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RESOLUTION OF THE LIGHT-DEPENDENT MODULATION SYSTEM OF PEA CHLOROPLASTS *

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The mechanism of the light-dependent inactivation of glucose-6-phosphate dehydrogenase and the light-dependent activation of NADP$^+$-malate dehydrogenase has been studied in partially purified extracts of pea (*Pisum sativum*) chloroplasts. Neither partially purified enzyme could be light modulated by washed thylakoids alone. However, a factor (mol. wt. 50 000) was present in the stroma which could, when added to purified enzyme and thylakoid membranes, reconstitute a light-dependent modulation of either glucose-6-phosphate dehydrogenase or NADP$^+$-malate dehydrogenase. This factor, which we term protein-modulating factor, is distinct from ferredoxin-thioredoxin reductase and from thioredoxin, the factors involved in another scheme for light modulation. The scheme proposed here for light modulation involves electron transfer from Photosystem I to a membrane-bound light-effect mediator and then to the soluble protein modulating factor which modulates chloroplast enzyme activity, probably by reduction of a regulatory disulfide bond.

Introduction

An increasing number of plant enzymes that exhibit altered activity upon illumination of the plant have been described (for reviews see Refs. 1 and 2). This modulation is not a transient allosteric modulation but apparently the result of a covalent modification of preexisting enzyme molecules. The modulation (which is mimicked by reducing agents such as dithiothreitol) appears to involve a change in the thiol groups of the modulated enzyme, probably the reduction of a cystine residue as the result of photosynthetic electron flow. This light-dependent modulation is responsible, in part, for the coordinated changes in metabolic pathways that are necessary upon illumination. Thus, some of the enzymes of the reductive pentose phosphate cycle as well as NADP$^+$-malate dehydrogenase are activated while enzymes of starch degradation are inactivated in the light [3].

Anderson and Avron [4] demonstrated that the light modulation of a variety of enzymes could occur in intact chloroplasts or in a broken chloroplast system with the thylakoids acting as the photoreceptor and the stromal fraction as the source of modulated enzyme. Various experiments showed that light modulation of NADP$^+$-malate dehydrogenase and glucose-6-phosphate dehydrogenase required electron flow through PS I [4,5] to an arsenite- and sulfite-sensitive pre-ferredoxin component called LEM_I (light-effect mediator) [4]. We have attempted to define further this light modulation system by purifying the components necessary for a minimal

* Preliminary accounts of this work have been published [7,8].

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Abbreviations: DTNB, dithionitrobenzoate; PS I, Photosystem I; Chl, chlorophyll; Tricine, *N*-tris(hydroxymethyl)-methylglycine; Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid.

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light-dependent enzyme-modulation system. We have used two enzymes as 'substrates' for the light-modulation system, glucose-6-phosphate dehydrogenase which is inactivated in the light and $NADP^+$-malate dehydrogenase which is activated in the light. The opposite light-dependent responses of these two enzymes provided a control for the specificity of the reconstituted light-modulation system. Our approach to elucidating the mechanism of light modulation, using thylakoid membranes as the photoreceptor and fractionating stroma, is broader in principle than the approach which assumes that light-dependent regulation occurs via dithiothreitol-reducible factors.

We present evidence here that the light inactivation of glucose-6-phosphate dehydrogenase and the light activation of $NADP^+$-malate dehydrogenase are mediated by a soluble factor, the protein-modulating factor. This light-modulation system differs from the ferredoxin-thioredoxin system described by Buchanan and co-workers [6].

Materials and Methods

Pea (*Pisum sativum* L., var. Little Marvel) plants were grown in vermiculite in a greenhouse. Chloroplasts were prepared as described by Cockburn et al. [9], but in the absence of sodium isoascorbate, and were washed once in 0.33 M sorbitol, 50 mM Hepes (K^+), 2 mM $MgCl_2$ and 2 mM EDTA (K^+), pH 7.2. Chloroplasts were lysed by resuspension in distilled H_2O and adjusted to 50 mM Hepes (K^+), 1 mM EDTA, 2 mM $MgCl_2$ and 10 mM KCl, pH 7.4 (Hepes-KCl buffer) by addition of concentrated buffer. Unless metioned otherwise, all purification steps were conducted at 0–4°C.

