Oxidized Amino Acid Residues in the Vicinity of QA and PheoD1 of the Photosystem II Reaction Center: Putative Generation Sites of Reducing-Side Reactive Oxygen Species

Laurie K. Frankel
Louisiana State University

Larry Sallans
University of Cincinnati

Patrick A. Limbach
University of Cincinnati

Terry M. Bricker
Louisiana State University

Follow this and additional works at: https://digitalcommons.lsu.edu/biosci_pubs

Recommended Citation
Frankel, L., Sallans, L., Limbach, P., & Bricker, T. (2013). Oxidized Amino Acid Residues in the Vicinity of QA and PheoD1 of the Photosystem II Reaction Center: Putative Generation Sites of Reducing-Side Reactive Oxygen Species. PLoS ONE, 8 (2) https://doi.org/10.1371/journal.pone.0058042

This Article is brought to you for free and open access by the Department of Biological Sciences at LSU Digital Commons. It has been accepted for inclusion in Faculty Publications by an authorized administrator of LSU Digital Commons. For more information, please contact ir@lsu.edu.
Oxidized Amino Acid Residues in the Vicinity of QA and PheoD1 of the Photosystem II Reaction Center: Putative Generation Sites of Reducing-Side Reactive Oxygen Species

Laurie K. Frankel1, Larry Sallans2, Patrick A. Limbach2, Terry M. Bricker1*

1 Department of Biological Sciences, Division of Biochemistry and Molecular Biology, Louisiana State University, Baton Rouge, Louisiana, United States of America, 2 The Rieveschl Laboratories for Mass Spectrometry, Department of Chemistry, University of Cincinnati, Cincinnati, Ohio, United States of America

Abstract

Under a variety of stress conditions, Photosystem II produces reactive oxygen species on both the reducing and oxidizing sides of the photosystem. A number of different sites including the Mn$_{4}$O$_{5}$Ca cluster, P$_{680}$, Pheo$_{D1}$, QA, Q$_{b}$ and cytochrome b$_{559}$ have been hypothesized to produce reactive oxygen species in the photosystem. In this communication using Fourier-transform ion cyclotron resonance mass spectrometry we have identified several residues on the D1 and D2 proteins from spinach which are oxidatively modified and in close proximity to QA (D1 residues 239F, 241Q, 242E and the D2 residues 238P, 239F, 242E and 242M) and Pheo$_{D1}$ (D1 residues 138E, 139L and 139F). These residues may be associated with reactive oxygen species exit pathways located on the reducing side of the photosystem, and their modification may indicate that both QA and Pheo$_{D1}$ are sources of reactive oxygen species on the reducing side of Photosystem II.

Introduction

In higher plants and cyanobacteria the Photosystem II (PS II) complex contains more than twenty polypeptide subunits. At the core of the photosystem, six intrinsic membrane proteins are unequivocally required for oxygen evolution: the D1 and D2 proteins, the CP43 and CP47 proteins and the $\alpha$- and $\beta$- subunits of cytochrome b$_{559}$. The genetic deletion of these components results in loss of the assembly of the photosystem. A number of different sites including the Mn$_{4}$O$_{5}$Ca cluster, P$_{680}$, Pheo$_{D1}$, QA, Q$_{b}$ and cytochrome b$_{559}$ have been hypothesized to produce reactive oxygen species in the photosystem. In this communication using Fourier-transform ion cyclotron resonance mass spectrometry we have identified several residues on the D1 and D2 proteins from spinach which are oxidatively modified and in close proximity to QA (D1 residues 239F, 241Q, 242E and the D2 residues 238P, 239F, 242E and 242M) and Pheo$_{D1}$ (D1 residues 138E, 139L and 139F). These residues may be associated with reactive oxygen species exit pathways located on the reducing side of the photosystem, and their modification may indicate that both QA and Pheo$_{D1}$ are sources of reactive oxygen species on the reducing side of Photosystem II.

