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Photophysical properties of lycopene organized in Langmuir–Blodgett films: formation of aggregates

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Abstract

Mixed films of lycopene and stearic acid (SA) form highly stable floating monolayers at the air–water interface which may be easily transferred to glass or quartz substrates with a high transfer ratio. The surface pressure vs. area per molecule isotherms indicate that the lycopene molecules have a preferred orientation at the air–water interface. Polarized absorption studies support such an orientation of lycopene molecules in Langmuir–Blodgett (LB) films. Repulsive-type interactions between lycopene and SA have been observed by Gibbs free energy measurements. Spectroscopic studies of lycopene in solution, binary solvents and LB films suggest the formation of both J- and H-type aggregates. © 1997 Elsevier Science S.A.

Keywords: Aggregates; Langmuir–Blodgett films; Lycopene; Stearic acid

1. Introduction

The photophysical and photochemical properties of carotenoids have received considerable attention due to their wide ranging role in photobiology, photochemistry and photomedicine [1]. During photosynthesis, carotenoids are involved in the absorption of solar energy and in the transfer of the energy to lower energy states at which chlorophyll pigments absorb [2–4]. Investigation of the excited states of these molecules may provide a better understanding of the energy transfer mechanism in biological systems. In symmetrical, conjugated, double-bond carotenoids, the energy level responsible for apparent light absorption is an optically allowed transition from the ground state $S_0(1A_g)$ to the second excited $S_2(1B_u)$ state, and the molecule has a dipole-forbidden $S_1(2A_g)$ state [5–8] which can be located close to the energy state of the acceptor molecules [3,9]. The low fluorescence quantum yields of these carotenoid molecules make it difficult to study the photophysical properties in vitro. However, the number of conjugated π bonds in polyenes plays a crucial role in determining the excited states from which the emission originates. β-Carotene and other linear polyenes show S_2 emission [10,11], whereas crocetindialdehyde [12], fucoxanthin and β-apo-8′-carotenal [13] emit dual fluorescence from both S_2 and S_1 states.

Langmuir–Blodgett (LB) films with fatty acid bilayers mimic biological membranes with lipid bilayers, and carotenoids in LB films with stearic acid (SA) may provide features of the structural organization of the photosynthetic unit. The self-organization of carotenoids [14,15] has an effect on the excited state energies and, consequently, on the energy and electron transfer efficiencies of such molecules in photosynthesis. The structure of carotenoid aggregates has not been determined with any certainty. Therefore spectroscopic and structural investigations of carotenoid aggregates in LB films may provide a better understanding of the photophysical processes involving these carotenoids. In this paper, we report the spectroscopic and orientational properties of aggregates of lycopene organized in lycopene–SA LB films.

2. Experimental details

Lycopene was obtained as a gift from Hoffman la Roche Co. (Switzerland) and SA was purchased from Sigma Chemical Co. (USA). They were used as received. The purity of the samples was checked by UV–visible absorption spectroscopy. All solvents used were of spectroscopic grade. An alternate layer LB deposition trough (Joyce Loebl, Model 4, UK) was used for the deposition of LB films. Triply distilled water purified by a Milli-Q Plus water purification system (Millipore Co., USA) with a resistivity of 18.2 MΩ cm was used

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as the subphase. The pH of the subphase was 6.4 and the temperature of the subphase was maintained by a controller at 23 °C. The surface pressure was monitored by a Wilhelmy plate, which was attached to a microbalance, and the output was fed to an IBM PC which also controlled the film compression rate and dipping speed. A small amount of chloroform solution containing lycopene and SA in a predetermined ratio was spread on the clean water surface of the LB trough. After allowing the solvent to evaporate for 30 min, the film was compressed at a speed of 2×10^{-3} nm^2 molecule^{-1} s^{-1} to record the isotherm data. Monolayer characterizations were made by analysing the isotherm data with software from Joyce-Loebl, UK. Y-Type monolayer and multilayer depositions of lycopene mixed with SA at different molar ratios and different surface pressures were obtained. Fluorescent grade quartz slides were used for spectroscopic measurements. Steady state absorption and emission spectra were recorded by a Shimadzu 2101 UV–visible spectrophotometer and a Hitachi F-4500 fluorescence spectrophotometer respectively. Dichroic sheet polarizers (03 FPG 005) obtained from Melles Griot were used.

