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# POTENTIOMETRIC TITRATION OF PHOTOSYSTEM II FLUORESCENCE DECAY KINETICS IN SPINACH CHLOROPLASTS

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**The fluorescence yield of chloroplasts reflects the redox state of the electron acceptor of the Photosystem II reaction center, with increasing yield as the acceptor is reduced. Chemical reductive titrations of fluorescence yield in chloroplasts at room temperature indicate two distinct midpoint potentials, suggesting the possibility of Photosystem II electron acceptor heterogeneity. We have carried out a potentiometric titration of the fluorescence decay kinetics in spinach chloroplasts using a continuous mode-locked dye laser with low-intensity excitation pulses and a picosecond-resolution single-photon timing system. At all potentials the fluorescence decay is best described by three exponential components. As the potential is lowered, the slow phase changes 30-fold in yield with two distinct midpoint potentials, accompanied by a modest (3-fold) increase in the lifetime. The titration curve for the slow component of the fluorescence decay of spinach chloroplasts is best characterized by two single-electron redox reactions with midpoint potentials at pH 8.0 of +119 and −350 mV, with corresponding relative contributions to the fluorescence yield of 49 and 51%, respectively. There is little change in the fast and middle components of the fluorescence decay. We found that the oxidized form of the redox mediator 2-hydroxy-1,4-naphthoquinone preferentially quenches the fluorescence, causing an anomalous decrease in the apparent midpoint of the high-potential transition. This effect accounts for a significant difference between the midpoint potentials that we observe and some of those previously reported. The selective effect of reduction potentials on particular fluorescence decay components provides useful information about the organization and distribution of the Photosystem II electron acceptor.**

## Introduction

The fluorescence yield of chloroplasts at room temperature reflects the redox state of the secondary electron acceptor, Q, of the PS II reaction center, with increasing yield as the acceptor is reduced [1]. In general, chemical potentiometric titrations of fluorescence yield in chloroplasts at room temperature have indicated two distinct redox transitions, establishing the existence of two quenchers of PS II fluorescence [2–9]. The source of the PS II electron acceptor heterogeneity remains to be established. Various models of the PS II photosynthetic unit suggest that the quenchers represent either alternative acceptors in the same PS II reaction center [4,5,10] or identical acceptors of physically distinct PS II reaction centers [4,10,11].

Abbreviations: DCMU, 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea; Hepes, N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid; $E_m$, reduction potential midpoint; PS, photosystem; Chl, chlorophyll.

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Conflicting measurements of the potentiometric titration curve of PS II chlorophyll fluorescence render the interpretation of Q heterogeneity difficult. All reports agree on the biphasic character of the curve. However, there are considerable differences in the fluorescence parameters used to determine midpoint potentials and in the actual midpoint potentials measured. The titration curve obtained by Horton and Croze [4] for pea chloroplasts in the presence of redox mediators used the ratio of variable to maximum fluorescence, $F_{var}/F_{max}$, to characterize the titration of Q. The curve consisted of two transitions with midpoint potentials at pH 7.8, $E_{m,7.8}$, of $-45$ mV for a high-potential component accounting for 70% of the total variable fluorescence and $-247$ mV for a low-potential component attributed to the remaining 30% of variable fluorescence. A titration of the initial level of fluorescence upon illumination, $F_i$, of pea chloroplasts with comparable redox mediators was reported by Malkin and Barber [5] and provided evidence for two components contributing equally to the variable fluorescence, with $E_{m,7.6}$ values of $+25$ and $-270$ mV. Golbeck and Kok [6] also used $F_i$ to monitor Q reduction in chloroplasts from *Scenedesmus* mutant No. 8 in the presence of redox mediators. They observed a high-potential component with $E_{m,7.2} = +68$ mV, accounting for 67% of Q, and a low-potential component with an $E_{m,7.2}$ near $-300$ mV, accounting for the remaining 33% of Q. In a potentiometric titration of the ratio $F_{max}/F_i$ in tobacco chloroplasts in the absence of redox mediators, Thielen and Van Gorkom [9] assigned an $E_{m,8.3}$ value of $+115$ mV to the high-potential transition and an $E_{m,8.3}$ below $-300$ mV to the low-potential transition.