Copyright: © 2013 Frankel et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: Funding was provided by the Division of Chemical Sciences, Geosciences, and Biosciences, Office of Basic Energy Sciences of the U.S. Department of Energy through grant DE-FG02-98ER20210 to T.M.B and L.K.F., which supported the protein chemistry aspects and data analysis for this manuscript, and the National Institutes of Health Grants GM58843 and R019900 to P.A.L., which supported the mass spectrometry experiments of this manuscript. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: btbric@lsu.edu
the CP43 (356W) and D1 (317W) proteins which appear to be targets for oxidizing side ROS \([21,22,23]\). Additionally, reductants produced by PS II, such as Qb− \([24]\), PheoD1− \([25]\), Qα− \([26]\), and, possibly, reduced low potential cytochrome b582 \([27,28]\), appear to have redox potentials and lifetimes sufficient to reduce molecular oxygen and have been hypothesized to be sources of ROS. Sharma et al. \([29]\) had previously identified a D1 peptide \([130E–136R]\) which lies in the vicinity of PheoD1 and which contained a single oxidative modification on an unidentified residue.

One would predict that amino acid residues in the vicinity of the sites of ROS production should be particularly susceptible to ROS modification. The identification of such oxidatively modified residues in the photosystem should serve to identify both the putative sites of ROS generation and possibly the putative pathway for ROS exit from PS II. In this communication we report the presence of natively oxidized amino acids in the vicinity of both Qα and PheoD1 in PS II membranes isolated from field-grown spinach. These modifications are apparently normally present in the PS II isolated from field-grown material and our findings suggest that Qα and PheoD1 may be sources of ROS under native growth conditions.

### Results

Fig. 1 illustrates the quality of the mass spectrometry data used for the identification of oxidized amino acid residues in the D1 and D2 proteins. In this figure the MS/MS data collected for the D2 peptide \(235A–252R\) are illustrated. In Fig. 1A, the data from the unmodified peptide are illustrated, while in Fig. 1B, data from the peptide bearing oxidized \(247M\) are shown. In this example and others (Figs. S1–S2), both modified and unmodified versions of the target peptide were identified; in another example only the modified peptide was detected (Fig. S3). Using a \(p\) value \(<0.00001\) assured extremely high quality peptide identifications with nearly complete y- and b-ion series being observed.

In this communication, we have focused on the domains of the D1 and D2 proteins located at or near redox active cofactors located on the stromal face of the thylakoid membrane. A total of 10 oxidatively modified amino acid residues on the D1 and D2 proteins were observed in the vicinity of these cofactors. These are summarized in Table 1 along with the type of oxidative modifications and the residue location (surface or buried and not in contact with cavity or channel) within the \(T.\) vulcanus crystal structure. Please note that these residues were originally reported to be modified in Frankel et al. \([20]\), however, their close association with the reducing-side cofactors was not discussed in that communication.

In general, mass spectrometry coverage of intrinsic membrane proteins is quite challenging. The overall sequence coverage observed in this study for the proteins examined was 24% for D1 and 27% for D2, values which are quite comparable to that observed for these proteins by other investigators (see, for instance Nakamura et al. \([30]\)). However, the coverage of the residues located in the stromally exposed domains of these proteins was significantly higher, 67% for D1 and 48% for D2. These are the domains of principal interest in this study, as ROS produced on the reducing side of the photosystem must transit these regions to exit the photosystem. Within this context it should be noted that most oxidative modifications to amino acid residues lying in the transmembrane helices of these proteins would be difficult to identify and many would escape detection due to their high hydrophobicity and consequent expected poor resolution during reversed-phase chromatography.

