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Action of the allelochemical, fischerellin A, on photosystem II

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Abstract

The cyanobacterium, *Fischerella muscicola*, produces a secondary metabolite named fischerellin A (FS) that strongly inhibits the growth of cyanobacteria and other photosynthetic organisms. The compound exhibits a unique structure and is composed of two cyclic amines and a C_{15} substituent that contains a double bond in the (*Z*) configuration and two triple bonds [L. Hagmann, F. Jüttner, Tetrahedron Lett., 37 (1996) 6539–6542]. The site of FS action is located in photosystem II (PSII). The chlorophyll fluorescence induction transient and O_2 evolution methods have been used to determine the site of action of FS in PSII. FS affects the fluorescence transients, as well as O_2 evolution by the cyanobacterium, *Anabaena P9*. The green alga, *Chlamydomonas reinhardtii*, and higher plants were also affected by FS in a concentration- and time-dependent fashion. FS acts at several sites which appear with increasing half-time of interaction in the following sequence: (1) effect on the rate constant of Q_A^- reoxidation; (2) primary photochemistry trapping; (3) inactivation of PSII reaction center; and (4) segregation of individual units from grouped units. FS does not affect the photosynthetic activity of purple bacteria, *Rhodospirillum rubrum*.

Keywords: Cyanobacterium; Fischerellin A; Photosystem II; Reaction center

1. Introduction

Cyanobacteria are photoautotrophic prokaryotes that are important plankton organisms in the sea and freshwater lakes. Benthic and terrestrial cyanobacteria form biofilms on solid surfaces which are well-known as cyanobacterial mats. The structural limitation of prokaryotic life favors the growth form of mats, and does not allow an efficient advance into the third dimension. Therefore, the availability of surfaces is an essential resource for benthic and terrestrial cyanobacterial growth. Competitors for surfaces are related cyanobacteria rather than other organisms, and it is not surprising that some cyanobacteria have developed mechanisms to combat competing

Abbreviations: ABS: absorption; Chl *a*: chlorophyll *a*; CS: cross-section of the sample; DCMU: 3-(3,4-dichlorophenyl)-1, 1-dimethylurea; D: density of RC; ET: energy flux for electron transport; F_0, F_m: initial and maximum Chl *a* fluorescence; FS: fischerellin A; J, I: intermediate steps of Chl *a* fluorescence rise between F_0 and peak (*P*); k_{ab}: rate constants for electron transport per trapped energy; k_N^-: non-photochemical de-excitation rate constant; k_P: photochemical de-excitation rate constant; LHC: light harvesting complex; MIC: minimum inhibitory concentration; PSI: photosystem I; PSII: photosystem II; Φ_{Eo}: probability that an absorbed photon will move an electron into the electron transport chain; Φ_{Po}: maximum quantum yield of primary photochemistry; Ψ_o: efficiency with which a trapped exciton can move an electron into the electron transport chain; Q_A: primary bound plastoquinone; Q_B: secondary bound plastoquinone; RC: reaction center; TR: energy flux for trapping

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cyanobacteria through chemical means by producing secondary metabolites. Several groups have been involved to understand the substance or substances responsible for inhibitory or stimulatory activity of these secondary metabolites [1–3]. It has been shown that cyanobacterin from *Scytonema hofmanni* inhibits electron transport in photosystem II (PSII) [4,5].

Recently, we have isolated from *Fischerella muscicola* a most active compound, called fischerellin A (FS). It exhibits a minimum inhibitory concentration (MIC) of up to 14 nM against other cyanobacteria and has interesting anti-fungal and herbicidal activity. The molecule, as determined by nuclear magnetic resonance spectrometry, contains no chlorine and has novel basic structural elements not found so far in natural products [6]. Since preliminary experiments have shown that this allelochemical has action sites in PSII [7], a more detailed biochemical study was undertaken to investigate the site and mode of action of this natural photosynthetic inhibitor. We have focused on the effect of FS on PSII activity by measuring the fast chlorophyll *a* (Chl *a*) fluorescence transients of cyanobacteria (*Anabaena*), green algae (*Chlamydomonas reinhardtii*) and higher plants (pea, spinach). Fast Chl *a* fluorescence induction kinetics provides information on the filling-up of the plastoquinone (PQ) pool which is affected by both the electron donor and acceptor sites of PSII [8–14]. The maximal rate of photochemical reaction, when all reaction centers (RCs) are open, can be determined very precisely by measuring the very initial slope. The effect of FS on PSII has been quantified by using the Theory of Energy Fluxes in Biomembranes [15,16]. The parameters calculated are the energy fluxes for absorption (ABS), trapping (TR) and electron transport per reaction center. The flux ratios, for example, (1) Φ_{Po}, maximum quantum yield of primary photochemistry, (2) Ψ_o, the efficiency by which a trapped exciton can move an electron into the electron transport chain after Q_A^- and (3) Φ_{Eo}, the probability that an absorbed photon will move an electron into the electron transport chain, have been calculated. Based on the same theory, relative values for the photochemical (k_P) and the non-photochemical (k_N) de-excitation rate constants have been calculated. Effect of FS on the photosynthetic activity of purple bacteria (*Rhodospirillum rubrum*) has also been tested.

The present paper demonstrates that the first site of action of FS is the inactivation of the RC of PSII, followed by changes in the architecture of the PSII antenna, which affects the energy migration properties within the photosynthetic units.

