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Adaptation of photosynthesis of *Chlorella* spp. to light conditions: changes in efficiency of charge separation detected by *in vivo* delayed fluorescence excitation spectroscopy

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Introduction

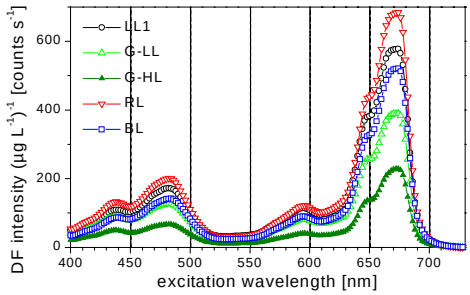
Delayed fluorescence (DF) occurs in photosynthetically active material. It originates from re-combination of an electron at the oxidized reaction center P680 in the dark. Research on the complex behaviour of the DF (Gerhardt and Bodemer 2000 and references cited therein) has led to the conclusion that DF can be used to determine concentrations of photosynthetically active pigments and to analyse phytoplankton compositions in freshwaters (Bodemer 1998, Bodemer et al. 2000). DF excitation spectroscopy is also a fast *in vivo* method to investigate processes occurring within the antenna of PSII and within the electron transport chain (Gerhardt and Bodemer 2001). Measuring DF excitation spectra of algal cultures have shown changes in pigment composition (Gerhardt and Bodemer 2000). It was investigated whether growth light conditions cause these changes.

Materials and methods

Culture growth conditions. *Chlorella* spp. were grown under various light conditions: constant white low light (**LL1** (n=number of samples=11): 4 µEinst m^{-2} s^{-1}, **LL2** (n=17): 30 µEinst m^{-2} s^{-1}), constant white high light (**HL** (n=25): 285 µEinst m^{-2} s^{-1}), constant red light (610 nm, **RL** (n=16): 10 µEinst m^{-2} s^{-1}), constant blue light (482 nm, **BL** (n=13): 3 µEinst m^{-2} s^{-1}) and daylight (greenhouse, no air-condition, **G-HL** (n=10): 430 µEinst m^{-2} s^{-1} (∅), **G-LL** (n=16): 70 µEinst m^{-2} s^{-1} (∅)). All cultures were grown as semi-continuous cultures, bubbled with air. Growth temperature was 21-25°C, except for the greenhouse cultures (8-25°C).
Inhibitor treatment. Lincomycin (10 ml of 1 mM solution) and dithiothreitol (DTT) (10 ml of 1 mM solution) were added to LL and HL cultures (750 ml, 100 µg L^{-1} [chla]) and exposed to low (30 µEinst m^{-2} s^{-1}) and high (285 µEinst m^{-2} s^{-1}) light, respectively for one hour (**LL-lin10**, **HL-lin10**, **LL-dtt10**, **HL-dtt10**).
DF measurement. DF excitation spectra were taken at 21°C. Details of the method are described in Gerhardt and Bodemer 2000.

Results and Discussion

The DF excitation spectra (normalized to 1 µg L^{-1} chlorophyll [chl*a*]) of *Chlorella* spp. cultures grown under different light conditions are shown in Fig. 1 (statistical data: see Fig. 2).



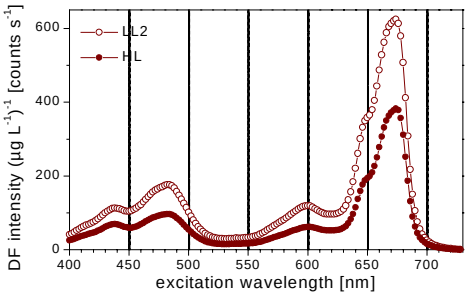


Fig.1.a, b. DF excitation spectra (means, normalized to 1 µg L^{-1} [chl*a*]) of *Chlorella* spp. cultures grown under different light conditions (number of measurements see Fig. 2)