The D1 and D2 proteins are highly homologous between higher plants and cyanobacteria (>95% similarity). Consequently, we can directly map the modified residues observed to be modified in spinach onto the homologous residues in the \(T.\) vulcanus crystal structure. These results are shown in Fig. 2. The close proximity of oxidatively modified residues to Qα and PheoD1 is evident in this illustration. Additionally, it is apparent that these residues appear to form two rather distinct groups. The first group appears associated with Qα, consists of both D1 and D2 residues, and leads in a nearly continuous manner from the cofactor to the surface of the complex. The second group is associated with PheoD1, consists solely of D1 residues, also forming a nearly continuous grouping of modified residues. This group of residues, however, does not reach the surface of the PS II complex.

### Discussion

It must be stressed that this is an observational study of the oxidative modifications naturally occurring in PS II isolated from field grown-spinach. We cannot comment on the field conditions which lead to these oxidative modifications (high light intensity, high temperatures, nutrient limitations, presence of heavy metals, etc.) nor on the chemical identification of the ROS responsible. Studies addressing some of these questions will be the topic of additional communications.

The oxidative modification of these D1 and D2 residues indicates that they have been modified by ROS. The proximity of these residues to Qα (D1 residues: \(239F, 241Q, 242E\) and the D2 residues: \(238P, 239T, 242E, \) and \(247M\) and PheoD1 (D1 residues: \(130E, 133L, \) and \(135F\)) supports the hypothesis that these two cofactors are sites of ROS production on the reducing side of PS II.

With respect to the residues in the vicinity of Qα in \(T.\) vulcanus, it should be noted that there is a single amino acid deletion at residue 10 in the D2 sequence with respect to the spinach sequence (Table 1). Consequently, in \(T.\) vulcanus:

\[
\text{Qα} - 2.9A-D2:24M - D1:23F - D1:241Q - D1:242E - D2:244E.
\]

These residues appear to form a near contiguous chain of oxidized residues leading from Qα (Fig. 3). Two additional oxidized residues D2:257P and 239T may also be part of this oxidized residue complex but these are more distantly located. The mass spectra identifying these modified residues are shown in Figs. 1, S1 and S2. All of these residues are at least partially surface-exposed. D2:246M, however, exhibits very limited contact with the bulk solvent. It should be noted that the oxidized D2 residues 257P and 239T are also in relatively close proximity to Qb (9 Å and 13 Å, respectively, Fig. 3). While no additional oxidized residues in the immediate vicinity of Qb were observed leading to the surface-located residues D2:257P and 239T, it is formally possible that oxidized residues are present in the region near Qb, but that they escaped detection in our experiments.

With respect to the residues in close proximity to PheoD1, D1:130E in spinach and \(Chlamydomonas\) is replaced by D1:130Q in the \(T.\) vulcanus structure. It should be noted that in \(T.\) vulcanus, D1:130Q is present in the constitutively expressed D1–1 isoform while the D1–2 and D1–3 isoforms, which are expressed only under certain environmental conditions, contain D1:130E \([31,32]\). These residues have been reported to be hydrogen- bonded to PheoD1 \([33,34]\). Additionally, in \(T.\) vulcanus, D1:135Y is replaced by D1:135F (Table 1). Consequently, in the \(T.\) vulcanus structure: PheoD1: −2.9A – D1:130Q – D1:133L – D1:135F (Fig. 4). The mass spectrum identifying this group of modified residues is shown in Fig. S3. These oxidized residues may be adjacent to a putative ROS exit pathway leading from...
It should be noted that in the static crystal structure, none of these residues are surface-exposed nor are they in contact with any apparent cavities or channels. Other residues which were not detected in our studies may be associated with the putative pathway, completing a pathway to the surface of the complex. It is unclear at this time how molecular oxygen penetrates into the protein structure to reach the vicinity of PheoD1. While no channels or cavities are present in the static protein structure in the vicinity of PheoD1, it is possible that these form transiently either due to thermal motion of the PS II complex on a nsec timescale or as a result of conformational changes occurring during the S-state transitions. It is also possible that oxygen can diffuse directly into the protein matrix as has been demonstrated in other systems [35]. In any event, our observation that the D1 residues 130E, 133L and 135F are oxidatively modified strongly suggests that molecular oxygen can penetrate the PS II structure and become partially reduced to an ROS by PheoD1.