2. Materials and methods

Experiments were performed with 3–4 weeks old, fully matured pea (*Pisum sativum* L.) leaves. Pea plants were grown in the green house at 22°C/18°C (day/night) under natural sunlight as described before [10]. The green alga, *C. reinhardtii*, was grown mixotropically in Tris–acetate-phosphate medium at 20°C and illuminated for 12 h with white light. The experiments with *C. reinhardtii* were done with 3-day old cultures during the log phase of their growth [12]. The cyanobacterium, *Anabaena P9*, was grown in cyanobacterial medium [17] at 25°C, bubbled with a 0.27% (v/v) CO_2–air mixture and illuminated with a fluorescent tube. The bacterial cells of *R. rubrum* were grown anaerobically and photoheterotropically in M-medium, with succinate as carbon source as described earlier [18]. Thylakoid membranes were isolated from market spinach leaves [19]. Freshly prepared thylakoid membranes were used for the experiments. The chlorophyll concentration of cells and thylakoid membranes was determined according to Porra [20].

Chl *a* fluorescence transients were measured by a plant efficiency analyser (PEA; Hansatech, King's Lynn, Norfolk, UK). Light was provided by an array of six light-emitting diodes (peak 650 nm) focused on the sample surface to provide homogenous illumination over the exposed area of the sample (about 4 mm in diameter). Chl *a* fluorescence signals were detected using a PIN photocell after passing through a long-pass filter (50% transmission at 720 nm), as described before [10].

An intact pea leaf was directly attached to the PEA head with the help of a leaf clip. Experiments with cells or thylakoid membranes were performed in 1-cm diameter vials containing 500 μl of suspension, which in turn contains 20–25 μg of chlorophyll. The optical thickness of the sample was 5 mm. The samples were dark-adapted for 5 min and then, in darkness, FS or DCMU [3-(3,4-dichlorophenyl)-1,

1-dimethylurea] was added, followed by vortexing the sample in the dark. The samples were incubated for different times, as indicated in the legends of individual experiments, before fluorescence measurements began. Chl *a* fluorescence signals were recorded in a time span of 10 μs to 2 min, with a data acquisition rate of 10 μs for the first 2 ms and 12 bit resolution. The fluorescence signal at 50 μs has been considered as F_o, since only data points after 40 μs were reliable and free from the artifacts of the instrument electronics (control measurement with a fast digital oscilloscope).

For the O_2 evolution measurements, *Anabaena* cells were first incubated for 5 min in the dark, then further incubated with different concentrations of FS or DCMU for different times in a DW-1 Hansatech cuvette in the dark. Cells were illuminated with 250 W m^{-2} of red actinic light from a Schott lamp (Schott Electronics KL1500, with red filter RG610). The rate of O_2 evolution was measured during the first minute after illumination.

FS was extracted with methanol from lyophilized cells of *F. muscicola* as described by Hagmann and Jüttner [6] and Gross et al. [7]. The purity was $>$ 97%, as determined by HPLC and UV (ultraviolet) detection. For all the experiments, stock solution (1 mM) of FS was prepared in ethanol, which was further diluted in water.

3. Results

3.1. Effect of FS on cyanobacteria

Fast Chl *a* fluorescence transients of *Anabaena* cells, incubated either with different concentrations of FS for 10 min (Fig. 1) or with 1 μM of FS for different periods of time (curves not shown), were measured. The fluorescence transients obtained from control cells (Fig. 1, the lowest curve) show two steps, J (about 2 ms) and I (about 30 ms), in between O and P. With increasing concentrations of FS, the major changes observed were (1) an increase in the J level (V_J = relative variable fluorescence at J level); (2) a decrease or a dip in between I and P levels; (3) a decrease in the variable fluorescence due to a sharp increase in the F_0 and a smaller increase in F_m values (Fig. 2). The cells showed the same results

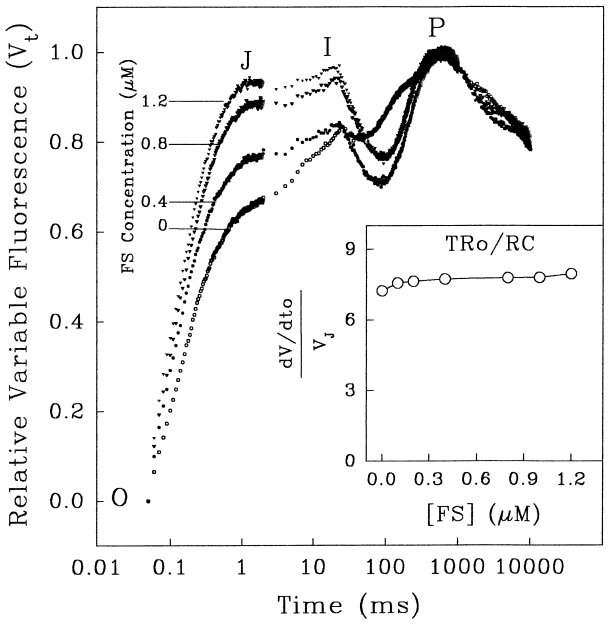

Fig. 1. Effect of FS on the relative variable fluorescence at time, t, ($V_t = (F_t - F_0)/(F_m - F_0)$) of *Anabaena P9*. Transients were obtained after incubating 0.5 ml of cell suspension (about 20 μg of chlorophyll) in different concentrations of FS for 10 min in darkness, before exposing them to 600 W m^{-2} of red actinic light. The insert shows the effect of FS on TRo/RC (= (dV/dto)/V_J) of *Anabaena* cells after 10 min of incubation.

