Cerulenin-Induced Modifications in the Fatty Acid Composition Affect Excitation Energy Transfer in Thylakoids of *Petunia hybrida* Leaves

Josef A. Graf, Karin Witzan

Biologisches Institut, Universität Stuttgart, Ulmer Straße 227, D-7000 Stuttgart 60, Bundesrepublik Deutschland

and

Reto J. Strasser

Bioenergetics Laboratory, University of Geneva, CH-1254 Lullier-Geneva, Switzerland

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Dedicated to Professor Ulrich Kull, Stuttgart, on the occasion of his 50th birthday

Cerulenin, Fatty Acid Synthesis, Excitation Energy Distribution

Cerulenin-induced modifications in the fatty acid composition have been used to investigate the influence of acyl lipids on excitation energy distribution in thylakoid membranes of *Petunia hybrida* by means of 77 K fluorescence spectroscopy. Although cerulenin has no effect on relative contents of chlorophyll and acyl lipids, changes in the fatty acid composition of all thylakoid acyl lipids have been observed. The main cerulenin effect seems to be an increase in linoleic acid at the expense of saturated and monounsaturated C_{16}- and C_{18}-fatty acids resulting most likely in an increase in acyl lipid species containing both linoleic and linolenic acid. Low temperature (77 K) fluorescence kinetics reveal a remarkable decrease in the ratio of the variable divided by the maximal fluorescence of photosystem II ($F_{2(v)}/F_{2(M)}$), taken as indicator for cerulenin-induced changes in this photosystem. Calculations of the excitation energy distribution terms based on a grouped bipartite model of photosynthesis suggest that a decrease in this ratio is caused by changes in energy transfer probabilities responsible for both, photochemical trapping of photosystem II and energetic cooperativity (grouping) between different photosystem II-light harvesting complex-units. Moreover, changes in the conformation responsible for spillover energy transfer are most likely to occur. Correlations between cerulenin-induced modifications of fatty acid composition and energy distribution support the assumption that excitation energy transfer depends on the structural state of the lipid matrix.

Introduction

Thylakoid acyl lipids have been the subject for intensive studies because of their high contents of polyunsaturated acyl compounds and their importance in maintaining structure and function of photosynthetic membranes. In order to coordinate complex photosynthetic processes like exciton transfer and charge separation, a high degree of membrane organization is necessary. To study the influence of acyl lipids on structural and functional aspects of thylakoid membranes, several methods have been employed such as liposome- and reconstitution techniques [1], digestion of lipids with lipolytic enzymes [2, 3], isolation of pigment-protein complexes including their lipid micro-environment [4, 5] and *in vivo* modification of the lipid matrix [6–10]. Considering excitation energy distribution in particular, a structure-function relationship between the surrounding lipid environment and pigment-protein complexes in thylakoids has been suggested [7, 8]. Besides inhibition of the desaturation process leading to polyunsaturated fatty acids using the pyridazinone compound BAS 13-338 (SAN 9785), partial inhibition of *de novo* fatty acid biosynthesis with cerulenin may be a useful tool in studying the influence of acyl lipids on excitation energy distribution in thylakoids. Cerulenin is an antibiotic produced by the fungus *Cephalosporium caerulens* and has been demonstrated to be a specific inhibitor of the β-ketoacyl-ACP-synthetase of bacteria, fungi and higher plants [11–14].

Abbreviations: PS I, II, photosystem I, II; RC I, II, reaction center I, II; LHC, light harvesting chlorophyll *a*/*b* complex; $F_{1(0)}$, $F_{1(v)}$, $F_{1(M)}$, initial, variable and maximal fluorescence of PS I; $F_{2(0)}$, $F_{2(v)}$, $F_{2(M)}$, initial, variable and maximal fluorescence of PS II; V, normalized variable fluorescence of PS II; s, integral of non-fluorescence of PS II; MGDG, monogalactosyldiacylglycerol; DGDG, digalactosyldiacylglycerol; SQDG, sulphoquinovosyldiacylglycerol; PG, phosphatidylglycerol; PL, other phospholipids; cerulenin, (2*S*,3*R*)-2,3-epoxy-4-oxo-7,10-dodecadienylamide.

Reprint requests to Dr. Josef A. Graf.

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In this study we show the effects of cerulenin on acyl lipid composition and excitation energy distribution in thylakoids of *Petunia hybrida*. A proposed relationship between fatty acid desaturation and excitation energy distribution is discussed.

