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The effects of ultraviolet-B radiation on the CF_0F_1-ATPase

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Abstract

The effects on the amount and activity of membrane-bound CF_0F_1-ATPase and isolated CF_1-ATPase after exposure of pea plants to supplementary UV-B have been studied. On a chlorophyll basis, the ATPase activity of thylakoids decreased by 25%, whereas the amount of the CF_1-ATPase protein in the membranes declined by 60% after 4 days of UV-B exposure. Thus, the activity of the remaining functional enzyme molecules almost doubled over the experimental period. An activation was also found when the ATPase activity of isolated CF_1 was measured. In addition, the abundance of mRNA transcripts for some of the CF_1-ATPase subunits and other photosynthetic components was studied. Even after one day of supplementary UV-B, no mRNA transcripts for the nuclear-encoded γ subunit and chlorophyll *a/b*-binding protein of the light-harvesting antenna complex could be found. The amounts of mRNA for the plastid-encoded β and ϵ CF_1-ATPase subunits and the cytochrome *b* and subunit IV of the cytochrome *b/f* complex were also substantially lowered but were still present after 4 days of UV-B treatment.

Key words: ATPase, CF_0F_1-; Cytochrome *b/f* complex; Ultraviolet-B; Gene expression; (*P. sativum*)

1. Introduction

The study of ultraviolet-B radiation (UV-B) effects on plant function has been prompted by the anticipated increased influx of UV-B to the earth due to depletion of the stratospheric ozone layer. Although the impact of UV-B on physiological parameters and morphological features has been extensively studied [1], our knowledge about effects of UV-B at the biochemical and molecular level in plants is very limited [2]. This is true also for photosynthesis and the components participating in this process.

The effects of UV-B on chloroplast components are of major importance. Inhibition of photosynthesis results from the reduced efficiency of some important protein complexes, e.g., Photosystem II, the CF_0F_1-ATPase (ATP synthase) and ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) [3]. In contrast, the activities of Photosystem I and the cytochrome *b/f* complex are much less affected. It has been shown [4] that the decrease in the amounts of Rubisco protein ($\approx 35\%$) after 3 days of UV-B exposure was much smaller than the decrease of the corresponding activity ($\approx 65\%$). This suggests that a large proportion of the Rubisco proteins were inactivated or that all were partially activated. This is supported by the in vivo activation of active Rubisco, which was increased by approx. 35%.

In recent studies on pea, *Pisum sativum*, we have shown that accumulation of mRNA transcripts for some of the chloroplast proteins involved in photosynthesis are severely affected by UV-B. This is true for both chloroplast and nuclear-encoded proteins [4,5]. The genes studied were *psb A* (chloroplast-encoded), which encodes the D1 reaction centre protein of Photosystem II, the *cab* gene (nuclear-encoded) for the chlorophyll *a/b* protein of the light-harvesting antenna complex II

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Abbreviations: CF_0F_1-ATPase, ATP synthase of the thylakoid membrane; CF_1-ATPase, the catalytic part of the ATP synthase; Rubisco, ribulose-1,5-bisphosphate carboxylase/oxygenase; UV-B, ultraviolet radiation between 280 and 320 nm.

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[5] and the *rbc S* (nuclear-encoded) and *rbc L* (chloroplast-encoded) genes [4], which encode the small and large subunits of Rubisco, respectively. That the decrease in abundance of mRNA for photosynthetic genes is not entirely a result of damage to the transcription mechanism of DNA is shown by the concomitant increase in expression of defence genes: the *chs* genes, which encode the different isoforms of chalcone synthase (the first enzyme dedicated to the biosynthesis of flavonoids [6,7]) and the glutathione reductase gene [7]. Flavonoids are UV-screening pigments that are produced and accumulated as a result of exposure to supplementary UV-B [8–10], and glutathione reductase is an enzyme involved in detoxification of potentially damaging oxidative chemical species [11]. The abundance of mRNA transcripts for another defence gene, encoding the radical scavenging chloroplast-localized superoxide dismutase, was, however, substantially decreased by UV-B [11].

The decrease in the amounts of mRNA transcripts for photosynthetic genes is likely to be an important factor in reducing the efficiency of the photosynthetic apparatus, particularly since the function of some polypeptides is dependent upon a rapid turnover (e.g., the D1 polypeptide of Photosystem II; see Ref. [12]).