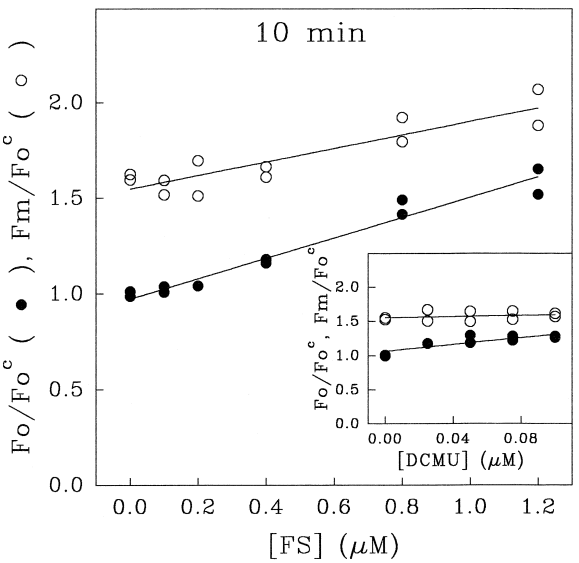

Fig. 2. Changes in the dark (F_0) and the maximum fluorescence (F_m) of *Anabaena P9* cells after incubating them with different FS concentrations for 10 min. All the values are normalized to the F_0 of control cells (F_0^c). Insert shows the same experiment, but the cells were incubated with DCMU. Note that the *x*-axes for (DCMU) and (FS) are different.

when they were incubated with DCMU. On a concentration basis, DCMU was about ten times more effective than FS (insert in Fig. 2). The effect of FS on *Anabaena* was found to be concentration- (Figs. 1 and 2) and time-dependent (Fig. 3). When the cells were incubated with 1 μM of FS, the maximum effects on F_0 or F_v were observed within 10 min of incubation (Fig. 3). After longer incubation, an increase in F_0 was observed. TRo (energy flux for trapping)/RC (detailed explanation in JIP-Test), which corresponds to the electron donation from the oxygen-evolving complex of PSII, seems to be unaffected by FS (insert in Fig. 1).

Fig. 4 shows the effect of FS and DCMU on O_2 evolution. Like the DCMU effects on fluorescence transients, DCMU inhibits O_2 evolution much faster and at lower concentrations in comparison to FS. More than 75% decrease in O_2 evolution was observed when the *Anabaena* cells were exposed to 0.1 μM of DCMU. FS also inhibits the O_2 evolution from *Anabaena* cells, but slower than DCMU. The effect of FS on O_2 evolution was time- and concentration-dependent. These results indicate that the uptake of FS is slower than that of DCMU.

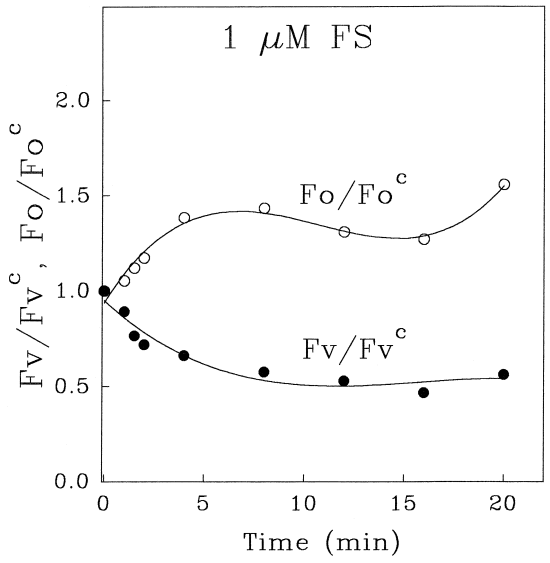

Fig. 3. Changes in the dark fluorescence (F_0) and variable fluorescence ($F_v = F_m - F_0$) of *Anabaena P9* cells after incubating them for different times in the presence of 1 μM of FS. The F_0 and F_v are normalized to the F_0 (F_0^c) and F_v (F_v^c) of the control cells.

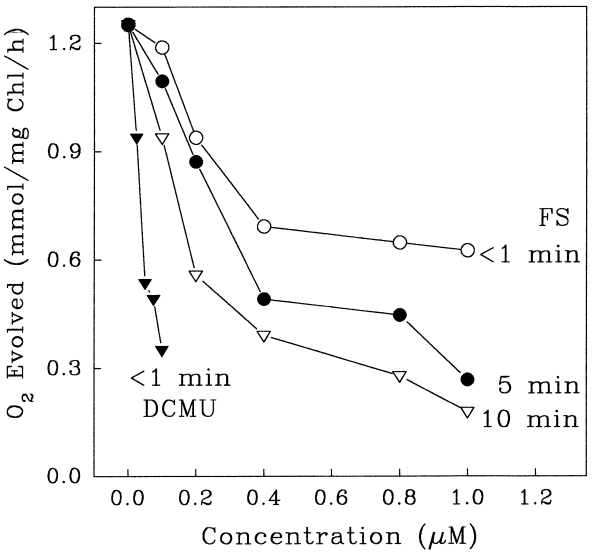

Fig. 4. Effect of FS and DCMU on the rate of O_2 evolution of *Anabaena P9*. Cells (about 20 μg of chlorophyll) were first incubated in 1 ml of cyanobacterial medium in DW-1 Hansatech cuvette with constant stirring at 25°C. After 5 min of dark adaptation, various concentrations of FS or DCMU were added. Oxygen evolution was measured from the cells immediately after DCMU addition, but in the presence of FS, from the cells either immediately after FS addition or after 5, 10 min of incubation, evolution was measured. Rate of O_2 evolution was recorded during the first minute after illumination with 80 W m^{-2} red actinic light.