Materials and Methods

Fully developed 6 week old *Petunia hybrida* plants (cyanidine type) were detached from the roots and incubated in solutions of 4.5 μM cerulenin in glass chambers under 15 W/m^2 light intensity, 20 ± 2 °C growth temperature and 70 ± 10% relative humidity for 5 to 7 days. Control plants were kept under the same conditions without cerulenin.

77 K fluorescence kinetic measurements (resolution 2 addresses/ms) of leaf discs taken from the plants were carried out using excitation light of 633 nm (HeNe laser, 20 W/m^2), a multibranched fiber optic and 2 photomultipliers. Fluorescence signals measured simultaneously at 735 nm (PS I, filters CS 9830/CS 2600) and at 695 nm (PS II, filters IF Balzers B 40690/CS 2030) were digitized on line (IMSAI 8080 computer) and stored in core memory for data handling. 77 K fluorescence emission spectra of diluted leaf powder samples [15] measured from 600 to 800 nm (monochromator Jobin Yvon H 10 vis, 0.5 mm slit, equipped with scan controller) were used to correct the $F_{(0)}$- and $F_{(M)}$-values obtained from the kinetics.

The characteristics of excitation energy distribution *i.e.* photochemical trapping, grouping, spillover and dissipation were analyzed for a grouped bipartite model of photosynthesis as described elsewhere [16] and expressed in quantum yields of PS II and PS I φ_{ij}, energy transfer probabilities p_{ij}, relative de-excitation rate constants k_{ij} and relative distances R_{ij} between different pigment-protein complexes. The indices i and j generally indicate the direction of excitation energy transfer from a locus i to a locus j, where 2 stands for PS II (RC and LHC), 1 for PS I (RC and LHC), D for dissipation (heat and fluorescence), b and a for P_{680} and P_{700}, respectively.

Chlorophyll content was estimated using extinction coefficients of [17]. Lipids were extracted from purified thylakoids as described elsewhere [18] and isolated by TLC on silica gel 60 (Merck) using acetone : benzene : water system (91:30:8, v/v/v) according to [19]. Quantitative analysis of glyco- and phospholipids was carried out as described in [20] and [21]. For fatty acid analysis, individual thylakoid lipids were transmethylated according to [22] in the presence of methylpentadecanoate standard. After extraction, aliquots of acylmethylesters were separated by GC in 10′ × ⅛″ steel columns packed with 10% EGSS-X on Chromosorb W/AW-DCMS (80–100 mesh) using a Varian Aerograph 2700 fitted with FID and CDS 111 integrator. Acyl lipid bands and acylmethylesters were identified by comparing them with authentic standards.

Results

As shown in Table I, relative contents of chlorophylls as well as the relative amounts of glyco- and phospholipids of thylakoids remain nearly constant following 5 days of treatment of *Petunia hybrida* with either 4.5 or 13.5 μM cerulenin; although there is a decrease in lipid content based on fresh weight of the leaves (data not shown). On the other hand, changes in the fatty acid composition have been observed. Within all acyl lipid fractions the amounts of saturated and monounsaturated C_{16}- and C_{18}-fatty acids are decreasing, while the amount of linoleic acid is increasing as compared to the control samples. Moreover, linolenic acid remains constant in MGDG, PG and PL and slightly increases in DGDG- and SQDG-fractions of cerulenin-treated samples (Table II). In addition, a significant increase of palmitic acid has been found in SQDG. Statistical calculations suggest an increase of the probability for combinations of linoleic acid with linolenic acid in all thylakoid lipid fractions by the factor 1.5 to 2. These observations clearly indicate that during cerulenin-induced inhibition of *de novo* fatty acid synthesis

Table I. Relative composition (% w/w) of the lipophilic fractions of purified thylakoid membranes following 5 days of treatment with 4.5 μM cerulenin. The values are mean ± SD obtained from 3 experiments.

