Inhibition of State Transition and Light-Harvesting Complex II Phosphorylation-Mediated Changes in Excitation Energy Distribution in the Thylakoids of SANDOZ 9785-Treated Plants

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Z. Naturforsch. **50 c,** 77–85 (1995); received August 3/September 28, 1994

Pyridazinone, Photosystems, State Transition, LHC II Phosphorylation, Energy Redistribution

Thylakoids isolated from SAN 9785 (4-chloro-5-dimethylamino-2-phenyl-3(2H)-pyridazinone)-treated pea plants showed an inhibition of "state transition" and the light-harvesting complex II (LHC II) phosphorylation-mediated changes in the energy distribution between photosystem II (PS II) and photosystem I (PS I) as measured by a decrease in PS II and an increase in PS I fluorescence yield. Interestingly, in these thylakoids the extent of phosphorylation-induced migration of light-harvesting complex (LHC II-P) to non-appressed membrane regions was only marginally inhibited. We propose that the suppression in the ability for "state transition" by SANDOZ 9785 (SAN 9785) treatment occurs due to a lack of effective coupling of the migrated LHC II-P and PS I. Since we observed a decrease in the antenna size of PS I of the treated plants, the lack of effective coupling is attributed to this decrease in the antenna size of PS I.

Introduction

Photosynthetic system of higher plants and green algae possess some structural characteristics by which they can respond to the changes in light quality (spectral composition) (Bonaventura and Myers, 1969; Murata, 1969; Mohanty *et al.,* 1973). Light predominantly absorbed either by PS I or PS II may create an imbalance in excitation energy distribution between the photosystems which leads to a physiological adjustment towards one of the two possible states, "state I" or "state II" in order to correct for the imbalance (Chow *et al.,* 1981; Fork and Satoh, 1986; Malkin *et al.,* 1986). The state I to state II change ("state transition") in higher plants is most probably mediated by phosphorylation-induced migration of a fraction of LHC II complexes from the PS II-enriched appressed region to the non-appressed region of the thylakoids (Bennett *et al.,* 1980; Steinback *et al.,* 1982, Anderson, 1986; Telfer *et al.,* 1983). This causes an increase in the average antenna size of PS I (Bennett *et al.,* 1980; Horton and Black, 1981).

Excitation energy distribution between PS II and PS I is a function of the structure as well as the composition of the thylakoid membranes. One of the ways to study this relationship is to modulate the structure and composition of the thylakoids by various treatments. It has been shown earlier (St. John, 1976) that the ratio of saturated to unsaturated fatty acids increased in the thylakoids when wheat plants were grown in the presence of SANDOZ 9785 (4-chloro-5-dimethylamino-2-phenyl-3(2H)-pyridazinone), a PS II inhibitor of electron transport (Hilton *et al.,* 1969; Tischer and Strotmann, 1977). Later, it was also shown that the structure of the photosynthetic apparatus gets altered in terms of Chl *a/b* ratio and presumably of PS II/PS I ratio in the thylakoids

Abbreviations: LHC II light-harvesting chlorophyll *a/b*-protein complex of photosystem II; LHC I, light-harvesting chlorophyll *a/b*-protein complex of photosystem I; Tricine, N-tris(hydroxymethyl)ethylglycine; Tris, N-tris(hydroxymethyl)aminomethane; PS I, photosystem I; PS II, photosystem II; Chl *a*, chlorophyll *a*; LDS, lithium dodecyl sulphate; DCPIP, dichlorophenol indophenol; MV, methylviologen; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; HEPES, N-2-hydroxyethylpiperazine-N′-2-ethanesulphonic acid; SAN 9785, SANDOZ 9785 (4-chloro-5-dimethylamino-2-phenyl-3(2H)-pyridazinone).