3.2. Effect of FS on green algae and green leaves

To test the effect of FS on an intact pea leaf, a drop of 50 μl of 100 μM FS was added on the abaxial surface. Changes in the Chl *a* fluorescence transient were measured during the infiltration time of FS into the thylakoids of the pea leaf (Fig. 5). A rise in the J level was observed with the penetration of FS into the leaf. After 15 min of incubation, the J level was much higher, followed by a big dip in between J and I steps. In about 30 min, J became the dominant step of the transient. Usually in a control leaf, the J level is reached after 2 ms, but by increasing the FS infiltration, the time taken to reach the J level is longer (e.g., 5 ms and 10 ms after 60 and 120 min of infiltration, respectively). A further rise from the I to the P level was observed even after FS infiltration, which is in contrast to the DCMU effect, where the fluorescence transient reaches its maximum within 2 ms after DCMU infiltration [10].

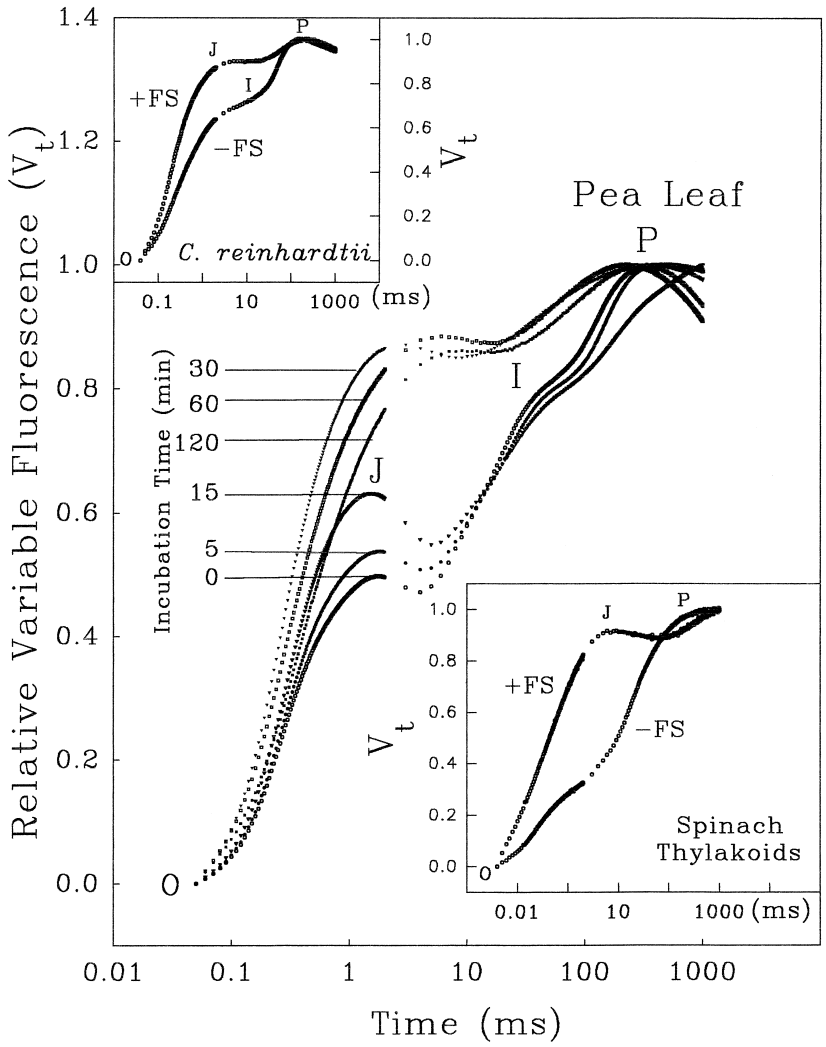

Fig. 5. Effect of duration of FS penetration on the relative variable fluorescence of an attached pea leaf. A drop of 50 μl of 100 μM FS was added on the abaxial surface of the leaf, and the fluorescence transients were measured after different times on the same area. The figures in the upper and the lower inserts show the effect of 1 μM of FS on the fluorescence transients of *C. reinhardtii* cells and spinach thylakoids, respectively. Cells and thylakoids were incubated with FS for 10 min before measurements.

A similar type of effect of FS was observed when the thylakoid membranes (lower insert in Fig. 5) or the cells of *C. reinhardtii* (upper insert in Fig. 5) were exposed to FS. The effect of FS on the thylakoid membranes and the *C. reinhardtii* cells also showed time- and concentration-dependent phenomena (data not shown), as observed with *Anabaena* cells and the pea leaf.

The slope of the fluorescence transient at the origin (between 0 to 300 μs) gives information about the organization of photosynthetic units within the thylakoid membrane. The fluorescence rise (on a linear time scale) is sigmoidal if energy transfer among PS units is happening (cooperativity, grouping); or exponential if no or very low energetic cooperativity among photosynthetic units exits (separate packages). Insignificant differences in the fluorescence rise at the origin was observed up to 15 min of incubation (data not shown). The sigmoidal shape of the curve was also retained up to 15 min of infiltration. After longer infiltration, the sigmoidal shape of the fluorescence rise changed into an expo-

nential rise, indicating decrease in the energetic cooperativity (grouping) between the antenna of photosynthetic units (data not shown).

3.3. The JIP test

A highly simplified model of the electron transport in the photosynthetic apparatus is shown in Fig. 6. ABS refers to the photon flux absorbed by the antenna pigments, Chl∗. A part of this excitation energy is dissipated, mainly as heat, less as fluorescence and exciton migration (spill-over and grouping). Another part is trapped (trapping flux, TR) to the RC. There, the excitation energy is converted into redox energy by reducing an electron acceptor Q_A to Q_A^-, which is then reoxidized to Q_A by donating the electron to Q_B. In this way, an electron transport flux, ET (electron transported beyond Q_A^- per time unit), is created.

