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EXCITATION ENERGY TRANSFER BETWEEN CHLOROPHYLL A  
AND CHLOROPHYLL B OBTAINED  
FROM FLUORESCENCE MEASUREMENTS

In this study, the fluorescence lifetime of chlorophyll a, chlorophyll b and their mixtures, and the possibility of migration of energy absorbed by chlorophyll a to chlorophyll b was examined.

With an aim to evaluate the yield of excitation energy transfer between ordered chlorophyll a and chlorophyll b molecules the fluid oriented medium-nematic liquid crystal (LC) mixture MBBA (p-methoxybenzylidene p-butylaniline) and EBBA (p-ethoxybenzylidene p-butylaniline) is used. LC is oriented by deposition of thin layer of sample between stretched polyvinyl alcohol films [1] or between silicon oxide orienting layers; pigment molecules are oriented similarly by strong interaction with LC molecules [2]. Chlorophylls in LC are monomeric except the highest concentration used ( $10^{-2}$  M/l).

The results of photoacoustic spectra suggest the excitation energy transfer from chl a to chl b in investigated system [3]. In order to check this unexpected result the mean lifetimes of chl a, chl b and their mixture were measured. Lifetimes were measured for chl a and chl b solutions and for their mixture in the same solvent. Table 1 gathers the results of lifetimes measurements for chl a chl b and their mixture in the same LC. Every one results from Tab. 1 is obtained by averaging of three or four independent measurements. As can be seen from

Table 1

Fluorescence lifetime of chl a, chl b  
and chl a + chl b mixture in MBBA + EBBA  
( $C_a = 1.7 \cdot 10^{-2}$  M/l,  $C_b = 3.6 \cdot 10^{-3}$  M/l)

Czas życia fluorescencji chl a, chl b  
i mieszaniny chl a + chl b w MBBA + EBBA  
( $C_a = 1,7 \cdot 10^{-2}$  mole/l,  $C_b = 3,6 \cdot 10^{-3}$  mole/l)

Sample	Wavelength		Lifetime [nm]
	of excitation [nm]	of fluorescence [nm]	
Chl a	405	> 620	$5.56 \pm 0.03$
	405	> 695	$5.53 \pm 0.02$
	436	> 620	$5.59 \pm 0.02$
	466	> 620	$5.35 \pm 0.02$
	491	> 620	$5.17 \pm 0.02$
Chl b	405	> 620	$8.61 \pm 0.03$
	405	> 695	$8.91 \pm 0.03$
	436	> 620	$8.81 \pm 0.03$
	466	> 620	$8.75 \pm 0.03$
	491	> 620	$8.46 \pm 0.03$
Chl a + chl b	405	> 620	$7.82 \pm 0.03$
	405	> 695	$8.01 \pm 0.02$
	436	> 620	$7.99 \pm 0.03$
	436	> 695	$8.13 \pm 0.02$
	466	> 620	$7.65 \pm 0.03$
	466	> 695	$7.72 \pm 0.02$
	491	> 620	$7.49 \pm 0.05$
	491	> 695	$7.70 \pm 0.04$

Tab. 1 for chl a + chl b mixture is much closer to that of chl b alone, then for the chl a solution in LC, even at higher chl a than chl b concentration. Measurement were done using two regions of fluorescence spectra:

1) using filter transmitting wavelengths  $\lambda > 620$  nm e.g. including whole chl a and chl b emission bands;

2) through the filter transmitting  $\lambda > 695$  nm which gives rise to chl a, then chl b fluorescence.

Comparing all results gathered in Tab. 1 one has to conclude, that because of strong overlapping of emission spectra of chl a chl b and their aggregates it is not possible to measure the belonging to every one of the pigments located in pigment mixture. The result of  $\tau$  for pigment mixture can be related with two effects:

1) the independent excitation of every one pigment followed by the overlapping of their emissions characterized by different  $\tau$ ;

2) the change in the fractions of emitting pigments by the excitation energy transfer.

First effect is trivial. To check if second effect plays important role in our system we analysed first lifetimes of pigment mixture using Pearlstein, Tumerman and Sordrin formula [4]

$$\tan \delta = \frac{(1 + \tan^2 \delta_2) f_1 \Phi_1 \tan \delta_1 + (1 + \tan^2 \delta_1) f_2 \Phi_2 \tan \delta_2}{(1 + \tan^2 \delta_2) f_1 \Phi_1 + (1 + \tan^2 \delta_1) f_2 \Phi_2} \quad (1)$$

where:  $\delta$  - observed phase shift for pigment mixture;

$\delta_1, \delta_2$  - phase shifts for the pigments in separated samples;

$f_1, f_2$  - fractions of light absorbed by chl a and chl b respectively;

$\Phi_1, \Phi_2$  - the yields of fluorescence of chl a and chl b

$$\tan \delta = -\omega\tau; \quad \omega = 7.36 \cdot 10^7 \text{ s}^{-1}$$

$\omega$  is 2Hv frequency of light modulation used in phase fluorometer. It was found that  $\delta$  obtained from experiment is different from those calculated from formula (1). For example at  $\lambda_{\text{exc}} = 491$  nm  $\delta_{\text{cal}} = -0.41$ ; whereas the experimental value is

$$\tan \delta_{\text{exp}} = -0.56$$

It is an evidence that in our sample the emission is at least partially excited by ET.