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0939–5075/95/0100–0077 $ 06.00

of SAN 9785-treated plants (Bose *et al.*, 1984; Mannan and Bose, 1985; Leech *et al.*, 1985). Furthermore, it has also been shown that cation-induced reversible changes in excitation energy distribution are inhibited in SAN 9785-grown plants (Mannan and Bose, 1987; Bose *et al.*, 1992). Thus thylakoids of SAN 9785-treated plants showed an altered structure-function relationship as compared to normally grown plants. Since state change is one of the most important dynamic characteristics of the photosynthetic apparatus of plants, and is likely to be governed by the structure-function relationship of thylakoids, it was felt that studies aimed at investigating the state transition in the SAN 9785-treated plants would reveal information regarding this process in altered membrane systems. We have, therefore, investigated the status of LHC II phosphorylation-dependent changes in terms of migration of phosphorylated LHC II to non-appressed regions, and consequent excitation energy redistribution between PS II and PS I in the thylakoids isolated from treated pea plants.

Materials and Methods

Seeds of *Pisum sativum* cv. Bonneville were obtained from National Seeds Corporation, IARI Campus, New Delhi. Seeds were scarified in concentrated H_2SO_4 for 5 min and subsequently washed under running tap water for 15–20 h. These seeds were then germinated in dark in acid-washed river sand inside a plant growth chamber (Environmental shaker model 3957, Labline, U.S.A.) at 20 °C. Three- to four-day-old seedlings were transferred to hydroponic system which contained 0.5 S Hoagland solution as the liquid nutrition medium and were allowed to grow for further 7 days under 12 h light ($80\ \mu E \cdot m^{-2} \cdot s^{-1}$)/12 h dark cycle.

SAN 9785 treatment was performed by transferring the 11-day-old seedlings to 0.5 S Hoagland medium containing 125 μM SAN 9785. Plants were allowed to grow in presence of SAN 9785 for 84 h, after which leaves were harvested for experiments.

Type A chloroplasts were isolated using the procedure of Nakatani and Barber (1977), with some modification. The fresh leaves were homogenized in ice-chilled isolation medium containing 0.4 M sorbitol, 15 mM tricine (pH 7.8) and 10 mM NaCl (buffer A) using a Polytron homogenizer PT 3000 (Kinematica AG). Pulses of 20–30 s duration were used. The homogenate was filtered through four layers of miracloth and centrifuged at $2100 \times g$ for 2 min. The rest of the pellet was washed in buffer having 10 mM Tricine (pH 7.8), 10 mM NaCl and 5 mM $MgCl_2$ (buffer B) and finally resuspended in the reaction buffer containing 0.1 M sorbitol, 10 mM Tricine (pH 7.8), 10 mM NaCl and 5 mM $MgCl_2$ (buffer C) to a concentration of 2 mg Chl · ml^{-1}. Intact chloroplasts used for "state transition" measurements, were isolated according to Fish and Jagendorf (1982). Isolation buffer (buffer A) was replaced by a buffer (buffer D) containing 350 mM sorbitol, 35 mM HEPES-KOH (pH 8.3), 2 mM EDTA, 1 mM $MgCl_2$ and 1 mM DTT. Chloroplasts were resuspended in a minimal volume (0.3–0.5 ml) of the buffer D and overlayered onto a 10 ml linear 25 to 92% gradient of Percoll containing the same ingredients as the buffer D, 0.6 mM glutathione, and gradients of 0.7 to 2.7% (w/v) of polyethylene glycol 4000 and 0.25 to 0.92% of both BSA and Ficoll. The gradient tubes were centrifuged for 7 min at 9000 rpm in the HB-4 rotor, and the lower green band of intact chloroplasts were collected and diluted with 30 ml of resuspension buffer containing 375 mM sorbitol, 35 mM HEPES-KOH (pH 8.3), 2 mM EDTA, 1 mM $MgCl_2$ and 1 mM DTT. The intact chloroplasts were pelleted for 3 min at 5000 rpm in the HB-4 rotor at 4 °C. The pellet of intact plastids was resuspended in the isolation buffer at a Chl concentration of 3 mg · ml^{-1}, and stored in ice. The Chl concentration was measured according to Arnon (1949).