Lipophilic fraction	Control	Cerulenin
MGDG	26.7 ± 2.1	24.0 ± 1.9
DGDG	19.5 ± 1.2	19.9 ± 1.2
SQDG	22.4 ± 1.8	18.4 ± 1.4
PG	3.1 ± 0.2	3.3 ± 0.2
PL	4.4 ± 0.3	3.3 ± 0.2
Chlorophyll *a*	14.1 ± 0.7	12.4 ± 0.6
Chlorophyll *b*	5.5 ± 0.3	5.2 ± 0.3
Chlorophyll *a*/*b*	2.6 ± 0.2	2.4 ± 0.2

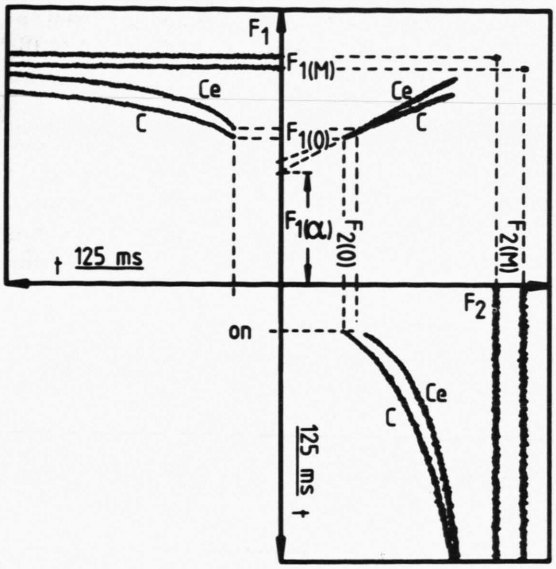

Fig. 1. Fluorescence kinetics of PS I and PS II at 77 K after treating *Petunia hybrida* plants with 4.5 μM cerulenin for 5 days. In order to get all reaction centers oxidized the leaf discs taken from the plants were kept 30 sec in the dark before freezing to 77 K. The fluorescence signals were simultaneously measured at 735 nm (the emission maximum of PS I) and at 695 nm (the emission maximum of PS II). Fluorescence intensities of PS I (735 nm) and PS II (695 nm) are symbolized by the terms F_1 and F_2, respectively. Time axis is indicated by t. The curves at maximal fluorescence, *i.e.* the assymptotes $F_{1(M)}$ and $F_{2(M)}$ were taken 10 sec following light on. The time-independent plots PS I-fluorescence *versus* PS II-fluorescence (F_1 *versus* F_2) reveal straight lines with different slopes (slope control = 0.38, slope cerulenin = 0.5) for control- and cerulenin samples, respectively. The intercept $F_{1(\alpha)}$ means that part of excitation energy in PS I due to light absorption by that photosystem itself. C = control, Ce = cerulenin.

desaturation reactions take place bringing about changes in the acyl composition of thylakoid lipids.

As estimated by ultra-red absorption spectroscopy, carbon dioxide fixation rate declined within the first few hours after starting incubation with 4.5 μM cerulenin and recovered thereafter to normal control values (data not shown).

Experimental 77 K fluorescence kinetics indicate that *Petunia hybrida* plants treated either with 4.5 or 13.5 μM cerulenin for 5 to 7 days exhibited a decrease in the ratio $F_{2(v)}/F_{2(M)}$ from 0.72 to 0.53 as compared to the control leaves (Fig. 2). Since this ratio has been demonstrated to be proportional to the probability that a exciton in the antennae pigments of PS II

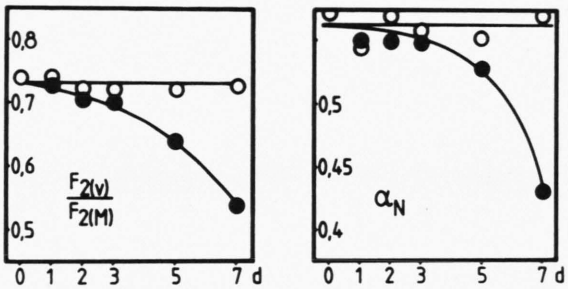

Fig. 2. Alterations in the ratio $F_{2(v)}/F_{2(M)}$ taken as indicator for changes in PS II and alterations in α_N the fraction of excitation energy in PS I due to light absorbed by that photosystem itself, of *Petunia hybrida* with 4.5 μM cerulenin for 7 days. α_N-Values are shown for maximal fluorescence levels *i.e.* when all reaction centers are reduced. α_N is defined as $F_{1(\alpha)}/F_{1(M)} = 1 - [F_{2(M)}(F_{1(M)} - F_{1(0)})/F_{1(M)}(F_{2(M)} - F_{2(0)})]$; ○ control, ● cerulenin.