The discrepancies in these measurements of the redox titration of the PS II electron acceptor Q are further confused by the heterogeneity observed in the kinetics of the fluorescence induction curve in chloroplasts inhibited with DCMU [1,12–18]. The shape of the induction curve is not characteristic of a first-order single-electron transfer, but instead consists of two phases, a slow exponential phase and a fast sigmoidal phase. The slow exponential phase, attributed to PS II reaction centers of low photochemical efficiency ($\beta$-centers) [15], titrates as a one-electron component with an $E_{m,8.3}$ of approx. $+115$ mV [9] and an $E_{m,7}$ of $+120$ mV [7]. One-electron reduction of the component responsible for the fast sigmoidal phase of fluorescence induction, a component ascribed to PS II reaction centers of high photochemical efficiency ($\alpha$-centers), occurs at about $-300$ mV [9]. The functional heterogeneity of Q in $\alpha$- and $\beta$-centers has been ascribed [15–19] to organizational differences. $\alpha$-Centers are characterized as PS II reaction centers randomly embedded in an array of Chl *a/b* light-harvesting antenna and thus capable of communication via excitation transfer, while the $\beta$-centers are isolated PS II reaction centers each with its own Chl *a/b* antenna complex and thus constituting a separate unit in terms of excitation transfer.

A correlation between the heterogeneity observed in the fluorescence induction kinetics and that measured in potentiometric titrations of fluorescence seems a logical means of attempting to explain the heterogeneity of Q [4,7–9,19]. The results of Thielen and Van Gorkom [9] are compatible with the assignment of $Q_H$ and $Q_L$ as the electron acceptors in $\beta$- and $\alpha$-centers, respectively; however, earlier investigations by others [7,8] were not.

In this paper we attempt to characterize more fully the nature of the PS II electron acceptor heterogeneity by extending the analysis to a potentiometric titration of the PS II fluorescence decay kinetics in spinach chloroplasts using low-intensity excitation pulses from a continuous mode-locked dye laser and a picosecond-resolution single-photon counting fluorescence lifetime system. The fluorescence decay kinetics of spinach chloroplasts at room temperature are best described by three exponential phases [20,21]. A fast phase of approx. 50–100 ps and a middle phase of approx. 400–800 ps arise from excitation lost prior to reaching the PS II reaction center. The fast phase is attributed to excitation lost from the Chl *a* antenna closely associated with PS II reaction centers and the middle phase reflects excitation lost from the Chl *a/b* light-harvesting antenna. There may also be a contribution from the antenna of PS I. A slow phase of 1–2 ns is attributed to fluorescence from the radical pair recombination of the oxidized primary electron donor in PS II, P-680$^+$, and the reduced pheophytin primary electron acceptor, denoted $I^-$, a recombination which arises as a consequence of a reduced state of the quinone secondary electron acceptor, Q. The changes in the fluorescence yields and lifetimes of the decay components as a function of redox potential reveal valuable information about Q organization and distribution. Furthermore, comparisons with steady-state potentiometric titrations of fluorescence yield will be made in an effort to explain the contradictions appearing in the literature.

## Materials and Methods

Broken chloroplasts were isolated from market spinach by grinding depetioled leaves in a blender for 10 s in a medium of 0.4 M sucrose, 50 mM Hepes-NaOH, pH 7.5, and 10 mM NaCl, followed by centrifugation at $6000 \times g$ for 7 min. A wash with fresh grinding medium was followed by centrifugation under the same conditions. The pellet was resuspended in a medium of 0.1 M sucrose, 10 mM Hepes-NaOH, pH 7.5, and 10 mM NaCl, and then centrifuged at $6000 \times g$ for 7 min. The isolated chloroplasts were resuspended in a medium of 0.1 M sucrose, 50 mM Hepes-NaOH, pH 7.5 or 8.0, 5 mM NaCl, and 5 mM $MgCl_2$ to give approx. 1 mg Chl/ml. For fluorescence measurements the chloroplast suspension was diluted with this final buffer, deaerated with argon gas, to a concentration of 17 $\mu$g Chl/ml.

For the potentiometric titrations the potential of the medium was measured by means of a platinum electrode with an Ag/AgCl electrode (saturated KCl solution) as reference, calibrated against a quinhydrone electrode. All reduction potentials were measured with a PAR model 173 potentiostat and are reported with respect to the standard hydrogen electrode (pH 0). The reduction potential of the suspension was adjusted under anaerobic conditions by additions of solid dithionite or small aliquots of 250 mM potassium ferricyanide (equilibration time – 15 min). Redox mediators, where present, included indophenol ($E_{m,7}$ = +228 mV); 1,4-naphthoquinone (+60 mV); duroquinone (0 mV); indigotetrasulfonate (−46 mV); 2,5-dihydroxybenzoquinone (−60 mV); indigotrisulfonate (−81 mV); indigodisulfonate (−125 mV); 2-hydroxy-1,4-naphthoquinone (−137 mV); anthraquinone-2,6-disulfonate (−184 mV); anthraquinone-2-sulfonate (−225 mV); and methyl viologen (−430 mV). Titrations performed in the absence of redox mediators required longer equilibration times (e.g., 20–30 min) to insure adjustment of the reduction potential by $Na_2S_2O_4$ or $K_3Fe(CN)_6$ alone.

