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Light-dependent degradation of the thylakoid 32 kDa Q_B protein in isolated chloroplast membranes of *Chlamydomonas reinhardtii*

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The 32 kDa Q_B protein of *Chlamydomonas reinhardtii* chloroplast membranes is rapidly and specifically degraded when a suspension of isolated thylakoids is exposed to high light intensity at 25°C, at pH 7.8. Loss of the 32 kDa Q_B protein correlates well with loss of Q_B-dependent electron-flow, does not require the addition of divalent cations, increases with light intensity and is enhanced by 650 nm light, is not inhibited by uncouplers but is partially inhibited by leupeptin and pepstatin when added in the presence of 0.15% (w/v) β-D-octylglucoside. The specificity of the light-dependent degradation toward the 32 kDa Q_B protein is gradually lost at increasing pH (8.5–9.5) or by the addition of β-D-octylglucose up to 1.0% (w/v), a concentration at which the thylakoids are completely solubilized. The reaction is significantly accelerated at high pH and detergent concentrations, and a marked degree of activity is observed under these conditions also in the dark. Pretreatment of thylakoids with trypsin at pH 7.8 does not abolish the membrane-bound specific proteolytic activity toward the trypsin-generated hydrophobic fragment of 17.5 kDa obtained from the 32 kDa Q_B protein which still contains the amino acid sequence of the herbicide- and quinone-binding sites. The light-dependent specific degradation of the 32 kDa Q_B protein by isolated thylakoids at pH 7.8 mimics in vitro the photoinhibition phenomena occurring in vivo, as described before (Kyle, D.J., Ohad, I. and Arntzen, C.J. (1984) Proc. Natl. Acad. Sci. USA 81, 4070–4074).

Introduction

It has been well established that the 32 kDa polypeptide of the chloroplast thylakoid membranes, which participates in the formation of the herbicide-binding site and the Q_B secondary electron acceptor of Photosystem II (PS II), turns over in the light but is stable in the dark [1–4]. We have recently shown that in vovo loss of PS II activity in algae exposed to high light intensity (photoinhibition) is caused by an accelerated degradation of the Q_B protein which surpasses the rate of de novo synthesis and integration of this polypeptide into functional PS II units [2,5]. Specific degradation of the Q_B protein could also be demonstrated in isolated thylakoids of pea chloroplast in vitro. The degradation of the 32 kDa Q_B protein in vitro

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Abbreviations: DCIP, dichlorophenolindophenol; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethyl urea; DPC, diphenylcarbazide; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; LDS, lithium dodecyl sulfate; PMSF, phenylmethylsulfonylfluoride; PS II, Photosystem II; $SiO_2 \cdot 12MoO_3$, silicomolybdate; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; LHC II light-harvesting chlorophyll *a*,*b*-protein complex of Photosystem II; PQ, plastoquinone.

was light-dependent and coincided with loss of Q_B-dependent electron-flow measured as reduction of dichlorophenolindophenol (DCIP), using H_2O as an electron donor, without significantly affecting Q_B-independent electron-flow via PS II, measured as reduction of silicomolybdate ($SIO_2 \cdot 12MoO_3$) with H_2O or diphenylcarbazide (DPC) as electron donors [6]. The light-dependent loss of the 32 kDa Q_B protein in this system did not require addition of chloroplast-soluble proteins. The degradation fragments could not be detected by autoradiography of ^{35}S-labeled thylakoid polypeptides separated by lithium dodecyl sulfate polyacrylamide gel electrophoresis nor by immunoblotting, using antibodies raised against a 23 kDa segment of the 32 kDa Q_B polypeptide, which reacted with the intact, complete antigen as well as with its trypsin-generated fragments [6].

In the present work, we have used isolated *Chlamydomonas reinhardtii* thylakoids in order to compare the activity of light-induced degradation in vitro of the 32 kDa Q_B protein with that of chloroplast membranes of pea reported before [6], and obtain additional information on the mechanism of the light-induced degradation of this polypeptide.

Materials and Methods

Radioactive labeling of the 32 kDa Q_B protein. It has been shown that *Chlamydomonas* cells exposed to the light preferentially label the 32 kDa Q_B protein due to its fast turnover [7,8]. The specificity of labeling depends on the rate of turnover which can be modulated by light intensity, the duration of the pulse and the physiological state of the cells. In non-dividing cells which are not involved in an active process of thylakoid synthesis, most of the radioactivity incorporated is found in the 32 kDa Q_B protein. It has also been shown that the newly synthesized polypeptide is not immediately integrated into functional PS II units where its light-dependent degradation occurs [7]. Based on these observations, the best protocol to follow in order to obtain thylakoids containing specifically labeled 32 kDa Q_B protein which will be degraded when whole cells or isolated membranes are exposed to the light, consists of a short pulse-labeling (30–90 min) of non-dividing cells in saturating light intensity (approx. 100–150 $W \cdot m^2$), followed by a chase period of 2–3 h [5,8].

