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Phenylglyoxal modification of the Photosystem II reaction center

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Time-resolved spectroscopic techniques, including optical flash photolysis and electron spin resonance (ESR), have been used in conjunction with fluorescence-induction and dye-reduction assays to monitor electron transport in Photosystem II (PS II) subchloroplast particles incubated with the covalent modifier, phenylglyoxal. Phenylglyoxal-modified digitonin (D-10) particles from spinach are characterized by (1) a high initial fluorescence yield (F_i) and an abolition of the variable component of fluorescence (F_v); (2) an inhibition of PS-II-mediated reduction of dichlorophenol indophenol (DPIP) by *sym*-diphenylcarbazide; (3) an abolition of flash-induced absorption transients ($t_{1/2} > 2$ μs) at 820 nm attributed to the primary electron donor, P-680$^+$; (4) the inhibition of photoreduction of the acceptor Q_a; and (5) the elimination of the ESR Signal 2_s and Signal 2_f. These observations suggest the critical participation of specific arginine residues on both the oxidizing and reducing sides of Photosystem II and also implicate phenylglyoxal as a quinone-binding site inhibitor (Golbeck, J.H. and Warden, J.T. (1984) Biochim. Biophys. Acta 767, 263–271).

Introduction

Current investigations in Photosystem II have focussed on the structure, peptide composition, chemical constituency and mechanisms associated with electron transport from water to the plastoquinone pool [1,2]. In contrast to earlier studies which were primarily phenomenological, recent advances in molecular biology, coupled with progress in biochemical and spectroscopic methodology, are enabling functional and mechanistic correlations with protein structure. Photosystem II (PS II) of photosynthesis is the site of oxygen evolution in green plants and algae, and is a membrane protein complex, consisting of 7–10 distinct polypeptides [3,4]. The core of PS II is proposed to consist of a complex of two peptides, 32 and 34 kDa, which functions as the host for the primary photoreactants: P-680, the primary electron donor, and pheophytin (Pheo), the primary electron acceptor [5]. The 32 kDa peptide is the locus for herbicide binding [6] on the reducing side of PS II and contains a binding site for the secondary, quinone acceptor, Q_b [7]. Peptide components assigned to the oxidizing side of PS II include a lysine-rich 33 kDa peptide; 10, 17, and 23 kDa peptides; and a 9 kDa component identified as cytochrome *b*-559 [8,9]. The 10, 17, 23 and

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Abbreviations: Chl, chlorophyll; DPIP, 2,6-dichlorophenol indophenol; Mes, 4-morpholinoethanesulfonic acid; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; D-10, digitonin Photosystem II particles; TSF-2, triton Photosystem II particles; PS I, Photosystem I; PS II, Photosystem II; F_i, initial fluorescence yield; F_{max}, maximum fluorescence yield; F_v, variable fluorescence yield; P-680, primary electron donor of Photosystem II; Q_a, primary quinone electron acceptor; Q_b, secondary quinone electron acceptor; Pheo, pheophytin.

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33 kDa componentes are extrinsic peptides and can be dissociated from the thylakoid membrane [10–12]. These proteins have been assigned to the oxygen-evolution complex; however, the exact function of each of these peptides is still uncertain.

Electron transport in PS II is initiated by the light-mediated oxidation of P-680 coupled with the reduction of pheophytin [13]. The pheophytin anion is oxidized by a primary, quinone acceptor, Q_a, in approx. 200 ps, with subsequent electron transfer to the secondary quinone acceptor, Q_b, in 200–600 μs [14]. The Q_b-binding site functions as a two-electron gate and is susceptible to a variety of inhibitors [7,15]. The pathway from P-680 to the oxygen-evolution complex of PS II has not been elucidated thoroughly [16,17]; however, two bound quinone sites have been assigned to the oxidizing side of PS II [18–20]. Thus the redox-span of the PS II reaction center is associated intimately with bound quinones, functioning in electron and proton transport.