Enzyme assays. The buffer used in assays for glucose-6-phosphate dehydrogenase and NADP-linked malate dehydrogenase was 25 mM Tricine, 1 mM EDTA adjusted to pH 8.3 with KOH. All enzyme activities were measured at room temperature (about 20°C) by following the absorbance change of pyridine nucleotide at 340 nm in the 1 ml reaction mixture using either a Cary 219 or a Gilford 2400 recording spectrophotometer or the fluorescence change using an Eppendorf 1100 photometer modified for fluorimetry.

The assay mixture for glucose-6-phosphate dehydrogenase (D-glucose-6-phosphate : $NADP^+$ 1-oxidoreductase, EC 1.1.1.49) was 1 mM in glucose-6-phosphate and 0.25 mM in $NADP^+$. The assay mixture for $NADP^+$-malate dehydrogenase (L-malate: $NADP^+$ oxidoreductase, EC 1.1.1.82) was 50 mM in L-malate (K^+), pH 8.3, and 0.25 mM in $NADP^+$ or 1 mM in oxaloacetate and 0.25 mM in NADPH. Under these conditions the reaction synthesizing malate is 5-fold faster than the reaction in the reverse direction. The reaction producing oxaloacetate (and NADPH), although slower, was the more sensitive when NADPH formation was followed fluorimetrically.

Assay of thioredoxin activity. Thioredoxin was measured by its ability to catalyze the dithiothreitol-dependent modulation of enzyme activity, i.e., activation of $NADP^+$-malate dehydrogenase or inactivation of glucose-6-phosphate dehydrogenase.

The 25-μl reaction mixture contained 10 μl of partially purified thioredoxin-free enzyme (10^{-3} U of either glucose-6-phosphate dehydrogenase or $NADP^+$-malate dehydrogenase, 10 μl sample containing thioredoxin and 5 μl dithiothreitol (25 mM for glucose-6-phosphate dehydrogenase inactivation or 250 mM for $NADP^+$-malate dehydrogenase activation) all in Hepes-KCl buffer. The reaction was started by addition of dithiothreitol, incubated for 15 min at 25°C and terminated by dilution into the appropriate enzyme assay mixture (1 ml final volume). Control assays to measure thioredoxin-independent activation ($NADP^+$-malate dehydrogenase) or inactivation (glucose-6-phosphate dehydrogenase) contained all components except thioredoxin.

Estimation of ferredoxin content of thylakoid membranes. Ferredoxin was measured by its ability to catalyze the photoreduction of $NADP^+$ by washed thylakoid membranes. The reaction mixture containing washed thylakoids (20 μg Chl/ml), an aliquot of stroma or buffer, 0.25 mM $NADP^+$, all in Hepes-KCl buffer was illuminated at 25°C. The increase in A_{340} was measured at 1 min intervals. For high-sensitivity measurements the thylakoid membranes were sedimented (10 000 $\times g$, 2 min) and the NADPH in the supernatant measured by spectrophotometry or fluorimetry. The photoreduction of $NADP^+$ by washed thylakoids in the absence of stroma (containing ferredoxin) was always at the detection limits of our assay system and thus estimates of the ferre-

doxin content of the thylakoids represent maximum possible values.

Light-modulation assays. Hepes-KCl buffer was used in all light-modulation assays. The assay mixture contained twice-washed thylakoid membranes (usually 0.3 to 1 mg Chl/ml), either partially purified $NADP^+$-malate dehydrogenase or glucose-6-phosphate dehydrogenase, and protein-modulating factor in 100 µl final volume. The mixture was illuminated ($59 \cdot 10^3$ lx) at 25°C, and the reaction terminated by dilution. All assays were conducted in air. Both light-modulated and chemically reduced $NADP^+$-malate dehydrogenase and glucose-6-phosphate dehydrogenase (data not shown) and chemically reduced $NADP^+$-malate dehydrogenase [10] are stable in air over the period of centrifugation before the enzyme assay. Two variations of this method were routinely used. The first (procedure 1) involved illumination in pyrex test-tubes (12 × 75 mm) and dilution with ice-cold H_2O followed by centrigugation using a Sorvall RC2B centrifuge (12 000 × *g* for 10 min at 4°C). A 0.5 ml aliquot of the supernatant solution was immediately added to the appropriate enzyme assay buffer and enzyme activity recorded. The second variation (procedure 2) involved illumination in 1.5 ml polypropylene centrifuge tubes, dilution directly into enzyme assay buffer (1 ml final volume) and centrifugation (10 000 × *g* for 1 min) in a Beckman microcentrifuge. The chamber of the microcentrifuge was precooled with solid CO_2 to ensure that the temperature of the chamber did not rise above room temperature during centrifugation. The enzyme activity of the supernatant solution was measured immediately.

