Proc. Natl. Acad. Sci. USA
Vol. 78, No. 3, pp. 1572–1576, March 1981
Biochemistry

The rapidly metabolized 32,000-dalton polypeptide of the chloroplast is the "proteinaceous shield" regulating photosystem II electron transport and mediating diuron herbicide sensitivity

(*Spirodela*/thylakoids/triazine/photosynthesis)

AUTAR K. MATTOO*†, URI PICK‡, HEDDA HOFFMAN-FALK*, AND MARVIN EDELMAN*

Departments of *Plant Genetics and ‡Biochemistry, The Weizmann Institute of Science, Rehovot, Israel

Communicated by Martin Gibbs, December 5, 1980

ABSTRACT **Mild trypsin treatment of *Spirodela oligorrhiza* thylakoid membranes leads to partial digestion of the rapidly metabolized, surface-exposed, 32,000-dalton protein. Under these conditions, photoreduction of ferricyanide becomes insensitive to diuron [3-(3,4-dichlorophenyl)-1,1-dimethylurea], an inhibitor of photosystem II electron transport. Preincubation of thylakoids with diuron leads to a conformational change in the 32,000-dalton protein, modifying its trypsin digestion and preventing expression of diuron insensitivity. Finally, light affects the susceptibility of the 32,000-dalton protein to digestion by trypsin. In other experiments, thylakoids specifically depleted in the 32,000-dalton protein were found to be deficient in electron transport at the reducing side of photosystem II but not at the oxidizing side or in photosystem I activities. Thus, the rapidly metabolized 32,000-dalton thylakoid protein in *Spirodela* chloroplasts fulfills the requirements of the hypothesized "proteinaceous shield" [Renger, G. (1976) *Biochim. Biophys. Acta* 440, 287–300] regulating electron flow through photosystem II and mediating diuron sensitivity.**

The use of mild trypsin digestion to probe the structure–function relationship of surface-exposed thylakoid membrane proteins led Renger (1) in 1976 to infer the existence of a "proteinaceous shield" covering the primary electron acceptor of photosystem II (PS II) and acting as a regulator of electron flow between PS II and photosystem I (PS I) in the chloroplast. In addition, the effect (2) of trypsin in preventing inhibition of PS II electron transport caused by the herbicide diuron [DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea] led Renger to postulate a reversible binding of the herbicide to the proteinaceous shield (1). Several authors have further investigated trypsin-induced, diuron-insensitive electron transport (3–7) as well as differential binding of herbicides to PS II components (8–10) in an attempt to focus on the identity of the PS II regulatory protein. In an intriguing communication (4) a thylakoid fraction enriched in PS II particles (identity of organism not supplied) displayed a markedly altered polypeptide pattern on polyacrylamide gels after treatment with trypsin. Prominent among the several changes noted was the disappearance of a band with a mobility on $NaDodSO_4$ gels corresponding to 32,000 daltons (Dal).§

We have been studying a rapidly synthesized thylakoid protein in *Spirodela oligorrhiza* that has a molecular weight of 32,000 on $NaDodSO_4$/polyacrylamide gels (12, 13). This polypeptide is the main membrane component synthesized by the chloroplast protein-synthesizing system and is produced in quantities approaching those of the very abundant large subunit of ribulose-bisphosphate carboxylase. However, the 32-kDal protein does not accumulate, turning over at a rate 50 to 60 times more rapid than that of the large subunit of carboxylase or the apoprotein of the chlorophyll *a*/*b* light-harvesting complex (LHCP) (14). In *Spirodela* this rapidly metabolized thylakoid protein is translated by a discrete poly(A)$^-$ plastid messenger RNA of ≈500 kDal (15) into a 33.5-kDal precursor molecule, which is speedily processed into the mature 32-kDal form (13). Differentiated thylakoids are a prerequisite for 33.5-kDal protein synthesis (16). In addition, the whole process of 33.5/32-kDal synthesis, maturation, and degradation is under tight inductive control by light (17, 18).

The rapidly metabolized 32-kDal thylakoid protein of *Spirodela* has its counterpart in other higher plants and algae. We have recently compared partial proteolytic digestion patterns of the photogene protein from maize (19), peak D from peas (20), and protein D-1 from *Chlamydomonas* (21) with the pattern of the 32-kDal protein in *Spirodela* and found them to be virtually identical (14).

The function of the 32-kDal thylakoid protein has, so far, eluded discovery. It does not appear to be involved in thylakoid biogenesis, being poorly synthesized in expanding juvenile tissue but vigorously synthesized in mature plants (22, 23). Although rapidly metabolized and light induced, this protein is not rate limiting for CO_2 fixation. Apparently, the dark reactions occurring in the stroma that limit the rate of photoassimilation of CO_2 do so even at 20% the normal level of the 32-kDal membrane protein, a factor not sufficiently appreciated previously (22). In the present report we present several lines of evidence correlating the rapidly metabolized 32-kDal polypeptide of *Spirodela* thylakoids with the regulatory PS II shield protein hypothesized by Renger (1). This protein is also shown to be responsive to the herbicide diuron.