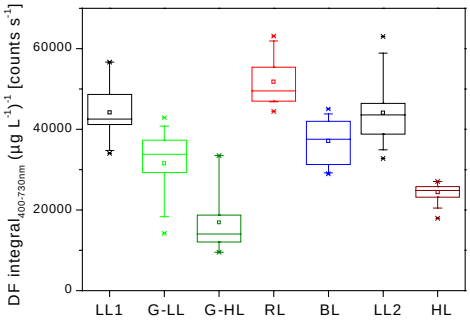


Fig.2. Statistical box diagram of DF integrals of the measured DF excitation spectra.
t-test of two independent populations:

LL1 (n=11)/G-LL	(n=16):	t = -3.87, p = 0.0006
LL1 (n=11)/G-HL	(n=10):	t = -7.77, p < 0.0001
LL1 (n=11)/RL	(n=16):	t = 2.629, p = 0.014
LL1 (n=11)/BL	(n=13):	t = -2.595, p = 0.017
G-LL (n=16)/G-HL	(n=10):	t = -4.181, p = 0.0003
LL2 (n=17)/HL	(n=25):	t = -11.367, p < 0.0001

Changes of efficiency of charge separation at P680

compared to light regime LL1 [%]

light regime	400-450 nm	450-510 nm	510-600 nm	600-660 nm	660-700 nm
G-LL	- 17.39	- 25.35	- 26.82	- 31.00	- 29.92
	- 54.44	- 61.00	- 64.76	- 64.10	- 60.30
G-HL	+ 22.91	+ 18.55	+ 7.03	+ 16.04	+ 22.65
RL	- 23.21	- 18.51	- 23.72	- 16.52	- 8.54
BL					

compared to light regime G-LL [%]

G-HL	- 44.85	- 47.75	- 51.84	- 47.98	- 43.35

compared to light regime LL2 [%]

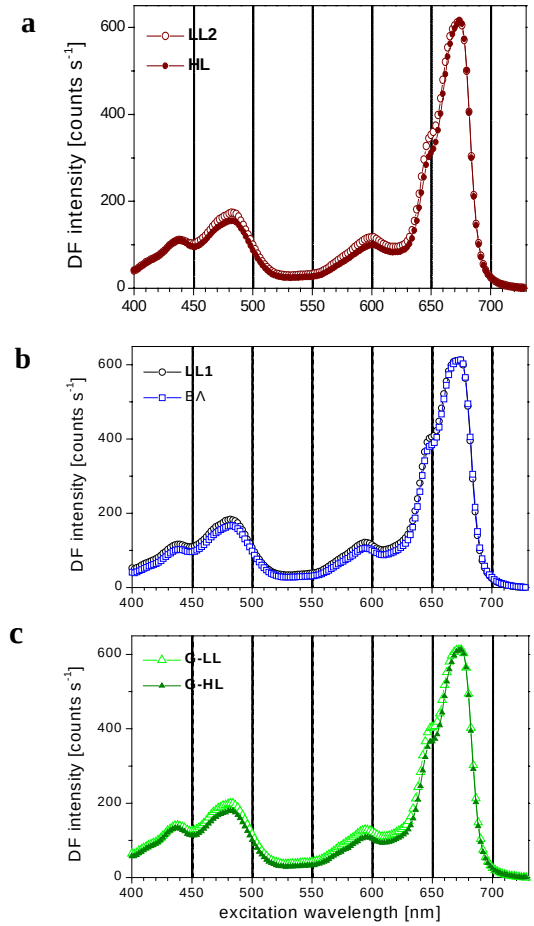
HL	- 39.42	- 45.16	- 48.96	- 46.04	- 39.71

Table 1. Changes in efficiency of charge separation at P680 of *Chlorella* spp. cultures grown under different light regimes calculated from wavelength intervals of the DF excitation spectra in Fig. 1.