It is the objective of the present study to extend our knowledge on the cellular responses of plants to UV-B radiation. The approach has been to study the abundance and activity of the ATP synthase complex and the expression of some of the genes that encode subunits of this enzyme

2. Materials and methods

Growth conditions

Pisum sativum L. (cv. Greenfeast) seedlings were grown in a growth chamber with a 12 h light, 12 h dark cycle (20°C). Incident irradiation was provided by white fluorescent tubes for 17 days. Half of the plants were then transferred into another chamber where the visible light was supplied by the similar fluorescent tubes, and supplementary UV-B radiation was provided by two UV-tubes (Philips TL 40 W/12 UV; 45 mW m^{-2} nm^{-1} at 297 nm and 70 mW m^{-2} nm^{-1} at 313 nm) during the 12 h light period. The plants were grown for another 5 days, during which unshaded leaves (both UV-B-treated and controls) were harvested at the beginning of each light period.

Chloroplast preparation

Chloroplasts were prepared by blending pea leaves in 70 ml of either 50 mM Mes-NaOH (pH 6.5); 5 mM $MgCl_2$; 10 mM NaCl; 0.4 M sorbitol; 0.2% BSA (ATPase measurements, electrophoresis) or 0.3 M sucrose; 10 mM $Na_4P_2O_7$, pH 7.4 (isolation of CF_1) in a mixer for 8 s. The homogenate was filtered through three layers of Miracloth (Calbiochem) and spun for 3 min at 3000 rpm in a Sorvall SS-34 rotor. The supernatant was discarded and remaining drops were immediately removed with a pasteur pipette. The pellet was solubilized in a small volume of the same buffer and the solution was transferred to a new tube which was filled up with the same buffer. The solution was re-spun for 5 min at 5000 rpm in the same rotor. The pellet was solubilized as above and kept on ice if the thylakoids were to be used immediately; otherwise they were frozen and kept in liquid nitrogen until use. The chlorophyll content was determined by the addition of 10 μl of chloroplast preparation to 5 ml of 80% acetone buffered with 25 mM Hepes-KOH (pH 7.5). The simultaneous equations of Porra et al. [13] were used to calculate chlorophyll concentration.

Immunological quantification of the ATP synthase

The amount of ATP synthase complexes was estimated by using rabbit antibodies against spinach CF_1. The cross-reactivity towards pea thylakoids was examined by Western-blotting. The thylakoid protein complexes were first separated with SDS-PAGE (12.5% acrylamide; Ref. [14]) by using a Midget PAGE unit (Pharmacia LKB Biotechnology). The protein was then transferred to nitrocellulose membranes in a semi-dry electroblotter (Pharmacia LKB Biotechnology). A continuous buffer system was used: 39 mM glycine/48 mM Tris/0.038% SDS (w/v)/20% methanol (w/v). The non-specific binding sites of the Western blots were blocked with 5% (w/v) fat-free dry milk powder in TBS-T (10 mM Tris-HCl (pH 8.0)/150 mM NaCl/0.05% Tween 20). Then, the blots were incubated with the rabbit anti-spinach-CF_1 antibodies (dilution 1:1000 in TBS-T), followed by goat anti-rabbit-IgG antibodies conjugated to alkaline phosphatase (Promega Protoblot; 1:7500 dilution in TBS-T), and colour was developed according to the manufacturers instructions. This method visualizes the CF_1 protein as blue bands. Quantitation of pea CF_1 was then accomplished by scanning the Western blots in a computer-interfaced LKB Ultroscan XL scanning laser densitometer by using the Pharmacia Gelscan XL software (Pharmacia LKB Biotechnology).

Isolation of the CF_1-ATPase

The thylakoids were washed (4°C) four times with 10 mM $Na_4P_2O_7$ (pH 7.4), to remove all Rubisco and other stromal proteins [15,16]. The membranes were then resuspended in 0.3 M sucrose/2 mM Tricine-NaOH (pH 7.8), and the solution was centrifuged at $35000 \times g$ for 10 min (20°C). The supernatant, which contains the CF_1, was concentrated in an Amicon cell YM-30 and either immediately trypsin-activated (see

below) or precipitated with ammonium sulfate and stored at 4°C for later use.

