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Lutein-Chlorophyll-*a* Energy Transfer in Detergent Micelles

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Abstract. Absorption, fluorescence and fluorescence excitation spectra were determined for equimolar mixed micellar detergent solutions of lutein and chlorophyll-*a* in the concentration range from $9 \cdot 10^{-6}$ to $1.8 \cdot 10^{-4}$ M, with detergent (triton-X100) concentrations from $3 \cdot 10^{-4}$ to $7 \cdot 10^{-3}$ M. In the range of detergent concentrations studied the pigments incorporated into the detergent micelles attained a high local concentration (0.1 to 0.01 M), reminiscent of pigment concentration within the chloroplast. A lutein → chlorophyll-*a* energy transfer with an efficiency of about 15% was found in these systems. In dilute ($9 \cdot 10^{-6}$ M) pigment solution with concentrated ($7 \cdot 10^{-3}$ M) detergent practically no transfer is observed. The extent of aggregation and the efficiency of transfer depend on the composition of the system. The aggregation of chlorophyll-*a* is partly inhibited by lutein molecules. It is shown that the energy transfer efficiency as function of distance follows an r^{-3} relationship, R_0 being 22 Å.

Key words: Lutein — Chlorophyll-*a* — Energy-Transfer — Micelles.

Introduction

The action spectra of photosynthesis show that light absorbed by carotenoids is utilized in photosynthesis, i.e. there exists a transfer of energy from the carotenoids to the chlorophyll molecules *in vivo*. Duysens [1, 2] found this transfer to have an efficiency of 50%. According to Goedheer [3, 4] the transfer from β-carotene to chlorophyll *in vivo* is very effective (near to 100%), while there is no transfer from xanthophylls to chlorophyll.

The transfer of electronic excitation energy from carotenoids to chlorophylls has already been studied in model systems [5 to 12]. The experiments suggest that the conditions for an effective carotenoid → chlorophyll energy transfer are high pigment concentration or/and the presence of mixed aggregates of chlorophyll and carotenoid [2, 13]. According to [14] effective transfer occurs in systems containing some type of "solid support surface", as is the case with detergent solutions of pigments, pigment layers and pigmented lipid membranes. The detergent micellar solution is an appropriate model for the study of energy transfer, because the local concentration of the pigment in the micelles can be very high. In addition, the pigment molecules in the micelles are incorporated in an ordered form promoting the transfer of energy.

The transfer of energy from xanthophyll to chlorophyll in detergent solutions of mixed pigments was first studied by Teale [10, 11]. Teale found an efficiency of 60% for the lutein → chlorophyll transfer. However, the characteristics of this system and a detailed study were not given.

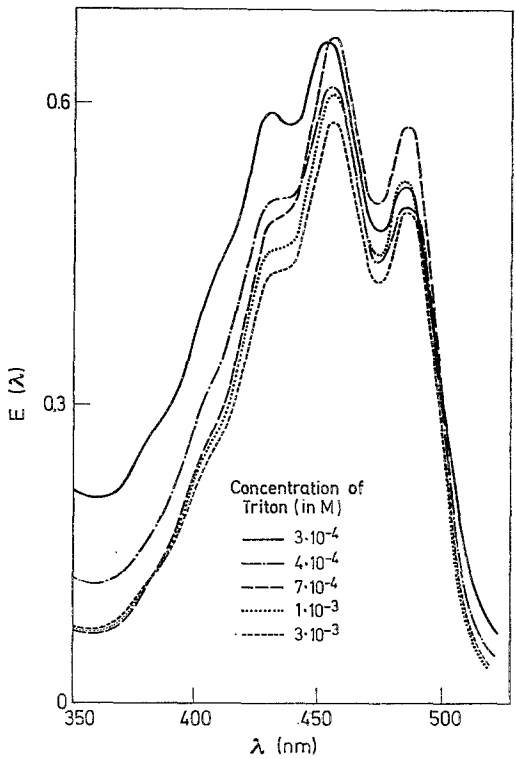

Fig. 1. Absorption spectra of $9 \cdot 10^{-6}$ M lutein in micellar solution with different concentrations of triton-X100 detergent at 10 °C (Layer thickness: 1 cm)

In this paper the transfer of electronic excitation energy from lutein to chlorophyll-*a* was studied in micellar solutions in order to find the conditions of applicability of these solutions for modeling energy transfer in living systems and to obtain information about the parameters influencing the efficiency of transfer.