MATERIALS AND METHODS

Isolation of Thylakoid Membranes. Axenic *Spirodela oligorrhiza* (Kurtz) Hegelm was cultured phototrophically (2150 lux, 25°C) in half-strength Hutner's medium (cf. ref. 24). Washed fronds (25 g), suspended in 3 vol of ice-cold 0.4 M

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: diuron, 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU); PS I, photosystem I; PS II, photosystem II; Dal, dalton; LHCP, chlorophyll *a*/*b* light harvesting complex; Tricine, *N*-[tris(hydroxymethyl)methyl]glycine; STN, sucrose/Tricine/NaCl; Chl, chlorophyll; Cl_2indophenol, 2,6-dichloroindophenol; FCCP, carbonyl cyanide *p*-trifluoromethoxy phenylhydrazone.

† Present address: Post Harvest Physiology Laboratory, Agricultural Research Center (West), U.S. Department of Agriculture, Beltsville, MD 20705.

§ In a lecture presented at the European Molecular Biology Organization Workshop on Chloroplast Membrane Polypeptides, Copenhagen, June 1979, C. J. Arntzen described a high-fluorescence mutant in maize (11) that lacked a 32-kDal polypeptide and certain PS II electron transport activities.

sucrose/0.01 M *N*-[tris-(hydroxymethyl)methyl]glycine (Tricine)–NaOH (pH 8 at 20°C)/0.01 M NaCl (STN solution) were blended in an Omnimixer (Sorvall) for 10 sec at maximal speed and 15 sec at half-maximal speed. The slurry was filtered through eight layers of cheesecloth and centrifuged at 500 × *g* for 30 sec. The supernatant was collected and centrifuged at 6000 × *g* for 7 min. The residue obtained was resuspended with a Teflon/glass homogenizer in 30 ml of STN solution containing 5 mM $MgCl_2$ (STN/Mg) and recentrifuged at 500 × *g* and at 6000 × *g* as described above. The resulting residue, containing thylakoid membranes, was suspended in a minimal volume (0.1 ml per g fresh weight of original tissue) of STN/Mg solution. Chlorophyll (Chl) was determined in 80% (vol/vol) acetone extract of thylakoid membranes (25).

Trypsin Digestion of Labeled Thylakoid Membranes. [^{35}S]Methionine-labeled thylakoid membranes, equivalent to 150–200 μg of Chl/ml, were digested with trypsin (Worthington) at 1.25–50 μg/ml in 10 mM Tris·HCl, pH 7.6, at 22°C for various times. The reaction mixtures were shaken intermittently during incubation. Trypsin digestion was stopped by addition of a 10-fold excess of soybean trypsin inhibitor (Worthington) and phenylmethylsulfonyl fluoride (Sigma) to a final concentration of 2 mM. The suspension was diluted 1:20 with ice-cold STN or STN/Mg solution and centrifuged at 10,000 × *g* for 15 min. The supernatant was discarded and the residue was dissolved at room temperature in a minimal volume of sample application buffer [30% (vol/vol) glycerol/15% (vol/vol) 2-mercaptoethanol/9% $NaDodSO_4$/0.19 M Tris·HCl (pH 6.8)/0.002% bromophenol blue] for gel electrophoresis (22).