The purity of the isolated CF_1 was determined by spectrofluorimetry [17,18]. The excitation wavelength was 280 nm. The ratio of the emission intensity at 305 and 350 nm was taken as a measure of purity. This ratio in pure homogeneous preparations should exceed 1.85 [17]. We routinely obtained a ratio of 2.1.

ATPase activity measurements

As previously described [19,20], the Mg^{2+}-dependent ATP-hydrolytic activity of thylakoids was assayed colorimetrically in the presence of octyl glucoside as an activator. The ATPase activity was taken as a measure of the functionality of the ATP synthase complex in order to avoid effects on the rate of ATP synthesis due to increased ion permeability of the thylakoid membrane after exposure to supplementary UV-B [21]. Full uncoupling of the thylakoids was obtained due to the inclusion of octyl glucoside in the medium.

The ATPase activity of trypsin-activated [17] isolated CF_1-ATPase was performed by a modification of the method of Rathbun and Betlach [22,23], $MgCl_2$ (1 mM) being the source of divalent cation, with 30 mM octyl glucoside present and the ATP concentration being 1 mM.

RNA isolation

RNA was purified by a modification of the methods described in Ref. [24]. Samples (circa 2.0 g fresh weight of pea leaves) were homogenized in $N_{2(liq.)}$ by using a mortar and pestle. The resulting powder was transferred to a test tube containing a 1:1 homogenized mixture of an aqueous and a phenol/chloroform phase. The aqueous buffer contained 50 mM Tris-HCl (pH 8.0)/4% (w/v) *p*-aminosalicylate (sodium salt)/1% (w/v) triisopropylnaphthalene sulfonate (sodium salt)/4% (v/v) mercaptoethanol. The phenol/chloroform phase consisted of equal volumes of phenol (containing 0.1% (w/v) hydroxyquinoline) and chloroform. The mixture containing the pulverized cells was blended by using an Ultra-Turrax for 2 min. The homogenate was partitioned by centrifugation at 7000 rpm for 20 min in a Sorvall SS-34 rotor. The phenol phase was re-extracted with buffer and the total nucleic acids within the combined aqueous phases were precipitated by the addition of 0.1 vols. of 3.0 M sodium acetate (pH 5.6) and 2.2 vols. of ethanol. After 2 h at −20°C, the nucleic acids were centrifuged at 11000 rpm for 30 min in a Sorvall GSA rotor. The pellet was resuspended in 500 μl of H_2O and LiCl was added to a final concentration of 2.7 M. The RNA was selectively precipitated at 4°C overnight and the tubes centrifuged in a microfuge for 10 min to collect the pellet. The RNA was washed sequentially in 3.0 M sodium acetate (pH 5.6) and 70% ethanol before it was dried and resuspended in water. The RNA absorbance was routinely checked at 320, 280, 260 and 230 nm and quantified by the absorbance at 260 nm (1 *A* corresponds to 40 μg RNA ml^{-1}) before storage at −70°C.

Electrophoresis, Northern blotting, slot-blotting and hybridization

RNA was separated in 1.2% agarose gels containing 6% (v/v) formaldehyde and transferred to Hybond-N membranes (Amersham International) by pressure blotting (Posiblot, Stratagene). Following transfer, the RNA was crosslinked to the nylon by UV irradiation in a Stratagene Stratalinker (dose 0.6 J m^{-2}). The filters were prehybridized for 2 h at 65°C and then hybridized overnight at the same temperature with cDNA probes labelled with [α-^{32}P]dCTP. Radiolabelling was carried out by using a Promega random primer kit according to the manufacturers instructions. Washing of nylon filters was briefly carried out at room temperature in 0.3 × SSC; 0.1% (w/v) SDS followed by 2 × 15 min at 65°C in the same solution, and if necessary, for 2 × 15 min at 65°C in 0.1 × SSC; 0.1% (w/v) SDS. Autoradiography of the filters was carried out at −70°C by using Fuji RX film with a single intensifying screen. Further details on these methods can be found in Refs [24] and [25].