A Spectra Physics synchronously pumped mode-locked dye laser (SP 171 argon ion laser, SP 362 mode locker, and modified SP 375 dye laser) was used for the fluorescence decay kinetics measurements. Samples were excited with pulses at 620 nm and with a full-width at half-maximum of 12 ps. The intensity of excitation was kept low to monitor the initial level of fluorescence and prevent appreciable steady-state reduction of Q. Fluorescence was detected at right angles at 680 nm. The single-photon timing system and numerical analysis methods have been described previously [20,21]. All fluorescence decay data were resolved into a sum of exponential decays with a lifetime resolution limit of 25 ps.

Fluorescence induction curves were recorded with an instrument similar in design to that previously constructed by Shimony et al. [22]. The chloroplast samples were excited in the blue region (Corning 3-67 and 4-96 filters in series) with low-intensity light (less than 0.05 $J \cdot m^{-2} \cdot s^{-1}$), and fluorescence was detected at wavelengths longer than 620 nm (Corning 2-60 and 2-61 filters in series) using a Hamamatsu R928 photomultiplier tube. Induction curves were stored with a Nicolet Explorer IIIA digital oscilloscope with 500 $\mu$s/address sweep timing. Chloroplasts were incubated in darkness at different redox potentials, and then 10 $\mu$M DCMU was added 2 min prior to recording the induction curve. The initial level of fluorescence upon illumination is denoted $F_i$; the minimum level of $F_i$ is observed when all PS II reaction centers are open (Q oxidized) and is denoted $F_0$. The maximum level of fluorescence upon continuous illumination is labelled $F_{max}$, and the quantity $F_{max} - F_i$ is termed the variable fluorescence, $F_{var}$.

## Results

The fluorescence induction curve in chloroplasts inhibited by DCMU was determined at different reduction potentials. Fig. 1 shows the

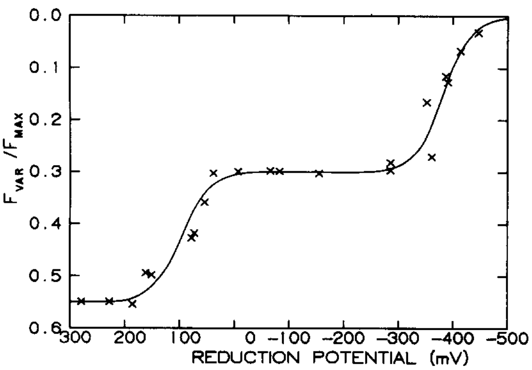


Fig. 1. Redox titration of the ratio of variable to maximum fluorescence, $F_{var}/F_{max}$, of chloroplasts at pH 7.5 in the presence of 10 $\mu$M DCMU and in the absence of redox mediators.

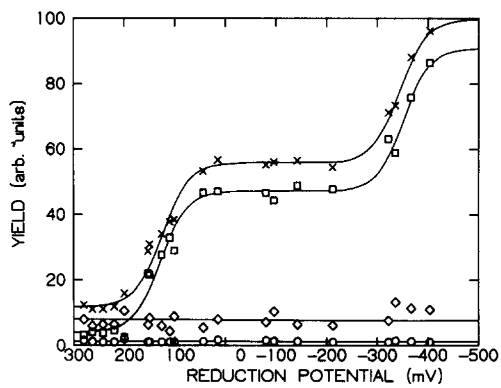


Fig. 2. Potentiometric titration of the total fluorescence yield and the yield of the components of the fluorescence decay in spinach chloroplasts at pH 8.0 in the absence of DCMU. Redox mediators present at 2.5 $\mu$M included indophenol; 1,4-naphthoquinone; duroquinone; indigotetrasulfonate; 2,5-dihydroxybenzoquinone; indigotrisulfonate; indigodisulfonate; anthraquinone-2,6-disulfonate; and methyl viologen. (×) Total fluorescence yield, (□) yield of the slow phase, (◇) yield of the middle phase, and (○) yield of the fast phase.