For a dark-adapted sample, assuming that all RCs are open, the ratio of the maximum exciton trapping and the absorption, maximum quantum yield, Φ_{Po}, can be calculated with the experimental measurements, F_0 and F_m, only [21,22].

$$\Phi_{Po} = \mathrm{TRo}/\mathrm{ABS} = 1 - (F_0/F_m) = F_v/F_m, \text{ where } F_v = F_m - F_0. \quad (1)$$

The correlation between the Φ_{Po} and the architecture of the PSII antenna has been expressed in Eq. (2) according to the energy flux theory of Strasser [15,16,23,24].

$$\Phi_{Po} = \mathrm{TRo}/\mathrm{ABS} = 1 - (F_0/F_m) = F_v/F_m = T/((1-C)(1-G)), \quad (2)$$

where T is the average probability that an exciton in the core antenna reaches a closed reaction center and excites again a core antenna chlorophyll. So, for open RC, $T \cong 0$, and for closed RC, $T \cong 1$. C is the average probability of energy cycling between the LHCs (light harvesting complex) and core antenna, and G is the average probability of energy cycling between the connected antenna of PSII units (grouped PSII units). Therefore, Φ_{Po} can be expressed in terms of these de-excitation probabilities which depend entirely on the de-excitation rate constants of the pigment protein complexes [25].

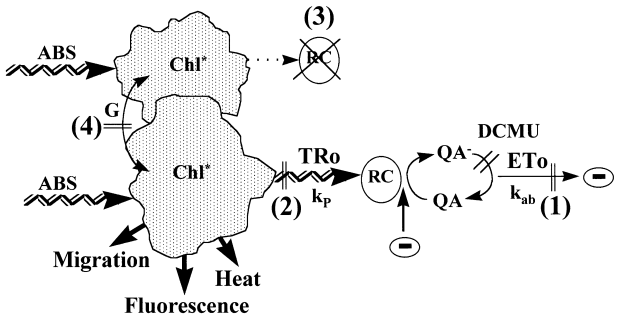

Fig. 6. A simplified model of PSII showing the energy conversion in a dark adapted sample. ABS refers to the fluxes of photons absorbed by the antenna pigments, Chl; Chl∗, excited pigment; TRo, fluxes of excitons channeled to the RC; ETo, flux of the electron transport chain which maintain the metabolic reactions; (−), the electrons; k_{ab}, rate constants for the reoxidation of Q_A^- to Q_A and an electron which participates in the electron transport; k_p, photochemical rate constant; (1), (2), (3), (4) refer to different sites of FS action on the electron transport of PSII with increasing half-time of interaction.

Using the JIP test by Strasser and Strasser [26] and Strasser et al. [11], other expressions like specific activity, phenomenological changes, antenna size, and density of the RCs per cross section, can be derived as follows [27].

TRt/RC expresses the specific rate by which an exciton trapped by an open RC allows the reduction of Q_A to Q_A^-. If we consider that at time zero, all the RCs are open, then the TRo/RC will be maximum. It has been shown that this maximum trapping flux TRo/RC is proportional to the original slope of the relative variable fluorescence dV/dto. The $1/V_J$ is used as the proportionality factor if no cooperativity is assumed [11,27].

$$\mathrm{TRo}/\mathrm{RC} = (\mathrm{d}V/\mathrm{dto})/V_J \quad (3)$$

The efficiency that a trapped exciton (TRo) can move an electron (ETo) further than Q_A^- is given by Eq. (4) [11,27].

$$\Psi_o = \mathrm{ETo}/\mathrm{TRo} = 1 - V_J \quad (4)$$

Thus, the probability that an absorbed photon (ABS) will move an electron (ETo) into the electron transport chain is the product of Φ_{Po} and Ψ_o.

$$\Phi_{Eo} = \Phi_{Po} \cdot \Psi_o = \mathrm{ETo}/\mathrm{ABS} = \mathrm{TRo}/\mathrm{ABS} \cdot \mathrm{ETo}/\mathrm{TRo} = F_v/F_m \cdot (1 - V_J) \quad (5)$$

Equation 8: ELECTRON TRANSPORT

$$\frac{ETo}{RC} = \underset{\text{eq. 4}}{\frac{ETo}{TRo}} \cdot \underset{\text{eq. 2}}{\frac{TRo}{ABS}} \cdot \underset{\text{def.}}{\frac{CS}{RC}} \cdot \underset{\text{def.}}{\frac{ABS}{CS}} = \underset{\text{eq. 3}}{\frac{TRo}{RC}} \cdot \underset{\text{eq. 4}}{\frac{ETo}{TRo}}$$

$$\Psi_o \cdot \Phi_{Po} \cdot \frac{1}{D} \cdot [Chl] = (k+1)\,\frac{dV/dto}{V_J} \cdot (1-V_J)$$

$$\underset{(1)}{k_{ab}} \cdot \underset{(2)}{k_P} \cdot \underset{(3)}{D} \cdot \underset{\text{Constant}}{} \underset{(4)}{G}$$

Fig. 7. Eq. 8 expresses the specific activity of maximum electron transport per reaction center, ETo/RC. Expressions in the second line show the influence of the rate of electron transport per RC, according to Eqs. (2)–(4) (see text). The density, *D*, of reaction center per cross-section (RC/CS), and the chlorophyll concentration per cross section (ABS/CS) are proportional to the ETo/RC. The far right part of Eq. 8 shows the ETo/RC expressed in the experimental signal, such as relative variable fluorescence at 2 ms (V_J), normalized slope of the fluorescence rise at the origin (dV/dto), and the shape of the fluorescence rise as sigmoidal ($k > 0$) or exponential ($k = 0$), which allows to measure energy transfer among the antenna of photosynthetic units (cooperativity, grouping, G). k_{ab} is the rate constant for the reoxidation of Q_A^- to Q_A and an electron which participates in the electron transport. k_P is the de-excitation rate constant that an exciton of an antenna is reaching a RC. (1), (2), (3), (4) refer to sites of action of FS such as k_{ab}, k_P, D and G with increasing half-time of interaction.

