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Oxygen Evolution in the Absence of the 33-Kilodalton Manganese-Stabilizing Protein†

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ABSTRACT: There has been a considerable amount of controversy concerning the ability of photosystem II to evolve oxygen in the absence of the 33-kDa, manganese-stabilizing protein. Early reports indicated that some capacity for oxygen evolution existed in manganese-stabilizing protein-depleted membranes while more recent studies have suggested that the observed oxygen evolution activity arose from residual manganese-stabilizing protein present in the salt-washed preparations. In this paper, it is conclusively demonstrated that significant rates of steady-state oxygen evolution are observed in oxygen-evolving photosystem II membranes in the absence of detectable quantities of the manganese-stabilizing protein. More then 99% of the manganese-stabilizing protein was removed by either one $CaCl_2$ or two NaCl–urea washes. The amount of manganese-stabilizing protein removed was quantified immunologically using mouse polyclonal antibodies. Oxygen evolution rates of 115–140 μmol of O_2 (mg of Chl)$^{-1}$ h^{-1} were observed in the NaCl–urea-washed preparations. These rates represent about 24% of the rate observed in untreated membranes [450–600 μmol of O_2 (mg of Chl)$^{-1}$ h^{-1}]. Somewhat lower, although still significant rates were observed in the $CaCl_2$-washed preparations. Optimal rates of oxygen-evolving activity in NaCl–urea-washed membranes which are devoid of the manganese-stabilizing protein required high concentrations of calcium and chloride.

Photosystem II (PS II)[1] is a multisubunit thylakoid membrane protein complex which catalyzes the light-driven oxidation of water to molecular oxygen and the reduction of plastoquinone to plastoquinol. This complex consists of both intrinsic and extrinsic protein subunits. Intrinsic polypeptides with apparent molecular masses of 49 (CPa-1), 45 (CPa-2), 34 (D1), 32 (D2), 9 and 4.5 (α and β subunits of cytochrome b_{559}), and 4 kDa (*psb*I gene product) in association with an extrinsic 33-kDa polypeptide have been assumed to form the minimum complex capable of photosynthetic oxygen evolution (Ghanotakis et al., 1987).

In higher plants, two additional extrinsic protein components with apparent molecular masses of 24 and 17 kDa are associated with the oxygen-evolving complex. Removal of these proteins by salt-washing (usually 1.0 M NaCl) dramatically lowers the oxygen-evolving capacity of PS II vesicles (Akerlund et al., 1982) and PS II membranes (Kuwabara & Murata, 1982). Much of the lost activity can be recovered by reconstitution with the 24- and 17-kDa proteins (Akerlund et al., 1982) or by the addition of moderate concentrations of calcium (Ghanotakis et al., 1984) and chloride (Andersson et al., 1984). These proteins are assumed to play a role in the regulation of calcium and chloride concentrations within the PS II complex.

The extrinsic 33-kDa protein is much more tightly associated with the intrinsic PS II proteins than are the 24- and 17-kDa proteins. Removal of this protein requires treatment with high concentrations of alkaline–Tris (Yamamoto et al., 1981), $CaCl_2$ (Ono et al., 1983), or NaCl–urea (Miyao & Murata, 1984). Treatment with alkaline–Tris also leads to the loss of the manganese cluster associated with the active site of PS II (Kuwabara & Murata, 1982). This was initially taken as evidence that the manganese cluster was associated with this

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[1] Abbreviations: Chl, chlorophyll; DCBQ, 2,6-dichloro-*p*-benzoquinone; Mes, 2-(*N*-morpholino)ethanesulfonic acid; PS II, photosystem II; TMACl, tetramethylammonium chloride; Tris, tris(hydroxymethyl)-aminomethane.

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extrinsic protein. $CaCl_2$ and NaCl–urea washes, however, efficiently remove the 33-kDa protein without the concomitant loss of the manganese cluster. In the absence of the 33-kDa protein, high concentrations of chloride are required to maintain the integrity of the manganese cluster (Miyao & Murata, 1984). At chloride concentrations below 100 mM, two of the four manganese associated with PS II rapidly become paramagnetically uncoupled and then dissociate from PS II membranes (Mavankal et al., 1987). These studies indicate that the extrinsic 33-kDa protein acts as a manganese-stabilizing protein for PS II.