Earlier studies identified domains containing oxidatively modified D1 and D2 residues. Sharma et al. [36] determined that the D1 peptide 235AFNPTQAEETYSMVTAN252R contained an oxidative modification, however the actual residue(s) modified and its spatial relationship with PheoD1 were not determined. Our observation that oxidative modification occurs on the D1 residues 130E, 133L and 135F fully confirms this observation of Sharma et al. [36]. These authors also identified a number of other peptides containing putative oxidative modifications on both the D1 and D2 proteins. However, none of the other residues that we observe to be oxidatively modified on the stromal domain lie in these additional peptides.

PS II, particularly when under stress, apparently can produce a variety of ROS at a variety of sites [16]. Several studies have identified the production of ROS, particularly the short-lived OH•, by the donor side of PS II [37,38]. 1O2 produced by the reaction of molecular oxygen with 3P680 has also been observed [17,18,19]. Interestingly, no oxidative modifications in the vicinity of P680 have been observed [20]. Since the production of 1O2 by PS II is well established [39], the lack of observed modifications in the vicinity of P680 strongly suggests that molecular oxygen can penetrate the PS II structure and become partially reduced to an ROS by PheoD1.

Figure 1. Example Mass Spectrometry Data from the Unmodified Peptide. 235AFNPTQAEETYSMVTAN252R and the Oxidatively Modified Peptide 235AFNPTQAEETYS130MVTAN252R of the D2 Protein A. Top, spectrum of the collision-induced dissociation (CID) of the unmodified peptide 235AFNPTQAEETYSMVTAN252R. Various identified ions are labeled. Bottom, table of all predicted masses for the y- and b- ions generated from this peptide sequence. Ions identified in the CID spectrum (above) are shown in red. The b′+, b′+y′+ and y′+ ions are generated by the neutral loss of water while the b′++, b′+y′++ and y′++ ions are generated from the loss of ammonia. A. Top, spectrum of the CID dissociation of the modified 235AFNPTQAEETYS130MVTAN252R. Various identified ions are labeled. Bottom, table of all predicted masses for the y- and b- ions generated from this peptide sequence. Ions identified in the CID spectrum (above) are shown in red. The b′+, b′+y′+ and y′+ ions are generated by the neutral loss of water while the b′++, b′+y′++ and y′++ ions are generated from the loss of ammonia. For comparison the b13–b17 ions of the unmodified peptide are highlighted in blue and those of the modified peptide are highlighted in cyan. All b ions longer than b12 in the modified peptide are 16 Da larger than the corresponding ions observed from the unmodified peptide. This indicates that 130M contains an oxidative modification. Additionally, the y6–y15 ions of the unmodified peptide are highlighted in green, while those of the modified peptide are highlighted in yellow. All y ions longer than y5 in the modified peptide are 16 Da larger than the corresponding ions observed from the unmodified peptide. This verifies that 130M contains an oxidative modification. The p values for the unmodified and modified peptide were 10−13 and 10−11, respectively.

doi:10.1371/journal.pone.0058042.g001

PheoD1 to residues near or at the surface of the complex. It should be noted that in the static crystal structure, none of these residues are surface-exposed nor are they in contact with any apparent cavities or channels. Other residues which were not detected in our studies may be associated with the putative pathway, completing a pathway to the surface of the complex.