ABS/RC has been derived in Eq. (6) using Eqs. (2) and (3).

$$TRo/RC = (TRo/ABS) \cdot (ABS/RC) = \Phi_{Po} \cdot (ABS/RC) \quad (6)$$

$$\text{Thus, } ABS/RC = (TRo/RC) \cdot (1/\Phi_{Po}) \quad (7)$$

Since only red actinic light has been used, the absorption is proportional to the chlorophyll concentration. The expression ABS/RC can be taken as a calculated average amount of chlorophyll which channels excitation energy into RC. Therefore, ABS/RC can be taken as a measure for an average antenna size.

We have observed that in the given time period, there was no difference in the chlorophyll concentration per active leaf cross-section. Therefore, we consider that the ABS/CS remains constant. Considering all the parameters, the maximum rate of electron transport per reaction center (ETo/RC) can be derived from Eqs. (2)–(7), as shown in Eq. 8 (Fig. 7). The specific activity indicated as maximum rate of electron transport per RC of PSII can be derived from the experimentally obtainable fluorescence signals as reported earlier [27].

3.4. Effect of FS on the quantum yield of excitation energy trapping and the electron transport of PSII

As discussed above, the expression Φ_{Po} expresses the quantum yield of excitation energy trapping of photosystem II [21]. An insignificant difference in the Φ_{Po} was observed up to 15 min of infiltration of FS in the pea leaf. But by further increasing the infiltration time, the Φ_{Po} decreased. In contrast to Φ_{Po}, the electron transport fluxes, electron transport per en-

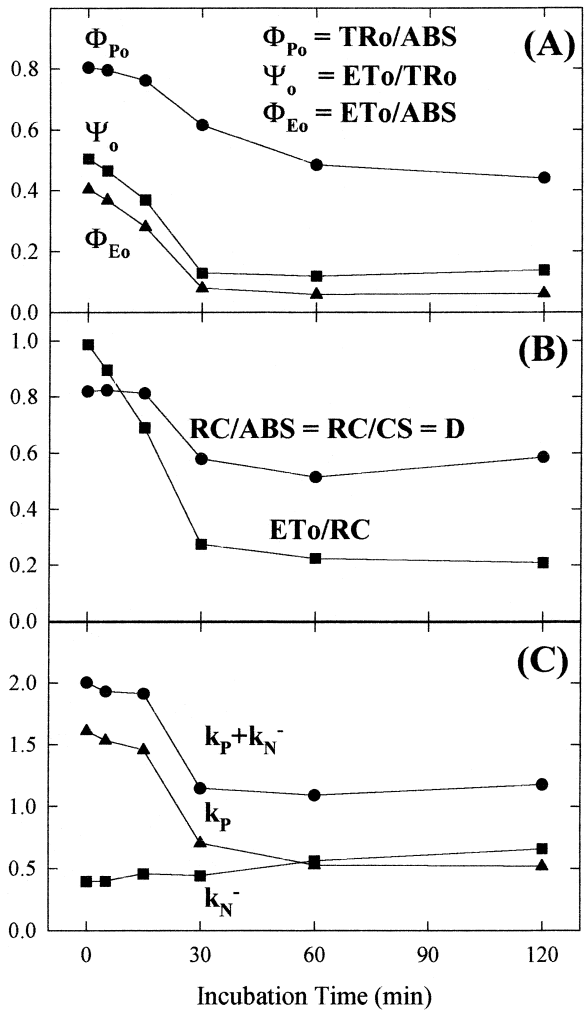

Fig. 8. Effect of FS infiltration into the pea leaf on the yields (A), some selected functional parameters (B), and the de-excitation rate constants (C). Data were obtained from Fig. 5.

ergy trapped (Ψ_o) and the quantum yield of electron transport (Φ_{Eo}) decreased even after 5 min of infiltration (Fig. 8A).

The electron transport per RC (ETo/RC) decreased sharply after FS infiltration (Fig. 8B). Insignificant differences in the RC per energy absorbed (RC/ABS) were observed up to 15 min, which were decreased by further infiltration with FS (Fig. 8B).

Fig. 8C shows changes in the k_P, de-excitation rate constant for photochemistry, and k_N^-, the sum of all non-photochemical rate constants minus the rate constant for excitation energy transfer between a group of photosynthetic units (k_G). These rate constants are calculated according to Havaux et al. [28], as follows:

$$k_N^- = I_{exc} \cdot k_F(1/F_m), \quad (9)$$

$$k_P + k_N^- = I_{exc} \cdot k_F(1/F_0), \quad (10)$$

$$k_P = I_{exc} \cdot k_F\{(1/F_0) - (1/F_m)\} \quad (11)$$

where I_{exc} and k_F correspond to the light absorbed and the rate constant for the fluorescence, respectively. These values have been considered as constant. Insignificant difference in the k_N was observed up to 15 min of infiltration of FS into the pea leaf. Even after longer incubation, a slight increase in the K_N^- was observed. A very small decrease in k_P was observed up to 15 min, but after longer infiltration, a distinct decrease in k_P was observed. Our results, showing that Φ_{Po} and RC/ABS declined approximately linearly with k_P, are consistent with the idea that inactivation of PSII RC is associated with a decrease of the trapping rate constant [29]. TRo/RC, which also corresponds to the electron donation from the oxygen-evolving complex of PSII, is not affected by the FS treatment (insert in Fig. 2 and Table 1).