There has been a considerable amount of controversy concerning the ability of PS II to evolve oxygen in the absence of the 33-kDa, manganese-stabilizing protein. Early reports indicated that some capability for oxygen evolution existed in manganese-stabilizing protein-depleted membranes (Ono & Inoue, 1984; Miyao & Murata, 1984; Kuwabara et al., 1985; Miyao et al., 1987). These reports indicated that $CaCl_2$ or NaCl–urea washing removed more than 95% of the manganese-stabilizing protein from PS II membranes and that these protein-depleted membranes retained 14–40% of the oxygen-evolving capacity of control membranes. One critical problem in these studies is the failure of the authors to satisfactorily document the extent of manganese-stabilizing protein depletion. These authors used scanning densitometry of Coomassie blue-stained gels to quantitate the residual manganese-stabilizing protein associated with PS II. Since, depending on the electrophoretic system used, other protein species may comigrate with the manganese-stabilizing protein (Odom & Bricker, 1990) and since, at low protein concentrations, the manganese-stabilizing protein does not appear to exhibit quantitative staining (Camm et al., 1987), these results have been called into question.

More recent studies have suggested that the observed oxygen evolution activity arose from residual manganese-stabilizing protein present in the salt-washed preparations (Camm et al., 1987; Hunziker et al., 1987). Camm et al. (1987) found substantial quantities of the manganese-stabilizing protein remaining after either $CaCl_2$ or NaCl–urea washing. They also found that the amount of PS II activity remaining after salt-washing was correlated to the amount of residual manganese-stabilizing protein associated with the membranes. Hunziker et al. (1987) also found that a significant quantity of the manganese-stabilizing protein remained associated with PS II membranes after 1.0 M $CaCl_2$ treatment. Additionally, these authors reported that both the steady-state oxygen evolution rates and the yield of the S_2 multiline signal of manganese-stabilizing protein-depleted PS II membranes were correlated with the amount of residual protein that remained associated with the membrane. They concluded that manganese centers which were no longer associated with the manganese-stabilizing protein were incapable of oxidizing water to oxygen.

My findings disagree with those presented by Camm et al. (1987) and Hunziker et al. (1987). In this paper, it is demonstrated that significant rates of steady-state oxygen evolution are observed in oxygen-evolving PS II membranes which lack detectable quantities of the manganese-stabilizing protein. These findings conclusively demonstrate that manganese centers which lack the 33-kDa, manganese-stabilizing protein are capable of oxygen evolution.

Materials and Methods

Chloroplasts were isolated from market spinach as described previously (Bricker et al., 1985). Oxygen-evolving PS II membranes were isolated as described by Berthold et al. (1981) with the modifications described by Ghanotakis and Babcock (1983). Typical preparations had a Chl a/b ratio of 1.8–2.0 and evolved oxygen at rates of 450–700 μmol (mg of Chl)$^{-1}$ h^{-1}. Chl concentration was determined by the method of Arnon (1949). Two treatments were used to remove the manganese-stabilizing protein from PS II. Isolated PS II membranes were washed either with 1.0 M $CaCl_2$, 20 mM Mes–Tris, pH 6.0, and 0.3 M sucrose or with 2.6 M urea, 0.2 M NaCl, 50 mM $CaCl_2$, 20 mM Mes–Tris, pH 6.0, and 0.3 M sucrose. In either case, the membranes were suspended in the appropriate buffer with glass homogenization to a Chl concentration of 1.0 mg/mL and incubated for 1 h at 4 °C. After salt treatment, the membranes were pelleted at 40000g for 10 min and then washed once with 50 mM $CaCl_2$, 20 mM Mes–Tris, pH 6.0, and 0.3 M sucrose at a Chl concentration of 0.5 mg/mL. After being pelleted at 40000g, the membranes were resuspended in this same buffer at a Chl concentration of 3–4 mg/mL. Electrophoresis, "Western blotting", and antibody probing were performed as previously described (Bricker et al., 1988). The polyclonal anti-manganese-stabilizing protein antibody used in this study was produced by immunization of mice with manganese-stabilizing protein purified by the procedure of Kuwabara et al. (1985). This purified protein also cross-reacts with a monoclonal antibody, FCC4, which recognizes the manganese-stabilizing protein (Frankel & Bricker, 1990).