DNA sequences

The *atp C* sequence was a pea cDNA clone encoding most of the γ-subunit of the CF_0F_1-ATPase (Gray et al., unpublished). The *atp BE* probe was a plastid genomic clone (C-96) from pea and corresponds to parts of the β subunit and the whole ε subunit of the CF_0F_1-ATPase [26]. The *pet BD* probe was a plastid sequence (C-109), which encodes parts of the genes for cytochrome *b* and subunit IV of the cytochrome *bf* complex of the thylakoid membrane [26]. The *cab* cDNA clone (pAB96), which corresponds to the gene encoding the chlorophyll *a*/*b*-binding protein, has previously been described by Coruzzi et al. [27].

3. Results

Amount and activity of the enzyme

To ascertain that any changes in the permeability of the thylakoid membranes due to UV-B exposure should not interfere with our measurements of the enzyme activity, we chose to measure ATP hydrolysis rather than ATP synthesis. Fig. 1 shows the ATP-hydrolytic activity of thylakoids isolated from control and UV-B-exposed pea leaves between day 17, i.e., the day of onset of supplementary UV-B, and day 21, the last day of UV-B treatment. During this period, the ATPase activity was lowered by 25% over the 5 days of UV-B exposure in irradiated leaves.

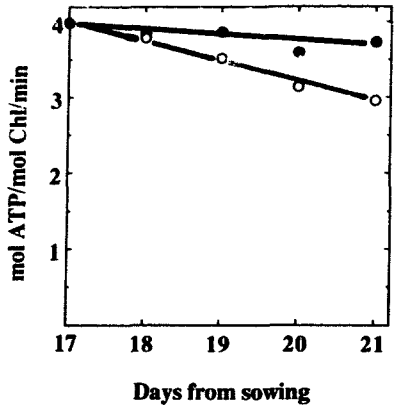

Fig. 1. ATPase activity of thylakoids after exposure of pea plants to supplementary UV-B radiation. Plants were grown for 17 days after sowing in a control light chamber. On day 17, half of the plants were transferred to a chamber with identical visible light supplemented with UV-B. Controls are indicated with (●) and UV-B samples with (○).

The antibodies raised against CF_1 of spinach thylakoids showed strong cross-reactivity towards pea thylakoids (Fig. 2A), mainly labelling the α and/or β subunits of CF_1 in both types of thylakoids. The labelling of different amounts of pea thylakoids with the rabbit anti-spinach-CF_1 antibody is shown in Fig. 2B. A clear linear relationship between the amount of Chl and the anti-IgG alkaline phosphatase labelling of the CF_1-ATPase was found (Fig. 2C). This is a prerequisite for the use of the antibodies for quantification of the enzyme in the membranes.

Compared with the controls, the irradiated samples showed a decrease in CF_1 content of about 60% over the 5 days of UV-B exposure (Fig. 3A), i.e., the amount of protein decreased more than the ATPase activity of the thylakoids. The differential effect of UV-B on the amount of enzyme protein present in the thylakoid membranes and its activity is partly compensated for by the activation of the remaining functional enzyme complexes (Fig. 3B). On day 21, after 4 days of UV-B irradiation, the activation was almost 90%.

Activity of the isolated CF_1

To determine whether the absorption of UV-B radiation by leaves, and by the CF_1-ATPase, caused any apparent changes in the UV-fluorescent properties of the CF_1-ATPase, and thus any modification of the protein, the enzyme was isolated and purified and its very distinct tyrosine-dominated fluorescence spectrum [17,18] was studied. However, no apparent difference in spectra of CF_1 from control leaves and UV-B-exposed samples could be found (not shown).

By using the ratio of the absorbances at 305 and 350 nm, which in a pure enzyme preparation should be greater than 1.85 [17], the present preparation was judged to be pure (ratio 2.1). In Fig. 4 is shown the comparison between the activity of the trypsin-activated CF_1-ATPase (isolated from control and UV-B-exposed plants after 2 and 4 days of treatment, i.e., 19 and 21 days after sowing, respectively) and the protein content of these preparations, which was determined from the fluorescence spectrum (excitation at 280 nm; peak at 305 nm). These results underline the finding above that the amount of CF_1 decreased more rapidly than the activity, and implies that an activation of the functional enzyme molecules takes place. The activation was not reflected in any change in the UV-fluorescent properties of the enzyme.

