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FLUORESCENCE EXCITATION PROFILES OF BETA-CAROTENE IN SOLUTION AND IN LIPID/WATER MIXTURES

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Intrinsic fluorescence from all-trans β-carotene molecules in solution and embedded in lipid/water mixtures has been observed under laser excitation and its excitation profiles measured. The profiles closely correspond to the absorption spectra. The observations can be explained in terms of a low-lying ^1A_g excited state.

The Resonance Raman spectrum of all-trans β-carotene has been the subject of extensive studies in recent years [1-11] and is theoretically well understood. At the same time, there have been conflicting reports about the observation of intrinsic fluorescence in the visible region [12-15]. However, examination of the experimental Resonance Raman spectra reveals the presence of a strong, broad background signal. This signal is absent only when the spectra are obtained either on irradiation far from the absorption bands [9] or at low temperatures [4].

This background signal can be attributed to emission by impurities in the sample or even to photodegradation products of the β-carotene molecules themselves. It can also be due, however, to intrinsic fluorescence of intact all-trans β-carotene. We have examined the latter possibility by measuring the excitation profiles of these background signals from β-carotene molecules in solution and embedded in lipid bilayers.

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Our results show that the signal is due to intrinsic emission from intact β-carotene molecules and that it can be explained in terms of a low-lying ^1A_g excited state following the scheme proposed by Thrash et al. [16].

Materials and Methods

All-trans β-carotene (from carrots, type III), as well as dimyristoyl lecithine, dioleoyl lecithine and egg yolk lecithine in hexane were obtained from Sigma. The β-carotene was used without further purification. Ethanol, 'AR' was purchased from Baker. Freshly distilled chloroform and deionized and doubly distilled water were used for sample preparation.

The purity of the materials was checked chromatographically. The absence of any fluorescence background from solvents, water and of quartz cells and plates was confirmed by recording their Raman spectra. All solutions were freshly prepared.

Lecithine-β carotene mixtures, molar ratio 10^3:1, were prepared by adding β-carotene dissolved in chloroform. The samples were dried by flushing with nitrogen gas. Water was added gravimetrically to the dry material to form a 30% (w/w) mixture. The sample was then allowed to equilibrate for 5 hours at room temperature under a humidified nitrogen atmosphere.

The hydrated lipid mixture was sandwiched between two quartz plates (thickness 1.2 mm, diameter 30 mm) and microscope coverslips (thickness 175 μm) were used as spacers.

The emission spectra were obtained under excitation with either a Kr^+-laser (Coherent Model CR3000K) or with a dye-laser (Coherent CR-599) using stilbene 3 (fluorescence maximum at 420 nm). An Anaspec-300S premonochromator was used to filter the plasma lines or the side-bands of the Lyot tuning elements of the dye-laser [17].

The sample was irradiated with a parallel beam (diameter 3 mm) and a laser power of 50 mW at the sample position. A 90° detection geometry was used and the scattered radiation was passed through a Jobin-Yvon Ramanor HG-2S double monochromator to a peltier-cooled, blue-sensitive EMI 9816A P.M. tube operating at -1000 V. Lock-in detection was used. Single scans were recorded at a rate of 2000 cm^{-1}/min with a slitwidth of 14 cm^{-1} at 20 000 cm^{-1}.

Results

Excitation profiles of the broad background emission were obtained at room temperature by measuring the intensity of the signal maximum as a function of the excitation wavelength. The results for solutions of β-carotene in chloroform and ethanol are shown in figs. 1 and 2 respectively. The absorption spectra are also given for comparison. No changes were found between absorption spectra obtained before and after the measurement series. Furthermore no changes in the spectra were observed after illuminating the samples with high intensity for a period of two hours. This can be explained as due to convection and diffusion processes in the solutions which remove material from the illuminated volume.

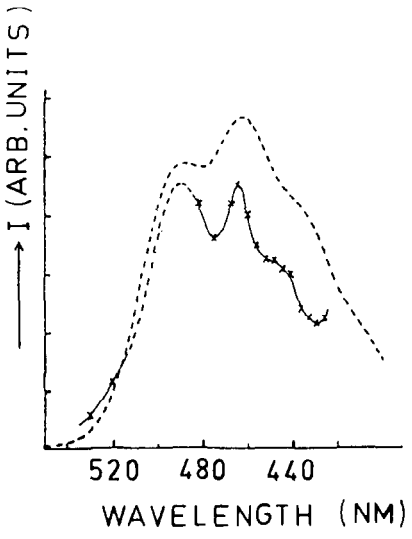

fig. 1 Fluorescence excitation profile (—) and absorption spectrum (--) of 1.5×10^{-5} M solution of beta carotene in chloroform. Fluorescence maximum: 540 ± 2 nm. Absorption maximum: 463 ± 1 nm.

