Alterations of the Oxygen-Evolving Apparatus Induced by a ^{305}Arg → ^{305}Ser Mutation in the CP43 Protein of Photosystem II from *Synechocystis* sp. PCC 6803 under Chloride-Limiting Conditions†

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ABSTRACT: The *psb*C gene encodes CP43, a component of Photosystem II (PSII) in higher plants, algae, and cyanobacteria. Previous work demonstrated that alteration of an arginine residue occurring at position 305 to serine produced a strain (R305S) with altered PSII activity (Knoepfle, N., Bricker, T. M., and Putnam-Evans, C. (1999) *Biochemistry 38*, 1582−1588). This strain grew at wild-type rates in complete BG-11 media (480 μM chloride) and evolved oxygen at rates that were 60−70% of the observed wild-type rates. The R305S strain assembled approximately 70−80% of the functional PSII centers contained in the control strain, and these PSII centers were very sensitive to photoinactivation at high light intensities. We recently observed that the R305S mutant exhibited a pronounced chloride effect. When this mutant was grown in media depleted of chloride (30 μM chloride), it exhibited a severely reduced photoautotrophic growth rate. The effect of chloride depletion on the growth rate of the mutant was reversed by the addition of 480 μM bromide to the chloride-depleted BG-11 media. Oxygen evolution rates for the mutant were further depressed to about 22% of that observed in control cells under chloride-limiting conditions. Addition of bromide restored these rates to those observed under chloride-sufficient conditions. The mutant exhibited a significantly lower relative quantum yield for oxygen evolution than did the control strain, and this was exacerbated under chloride-limiting conditions. Fluorescence yield measurements indicated that both the mutant and the control strains assembled fewer PSII reaction centers under chloride-limiting conditions. The reaction centers assembled by the mutant exhibited an enhanced sensitivity to photoinactivation under chloride-limiting conditions, with a $t_{1/2}$ of photoinactivation of 2.6 min under chloride-limiting conditions as compared to a $t_{1/2}$ of 4.7 min under normal growth conditions. The mutant also exhibited an enhanced stability of its S_2 state and increased number of centers in the S_1 state following dark incubation. These results indicate that the mutant R305S exhibits a defect in its ability to utilize chloride in support of efficient oxygen evolution in PSII. This is the first mutant of this type described in the CP43 protein.

The light-driven oxidation of water to molecular oxygen and concomitant reduction of plastoquinone to plastoquinol is catalyzed by the Photosystem II (PSII)[1] complex. PSII consists of both intrinsic polypeptide subunits embedded within the thylakoid membrane and extrinsic components exposed to the thylakoid lumen. The intrinsic polypeptide components necessary for the formation of a PSII complex competent in oxygen-evolution are CP47, CP43, D1, D2, the α and β subunits of cytochrome b_{559}, the 4 kDa *psb*I gene product, and a number of other low molecular mass components. In higher plants, three extrinsic proteins with apparent molecular masses of 33 (manganese-stabilizing protein, MSP), 24, and 17 kDa are required for maximal rates of oxygen evolution under physiological ionic conditions. The 24 and 17 kDa proteins are absent in cyanobacteria. These proteins appear to be functionally replaced by cytochrome c_{550} and a 12 kDa protein (*1*). In the absence of the extrinsic proteins, PSII complexes retain the ability to evolve oxygen but at significantly reduced rates (*2*, *3*). This ability to evolve oxygen is dependent upon the presence of high nonphysiological concentrations of calcium and chloride. The extrinsic PSII proteins may function as a diffusional barrier that sequesters chloride and calcium in the vicinity of the oxygen-evolving complex (*4*).

Chloride is required for both the assembly and the stability of the oxygen-evolving complex. PSII membranes lacking MSP show an absolute requirement for chloride for photoactivation (*5*). In isolated PSII membranes depleted of MSP and in the Δ*psb*O mutant of *Synechocystis*, which lacks MSP, two of the so-called chloride-sensitive manganese are lost at low (<100 mM) chloride concentrations (*6*, *7*). Chloride has been proposed to bind directly to the manganese cluster

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[1] Abbreviations: PSII, Photosystem II; kb, kilobase pair(s); DCBQ, 2,6-dichloro-*p*-benzoquinone; PCR, polymerase chain reaction; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; kDa, kilodaltons; LDS, lithium dodecyl sulfate; bp, base pairs.