Recently we have embarked on a program of characterizing secondary electron transport of Photosystem II particles, utilizing kinetic, potentiometric, spectroscopic and inhibition techniques. These studies have defined a general class of inhibitors, designated as quinone-binding site inhibitors, which function, we hypothesize, by displacement of native quinone from quinone-binding proteins associated with both the reducing and oxidizing sides of PS II [21,22]. Structurally divergent compounds such as the fatty acid, linolenic acid [21], and salicylaldoxime [22] appear to function as reversible, quinone-binding site inhibitors. To map and localize the site(s) of quinone binding in PS II, one requires an irreversible, preferably covalent, inhibitor with specificity for the quinone site. Of particular importance is the observation by Gardner et al. [23] that the peptide-modification reagent, phenylglyoxal, inhibits dichlorophenol indophenol reduction in chloroplasts and additionally abolishes the specific binding of herbicides to the 32 kDa peptide. In this communication we extend the initial studies of Gardner et al. and demonstrate that phenylglyoxal is a general, covalent inhibitor of both the oxidizing and reducing loci of PS II, and specifically is a quinone-binding site inhibitor.

Materials and Methods

Digitonin particles (D-10) were isolated from spinach thylakoid membranes according to the procedure of Boardman [24]. Triton Photosystem II particles (TSF-2) were isolated by the procedure reported previously [21]. All preparations were stored frozen in 20% glycerol prior to use.

To induce covalent modification Photosystem II particles (200 μg per ml chlorophyll *a*) were incubated in the dark with the specified concentration of phenylglyoxal in 0.1 M Hepes-NaOH buffer (pH 7.9) at 30°C. Aliquots (200–250 μl) were removed from the incubation medium at designated times and were assayed for (i) PS-II-mediated 2,6-dichlorophenol indophenol reduction and (ii) flash-induced $P\text{-}680^+$ kinetics.

The PS-II-mediated reduction of 2,6-dichlorophenol indophenol (DPIP) by the donor *sym*-diphenylcarbazide was monitored at 600 nm utilizing an Aminco DW-2 spectrophotometer in the dual-beam mode.

Kinetics of $P\text{-}680^+$ reduction were monitored at 820 nm with a laboratory-constructed, flash-photolysis spectrometer interfaced to a Digital Equipment Corp. PDP 11/23 processor [21]. Actinic flashes (2.5 μs FWHM) were provided by a Photochemical Research Associates 610B xenon flash source. Flash-photolysis studies were performed on 3 ml samples containing PS II particles at 20 $\mu g \cdot ml^{-1}$ in chlorophyll, and at the pH, buffer and inhibitor concentration specified in the text. Potassium ferricyanide (1 mM) was added to the cuvette 30 s prior to flash photolysis. Each trace is the average of eight repetitive flashes. Absorption transients at 320 nm were obtained with a dedicated flash-photolysis spectrometer, utilizing repetitive 200 ns actinic flashes (660 nm) supplied by a Phase-R DL1100 flashlamp-pumped dye laser. These ultraviolet flash-photolysis studies employed a cuvette with a 2 mm pathlength and the chlorophyll concentration was typically 100 $\mu g \cdot ml^{-1}$.

Chlorophyll *a* fluorescence rise-curves were obtained with a purpose-constructed fluorometer. The particles were suspended in buffer and subjected to a 5-min dark-adaptation period prior to measurement. The 3 ml cuvette was illuminated by 450 nm light (intensity, 560 mW/m^2) selected

from a 250 W tungsten-halide lamp by a Bausch and Lomb 250 mm monochrometer. All fluorescence studies were performed on samples containing 10 μg per ml chlorophyll.

Electron spin resonance studies were achieved with a Varian E-9 spectrometer interfaced to the PDP 11/23 computer. Saturating actinic illumination was provided by a 1000 W tungsten lamp (Oriel). Specific ESR operating conditions are provided in the figure captions.

Phenylglyoxal was obtained from Aldrich Chemical Company and used as received.