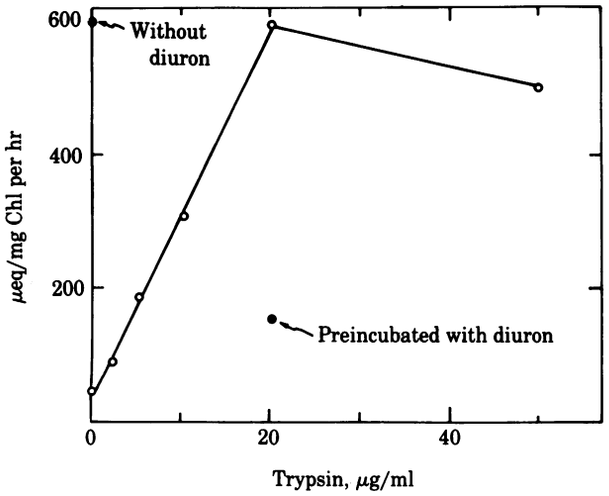

FIG. 1. Trypsin-induced, diuron-insensitive ferricyanide reduction by *Spirodela* thylakoids. Thylakoids equivalent to 50 μg of Chl were incubated with 30 mM Hepes–NaOH, pH 7.0/30 mM KCl/5 mM $MgCl_2$/5 mM NaN_3/3 μg of gramicidin/0.6 mM $K_3Fe(CN)_6$ and trypsin at the indicated concentrations in a total volume of 3 ml (○). A sample was preincubated for 5 min with 10 μM diuron before the addition of trypsin (final concentration 20 μg/ml) (●, preincubated with diuron). The reaction mixtures were incubated in the dark for 5 min at 23°C. Trypsin digestion was stopped by adding 5 μl of soybean trypsin inhibitor solution to a 10-fold excess over trypsin concentration. Ferricyanide reduction, followed as the light-induced decrease in absorbance at 420 nm, was measured in the presence of 2 μM diuron in a Cary 14 spectrophotometer. Illumination was provided from a tungsten lamp fitted with a Corning CS 2-62 filter. The photomultiplier was protected from the actinic light by Wratten 34 and Corning CS 4-96 filters. The intensity of the excitation light in the cuvette was 0.039 J·cm^2·sec^{-1}. The rates of activity of control samples that were not treated with trypsin were also measured in the absence (●, without diuron) and presence of 2 μM diuron (○, on *y* axis).

RESULTS

Photosynthetic membrane preparations isolated from higher plants are capable of reducing ferricyanide, an artificial PS II electron acceptor. Addition of diuron inhibits this reaction. The herbicide effect can be eliminated, however, by a preincubation of the thylakoid preparations with trypsin (cf. ref. 1). The behavior of *Spirodela* thylakoid membranes in these reactions is shown in Fig. 1. Thylakoid preparations isolated from light-grown plants reduced ferricyanide to ferrocyanide at a rate of 600 μeq/mg of Chl per hr; addition of 2 μM diuron severely inhibited this reduction to a rate of 40 μeq/mg of Chl per hr. Incubation of the thylakoid preparations with increasing concentrations of trypsin prior to the addition of diuron resulted in virtual cancellation of the inhibition at a concentration of 20 μg of enzyme per ml. Thus, *Spirodela* thylakoids behave in a classical Renger fashion (1) as regards trypsin-induced, diuron-insensitive electron transport.

Fig. 1 also shows that preincubation of the *Spirodela* thylakoid membranes with diuron prevents, to a large extent, the effect of the proteolytic enzyme. We have repeatedly observed this protective effect of diuron at a concentration of trypsin (20 μg/ml) optimal for expression of diuron insensitivity. Thus, diuron inhibition and trypsin digestion appear to be acting in close concert, possibly through an effect on a common proteinaceous entity. Considering these results, we thought it worth investigating whether trypsin digestion of the thylakoid membranes might be hampered or altered in some demonstrable

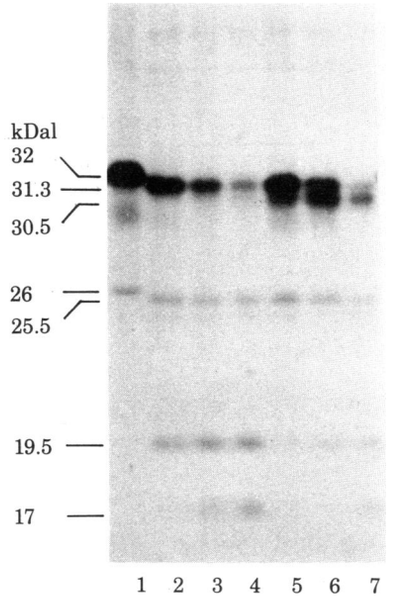

FIG. 2. Polyacrylamide gel fractionation of [^{35}S]methionine-labeled thylakoid proteins after trypsin digestion in the absence and presence of diuron. Steady-state, light-grown (3230 lux, 25°C) *Spirodela* plants were placed for 2 hr in medium containing 100 μCi (3.7 × 10^6 becquerels) of [^{35}S]methionine per ml, washed, and mixed with an equal weight of unlabeled plants. Thylakoid membranes were isolated and samples (200 μl) were preincubated for 10 min in the dark or in the light with diuron in methanol (25 μM diuron per mg of Chl) (lanes 5–7) prior to trypsin digestion. Control samples lacking diuron received equal volumes of methanol (lanes 2–4). Thylakoid membranes (0.15 mg of Chl per ml) suspended in STN/Mg solution were incubated in the light or in the dark at 23°C in 10 mM Tris·HCl, pH 7.6, with trypsin (50 μg/ml; trypsin-to-Chl weight ratio 0.33) for 10 (lanes 2 and 5), 30 (lanes 3 and 6), and 60 (lanes 4 and 7) min. The untreated (lane 1) and trypsin-treated samples (lanes 2–7) were fractionated on $NaDodSO_4$/polyacrylamide slab gels and fluorographed. Only dark-incubated samples are shown.

fashion in the presence of diuron. Our approach was to screen newly synthesized (radiolabeled) thylakoid proteins rather than simply the most abundant ones. The radioactive tracer approach is a sensitive one, and in addition we were guided by studies showing a close relationship between light-induced electron flow and rapid synthesis and metabolism of certain thylakoid proteins in *Spirodela* (18).