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or to a site close to it (*8*, *9*). EXAFS experiments have suggested that at least one chloride may act as a terminal ligand in the manganese cluster (*10*). Studies in higher plants indicate that a single chloride ion is required at the oxygen-evolving site (*11*, *12*). In studies using chloride-depleted PSII membranes retaining MSP, stabilization of the S_2 and S_3 states and retardation of the $S_3 \rightarrow [S_4] \rightarrow S_0$ transition have been observed (*13*−*15*). Recent studies show that chloride can bind at or near the manganese cluster in both the higher (S_2 and S_3) and the lower (S_0 and S_1) S states (*16*, *17*). With regards to mechanism, chloride may act as a bridging ligand between manganese atoms within the manganese cluster (*8*, *18*, *19*) or possibly between manganese and a positively charged amino acid (*20*). Alternatively, the binding of chloride may alter the conformation of proteins that bind the manganese cluster and thus indirectly affect the stability of the PSII active site (*21*). Proteins that bind the functional chloride within PSII have not yet been identified.

The product of the *psb*C gene, CP43 is an integral thylakoid protein and component of the proximal antennae of PSII (*22*). Hydropathy analysis predicts that CP43 contains six transmembrane α helices (*23*). This has been confirmed by the recent 3.8 Å resolution crystal structure of PSII from *Synechococcus elongatus* (*24*). In addition to the six membrane-spanning regions, CP43 also contains five hydrophilic loops that connect the membrane-spanning domains. One of these loops, the large extrinsic loop E, spans amino acid residues ^{278}Asn−^{410}Trp and is located between the fifth and sixth membrane-spanning helices. This large extrinsic loop is exposed to the lumenal side of the thylakoid membrane (*25*). While CP43 functions as an interior chlorophyll-*a* light-harvesting antenna for PSII, it has become increasingly clear that CP43 also plays a role in the stable assembly of PSII and in the oxygen-evolving process. CP43 is a necessary component for optimal oxygen-evolving activity in isolated PSII preparations (*26*). *Chlamydomonas* mutants bearing alterations in *psb*C that affect either the synthesis or the stability of CP43 are deficient in PSII activity (*27*). In these mutants, the levels of other PSII core proteins within the thylakoid are severely reduced. Following nitrosoguanidine mutagenesis, Dzelzkalns and Bogorad (*28*) recovered a *Synechocystis* mutant unable to evolve oxygen or support PSII electron transport from water to either dichlorobenzoquinone (DCBQ) or methyl viologen. Further characterization of this mutant showed that it contained a short deletion within the *psb*C gene. Additionally, isolated thylakoid membranes from this mutant contained decreased levels of the reaction center protein D2. Site-directed mutagenesis has also been used to probe the function of CP43 in PSII. For these studies the *Synechocystis* sp. PCC6803 system has been exploited. Interruption of the *psb*C gene by insertional mutagenesis produced a mutant incapable of evolving oxygen but which exhibited primary charge separation (*29*). Deletion of *psb*C in *Synechocystis* produces mutants that accumulate PSII core complexes to only 10% of wild-type levels and do not grow photoautotrophically or evolve oxygen (*30*, *31*). Eight short deletions were introduced within the large extrinsic loop E of CP43 in *Synechocystis* by Kuhn and Vermaas (*32*). Significantly, all resulting mutants showed complete loss of both photoautotrophic growth and ability to evolve oxygen. These mutants also contained decreased levels of the PSII reaction center proteins D1, D2, and CP47. Site-directed mutagenesis of an arginine residue at position 305 in the large extrinsic loop of CP43 produced a mutant that exhibited decreased rates of steady-state oxygen evolution, enhanced rates of photoinactivation, an increased fluorescence rise time, and decreased numbers of fully functional PSII centers (*33*). These data collectively suggest that CP43 is essential for normal PSII assembly and function.

Examination of the Zouni et al. crystal structure (*24*) indicates that it is the assigned D1/CP 43 side of the photosystem over which cytochrome c_{550} appears to be positioned, while the electron density assigned to the manganese-stabilizing protein appears to be positioned over the assigned D2/CP47 side of the reaction center. At the current resolution, however, it is difficult to unambiguously trace the backbone of the intrinsic membrane proteins to determine the domains of interaction between the intrinsic and the extrinsic components of the photosystem.