3. Results and discussion

3.1. Surface pressure–area isotherms

A small amount of lycopene dissolved in chloroform was spread on the pure water subphase. By slowly compressing the film at a speed of 2×10^{-3} nm^2 molecule^{-1} s^{-1}, it was observed that the pressure did not increase beyond 15 mN m^{-1}. A further increase in the surface pressure or the addition of larger amounts of material result in the formation of microcrystals at the air–water interface. It is interesting to note that, on relaxation of the surface pressure, the microcrystals do not disintegrate into molecules, but form smaller islets. However, by mixing lycopene with SA, a highly stable uniform floating layer was obtained at the air–water interface.

Fig. 1 shows the π–A isotherms for a range of different molar solutions. The area per molecule for pure lycopene is 0.2 nm^2, which is much smaller than the area predicted by the space filling model, suggesting that the lycopene molecules do not lie flat, but may have a preferred orientation at the air–water interface, or may form multilayers or aggregates. In this context, it may be pointed out that a similar molecule, namely β-carotene [16], makes an angle of 69° at the air–water interface. A closer look at the π–A isotherm curves (Fig. 1) reveals that pure SA shows the standard isotherm with an area per molecule of 0.24 nm^2. With increasing mole fraction of lycopene in the mixture, the area per molecule of the mixed system increases, reaches a maximum at a mole fraction of 0.1 and then decreases with a further increase in lycopene. With increasing lycopene concentration in the mixture, the apparent loss of lycopene molecules at the air–water interface may be due to the formation of aggregates or microcrystals. These aggregates may subsequently be precipitated into the bulk of the subphase due to the high hydrophobicity and low solubility of lycopene molecules, or they may form larger aggregates on the monolayer. Alternatively, the lycopene molecules or aggregates may be located within the SA matrix above the air–water interface sandwiched between adjacent chains of SA or on the surface of the monolayer. The latter has been observed for various polyaromatic hydrocarbons [17].

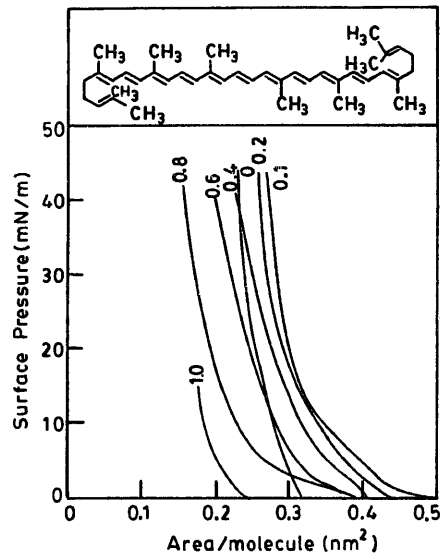

Fig. 1. Surface pressure vs. area per molecule isotherms of a mixed lycopene–SA monolayer on a water subphase at different molar ratios. Inset shows the structure of the lycopene molecule.

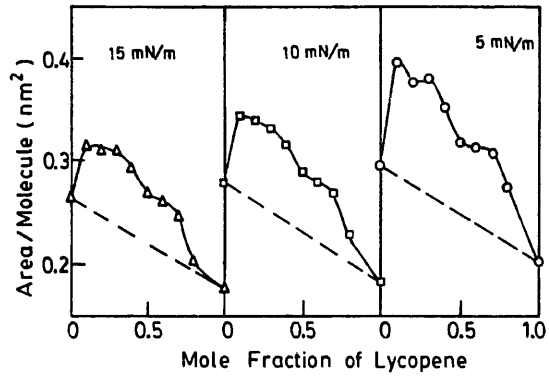

Fig. 2. Plots of the area per molecule as a function of the lycopene concentration at surface pressures of 15 mN m^{-1}, 10 mN m^{-1} and 5 mN m^{-1}.