Results

Donor-mediated Photosystem II electron transport

Gardner et al. [23] demonstrated that phenylglyoxal abolishes the ability of pea chloroplasts to catalyze electron transport from water to DPIP. Although these workers identified an inhibition site in the locus of the herbicide (i.e., atrazine) binding site, their data does not exclude additional inhibition sites in PS II. To characterize further the mode and site(s) of interaction of the covalent modifier with PS II, we have utilized digitonin particles that are devoid of oxygen evolution and sustain electron transport to an exogenous acceptor only in the presence of an efficient donor, such as diphenylcarbazide. The time dependent decrease in PS II mediated DPIP photoreduction as a function of phenylglyoxal concentration is illustrated in Fig. 1. Treatment in the dark of D-10 particles with 50 mM phenylglyoxal for 60 min (pH 7.9, 30°C) inhibits completely the capacity of these particles to reduce DPIP (Fig. 1c). Additionally all PS II electron-transport activity is lost in 10 min when incubations are performed with 100 mM inhibitor (data not shown). Similar patterns of inhibition are observed when incubations of the PS II particles are performed in ambient room light. However, control D-10 particles, incubated for 90 min in the absence of the inhibitor, exhibit only a minimal decrease in diphenylcarbazide-supported photoreduction of DPIP (Fig. 1a).

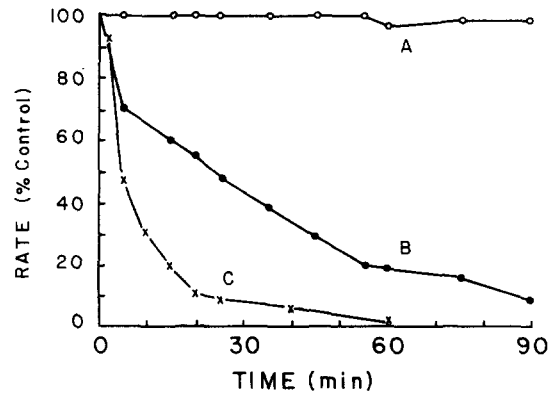

Fig. 1. Inhibition of electron transport in spinach D-10 particles by phenylglyoxal. D-10 particles (200 $\mu g \cdot ml^{-1}$) in 5 ml of 0.1 M Hepes-NaOH buffer (pH 7.9) were incubated with the indicated amounts of the covalent modifier. Aliquots (250 μl) were withdrawn at specified times and diluted into 3.0 ml of 0.1 M Mes-NaOH (pH 6.5) for assay of the PS-II-mediated photoreduction of DPIP (60 μM) by diphenylcarbazide (0.5 mM). (A) Control sample; (B) 25 mM phenylglyoxal; (C) 50 mM phenylglyoxal. Control activity (100%) corresponds to 220 μmol DPIP reduced per mg Chl per h.

Fluorescence yields

Klimov et al. [25], and more recently Sauer and coworkers [26], have proposed that Chl *a* variable fluorescence (F_v) in Photosystem II arises from recombination luminescence when the reaction centers are in the state $P\text{-}680^+Pheo^-Q_a^-$. Additionally we have suggested that inhibitors which block electron transfer between pheophytin and Q_a induce a high initial fluorescence yield, which results from the backreaction between $P\text{-}680^+$ and $Pheo^-$ [21]. Such an effect has been demonstrated with the inhibitors linolenic acid and salicylaldoxime [21,22].

Fig. 2a illustrates the fluorescence induction curve in spinach D-10 particles in the presence of 500 μM diphenylcarbazide, a PS II electron donor. Since these particles retain a fraction of the plastoquinone pool, the fluorescence rise curve in Fig. 2a represents the composite reduction of Q_a and the additional plastoquinone acceptors. The induction curve is characterized by an initial low level of fluorescence (F_i) with a subsequent rise to the maximum fluorescence yield (F_{max}). Addition of 50 mM phenylglyoxal to a dark-adapted sample results in a temporary and partial suppression (14%) of F_i and F_v (Fig. 2b). Longer term incubation of the sample with the covalent modifier is characterized by the elimination of F_v and the development of an F_i comparable to F_{max} in control particles (Fig. 2c). Incubation periods with the