C. reinhardtii y-1 cells were grown as described before, harvested at the end of the logarithmic phase of growth, washed and resuspended in fresh growth medium, without sulfate, at a final cell concentration of 10^7 cells/ml (non-dividing conditions) [8,9], and incubated in white light provided by six fluorescent lamps of 60 W (incident light on the surface of the cell suspension, approx. 120 $W \cdot m^{-2}$). $^{35}SO_4^{2-}$ was added (300 μCi/μmol, 1–5 μCi/ml) and incubation continued for 90 min, when non-radioactive sulfate was added to reduce the specific radioactivity to 1 μCi/μmol, and incubation continued for an additional period of 3 h. The cells were then harvested, washed free of radioactive $^{35}SO_4^{2-}$, and thylakoid membranes were prepared as described [8]. The thylakoids were washed in a buffer solution containing sodium Tricine (50 mM, pH 7.8), 10 mM NaCl and 5 mM $MgCl_2$ (buffer 1), pelleted in microfuge tubes (100 μg chlorophyll/tube) and stored at −80°C for up to 3 weeks.

Photoinhibition in vitro and electrophoretic separation of thylakoid polypeptides. Frozen membranes were thawed by the addition of cold buffer 1, as above, to give a final concentration of 500 μg chlorophyll/ml, and exposed, in translucent microfuge tubes (30–50 μl/tube) immersed in a transparent bath at 25°C, to white light of various intensities: 1200, 600 or 200 $W \cdot m^{-2}$ (high, medium or low light intensities, respectively) measured at the level of the tube with a YSI Kettering model 65 radiometer probe. Incubation was continued for various periods of time, and terminated by chilling in ice and addition of the electrophoretic sample buffer containing LDS. Samples were then electrophoresed on 10–17.5% polyacrylamide, 0.7 mm thick gel slabs, using LDS and 4 M urea as described elsewhere [2]. The gels were stained with Coomassie brilliant blue R, dried and autoradiographed by conventional procedures. Equal amounts of chlorophyll (10–15 μg) were loaded on each slot. The radioactivity incorporated corresponded to about 4000 cpm/μg protein. About 40–60% of the radioactivity was usually found in the 32 kDa Q_B polypeptide band, which amounts to less than 2% of the total mem-

brane protein. Autoradiograms were exposed at −80°C for 3–5 days.

Chlorophyll was measured as described by Arnon [10]. Measurements of reduction of DCIP and $SiO_2 \cdot 12MoO_3$ were carried out spectrophotometrically as described before [2]. All reagents used in this work were of analytical grade. $^{35}SO_4^{2-}$ was purchased from Amersham International, U.K.

Results

Effect of divalent cations on the light-dependent degradation of the 32 kDa Q_B protein

As reported before, the 32 kDa Q_B protein is degraded in vitro when isolated thylakoids are exposed to light [6]. The incubation system is the previous work contained 5 mM $MgCl_2$. When $CaCl_2$ was used instead of $MgCl_2$, the degradation of the 32 kDa Q_B polypeptides was only slightly reduced (Fig. 1A). Addition of 5 mM EGTA did not have any significant effect, and removal of EGTA by washing, followed by addition of $MgCl_2$, resulted in a slightly higher degradation activity. However, the differences between the various treatments were minute, and it appears that divalent cations are not an absolute requirement for the activity of the degradation system. Nevertheless, since other thylakoid activities such as phosphorylation and electron-flow are measured in their presence, Mg^{2+} cations were routinely added to all the assays performed. Measurements of DCIP and $SiO_2 \cdot 12MoO_3$ reduction showed that, as reported for isolated pea thylakids [6], the reduction of DCIP was completely inhibited after 20 min of incubation in the light, while only 10–15% loss of DCIP reduction was observed in the dark, control. Practically no loss of $SiO_2 \cdot 12MoO_3$ reduction occurred in *Chlamydomonas* isolated thylakoids exposed to high light intensity (Fig. 1B). The ob-

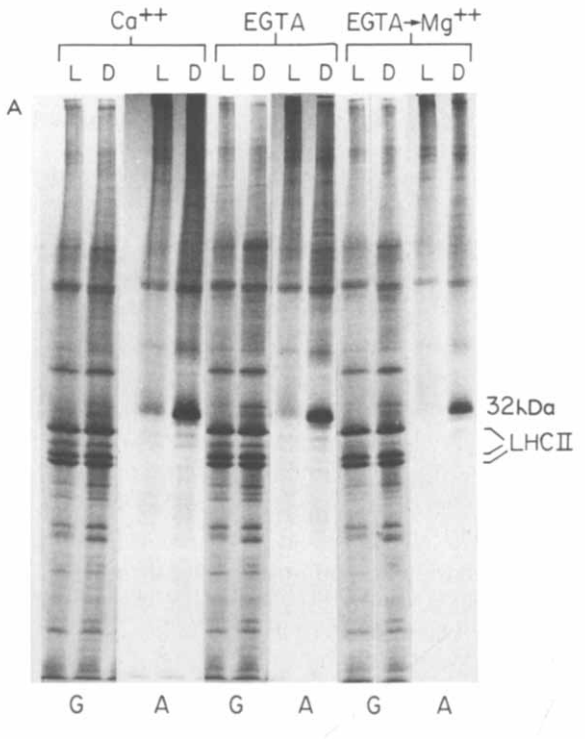

Fig. 1. Light-induced loss of Q_B-dependent electron-flow and degradation of the 32 kDa Q_B protein in vivo. (A) Degradation of thylakoid polypeptides: effect of Ca^{2+} and Mg^{2+}. Thylakoids were washed in 50 mM sodium-Tricine buffer (pH 7.8) and 10 mM NaCl, with or without addition of 5 mM EGTA. Ca^{2+} or Mg^{2+} was added at a final concentration of 5 mM. The chlorophyll concentration was 500 μg/ml. Incubation was carried out for 30 min in the dark (D) or light (L, 1200 $W \cdot m^{-2}$) at 25°C in translucent microfuge tubes. The reaction was stopped by addition of electrophoresis sample buffer. Samples equivalent to 10 μg chlorophyll were loaded on each slot. G, stained gel: A, autoradiogram; LHC II; the polypeptides of the light-harvesting chlorophyll *a,b* complex; 32 kDa, the 32 kDa Q_B protein. (B) Loss of Q_B-dependent electron-flow as a function of time of photoinhibition. Same experimental conditions as in (A); at times as indicated, samples were taken and Q_B-dependent (reduction of DCIP) and Q_B-independent (reduction or $SiO_2 12MoO_3$ (SIMO)) were measured.