In this communication we demonstrate that the phenotype of the R305S mutant is significantly exacerbated when the strain is grown under chloride-limiting conditions. The mutant loses much of its ability to grow photoautotrophically, it assembles fewer functional PSII reaction centers, and the centers that do assemble are more defective and exhibit higher rates of photoinactivation. Additionally, the mutant exhibits a stabilization of its S_2 state. This is the first mutant in the CP43 protein to show such effects when propagated under chloride-limiting conditions.

MATERIALS AND METHODS

Growth Conditions. The construction of the kanamycin-resistant control strain and the R305S mutant were described previously (*33*, *34*). Control and mutant *Synechocystis* sp. PCC 6803 were grown in liquid BG-11 media (*35*) at 30 °C and a light intensity of 25 μmol photons m^{-2} sec^{-1}. Cultures were shaken on a rotary shaker at 200 rpm. Where appropriate, glucose was added to cultures at a final concentration of 5 mM. Antibiotics were added to the media to a final concentration of 10 μg/mL. Cultures maintained on plates in BG-11 media were supplemented with 1.5% agar, 0.3% sodium thiosulfate, 10 mM TES/KOH (pH 8.2), and 10 μM DCMU. For growth under chloride-limiting conditions, calcium nitrate, cobalt nitrate, and manganese sulfate were added to the micronutrient component of BG-11 media in place of calcium chloride, manganous chloride, and cobalt chloride. These alterations did not change the total nitrate or sulfate concentrations significantly. Polycarbonate flasks and carboys were used to grow liquid cultures to prevent the leaching of chloride from the glass. A total of 480 μM sodium bromide was added to chloride-deficient media where cells were grown in the presence of bromide.

Oxygen Evolution Assays. PSII activity was measured by O_2 polarography with a Hansatech oxygen electrode. Assays were performed at 25 °C on whole cells in BG-11 media using 1 mM DCBQ as an electron acceptor. A saturating light intensity of 2500 μmol photons m^{-2} sec^{-1} (white light) was used for these experiments. For the quantum yield experiments, cells were irradiated at low, nonsaturating light intensities during the oxygen evolution measurements. Light intensity was measured with a spectroradiometer equipped with a quantum probe (Li-Cor, Inc.). For photoinactivation experiments, cells were incubated in BG-11 media at a

chlorophyll concentration of 10 μg/mL at 5000 μmol photons m^{-2} sec^{-1} at 25 °C. At the indicated times, aliquots were removed and assayed for oxygen-evolving activity as described above. The chlorophyll concentration in all oxygen evolution assays was 10 μg/mL. Chlorophyll content was measured as in Williams (*35*).

Flash oxygen yield measurements were made on a bare platinum electrode (Artesian Scientific Co., Urbana IL) as described previously (*20*). Flashes were supplied by an integrated, computer-controlled Xenon flash lamp (20 μs width at 1/2 height). For the measurements of S state distributions and Kok parameters, cells were grown photoheterotrophically in BG-11 media. They were then washed twice with, and incubated under room light in, BG-11 media and applied to the bare platinum electrode as a thin paste. The cells were then incubated for 10 min in the dark, the electrode was polarized at 0.73 V for 20 s, and a series of 16 saturating flashes were applied. Data points were collected at 500 μs intervals during the duration of the flash train. The data were analyzed using a four-step, homogeneous model (*36*).

For S_2 and S_3 lifetime measurements, cells were harvested as described above. They were then incubated for 10 min in the dark, given a single saturating flash, incubated another 10 min, given either a single flash (S_2 measurements) or two flashes at a 300 ms interval (S_3 measurements), and then incubated in the dark for a variable length of time. Finally, the sample was given a train of fifty saturating flashes during which data were collected. Regardless of the length of the last dark incubation, the electrode was polarized for 20 s before data collection commenced. The oxygen yield on either the first flash (S_3 measurement) or the second flash (S_2 measurement) of the 50 flash series was normalized to the average yield obtained on the 47−50th flashes. The very first saturating flash and the second dark incubation were included to poise the maximum number of PSII centers in the S_1 state prior to subsequent flashes. The data were analyzed by plotting the normalized oxygen yield of the first flash of the series for the S_3 measurement (or the second flash of the series for the S_2 measurement) versus delay time.