In purpose to calculate the yield and the direction of exci-

tation energy transfer between investigated pigments the formula proposed by Knox [4] is used:

$$\delta_{f_1, f_2} = \arctan \left[ \frac{(f_2 + \frac{A_1}{A_2} f_1) \omega}{K + f_2 K_1 + \frac{A_1}{A_2} f_1 K_2} \right] - \arctan \left( \frac{\omega}{K_2} \right) - \arctan \left( \frac{\omega}{K_1 + K} \right) \quad (2)$$

where:  $A_1, A_2$  are natural radiative rate of chl b and chl a ( $A_1 = \frac{1}{\tau_{01}}, A_2 = \frac{1}{\tau_{02}}$ );  
 $K_1, K_2$  - total decay constants for uncoupled systems;  
 $F$  - rate excitation energy transfer. It is supposed in calculation that energy is transferred from chl b to chl a;

$\delta_{f_1, f_2}$  - phase shift predicted when absorbed fraction are  $f_1$  and  $f_2$ .

Data used is  $f_1, f_2$  calculation for two wavelengths of exciting light are gathered in Tab. 2.

$A_1$  and  $A_2$  are obtained from red absorption bands of chl b and chl a respectively using formula:

$$A = \frac{1}{\tau_0} = 3.10^{-9} \tilde{\nu}^2 \Delta \tilde{\nu} E_{\max}$$

where:  $\tilde{\nu}$  - position of band maximum in  $\text{cm}^{-1}$ ;

$\Delta \tilde{\nu}$  - half band width (in  $\text{cm}^{-1}$ );

$E_{\max}$  - excitation at maximum of absorption  $\text{M}^{-1} \text{cm}^{-1}$ .

$K_1 = \frac{1}{\tau_{\text{chl b}}}$  and  $K_2 = \frac{1}{\tau_{\text{chl a}}}$  are obtained from measured lifetimes of separated pigments.  $f_1, f_2$  and  $\omega$  are defined as in formula (1).

Data used in calculations and results are gathered in Tab. 2. It was found that in all cases  $K_{\text{chl b} \rightarrow \text{chl a}}$  - rate of ET from chl b to chl a is negative. It means that rate of ET from chl a to chl b predominates in investigated system. Therefore the yield of ET from chl a to chl b is calculated as  $\Phi_{\text{chl a} \rightarrow \text{chl b}} = -K_{\text{chl b} \rightarrow \text{chl a}} \cdot \tau_{\text{chl a}}$ . The accuracy of calculation is better for not very low every one  $f_1$  and  $f_2$ , therefore such results are shown in Tab. 2.



Table 2

Values used in the calculation of the efficiency of the excitation energy transfer on the basis of formula (2)

Wartości użyte do obliczeń wydajności transferu migracji energii według formuły (2)

Wavelength of excitation (nm)	pigment	A (s <sup>-1</sup> )	K (s <sup>-1</sup> )	f	$\tau$ (ns)	$\tan \delta$ $f_1 f_2$	K chl b-chl a	$\Phi$ chl a-chl b
466	chl a (2)	$2.78 \times 10^7$	$18.7 \times 10^7$	0.57	5.35	-0.5630	$-2.2 \times 10^7$	0.12
	chl b (1)	$2.83 \times 10^7$	$11.4 \times 10^7$	0.43	8.75	-	-	-
491	chl a (2)	$2.78 \times 10^7$	$19.3 \times 10^7$	0.71	5.17	-0.5513	$-5.0 \times 10^7$	0.26
	chl b (1)	$2.83 \times 10^7$	$11.8 \times 10^7$	0.29	8.46	-	-	-

