite 545) (1 : 2, v/v). Elution from the petroleum ether equilibrated column was with petroleum ether containing 0.5–1.0 % acetone. Saponifiable material and sterols were removed using established procedures [8], followed by crystallization from benzene/methanol at −20 °C.

The α-carotene and β-carotene components were identified by their absorption spectra and by their chromatographic properties. Purity was established by fast Fourier transform ^{13}C-NMR spectroscopy and by their specific absorption coefficients. Samples used in these experiments were dissolved in oxygen-free chloroform (N_2 atmosphere) to a concentration of $6 \cdot 10^{-2}$ M. They were maintained at 5 °C until ready for use.

A densitometer trace showing the dependence of the fluorescence intensity with time for α-carotene is reproduced in Fig. 1. The risetime of the fluorescence is less than 10 ps, the resolution time of the instrument, while the falltime, which is nearly exponential, is measured to be 55±10 ps. Similar results were obtained for β-carotene at the same concentration.

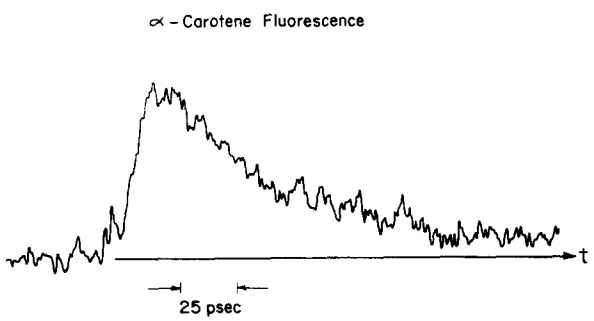

Fig. 1. Densitometer trace of streak camera photograph showing time dependence of α-carotene fluorescence. The sample was excited with a 5-ps, 5300 Å light pulse. Careful calibration yields a decay time of 55±10 ps.

The carotenes have been reported [9] to be present in chloroplasts in approximately the same concentrations we have used in our experiment. At these concentrations, what is commonly called concentration quenching [10] is probably responsible for the ultrashort fluorescence lifetimes we observe.

For picosecond studies, the streak camera technique used here has many advantages over an optical gate [11]. Among the advantages are the simplicity of the experimental arrangement, and the use of a single laser shot to obtain the data. As a result, fluctuations in the data are small, and complicated unravelling of numerous signals is unnecessary.

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