It is unclear at this time how molecular oxygen penetrates into the protein structure to reach the vicinity of PheoD1. While no channels or cavities are present in the static protein structure in the vicinity of PheoD1, it is possible that these form transiently either due to thermal motion of the PS II complex on a nsec timescale or as a result of conformational changes occurring during the S-state transitions. It is also possible that oxygen can diffuse directly into the protein matrix as has been demonstrated in other systems [35]. In any event, our observation that the D1 residues 130E, 133L and 135F are oxidatively modified strongly suggests that molecular oxygen can penetrate the PS II structure and become partially reduced to an ROS by PheoD1.
vicinity of P680 could be a detection issue, as the peptides in the vicinity of P680 are highly hydrophobic and difficult to resolve by reversed-phase HPLC. It is also possible that $^1O_2$ may be vectored away from P680 rapidly, giving rise to a low yield of oxidative modifications. Finally, oxidative modification of residues in the vicinity of P680 may trigger D1 turnover more effectively than oxidative modifications at other sites in vivo. We cannot distinguish between these and other possibilities at this time.

ROS may also be produced on the reducing side of the photosystem by the partial reduction of molecular oxygen, yielding long-lived $O_2^-$ and $H_2O_2$ and the very short-lived $OH^\cdot$. It should be stressed, however, that at this time we cannot discriminate between these or other mechanisms that produce the ROS responsible for the oxidative modifications that we observe. Using mass spectrometry, it is also very difficult, and in most cases impossible, to differentiate oxidative modifications of amino acid side chains produced by $OH^\cdot$, $^1O_2$, $O_2^-$ or other oxidative species.

The site of ROS production by PS II has been the subject of much discussion [16]. Earlier, we reported that CP43:354E, 355T, 356M and 357R, which are in close proximity to the Mn$_4$O$_5$Ca cluster, were oxidatively modified [20]. These results indicate that the manganese cluster itself appears to be a source of oxidizing-side ROS. On the reducing side of the photosystem, PheoD1,Q A, QB and low potential cytochrome b559 have all been suggested as sites of ROS production. In this communication we have reported the oxidative modification of residues in close proximity to PheoD1 and QA. These results support the hypothesis that both of these sites can produce ROS that lead to amino acid oxidative modification. Since these modifications were observed on PS II membranes isolated from market spinach, it appears that they can accumulate to detectable levels within the D1 protein.

### Table 1. Oxidatively Modified Residues in the Vicinity of PheoD$_1$ and QA.

| Protein | Modified Spinach Residues | Corresponding Thermosynechococcus Residues |
|---------|---------------------------|------------------------------------------|
| D1      | $^{13}$H$_{13}$E + go     | $^{13}$O                           |
|         | $^{13}$L$_{13}$E + go     | $^{13}$L                           |
|         | $^{13}$F$_{13}$E + go     | $^{13}$Y                           |
|         | $^{13}$F$_{13}$E + go     | $^{13}$F                           |
|         | $^{24}$Q$_{24}$E + ca     | $^{24}$Q                           |
|         | $^{24}$E$_{24}$E + gam    | $^{24}$E                           |
| D2      | $^{23}$P$_{23}$P + ca     | $^{23}$P                           |
|         | $^{23}$T$_{23}$T + go     | $^{23}$T                           |
|         | $^{24}$E$_{24}$E + gam    | $^{24}$E                           |
|         | $^{24}$M$_{24}$M + go     | $^{24}$M                           |

Individual residues are listed along with the modifications observed. For a complete list of oxidative modification types, the amino acids targeted, and mass modifications searched for in this study, as well as structures arising from these oxidative modifications, see [50,51]. Key: ca, carbonyl addition (+14 Da); gam, Glu/Asp modification (decarboxylation and oxidation, ~ 30 Da); go, general oxidation (+16 Da). Oxidative modification of these residues was originally reported in Frankel et al. [20].