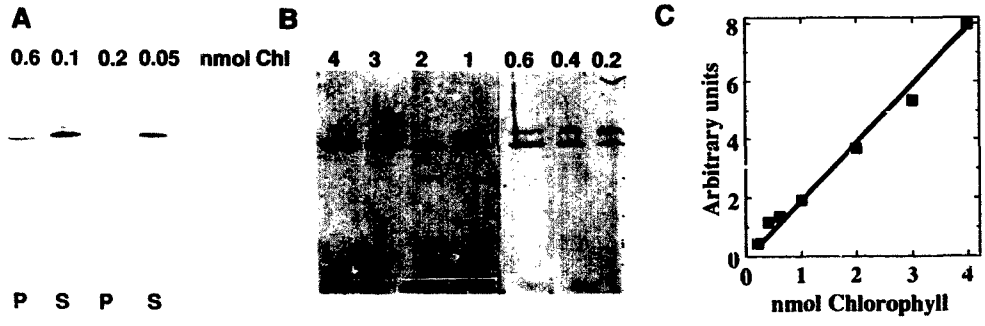

Fig. 2. The suitability of rabbit anti-spinach CF_1 antibodies as a means for determining the amounts of CF_1 in pea thylakoids. (A) Western blot of a 12.5% Laemmli PAGE gel on which 0.05 and 0.1 nmol Chl of spinach (S) thylakoids or 0.2 and 0.6 nmol Chl of pea (P) thylakoids had been loaded. The Western blot was blocked and incubated with 1:1000 dilution of rabbit anti-spinach CF_1 antibodies. The second antibody, goat anti-rabbit IgG conjugated to alkaline phosphatase, was then added and colour developed according to the manufacturers instructions. The α and β subunits ran to a similar position in this gel system and the specifically labelled band on the spinach blot corresponds to either of these subunits or both. In pea, however, it is more likely that the β subunit is labelled, since it shows stronger homology to the corresponding spinach subunit than does α. (B) Western blot of a 12.5% Laemmli PAGE gel on which 0.2 to 4 nmol Chl of pea thylakoids had been loaded. (C) The Western blot in (B) was scanned in a Pharmacia LKB Ultroscan XL and the absorption of the lower coloured band was evaluated by using the Pharmacia LKB GelScan XL software. The absorption was plotted against the amount of chlorophyll of the pea thylakoids loaded on to the gel.

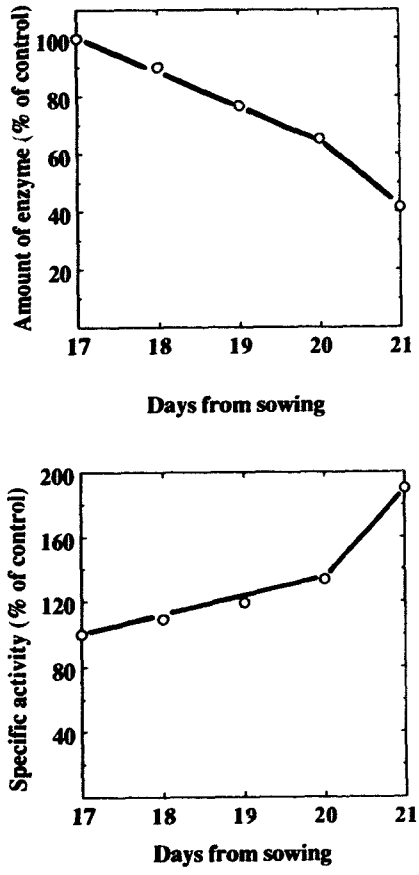

Fig. 3. Amount and relative specific ATPase activity of CF_1 of pea thylakoids from pea plants which had been exposed to supplementary UV-B radiation. Plants were grown for 17 days after sowing in a control light chamber. On day 17, half of the plants were transferred to a chamber with identical visible light supplemented with UV-B. (A; top) Amount of CF_1 in UV-B treated thylakoids relative to the controls as determined by the use of rabbit anti-spinach CF_1 antibodies. (B; bottom) The relative specific ATPase activity of remaining CF_1 molecules after supplementary UV-B. Derived from Fig. 1 and part (A) of this figure.