Preparation of glucose-6-phosphate dehydrogenase. Glucose-6-phosphate dehydrogenase from pea chloroplasts was prepared as described previously [11] but for the gel filtration step Sephacryl S-200 was replaced by Sepharose CL-6B.

Preparation of $NADP^+$-malate dehydrogenase and thioredoxin. The fraction of stroma precipitating between 45 and 70% $(NH_4)_2SO_4$ saturation was redissolved in Hepes-KCl buffer and further purified by gel filtration on Sephacryl S-200 equilibrated in Hepes-KCl buffer. The $NADP^+$-malate dehydrogenase elutes as a single peak corresponding to a molecular weight of 100 000. Fractions containing $NADP^+$-malate dehydrogenase were pooled and frozen at −20°C in small aliquots until needed for thioredoxin assays.

Thioredoxin, which elutes as a molecular weight species of 10 000–20 000, was obtained from the same Sephacryl S-200 gel filtration. This thioredoxin fraction catalyzes the dithiothrietol-dependent activation of $NADP^+$-malate dehydrogenase and inactivation of glucose-6-phosphate dehydrogenase.

Protein and chlorophyll. Protein was measured by the procedure of Scopes [12] and chlorophyll by its absorbance at 652 nm in 80% acetone [13].

Materials. Sephacryl S-200 was obtained from Pharmacia, Piscataway, NJ. Biochemicals and Hepes were obtained from Sigma, St. Louis, MO, while all other chemicals were analytical reagent grade. Pea seeds were obtained from Northrup and King, Chicago.

Results

When glucose-6-phospate dehydrogenase was purified by gel filtration it could no longer be inactivated in the presence of light and thylakoid membranes. The glucose-6-phosphate dehydrogenase could still be inactivated by dithiothreitol alone or by light if it was incubated in the presence of stroma, indicating that the purified enzyme could still be inactivated in the same way as the enzyme of crude extracts. By combining illuminated thylakoids and glucose-6-phosphate dehydrogenase with fractions from the Sephacryl S-200 column, the system for light inactivation of glucose-6-phosphate dehydrogenase could be reconstituted. The profile in Fig. 1 shows that light inactivation of glucose-6-phosphate dehydrogenase is catalyzed by fractions 27 and 28. Fig. 2 shows that light inactivation is a time-dependent process resulting (in this case) in loss of 64% of the original glucose-6-phosphate dehydrogenase activity after 20 min illumination. Thioredoxin activity, catalyzing the dithiothreitol-dependent inactivation of glucose-6-phosphate dehydrogenase, could also be detected in the Sephacryl S-200 profile.

A time course of glucose-6-phosphate dehydrogenase inactivation catalyzed by the thioredoxin fraction is shown in Fig. 3. The inactivation of glucose-6-phosphate dehydrogenase is not absolutely dependent upon the presence of thioredoxin, since inactivation can occur in the presence of dithiothreitol alone. This

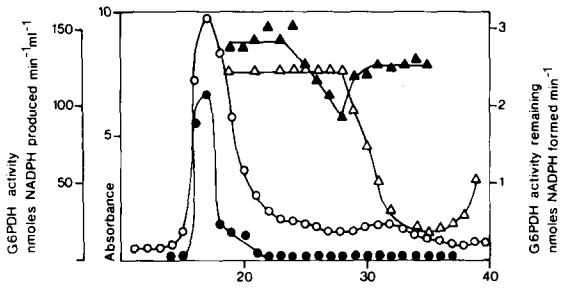

Fig. 1. Gel filtration profile of glucose-6-phosphate dehydrogenase (G6PDH)-inactivating factors. A 0–70% saturated $(NH_4)_2SO_4$ fraction (4.4 ml, 51 mg protein/ml) was chromatographed on a Sephacryl S-200 column (4.9 cm^2 X 43 cm, 5.25-ml fractions). The assay mixture for light inactivation of glucose-6-phosphate dehydrogenase contained washed thylakoids (2.2 mg Chl/ml), glucose-6-phosphate dehydrogenase and 25 μl of the appropriate fraction in a final volume of 100 μl: This assay followed procedure 2 described in Materials and Methods. The mixture was illuminated for 10 min at 25°C. A_{280}, ○; stromal glucose-6-phosphate dehydrogenase activity, ●; glucose-6-phosphate dehydrogenase activity remaining after thioredoxin-catalyzed inactivation, △; glucose-6-phosphate dehydrogenase activity remaining after light inactivation of a glucose-6-phosphate dehydrogenase fraction, ▲.