Material and Methods

Chlorophyll-*a* was prepared from fresh spinach leaves according to [15]. Lutein was obtained by thin-layer chromatography [16]. The detergent, triton-X100 produced by Röhm and Haas Company was applied. The solutions were prepared as follows. Lutein and chlorophyll-*a* were dissolved in acetone. An appropriate amount of acetone solution was added to a concentrated detergent solution under stirring, and then water was added to the system. The concentration of acetone in the final solution was 0.8 Vol.-%. After preparation the solutions were kept at 40 to 45 °C for an hour, and then allowed to cool rapidly to 4 to 5 °C. In this way optically stable and reproducible solutions were obtained. Thereafter the solutions were stored at 4 °C in the dark. Absorption, fluorescence and fluorescence excitation spectra of chlorophyll, lutein (absorption spectra only) and their equimolar mixed solutions were measured at 10, 30 and 45 °C. The *absorption spectra* were measured with an Unicam SP 1800B spectrophotometer in a sample

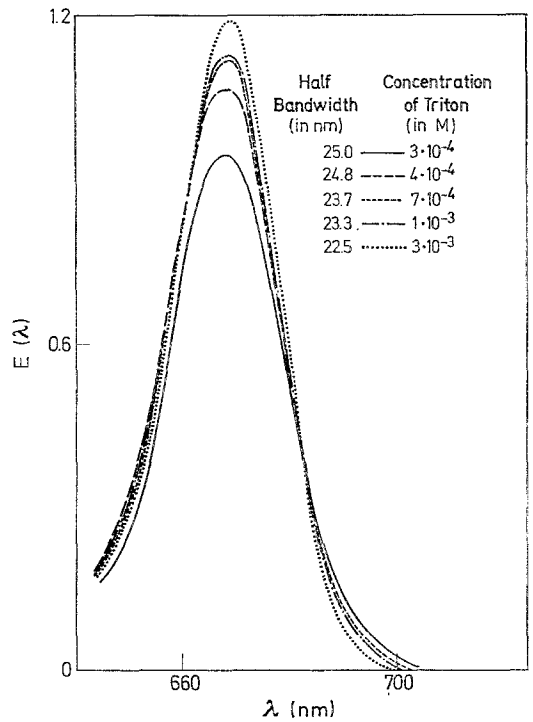

Fig. 2. The red bands of the absorption spectra of $9 \cdot 10^{-6}$ M chlorophyll-*a* micellar solution with different concentrations of triton-X100 detergent at 30 °C (Layer thickness: 1 cm)

position near the detector. The *fluorescence* and *fluorescence excitation spectra* were measured with a Perkin-Elmer MPF3 spectrofluorimeter. Front surface observation was applied. The bandwidths of excitation and observation were 10 and 8 nm, respectively. In the case of fluorescence measurements the cell thickness was chosen to give a maximum extinction less than 0.1. In this way the inactive absorption of lutein [5], the effect of secondary fluorescence and of reabsorption of fluorescence are negligible [17, 18]. The fluorescence spectra are corrected for the response of the instrument.

Results

The *absorption spectra* of lutein (Fig. 1) depend on the detergent concentration. The absorption spectra obtained at high detergent concentrations are similar to those in nonpolar solvents [19]. The location of the band maxima in micellar solutions is shifted 8 to 10 nm towards longer waves as compared to acetone solutions. The red absorption spectrum of chlorophyll-*a* depends also on the detergent concentration (Fig. 2). The half bandwidth decreases, and the extinction increases with the increase of the detergent concentration. The maximum absorption at 668 nm is at longer waves than in the usual organic solvents. At low detergent concentrations the red band of the chlorophyll-*a* in pure chlorophyll-*a* detergent solutions is broader than in mixed chlorophyll-*a* lutein detergent solutions (Fig. 3). At high detergent concentrations no difference is observable.