redox titration at pH 7.5 of the ratio $F_{var}/F_{max}$, in the absence of redox mediators, indicating the two distinct one-electron transitions previously attributed to the high- and low-potential components of Q, $Q_H$ and $Q_L$. The ratio $F_{var}/F_{max}$ has a maximal value (about 0.6) when $Q_H$ and $Q_L$ are both completely oxidized and a minimum (0) when both acceptors are fully reduced. The $E_{m,7.5}$ values are +98 and −381 mV for $Q_H$ and $Q_L$, respectively, with $Q_H$ accounting for 45% of the variable fluorescence and $Q_L$ for 55%.

Analysis of the fluorescence lifetime decay kinetics of spinach chloroplasts as a function of reduction potential resulted in a characterization of the decay as a sum of three exponential phases. Fig. 2 presents the potentiometric titration of the total fluorescence yield and that for each decay component at pH 8.0 in the presence of the specified mediators. Data were obtained for both oxidative and reductive titrations; the process appears to be completely reversible. The yields of the fast and middle lifetime components of the fluorescence decay are essentially insensitive to the state of the PS II reaction center. The slow phase, however, significantly increases in yield as the potential is lowered, with two distinct redox transitions. Thus, the two-step increase in the total fluorescence yield reflects the two reductive titrations of the components responsible for the slow phase. The curve through the experimental total yield data represents a composite of two Nernst equations for one-electron redox reactions consistent with midpoint reduction potentials $E_{m,8.0}$ = +116 and −343 mV and with relative contributions to the variable fluorescence yield of 50% each. A similar attempt to fit the experimental data for the slow phase to two one-electron Nernst equations results in midpoint potentials $E_{m,8.0}$ = +119 and −350 mV for $Q_H$ and $Q_L$, respectively, with corresponding relative contributions to the fluorescence yield of 49 and 51%.

The lifetimes of each component of the fluorescence decay as a function of reduction potential are plotted in Fig. 3. The slow phase shows an increase in lifetime during both $Q_H$ and $Q_L$ reduction. A modest increase in the lifetime of the middle component occurs only during $Q_H$ reduction, while the lifetime of the fast phase is apparently insensitive to the state of the reaction center.

Fig. 4 presents an analogous potentiometric titration of the total fluorescence yield in the absence of mediators, but buffered at pH 7.5. The titration curve is well characterized by two single-electron redox reactions making equal contributions to the total variable fluorescence, with midpoint potentials $E_{m,7.5}$ = +106 and −384 mV. The small differences in the corresponding $E_m$ values for the high- and low-potential transitions of the total fluorescence yield in Figs. 2 and 4

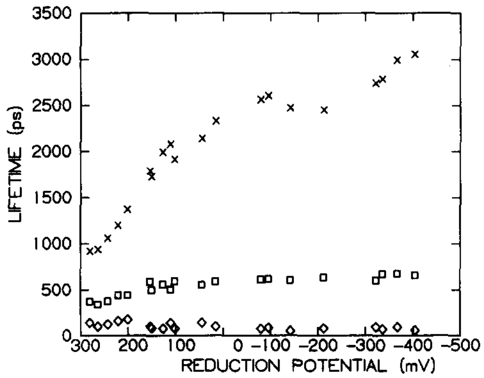


Fig. 3. Lifetimes of the components of the fluorescence decay in spinach chloroplasts at pH 8.0 in the absence of DCMU as a function of the reduction potential of the medium. Redox mediators as in Fig. 2. (×) Lifetime of the slow phase, (□) lifetime of the middle phase, and (◇) lifetime of the fast phase.

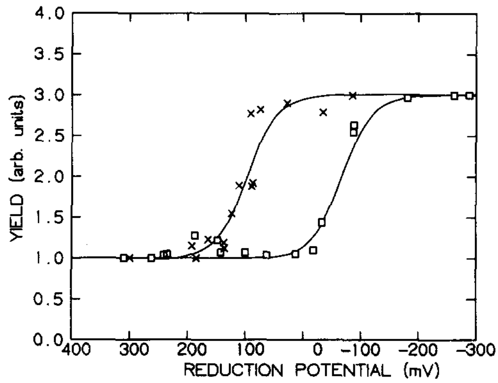


Fig. 5. Potentiometric titration of the total fluorescence yield in spinach chloroplasts at pH 7.5 in the presence (□) and absence (×) of the redox mediator 2-hydroxy-1,4-naphthoquinone.

most likely result from pH differences, slight mediator artifacts, and the estimated error in the midpoint potential. The lifetime behavior of each component as a function of reduction potential is similar to that found in the presence of mediators (Fig. 3) and, therefore, is not shown.