servation is at variance with previously reported data showing that $SiO_2 \cdot 12MoO_3$ reduction is partially photoinhibited in isolated pea thylakoids [6]. This might be due to the difference between *Chlamydomonas* and pea thylakoids.

Effect of light intensity

The effect of light intensity on the time-course of the in vitro degradation of the 32 kDa Q_B polypeptide is shown in Fig. 2. The rate of degradation increases with light intensity; however, at equal degrees of degradation of the 32 kDa Q_B polypeptide, the same pattern of loss of radioactivity is observed. The degradation seems to be specific for the 32 kDa Q_B, as shown by examination of the autoradiograms and pattern of stained polypeptides. However, at advanced degradation stages, a loss of stained polypeptides in the 32–33 kDa range can also be observed (Fig. 2). When the effect of equal light intensities at 650 or 710 nm were compared (100 $W \cdot m^{-2}$, Baird-Atomic interference filters, 40 nm half-bandwidth), a more pronounced loss of radioactivity from the 32 kDa Q_B polypeptides was observed when 650 nm light was used (data not shown). The highest absorption of the thylakoids at 650 nm, as compared to 710 nm, might account, at least in part, for this effect.

Effect of pH on the in vitro degradation of the 32 kDa Q_B polypeptide

The in vitro degradation of the labeled 32 kDa Q_B polypeptide was found to be strongly influenced by the pH of the incubation system (Figs. 3 and 4). The activity increased with the rise in pH from 6.0 to 9.5. With the increase in pH, the specificity of the in vitro system for the light-de-

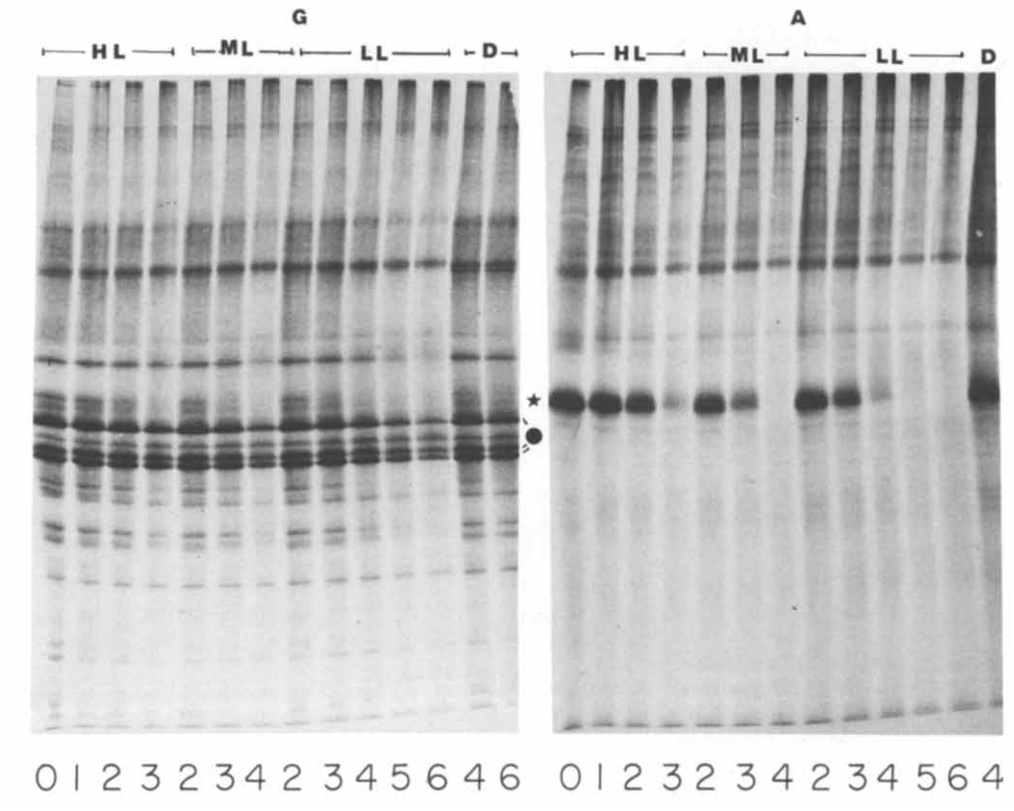

Fig. 2. Effect of light intensity on the degradation of thylakoid polypeptides. Same experimental conditions as in Fig. 1. The membrane suspension was incubated in the dark (D) or high (HL), medium (ML) and low (LL) light intensity, as described in Materials and Methods. Asterisk, the 32 kDa Q_B protein; black dot, the polypeotides of the LHC II; samples were taken at 0 time (0), 8 min (1), 15 min (2), 30 min (3), 60 min (4), 90 min (5) and 120 min (6). G, stained gel; A, autoradiogram.