thioredoxin activity, however, was resolved from the light-inactivating factor, thioredoxin activity being most abundant in fraction 35 and not detectable in the fractions containing light-inactivating factor. The light-inactivating factor obtained from the Sephacryl S-200 column eluted in the same position after rechromatography under identical conditions where any residual thioredoxin contamination would be

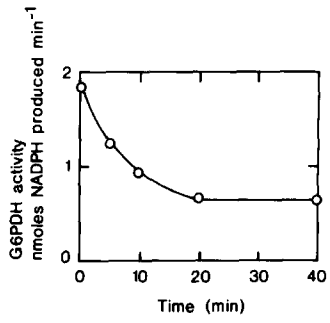

Fig. 2. Light-dependent inactivation of glucose-6-phosphate dehydrogenase (G6PDH) catalyzed by protein-modulating factor. The light inactivation of glucose-6-phosphate dehydrogenase was conducted as described in the legend to Fig. 1 using fraction 27 as source of the protein-modulating factor.

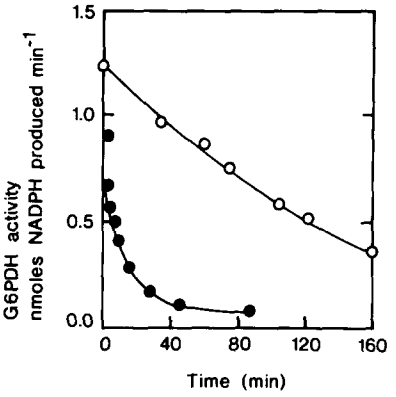

Fig. 3. Inactivation of glucose-6-phosphate dehydrogenase (G6PDH) by dithiothreitol and thioredoxin. The thioredoxin assay was conducted as described in Materials and Methods. Aliquots were removed at the appropriate time and assayed immediately for glucose-6-phosphate dehydrogenase activity. Thioredoxin plus dithiothreitol, •; dithiothreitol alone, ○.

removed. Incubation of glucose-6-phosphate dehydrogenase with fractions from the Sephacryl S-200 column in the absence of dithiothreitol or light plus thylakoids caused no inactivation of the glucose-6-phosphate dehydrogenase.

The addition of thioredoxin to this reconstituted

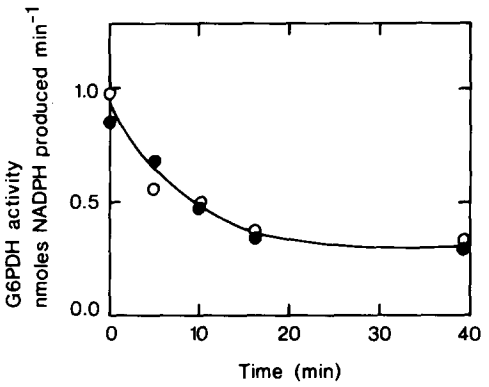

Fig. 4. Effect of added thioredoxin on light-dependent inactivation of glucose-6-phosphate dehydrogenase (G6PDH). The light inactivation was conducted using procedure 1 (Materials and Methods) in any assay mixture containing 0.22 mg Chl/ml. The thioredoxin included in the assay was sufficient to inactivate more than 95% of the glucose-6-phosphate dehydrogenase within 15 min in the standard thioredoxin assay. Inactivation in the presence of protein-modulating factor, ○; and of protein-modulating factor plus thioredoxin, •.

system did not alter the rate of light inactivation of glucose-6-phosphate dehydrogenase (Fig. 4). The amount of thioredoxin added would have inactivated more than 95% of the glucose-6-phosphate dehydrogenase if it had been reduced in this system.