Fluorescence Measurements. Fluorescence yield measurements were performed on a Walz PAM 101 fluorimeter as described previously (*37*, *38*). Samples (10 μg/mL chlorophyll) were incubated in the dark for 5 min in the presence of 1mM potassium ferricyanide and 330 μM DCBQ. DCMU was added to a final concentration of 40 μM followed 1 min later by the addition of hydroxylamine hydrochloride (pH 6.5) to a concentration of 20 mM. After 20 s, the weak monitoring flashes were turned on followed 1 s later by continuous actinic illumination (1000 μmol photons m^{-2} sec^{-1}). The variable fluorescence, F_v, was measured. F_{max} was measured 5 s after the onset of actinic illumination.

RESULTS

Growth Characteristics. The effects of the R305S mutation on growth rates were examined under photoautotrophic and photomixotrophic growth conditions. Control and mutant strains were grown in complete BG-11 media (480 μM chloride), chloride-depleted BG-11 media (30 μM chloride), and chloride-depleted BG-11 media supplemented with 480 μM sodium bromide. The control strain was a strain of

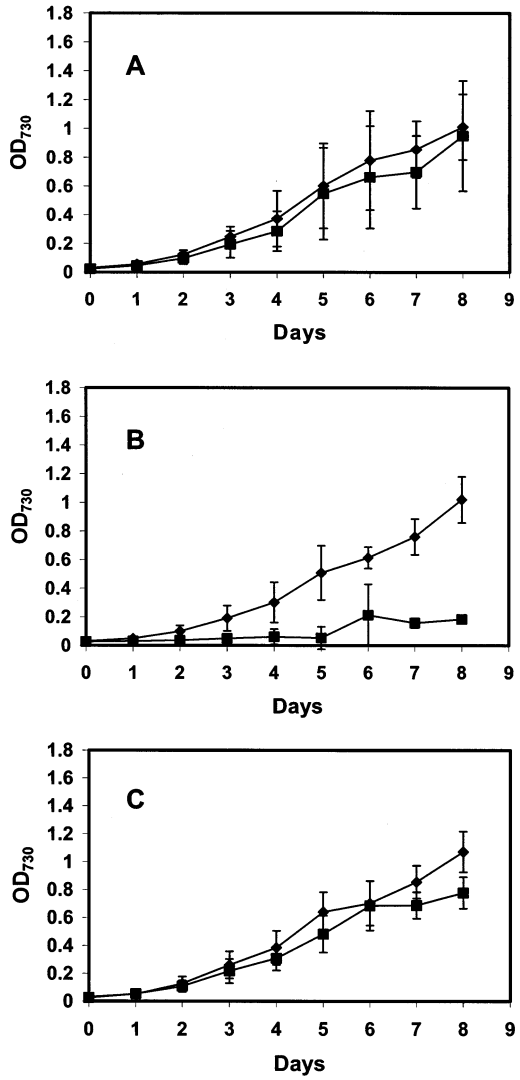

FIGURE 1: Photoautotrophic growth characteristics of the control strain (closed diamonds) and mutant R305S strain (closed squares). (A) Growth under chloride-sufficient (480 μM) conditions. (B) Growth under chloride-limiting (30 μM) conditions. (C) Photoautotrophic growth in the presence of 480 μM bromide and under chloride-limiting conditions. Each growth curve represents the average of at least three independent experiments. The error bars are ± one standard deviation.

Synechocystis containing a kanamycin-resistance gene in the 3′ noncoding region of CP43 but containing no site-directed mutations. The data shown in Figure 1 illustrate the photoautotrophic growth of the mutant strain as compared to growth of the control strain. The R305S mutant and control strain grew at similar rates in complete media (Figure 1A). Under conditions of chloride depletion, however, the R305S mutant exhibited a barely perceptible rate of photoautotrophic growth (Figure 1B). Growth was restored to rates comparable to that of the control by the addition of bromide to the chloride-depleted media (Figure 1C). These data demonstrate that bromide can effectively replace chloride in supporting photoautotrophic growth in both the control and the mutant strains. Previous work (*10*) has shown that bromide will functionally replace chloride at the oxygen-evolving site in cyanobacteria grown on bromide-containing media. Additionally, it is well-known that bromide can replace chloride in supporting oxygen evolution in isolated thylakoids (*39*) and PSII membranes (*8*). When grown photomixotrophically,