Table II. Effect of 13.5 μM cerulenin on fatty acid composition of thylakoid lipids from *Petunia hybrida* leaves in percentage of total fatty acid content. The values are mean ± SD obtained from 3 experiments.

Acyl lipid	Treatment	16:0	16:1	16:u	18:0	18:1	18:2	18:3	18:4	DBI[a]
MGDG	Control	3.9 ± 0.2	1.2 ± 0.1	1.5 ± 0.1	1.4 ± 0.1	1.2 ± 0.1	4.5 ± 0.1	78.1 ± 0.8	7.3 ± 0.7	2.8 ± 0.04
	Cerulenin	3.0 ± 0.2	0.9 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	9.6 ± 0.1	78.5 ± 0.8	5.5 ± 0.6	2.8 ± 0.04
DGDG	Control	11.8 ± 0.7	1.7 ± 0.1	2.4 ± 0.1	2.4 ± 0.1	2.0 ± 0.1	3.3 ± 0.1	71.3 ± 0.7	3.6 ± 0.2	2.4 ± 0.05
	Cerulenin	9.6 ± 0.6	1.3 ± 0.1	1.3 ± 0.1	1.6 ± 0.1	1.3 ± 0.1	6.5 ± 0.1	74.7 ± 0.7	3.4 ± 0.2	2.6 ± 0.06
SQDG	Control	30.8 ± 0.6	3.8 ± 0.4	3.2 ± 0.6	5.3 ± 0.3	3.6 ± 0.2	5.5 ± 0.4	38.9 ± 0.9	5.3 ± 0.2	1.6 ± 0.06
	Cerulenin	37.7 ± 0.7	2.2 ± 0.2	0.9 ± 0.2	2.9 ± 0.2	1.6 ± 0.1	7.1 ± 0.5	43.4 ± 1.0	3.0 ± 0.1	1.6 ± 0.06
PG	Control	28.6 ± 1.6	19.3 ± 0.8[b]	3.5 ± 0.1	5.2 ± 0.4	4.2 ± 0.3	6.7 ± 0.6	26.0 ± 0.9	1.1 ± 0.1	1.2 ± 0.04
	Cerulenin	28.4 ± 1.6	15.5 ± 0.6[b]	1.6 ± 0.1	3.4 ± 0.3	6.1 ± 0.5	13.6 ± 1.1	25.6 ± 0.9	1.5 ± 0.1	1.4 ± 0.05
PL	Control	28.3 ± 1.8	4.5 ± 0.3	4.8 ± 0.4	6.4 ± 0.6	4.2 ± 0.2	14.1 ± 0.8	29.3 ± 1.1	1.1 ± 0.2	1.6 ± 0.08
	Cerulenin	27.1 ± 1.8	4.1 ± 0.3	3.6 ± 0.3	3.9 ± 0.4	4.7 ± 0.2	20.7 ± 1.1	31.2 ± 1.2	2.8 ± 0.4	1.6 ± 0.08

[a] Double bond index.
[b] *trans*-3-Hexadecenoic acid.

reaches a RC II [23], it was taken as an indicator for cerulenin-induced changes in PS II. However, the data presented in this paper are derived from a grouped bipartite model of photosynthesis [16], where not only photochemical trapping of PS II but also grouping between different PS II-LHC-units contribute to the ratio of $F_{2(v)}/F_{2(M)}$. Therefore, a decrease in this ratio suggests that the probabilities for photochemical trapping of PS II and/or for the cooperativity of different PS II-LHC-units are influenced during cerulenin treatment of *Petunia hybrida* plants.

As shown in Fig. 1 the continuous plot F_1 (735 nm) *versus* F_2 (695 nm) yields a straight line the extrapolation of which gives an intercept on the F_1-axis termed $F_{1(\alpha)}$. Normalized to $F_{1(M)}$, the terms $\alpha_N = F_{1(\alpha)}/F_{1(M)}$, and $\beta_N = 1 - \alpha_N$ indicate the fractions of excitation energy available in PS I. α_N implies the fraction of excitation energy due to its own light absorption, whereas β_N stands for the fraction of excitation energy in PS I due to spillover. The experimental data clearly indicate a decrease in α_N from 57 to 43% and a concomitant increase in the spillover term β_N within 7 days of cerulenin treatment. Moreover, it has been proposed that the slopes of the straight lines in Fig. 1 are proportional to the de-excitation rate constant k_{21} responsible for spillover energy transfer [16]. Since there is a remarkable increase in the slopes of cerulenin-treated samples compared to the controls (Fig. 1), an increase in the spillover de-excitation rate constant k_{21} may be expected. The same results were observed using either 4.5 or 13.5 μM cerulenin.