Abundance of mRNA transcripts

To examine whether the decrease in the amount of ATP synthase complexes was reflected in a decrease in the abundance of mRNA for some of the polypeptides of the enzyme, the amounts of mRNA for the β, γ and ϵ subunits were determined. The effect of the treatment of pea plants with supplementary UV-B on the amount of transcripts for the β and ϵ subunits was analyzed with a plastid DNA fragment containing part of both the *atp B* and *atp E* genes. The levels of the mRNA transcripts for the γ subunit were probed with a near full-length cDNA clone for the nuclear *atp C* gene. For detection of significant amounts of mRNA transcripts for the CF_1 subunits, it was of great importance that young tissue was used. In the two left lanes of both Fig. 5A and 5B is compared the expression of *atp BE* and *atp C*, respectively, in the second and the fourth leaf pair from the base of the plant. Obviously, much less of the corresponding mRNA transcripts were found in the older tissue than in the younger.

Six different transcripts were found by hybridization with the *atp BE* probe (of which five are clearly visible in Fig. 5A); they ranged in size from 0.7 to 2.8 kb. This is consistent with the pattern found by Woodbury et al. [28]. Only the larger transcripts could correspond to the entire dicistronic mRNA, or be the full-length mRNA for the β subunit. The smallest and most strongly labelled transcripts probably correspond to the ϵ subunit mRNA [28]. The poor labelling of the transcripts corresponding to the β subunit, as compared to the shorter transcripts, could be due either to a lower turnover of the larger subunit, or rather, to the fact that only part of the *atp B* gene is included in the probe, leaving long stretches of DNA unable to hybridize under the stringent conditions used and thus giving rise to an unproportionally weak signal.

A substantial decrease in *atp BE* mRNA transcripts was seen even after one day of UV-B exposure (lanes 3 and 4 of Fig. 5A). The amount of mRNA corresponding to *atp C* was below the level of detection after one day of UV-B exposure (Fig. 5B lanes three and four).

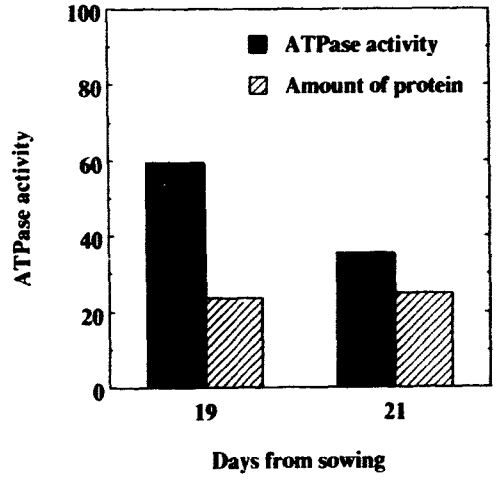

Fig. 4. The relative amount and ATPase activity of isolated CF_1 from plants that had been exposed to supplementary UV-B radiation. Plants were grown for 17 days after sowing in a control light chamber. On day 17, half of the plants were transferred to a chamber with identical visible light supplemented with UV-B. After 2 and 4 days of UV-B exposure, 19 and 21 days after sowing, respectively, leaves were harvested and CF_1 was isolated. The ATPase activity of trypsin-activated CF_1 was determined and the amount of protein was estimated from the peak of emission spectrum after excitation of the preparation at 280 nm. The amount and activity are shown relative to controls.

In a previous study [3] it was shown that the electron transport activity of the cytochrome *bf* complex in pea leaves was much less affected by UV-B treatment than for example the ATPase and Photosystem II activities. For a comparison with the effects on the mRNA transcripts of the genes for CF_1 subunits, the abundance of the mRNA transcripts for the *pet BD* gene cluster, which encodes the genes for cytochrome *b* and subunit IV of the cytochrome *bf* complex, was also determined. The dependence on the age of the tissue was not as crucial as with the CF_1 subunit transcripts (lanes 1 and 2 in Fig. 6A). However, the decrease in mRNA for the *pet BD* gene as a result of UV-B exposure was as rapid as the decrease in *atp BE* (lanes 3 and 4, Fig. 6A).