Fig. 2 shows the $NaDodSO_4$/polyacrylamide gel pattern of [^{35}S]methionine-labeled proteins from thylakoid membranes of *Spirodela* treated with trypsin in the absence and presence of diuron. The major labeled polypeptide in the nondigested control (lane 1) is the chloroplast-translated 32-kDal protein (13, 17). The band of 26 kDal is the apoprotein of the LHCP. At 50 μg of trypsin per ml the 32-kDal protein in the thylakoids is attacked, yielding (Fig. 2, lanes 2, 3, and 4) a digestion product of 31.3 kDal, which, with increasing times of incubation, is further digested to fragments of 19.5 kDal and then 17 kDal. In addition to the products formed from digestion of the 32-kDal protein, trypsin treatment of the 26-kDal LHCP yields a band of 25.5 kDal. The origin of other, fainter, bands (e.g., that at 24.5 kDal, Fig. 3*A*) has not been explored.

When similar thylakoid samples were preincubated in the dark or in the light with 10 μM diuron for 10 min and then trypsin at 50 μg/ml was added as above, a specific change in the digestion pattern of the 32-kDal protein resulted. As seen in Fig. 2 (lanes 5, 6, and 7), in addition to the 31.3-kDal digestion product, a prominent polypeptide of 30.5 kDal appears. The accumulation of this digestion product is at the expense of the 19.5-kDal and 17-kDal fragments, whose amounts are less in these lanes than in the samples not incubated with diuron.

Similar experiments were carried out at two trypsin concentrations (1.25 and 20 μg/ml) chosen to represent the extremes in the rate of diuron-insensitive ferricyanide reduction obtained with enzyme-treated thylakoids (cf. Fig. 1). These results are shown in Fig. 3*A*. Only at 20 μg of trypsin per ml was the 32-kDal protein digested to 31.3 kDal (lanes 8–11) and, in the presence of diuron, protected from further digestion (lanes 12–15). At 1.25 μg of trypsin per ml no proteolytic effect or effect of diuron on the 32-kDal polypeptide could be discerned (compare lanes 2–7 with the controls in lanes 1 and 16). Thus, concentration dependence of the trypsin effect on diuron-insensitive ferricyanide reduction parallels that for digestion of the 32-kDal protein. The specificity of this effect is underlined by comparing the changes occurring in the same samples to LHCP at the two enzyme concentrations. At 1.25 μg of trypsin per ml the 26-kDal band is already partially converted to the 25.5-kDal product (compare lanes 2–4 with lane 1), the process reaching completion with 20 μg of trypsin per ml (lanes 8–10). Thus at enzyme concentrations that do not affect the ferricyanide reduction (cf. Fig. 1) but that do affect the ability of the thylakoids to stack (26, 27), the digestion of 26-kDal protein commences. Fig. 3*A* also indicates a slight retardation in the proteolysis of 26-kDal protein in diuron-treated thylakoids (e.g., compare lanes 3 and 4 with lanes 6 and 9). In overexposures of the fluorographs from both Figs. 2 and 3*A*, more than 20 bands could be visualized. Aside from those at 32 and 26 kDal, no other