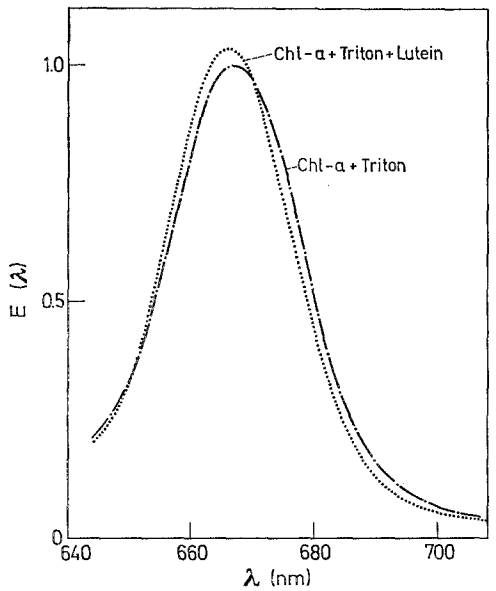

Fig. 3. The red bands of the absorption spectra of a chlorophyll-*a* and a $9 \cdot 10^{-6}$ M equimolar mixture of chlorophyll-*a* and lutein micellar solutions with $3 \cdot 10^{-4}$ M detergent

The *fluorescence spectrum* also depends on the detergent concentration and, in addition, on the temperature (Fig. 4). The fluorescence maxima are shifted from 672 nm (the maximum position observed in acetone solution) to 680 nm and the band at 740 nm decreases with the increase of temperature and detergent concentration. In the fluorescence spectrum of a mixture of chlorophyll and lutein the short-wave maximum is more intense than for a solution containing chlorophyll-*a* only.

Excitation of the fluorescence of chlorophyll-*a* at any wavelength for which the absorption of chlorophyll-*a* is low compared to that of lutein (from 440 to 550 nm) leads to an enhancement of the intensity of fluorescence (Table 1). The intensity in mixed solutions is greater than in a solution of chlorophyll-*a* only. The enhancement decreases with increasing detergent concentration.

The *fluorescence excitation spectra* were taken observing at 680 nm. In the maximum at 625 nm, where lutein does not absorb at all, the spectra were arbitrarily taken as being of unit height (Fig. 5). The excitation spectra of chlorophyll-*a* solutions with different detergent concentrations (not shown in the Figure) do not change in the region of 450 to 650 nm (in the Soret-band, however, changes can be observed). The excitation spectra of mixed solutions (Fig. 5) differ from those of pure chlorophyll-*a* solutions: in the region of lutein absorption (from 520 nm to shorter waves) the spectrum depends on the detergent concentration. The difference decreases with increasing detergent concentration and is virtually absent in acetone solution. From the excitation and absorption spectra of mixed solutions (Fig. 6) the efficiency of energy transfer η can be calculated. By taking the fluorescence excitation spectra with the same height at 625 nm, the dis-

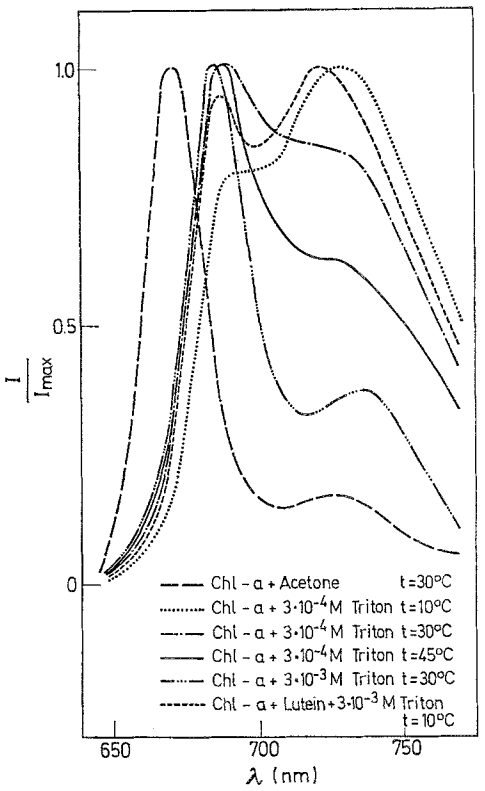

Fig. 4. Fluorescence spectra of $9 \cdot 10^{-6}$ M chlorophyll-*a* in acetone, in $3 \cdot 10^{-3}$ and $3 \cdot 10^{-4}$ M triton-X100 detergent solution, in $3 \cdot 10^{-4}$ M detergent together with $9 \cdot 10^{-6}$ M lutein at 10, 30 and 45 °C. The maximum of each fluorescence spectrum was taken as unit height