The similarities of Figs. 2 and 4 suggest the absence of any significant fluorescence quenching by the specified redox mediators at the low concentration level of 2.5 $\mu$M. However, sizable fluorescence quenching was detected in the presence of the oxidized form of 2-hydroxy-1,4-naphthoquinone, even at low concentrations. Fig. 5 illustrates the effect of 2.5 $\mu$M 2-hydroxy-1,4-naphthoquinone in producing a significant lowering of the midpoint potential for $Q_H$, from +96 to −68 mV. This is attributed to strong quenching of the $Q_H$ fluorescence by the oxidized form of the mediator. Malkin and Barber [5] observed quenching of chlorophyll fluorescence by this mediator in similar steady-state redox titrations.

Fig. 6 presents the potentiometric titration of the fluorescence decay components in the presence of DCMU. The curve for the total fluorescence yield is described by two single-electron transitions with $E_{m,7.5}$ values of +58 and −386 mV, contributing 31 and 69%, respectively, to the total variable fluorescence. Only the slow phase yield is

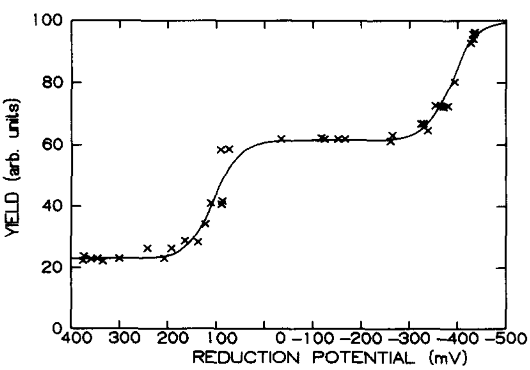


Fig. 4. Potentiometric titration of the total fluorescence yield in spinach chloroplasts at pH 7.5 in the absence of redox mediators and DCMU.

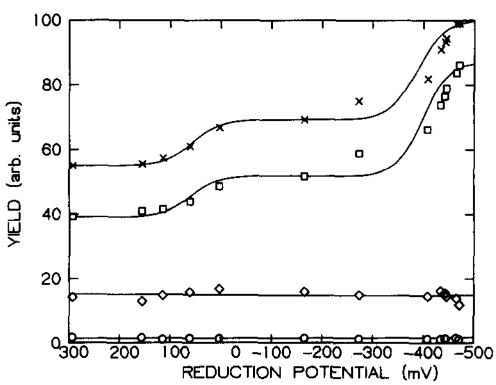


Fig. 6. Potentiometric titration of the total fluorescence yield and the yield of the components of the fluorescence decay in spinach chloroplasts at pH 7.5 in the absence of redox mediators and in the presence of 10 $\mu$M DCMU. (×) Total fluorescence yield, (□) yield of the slow phase, (◇) yield of the middle phase, and (○) yield of the fast phase.

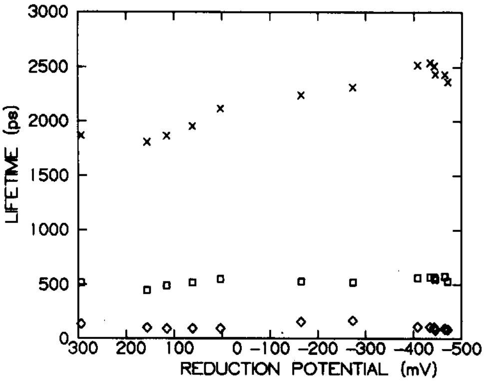


Fig. 7. Lifetimes of the components of the fluorescence decay in spinach chloroplasts at pH 7.5 in the presence of 10 $\mu$M DCMU as a function of reduction potential of the medium. (×) Lifetime of the slow phase, (□) lifetime of the middle phase, and (◇) lifetime of the fast phase.