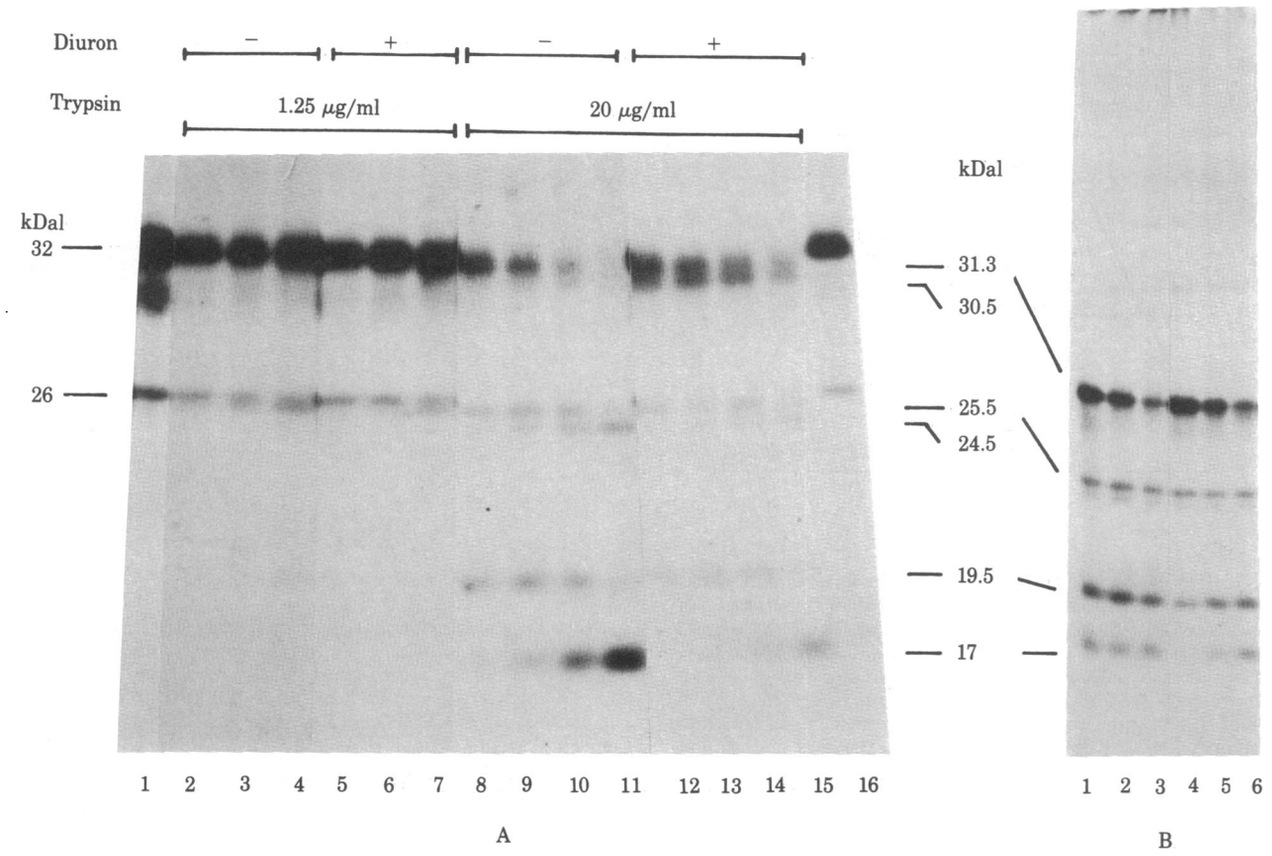

FIG. 3. (*A*) Polyacrylamide gel fractionation of [^{35}S]methionine-labeled proteins from thylakoid membranes treated with trypsin at two concentrations. Samples not treated with trypsin are in lanes 1 and 16. Trypsin digestion of thawed thylakoid membranes equivalent to 75 μg of Chl per ml (trypsin-to-Chl weight ratios 0.016 and 0.26) was carried out for 5 (lanes 8 and 12), 10 (lanes 2, 5, 9, and 13), 30 (lanes 3, 6, 10, and 14), and 60 (lanes 4, 7, 11, and 15) min. Samples in lanes 5–7 and 12–15 were preincubated with diuron. For other details see legend to Fig. 2. (*B*) Influence of light on trypsin digestion of the 32-kDal protein in the thylakoid. Lanes 1–3, incubation in the light (3230 lux); lanes 4–6, incubation in the dark. Incubation times are 10 (lanes 1 and 4), 30 (lanes 2 and 5), and 60 (lanes 3 and 6) min.

polypeptide band exhibited differential proteolysis upon addition of diuron (28).

Trypsin digestion of the 32-kDal protein in the thylakoid is enhanced in the light. This can be noted in Fig. 3*B*, particularly in the 17-kDal region of the polyacrylamide gel. For example, the 17-kDal band is already present at the shortest incubation time in the light (lane 1) while it is absent from the corresponding digestion pattern in the dark (lane 4).

The possible relationship between PS II electron transport and the 32-kDal membrane protein was further studied by comparing control and "32-kDal-depleted" thylakoids. In *Spirodela*, advantage can be taken of the rapid rate of 32-kDal-protein turnover and the inhibitory effect of chloramphenicol on chloroplast protein synthesis to obtain thylakoids selectively reduced in 32-kDal protein to a level 20% of that in untreated plants (22). The ability of the control and 32-kDal-depleted thylakoids to catalyze electron transport reactions via different sections of the electron transport chain is shown in Fig. 4. The calculated initial rates for the photoreactions measured are summarized in Table 1. Electron transport activity on the oxidizing side of PS II, from water to silicomolybdate, was the same in both types of thylakoids. Similarly, totally PS I-dependent electron transport reactions, from ascorbate plus 2,6-dichloroindophenol (Cl_2indophenol) or diaminodurene to methyl viologen, were not hindered in the 32-kDal-depleted thylakoids. In contrast, electron transport reactions dependent on PS II, from water to ferricyanide and from water to methyl viologen (which includes the PS II to plastoquinone section), were markedly decreased in the 32 kDal-depleted thylakoids (Fig. 4, Table 1).