In addition to the 0.9 kb transcript, very strongly labelled by the C-109 probe, at least three longer transcripts (1.2, 1.7 and 2.3 kb) appeared after prolonged autoradiography. Due to saturation of the film, the differences in the 0.9 kb band between controls and UV-B samples was diminished, and thus these results are not shown. All labelled transcripts correspond well to transcripts which were found to be labelled with this probe in the initial study [28]. The intensities of the longer transcripts decreased similarly to the 0.9 kb transcript after UV-B exposure.

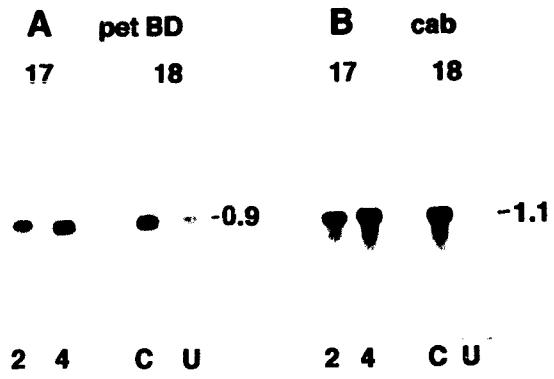

Fig. 6. Northern blots showing the amounts of mRNA transcripts for two subunits of the cytochrome *b/f* complex and for the chlorophyll *a/b*-binding complex of the Photosystem II of UV-B-exposed pea plants. Plants were grown for 17 days after sowing in a control light chamber. On day 17, half of the plants were transferred to a chamber with identical visible light supplemented with UV-B. To determine the dependence of the abundance of mRNA transcripts on leaf age, the second (2) and fourth (4) leaf pairs of control plants were sampled on day 17 after sowing. On day 18, after one day of UV-B exposure, the 4th leaf pair was sampled from both control (C) and UV-B-exposed (U) plants. The approximate sizes (in kb) of the transcripts are shown to the right of the figures. (A) Total RNA (10 μg in each lane) probed with the plastid C-109 probe containing parts of genes encoding the cytochrome *b* and subunit IV of the cytochrome *b/f* complex; (B) Total RNA (10 μg in each lane) probed with a cDNA probe (pAB96) containing the entire nucleotide sequence encoding the chlorophyll *a/b*-binding complex of the Photosystem II.

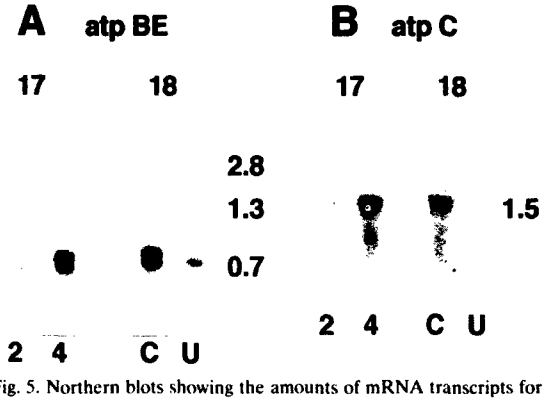

Fig. 5. Northern blots showing the amounts of mRNA transcripts for different subunits of the CF_1-ATPase of UV-B-exposed pea plants. Plants were grown for 17 days after sowing in a control light chamber. On day 17, half of the plants were transferred to a chamber with identical visible light supplemented with UV-B. To determine the dependence of the abundance of mRNA transcripts on leaf age, the second (2) and fourth (4) leaf pairs of control plants were sampled on day 17 after sowing. On day 18, after one day of UV-B exposure, the 4th leaf pair was sampled from both control (C) and UV-B-exposed (U) plants. The approximate sizes (in kb) of the transcripts are shown to the right of the figures. (A) Total RNA (10 μg in each lane) probed with the plastid C-96 probe containing parts of genes encoding the β and ε subunits of the CF_1-ATPase; (B) Total RNA (10 μg in each lane) probed with a cDNA probe containing the entire nucleotide sequence encoding the γ subunit of the CF_1-ATPase.

For comparison, the cab gene mRNA level was determined as it had previously been shown to be down-regulated [5]. No mRNA transcripts at all could be detected for the *cab* gene, which encodes the chlorophyll *a/b*-binding protein of the light-harvesting antenna complex associated with Photosystem II (Fig. 6B, lanes 3 and 4), after 1 day of UV-B exposure. Furthermore, leaf age played a much smaller role for the abundance of *cab* mRNA transcripts than for the other genes in this study (lanes 1 and 2, Fig 6B).