---

![Figure 2](https://example.com/figure2.png)

**Figure 2. Oxidized Residues Identified on the Stromally Exposed Regions of the D1 and D2 Proteins in the Vicinity of QA and PheoD$_1$.** The *T. vulcanus* residues corresponding to the oxidatively modified spinach residues (Table 1) are highlighted. These oxidized residues are shown as spheres superimposed on monomer I of the *T. vulcanus* structure. For clarity, only the D1 and D2 proteins and their associated cofactors are shown. A. the view from outside Monomer I, looking towards the dimeric complex from within the plane of the membrane. B. the view from Monomer II looking towards its interface with Monomer I within the plane of the membrane. The D1 protein is shown in pale green and the D2 protein is shown in pale yellow. The oxidatively modified residues of D1 are shown in dark green while those of D2 are shown in orange. Various cofactors of both D1 and D2 are labeled and colored pale green or yellow, respectively. PheoD$_1$ is shown in bright green. The non-heme iron is shown in bright red. The Mn$_4$O$_5$Ca cluster and its associated chloride ions are labeled as the OEC. Figs. 2–4 were produced using PYMOL [53].

doi:10.1371/journal.pone.0058042.g002
We cannot rule out, at this time, the possibility that the accumulation of such putative oxidative modifications may trigger D1 turnover and, consequently, limit the detection of D2 proteins in the vicinity of this cytochrome, we speculate on the relative rate of ROS production by PheoD1 or QA. These modifications in the vicinity of the metal cluster (or, perhaps, P680) may trigger D1 turnover and, consequently, limit the detection and/or accumulation of such putative oxidative modifications.

While no modified residues were observed in the immediate vicinity of Qβ, we cannot rule out, at this time, the possibility that this site could also contribute to reducing-side ROS production. Additionally, since we did not collect mass spectrometry data on the cytochrome b6f α and β subunits or on the other low molecular mass subunits in the vicinity of this cytochrome, we cannot comment on their ability to produce ROS. We also cannot speculate on the relative rate of ROS production by PheoD1 or QA (or other putative ROS-producing sites). We have no quantitative data as to the proportion of modified amino acid residues present at any of the observed positions. Indeed, such quantification would be difficult to obtain given the different hydrophobicity of the unmodified α subunit residues and their subsequent differential resolution by reversed-phase chromatography. Elucidation of the time-course for modification of these oxidized residues using 18O2, however, may provide valuable evidence bearing on the relative importance of ROS production by PheoD1 and QA. These experiments are currently underway.

Materials and Methods

PS II membranes were isolated from market spinach [43,44]. The PS II membranes were suspended at 2 mg chlorophyll/ml in 50 mM Mes-NaOH, pH 6.0, 300 mM sucrose, 15 mM NaCl buffer and frozen at −80°C until use. The proteins in the samples were separated on a 12.5–20% polyacrylamide gradient by lithium dodecyl sulfate-polyacrylamide gel electrophoresis [45] with the modifications outlined by Rabilloud et al. [46] and Sun et al. [47]. Electrophoresis was performed for 16 hrs at 1 W at 4°C. Upon completion of electrophoresis, the gels were stained with Coomassie Blue, destained, and protein bands containing D1 and D2 proteins were excised. These proteins (along with a number of other protein components of PS II) were then subjected to mass analysis using MassMatrix, max(pp1, ppm) was 8.5 and ppconcert≥ 5.0 [48,49]. These parameters yield a p value of ≤0.00001; only oxidized peptides which exhibited this extremely low p value were considered. Since the data was of very high quality, the union of the replicate data sets was examined [20]. Since the D1 and D2 proteins present in spinach and T. vulcanus are highly homologous, the PYMOL software suite [53] was used to map the oxidatively modified residues observed in spinach onto the T. vulcanus PS II structure [8] of the D1 and D2 proteins. It should be noted that protein electrophoresis is the principal source of protein oxidation artifacts in many biochemical studies. The ammonium persulfate catalyst (and the TEMED activator) typically used in this method for the polymerization of the acrylamide-bis acrylamide monomers generates sulfate radicals. This radical can react with water to produce both O2− and OH− [54], both of which can oxidatively modify proteins. To alleviate this problem the acrylamide gels used in this study were thoroughly degassed and photopolymerized with flavin mononucleotide, diphenyliodonium chloride and sodium toluenesulfonic acid [46]. Additionally, the cathode buffer contained thioglycolate [47]. This electrophoretic system had been shown to completely
eliminate artifactual electrophoresis-associated oxidative modifications of cytochrome c [47] and greatly minimize apparent electrophoresis-induced oxidative modifications in PS II (see [20] Fig S1). Subsequent to electrophoresis, the protein and peptide samples were maintained under reducing conditions (presence of dithiothreitol and/or low pH) to minimize artifactual oxidative modifications. Staining was performed in the presence of acetic acid, the excised protein bands were reduced with dithiothreitol (and then blocked with iodoacetic acid), and after tryptic digestion the peptides were brought to 0.1% formic acid and frozen at −80°C. Reversed phase HPLC was performed in the presence of 0.1% formic acid. The sheath and auxiliary gas for electrospray ionization was N2 [20].