From the 77 K fluorescence emission spectra of diluted leaf powder samples [15] an increase in the ratios F_{695}/F_{735} and F_{685}/F_{735} have been found. Furthermore, a shift in PS I emission peak of about 5 nm towards shorter wave-lengths has been detected (data not shown) as described previously for barley [10].

Analysis of grouping probability p_{22} (Fig. 3) was conducted to determine whether or not the ratio $F_{2(v)}/F_{2(M)}$ was affected by changes in the cooperativity of PS II-LHC-units. As described in [16], the normalized variable fluorescence of PS II *V versus* the integral *s* of the non-fluorescence of PS II yield horizontal hyperbolic functions, the curvatures of which are a measure for the energetic cooperativity between different PS II-LHC-units. Therefore, the grouping probability p_{22} can be determined for each sample from the intercepts V_i after transformation of the hyperbolic functions to straight lines and extra-

Table III. Excitation energy distribution terms derived from the experimental 77 K fluorescence data as described in [31] following treatment with 4.5 μM cerulenin for 7 days. Relative values are calculated from the mean of 3 experiments. Quantum yields φ_{ij} may be greater than 1. This fact is either due to grouping or spillover. *E.g.* excitation rates of PS II for both controls (1.51) and cerulenin-treated plants (2.32) indicate that in control plants one photon absorbed by PS II-LHC-units creates 1.51 excitation events per time in these complexes, while in cerulenin-treated samples one photon absorbed by PS II-LHC-units creates 2.32 excitation events per time due to increased energetic co-operativity (grouping) between these complexes.

Energy distribution	Term	Control	Cerulenin	Energy distribution	Term	Control	Cerulenin
Quantum yields φ_{ij} of PS II or PS I				Relative de-excitation rate constants k_{ij}/k_{2D}			
Excitation rate PS II	φ_2	1.51	2.32	Trapping PS II	k_{2b}/k_{2D}	7.69	3.37
Trapping PS II	φ_{2b}	0.72	0.53	Grouping PS II-LHC-units	k_{22}/k_{2D}	5.47	8.22
Grouping PS II-LHC-units	φ_{22}	0.51	1.32	Spillover PS II → PS I	k_{21}/k_{2D}	1.96	1.87
Dissipation PS II	φ_{2D}	0.09	0.16	Trapping PS I	k_{1a}/k_{2D}	2.36	2.13
Spillover PS II → PS I	φ_{21}	0.18	0.30				
Dissipation PS I	φ_{1D}	0.36	0.51	Relative distances R_{ij}/R_{2D}			
Trapping PS I	φ_{1a}	0.86	1.09	PS II ↔ RC II	R_{2b}/R_{2D}	0.71	0.82
Excitation rate PS I	φ_1	1.22	1.60	PS II-LHC ↔ PS II-LHC	R_{22}/R_{2D}	0.75	0.70
				PS II ↔ PS I	R_{21}/R_{2D}	0.89	0.90
Energy transfer probabilities p_{ij}				PS I ↔ RC I	R_{1a}/R_{2D}	0.87	0.88
Trapping PS II	p_{2b}	0.48	0.23				
Grouping PS II-LHC-units	p_{22}	0.34	0.57	$F_{2(v)}/F_{2(M)}$		0.72	0.53
Dissipation PS II	p_{2D}	0.06	0.07	α_N at maximal fluorescence		0.56	0.43
Spillover PS II → PS I	p_{21}	0.12	0.13	β_N at maximal fluorescence		0.44	0.57
Dissipation PS I	p_{1D}	0.30	0.32	α_A absorption fraction of PS I		0.46	0.33
Trapping PS I	p_{1a}	0.70	0.68	β_A absorption fraction of PS II		0.54	0.67