Oxygen evolution assays were performed polarographically with a Hansatech oxygen electrode in a volume of 1.0 mL at a light intensity of 1500 μmol of photons m^{-2} s^{-1} of white light and at 25 °C. The reaction buffer contained 20 mM Mes–Tris, pH 6.0, 0.3 M sucrose, and varying amounts of calcium and chloride. The calcium concentration was varied by the addition of either $CaCl_2$ or Mes–$Ca(OH)_2$ while the chloride concentration was varied by the addition of TMACl (Waggoner et al., 1989). All assays were performed at a Chl concentration of 10 μg/mL with 250 μM DCBQ as an electron acceptor. For the light saturation experiments, the light intensity was measured using a Li–Cor spectroradiometer equipped with a quantum probe.

For the time course experiments, PS II membranes were suspended as described above either in 2.6 M urea, 0.2 M NaCl, 50 mM $CaCl_2$, 20 mM Mes–Tris, pH 6.0, and 0.3 M sucrose or in 300 mM sucrose, 50 mM $CaCl_2$, and 20 mM Mes–Tris, pH 6.0, at a Chl concentration of 1.0 mg/mL. At the various time points, 1.0-mL aliquots were removed and pelleted at 14000g for 1 min, washed once with 1.0 mL of 50 mM $CaCl_2$, 20 mM Mes–Tris, pH 6.0, and 0.3 M sucrose, pelleted at 14000g for 1 min, and finally resuspended in 1.0 mL of 50 mM $CaCl_2$, 20 mM Mes–Tris, pH 6.0, and 0.3 M sucrose. The membranes were assayed for oxygen-evolving activity immediately after resuspension. Control membranes were treated identically with initial suspension in 50 mM $CaCl_2$, 20 mM Mes–Tris, pH 6.0, and 0.3 M sucrose. Aliquots of these samples were electrophoresed, and the amount of manganese-stabilizing protein which remained associated with the membranes at each time point was estimated from "Western blots".

Results and Discussion

Figures 1 and 2 illustrate the efficiency of $CaCl_2$ and NaCl–urea washing in the removal of the manganese-stabilizing protein. In these figures, the amount of the manganese-stabilizing protein remaining after one salt wash (Figure 1) and two salt washes (Figure 2) can be estimated by comparing the antibody staining intensity of the salt-washed lanes with the staining observed in a dilution series of control PS

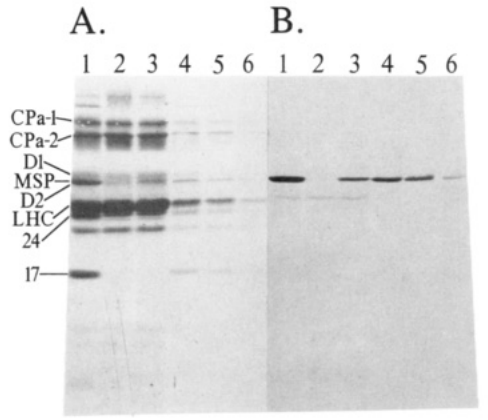

FIGURE 1: "Western blot" analysis of PS II membranes washed once with salt. (Panel A) Coomassie blue-stained; (panel B) probed with an anti-manganese-stabilizing protein antibody. Lane 1, control membranes, 10 μg of Chl; lane 2, $CaCl_2$-washed membranes, 10 μg of Chl; lane 3, NaCl–urea-washed membranes, 10 μg of Chl; lane 4, 10% of lane 1, 1 μg of Chl; lane 5, 5% of lane 1, 0.5 μg of Chl; lane 6, 1% of lane 1, 0.1 μg of Chl. PS II proteins are labeled; MSP, manganese-stabilizing protein; LHC, light-harvesting chlorophyll–protein II. The faint bands located below the manganese-stabilizing protein in panel B are green, chlorophyll–protein bands.