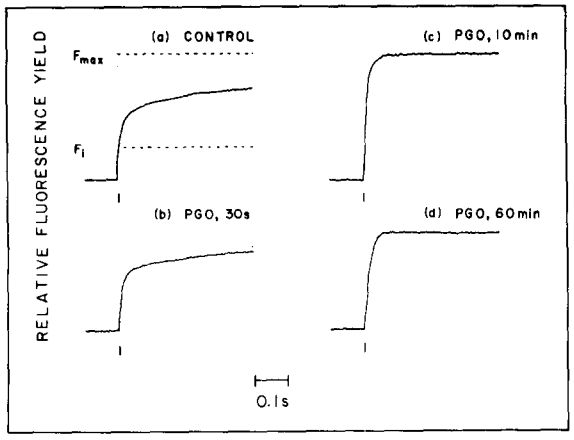

Fig. 2. Fluorescence induction curves of spinach D-10 particles incubated with 50 mM phenylglyoxal. Incubation with the inhibitor for the indicated time was performed as in Fig. 1 and samples were diluted 5 min prior to measurement into 0.05 M Tricine (pH 7.2)/0.01 $MgCl_2$ buffer. In all cases 0.5 mM diphenylcarbazide was added to the sample immediately prior to measurement. The initial (F_i) and the maximum (F_{max}) levels of fluorescence for the control sample are designated. The vertical bars indicate the onset of the actinic light.

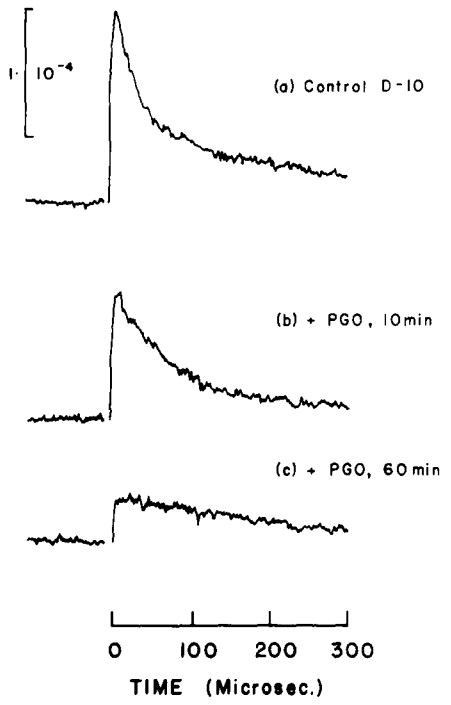

Fig. 3. Inhibition of P-680 photooxidation in D-10 particles incubated with 50 mM phenylglyoxal. Incubation conditions were as specified in Fig. 1 and flash photolysis was performed on particles at a chlorophyll concentration of 20 $\mu g \cdot ml^{-1}$ in Mes-NaOH buffer (0.1 M, pH 5.0). Samples were dark adapted 2 min prior to photolysis and contained 1 mM potassium ferricyanide. The bar on the vertical axis represents an absorbance change of 10^{-4} absorbance units at 820 nm.

inhibitor exceeding 30 min result in the gradual decline of fluorescence yield (Fig. 2d).

P-680 absorption kinetics

In control D-10 particles at pH 5.0 and in the presence of 1 mM potassium ferricyanide, the reduction kinetics of photo-oxidized $P\text{-}680^+$ exhibit biphasic character [21]: a fast phase with a half-life of 70 μs accounting for 79% of the absorption change at 820 nm (extrapolated to the onset of the actinic flash) and a slow component with a half-life of approx. 250 μs (Fig. 3a). Phenylglyoxal modified D-10 particles display a time-dependent disappearance of the fast component of the $P\text{-}680^+$ absorption transient (Fig. 3b and c). The amplitude of this transient, when monitored as a function of incubation time, shows a monotonic decrease, whereas the decay kinetics remain essentially unaltered. With 50 mM covalent modifier, 60% of the $P\text{-}680^+$ absorption is abolished in 15 min; and by 60 min all of the fast absorption transient is inhibited. The slow phase is more resistant to the action of the inhibitor; however, incubation periods of 90 to 120 min results in the elimination of this component.