Supporting Information

Figure S1 Mass Spectrometry Data from the Unmodified Peptide. 235AFNPTQAEETYSMVTAN252R and the Oxidatively Modified Peptide 235AFN238P16 239T16 QA242E-30 ETYSMVTAN252R of the D2 Protein A. Top, spectrum of the CID dissociation of the unmodified peptide 235AFNPTQAEETYSMVTAN252R. Various identified ions are labeled. Bottom, table of all predicted masses for the y- and b- ions generated from this peptide sequence. Ions identified in the CID spectrum (above) are shown in red. The b∗++, b′++ y∗++ and y′++ ions are generated by the neutral loss of water while the b∗++, b′++ y′++ and y′++ ions are generated from the loss of ammonia. B. Top, spectrum of the CID dissociation of the modified peptide 235AFN238P16 239T16 QA242E-30 ETYSMVTAN252R. Various identified ions are labeled. Bottom, table of all predicted masses for the y- and b- ions generated from this peptide sequence. Ions identified in the CID spectrum are shown in red. The b∗++, b′++ y∗++ and y′++ ions are generated by the neutral loss of water while the b∗++, b′++ y′++ and y′++ ions are generated from the loss of ammonia. The p values for the unmodified and modified peptide were 10−12 and 10−9, respectively.

(DOCX)

Figure S2 Mass Spectrometry Data from the Unmodified Peptide. 239GFQEEETYNHAAHGYFG257R and the Oxidatively Modified Peptide 239FG241Q16 239E242E-30 EETYNHAAHGYFG257R of the D1 Protein A. Top, spectrum of the CID dissociation of the unmodified peptide 239GFQEEETYNHAAHGYFG257R. Various identified ions are labeled. Bottom, table of all predicted masses for the y- and b- ions generated from this peptide sequence. Ions identified in the CID spectrum (above) are shown in red. The b∗++, b′++ y∗++ and y′++ ions are generated by the neutral loss of water while the b∗++, b′++ y′++ and y′++ ions are generated from the loss of ammonia. B. Top, spectrum of the CID dissociation of the modified peptide G241Q16 242E-30 EETYNHAAHGYFG257R. Various identified ions are labeled. Bottom, table of all predicted masses for the y- and b- ions generated from this peptide sequence. Ions identified in the CID spectrum are shown in red. The b∗++, b′++ y∗++ and y′++ ions are generated by the neutral loss of water while the b∗++, b′++ y′++ and y′++ ions are generated from the loss of ammonia. The p values for the unmodified and modified peptide were 10−6.

(DOCX)

Author Contributions

Conceived and designed the experiments: LKF TMB. Performed the experiments: LKF LS. Analyzed the data: LKF TMB. Contributed reagents/materials/analysis tools: LKF TMB. Wrote the paper: LKF TMB.
34. Dorlet P, Xiong L, Sayre RT, Un S (2001) High field EPR study of the
structure characterization of the Photosystem II D1 and D2 subunits. J Biol
Chem 276: 22313–22316.