For state transition studies, the intact chloroplasts were suspended in buffer A at a Chl concentration of 100 μg · ml^{-1}. Chloroplasts suspension was first adapted to state by illuminating with far-red light ($150\ \mu E \cdot m^{-2} \cdot s^{-1}$), which was obtained by passing the white light through a red interference filter (711 nm; half maximum-full width, 21 nm, Baird Atomic Inc., U.S.A.) for 5 min at 25 °C. The same suspension was then irradiated for transition to state II with a weak intensity ($75\ \mu E \cdot m^{-2} \cdot s^{-1}$) blue actinic light (PS II light) under the similar conditions. Blue light was obtained using a blue interference filter (437 nm; half maximum-full width, 10.7 nm, Baird Atomic Inc., U.S.A.). An aliquot was taken from different set of samples at various time intervals and diluted to

a 1 μg Chl·ml^{-1} with buffer C having 30% glycerol. The resultant suspension was then frozen immediately to 77 K and maintained at this temperature till the necessary dark incubation and spectral measurements are over.

For phosphorylation of LHC II, and fractionation of appressed and non-appressed membrane regions of phosphorylated and non-phosphorylated thylakoids, the method as described by Larsson *et al.* (1987) was used.

Thylakoid samples were prepared for polyacrylamide gel electrophoresis according to Fish and Jagendorf (1982). Sample equivalent to 10 μg of Chl was loaded onto the gels. Electrophoresis was performed for 12 h at constant voltage (100 V) on 1 mm thick, 10–15% (w/v) polyacrylamide linear gradient resolving gel containing a 5.5 to 11.0% linear sucrose gradient. The gels were run using a vertical electrophoretic apparatus, model AE-6200, Atto Corporation, Japan. The gels were stained and destained as described by Fish and Jagendorf (1982). Immediately after the run, the gels were scanned densitometrically at a wavelength 700 nm using GS 300 densitometer from Hoefer Scientific Instruments, U.S.A.

Thylakoid membranes were resolved into its pigment-protein complexes (CP I, CP II and FP) on a non-denaturing polyacrylamide gel using the method similar to Thornber and Highkin (1974). Immediately after the run, gels were scanned densitometrically at a wavelength 632 nm using Ultrascan *TM* XL densitometer (Pharmacia, Sweden). The amount of individual pigment-protein complexes was measured by measuring the area under the peak in densitometric scan. Room temperature Chl *a* fluorescence emission yield of thylakoid membrane suspension was measured using a spectrofluorimeter (Luminescence spectrophotometer, Model L.S. 5, Perkin-Elmer, U.S.A.). Thylakoid membranes equivalent to Chl concentration of 2 μg·ml^{-1} were suspended in buffer C and dark-incubated for 5 min before measurements. Low temperature Chl *a* fluorescence emission spectra of isolated thylakoids were recorded on the same instrument using a low temperature luminescence accessory (PE part No. 5212–6000) at a temperature 77 K. Thylakoid membranes equivalent to 1 μg Chl·ml^{-1} were taken in glass capillary tubes. The capillary tubes were positioned on the free end of a high purity copper rod, which was kept immersed in liquid nitrogen. The sample was dark-incubated for 5 min before measurements. Excitation slit used was 5 nm whereas emission slit was 2.5 nm. Buffer C containing 30% glycerol was used as suspending medium for the spectral measurements. Spectra have not been corrected for the spectral sensitivity of the instrument.

PS I-catalyzed electron transport capacity of thylakoids were assayed using $DCPIPH_2$ as electron donor and MV as acceptor. The activity was monitored polarographically in terms of O_2 consumption at 25 °C using an oxygen electrode assembly (Hansatech Ltd., model DW 2). The reaction mixture contained 100 mM sorbitol, 10 mM Tricine (pH 7.8), 5 mM $MgCl_2$, 10 mM NaCl, 5 mM sodium ascorbate, 5 mM sodium azide, 0.1 mM DCPIP, 0.05 mM MV, 0.02 mM DCMU and thylakoids equivalent to 20 μg Chl·ml^{-1}. The intensity of light used for saturating the PS I activity was 1000 μE·m^{-2}·s^{-1}.