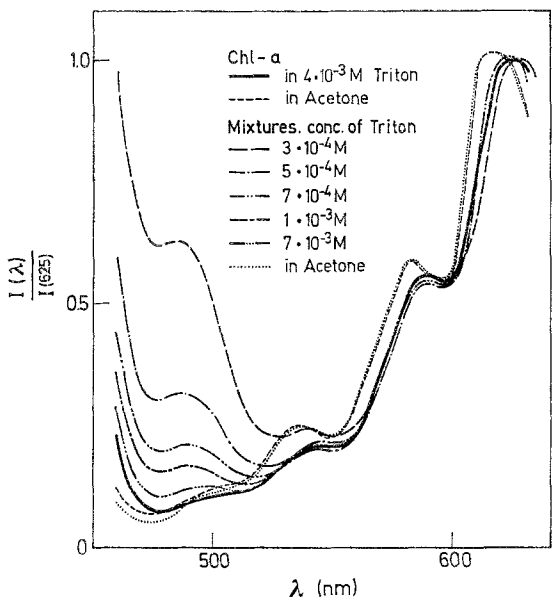

Fig. 5. Fluorescence excitation spectra of chlorophyll-*a* in acetone, and lutein and chlorophyll-*a* equimolar ($4.7 \cdot 10^{-5}$ M) mixed solutions at different triton-X100 concentrations. The fluorescence intensity at 625 nm was taken as unit height

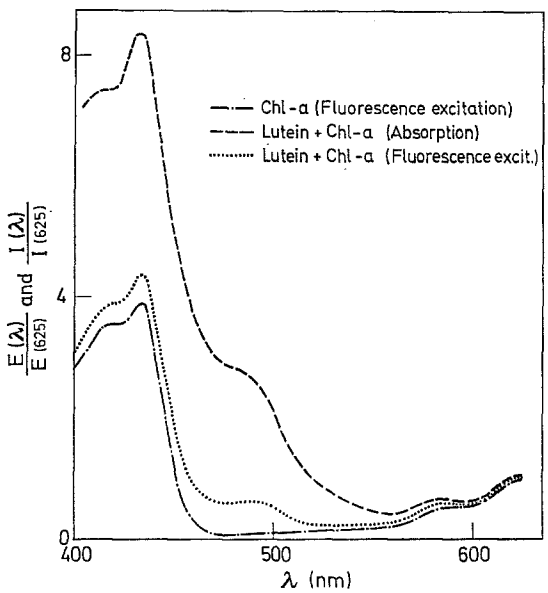

Fig. 6. Absorption and fluorescence excitation spectrum of equimolar ($4.7 \cdot 10^{-5}$ M) mixed micellar solution of lutein and chlorophyll-*a*. The intensity of absorption and fluorescence at 625 nm was taken as unit height

Table 1. Arbitrary intensities of fluorescence of $9 \cdot 10^{-6}$ M chlorophyll-*a* and mixed equimolar lutein chlorophyll-*a* micellar solutions excited at 486 nm at different detergent concentrations

Solution	Arbitrary intensities of fluorescence at 680 nm				
	Concentration of detergent in M				
	$3 \cdot 10^{-4}$	$4 \cdot 10^{-4}$	$7 \cdot 10^{-4}$	10^{-3}	$3 \cdot 10^{-3}$
Chlorophyll-*a*	0.009	0.063	0.521	0.907	1.41
Chlorophyll-*a* + lutein	0.134	0.237	0.776	0.861	1.42

aggregation effect of lutein upon chlorophyll-*a* aggregates (see Discussion) can be accounted for. For calculation the following formula can be used [12]:

$p = p_0 (l + k\eta)$. Here $k = \varepsilon_{\text{lut},\lambda} \cdot c_{\text{lut}} / \varepsilon_{\text{chl-}a,\lambda} \cdot c_{\text{chl-}a} = E_{\text{lut},\lambda} / E_{\text{chl-}a,\lambda}$,

$p = I_{\text{mix},\lambda} / I_{\text{mix},625}$ and $p_0 = \varepsilon_{\text{chl-}a,\lambda} / \varepsilon_{\text{chl-}a,625}$.