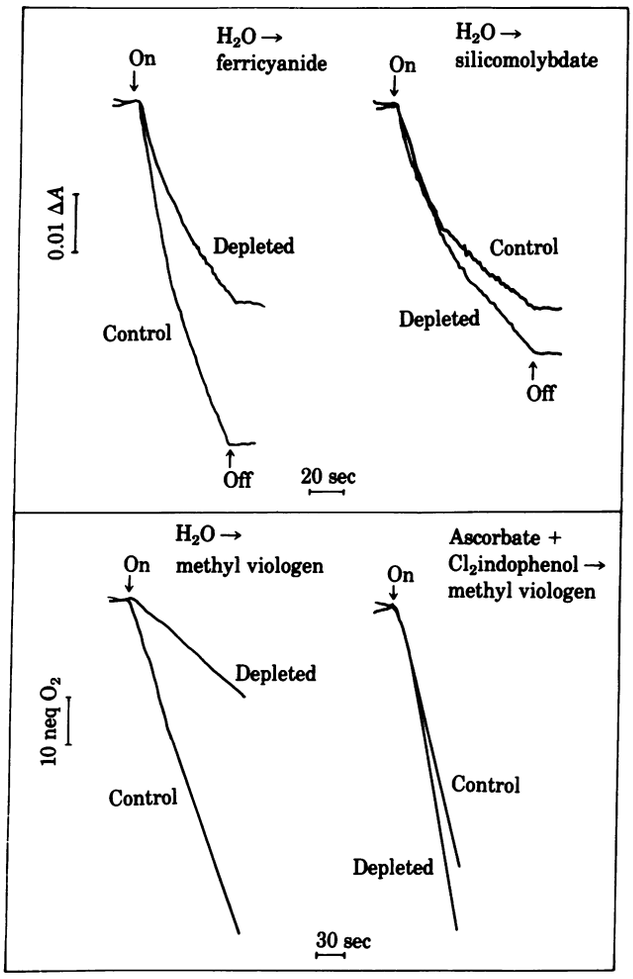

FIG. 4. Light-dependent electron transport in control and 32-kDal-depleted thylakoids of *Spirodela oligorrhiza*. Thylakoid membranes were isolated from normally grown cultures (control) and 32-kDal-depleted cultures (depleted) as described in the text. Ferricyanide reduction was determined in the absence ($H_2O \rightarrow$ ferricyanide) and presence ($H_2O \rightarrow$ silicomolybdate) of 0.2 mg of silicomolybdate in the reaction mixture described in the legend to Fig. 1, omitting trypsin and diuron. Oxygen uptake was measured with a Clark oxygen electrode (Yellow Springs Instrument). Illumination was provided from a tungsten lamp fitted with a Schott 00-530 filter. The light intensity was 0.17 $J \cdot cm^{-2} \cdot sec^{-1}$. Oxygen uptake from H_2O to methyl viologen was measured in a reaction mixture that contained, in 2.0 ml: 30 mM Tricine/NaOH (pH 7.2), 30 mM KCl, 5 mM $MgCl_2$, 5 mM NaN_3, 5 μM carbonyl cyanide *p*-trifluoromethoxy phenylhydrazone (FCCP), 0.2 mM methyl viologen, and chloroplasts equivalent to 70–80 μg of Chl. Oxygen uptake from ascorbate and Cl_2indophenol to methyl viologen was measured in a reaction mixture that contained, in 2.0 ml: 30 mM Hepes/NaOH (pH 7.0), 30 mM KCl, 5 mM $MgCl_2$, 5 mM NaN_3, 5 μM FCCP, 50 μM Cl_2indophenol, 4 mM sodium ascorbate, 10 μM diuron, and chloroplasts equivalent to 50 μg of Chl.

Table 1. Electron transport in control and 32-kDal-depleted thylakoids of *Spirodela oligorrhiza*

Reaction measured	Initial rate of electron transport, μeq/mg Chl per hr*		
	Control thylakoids	32-kDal-depleted thylakoids	Depleted/control
H_2O to silicomolybdate†	339	474	1.4
H_2O to ferricyanide†	705	385	0.6
H_2O to methyl viologen†	204	92	0.5
Diaminodurene + ascorbate to methyl viologen‡	854	819	1.0
Cl_2indophenol + ascorbate to methyl viologen†	660	864	1.3

* The rate of each partial reaction was calculated from the slope of the initial linear phase of traces (e.g., see Fig. 4). For the reactions with methyl viologen, μeq refers to O_2.

† Determinations were made in the reaction mixtures described in the legends to Figs. 1 and 4.

‡ O_2 uptake was measured in a 2.0-ml reaction mixture that contained: 30 mM Tricine/NaOH (pH 7.2), 30 mM KCl, 5 mM $MgCl_2$, 5 mM NaN_3, 5 μM diaminodurene, 5 μM FCCP, 10 μM diuron, 2 mM sodium ascorbate, 0.2 mM methyl viologen, and chloroplasts equivalent to 75 μg of Chl.