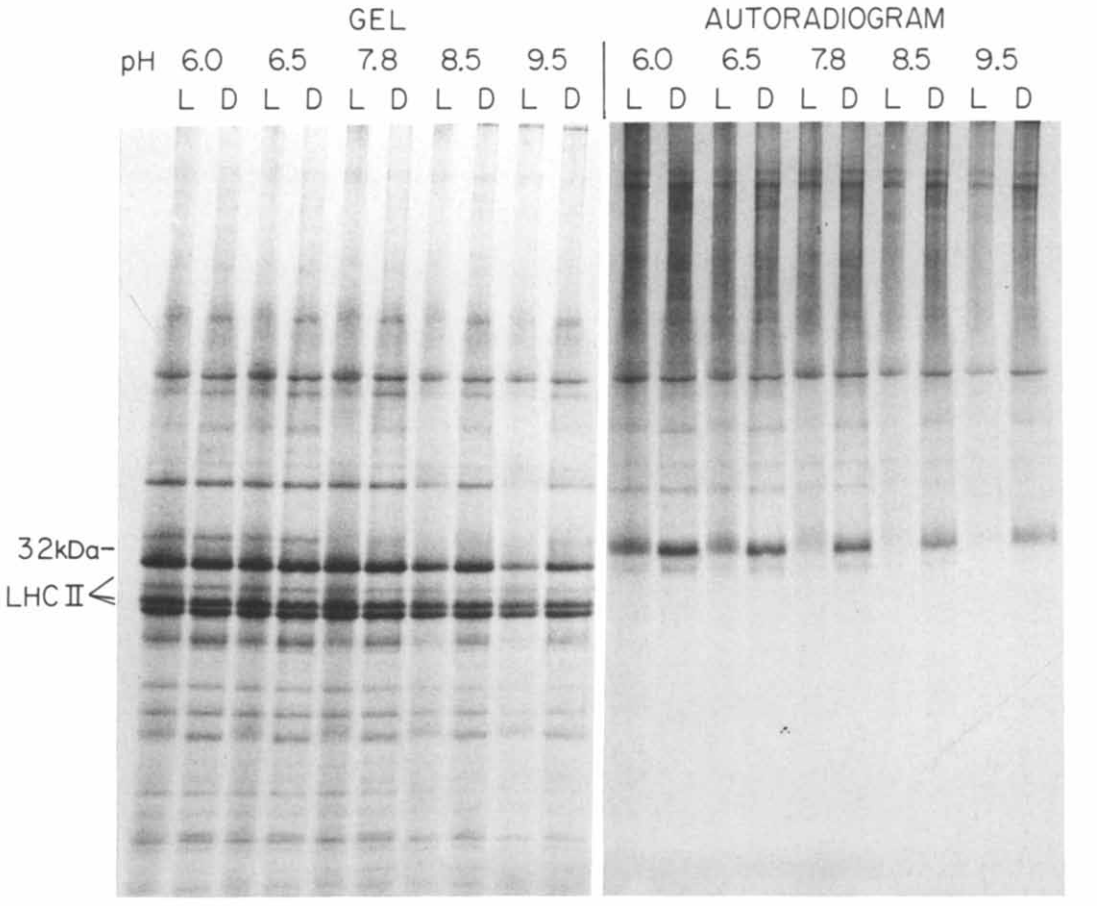

Fig. 3. Effect of pH on the light-dependent degradation of thylakoid polypeptides. Same experimental conditions as in Fig. 1. The thylakoids were pelleted and resuspended in either sodium-Tricine buffer (pH 7.8–9.5) or Hepes (pH 6.0–6.5), with the addition of 10 mM NaCl and 2 mM $MgCl_2$. Incubation was performed at 1200 $W \cdot m^{-2}$ for 25 min at 25°C. 32 kD, the 32 kDa Q_B protein; L, D, light- or dark-incubated samples, respectively; equal amounts of membranes were loaded on each slot, equivalent to 10 μg chlorophyll.

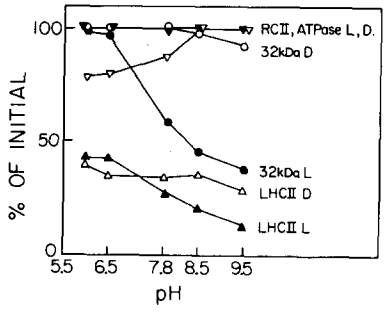

Fig. 4. Specificity of thylakoid protein degradation as a function of pH. An autoradiogram obtained from the experiment in Fig. 3 was scanned using a soft laser scanning densitometer, Model SL-TRFF Biomed Instrument, Inc., CA, and the loss of radioactivity of various polypeptides measured as peak hights after 25 min of incubation at 25°C in the light (L, 1200 $W \cdot m^{-2}$) or dark (D) was plotted relative to that present in the dark-incubated sample at pH 6.0. RC II, combined radioactivity of polypeptides of 44 and 47 kDa (▽); ATPase, the combined peaks of the α, β subunits (△); 32 kD, the 32 kDa Q_B polypeptide (○, ●); the various polypeptides are identified from their electrophoretic mobility.

pendent degradation of the 32 kDa Q_B was progressively lost and some degradation was also observed in the dark-incubated samples. Specificity and relatively fast degradation (20–30 min) were obtained best at pH 7.5–7.8 (Fig. 4). The effect of incubation time in the light at pH 7.8 and 9.5 is shown in Fig. 5. While a progressive loss of the radioactivity of the 32 kDa Q_B polypeptide was