Spinach TSF-2 particles exhibit similar behavior toward phenylglyoxal inhibition, except that the time required for inhibition of the P-680 absorption transient is decreased (e.g., 50 mM phenylglyoxal (pH 7.9, 30°C) yields complete inactivation in less than 20 min).

Absorption transient at 320 nm

When TSF-2 particles are examined at 320 nm during microsecond flash photolysis (Fig. 4a), a characteristic positive absorption change is observed which has been ascribed to the formation of a plastoquinone radical anion [27,28]. Since these triton particles are deficient in the secondary electron acceptor, Q_b, as determined by fluorescence induction studies (data not shown), this ultraviolet transient is presumed to originate predominantly from the reduction of Q_a, the primary

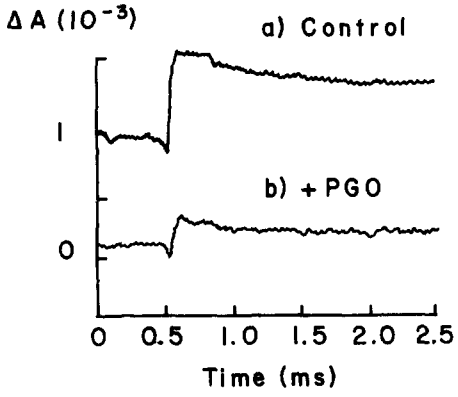

Fig. 4. Absorption transients at 320 nm in control and phenylglyoxal inhibited TSF-2 particles. After incubation (15 min) with 50 mM inhibitor as per the protocol in Fig. 1, the sample was pelleted and resuspended in phenylglyoxal-free buffer (0.1 M Mes-NaOH, pH 5.0). Chlorophyll concentration was 100 $\mu g \cdot ml^{-1}$ and 100 μM potassium ferricyanide was added to the cuvette prior to photolysis. Each kinetic trace represents the average of 16 flashes spaced 2 s apart.

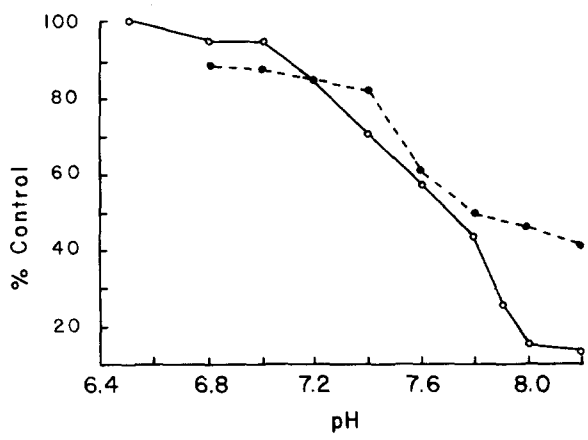

Fig. 5. The pH dependence of the inactivation of spinach D-10 particles by 50 mM phenylglyoxal. Incubations were as given in Fig. 1 with the exception that the pH was varied systematically. Aliquots were sampled after 15 min incubation with the inhibitor and assayed (as in Fig. 1) for DPIP reduction activity (○——○) and P-680 photooxidation capacity (●——●).

quinone acceptor of PS II. The total absorbance change corresponds to one Q_a reduced per 295 chlorophylls, as determined using a differential molar absorptivity for the semiquinone anion of 13000 $M^{-1} \cdot cm^{-1}$ [27]. This Triton preparation has been shown to contain approx. one P-680 per 245 chlorophyll molecules [29], hence the absorption change at 320 nm represents photoreduction of Q_a in approx. 83% of the reaction centers. The reoxidation of Q_a^- occurs in the millisecond time domain and presumably proceeds bimodally via recombination with D_1, the primary donor to P-680$^+$, and the competitive electron transfer to the oxidant ferricyanide [30]. Incubation of the particles for 15 min with 50 mM phenylglyoxal (Fig. 4b), results in a significant decrease in transient absorption at 320 nm, with the magnitude of decrease in qualitative agreement with the corresponding inhibition of the P-680$^+$ absorption transient.