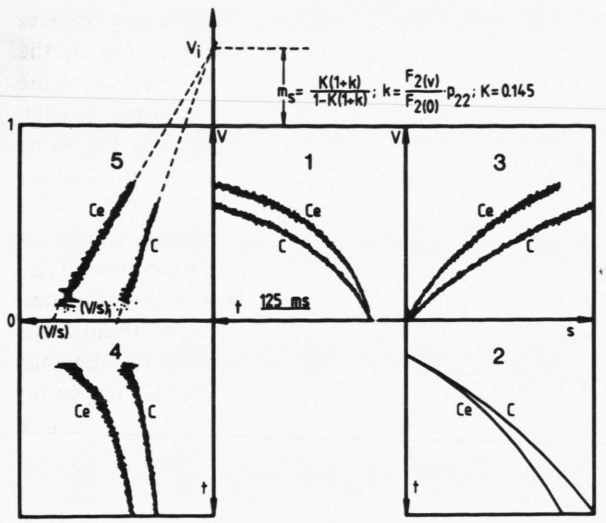

Fig. 3. Methods used to calculate the grouping probabilities p_{22} according to [16]. This calculation connects V the variable fluorescence of PS II to the area growth curve s (area between the variable fluorescence and the maximal fluorescence) where s is a measure for the excitation energy flux transferred from the antennae pigments of PS II to the reaction center P_{680}. Square 1 shows the normalized variable fluorescence V of PS II (695 nm) as a function of time t; the definition of V is: $V = (F_{2(M)} - F_{2(t)})/(F_{2(M)} - F_{2(0)})$; square 2 indicates the area growth curve s ($s = {}_0\int^t (1-V)dt$) as a function of time t; square 3 reveals the relationship between the variable fluorescence V of PS II and the area growth curve s (the measure for the energy flux transferred to P_{680}); the plot V *versus* s is a hyperbolic function, the curvature of which contains the information of grouping probability p_{22}; the plots in squares 4 and 5 are used to prove the hyperbolic functions: square 4 shows V divided by s as a function of time t in order to get the transformations of the hyperbolic functions in square 3 into the functions of square 5; square 5 shows straight lines as revealed in V *versus* V/s plots. Following these transformations the grouping probabilities p_{22} can be calculated from the intercept V_i. $F_{2(0)}$ means the initial fluorescence of PS II *i.e.* when all reaction centers are oxidized, $F_{2(M)}$ indicates the maximal fluorescence of PS II *i.e.* when all reaction centers are reduced. $F_{2(v)}$ means the difference $F_{2(M)} - F_{2(0)}$. K, the equilibrium constant for charge transfer from a reduced reaction center of PS II to internal acceptors (pheophytine, Q_A) has the value 0.145 [7, 8, 24]. k is defined as $p_{22} \cdot F_{2(v)}/F_{2(0)}$. C = control, Ce = cerulenin.

polation to the V-axis where $V_i = 1/[1 - K(1+k)]$. In this equation, k is equal to $p_{22} \cdot F_{2(v)}/F_{2(0)}$ and K signifies the equilibrium constant for charge transfer from a reduced RC II to oxidized internal acceptors (pheophytine, Q_A). As shown in [7, 8, 24] K has been calculated to have a value of 0.145 valid not only for *Petunia hybrida* but also for *Pisum sativum* and *Picea alba*. Since cerulenin does not interfere with the Q_B-site of PS II [25, 26], changes in K are not expected.

In Table III excitation energy distribution terms are presented. Quantum yields calculated for the different energy fluxes of PS II indicate a significant increase in grouping, dissipation, spillover and in the excitation rate of PS II, while the quantum yield for photochemical trapping of PS II is decreased following 7 days of treatment with 4.5 μM cerulenin. Quantum yields obtained for PS I show the same effects concerning dissipation and excitation rate of PS I, whereas the quantum yield for photochemical trapping of PS I is increased. Significant changes in energy transfer probabilities during treatment of *Petunia hybrida* with cerulenin have only been observed for photochemical trapping of PS II and grouping between different PS II-LHC-units. An increase in grouping probability p_{22} is accompanied by a decrease in trapping probability p_{2b} of PS II.

De-excitation rate constants k_{ij} are a relative measure of the conformation responsible for distinct energy fluxes of pigment-protein complexes in thylakoid membranes and related to relative distances R_{ij} between these complexes (Table III). As the de-excitation rate constants k_{ij} decrease the relative distances R_{ij} increase by the assumed FÖRSTER-relationship: $k_{ij}^{-1} = R_{ij}^6$ [27]. In *Petunia hybrida* plants treated with cerulenin, it has been found that the de-excitation rate constant k_{2b} responsible for photochemical trapping of PS II is decreased, whereas the de-excitation rate constant k_{22} responsible for grouping is increased. The opposite effects are true considering the relative distances R_{2b} and R_{22}, respectively. Assuming the total absorption flux of PS I and PS II equal to 1 ($\alpha_A + \beta_A = 1$) a shift in the absorption flux of PS II relative to PS I has been detected in favour of PS II.