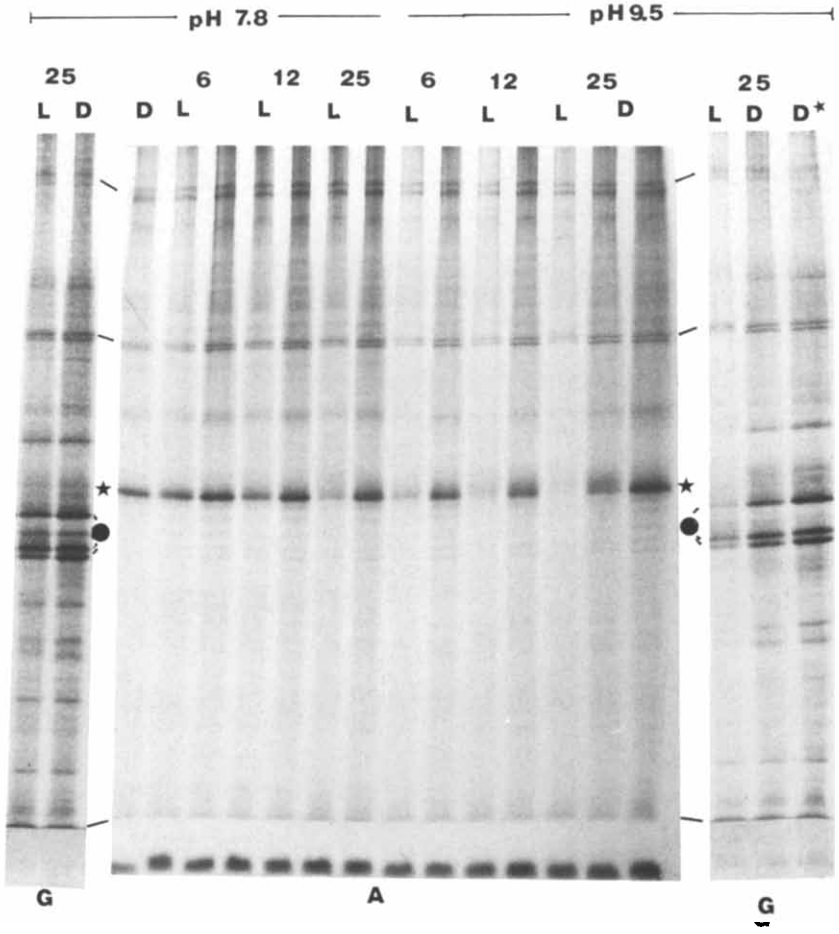

Fig. 5. Time-course of the light-dependent degradation of thylakoid proteins at pH 7.8 and 9.5. Same experimental conditions as in Fig. 3. The thylakoid suspension was incubated at 25°C in the light (L), dark (D or unmarked) or dark at 0°C (D*) for times (in minutes) as indicated by the number appearing on the upper part of each panel. G, stained gel; A, autoradiogram; asterisk, the 32 kDa Q_B protein; dark not, the LHC II polypeptides.

observed at pH 7.8 up to 25 min of incubation, almost complete degradation was already observed after 6 min of incubation in the light at ph 9.5. As shown in Fig. 3, a partial loss of radioactivity of the 32 kDa Q_B polypeptide occurred also in membranes incubated in the dark at pH 9.5. Loss of radioactivity and stained polypeptide bands at pH 9.5 did not occur at 0°C in the dark (Fig. 5).

Effect of reducing agents, electron acceptors and donors, detergents and trypsin treatment

It has been previously proposed that the 32 kDa Q_B polypeptide is modified following interaction with quinone anion radicals. The modified protein is then recognized by a membrane-bound degradation system and removed in order to allow insertion of a newly synthesized polypeptide and its integration into functional PS II units [2,4,11,12]. The results presented above indicate that alkaline pH-induced changes in the organization of membrane polypeptide(s) results in the partial activation of the membrane-bound degradation system in the dark. However, attempts to activate the degradation system in the dark by reduction of the thylakoid quinones via addition of reduced duroquinone failed, as reported before, when sodium dithionite was used as a reducing agent for pea thylakoids [6]. Blocking electron-flow

via Q_B with DCMU slightly protected against light-induced degradation at pH 7.8, and a similar effect was observed when $SiO_2 \cdot 12MoO_3$ was used as an electron acceptor at the Q_A site (data not shown). Addition of uncouplers such as NH_4^+ or FCCP had no effect.

The effect of detergents as a means to activate the degradation system was also tested. The results are shown in Figs. 6 and 7. Since addition of β-D-octylglucoside causes at least partial solubilization of thylakoids, the activity of the degradation system was assessed at various detergent concentrations on the total incubation mixture and on the pellet or supernatant obtained after centrifugation of the detergent-treated thylakoids in a Beckman microfuge for 2 min. The results show that the light-dependent degradation system is not inactivated by progressive solubilization of the membranes at increasing β-D-octylglucoside concentrations, and the activity is found in both the pellet and the soluble fraction (Fig. 6). Addition of 0.5% β-D-octylglucoside to the incubation system caused a drastic acceleration of the 32 kDa Q_B polypeptide degradation in the light. The light-dependent degradation was not inhibited, even by the addition of 1% β-D-octylglucoside (Fig. 7). As in the case of activation of the degradation system at