pH dependence of phenylglyoxal inhibition

The residual DPIP photoreduction rate and the amplitude of the P-680 absorption change, monitored after a 15 min incubation with 50 mM phenylglyoxal, are presented as a function of pH in Fig. 5. For these pH studies the incubations were carried out as described in the Materials and Methods section, except that the pH was varied over a range of 6.8–8.2. These data indicate that significant inhibition of P-680$^+$ photo-oxidation, as well as DPIP reduction capability, is lost in the region of pH 7.5. An interesting observation is that at pH 8.2 approx. 40% of P-680 photoactivity is retained in contrast to only 15% of the initial DPIP reduction capability. This observation of differential sensitivity to phenylglyoxal suggests that multiple sites in PS II are being modified by the covalent inhibitor: site(s) associated with DPIP reduction (Q_b), diphenylcarbazide oxidation (D_1) and Q_a, the stable photoproduct of electron transfer from P-680.

Electron spin resonance Signals 2_s and 2_f

Electron spin resonance Signal 2_s and Signal 2_f are spectroscopic markers for quinoidal, electron-transport components on the oxidizing side of Photosystem 2. Signal 2_s, a stable paramagnetic species ($t_{1/2} \approx 2$ h), has no known function in PS II [31]. However, the transient Signal 2_f ($t_{1/2} \approx 1$ s) has been attributed to D_1, the electron donor to P-680$^+$ in preparations lacking oxygen evolution capability [32,33]. Fig. 6a illustrates Signal 2_s in a control D-10 preparation as observed in the dark, following a 90 s preillumination. Illumination of

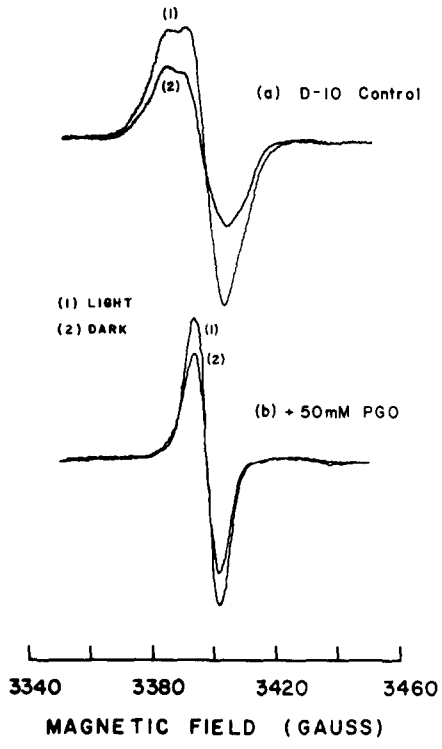

Fig. 6. Electron spin resonance spectra of control and phenylglyoxal-inhibited D-10 particles. (a) Control sample in Hepes-NaOH buffer (0.1 M, pH 7.2) at a chlorophyll concentration of 5.4 mg·ml^{-1}. (b) Same as (a), except that the sample was incubated with 50 mM phenylglyoxal for 60 min (conditions as in Fig. 1), pelleted and resuspended in inhibitor-free buffer. Both control and inhibited samples contained 5 mM potassium ferricyanide. ESR conditions: scan dwell time, 1.0 s per point; time constant, 1.0 s; modulation amplitude, 4 Gauss; receiver gain, 10000; microwave power, 25 mW; microwave frequency, 9522 MHz. The vertical axis represents ESR signal intensity in arbitrary units.

this sample results in the formation of Signal 2_f, as evidenced by the increased signal amplitude in the vicinity of 3384 G. Incubation of the PS II preparation with 50 mM phenylglyoxal for 60 min causes the elimination of both Signal 2_s and Signal 2_f. Neither one of these resonances can be restored by illumination. These data are presented in Fig. 6b. The ESR resonance (centered at $g \approx 2.0026$) remaining after incubation with the inhibitor can be assigned to contaminant chlorophyll cation radicals associated with P-700$^+$ in PS I (Signal 1).