Light activation of NADP$^+$-malate dehydrogenase

As was found for glucose-6-phosphate dehydrogenase, when malate dehydrogenase had been partially purified by gel filtration it could no longer be activated by light in the presence of thylakoid membranes but could be activated by incubation with dithiothreitol. A search of the Sephacryl S-200 profile for fractions that could reconstitute light activation of malate dehydrogenase revealed a peak of activity eluting after malate dehydrogenase (Fig. 5). The time course of light-dependent activation of malate dehydrogenase, shown in Fig. 6, was determined by measuring malate dehydrogenase fluorimetrically to enable the dark control malate dehydrogenase activity to be measured more precisely relative to the light-activated controls. It is apparent that the NADP$^+$-malate dehydrogenase is absolutely dependent upon light (or dithiothreitol in vitro) for activity. The activation obtained varies but is largely dependent upon residual active enzyme in the malate dehydrogenase preparation.

Thioredoxin activity was largely resolved from the fractions catalyzing the light-dependent activation of malate dehydrogenase. The apparent thioredoxin

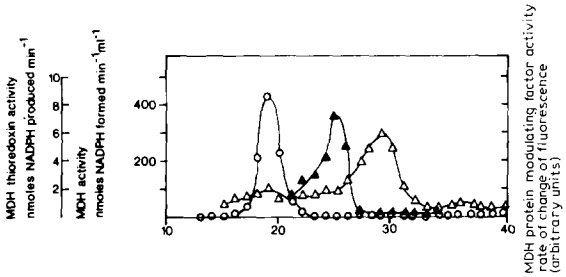

Fig. 5. Gel filtration profile of malate dehydrogenase activating factors. A 0–70% saturated $(NH_4)_2SO_4$ fraction was chromatographed on a Sephacryl S-200 column ($4.9^2 \times 38$ cm, 4.9-ml fractions). The assay for light activation of malate dehydrogenase contained washed thylakoids (0.4 mg Chl/ml) and followed procedure 1 as described in Materials and Methods. The mixture was illuminated for 35 min at 25°C. Malate dehydrogenase, ○; protein-modulating factor, ▲; thioredoxin, △; MDH, NADP-linked malic dehydrogenase.

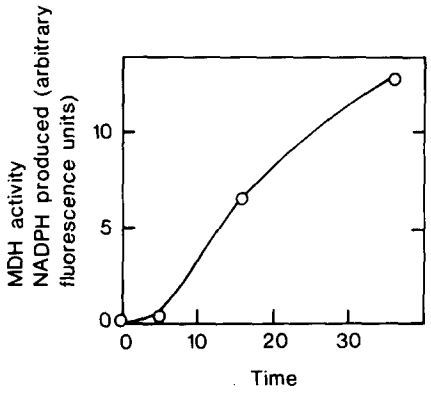

Fig. 6. Time course of the light-dependent activation of malate dehydrogenase (MDH). The activation was conducted as described in Materials and Methods using procedure 1 and the malate dehydrogenase activity was followed fluorometrically. The assay mixture contained 1.1 mg Chl/ml.

activity eluting as high molecular weight material is probably due to simple dithiothreitol-dependent activation of malate dehydrogenase present in that part of the profile. Rechromatography of the protein-modulating factor on Sephacryl S-200 resulted in no change in the elution volume of the factor but did resolve it from residual thioredoxin. As has been reported for NADP$^+$-malate dehydrogenase from other plants [10,14,15], pea leaf NADP$^+$-malate dehydrogenase is activated in the presence of dithiothreitol and thioredoxin (data not shown). This activation is not absolutely dependent upon thioredoxin, since incubation with dithiothreitol alone causes a slow activation of the pea enzyme.

Estimates of ferredoxin content of thylakoids

Washed thylakoid membranes were routinely used as the photoreceptor for the reconstitution studies described above. Only 1% of the total chloroplast ferredoxin was found to be associated with these washed thylakoids when ferredoxin was estimated by measuring rates of NADP$^+$ photoreduction by the washed thylakoids with and without an aliquot of the stroma derived from these thylakoids (data not shown). The supernatant solution from the $(NH_4)_2SO_4$ fractionation of the stromal fraction probably contained most of the soluble ferredoxin, since it was a good source of that protein [16]. Any residual ferredoxin would have come off the columns in the 12 000 molecular weight range fractions. It seems