Fig. 2 shows plots of the area per molecule of the mixed monolayer of lycopene and SA vs. the lycopene concentration at different surface pressures. The broken lines in Fig. 2 correspond to ideal curves for a mixed two-component system. The experimental curves are not monotonic, but show a positive deviation from the ideal curves predicted by the additivity rule. It is evident from Fig. 2 that the experimentally obtained area per molecule of the mixed system is greater than the sum of the areas of the pure components, suggesting the existence of a repulsive-type interaction between the lycopene and SA molecules. A similar observation has been reported for chrysene [17], valinomycin [18], biphenyl

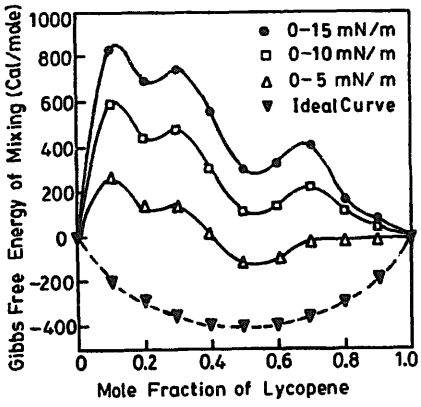

Fig. 3. Plots of the Gibbs free energy as a function of the lycopene concentration in mixed monolayers obtained at different ranges of surface pressure. The lowermost line (▼) shows the curve calculated using Eq. (3).

derivatives [19] and crocetindialdehyde [12] mixed with fatty acids.

The Gibbs free energy, an important thermodynamic parameter of mixing, has been calculated from our isotherm data using the following equations [20–22]

$$G_M^E = \int_0^{\pi} (A_{SALY} - N_{LY}A_{LY} - N_{SA}A_{SA})\,d\pi \quad (1)$$

$$G_M^I = RTN_{LY}(\ln N_{LY}) + RTN_{SA}(\ln N_{SA}) \quad (2)$$

$$G_M = G_M^E + G_M^I \quad (3)$$

where G_M^E is the excess free energy of mixing, G_M is the free energy of mixing, G_M^I is the free energy of mixing for an ideal system, R is the universal gas constant, π is the surface pressure of deposition and T is the temperature in kelvin.

Plots of the Gibbs free energy of mixing (G_M) vs. the mole fraction of lycopene in the mixed monolayer at different surface pressures are shown in Fig. 3. A similar positive deviation is observed in this case also, which indicates molecular association. It is probable that the lycopene molecules are incorporated in the SA matrix which provides a hydrophobic microphase. The dissimilar nature of lycopene and SA may result in phase separation of the individual components as aggregates or microcrystalline domains. Various workers have reported the formation of such aggregates or crystalline domains in LB films. By employing different methods, namely epifluorescence [23], transmission electron microscopy (TEM), cryo-TEM and Brewster angle microscopy, two-dimensional and three-dimensional crystalline structures and fractal-like structures have been reported.

3.2. Deposition of films

Y-Type deposition was obtained on a variety of substrates by slowly lifting the substrate through the floating mixed monolayer at a speed of 5 mm min^{-1} at different surface pressures. The transfer ratio of the mixed monolayer was found to be 0.9 at a constant surface pressure of 30 mN m^{-1}.

3.3. Absorption spectroscopy

Fig. 4 shows the electronic absorption spectra of lycopene in pure ethanol solution, an ethanol–water mixture and an LB film at room temperature. The absorption spectrum of lycopene shows an $S_0 \rightarrow S_2$ transition, since the $S_0 \rightarrow S_1$ transition is symmetry forbidden [4] and can only be observed in two-photon spectroscopy. This is typical of unsubstituted carotenoids. The assignment of the electronic absorption bands of lycopene in ethanol solution in the spectral range 420–510 nm is shown in Table 1. The vibrational structure of the spectrum can be assigned to the C–C stretching mode in the excited state, which is expected to have a higher frequency [24] than that in the ground state [25]. The high energy band at 294 nm corresponds to the cis isomer of the lycopene molecule. The absorption spectrum of lycopene in the LB film shows a remarkable change. The band system in the 420–510 nm range in solution is decreased significantly in intensity and appears as a weak, structured, red-shifted band system in the spectral region 430–570 nm. In addition, a very intense fairly sharp band appears at 348 nm. The cis band, observed at 294 nm in solution, is blue shifted to 272 nm. The redissolved LB film reproduces the solution absorption spectrum. The broadening and shift of the absorption band maxima with respect to those in solution indicate strong dipole–dipole interactions between the molecules in the aggregated state, and new exciton bands are created due to the interaction between the molecular transition dipoles [26]. The new bands may be located above and/or below the monomeric state depending on the orientation of the two interacting transition dipoles. In J aggregates, the dipoles have a head-to-tail arrangement and these spectra show red-shifted