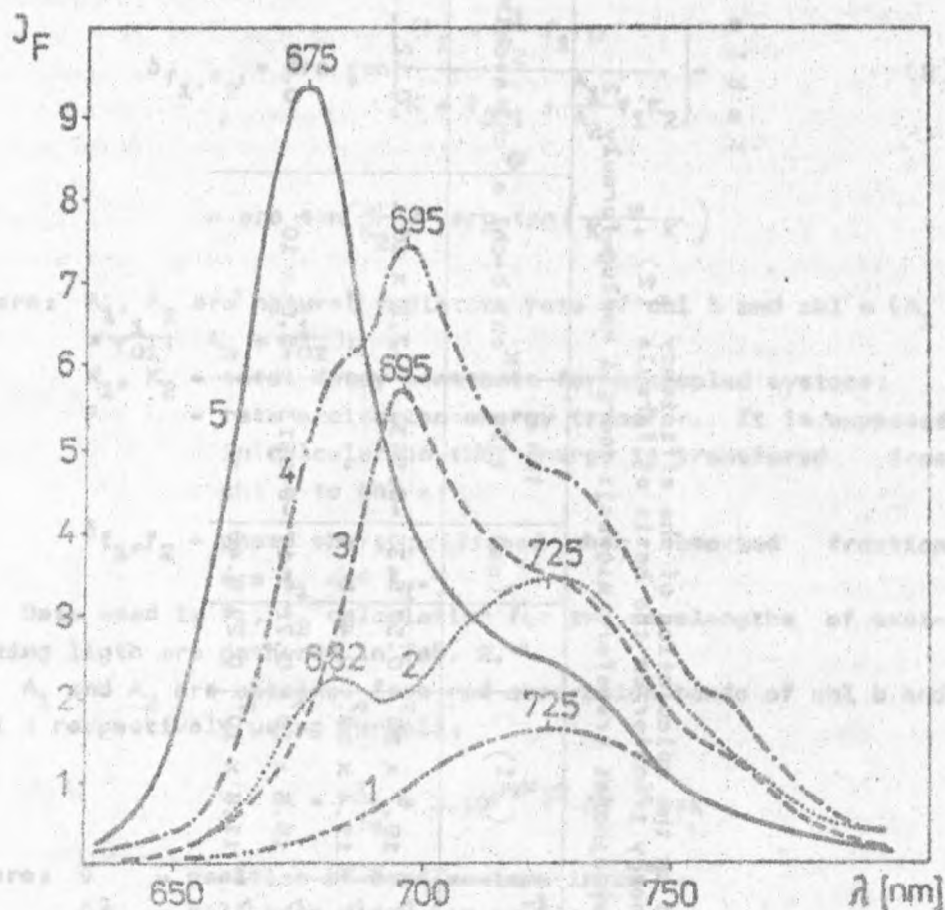


Fig. 1. Fluorescence spectra of chl a ( $C_a = 2.3 \cdot 10^{-2} M$ )  $\lambda_{exc} = 470$  nm (curve 1);  $\lambda_{exc} = 430$  nm (curve 2); chl b ( $C_b = 5.2 \cdot 10^{-3} M$ )  $\lambda_{exc} = 470$  nm (curve 5); chl a + chl b mixture ( $C_a = 1.7 \cdot 10^{-2} M$ ;  $C_b = 3.6 \cdot 10^{-3} M$ )  $\lambda_{exc} = 430$  nm (curve 3);  $\lambda_{exc} = 480$  nm (curve 4)

Widma fluorescencji chl a ( $C_a = 2,3 \cdot 10^{-2}$  mola)  $\lambda_{exc} = 470$  nm (krzywa 1);  $\lambda_{exc} = 430$  nm (krzywa 2); chl b ( $C_b = 5,2 \cdot 10^{-3}$  mola)  $\lambda_{exc} = 470$  nm (krzywa 5); chl a + chl b mieszanina ( $C_a = 1,7 \cdot 10^{-2}$  mola,  $C_b = 3,6 \cdot 10^{-3}$  mola)  $\lambda_{exc} = 430$  nm (krzywa 3);  $\lambda_{exc} = 480$  nm (krzywa 4)

All used excitation wavelengths are located in a region of chlorophylls Soret bands. The difference  $\Phi_{\text{chl a} \rightarrow \text{chl b}}$  obtained for various  $\lambda_{\text{exc}}$  are related with different fractions of monomers and aggregates of pigment absorbing at various excitations.

One can conclude, that similarly as from PAS results, analysis of lifetimes of fluorescence of pigment mixture suggests some way of migration of energy observed by chl a molecules to chl b. The lifetime of aggregate has to be longer than that of chl a, closer to  $\tau$  of chl b.

The thermal deactivation of excitation energy in aggregate has to be higher than that of chl a.

In fluorescence spectrum of pigment mixture additional maximum located at 695 nm was found Fig. 1. This maximum is not occurring in spectra of separate pigments, therefore it is probably connected with mixed aggregates of both and LC molecules.

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PRZEKAZYWANIE ENERGII POBUDZENIA  
POMIĘDZY CHLOROFILEM A I CHLOROFILEM B  
W ŚWIETLE POMIARÓW FLUORESCENCJI

Badania czasu życia fluorescencji chlorofilu a i chlorofilu b oraz mieszanin chlorofilu sugeruje możliwość migracji energii absorbowanej przez chlorofil a do chlorofilu b.