Results

A prolonged treatment of plants with sublethal concentration of SAN 9785 in the present study resulted in alterations in the pigment composition of plants. The plants treated with SAN 9785

Table I. Alterations in the pigment-protein complexes and PS I-catalyzed electron transport rates in the thylakoids of SAN 9785-treated plants. Thylakoids were isolated and resolved into various pigment-protein complexes on a non-denaturing acrylamide gel. Rate of PS I electron transport was measured in the saturating intensities of light. Results are representative of six independent experiments.

	mg Chl·g^{-1} fresh weight	Chl *a/b*	CP I [%]	CP II [%]	PS I activity (DCPIP → MV) μmol O_2 mg^{-1}·Chl·h^{-1}
Control	2.7 ± 0.38	2.8 ± 0.12	21.0 ± 2.0	42.0 ± 1.8	532 ± 29
Treated	2.1 ± 0.43	2.5 ± 0.11	15.0 ± 2.6	52.0 ± 2.7	518 ± 15

showed 20% decrease in the chlorophyll content as compared to control plants (Table I). Treated plants also showed a 11% decrease in the ratio of Chl *a* to Chl *b* (Table I). To further analyze how such changes reflect in the composition of thylakoids of treated plants in terms of pigment-protein complexes, we determined the amount of individual pigment-protein complexes by resolving them on a non-denaturing polyacrylamide gel. SAN 9785 treatment effected a decrease in CP I content leading to a 60% increase in CP II: CP I, relative to the thylakoids of control plants (Table I). A decrease in Chl *a/b* and an increase in CP II/CP I ratio indicates a structural change in thylakoids of plants and an increase in the antenna size of PS II. These results are in agreement with the results of previous studies conducted with SAN 9785-treated plants (Bose *et al.*, 1984; Mannan and Bose, 1985; Leech *et al.*, 1985; Mannan and Bose, 1987; Bose *et al.*, 1992).

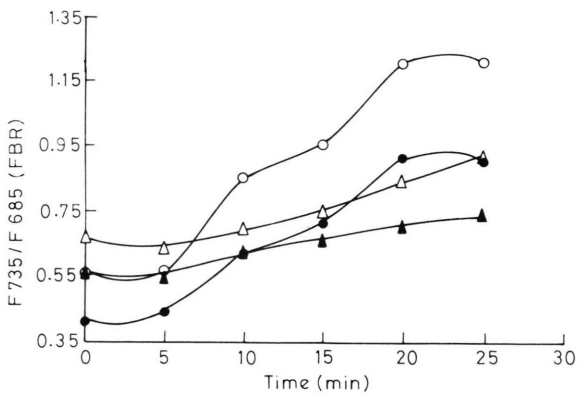

Fig. 1. Light-induced transition of state I to state II in the intact chloroplasts of control and SAN 9785-treated plants as evaluated by ratio F735/F685. Freshly harvested chloroplasts were suspended in buffer A at 100 μg Chl·ml^{-1}. The chloroplasts suspension was first irradiated with weak intensity (150 μE·m^{-2}·s^{-1}) red light for 5 min at 25 °C to attain state I (time point 0.0) and then in weak intensity (75 μE·m^{-2}·s^{-1}) actinic blue light to attain state II. At specific intervals of time, an aliquot from incubation mixture was taken and diluted to Chl concentration equivalent to 1 μg·ml^{-1} in the incubation buffer containing 30% glycerol and frozen immediately to 77 K. Fluorescence emission spectra at 77 K was then determined for the samples frozen at various intervals of time. Fluorescence band ratio, FBR (F735/F685) was measured from spectra as described in text as a parameter to assess the status of state II transition. Control (○—○) exci. 440 nm, (●—●) exci. 480 nm; SAN 9785-treated (△—△) exci. 400 nm, (▲—▲) exci. 480 nm.

The time course of "state I" to "state II" transition measured in terms of an increase in PS I fluorescence yield (F735) relative to PS II yield (F685) in the isolated intact chloroplasts of control and treated plants is shown in Fig. 1. The ratio of PS I emission yield at 77 K to that of PS II, represented as "fluorescence band ratio" (FBR) of F735/F685, has been used as an indicator of excitation energy distribution between PS I and PS II (Murata, 1969; Fork and Satoh, 1986). Chloroplasts from treated plants showed a higher ratio of F735/F685 than the control plants in their state I (see FBR at time point zero). This indicated that under identical conditions of excitation, a greater part of excitation energy was distributed in favour of PS I in the thylakoids of treated plants than in control plants. Chloroplasts were slowly converted to state II by illuminating them with blue actinic light of weak intensity. The transition to state II in the chloroplasts of treated plants proceeded slower than in the chloroplasts of control plants (Fig. 1). Moreover, chloroplasts of treated plants did not attain a value of F735/F685 as high as that attained by the chloroplasts of control plants when the process of transition to state II was almost complete (at ≈25 min after actinic illumination). This indicated that the transition to state II is inhibited in SAN 9785-treated plants.