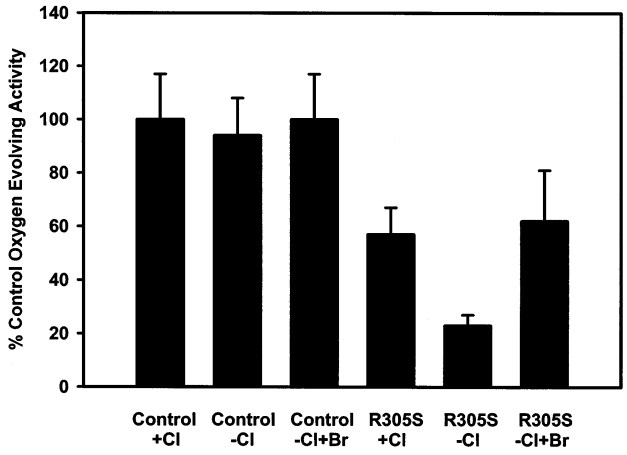

FIGURE 2: Oxygen evolution (water to DCBQ) of the control strain and R305S mutant strain. Cells were grown in liquid BG-11 media containing 5 mM glucose. The data represents the average of three independent experiments. The error bars are ± one standard deviation. Oxygen evolution rates for the control strain averaged 517 μmol O_2 mg chlorophyll^{-1} hr^{-1}.

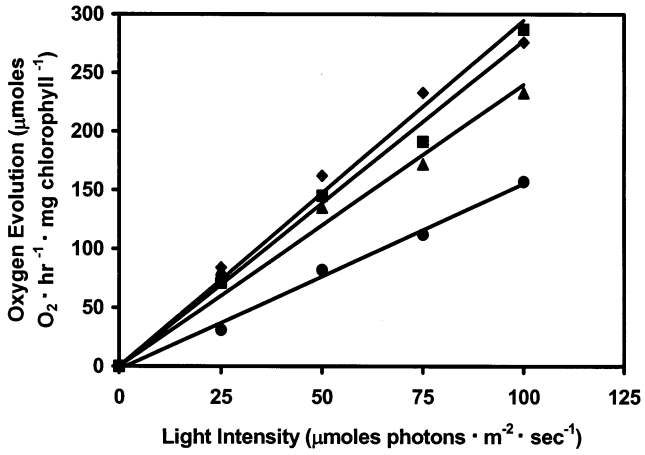

FIGURE 3: Relative quantum yield for oxygen evolution (Φ) estimation for the control strain and R305S mutant grown in complete and chloride-depleted media. The data were fit by linear regression to obtain the relative quantum yields of the two strains under the different growth conditions. Closed diamonds, control strain grown in chloride-sufficient media ($\Phi = 1.0$); closed squares, control strain grown in chloride-depleted media ($\Phi = 0.9$); closed triangles, R305S mutant grown in chloride-sufficient media ($\Phi = 0.8$); and closed circles, R305S grown in chloride-depleted media ($\Phi = 0.56$). These data represent the average of four independent experiments. The error in the data ranged from 5 to 20% for each data point.

the mutants exhibited rates of growth comparable to that of wild-type regardless of the chloride concentration in the media (data not shown). These data indicate that the mutation introduced at position 305 of the large extrinsic loop E of CP43 is associated with an alteration of the chloride requirement of PSII.

PSII Characterization. Steady-state rates of oxygen evolution were measured from water to DCBQ in mutant and control strains grown under photomixotrophic conditions. Figure 2 shows steady-state oxygen evolution rates as a percentage of control rates where the control strain was grown in complete media. Control oxygen evolution rates averaged 517 μmol O_2 mg chlorophyll^{-1} hr^{-1}. Under chloride-limiting conditions, the oxygen evolution rate for the control strain was 94% of that observed under chloride-sufficient conditions. When control cells were grown under conditions where bromide was substituted for chloride, the observed oxygen evolution rate was 100% of the control rate. The R305S mutant grown under chloride-sufficient conditions demonstrated a decreased rate of oxygen evolution to approximately 60% that of the control strain. This had been previously reported by Knoepfle et al. (*33*). Interestingly, the R305S mutant grown under chloride-limiting conditions evolves oxygen at a much lower rate, approximately 20% of that of the control (Figure 2). This effect could not be prevented by the addition of chloride to the reaction vessel just prior to assay (data not shown). Addition of bromide to the chloride-depleted media during growth of this strain restored the oxygen evolution rate to the level observed when the R305S strain was grown in complete media.