DISCUSSION

The main theme of this work has been the macromolecular identity of the proteinaceous shield, postulated by Renger (1) to regulate PS II activity and responsible for the inhibitory effects of diuron-type herbicides in plants (6). In Renger's model, trypsin is assumed to effectively digest the proteinaceous shield in isolated thylakoids. This leads to a functional inhibition of electron transport between the primary electron acceptor of PS II and the plastoquinone pool. At the same time digestion of the shield bares the primary acceptor of PS II to external redox agents. It is assumed that light-induced conformational changes occur *in vivo* in the proteinaceous shield, permitting allosteric regulation of electronic interaction between the primary electron acceptor of PS II and the plastoquinone pool. Renger (1) further hypothesized that the well-known inhibitory effect of diuron was a result of reversible binding of the herbicide to a site on or close to the shield, leading to allosteric inhibition of electron transfer from the primary acceptor to its connector molecule (plastoquinone B).

The results presented in this paper identify the proteinaceous shield as the rapidly metabolized 32-kDal protein in *Spirodela* chloroplasts. As required by Renger's hypothesis: (*i*) The 32-kDal protein is located at the outer surface of the thylakoid membrane. This can be seen from its partial susceptibility in membrane preparations to proteolytic digestion by the large, water-soluble enzyme trypsin (also see ref. 19). (*ii*) In isolated thylakoids the 32-kDal protein is digested by trypsin specifically at concentrations that induce diuron-insensitive PS II electron transport. (*iii*) Its availability as a substrate for proteolytic digestion is modulated by light. (*iv*) In thylakoid preparations largely depleted of the 32-kDal protein, electron transfer is specifically inhibited at the reducing side of PS II and between the two photosystems. (*v*) Incubation of isolated thylakoids with the herbicide diuron markedly alters trypsin digestion of the 32-kDal protein and protects the electron transport system from the trypsin digestion effects.

Fractionation of Triton-dissolved thylakoids in sucrose gradients has shown that the rapidly labeled 32-kDal protein from *Spirodela* does not copurify with polypeptides of PS I particles or the CF_1-CF_0 ATPase complex (unpublished data). Instead, using two separate methods (4, 27), we find an association of the 32-kDal protein with PS II-related particles and the LHCP (unpublished data). In this regard we note the dual conformational effects on the 32-kDal protein and LHCP brought about by diuron (cf. Fig. 3), which may also indicate a certain proximity of the two in the thylakoid. Taking all of these data into consideration, the 32-kDal protein from *Spirodela* seems to answer the description of the proteinaceous shield regulating PS II electron flow.

On the basis of the trypsin digestion and electron transport data, an initial delineation of the functional domains of the 32-kDal protein can be made: A 17-kDal section of the molecule acts as a tightly bound membrane anchor. This half of the polypeptide is impervious to prolonged incubation with trypsin at 20–50 μg/ml (cf. Fig. 3, lane 11). However, once the protein is removed from the photosynthetic membranes by detergent, proteolysis of the 17-kDal segment becomes rapid, even at much lower enzyme concentrations (not shown). An additional 2.5-kDal piece of the molecule, contiguous with this anchor section, is partially protected from trypsin digestion (i.e., the 19.5-kDal fragment). The remaining 12.5-kDal piece, on the other hand, is more prone to proteolysis and, hence, is postulated to extend beyond the outer thylakoid surface into the stroma (see also ref. 19). Within this "stromal domain" there lies, however, at least one trypsin site that is normally protected from digestion. This residue [most probably arginine, because in *Spirodela* the 32-kDal protein appears to be devoid of lysine (13)], defining a 30.5-kDal fragment, becomes susceptible to proteolysis upon incubation of the thylakoids with the herbicide diuron. Indeed, in the presence of diuron, the 32-kDal protein apparently undergoes a conformational change such that as one site (where cleavage produces a 30.5-kDal fragment) is newly revealed, other sites (where cleavage yields 19.5- and 17-kDal fragments) become more resistant to enzymatic attack. Finally, less than 1 kDal from an end of the 32-kDal molecule, a trypsin site exists whose proteolysis (resulting in a 31.3-kDal fragment) opens the way for external redox agents to reach the primary electron acceptor for PS II such that flow of electrons through the system is possible even in the presence of diuron.

The present experiments were not designed to determine if the herbicide diuron exerts its conformational effect on the 32-kDal protein by binding directly to it or indirectly via another polypeptide in its vicinity. The secondary electron acceptor for PS II, plastoquinone B, has been suggested by some as the site for diuron binding (3, 8). The relationship of triazine-type herbicides to the 32-kDal protein also seems ripe for investigation. Atrazine [2-chloro-4-(2-propylamino)-6-ethylamino-*s*-triazine] appears to produce an effect, albeit weaker, on the trypsin digestion pattern of rapidly labeled thylakoid proteins of *Spirodela* similar to that produced by diuron (unpublished data). Pfister *et al.* (8) have suggested that the sites of action of diuron and atrazine partially overlap. In studies to be reported elsewhere, we showed that the 32-kDal protein is vigorously synthesized in both atrazine-resistant and atrazine-susceptible biotypes of *Solanum nigrum*, *Chenopodium album*, and *Brassica campestris*. Thus, if both diuron and atrazine are indeed affecting the 32-kDal thylakoid protein, resistance in these cases could be related to a slight structural alteration or conformational change in the molecule.