The response of thylakoids to LHC II phosphorylation was monitored by measuring the yield of Chl *a* fluorescence in isolated thylakoids of control and treated plants. The emission intensity at 685 nm (F685) at 25 °C monitored from the phosphorylated thylakoids of control plants relative to that of the non-phosphorylated thylakoids decreased by 23% with 440 nm and 27% with 480 nm excitation (Table II). The decrease in corresponding values of treated plants was only 3 and 11%, respectively (Table II). Thus, the phosphorylation-dependent decrease of Chl *a* fluorescence at room temperature was markedly inhibited in the thylakoid membranes of treated plants.

Earlier, it has been shown that the phosphorylation-mediated lowering of PS II fluorescence is accompanied by an increase in PS I fluorescence indicating an increased energy distribution in favour of PS I (Fork and Satoh, 1986; Horton and Black, 1981). This redistribution of excitation energy has been correlated to the phosphorylation of LHC II in the thylakoids (Fork and Satoh, 1986;

Table II. Phosphorylation-mediated change in the relative emission of Chl *a* fluorescence in the thylakoids isolated from control plants or plants treated with SAN 9785. Fluorescence measurements were done at 25 °C. Excitation wavelength of 440 nm (λ exci. 440) or 480 nm (λ exci. 480) was used to detect fluorescence emission at 685 nm (F685). The values shown in the table are representative of six independent experiments.

Thylakoid preparation	F685 λ exci. 440 (rel. units) Control	Treated	F685 λ exci. 480 (rel. units) Control	Treated
Non-phosphorylated	75	80	66	74
Phosphorylated	58	78	48	66
% decrease upon phosphorylation	23	3	27	11

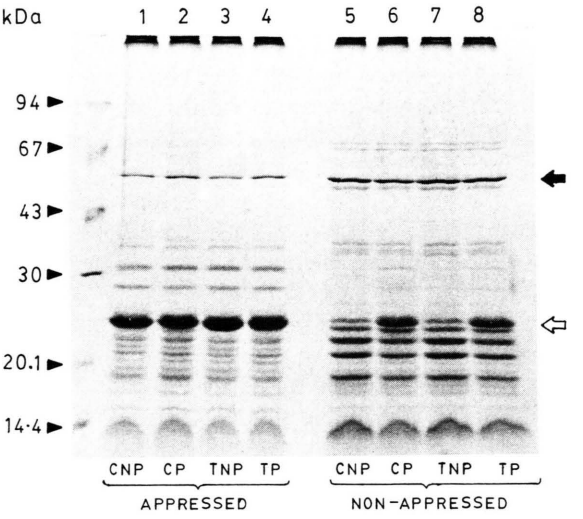

Fig. 2. Phosphorylation-dependent lateral migration of LHC II from appressed lamellae to non-appressed ones. After phosphorylation, thylakoids were fractionated by addition of digitonin and appressed and non-appressed membranes were isolated by differential centrifugation. Polypeptides of appressed and non-appressed membrane fractions were resolved on a 10–15% gradient SDS-PAGE. Open (⇧) and closed arrow (⬆) indicate LHC II and PS I RC, respectively. Lane 1: control non-phosphorylated (CNP); lane 2: control phosphorylated (CP); lane 3: treated non-phosphorylated (TNP); lane 4: treated phosphorylated (TP); lanes 5, 6, 7, 8 show similar preparations from non-appressed membranes.