Figs. 1.a and 1.b shows that *Chlorella* spp. grown in different light regimes exhibit very different DF intensities when normalized to 1 µg L^{-1} [chl*a*]. Differences in DF integrals (per µg L^{-1} [chl*a*]) between LL1 and G-LL, G-HL, RL and BL cultures, between G-LL and G-HL cultures and between LL2 and HL cultures are highly significant (Fig. 2). The efficiency of charge separation at P680 is changed by the different light treatments (Table 1): This efficiency is only 50 % in G-HL cultures compared to G-LL cultures, in RL cultures it is about 20 % higher and in BL cultures it is about 20 % lower than in LL1 cultures, and it is about 45 % lower in HL cultures compared to LL2 cultures.

Fig. 3 presents DF excitation spectra normalized at the chl *a* peak (674 nm). It is obvious that the excitonic energy of antenna pigments produced by light absorption in the different wavelength regions does not lead to charge separation at P680 proportionally: e.g. in HL cultures charge separation at P680 due to energy transfer from pigments absorbing light from 400-500 nm is reduced compared to LL2 cultures (Fig. 3.a). Calculating the ratios of the DF excitation spectra – as shown in Fig. 4 – these differences become more distinct. The results in Table 1 also shows this disproportionality: the efficiency of charge separation at P680 due

to light absorption is wavelength-specific, varying e.g. in RL cultures compared to LL1 cultures from an increase of 22.9 % (400-450 nm) to 7 % (510-600 nm) or in G-LL cultures



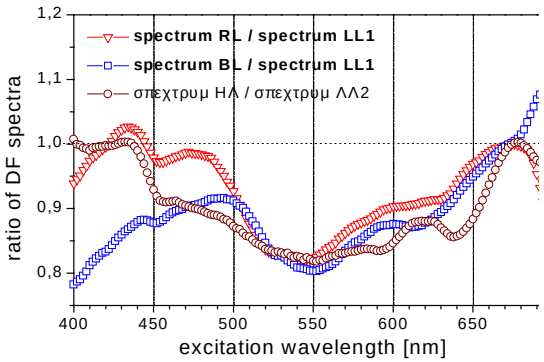


Fig. 4. Ratios of DF spectra (normalized at 674 nm) of three different light regimes.

Fig. 3a, b, c. DF excitation spectra normalized at 674 nm.

compared to LL1 cultures from a decrease of 31 % (600-660 nm) to 17.4 % (400-450 nm).

The observed changes in the DF excitation spectra of cultures grown under different light conditions can be explained by three processes occurring in the antenna of PSII: **1**) Inhibition of D1 protein synthesis leads to a decrease in efficiency of charge sepa-ration at P680 over the total excitation wavelength range (400-730 nm) in those cases when destruction exceeds *de-novo* synthesis of this protein (Fig. 1). **2**) Development or activation of zeaxanthin, respectively, causes a decrease in efficiency of charge separation in the wavelength region 400-500 nm. This shading effect protects reaction centers against high energy photons at high light intensities (Fig.3 and 4). **3**) Changes in Chl *a/b* ratio superimpose the effects of the processes 1) and 2).

It was shown that LL and HL grown plants differ in D1 protein content resulting in an increase in non-functional PSIIs in HL plants (Anderson et al. 1998, Baroli and Melis 1998) and that HL samples contain more xanthophyll cycle pigments violaxanthin, antheraxanthin and zeaxanthin (Demmig-Adams et al. 1996), the latter leading to energy dissipation to protect PSII against photodamage (Frank et al.1994). The different Chl *a/b* ratios of LL and HL plants are well known (e.g. Kim et al. 1993).

The results obtained by *in vivo* DF excitation spectroscopy correspond to these findings:

Decrease of DF intensity in HL and G-HL cultures (compared to LL2 and G-LL cultures, respectively) are mainly due to destruction of D1 protein. Xanthophyll cycle pigments additionally change the DF intensities wavelength-specific (400-500 nm).

cultures exhibit the highest DF intensity. Red photons lead to a very efficient charge separation at P680 because no zeaxanthin is necessary to dissipate high energy of blue photons and the turnover of D1 protein is working.