Since the yield of fluorescence is constant in the spectrum range studied $p_0 = I_{\text{chl-}a,\lambda} / I_{\text{chl-}a,625}$. I is the intensity of fluorescence and the subscripts refer to *mix*ture and pure *chl*orophyll-*a* solutions excited at 625 or λ nm. This formula can be rewritten, if $p_{\max} = p_0 (l + k)$ is introduced (with $\eta = 1$). We obtain

$$\eta = \frac{p - p_0}{p_{\max} - p_0} . \tag{1}$$

where $p_{\max} = p E_{\text{mix},\lambda} / E_{\text{chl-}a,\lambda} = I_{\text{chl-}a,\lambda} \cdot E_{\text{mix},\lambda} / I_{\text{chl-}a,625} E_{\text{chl-}a,\lambda}$. At small optical densities $I_{\text{chl-}a,\lambda} / I_{\text{chl-}a,625} = E_{\text{chl-}a,\lambda} / E_{\text{chl-}a,625}$ and thus $p_{\max} = E_{\text{mix},\lambda} / E_{\text{chl-}a,625}$. Formula

Table 2. The efficiencies (in per cent) of the lutein → chlorophyll-*a* energy transfer in equimolar detergent micellar solutions, calculated with Eq. (1)

Concentration of triton-X100 (M. 10^4)	Concentration of chlorophyll-*a* and lutein (M)			
	$9 \cdot 10^{-5}$	$4.7 \cdot 10^{-5}$	$5.7 \cdot 10^{-5}$	$1.8 \cdot 10^{-4}$
3	20.7	18.5	—	17.9
4	8.1	—	22.0	—
5	—	9.1	—	17.5
7	4.0	4.1	—	12.5
10	2.6	5.0	7.7	11.6
20	—	2.9	—	11.6
30	0.6	—	1.8	—

(1) containing p_{max} is somewhat simpler to treat than the original formula involving k. (1) is valid only if the emission of excitation energy of primarily excited chlorophyll molecules and chlorophyll molecules with excitation from lutein occurs with the same fluorescence yield. The effectiveness of the lutein → chlorophyll-*a* energy transfer at various detergent and pigment concentrations, calculated with (1), are shown in Table 2.

The local concentration of the pigment within a micelle can be estimated by means of the approximate formula,

$$c_{loc} \cong \frac{c_{chl} \cdot d_{det}}{c_{det} \cdot M_{det}} \cdot 10^2 , \tag{2}$$

where c_{chl} and c_{det} are the molar concentrations of chlorophyll and detergent, respectively, d_{det} is the density of the detergent in g/cm^3 and M_{det} is the molecular weight of the detergent, and c_{loc} is the local molar concentration. This formula was obtained by assuming that all pigment molecules are incorporated into the micelles, the total volume of which (together with the water molecules within the micelles) is ten times greater than the volume of the same amount of detergent in the absence of water would be. This factor was taken to fit the value of c_{loc} to that of c_{loc} given by other methods [20]. According to Eq. 2 very high pigment concentrations can be obtained by proper choice of c_{chl} and c_{det} and the same c_{loc} can be reached with different c_{det}.

Discussion

Figs. 1 and 2 show that both lutein and chlorophyll-*a* are incorporated into the detergent micelles. At low detergent concentrations there exists an aggregation of pigments of different types, while at high detergent concentrations the pigments are mainly in monomeric form. This can be concluded from the decrease in the half bandwidth of chlorophyll-*a* absorption which occurs with increasing detergent concentration and from the decrease in the intensity of the band of the emission spectra at 740 nm. This band is attributed to the presence of fluorescing aggregates of chlorophyll-*a* [22]. The aggregates of chlorophyll-*a* are decomposed on raising the temperature and also in the presence of lutein. The enhancement of the intensity of fluorescence can also be explained by the dissolution of chlorophyll-*a*