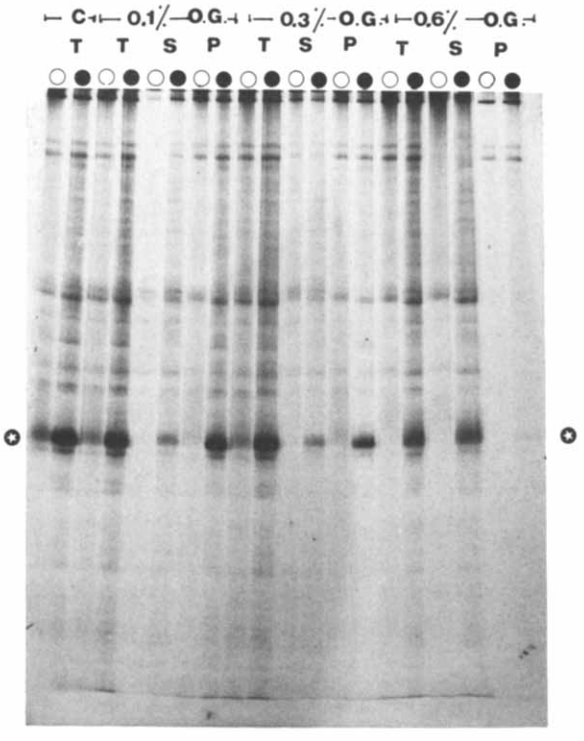

Fig. 6. Effect of thylakoid solubilization by β-D-octylglucoside on the degradation of thylakoid polypeptides. Thylakoids were suspended as described in Materials and Methods at a final concentration of 500 μg chlorophyll/ml in translucent microfuge tubes in ice; β-D-octylglucoside was added from a concentrated (10%) aqueous solution, to obtain final concentrations as indicated (w/v, %), and the suspensions were exposed to 1200 $W \cdot m^{-2}$ white light (open circles) or dark (closed circles) at 25°C for 25 min. Aliquots (10 μg chlorophyll) were taken for electrophoresis of the total mixture (T) or for the supernatant (S) or pellet (P) obtained after centrifugation in the cold for 3 min in a Beckman microfuge. C, control; O.G., detergent-treated samples; asterisk, the 32 kDa Q_B polypeptide. Only the autoradiogram is shown.

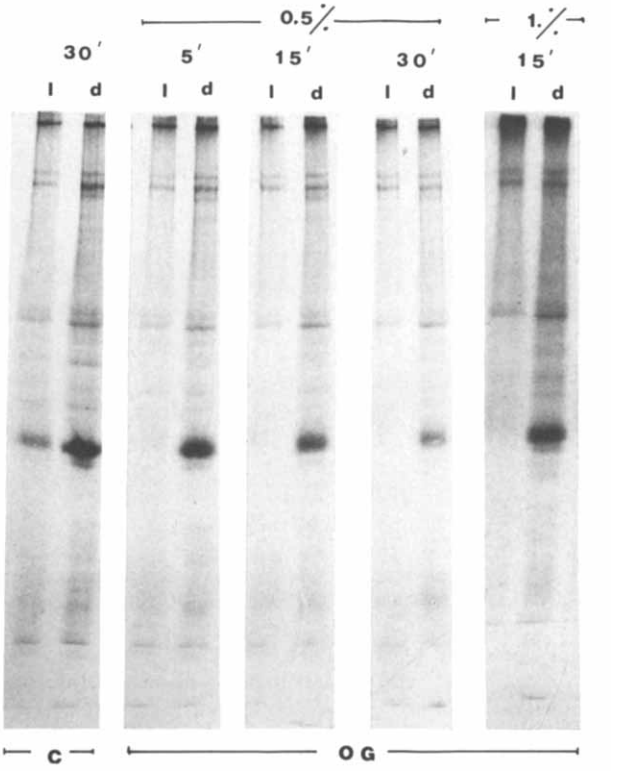

Fig. 7. Time-course of the light-dependent degradation of thylakoid polypeptides in the presence of β-D-octylglucoside. Same experimental conditions as in Fig. 6. The detergent concentrations was 0.5% (v/v) and incubation continued as shown for 5, 15 or 30 min. One time point at 1% concentration is also shown. Samples of the total incubation mixture (10 μg chlorophyll) were loaded on each slot. l, d, light- and dark-incubated samples, respectively; C, control; OG, detergent-treated samples. Only the autoradiogram is shown.

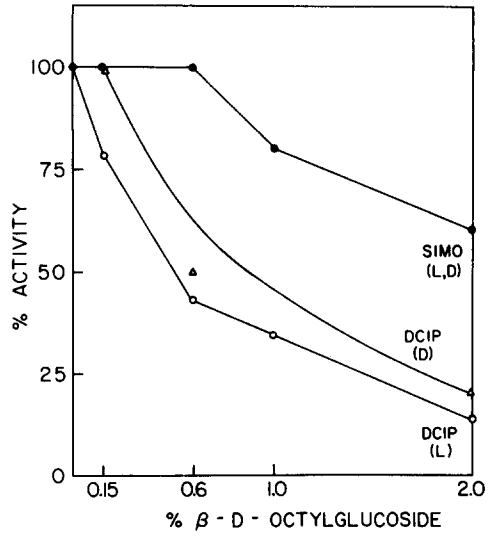

Fig. 8. Effect of increasing concentrations of detergent on the reduction of DCIP and $SiO_2 \cdot 12MoO_3$ (SIMO). Increasing concentrations of β-D-octylglucoside were added to a thylakoid suspension containing 500 μg chlorophyll in buffer 1. The suspension was incubated for 10 min in the dark (D) or light (L) (1200 $W \cdot m^{-2}$), and samples were taken and activity measured as described in Materials and Methods.

alkaline pH in the dark, a slight degree of degradation in the dark was also observed in the presence of detergent, up to 0.6% of β-D-octylglucoside but not at 1%. Measurements of DCIP reduction following incubation of thylakoids in the presence of increasing β-D-octylglucoside concentrations for 10 min in the light or dark, showed that electron-flow was not completely inhibited by this detergent even at concentrations which solubilized the membranes (Fig. 8). Loss of DCIP reduction was faster in the light. The effect of the detergent on the reduction of $SiO_2 \cdot 12MoO_3$ was significantly less pronounced and no difference was observed between the light- or dark-treated samples (Fig. 8).