The lower DF intensity of BL cultures (compared to LL1 cultures) is caused – despite similar low light intensity – by destruction of D1 protein **and** formation of protection pigment zeaxanthin to dissipate light energy absorbed in the blue wavelength region. The turnover of D1 protein might work less efficiently due to the very low light intensity from which energy for all metabolic processes has to be drawn.

First experiments adding lincomycin, an inhibitor of D1 protein synthesis, and dithiothreitol (DTT), an inhibitor of zeaxanthin synthesis, support these conclusions. Lincomycin leads in HL as well as in LL cultures to a decrease of DF intensity (Fig. 5.a), i.e. to an inactivation of functional PSIIs of about 25 % in HL and about 10 % in LL cultures which corresponds in principle to results with chloramphenicol-treated *Dunaniella salina* (Kim et al. 1993) and lincomycin-treated leafs (Anderson et al. 1998). DTT treatment should lead to an inhibition of zeaxanthin synthesis causing decreased energy dissipation (Bilger and Björkman 1990, Wild et al. 1995, Demmig-Adams et al. 1996). The effects on DF intensity by adding DTT to HL and LL cultures could be interpreted as follows (Fig. 5.b): In LL cultures (open Δ) there occurs no or almost no change in efficiency of charge separation at P680 (ratio = ± 0.99) because no or only few zeaxanthin is present or built up under the used low light conditions. Therefore, an inhibition of zeaxanthin synthesis by DTT will cause no changes. However, in HL cultures (open o) an effect of DTT treatment could be observed: The DF intensity is reduced. Decreased energy dissipation due to less zeaxanthin is not evident (DF ratio should be >1 or between 400 and 500 nm higher than in the remaining spectral range). The decrease in DF intensity seems to be caused by an increased destruction of D1 reaction center protein and (like in the lincomycin-treated HL culture) the efficiency of charge separation at P680 decreases (compare shapes of the lincomycin-treated (□ in Fig. 5.a) and DTT-treated (o in Fig. 5.b) HL cultures). This interpretation corresponds to the observed increased susceptibility to photoinhibition in DTT-treated leaves (Bilger and Björkman 1990).
Further investigations, especially comparing DF and HPLC results, will elucidate the observed processes within the antenna of PSII.

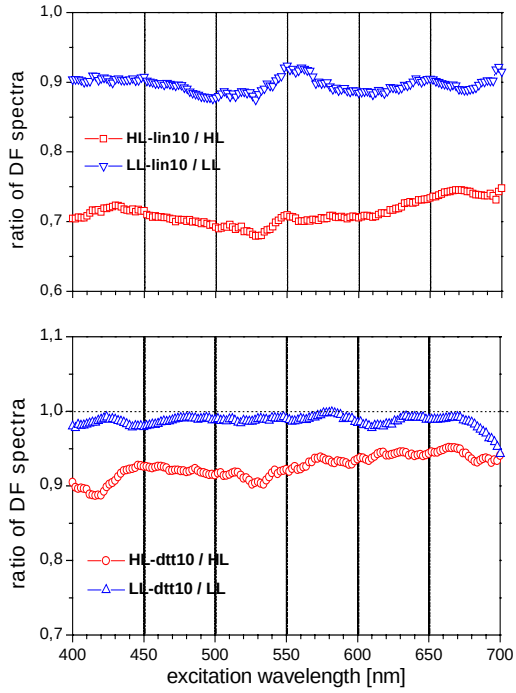


Fig. 5. Ratios of DF spectra. **a**. Lincomycin-treated HL and HL *Chlorella* spp. cultures (HL-lin10 / HL, □) and Lincomycin-treated LL and LL *Chlorella* spp. cul-tures (LL-lin10 / LL, ∇). **b.** DTT-treated HL and HL *Chlorella* spp. cultures (HL-dtt10 / HL, o) and DTT-treated LL and LL *Chlorella* spp. cultures (LL-dtt10 / LL, Δ). 10 ml 1mM of the inhibitors was added to 750 ml culture of 100 µg L^{-1} [chl*a*].

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