3.5. Effect of fischerellin A on bacteria

The photosynthetic reaction centers of purple bacteria and that of photosystem II of higher plants, green algae and cyanobacteria are very similar. In

Table 1
Effect of FS on different parameters of the fast fluorescence rise, OJIP

Time (min)	F_0	F_m	M_o / dV/dto	V_J / $(F_J - F_0)/F_v$	TRo/ABS / Φ_{Po} / $1 - F_o/F_m$	ETo/TRo / Ψ_o / $1 - V_J$	ETo/ABS / Φ_{Eo} / $\Phi_{Po} * \Psi_o$
0	499	2532	0.972	0.496	0.803	0.504	0.404
5	518	2516	1.034	0.536	0.794	0.464	0.368
15	523	2184	1.182	0.632	0.761	0.369	0.280
30	873	2269	1.853	0.871	0.615	0.129	0.079
60	917	1774	1.657	0.881	0.483	0.119	0.058
120	849	1515	1.296	0.862	0.440	0.138	0.061

Time (min)	$k_P + k_N^-$ / $(1000/F_o)$	k_N^- / $(1000/F_m)$	k_P / $1/F_0 - 1/F_m$	TRo/RC / M_o/V_J	ETo/Rc / $(M_o/V_J)\Psi_o$	ABS/RC / $(M_o/V_J)/\Phi_{Po}$	RC/ABS / $\Phi_{Po}/(M_o/V_J)$
0	2.0040	0.3949	1.6091	1.9591	0.9868	2.4399	0.4098
5	1.9305	0.3975	1.5330	1.9298	0.8954	2.4301	0.4115
15	1.9120	0.4579	1.4542	1.8718	0.6897	2.4611	0.4063
30	1.1455	0.4407	0.7048	2.1271	0.2742	3.4573	0.2892
60	1.0905	0.5637	0.5268	1.8810	0.2240	3.8936	0.2568
120	1.1779	0.6601	0.5178	1.5042	0.2079	3.4217	0.2922

The pea leaf was attached to the PEA head with the help of a leaf clip, with the abaxial side in front of the light source. A drop of 50 μl of 100 μM FS was added between the PEA head and the leaf surface. Measurements of 1-s duration were done after different incubation times of FS. In the first line, the energy fluxes are indicated as ABS for absorption; TRo for trapping; and ETo for electron transport flux at time zero. An estimation for the de-excitation rate constant is indicated as k_P, the rate constant for photochemistry; and k_N^-, the sum of all non-photochemical rate constants are taken into account, except the one for unit-unit energy transfer. In the second line, symbols are indicated, which are defined by the experimental signals, as indicated in the third line. V_J corresponds to the relative variable fluorescence at step J and dV/dto is the slope at the origin of the relative variable fluorescence.

both types of reaction centers, the core is formed by two integral membrane protein sub-units, L and M, in bacteria and D1 and D2 in PSII. The pigments that mediate the electron transfer across the membrane are bound to these proteins. The homology between the RC from PSII and purple bacteria is considerable for the electron acceptor complex, which is formed by two quinone molecules, Q_A and Q_B, and non-heme iron in both systems. One common characteristic is the accessibility of the Q_B binding site for a variety of chemically different substances. However, some classic inhibitors of PSII, such as phenolics and ureas, are inactive in the RC of purple bacteria. Like DCMU, FS was found to be ineffective on photosynthetic electron transport of *R. rubrum*. An insignificant difference in the fluorescence transient was observed even after incubating *R. rubrum* cells for 30 min in the presence of an extremely high concentration of 10 μM of FS or DCMU (data not shown). A triazin-resistant mutant T4 from *Rhodopseudomonas viridis*, which has the tyrosin residue at position 222 on the L sub-unit substituted for phenylalanine (TyrL222Phe), is sensitive to DCMU [30]. The EPR signal of Q_B^- in T4 looked completely different from