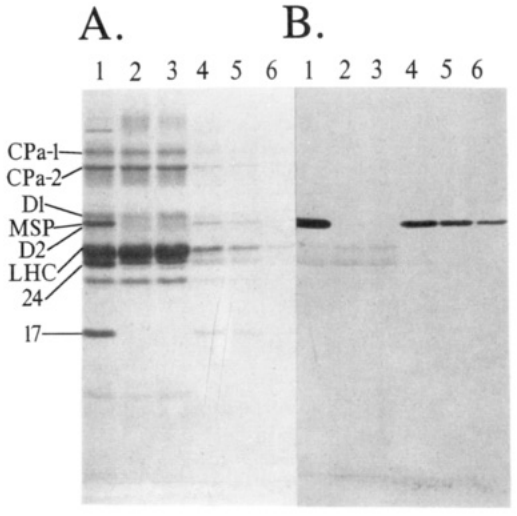

FIGURE 2: "Western blot" analysis of PS II membranes washed twice with salt. (Panel A) Coomassie blue-stained; (panel B) probed with an anti-manganese-stabilizing protein antibody. Lane 1, control membranes, 10 μg of Chl; lane 2, $CaCl_2$-washed membranes, 10 μg of Chl; lane 3, NaCl–urea-washed membranes, 10 μg of Chl; lane 4, 10% of lane 1, 1 μg of Chl; lane 5, 5% of lane 1, 0.5 μg of Chl; lane 6, 1% of lane 1, 0.1 μg of Chl. PS II proteins are labeled; MSP, manganese-stabilizing protein; LHC, light-harvesting chlorophyll–protein II. The faint bands located below the manganese-stabilizing protein in panel B are green, chlorophyll–protein bands.

II membranes. These figures demonstrate that one $CaCl_2$ wash removes more than 99% of the manganese-stabilizing protein from PS II membranes while one NaCl–urea wash removes about 95% of this protein. Two sequential NaCl–urea washes remove more than 99% of the manganese-stabilizing protein from these membranes.

These results differ dramatically from those obtained by Camm et al. (1987) and Hunziker et al. (1987). Camm et al. (1987), also using immunological detection, found that after 1.0 M $CaCl_2$ washing about 50% of the manganese-stabilizing protein remained associated with PS II membranes. After NaCl–urea washing, they reported that almost 10% of this protein remained associated with the membranes. Hunziker et al. (1987) estimated that 33% of the manganese-stabilizing protein remained associated with PS II membranes after 1.0 M $CaCl_2$ treatment. In this study, a heat-shock treatment was used to remove residual manganese-stabilizing protein from the $CaCl_2$-treated PS II membranes. The amount of heat-shock-released manganese-stabilizing protein was then estimated from densitometry of Coomassie blue-stained polyacrylamide gels.

Table I: Oxygen Evolution Rates of Manganese-Stabilizing Protein-Depleted PS II Preparations

membrane type	rate [μmol of O_2 (mg of Chl)$^{-1}$ h^{-1}]	% control rate
control	575	100
1 $CaCl_2$ wash	91	16
1 NaCl–urea wash	178	31
2 NaCl–urea washes	137	24

It is unclear exactly why the findings of Camm et al. (1987) and Hunziker et al. (1987) differ so dramatically from the results presented in this paper. One possibility is a possible difference in the salt-washing and resuspension protocol. In my preparations, glass homogenization was used to obtain a homogeneous suspension of membranes during the salt wash and rinsing steps. Gentle resuspension with a small paintbrush, which is often used in photosynthetic studies, generates an inhomogeneous suspension of PS II membranes from which the manganese-stabilizing protein would be inefficiently extracted by the salt washes. It is probable that glass homogenization is essential for obtaining quantitative release of the manganese-stabilizing protein from PS II membranes. It is unlikely that the length of incubation, 1 h in this study versus 30 min in the other studies, is important. PS II membranes which are more than 99% depleted of the manganese-stabilizing protein were consistently obtained with two 20-min NaCl–urea washes (data not shown).