We thank Mordehay Avron for many fruitful discussions, suggestions, and critical reading of the manuscript. We also thank Jonathan Gressel, Karl Jakob, Robert Fluhr, and Jonathan Marder for helpful comments concerning the manuscript. A.K.M. is a recipient of a Deutscher Akademischer Austauschdienst Fellowship. This study was supported in part by a United States–Israel Binational Science Foundation Research Grant to M.E.

1. Renger, G. (1976) *Biochim. Biophys. Acta* **440**, 287–300.
2. Regitz, G. & Ohad, I. (1976) *J. Biol. Chem.* **251**, 247–252.
3. Trebst, A. (1979) *Z. Naturforsch.* **34C**, 986–991.
4. Croze, E., Kelly, M. & Horton, P. (1979) *FEBS Lett.* **103**, 22–26.
5. Steinback, K. E., Burke, J. J. & Arntzen, C. J. (1979) *Arch. Biochem. Biophys.* **195**, 546–557.
6. Van Rensen, J. J. S. & Kramer, H. J. M. (1979) *Plant Sci. Lett.* **17**, 21–27.
7. Böger, P. & Kunert, K.-J. (1979) *Z. Naturforsch.* **34C**, 1015–1020.
8. Pfister, K., Radosevich, S. R. & Arntzen, C. J. (1979) *Plant Physiol.* **64**, 995–999.
9. Arntzen, C. J., Ditto, C. L. & Brewer, P. E. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 278–282.
10. Trebst, A. & Draber, W. (1979) in *Advances in Pesticide Science*, ed. Greissbuhler, H. (Pergamon, Oxford), Part 2, pp. 223–234.
11. Leto, K. J. & Miles, D. (1980) *Plant Physiol.* **66**, 18–24.
12. Edelman, M. & Reisfeld, A. (1978) in *Chloroplast Development*, eds. Akoyunoglou, G. & Argyroudi-Akoyunoglou, J. H. (Elsevier/North-Holland, Amsterdam), pp. 641–652.
13. Edelman, M. & Reisfeld, A. (1980) in *Genome Organization and Expression in Plants*, ed. Leaver, C. J. (Plenum, New York), pp. 353–362.
14. Hoffman-Falk, H., Mattoo, A. K., Marder, J., Fluhr, R. & Edelman, M. (1980) *Proceedings of the 13th FEBS Meeting*, p. 22 (abstr.).
15. Reisfeld, A., Jakob, K. M. & Edelman, M. (1978) in *Chloroplast Development*, eds. Akoyunoglou, G. & Argyroudi-Akoyunoglou, J. H. (Elsevier/North-Holland, Amsterdam), pp. 669–674.
16. Mattoo, A. K., Edelman, M. & Gressel, J. (1980) *Plant Physiol.* Suppl. **65**, 21 (abstr.).
17. Reisfeld, A., Gressel, J., Jakob, K. M. & Edelman, M. (1978) *Photochem. Photobiol.* **27**, 161–165.
18. Mattoo, A. K., Edelman, M. & Gressel, J. (1980) *Proceedings of the 13th FEBS Meeting*, p. 22 (abstr.).
19. Grebanier, A. E., Coen, D. M., Rich, A. & Bogorad, L. (1978) *J. Cell Biol.* **78**, 734–746.
20. Blair, G. E. & Ellis, R. J. (1974) *Biochim. Biophys. Acta* **319**, 223–234.
21. Chua, N.-H. & Gillham, N. W. (1977) *J. Cell Biol.* **74**, 441–452.
22. Weinbaum, S. A., Gressel, J., Reisfeld, A. & Edelman, M. (1979) *Plant Physiol.* **64**, 828–832.
23. Silverthorne, J. & Ellis, R. J. (1980) *Biochim. Biophys. Acta* **607**, 319–330.
24. Posner, H. B. (1967) in *Methods in Developmental Biology*, eds. Witt, F. A. & Wessels, N. K. (Crowell, New York), pp. 301–317.
25. Arnon, D. I. (1949) *Plant Physiol.* **24**, 1–15.
26. Carter, D. P. & Staehlin, L. A. (1980) *Arch. Biochem. Biophys.* **200**, 374–386.
27. Burke, J. J., Ditto, C. L. & Arntzen, C. J. (1978) *Arch. Biochem. Biophys.* **187**, 252–263.
28. Hoffman-Falk, H. (1980) Dissertation (Weizmann Institute of Science, Rehovot, Israel).