35. Calhoun DB, Vanderkooi JM, Woodrow III GV, Englander S, W. (1983)
Penetration of dioxygen into proteins studied by quenching of phosphorescence
and fluorescence. Biochemistry 22: 1526–1532.

36. Sharma J, Panico M, Barber J, Morris HR (1997) Purification and
characterization and quantification of the alternative PsbA copies in
species in photosystem II membrane particles at elevated temperature: EPR
spectrometry. Proteomics 4: 2315–2326.

37. Summerfield TC, Toepel J, Sherman LA (2008) Low-oxygen induction of
normally cryptic psbA genes in cyanobacteria. Biochemistry 47: 12039–12941.

38. Yamashita A, Nijo N, Pospisil P, Morita N, Takenaka D, et al. (2008) Quality
control of Photosystem II: reactive oxygen species are responsible for the damage
to Photosystem II under moderate heat stress. J Biol Chem 283: 28380–28391.

39. Krüger-Lütkay A (2003) Singlet oxygen production in photosynthesis. J Exp Bot
56: 337–346.

40. Schöneich C, Sharov VS (2006) Mass spectrometry of protein modifications by
reactive oxygen and nitrogen species. Free Rad Biol Med 41: 1507–1520.

41. Sundby C, McCaffery S, Anderson JM (1993) Turnover of the Photosystem II
D1 protein in higher plants under photoinhibitory and nonphotoinhibitory
irradiance. J Biol Chem 268: 25476–25482.

42. Sundby C, McCaffery S, Anderson JM (1994) Turnover of the Photosystem II
D1 protein in higher plants under photoinhibitory and nonphotoinhibitory
irradiance. J Biol Chem 268: 25476–25482.

43. Berthold BA, Babcock GT, Yocum CF (1981) A highly resolved oxygen-
mediated by the low-potential form of cytochrome b559 in spinach thylakoids.
Photosyn Res 62: 273–279.

44. Ghastakts DF, Babcock GT (1983) Hydroxylamine as an inhibitor between Z
and P680 in Photosystem II. FEBS Lett 153: 231–234.

45. Delepelaire P, Chua N (1979) Lithium dodecyl sulfate/polyacrylamide gel
electrophoresis of thylakoid membranes at 4°C: Characterizations of two
additional chlorophyll a protein complexes. Proc Natl Acad Sci (USA) 76: 111–
115.

46. Rabilloud T, Vincon M, Garin J (1995) Micropreparative one- and
two-dimensional electrophoresis: Improvement with new polymerization
media. Electrophoresis 16: 1414–1422.

47. Sun G, Anderson VE (2004) Prevention of artificial protein oxidation
generated during sodium dodecyl sulfate-gel electrophoresis. Electrophoresis
25: 959–965.

48. Xu H, Freitas MA (2007) A mass accuracy sensitive probability based scoring
algorithm for database searching of tandem mass spectrometry data. BMC
Bioinform 8: 133–137.

49. Xu H, Freitas MA (2009) MassMatrix: A database search program for rapid
characterization of proteins and peptides from tandem mass spectrometry data.
Proteomics 9: 1548–1555.

50. Takamoto K, Chance MR (2006) Radiolytic protein footprinting with mass
spectrometry to probe the structure of macromolecular complexes. Ann Rev
Biophys Biomol Struct 35: 251–276.

51. Renzone G, Salzano AM, Arena S, D’Ambrosio C, Scaloni A (2007) Mass
spectrometry-based approaches for structural studies on protein complexes at
low-resolution. Curr Protein Peptide Lett 10: 1–16.

52. Elias JI, Haas W, Faherty BK, Gygi SP (2005) Comparative evaluation of mass
spectrometry platforms used in large-scale proteomics investigations. Nat Meth
2: 667–675.

53. Software The PyMOL Molecular Graphics System, Version 1.4 Schrodinger,
LLC.

54. Furman OS, Teel AL, Watts RJ (2010) Mechanism of base activation of
persulfate. Environ Sci Technol 44: 6423–6428.