Discussion

In vivo modification of the lipid matrix is one useful method to investigate the influence of acyl lipid compounds on the functions of pigment-protein complexes in thylakoid membranes. Inhibition of fatty acid desaturation with the pyridazinone BASF 13-338 (SAN 9785) as well as inhibition of fatty acid synthesis with cerulenin have been used to modify the lipid matrix of thylakoids *in vivo* [6–8, 10, 25, 26].

With the assumption that lipid packing properties bring about asymmetrical distribution of lipid classes and lipid species in membranes, changes in the desaturation of acyl compounds which might affect the lipid shape are expected to induce alterations in the organization of the lipid matrix [7, 8, 28].

In our analysis of primary photosynthetic processes by means of 77 K fluorescence spectroscopy, the variable fluorescence of PS II divided by the maximal fluorescence of PS II ($F_{2(v)}/F_{2(M)}$) may be considered a good indicator for functional changes in PS II. This term has been demonstrated to be proportional to the probability that a exciton in the antennae pigments of PS II reaches a RC II when based on a separate packed model of photosynthesis [23]. Since the fluorescence data presented in our paper are based on a grouped bipartite model [16], an analysis of the grouping probability p_{22} is necessary in order to elucidate, whether trapping or grouping or both contribute to a cerulenin-induced decrease in the ratio $F_{2(v)}/F_{2(M)}$. This ratio is proportional to the term $p_{2b}/(1-p_{22})$ for a grouped bipartite model of photosynthesis [16]. An analysis of the experimental data reveals that a decrease in the ratio $F_{2(v)}/F_{2(M)}$ is caused by a decrease in the trapping probability p_{2b} as well as by an increase in the grouping probability p_{22}.

As shown in Table III a marked increase in the excitation energy fraction β_N ($\beta_N = 1-\alpha_N$) derived from PS II to PS I energy transfer has been observed during cerulenin treatment. This effect may be caused not only by an increase in the excitation rate of PS II-LHC-units, but also by changes in the molecular organization responsible for spillover. This proposal is supported by increases in the slopes of F_1 *versus* F_2 plots but not by the relative de-excitation rate constants k_{21}/k_{2D}. Moreover, an increase in the absorption fraction of PS II β_A at the expense of the absorption fraction of PS I α_A as well as a shift in the fluorescence emission maximum of PS I (not shown) suggest cerulenin-induced changes in the structural organization of PS I. Shifts in the 77 K fluorescence emission peak of PS I towards shorter wave-lengths have also been reported for barley [10].

From the analysis of the energetic cooperativity between different PS II-LHC-units, it is most likely that cerulenin treatment brings about an increase in the grouping probability p_{22} provided that the equilibrium constant K for charge transfer from a reduced RC II to oxidized acceptors remains unchanged. This could be expected since cerulenin neither inhibits at the Q_B-site of PS II [10, 25] nor inhibits state-1 state-2 transitions [29]. A marked increase in the excitation rate of PS II as a result of an increase in the grouping probability p_{22} as well as an increase in the absorption flux of PS II has been observed.

Recently, it has been suggested that the co-operativity between different PS II-LHC-units is positively correlated to the degree of desaturation of acyl components mainly of MGDG and DGDG [7, 8, 24]. Since cerulenin is an inhibitor of *de novo* fatty acid synthesis, and since no other inhibitory effects has yet been described, it is suggested that the influence on excitation energy distribution is caused by an altered acyl composition of the lipid matrix. This is in agreement with the assumption dealing with the greening process in barley during cerulenin treatment and DCMU-insensitive oxygen evolution of cerulenin-treated *Chlorella* cells [10, 30].

It is most interesting to note that excitation energy transfer from LHC to PS II core complex seems to be also stabilized by high amounts of polyunsaturated fatty acids in MGDG when Triton X-100 treated protein particles are reconstituted in liposomes [1]. However, further investigations are required to get a more detailed insight into the correlations between structure of the lipid matrix and functions of pigment-protein complexes in thylakoid membranes.

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