Figure 3 shows the results of a relative quantum yield experiment performed on the control and R305S strains grown in the presence of glucose in chloride-containing media or media depleted of chloride. Measurement of oxygen evolution rates at limiting light intensities indicates that, under chloride-sufficient conditions, the mutant exhibits 80% of quantum yield found in the control strain. Under conditions of chloride depletion, the control strain exhibits a near normal (90%) quantum yield. However, the quantum yield of the R305S mutant under chloride-limiting conditions is reduced from about 80 to 56%. This result could be obtained in at least three ways. First, the absolute number of reaction centers could be lower under chloride-limiting conditions than under chloride-sufficient conditions. Second, the number of reaction centers could be similar under both growth conditions, but the assembled reaction centers in the mutant are less efficient in carrying out oxygen evolution under chloride-limiting conditions. Third, a combination of lower reaction center numbers with a lowered efficiency for oxygen evolution could be present.

Table 1: Variable Fluorescence Yields

strain[a]	media	F_v/F_o
control	BG-11	0.58 ± 0.03[b]
control	low Cl^-	0.43 ± 0.03
R305S	BG-11	0.41 ± 0.03
R305S	low Cl^-	0.26 ± 0.04

[a] Cells were grown in complete BG-11 media (480 μM Cl^-) supplemented with 5 mM glucose or in low chloride (30 μM Cl^-) media supplemented with 5 mM glucose. The electron donor was hydroxylamine. [b] Standard deviation, $n = 3$.

The third possibility appears to be the case. Measurements of the variable fluorescence yields for the control and R305S strain are shown in Table 1. These results indicate that while both the control and the mutant strains assemble somewhat fewer reaction centers under chloride-limiting conditions, the differential observed for the R305S mutant is probably insufficient to fully account for the relatively large difference observed in the relative quantum yield experiments (Figure 3) and in the steady-state oxygen evolution experiments (Figure 2).

Photoinactivation experiments directly demonstrate that the PSII centers that do assemble in the mutant are defective and do not exhibit normal PSII activity and/or stability. Figure 4 shows the results of experiments in which control and R305S cells were grown in the presence of glucose and either a normal chloride concentration or under conditions

Table 2: S State Distributions, Parameters, and Lifetimes

	S State Distribution[a]				S State Parameters				S State Lifetimes ($t_{1/2}$)[b]	
strain	S_0 (%)	S_1 (%)	S_2 (%)	S_3 (%)	α (%)	β (%)	γ (%)	δ (%)	S_2	S_3
control	45[c]	54[c]	0	1	9[c]	86[c]	1[c]	4	4 ± 1 s 44% 59 ± 2 s 56%	3 ± 1 s 35% 21 ± 2 s 65%
R305S	34[c]	63[c]	0	3	11[c]	84[c]	2[c]	3	19 ± 1 s 74% 143 ± 35 s 26%	3 ± 1 s 31% 13 ± 1 s 69%

[a] For the control, $n = 10$ and for R305S, $n = 13$. In both instances the standard errors of the mean were 0.2% or less. [b] The curves shown in Figure 5 were fit to a double-exponential decay function. Listed are the fast and slow decay components. For the control and R305S, $n = 3-4$. [c] Significantly different at the 99% confidence level using a two-tailed Student's *t* test.