Discussion

Although peptide-modification reagents possess great potential as structural and functional probes for the study of photosynthetic electron transport, few studies of systematic protein modification have been reported [34–36]. The use of phenylglyoxal, an arginine modifier [37], as a probe for the bicarbonate binding site was introduced by Vermaas et al. [34] and elaborated by Gardner et al. [23] in a study of atrazine binding by the 32 kDa herbicide binding peptide.

The efficacy of phenylglyoxal for inhibition of PS II has been demonstrated in our laboratory in a variety of preparations (spinach thylakoid membranes, D-10 and TSF-2 particles), with inhibition more rapidly expressed in the more purified particles. Inhibition of electron transport by phenylglyoxal in all preparations is irreversible and is equally efficient in the absence or presence of illumination. Thus light (or electron transport) is not required for inhibition. The data in Fig. 1 confirm and amplify the brief report by Gardner et al. that this covalent modifier abolishes the PS II-mediated reduction of DPIP. Kinetic analysis of the data of Fig. 1 suggests that peptide-modification by phenylglyoxal proceeds as a pseudo-first-order process, with observed rate constants clustering in the range of 0.03–0.3 min^{-1} (pH 7.9 and 30°C). Thus at 50 mM reagent, the half-time for inactivation of electron transport is approx. 6 min, a value similar to that reported by Gardner et al. for inhibition of atrazine binding [23].

Additional to the inhibition of net electron transport in PS II, the experiments described herein clearly establish that the mode of action of phenylglyoxal is multivalent; that is, the peptide-modifying reagent displays specificity for more than one site in the PS II peptide complex. For example, the data of Fig. 5 demonstrate a variance in the degree of inhibition by phenylglyoxal when monitored by DPIP reduction or by the extent of P-680 photooxidation. While the observation of P-680$^+$ kinetics with microsecond duration in these experiments requires the availability of functional Q_a, the maintenance of DPIP reduction capability is presumed also to be dependent on (1) the access of the dye to the Q_b site [15,39] and (2) the oxidation of diphenylcarbazide by D_1 (Signal 2_f)

[30]. Thus while the data of fig. 5 can be interpreted that the Q_b site is more facilely modified by phenylglyoxal at alkaline pH than is the Q_a site, one cannot exclude the possibility, based on these data alone, that covalent modification at the oxidizing side of PS II is rendering the diphenylcarbazide oxidation site nonfunctional. Indeed the observation that Signal 2_f (D_1) and Signal 2_s are irreversibly abolished in the presence of phenylglyoxal (Fig. 6) demonstrates that quinone binding sites on the oxidizing side of PS II are also susceptible to or influenced by the covalent modifier. The half life for disappearance of Signals 2_f and 2_s in D-10 particles incubated with 50 mM phenylglyoxal (pH 7.9, 30°C) is approx. 7 min, a value comparable to that observed for inhibition of DPIP-reduction capability (Fig. 1c) and the abolition of atrazine or 3-(3,4-dichlorophenyl)-1,1-dimethylurea binding [23]. These composite data are consistent with a concurrent inhibition by the covalent modifier on both the oxidizing and reducing sides of Photosystem II. This conclusion is supported further by the flash photolysis data presented in Fig. 3: inhibition of microsecond P-680$^+$ transients proceeds without significant alteration of the rereduction kinetics. In contrast, if phenylglyoxal modification occurred solely or preferentially at the diphenylcarbazide donation site (Signal 2_f), P-680$^+$ reduction would be significantly retarded with an enhancement of the kinetic component possessing a lifetime of 600–900 μs [21,40].

The fluorescence induction studies of Fig. 2 and the optical flash-photolysis data of Figs. 3 and 4 are best reconciled by noting that inhibition of electron transfer between pheophytin and Q_a is characterized by the dissipation of the charge-separated radical pair via nanosecond electron recombination between P-680$^+$ and pheophytin anion [38]. Thus phenylglyoxal inhibition at the Q_a site would induce an enhanced fluorescence yield (due to recombinant luminescence) and a concomitant abolition of absorbance transients from P-680$^+$ and Q_a^-.