Telfer *et al.*, 1983). Using various external fluorescent standards to take care of the problems of reabsorption, it has been established that this increase in F735/F685 ratio arises due to a decrease in F685 and a concomitant increase in F735 (Krause and Behrend, 1983; Saito *et al.*, 1983). Therefore, the ratio F735/F685 was used as a parameter to estimate the phosphorylation-dependent increase in PS I fluorescence yield. As shown in Table III the extent of increase in the ratio F735/F685 in the phosphorylated thylakoids of control plants relative to non-phosphorylated thylakoids was of 200%. The corresponding increase in the ratio F735/F685 was restricted to only 60% in case of treated plants.

The decrease in the fluorescence yield of PS II in phosphorylated membranes is believed to be linked to the lateral diffusion of the LHC II from appressed membranes to non-appressed zones (Bennett *et al.*, 1980; Steinback *et al.*, 1982; Anderson, 1986). To probe into the magnitude of the phosphorylation-dependent migration of LHC II, we monitored the increase of LHC II in the non-

Table III. Phosphorylation-dependent changes in the ratio of Chl *a* fluorescence emission characteristics and lateral rearrangements of thylakoid membranes. Fluorescence emission was monitored at 77 K with 480 nm excitation of thylakoids. LHC II : PS I RC ratio in non-appressed subfractions was taken as an indicator of the lateral rearrangement of thylakoids. The amount of LHC II and PS I RC was determined as described in Fig. 3.

Thylakoid preparation	F735 : F685 Control	Treated	LHC II : PS I RC Control	Treated
(Non-phosphorylated)	0.30 ± 0.08	0.47 ± 0.06	0.59	0.57
(Phosphorylated)	0.90 ± 0.12	0.075 ± 0.07	1.56	1.40
% increase upon phosphorylation	200	60	164	146

appressed region by quantifying LHC II polypeptides, directly. Fig. 2 and 3 show that amount of one of the apoproteins of LHC II increased significantly in the non-appressed region of thylakoids following phosphorylation. Since PS I is known to exist preferentially in the non-appressed regions (Andersson and Haehnel, 1982; Anderson and Malkin, 1982; Vallon *et al.*, 1985) and its amount is not affected by phosphorylation, we measured phosphorylation-induced increase in the amount of LHC II polypeptide in the non-appressed membrane region relative to PS I reaction centre protein (PS I RC) *i.e.* in terms of LHC II/PS I RC ratio. The data in Table III demonstrates that the ratio LHC II/PS I RC in the non-appressed membrane region increased by 164% in phosphorylated thylakoids relative to non-phosphorylated thylakoids in case of control plants. The corresponding increase in the thylakoids of treated plants was only marginally inhibited (Table III).

Even after appreciable extent of migration of phosphorylated LHC II to the non-appressed region (Fig. 2, 3; Table III), which brings it to the vicinity of PS I the increase in the yield of Chl *a* fluorescence emission at 735 nm (F 735) was suppressed in the thylakoids of treated plants (Table III). The phosphorylated LHC II in the thylakoids of treated plants, thus, does not seem to associate itself functionally to PS I as it does in the case of thylakoids of control plants. It is difficult to explain the reason for this because as such the factors determining the interaction of phosphorylated LHC II with PS I are not well understood (Allen, 1992). An inhibition of phosphorylation-dependent enhancement of PS I fluorescence yield has been correlated earlier (Bredenkamp and Baker, 1990) to the decrease in the antenna (LHC I) associated with PS I. Hence, our results prompted an investigation of change in the size of PS I antenna in SAN 9785-treated plants. Thylakoids from the treated plants contained lower amount of CP I in comparison to control plants (Table I). CP I as resolved on a native gel system used in this study consists of PS I reaction centre (P_{700}) and its antenna (LHC I) (Thornber and Highkin, 1974). A decrease in total amount of CP I in the thylakoids of treated plants may arise either due to a decrease in P_{700} or LHC I, or both. A possible decrease in P_{700} on Chl basis in the thylakoids of treated plants in comparison to that present in thylakoids of control plants is expected to result in a decrease in the rate of PS I-mediated