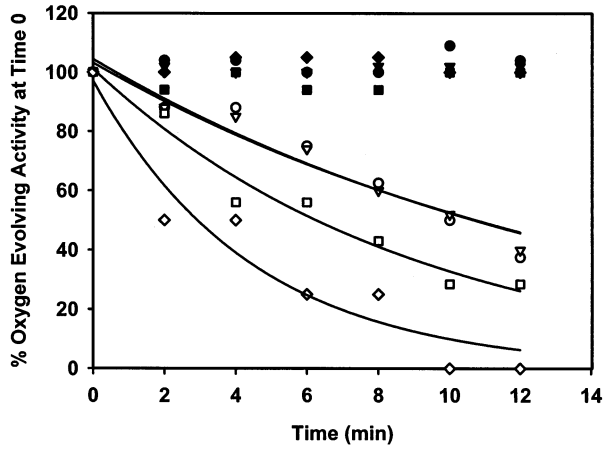

FIGURE 4: Photoinactivation experiment performed on the control strain and the site-directed mutant R305S grown in either complete media or under conditions of chloride depletion. Cells were assayed for oxygen-evolving activity (water to DCBQ) at the indicated times following exposure to 5000 μmol photons m^{-2} sec^{-1} white light. Open circles, control strain grown in complete media plus photoinactivating light; open triangles, control strain grown in chloride-depleted media plus photoinactivating light; open squares, R305S grown in complete media plus photoinactivating light; open diamonds, R305S grown in chloride-depleted media plus photoinactivating light. Closed symbols represent the corresponding dark controls. The data are the average of three independent experiments. The $t_{1/2}$ values were calculated from a fit of the data to a single-exponential decay. The standard deviations for all data averaged 14%.

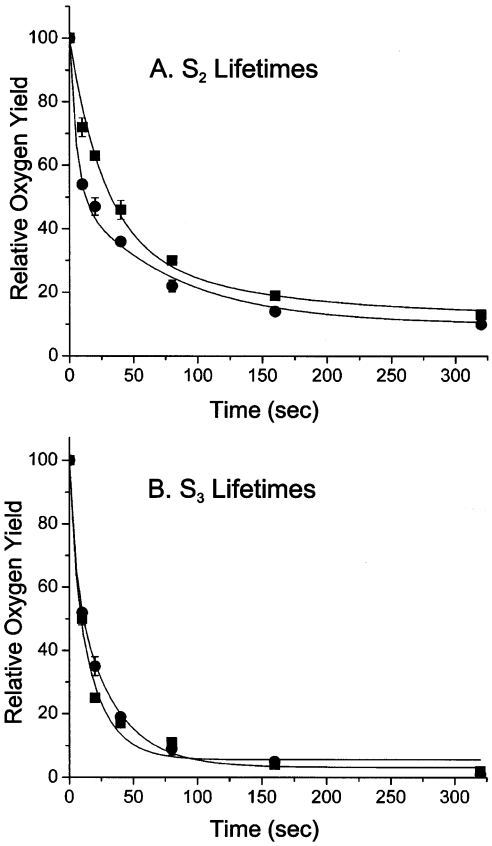

FIGURE 5: S_2 and S_3 lifetime measurements for the control strain and the R305S mutant grown in chloride-sufficient BG-11 media. Closed circles, the control strain; closed squares, the R305S mutant strain. Error bars are ± 1.0 standard error, $n = 3-4$; in some instances the error bars are smaller than the symbols. The lines drawn are fits generated from a double-exponential decay function. The parameters for these decays are shown in Table 2.

of chloride depletion. These strains were then exposed to a light intensity sufficient to cause photoinactivation (5000 μmol photons m^{-2} sec^{-1} of white light). At the indicated time intervals, cells were removed and assayed for oxygen-evolving activity. Control cells grown in either complete or chloride-depleted media had a $t_{1/2}$ for photoinactivation of 10 min. The R305S mutant photoinactivated significantly faster under normal growth conditions with a $t_{1/2}$ of 4.7 min (*33*). However, under conditions of chloride depletion, the mutant exhibited a markedly enhanced rate of photoinactivation. The $t_{1/2}$ for photoinactivation under these conditions was 2.6 min. A variety of oxidizing-side mutants show similar results (*40*−*42*). Photodamage to the reaction center proteins in these mutants may occur because of the presence of long-lived oxidizing species that accumulate in the absence of a fully competent oxygen-evolving complex. Interestingly, exposure of isolated PSII membrane preparations to a low chloride environment also leads to increased rates of photoinactivation (*43*).