A typical room temperature emission spectrum of β-carotene embedded in lecithine bilayers is shown in fig. 3. The background fluorescence intensity decreased exponentially by approximately 16% for the first two hours, but no further change was observed in the following 30 minutes of

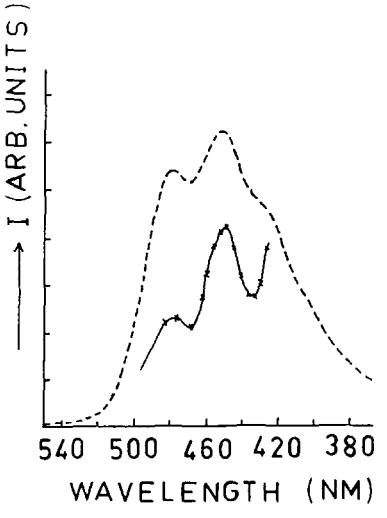

fig. 2 Fluorescence excitation profile (—) and absorption spectrum (--) of 1.1×10^{-5} M solution of beta carotene in ethanol. Fluorescence maximum: 527 ± 2 nm. Absorption maximum: 451 ± 1 nm.

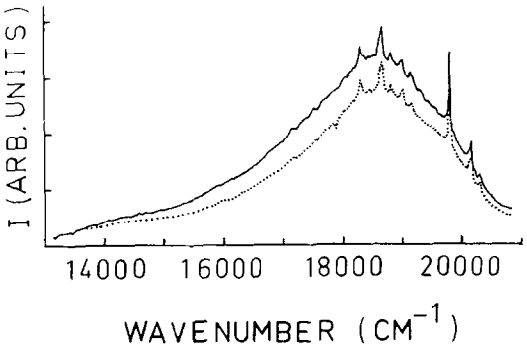

fig. 3 The influence of temperature change on the fluorescence of 1 mM beta carotene in dimyristoyl phosphatidylcholine. Lipid to water ratio 70:30 (w/w).

(—) Fluorescence intensity at time t=0 min and at time t = 17 hours after initial illumination.
(--) Fluorescence intensity at time t = 143 min.
Laser wavelength 468 nm. Power on sample 50 mW.

illumination. The loss of intensity could, however, be recovered on storing the samples at 5°C for 15 hours. In contrast, on cooling the samples to 15°C, reversible increases in signal intensities of about 10% were observed. The changes in the absorption spectra of the samples before and after illumination were less than 5%.

The excitation profiles for β-carotene embedded in lecithin bilayers were similar to those shown in figs. 1 and 2.

The positions of the maxima of the absorption and background emission spectra for β-carotene in the various systems studied here are summarized in table 1. The data show a red shift of 76±2nm of the emission relative to the corresponding absorption bands.

Discussion

The results presented above show that the absorption spectra of all-trans β carotene molecules in solution or embedded in lecithine bilayers correspond closely to the excitation profiles of the broad background emission observed in these systems. We have been unable to find any evidence of photodegradation of β carotene under our experimental conditions. Reversible changes in the emission from β carotene molecules embedded in lecithine bilayers were indeed observed, but were found to be dependent on the temperature of the samples.

Table 1
Absorption and fluorescence maxima of beta-carotene containing systems

system	absorption maximum (nm)	fluorescence maximum (nm)
chloroform solution (1.5×10^{-5} M)	463	540
ethanol solution (1.1×10^{-5} M)	451	527
dioleoyl phosphatidyl-choline (1.10^{-2} M)	457	534
dimyristoyl phospatidyl-choline (1.10^{-3} M)	457	534
egg yolk phosphatidyl-choline (1.10^{-3} M)	457	534

These results indicate that the background emission oberved under resonance excitation is due to intrinsic fluorescence from intact β carotene molecules.

The low absolute intensity of the emission as well as the position of the band maximum can be understood in terms of the energy-level scheme proposed by Thrash et al. [16] on the basis of excitation profiles of pre-Resonance Raman scattering. This scheme postulates the existence of a ^1A_g excited state, lying 3470 $\pm$ 100 cm^{-1} below the ^1B_u state. The electronic transition to this state from the ^1A_g ground state is symmetry forbidden to first order. On taking the maximum of the dipole allowed 0-1 $^1A_g \rightarrow {}^1B_u$ transition to be 457 nm (21904 cm^{-1}) [18,19], we would expect the $^1A_g \rightarrow {}^1A_g^*$ transition to have a maximum around 542 nm. This agrees remarkably well with the position of the maximum of the observed emission band.

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