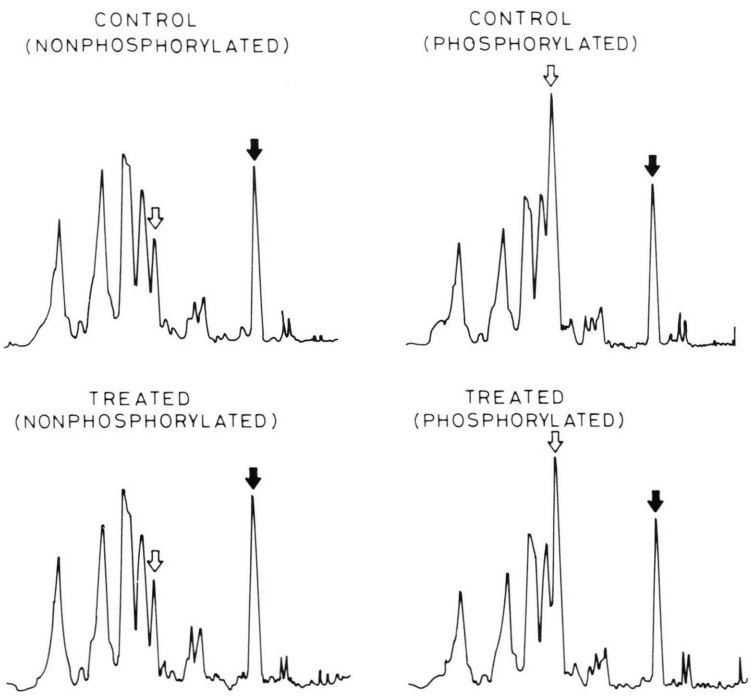

Fig. 3. The non-appressed membrane fractions of phosphorylated and non-phosphorylated thylakoid preparations showing the phosphorylation-dependent migration of LHC II. The portion of gel from Fig. 2 showing non-appressed membrane regions was densitometrically scanned using a densitometer (Hoefer Scientific Corporation, U.S.A.) at 700 nm. LHC II apoprotein (▽) which showed a significant increase in the non-appressed lamellae of phosphorylated samples, was quantitated by its peak area in the densitometric scan. For comparative purpose its amount was expressed in relation to PS I reaction centre polypeptide (PS I RC) (▼) which was also quantitated, similarly. The ratio LHC II/PS I RC was taken as a parameter to monitor the phosphorylation-induced migration of LHC II to non-appressed thylakoids (Table III).

electron transport at saturating intensities of light. However, on equal Chl basis, the rate of PS I-mediated electron transport under saturating intensities of light was observed to be the same in thylakoid membranes of control and treated plants (Table I). This suggests that decrease in CP I in treated plants specifically comes from LHC I and not in PS I reaction centres (P_{700}). LHC I is suggested to have a role in the functional coupling of phosphorylated LHC II to PS I RC (Bredenkamp and Baker, 1990). A decrease in the LHC I content in the thylakoids of treated plants may, therefore, affect the phosphorylation-dependent coupling of LHC II to PS I and, thereby, the associated excitation energy distribution in these membranes.

Discussion

Phosphorylation of LHC II has been proposed to be the mechanism underlying the "state I" to "state II" transition in higher plants and algae (Bennett *et al.*, 1980; Steinback *et al.*, 1982; Anderson, 1986). We investigated the ability of thylakoids of SAN 9785-treated plants to undergo light-induced state transition and LHC II phosphorylation. The state I to state II transition was inhibited in the thylakoids of treated plants (Fig. 1). The thylakoids of treated plants showed a suppression in the extent of excitation energy distribution between PS II and PS I (Table II and Table III) as well as a slower kinetics. The state I to state II transition is known to be brought about mainly by a decrease in PS II absorption cross section and subsequent increase in PS I excitation and is comparable to protein phosphorylation in presence of $MgCl_2$ carried out in isolated thylakoids (Allen *et al.*, 1981; Telfer *et al.*, 1983). Such a correlation of LHC II phosphorylation and state transition was, thus, also found to be true in the thylakoids of SAN 9785-treated plants.