TABLE I

EFFECTS OF DIAMIDE UPON LIGHT-ACTIVATED $NADP^+$-MALATE DEHYDROGENASE

Malate dehydrogenase activity was determined fluorometrically in the direction of oxaloacetate synthesis while light activation was conducted using procedure 1 described in Materials and Methods. The activation assay using whole chloroplasts contained 0.43 mg Chl/ml and was illuminated for 20 min while the broken chloroplast system contained 0.16 mg Chl/ml (35 min illumination). Diamide treament involved incubating the enzyme preparation with 10 mM diamide for 5 min at 20°C. Since diamide rapidly oxidizes NADPH (which is produced in the malate dehydrogenase assay), the excess diamide was destroyed by a further incubation with 30 mM 2-mercaptoethanol for 5 min before malate dehydrogenase activity was assayed and quantitated in arbitrary fluorescence units.

	Malate dehydrogenase activity (fluorescence units)			
	Dark	Light	Light + diamide	Dithiothreitol treated
Intact Chloroplasts	5.9	9.0	5.3	33.5
Broken Chloroplasts	2.7	5.6	2.6	–

unlikely that ferredoxin was present in the light-modulation assay or that ferredoxin is involved in light modulation of these two enzymes in the system described here.

Molecular weight determination of protein-modulating factor

Using the procedures described in detail by Ashton et al. [11], the molecular weight of the protein-modulating factor was estimated by gel filtration and using malate dehydrogenase as substrate to be approx. 50 000. The protein-modulating factor activities with both glucose-6-phosphate dehydrogenase and malate dehydrogenase as substrate elute together on Sephacryl S-200 columns indicating that the same or similar species modulate both glucose-6-phosphate dehydrogenase and malate dehydrogenase.

Effect of thiol oxidants on light modulation

Diamide is a relatively specific thiol-oxidizing agent widely used to oxidize cellular glutathione [17]. We have found that stromal malate dehydrogenase activated by dithiothreitol can be deactivated by treatment with 10 mM diamide. The diamide-inactivated enzyme can be reactivated either by dithiothreitol or by light. Diamide treatment of

TABLE II

EFFECT OF ARSENITE TREATMENT OF THYLAKOID MEMBRANES UPON PROTEIN-MODULATING FACTOR-CATALYZED INACTIVATION OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE

Thylakoid membranes were treated with arsenite in the light as described by Anderson and Avron [4]. The light-modulation assay was conducted according to procedure 2 except that the reaction was terminated by a 10-fold dilution with ice-cold water. The supernatant solution was assayed for glucose-6-phosphate dehydrogenase activity. Assays of control and arsenite-treated membranes contained equal concentrations of chlorophyll. Illumination was for 15 min at 25°C.

Expt. No.	Glucose-6-phosphate dehydrogenase activity (nmol NADPH formed/min per ml)				% inhibition of glucose-6-phosphate dehydrogenase inactivation
	Untreated membranes		Arsenite-treated membranes		
	Dark	Light	Dark	Light	
1	13.0	7.5	12.4	10.2	58
2	10.5	6.3	9.8	8.0	53

malate dehydrogenase, which had been light activated in either intact chloroplasts or a broken chloroplast system, reduced the malate dehydrogenase activity to that of the dark control (Table I).

Light modulation by arsenite-treated membranes

The light-dependent inactivation of glucose-6-phosphate dehydrogenase catalyzed by the protein-modulating factor is inhibited if arsenite-treated membranes are used as the photoreceptor. The results of these experiments are listed in Table II.

Discussion

The results presented here provide evidence that a soluble factor is required for the light-dependent modulation of chloroplast enzymes. If light modulation of enzymes is shown to be a reductive process then a more appropriate term may be protein reductase. The system we have described differs in several respects from the ferredoxin-thioredoxin system elucidated by Buchanan and co-workers [6]. The protein-modulating factor system can function in the absence of ferredoxin. This confirms the work of Anderson and Avron [4] which indicated that ferredoxin was not necessary for the light modulation of malate dehydrogenase and glucose-6-phosphate dehydrogenase in stromal extracts. The protein-modulating factor functions in the absence of thioredoxin, the ultimate mediator of the ferredoxin-thioredoxin system. The Sephacryl S-200 column resolves the factor from thioredoxin and, furthermore, the addition of thioredoxin to a light-modulation assay does not influence light inactivation of glucose-6-phosphate dehydrogenase. We have not assayed our column for the enzyme ferredoxin-thioredoxin reductase but the results of Buchanan and co-workers [6] show that this protein requires the presence of both ferredoxin and thioredoxin to achieve in vitro light modulation. Lara et al. [18,19] have recently described a factor from spinach which mediates the light-dependent activation of fructose-1,6-bisphosphatase in a ferredoxin-thoredoxin-independent process. In this respect the factor that they have described resembles our protein-modulating factor. It is not known, however, where ferralterin (the name given to their new factor by Lara et al. [19] interacts with the photosynthetic electron-transport chain. We do not know at this stage if the two factors are identical.