The data presented in this manuscript have documented that the arginine-specific reagent, phenylglyoxal, effectively inhibits Photosystem II at loci on both the oxidizing and reducing sides of the PS II reaction center. Of note is the observation that all of the inhibition sites (Q_a, Q_b, Signal 2_f and Signal 2_s) are associated with bound-quinone molecules. We suggest that common to the binding environment or topology of the quinone binding sites in Photosystem II exist critical arginine residues, that are accessible for modification by phenylglyoxal. In this regard, recent refinement of the X-ray structure analysis of the reaction center from *Rhodopseudomonas viridis* [41] by Michel and coworkers has revealed the presence of an arginyl residue closely associated with the Q_a site (Ref. 42, see also Michel, H., personal communication). The Q_a site arginine is adjacent in the peptide sequence to a tryptophan residue whose indole ring is parallel to and partially overlaps the benzenedione functional group of menaquinone, the native quinone resident in the Q_a site.

Recently the sequences for reaction-center subunits L and M from the photosynthetic bacteria *Rhodopseudomonas capsulata* [43] and *Rhodobacter sphaeroides* (formerly called *Rhodopseudomonas sphaeroides*) [44] have been published. Similarly, the nucleotide sequences for a number of the Photosystem II peptides have been determined, notably the 32 kDa 'herbicide-binding protein' [45] and the 34 kDa integral peptide (D_2) [46,47]. Several investigators [5,42,47] have discussed the parallel character of the bacterial L and M subunits in comparison to the 32 kDa and D_2 peptides, and have noted the possible correspondence of the L subunit to the PS II 32 kDa peptide and the similar relationship of the M and D_2 peptides. Utilizing the data of Michel and coworkers [41,42] as well as the published sequences for the bacterial peptides and those for the D_2 and 32 kDa components, we have sought regions of sequence homology containing arginines. Of particular interest is the segment gly, ile, his, arg which is conserved in both *Rb. sphaeroides* and *R. capsulata* L and M subunits (residues 227–230 in *Rb. sphaeroides* L peptide). The arginine of this sequence segment is conserved in the 32 kDa herbicide binding peptide (residue 269) and the D_2 component (residue 266). This position of homology is five residues toward the C-terminus from $serine_{264}$ which has been implicated in the 32 kDa 'herbicide-binding protein' as conferring sensitivity to the herbicides atrazine and diuron [48]. Additionally $arginine_{266}$ in the D_2

peptide is adjacent to a tryptophan residue, and therefore analogous to the Q_a site in *Rps. viridis*. Thus, based on these homologies and the crystallographic data of Michel and coworkers, we propose that arginine$_{266}$ (D_2) is associated with the Q_a site and arginine$_{269}$ (32 kDa) with the Q_b site.

The role of the proposed argininyl residues in the quinone binding environment is at present unknown. However, arginines are often associated with anion-binding sites [49]. Thus the positively charged guanidinium group may serve to stabilize the anionic semiquinone radical, upon reduction of Q_a or Q_b [50]. In this regard, Patthy and Thesz have proposed that the electrostatic environment of anion binding sites in proteins produces a significant decrease in the pK_a of the guanidinium moiety with a concomitant enhancement in its reactivity to dicarbonyl reagents [49]. Thus the specificity of phenylglyoxal as a covalent modifier is two-fold: (1) for the arginyl residue in general; but, more precisely, (2) for those arginines associated with binding an anionic ligand.

Caution should be exercised in the interpretation of the results of chemical modification studies, especially when the target residues are contained in a multipeptide complex. Of particular concern is the nonselective modification of residues, especially residues having no direct functional or structural role in PS II reactions. Although we cannot unequivocally exclude the occurrence of nonspecific modification, we consistently observe, as discussed previously, that each of the proposed modification sites associated with secondary electron transport can be differentiated from modification at the primary (Q_a) site on the basis of modifier concentration dependence and reaction rate. The utilization of [^{14}C]phenylglyoxal would resolve the issue of selectivity in that the specific target peptides and their degree of modification would be clarified.

Acknowledgements

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