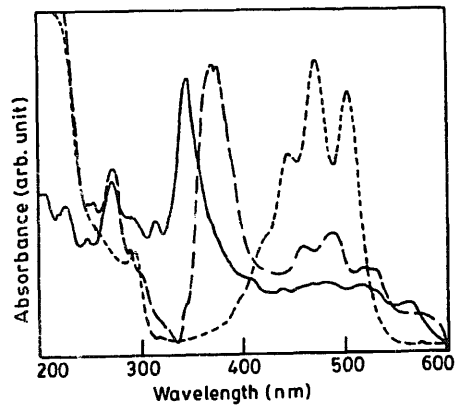

Fig. 4. Absorption spectra of lycopene in ethanol (- - -), lycopene in an ethanol–water mixture (volume fraction of water, 0.8) (— — —) and a ten-layer lycopene–SA LB film with a molar ratio of 1 : 5 (———).

Table 1
Assignments of the absorption bands of lycopene in ethanol solution

Position in nanometres (cm^{-1})	Difference	Assignment
504 (19841)	0	0–0 ($1^1A_g - 1^1B_u$)
472 (21186)	1345	0 + C–C stretching
446 (22421)	2580	0 + 2 × C–C stretching

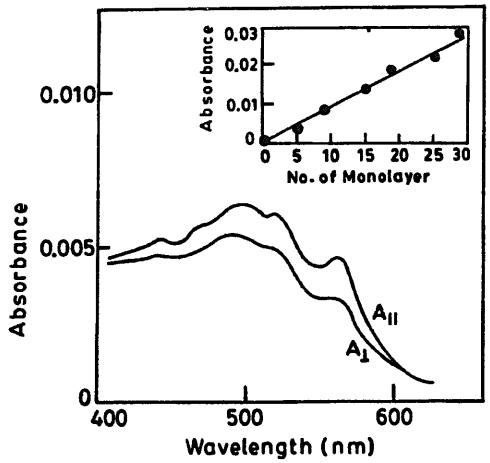

Fig. 5. Polarized absorption spectra of a mixed lycopene–SA LB film. Inset shows a plot of the absorbance vs. the number of layers.

absorption and emission bands. In H aggregates, the molecular dipoles are arranged in a pack-of-cards array. The spectral manifestation of such aggregates includes a blue-shifted absorption band and a red-shifted emission band. The band system in the 450–570 nm region (with the 0–0 band at 563 nm) in the LB film is considerably red shifted from the band position in solution and suggests the formation of J aggregates in the LB film. The new, intense, blue-shifted absorption band at 348 nm in the LB film suggests the formation of H aggregates. It therefore appears that, in the LB film, lycopene forms both J and H aggregates. The relative intensities of the long-wavelength and short-wavelength band systems depend on the relative populations of the two aggregate species. Recently, the coexistence of such grossly different species in LB films has been reported [27,28]. It is well established that, because of the strong water–water interaction and weak water–organic molecule interaction, organic molecules form aggregates on addition of water to their solutions. To induce aggregation of the lycopene molecules, water was slowly added to the lycopene solution in ethanol. Aggregation was characterized by a loss of visual colouration, but no precipitation or coagulation was observed. Such aggregation was found when the volume fraction of water was greater than 0.4. The resemblance between the absorption spectrum of lycopene in a binary ethanol–water mixture at a volume fraction of water of 0.8 (shown in Fig. 4) and that in the LB film also supports aggregate formation. Such a spectral change in binary mixtures and the formation of aggregates have been reported previously for other polyene molecules [29]. A plot of the absorbance vs. the number of layers in the LB film (shown in the inset of Fig. 5) is a straight line, which confirms that the films are reproducibly assembled during LB deposition.