It has been reported before that part of the 32 kDa Q_B polypeptide is exposed on the membrane surface and is subject to trypsin digestion, which results in the formation of a membrane-bound fragment of approx. 17–20 kDa [3,6,13,14] depending on the electrophoretic separation system. Electron-flow via Q_B and reduction of plastoquinone are lost following trypsin treatment of thylakoids [15,16] as well as the binding of herbi-

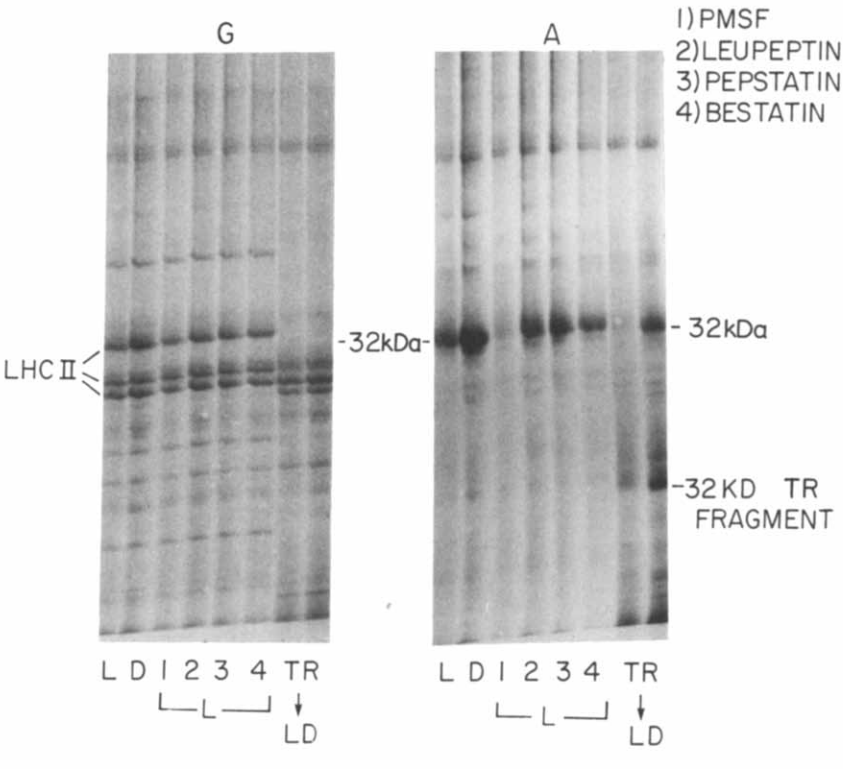

Fig. 9. Effect of proteinase inhibitors and trypsin on the degradation of thylakoid polypeptides. Thylakoids were suspended in buffer as described in Materials and Methods (500 μg chlorophyll/ml) and exposed for 25 min to 1200 $W \cdot m^{-2}$ white light (L) or dark (D) at 25°C, in the absence or presence of 1 mM PMSF (1) or 0.15% β-D-octylglucoside and 0.5 mg/ml leupeptin (2), pepstatin (3) or bestatin (4). To another sample (TR), 20 μg/ml trypsin were added (Sigma, type IV). The trypsin-containing sample was first incubated in the dark at 25°C for 30 min; then, 40 μg per ml trypsin inhibitor were added and the sample was divided into two parts. One part was incubated in the light and the other in the dark, as above. Equal samples of the total incubation mixture were loaded on the gel (10 μg chlorophyll). G, stained gel: A, autoradiogram; 32 kD, the 32 kDa Q_B polypeptide.

cides [17–19]. However, the trypsin-generated fragment is still susceptible to light-induced degradation in vitro (Fig. 9). It has been previously reported that protease inhibitors such as phenylmethylsulfonylfluoride (PMSF) or antipain had practically no effect on the in vitro degradation system of pea thylakoids [6]. Similar results are obtained with *Chlamydomonas* thylakoids (data not shown). However, when several protease inhibitors were tested in the presence of low amounts (0.15%) of β-D-octylglucoside added to facilitate solubilization, a significant degree of protection against the light-induced degradation of the 32 kDa Q_B polypeptide was obtained when using leupeptin and pepstatin, although PMSF and bestatin had no such protective effect (Fig. 9).

Discussion

The light-dependent turnover of the thylakoids 32 kDa Q_B protein, its physiological role in the process of photoinhibition and suggestions for the possible mechanism of this phenomenon have been reported before [2,3,5,20] and have been recently reviewed [4,12].