Table I documents the steady-state oxygen evolution capability of PS II membranes after the different salt washes. It is apparent that significant amounts of oxygen evolution can occur even in the absence of detectable quantities of the manganese-stabilizing protein. After one $CaCl_2$ wash, 16% of the control membrane oxygen evolution rate was observed, while after two NaCl–urea washes 24% of the control rate was observed. Camm et al. (1987) and Hunziker et al. (1987) also observed significant amounts of PS II activity in $CaCl_2$- and NaCl–urea-washed membranes but attributed this activity to the residual manganese-stabilizing protein that was present in their preparations. It should be pointed out that the PS II assays performed by Camm et al. (1987) were at suboptimal concentrations of both chloride and calcium for centers depleted in the manganese-stabilizing protein (see below). Since their reaction mixture included 25 mM chloride with no added calcium, they would not have observed any significant oxygen evolution from PS II centers lacking the manganese-stabilizing protein. Indeed, as they reported, all of the PS II activity that they observed can be attributed to the manganese-stabilizing protein that they were unable to remove from their membrane preparations.

Similar problems are evident in the report by Hunziker et al. (1987). In one series of experiments, they removed the manganese-stabilizing protein from their PS II preparations by treatment with 1.0 M NaCl under various pH conditions. Increasing the pH resulted in the release of increasing amounts of the manganese-stabilizing protein and a concomitant loss of oxygen evolution activity. I have found that the oxygen-evolving capacity of manganese-stabilizing protein-depleted PS II preparations is rapidly lost in the absence of high concentrations of calcium. Such conditions were not maintained in the above-mentioned experiments. Under the conditions

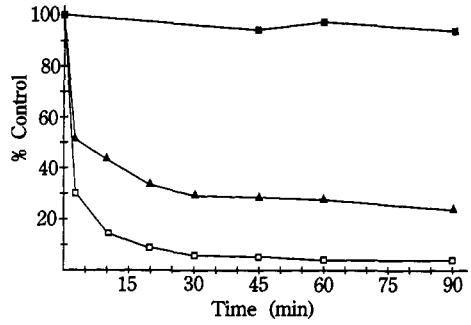

FIGURE 3: Time course illustrating the loss of PS II activity and the manganese-stabilizing protein during incubation with 200 mM NaCl and 2.6 M urea. Oxygen evolution rates are expressed as a percent of the rate exhibited by the control PS II membranes at time zero. The amount of manganese-stabilizing protein remaining at each time point was estimated from "Western blots" and is expressed as a percent of the amount of the manganese-stabilizing protein present in the control PS II membranes at time zero. No loss of the manganese-stabilizing protein was observed in the control membranes during the duration of the time course (data not shown). Solid squares, oxygen evolution activity of control PS II membranes incubated in 300 mM sucrose, 50 mM $CaCl_2$, and 20 mM Mes–Tris, pH 6.0. Solid triangles, oxygen evolution activity of PS II membranes incubated in 300 mM sucrose, 200 mM NaCl, 2.6 M urea, 50 mM $CaCl_2$, and 20 mM Mes–Tris, pH 6.0. Open squares, relative amount of the manganese-stabilizing protein remaining associated with the salt-washed PS II membranes. This is the result of a typical experiment. The 100% control PS II oxygen evolution rate was 550 μmol of oxygen (mg of Chl)$^{-1}$ h^{-1}.

which these authors used, oxygen evolution would only be observed from centers which contained the manganese-stabilizing protein. Centers which had lost the manganese-stabilizing protein would rapidly lose the ability to evolve oxygen.

Figure 3 shows the results of a time course experiment comparing the loss of the oxygen-evolving activity of control and NaCl–urea-washed membranes. Additionally, the loss of the manganese-stabilizing protein from the NaCl–urea-washed membranes was estimated. It is apparent that after 20 min of NaCl–urea treatment about 90% of the manganese-stabilizing protein had been removed. These manganese-stabilizing protein-depleted membranes, however, exhibited 37% of the control oxygen evolution rate. From this experiment, it can be concluded that, while the manganese-stabilizing protein is certainly required for high rates of oxygen-evolving activity, it is not required per se for oxygen evolution to occur.