3.4. Linear dichroism study

Linear dichroism has been used to study the orientation of molecules in the LB film [30]. We have studied the polarized absorption spectrum of the long-wavelength band of lycopene in the LB film. At normal incidence, no anisotropy of the absorption band is observed, which suggests that the transition dipole moments are uniformly and homogeneously distributed. Fig. 5 shows the polarized absorption spectra of 19 monolayers of lycopene molecules deposited at 20 mN m^{-1} surface pressure at an angle of incidence of 45°. The change in the absorbance value for parallel and perpendicular polarized light suggests that the lycopene molecules incorporated in the LB film have a specific orientation, supporting our isotherm studies. With a change in the angle of incidence from 0° to 45° or 65°, linear dichroism (D) is observed. For the 19 monolayer film (1 : 10 molar ratio), the value of D_{45} is 0.25, and the corresponding angle between the transition dipole moment and the plane of the solid support is 47°. The transition dipole for the long-wavelength absorption band ($^1A_g - {}^1B_u$) is along the long molecular axis. In J aggregates, the dipole for this transition will be along this specific direction. The orientation angle estimated is applicable for stacked/aggregated species of lycopene molecules.

3.5. Emission spectroscopy

The emission spectrum of lycopene in chloroform solution at room temperature is shown in Fig. 6. The spectrum consists of sharp bands with maxima at 535 and 558 nm and a hump at 600 nm. This emission spectrum exhibits good overlap with the absorption spectrum. The emission band in the spectral region 500–590 nm corresponds to the $S_2 \rightarrow S_0$ transition and originates from the 1^1B_u state. It is worth mentioning that the fluorescence quantum yield of lycopene molecules in solution is in the range 5×10^{-5}–5×10^{-4}. The emission spectrum of lycopene mixed with SA and assembled in LB films at room temperature, as shown in Fig. 6, is shifted considerably to the lower energy region and the band maxima in this case are at 614 and 625 nm with a hump at 670 nm. These red-shifted emission bands in LB films suggest the formation of aggregates. This emission also shows good overlap with the corresponding absorption spectrum. The emission spectrum of lycopene microcrystals has also been recorded for comparison (not shown in the figure). The crystal emission is similar to the LB film spectrum with the band maximum shifted to 635 nm. The emission spectrum of the

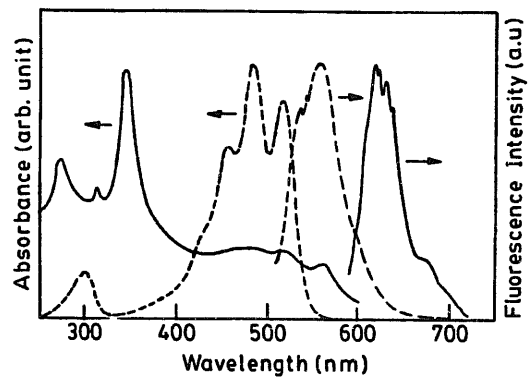

Fig. 6. Absorption and emission spectra of lycopene in chloroform (- - -) and a ten-layer lycopene–SA LB film with a molar ratio of 1 : 5 (———).

LB film is intermediate between the solution spectrum and the microcrystal spectrum, as expected for aggregates or low dimensional crystals. It is worth mentioning that, in carotenoids with ten conjugated double bonds or more ($N \geq 10$), $S_1 \rightarrow S_0$ emission has not yet been observed. Lycopene has 13 π bonds, which determine the energies of the electronic transitions. In this context, it should be noted that shorter polyenes containing fewer than nine double bonds generally fluoresce from the first excited singlet state $S_1(2^1A_g)$, whereas longer carotenoids containing more than eight bonds show an anti-Kasha emission from the $S_2(1^1B_u)$ state [31,32].

4. Conclusions

Monolayers and multilayers of lycopene molecules mixed with SA can be incorporated successfully as LB films. Our isotherm studies suggest that a repulsive-type interaction exists between the carotenoid and SA molecules. A comparison of the solution absorption spectrum with that of LB films indicates the presence of both J- and H-type aggregates. The similarity between the absorption spectra of lycopene in LB films and in an ethanol–water mixture confirms the formation of such aggregates. A linear dichroism study suggests that the aggregated species of lycopene molecules are oriented in a specific way in the LB film, such that the transition dipoles make an angle of 47° with the plane of the solid support. Our spectroscopic and isotherm studies at the air–water interface seem to complement each other and support the formation of aggregates of lycopene molecules in the LB film.

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