The aim of this work was to establish an in vitro assay system which could be used in the future to elucidate the actual mechanism of the specific light-dependent degradation of this protein, which appears to be a general phenomenon in all in vivo systems so far investigated, including higher plants [3,20], algae [5–7] and cyanophytes (Poplawsky, R., Cohen, Y. and Ohad, I., unpublished results). The obvious advantages of an in vitro system, to mention only a few, are: accessibility to reagents which may not penetrate various barriers in a whole organism or cause secondary effects due to their interaction with other subcellular compartments; ability to control the thylakoid environment and to modulate membrane composition by selective enzymatic degradation. The in vitro system will also avoid the complication of superimposed de novo synthesis and integration of the polypeptide on its process of degradation.

The obvious disadvantage is that following removal of the thylakoids from their natural environment, minute changes in their organization or interaction between their polypeptides and lipid components might hinder or unduly exaggerate processes that otherwise occur in a controlled manner in vivo.

Attempts to obtain an in vitro system using *Chlamydomonas* thylakoids were carried out before [8]. The system described in this work, consisting of isolated washed *C. reinhardtii* thylakoids, mimics quite efficiently the activity of the light-dependent degradation of the 32 kDa Q_B protein in vivo as far as the following parameters are concerned: specificity, light dependence, loss of Q_B-dependent electron-flow but retention of the reaction center II activity assessed as reduction of $SiO_2 \cdot 12MoO_3$ [6], and a comparable time-course which is, however, shortened, as expected in a system in which degradation is not accompanied by de novo synthesis and all the thylakoid electron carriers are fully reduced in the absence of added electron acceptors.

As previously reported for pea thylakoids [6], the results show that the degradation system is contained within the thylakoids. Furthermore, it responds to light absorbed preferentially by PS II, it is not affected by uncouplers but is partially susceptible to hydrophobic protease inhibitors, in agreement with the previously suggested possibility of the participation of a specific membrane protease acting on the 32 kDa Q_B protein degradation process [2,5,11]. However, a departure from this agreement between the expected results based on the data obtained in vivo and those of the in vitro system became apparent when the conditions of the assay were altered by alkalinization, addition of detergents or selective proteolysis. Thus, with the rise of the pH to 9.5, the specificity of the 32 kDa Q_B protein degradation was progressively lost. The degradation was also considerably accelerated in the light and was already detected in the dark. A rather similar situation was observed also when the thylakoids were treated with increasing concentrations of β-D-octylglucoside until complete solubilization.

It has been previously reported that following trypsinization of thylakoids, only a few polypeptides are affected including the LHC II polypeptides [15,16], the 33 kDa or Murata protein and the herbicide-binding 32 kDa or Q_B protein [15,16,19,21]. The latter generates a fragment of 20 kDa when electrophoresis is carried out in the absence of urea [3], but shows a mobility corre-

sponding to 17.5 kDa in the presence of 4 M urea [6]. This fragment is unable to sustain electron-flow to DCIP nor able to bind DCMU or atrazine [15,19], although it contains the amino acid sequence to which azido atrazine, and therefore possibly also the plastoquinone, binds [14]. The results presented here show that the trypsin 17.5 kDa fragment of the 32 kDa Q_B protein is still subject to specific light-dependent degradation in the isolated thylakoids.

The question thus arises as to whether the results of this work are still in agreement with the originally proposed hypothesis explaining the light-dependent degradation of this polypeptide as the result of an interaction with reduced quinone radicals from electron-flow, and recognition by a membrane-bound protease [11,12]. When the assay system is used at physiological pH (7.5–7.8), both in pea thylakoids [6] and in *Chlamydomonas* as shown here, there is a good agreement between the results obtained in vivo and in vitro. At low detergent concentrations when electron-flow via Q_B is not completely inhibited, the degradation process is stimulated by light and still specific for the 32 kDa polypeptide. It is possible that at higher detergent concentrations or pH, structural changes are induced in the thlakoids by alterations of protein-protein, protein-lipid or lipid–lipid interactions which might allow or promote the interaction of free radicals generated by excitation of disorganized chlorophylls (not necessarily those of reaction center II) and various thylakoid polypeptides, and activate the putative protease. Furthermore, the trypsin fragment generated by proteolysis of intact thylakoid in the standard assay system might still be able to interact with quinone radicals more specifically than other membrane proteins and thus retain its susceptibility to the light degradation system. However, the fact that the trypsin fragment is not recognized by the degradation system as being 'sufficiently modified' relative to the native polypeptide to be removed in the dark, remains to be explained. It should be noted that the degradation system proper is not subject to proteolysis and must be located within the hydrophobic phase of the membrane.

The failure to activate the 32 kDa Q_B degradation system in the dark by reducing agents such as dithionite [6] or reduced duroquinone (this work) which activate the thylakoid protein kinase to phosphorylate the LHC II polypeptides in the dark [22], is apparently at variance with the hypothesis that reduced quinones participate in the activation of the protease [2,5]. It should be considered, however, that the activation of the protein kinase does not require a complete reduction of all the thylakoids' quinone pool but only an increase in the ratio PQ_{red}/PQ_{ox}. Possibly, the experimental conditions used to reduced the thylakoids' quinones with dithionite or duroquinone are not sufficient to generate reduced quinone radicals to activate the system. The data presented here and elsewhere [6] indicate that the activation of a 32 kDa Q_B protein degradation system by light is not due to a direct effect on the putative protease. Nevertheless, this possibility cannot be excluded.

Activation of a thylakoid-bound enzyme, the CF1, as an ATP hydrolase by β-D-octylglucoside [23,24] or various lipids was previously reported [2,5]. The explanations presented here for the activation of the thylakoid-bound degradation system described in this work should be considered only as a working hypothesis to be further supported by experimental data.

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