Figure 4 demonstrates that the oxygen evolution rate of manganese-stabilizing protein-depleted PS II membrane exhibits a strong chloride concentration dependence with maximal rates of oxygen evolution observed at 100 mM chloride. In this experiment, the calcium concentration was held constant at 10 mM. The strong chloride dependence which is observed is probably due to the requirement of a high chloride concentration for the maintenance of manganese in a structurally functional state. Miyao and Murata (1984) have shown that at low chloride concentrations two of the four manganese associated with the oxygen-evolving site are rapidly lost from NaCl–urea-washed PS II membranes. Additionally, washing of manganese-stabilizing protein-depleted membranes with buffers which contain no chloride leads to a rapid and reversible loss of paramagnetic coupling of two of the four manganese associated with the oxygen-evolving site (Mavankal et al., 1986). These experiments were carried out with PS II membranes which contained 4 manganese per 300 Chl (Mavankal, 1989).

Figure 5 shows the stimulation of oxygen-evolving activity of manganese-stabilizing protein-depleted PS II membranes

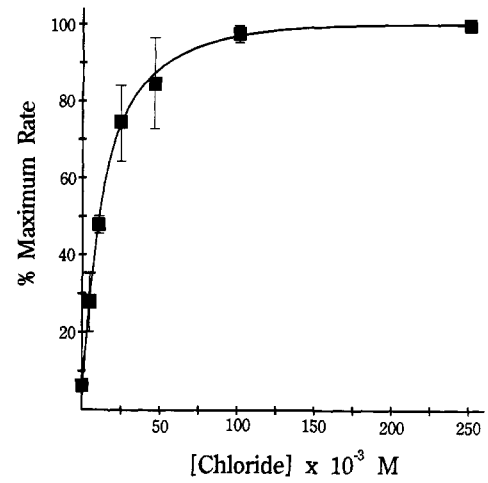

FIGURE 4: Effect of increasing chloride concentration on the oxygen evolution activity of manganese-stabilizing protein-depleted PS II membranes. In this experiment, the calcium concentration was held constant at 10 mM. The chloride concentration was varied by the addition of TMACl. The maximum rate observed was 110 μmol of oxygen (mg of Chl)$^{-1}$ h^{-1}. These are the average values obtained from two independent trials. Error bars = ±1.0 SD.

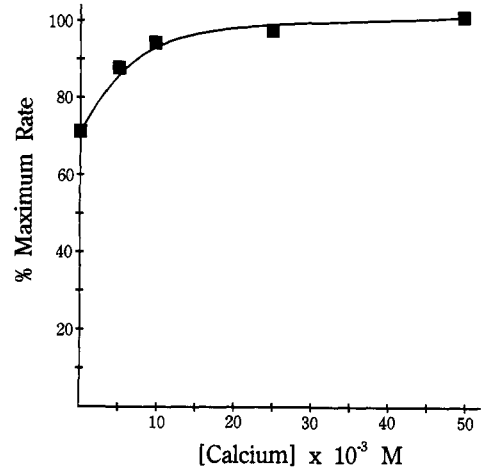

FIGURE 5: Effect of increasing calcium concentration on the oxygen evolution activity of manganese-stabilizing protein-depleted PS II membranes. In this experiment, the chloride concentration was held constant at 100 mM. The calcium concentration was varied by the addition of calcium chloride. The maximum rate observed was 115 μmol of oxygen (mg of Chl)$^{-1}$ h^{-1}. These are the average values obtained from two independent trials. The ±1.0 SD error bars in this experiment are smaller than the diameter of the symbols.

by calcium. In this experiment, the chloride concentration was held constant at 100 mM; 10 mM calcium was required for maximum stimulation of the PS II activity. Only a 30% stimulation of activity was observed. This is due to the fact that these protein-depleted membranes must be isolated in the presence of high calcium concentrations to maintain maximal rates of oxygen-evolving activity. A considerable amount of calcium remains associated with the PS II membranes. NaCl-washed membranes also exhibit a requirement for calcium (Waggoner et al., 1989) which appears to saturate at 5 mM. This indicates that the presence of the manganese-stabilizing protein lowers the requirement for calcium by at least a factor of 2 and may indicate that this protein is required to maintain a normal association of calcium with PS II.