The decrease in PS II fluorescence yield and a corresponding increase in PS I fluorescence in phosphorylated thylakoid membranes is associated with the lateral diffusion of LHC II from appressed to non-appressed membranes and this is expected to be dependent on the structural order of membrane lipid matrix (Barber, 1983). Graf *et al.* (1984) found a suppression in the ability of *Petunia hybrida* to undergo state transition after SAN 9785 treatment. The authors assumed that this suppression of state I to state II was brought about as a result of SAN 9875-induced changes in the composition of fatty acids, leading from an unsaturated (higher linoleic content) to a more saturated (higher linolenic acid content) state of the thylakoid membrane matrix. Graf *et al.* (1984) concluded that SAN 9785 inhibited the dynamic changes within and between the photosynthetic units during state I to state II transition by altering the matrix structure of the membrane. However, the studies done by Murphy *et al.* (1985) showed a variation in the susceptibility of different plant species to SAN 9785-induced fatty acid changes. Out of six angiospermic species that they examined, *P. sativum* exhibited least change in the fatty acid composition in response to SAN 9785 treatment. However, those species which showed resistance to such type of fatty acid changes in response to SAN 9785, did exhibit other typical changes which this herbicide induces in their photosynthetic behavior. On the basis of these observations these authors suggest that SAN 9785-induced changes in fatty acid composition and photosynthetic functions to be independent of each other. So, a correlation of photosynthetic changes and alteration of thylakoid membrane matrix to explain altered energy distribution in treated plants as suggested by Graf *et al.* (1984) does not hold at least for *P. sativum*. Our observations of phosphorylation-induced migration of LHC II to non-appressed membranes being almost similar in control and treated thylakoids (Fig. 2, 3 and Table III) support the view that in *P. sativum* these changes can not be explained on the basis of an suppressed movement of phosphorylated LHC II in a rigid membrane matrix as explained by Graf *et al.* (1984). Our results indicate that a decrease in the LHC I content of the thylakoids of treated plants affects the phosphorylation-dependent coupling of LHC II to PS I and thereby the associated excitation energy distribution in these membranes. In a study done by Bredenkamp and Baker (1990), a similar inhibition of phosphorylation-dependent enhancement of PS I fluorescence in developing base thylakoids as compared to mature tip thylakoids of wheat seedlings was also seen to be accompanied by a decrease in the LHC I. Thylakoids of treated plants showed an inhibition of phosphory-

lation-dependent decrease in PS II fluorescence (Table II). This seems inconsistent with the observations that thylakoids of treated plants did show phosphorylation-dependent migration of LHC II to non-appressed membrane region (Table III and Fig. 2, 3). The phosphorylation-dependent migration of phosphorylated LHC II requires uncoupling of a pool of LHC II from a fraction of PS II units and should, therefore, result in a decrease in the absorptive cross section of PS II (Bennett *et al.,* 1980; Horton and Black, 1981). Such a decrease in the absorptive cross section of PS II following phosphorylation in the thylakoids of treated plants, may be expected to manifest as a decrease in the yield of fluorescence emission at room temperature. The reason for this discrepancy can, however, be sought on the basis of an altered structure-function relationship of thylakoids in SAN 9785-treated plants resulting in an increase in CP II content in the thylakoids of treated plants (Table I). CP II resolved under the conditions of experiments used in this study is mainly comprised of LHC II. It seems, even after uncoupling of a fraction of LHC II in response to phosphorylation in the thylakoids of treated plants a significant pool of LHC II possibly remain bound to PS II, and consequently, the absorptive cross section of PS II in thylakoids of treated plants does not become limiting in the capture of excitation energy. In summary, the results of this study show that short-term changes (state transition and LHC II phosphorylation-mediated changes in excitation energy distribution between photosystems) were inhibited significantly in SAN 9785-treated plants. Such inhibition in the capability of thylakoids of treated plants to undergo short-term adaptations does not seem to occur because of an inhibited migration of pigment-protein complexes in a supposedly rigid membrane matrix in treated plants; instead they can be explained to occur as a result of an altered structure-function-relationship of thylakoids in treated plants.

Acknowledgements

We acknowledge the S. R. F. offered to M. K. J. by the CSIR, India. P. M. thanks DST B6.1/88 for financial support. Thanks are also due to Dr. Devaki Bhaya for her support and helpful discussions and to Dr. Madhulika Srivastava for the critical reading of the manuscript. SAN 9785 was a kind gift from SANDOZ Crop Protection, Des Plains, Ill., U.S.A.

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