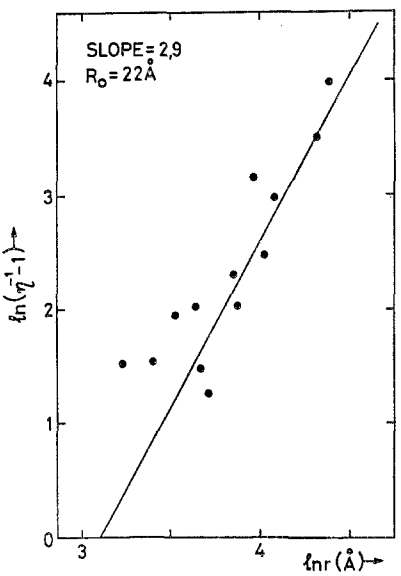

Fig. 7. The dependence of the efficiency of energy transfer (η) on distance (r). The slope of the plot is 2.9. The results of three series of measurements are plotted

aggregates. At low detergent concentrations the low intensity of fluorescence may be due to a concentration quenching resulting from the high local pigment concentration. The fluorescence enhancement in mixed solution can be attributed either to the higher amount of fluorescent monomeric chlorophyll molecules (caused by the presence of lutein) or to the lutein → chlorophyll-*a* energy transfer revealed by the fluorescence excitation spectra.

Since the excitation spectra of fluorescence of the mixed solutions lay under the absorption spectra, the efficiency of the transfer leading to fluorescence should be less than 100%. According to Table 2 the efficiencies of transfer depend on the concentrations of detergent, chlorophyll-*a* and lutein. At low and high detergent concentrations (below $4 \cdot 10^{-4}$ and above $2 \cdot 10^{-3}$ M) there is practically no dependence on the pigment concentration, because below the critical micelle concentration there are no regular micelles while above $2 \cdot 10^{-3}$ M the number of micelles is great and therefore the pigment molecules are highly distributed, leading to unfavourable conditions for energy transfer. Under normal conditions, e.g. in $5 \cdot 10^{-4}$ and $7 \cdot 10^{-4}$ M detergent solutions, the maximum transfer efficiency is 12.5 to 17.5% with about 0.1 M local concentrations of the pigment. This concentration should be very near to the concentration of pigments *in vivo*. The average distance of molecules at this concentration ([21], Eq. (47, 3) p. 227) — assuming a random distribution of the molecules — is about 25 Å. From Eq. (1) the efficiency of lutein chlorophyll-*a* energy transfer under these conditions should not be too far from the transfer efficiency *in vivo*, since a higher pigment concentration for the photosynthetic pigment within the chloroplast is not probable.

From the pigment (local) concentration dependence of transfer efficiency on the distance between molecules can be deduced. The efficiency of energy transfer as a function of distance is given by

$$\eta = \frac{(R_0/r)^j}{(R_0/r)^j + 1}, \tag{3}$$

where R_0 is the distance corresponding to 50% energy transfer, j is the exponent of the distance dependence [24]. R_0 and j can be obtained by plotting $\ln(\eta^{-1} - 1)$ versus $\ln r$ (Fig. 7), j and R_0 was found to be 2.9 and 22 Å. respectively. For 50% energy transfer about 0.037 M local concentration for both lutein and chlorophyll-a would be needed.

Since lutein has no measurable fluorescence the R_0 value obtained above can not be compared with an R_0 value obtained from fluorescence data of lutein. For another carotenoid → chlorophyll-a type energy transfer (β-carotene → chlorophyll-a) $R_0 = 12$ [1] and 8.5 Å [6, 11] is known on the basis of supposed fluorescence yield and spectrum [23], and also 18 to 25 Å on the basis of experimental data [6]. From the data presented it is concluded, that the lutein → chlorophyll-a energy transfer follows an r^{-3} distance dependence. This implies that effective transfer occurs between closely packed lutein and chlorophyll molecules. At high local pigment concentrations the efficiency of transfer does not closely follow the r^{-3} law, probably because chlorophyll aggregates are formed (see Fig. 7).

It may be concluded that the pigment detergent system at detergent concentrations around the critical micelle concentration is useful for modeling energy transfer between biological pigments active in photosynthesis.

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