4. Discussion

Exposure of plants and plant tissue to supplementary UV-B radiation gives rise to a multitude of changes at the biochemical level in plants. The impact on photosynthesis is of special interest [29]. One of the components participating in photosynthesis, the CF_0F_1-ATPase, was the subject of the present study. The amount and activity of the membrane-bound and isolated enzymes were examined as well as the expression of genes encoding some of the subunits of the complex.

In accordance with previous results [3], CF_1 activity decreased as a result of the UV-B treatment (Fig. 1). Interestingly, the amount of the CF_1-ATPase declined to an even greater extent than did the ATPase activity (Fig. 3A), which implies that an activation of the remaining functional enzyme complexes occurs (Figs. 3B and 4). The cause for the activation seen in this study is unknown. However, it has been found that a reduction of disulfide bridges of cystines within the γ subunit of the CF_1-ATPase in vitro gives rise to a more active enzyme [30,31]. Thioredoxin is thought to be the component responsible for reduction of these disulfides in vivo [32,33]. It is possible that the increase in the expression of genes involved in protection against oxidative damage, such as the glutathione reductase gene [7], could result in a reduction of disulfide bridges, either directly or via thioredoxin. Furthermore, the situation is similar to Rubisco [4], where the actual in vivo activation of the enzyme increased as a result of UV-B exposure [3,4].

A very interesting effect of the exposure of plant material to UV-B radiation is the rapid change in gene expression and mRNA abundance [34]. While the expression of genes encoding proteins involved in defence, such as glutathione reductase [7] and chalcone synthase [6,7] increased dramatically after UV-B treatment, the amounts of mRNA transcripts for some photosynthetic genes decreased [4,5]. This was also the case for the photosynthetic genes described in this paper, *atpBE*, *atpC* and *petBD*. Although the cytochrome *b/f* complex, together with Photosystem I, was the photosynthetic component least affected by UV-B [3], the mRNA transcripts for the cytochrome *b* and subunit IV genes, *petBD*, declined to an extent similar to the *atpBE* transcripts. Thus, the stability of the cytochrome *b/f* complex was not due to increased transcription of its genes or mRNA stability but rather the reflection of a greater intrinsic stability of the polypeptides towards UV-B radiation. It is important to note, however, that the level of cytochrome *b/f* complex was determined as activity, not protein [3]. Thus, there is still a possibility that the amount of this protein also decreased at the same time as an activation took place.

Furthermore, the genes for which the amounts of mRNA transcripts declined more rapidly are all encoded in the nuclear genome: *cab* (Refs. 5, 7, 34 and this study), *rbcS* (encoding the small subunit of Rubisco; Ref. [4]) and *atpC* (this study). The plastid-encoded genes, *atpBE* and *petBD* (this study), *rbcL* (encoding the large subunit of Rubisco; Ref. [4]) and *psbA* (encoding the D1-protein of Photosystem II; Ref. [5]), all possessed mRNA transcripts that were more stable than the nuclear-encoded genes. The mRNA transcripts for *psbA* were the most stable, a finding which possibly reflects the fact that this protein is the polypeptide in the chloroplast that is turned-over most rapidly and also the one most prone to damage by light [35]. These differences in the gene expression or abundance of mRNA transcripts may be a reflection of the differences in regulation of nuclear- and plastid-encoded genes, the expression of the former being controlled at the transcriptional level [36,37], the latter mainly post-transcriptionally [38].

In addition to the decrease in the abundance of mRNA transcripts of photosynthetic genes and to the corresponding increase for cytoplasmically localized proteins involved in plant defence, the amount of mRNA transcripts for the chloroplast-localized radical-scavenging superoxide dismutase was also decreased upon UV-B treatment [7]. This fact widens the issue of the effects of UV-B on gene expression from being a matter of decreases for photosynthetic genes and increases for defence genes, to a question of whether a general decrease in mRNA transcripts for chloroplast-localized proteins occurs. However, the expression of the glutathione reductase gene was also found to increase to a certain extent [7]. The activity of the corresponding protein is distributed mainly between cytosol and chloroplasts and it is not clear if up-regulation is manifested for the chloroplastic or cytosolic glutathione reductase.

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