Previous work in this laboratory suggested, on the basis of experiments with dithiothreitol- and dithionitrobenzoate-treated stroma, that the most probable mechanism of light modulation involved a thiol-disulfide exchange of a cysteine residue of the nodulated enzyme [20]. This interpretation has now been rendered ambiguous by the present demonstration of the protein-modulating factor which would have been present in the stromal fraction in those experiments.

Based on experiments in which the thiol oxidant diamide reverses the light activation of malate dehydrogenase as well as the activation of malate dehydrogenase by dithionitrobenzoate, it is most probable that reduction of a regulatory disulfide is necessary for the light activation of malate dehydrogenase. Also, the work of Scheibe and Anderson [21] has shown that oxidized thioredoxin can reverse the light-dependent modulation of enzyme activity in a stoichiometric rather than catalytic fashion. Such a finding is more consistent with modulation by reduction than thiol-disulfide exchange.

Based on this and previous work it seems that light modulation involves transfer of electrons within the membrane from PS I to the light-effect mediator and then to the soluble protein-modulating factor which reduces and thereby modulates the appropriate stromal enzyme (Fig. 7). The inhibition of protein-modulating factor-catalyzed inactivation of glucose-6-phosphate dehydrogenase by treatment of thylakoids with arsenite shows that the protein-modulating factor interacts with the previously described membrane-associated light-effect mediator of Anderson and Avron [4].

Since two pathways for light modulation have been described the question arises: Do both pathways for light modulation function in vivo or is one path predominant? In bacteria, for example, a precedent exists for thioredoxin participating in one of two parallel pathways. Ribonucleotide reduction can occur with thioredoxin as hydrogen donor or glutaredoxin, a polypeptide reduced by glutathione [22], and mutants lacking thioredoxin can reduce ribonucleotides. No rigorous evidence exists to decide the question posed above; however, the work of Scheibe and Anderson [21] does shed light on this question.

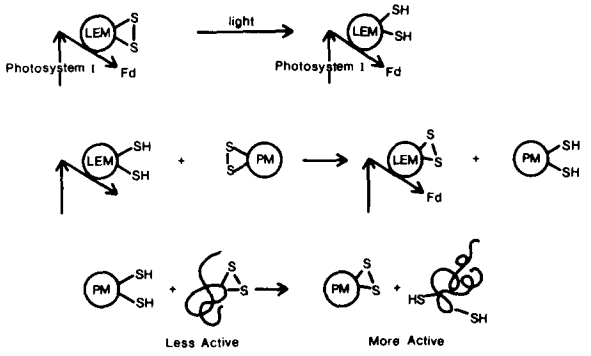

Fig. 7. Scheme for light activation of malate dehydrogenase activity in pea leaf chloroplasts. PM, protein-modulating factor. Electrons from the reducing side of PS I reductively activate light-effect rediator (LEM_I) which, in turn, activates (and probably reduces) protein-modulating factor. The factor then interacts with the inactive form of the enzyme, probably reduces a disulfide bond, and the enzyme undergoes a change in conformation. From Ref. 23, with permission. Fd, ferredoxin.

They found a soluble factor present in the stroma which could mediate the reversal of light modulation. This factor is apparently identical to thioredoxin and the 'dark' reaction that they describe is probably simply the reversal of the well known dithiothreitol-dependent modulation, the outcome of incubation of enzyme and thioredoxin thus dependending on the redox potential of the system. A significant feature of the work of Scheibe and Anderson [21] is that the protein-modulating factor fractions did not catalyze the dark reversal of light-modulated enzyme, suggesting that the protein-modulating factor reaction is not readily reversible. This situation is more in accord with other regulatory enzyme systems where separate enzymes catalyze modification and its reversal (e.g., protein kinase and protein phosphatase).

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