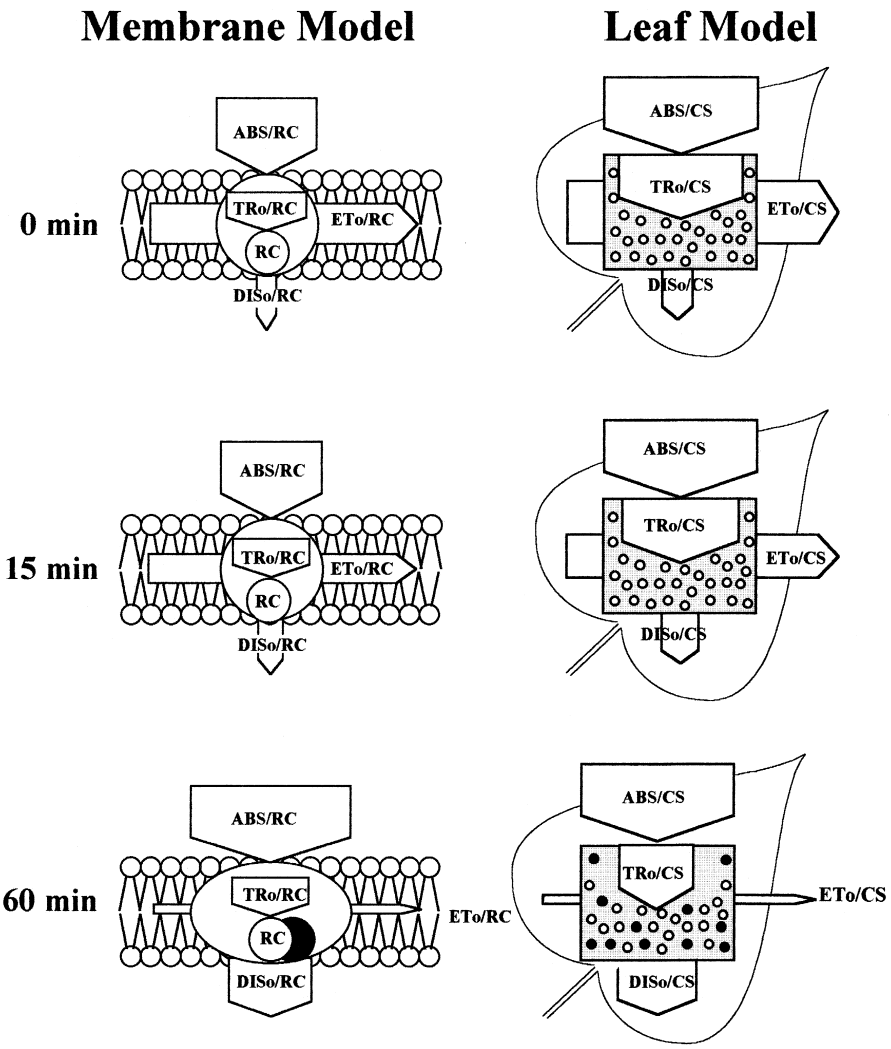

Fig. 9. Pipeline models showing relative changes in energy flows per reaction center (left panel) and per cross-sectional area (right panel) after 15 min (middle) and 60 min (lower) of FS treatment of a pea leaf. The response of each of the parameters can be seen in the relative change in the width of each arrow compared to the control sample (0 min). Active RCs are shown as open circles and inactive RCs are as filled black circles.

that of the wild type, but is very similar to that reported for PSII [31]. The size of the Q_B site in T4 seems to be just enough to allow DCMU to squeeze in Ref. [30]. Like DCMU, it is possible that FS could not bind in bacterial photosystem. Experiment using the bacterial T4 mutant of *R. viridis* or some other mutants could perhaps be helpful to find out the probable binding site of FS in the photosynthetic apparatus.

4. Discussions

Based on all results, which are summarized in Table 1, FS acts on green organisms at several sites (1, 2, 3, 4 with increasing half-time of interaction) as indicated in Figs. 6 and 7 and as follows.

(0–15 min): (a) The electron transport (ETo/RC) decreases; (b) yield for electron transport (Φ_{Eo}) decreases; (c) yield for Q_A^- reoxidation (Ψ_o) decreases; (d) O_2 evolution decreases; (e) trapping per reaction center (TRo/RC) constant; (f) yield for primary photochemistry (Φ_{Po}) constant; (g) energy distribution constants (k_P and k_N^-) are constant. Summarizing these parameters, it seems that during the first phase, FS affects the rate constant for Q_A^- reoxidation (k_{ab}).

(15 to 30 min): (a) Φ_{Po}, Φ_{Eo}, Ψ_o decreases; (b) ABS/RC increases; (c) TRo/RC constant; (d) k_P decreases. FS clearly affects the trapping of the primary photochemistry.

(15–60 min): FS basically inactivates photosynthetic RCs.

(30–120 min): The loss of the observed sigmoidicity corresponds to a decrease of cooperativity or grouping, G, which stimulates the formation of separate pack units out of grouped units. Therefore, FS directly affects the quantum yield of excitation energy trapping of the PSII according to Eq. (2).

The appearance of the action of FS on several sites of PSII, after different times of treatment, can be visualized by the energy pipeline model of photosynthetic apparatus as proposed by Strasser [32] and as demonstrated in Fig. 9. This is a dynamic model in which the value of each energy flux, changing as a function of time of FS treatment, is expressed by the appropriately adjusted width of the corresponding arrows. Two types of models have been shown; one deals with the specific energy (per RC, Membrane Model) and the another which refers to the cross-section of a leaf (per CS, Leaf Model). The fluxes of dissipated energy at time zero ($DIS_o = ABS - TR_o$) have also been indicated. In the membrane model, the antenna size, which corresponds to the ABS/RC, has been demonstrated. In the leaf model, the active RC per cross-section (RC/CS) is indicated by open circles, and the inactive ones by closed circles.

It is clear from these visual presentations (Fig. 9) that after 15 min of FS treatment, although there is a decrease in the electron transport per RC, FS does not affect the antenna size. After longer exposure, FS starts to inactivate the RC (see the closed circles in the models) which results in the associated increase in the ABS/RC, both as flux and as average antenna size. The models presented in Fig. 9 demonstrate that changes in the photochemistry do not always completely follow the quantity of active RC/CS. Several additional mechanisms are involved to regulate the photochemical rate constants after FS treatment, e.g., modified architecture of the antenna and the LHC [24].

For all oxygenic systems, even after FS treatment, the fluorescence rise from level I to level P remained, which is in contrast to the action of DCMU where the fluorescence rise levels off at 2 ms, which corresponds to step J.

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