Figure 6A shows the results of a quantum yield experiment performed with control and NaCl–urea-washed membranes. At low light intensities, examination of the first-order rate constants allows estimation of the relative quantum yield for

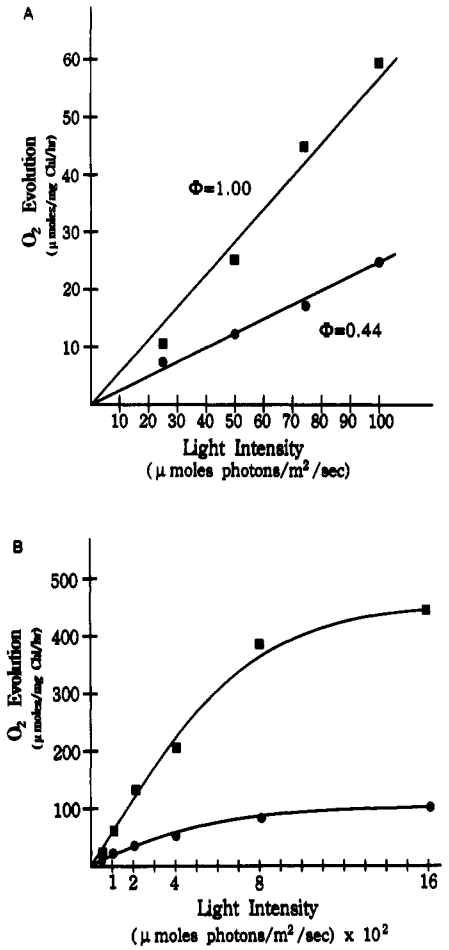

FIGURE 6: Typical light saturation experiment performed with control and NaCl–urea-washed PS II membranes. Figure 6A illustrates the light-dependent oxygen evolution observed at low light intensities. The relative quantum yields from control and salt-washed membranes are shown. Figure 6B shows the entire light saturation curve from this experiment. Closed squares, control membranes; closed circles, NaCl–urea-washed membranes.

oxygen evolution exhibited by these membranes. The NaCl–urea-washed membranes exhibit a relative quantum yield of 0.44 with respect to control PS II membranes. At least two interpretations are consistent with this result. First, these membranes may contain 44% of the active centers present in the control membranes. Since, under steady-state oxygen-evolving conditions, NaCl–urea-washed membranes exhibit 24% of the activity observed in control membranes (Table I), the PS II centers which are active in the NaCl–urea-washed membranes would then evolve oxygen at rates approaching 55% of control PS II centers. Alternatively, the lower quantum yield could be the result of the production of PS II centers which are less efficient in carrying out stable charge separation. If, for instance, removal of the manganese-stabilizing protein led to a substantially increased probability of charge recombination within the reaction center, a lowered quantum yield could be observed. Figure 6B shows the entire light saturation curve from this experiment.

Miyao et al. (1987) reported the isolation of manganese-stabilizing protein-depleted PS II preparations in which approximately 100% of the PS II centers were active. These preparations probably contained about 5–10% of the manganese-stabilizing protein present in control PS II membranes. The lower relative quantum yield which was observed in this paper is almost undoubtedly due to the harsher treatment conditions which were used to ensure complete removal of the manganese-stabilizing protein.

Burknap and Sherman (1991) have deleted the *psb*O gene, which encodes the manganese-stabilizing protein, in the cyanobacterium *Synechocystis* 6803. This mutant grows photoautotrophically and is capable of significant rates of PS II-dependent oxygen evolution (about 30% of wild-type). Interestingly, an analogous mutant produced in *Chlamydomonas* (Mayfield et al., 1987) cannot grow photoheterotrophically, does not evolve oxygen, and does not assemble functional PS II centers. These results either could be due to differences in the mechanism of oxygen evolution in cyanobacteria and green algae/higher plants or could be the result of different mechanisms being employed for PS II assembly in these two systems. My results indicate that, as in *Synechocystis*, oxygen evolution can occur in higher plants in the absence of the manganese-stabilizing protein. It is possible that in *Synechocystis* the in vivo concentrations of calcium and chloride are sufficient for the photoligation of the manganese cluster to occur, allowing the functional assembly of PS II centers. Tamura and Cheniae (1987) have shown that in the presence of 50 mM $CaCl_2$, photoligation of manganese can occur in vitro in the absence of the manganese-stabilizing protein. If in chloroplasts the in vivo concentrations of calcium and chloride were low, manganese ligation could not occur in the absence of the manganese-stabilizing protein, and it is unlikely that PS II assembly would take place.