S state distributions and parameters for the control strain and R305S are shown in Table 2. The R305S strain exhibits significantly more centers in the S_1 state and fewer centers in the S_0 state than does the control strain. Additionally, the R305S strain exhibits significantly more misses and double hits than the control strain. S_2 and S_3 state lifetime measurements for the wild type and the R305S mutant are shown in Figure 5. Both strains exhibit similar S_3 lifetimes under chloride-sufficient conditions (Table 2). The fast and slow components are very similar as are the proportions that they contribute to the decay. The R305S strain, however, exhibited a significantly slower S_2 state decay than was observed for the wild-type strain. Both the fast and the slow components of the decay are considerably longer than observed for the control strain. Additionally, a higher proportion of the decay can be attributed to the faster component. These results indicate a stabilization of the S_2 state in the mutant strain. It should be noted that neither of these experiments could be performed under true chloride-limiting conditions. Cells grown photoheterotrophically in chloride-depleted BG-11

media exhibited pronounced levels of photoactivation even after 200 preflashes of light (data not shown). We speculate that this may be due to enhanced rates of dark inactivation of the mutant.

To study the possible effects of the mutation on PSII assembly, the PSII complexes of both mutant and control strains grown in chloride-depleted media were isolated utilizing a His-tag present on the 3′ terminus of the CP47 protein (*51*). Immunoblots of the isolated PSII core particles (data not shown) demonstrated no significant alterations in the amounts of the PSII proteins examined (CP43, CP47, D1, and the manganese-stabilizing protein). These results are similar to those obtained previously using isolated thylakoids from control and R305S strains grown in chloride-sufficient media (*33*).

DISCUSSION

The R305S mutation results in a *Synechocystis* strain exhibiting a moderate alteration in PSII function when grown in complete media, but our results demonstrate that it is much more severely impaired when grown under chloride-limiting conditions. We hypothesize that R305S contains a lesion(s) either directly affecting the binding of chloride or affecting the sequestration of chloride in the vicinity of the manganese cluster. Alterations in the ability of PSII to bind chloride may lead to a relative destabilization of the manganese cluster. Under chloride-sufficient conditions, this would lead to the increased susceptibility to photoinactivation at high light intensities that we observe since, in those centers that lack a fully functional oxygen-evolving complex, Y_Z would not be reduced efficiently. These effects would be amplified under chloride-limiting conditions.

Chloride is required for normal S state advancement beyond S_2 (*44*, *45*). In chloride-depleted PSII, both the $S_2 \rightarrow S_3$ transition (*18*) and the $S_3 \rightarrow [S_4] \rightarrow S_0$ transition (*43*) are affected. Chloride depletion may be related to a lack of deprotonation on the $S_2 \rightarrow S_3$ transition, resulting in a blockage of charge accumulation because of electrostatic effects (*46*). It may also be possible that this type of inhibition can occur under conditions that do allow for S state advancement and electron donation to P_{680}^+, though at a reduced efficiency. This has been suggested to occur in cases where chloride depletion affects the $S_3 \rightarrow [S_4] \rightarrow S_0$ transition (*47*). Whatever the mechanism, alterations in the ability to bind chloride could affect the kinetics of S state transitions such that oxidizing-side radicals accumulate and photoinactivation occurs as described above. Our observation that the S_2 lifetime of the R305S mutant is significantly longer than that observed for wild type indicates that the mutant possesses a more stable S_2 state.

Another possibility is that the observed effects could be derived, in part, from impaired binding of the manganese-stabilizing protein or other extrinsic proteins associated with the photosystem. The functional characteristics of chloride-depleted PSII membranes are very similar to those of membranes from which the MSP has been removed and to mutants in which the *psb*O gene has been deleted (*4*). Our results indicate that MSP is structurally associated with PSII particles in both the control and the mutant under conditions of chloride-depletion; however, at this time we cannot rule out the possibility that a functional interaction between MSP and CP43 has been disrupted or that the association of other extrinsic proteins with PSII may be affected.

The R305S mutant is the first mutant identified in the CP43 protein of PSII that exhibits a chloride effect. However, several mutations in the CP47 protein, also within the large extrinsic loop E of that protein, have resulted in mutants that show similar effects (*20*, *41*, *48*−*50*). The phenotypes of all of these mutants are remarkably similar in that all show decreased rates of photoautotrophic growth, decreased oxygen-evolving activity, and enhanced rates of photoinactivation under normal growth conditions. Chloride depletion either entirely abolishes PSII function or severely reduces it. It therefore appears that the large extrinsic loops of both CP47 and CP43 may participate in chloride binding/sequestration within PSII.

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