A number of conclusions can be drawn from the experiments presented in this paper. First, $CaCl_2$ and NaCl–urea washes, if performed properly, are very efficient in removing the manganese-stabilizing protein from PS II membrane preparations. Second, significant rates of steady-state oxygen evolution can be observed in the absence of detectable amounts of the manganese-stabilizing protein. This indicates that PS II centers which are devoid of the manganese-stabilizing protein are capable of oxygen evolution. Third, the oxygen evolution observed in these protein-depleted membrane preparations is dependent on the presence of high quantities of chloride and calcium. Finally, quantum yield measurements indicate that PS II centers which lack the manganese-stabilizing protein evolve oxygen at about 55% of the rate of intact centers.

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Registry No. O_2, 7782-44-7; Ca, 7440-70-2; Cl^-, 16887-00-6.

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Free Fatty Acids Activate a High-Affinity Saturable Pathway for Degradation of Low-Density Lipoproteins in Fibroblasts from a Subject Homozygous for Familial Hypercholesterolemia†

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ABSTRACT: This paper describes a mechanism for degradation of low-density lipoprotein (LDL) in fibroblasts unable to synthesize the LDL receptor. In this cell line, long-chain free fatty acids (FFA) activated ^{125}I-LDL uptake; unsaturated FFA were the most efficient. The first step of this pathway was the binding of LDL apoB to a single class of sites on the plasma membrane and was reversible in the presence of ≥10 mM suramin. Binding equilibrium was achieved after a 60-90-min incubation at 37 °C with 1 mM oleate; under these conditions, the apparent K_d for ^{125}I-LDL binding was 12.3 μg/mL. Both cholesterol-rich (LDL and β-VLDL) and triglyceride-rich (VLDL) lipoproteins, but not apoE-free HDL, efficiently competed with ^{125}I-LDL for this FFA-induced binding site. After LDL bound to the cell surface, they were internalized and delivered to lysosomes; chloroquine inhibited subsequent proteolysis of LDL and thereby increased the cellular content of the particles. A physiological oleate to albumin molar ratio, i.e., 1:1 (25 μM oleate and 2 mg/mL albumin), was sufficient to significantly ($p < 0.01$) activate all three steps of this alternate pathway: for example, 644 ± 217 (25 μM oleate) versus 33 ± 57 (no oleate) ng of LDL/mg of cell protein was degraded after incubation (2 h, 37 °C) with 50 μg/mL ^{125}I-LDL. We speculate that this pathway could contribute to the clearance of both chylomicron remnants and LDL.

Low-density lipoproteins (LDL)[1] and chylomicron remnants bind with high affinity to the LDL receptor, which mediates endocytosis of both particles (Brown & Goldstein, 1986; Nagata et al., 1988). Besides this well-characterized receptor, less clearly delineated pathways significantly contribute to the removal of these lipoproteins (Shepherd et al., 1979; Goldstein & Brown, 1989). The issue of the mechanisms underlying these pathways has been addressed from two distinct perspectives: (1) a putative receptor for chylomicron remnants (Brown et al., 1991) and (2) a pathway for LDL receptor independent catabolism of LDL (Myant, 1990).

The normal rate of removal of chylomicron remnants in subjects homozygous for familial hypercholesterolemia (FH) (Rubinsztein et al., 1990) and in Watanabe heritable hyperlipidemic (WHHL) rabbits (Kita et al., 1982) led to the hypothesis that a specific receptor mediates the clearance of these particles. Recent reports have introduced (Herz et al., 1988) and support (Kowal et al., 1989, 1990; Beisiegel et al., 1989) the notion that the LDL receptor related protein (LRP) might be a chylomicron remnant receptor (Brown et al., 1991). This

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[1] Abbreviations: apo, apoprotein; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; FFA, free fatty acid(s); FH, familial hypercholesterolemia; HDL, high-density lipoprotein(s); LDL, low-density lipoprotein(s); LPDS, lipoprotein-deficient serum; PBS, phosphate-buffered saline; TCA, trichloroacetic acid; VLDL, very low density lipoprotein(s); WHHL, Watanabe heritable hyperlipidemic.

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