TABLE I

CHARACTERISTICS OF THE POTENTIOMETRIC TITRATION OF THE ELECTRON ACCEPTOR Q IN PS II

Chloroplasts, isolated as described in Materials and Methods, were suspended in a medium of 0.1 M sucrose, 50 mM Hepes-NaOH (buffered at the stated pH), 5 mM NaCl, and 5 mM $MgCl_2$ to give a chlorophyll concentration of 17 $\mu$g/ml. Fluorescence samples were dark adapted under anaerobic conditions for 15 min at the appropriate reduction potential before addition of DCMU or illumination.

| pH | [DCMU] ($\mu$M) | $E_m^a$ (mV) | Contribution to $F_{var}$ (%) |
|---|---|---|---|
| Fluorescence induction experiments, $F_{var}/F_{max}$ | | | |
| 7.5 | 10 | +98 [b] | 45 |
| | | −381 | 55 |
| Fluorescence decay kinetics, $F_i$ level | | | |
| 8.0 | 0 | +116 [c] | 50 |
| | | −343 | 50 |
| 7.5 | 0 | +106 [b] | 50 |
| | | −384 | 50 |
| 7.5 | 0 | +96 [c,e] | – |
| 7.5 | 0 | −68 [d,e] | – |
| 7.5 | 10 | +58 [b] | 31 |
| | | −386 | 69 |

[a] $E_m$ values relative to standard hydrogen electrode.
[b] No redox mediators present.
[c] Redox mediators present (2.5 $\mu$M); 2-hydroxy-1,4-naphthoquinone not included as a redox mediator.
[d] Redox mediators present (2.5 $\mu$M); 2-hydroxy-1,4-naphthoquinone included as a redox mediator.
[e] $E_m$ value determined for $Q_H$ only.

influenced by reduction potential, exhibiting two transitions at $E_{m,7.5} = +64$ and $-392$ mV with relative fluorescence yield contributions of 26 and 74%, respectively. A small effect of reduction potential on the slow phase lifetime is depicted in Fig. 7, while the lifetimes of the middle and fast phases are not altered by reduction potential.

Table I summarizes the results obtained for the potentiometric titrations of fluorescence induction and decay kinetics.

## Discussion

The major features of the potentiometric titration of the fluorescence decay kinetics in chloroplasts at room temperature in the absence of DCMU are: (1) the absence of any significant effect of reduction potential on the fluorescence yield of the fast and middle phases but the presence of two distinct redox transitions in the slow phase yield (and consequently in the total yield), with midpoint potentials $E_{m,7.5} = +106$ and $-384$ mV; and (2) a moderate increase in the lifetime of the slow phase during both $Q_H$ and $Q_L$ reduction and in the lifetime of the middle component during $Q_H$ reduction, but no effect of reduction potential on the lifetime of the fast phase.

The choice of redox mediators and their concentration is critical in order to monitor accurately changes in chlorophyll fluorescence induced by changes in the redox state of Q. The ability of a number of quinone mediators to quench chlorophyll fluorescence directly when in their oxidized forms is well documented [5,23–25]. Distortion of the titration curve is a possible consequence of such quenching. In this work the mediator 2-hydroxy-1,4-naphthoquinone was found to have a high quenching ability, significantly lowering the midpoint potential of the high-potential component in measurements of total fluorescence yield and the slow phase yield of fluorescence decay. Titrations in the absence of 2-hydroxy-1,4-naphthoquinone but either in the presence or absence of other redox mediators compare favorably in midpoint potentials and relative amplitudes of the two transitions.

Changes in the lifetime of the slow phase can be used to determine the extent of excitation energy transfer between reaction centers and thus discriminate between connected or isolated reaction centers. Energy transfer between photosynthetic units implies that Chl *a/b* light-harvesting antennae serve more than one reaction center. The lifetime of an excited state in the antenna of a closed reaction center is a function of the ability of that reaction center to communicate with other reaction centers. Communication via the Chl *a/b* light-harvesting antenna increases the lifetime of such an excited state; the extent of such an increase is dependent on the fraction of open reaction centers. Thus, we expect the lifetime of the slow phase of fluorescence decay of an $\alpha$-center, a PS II reaction center capable of communication via excitation transfer, to be dependent on the state of the reaction center as monitored by the effect of reduction potential. $\beta$-Centers, PS II reaction centers that are isolated in terms of excitation transfer, should exhibit a slow phase lifetime that is independent of reduction potential. On the basis of the lifetime of the slow phase of fluorescence decay in Fig. 3, both $Q_H$ and $Q_L$ act as electron acceptors in $\alpha$-centers. This result is in agreement with conclusions made by Horton [8] in fluorescence induction studies of pea chloroplasts. No distinction can be made here as to whether each $\alpha$-center contains both $Q_H$ and $Q_L$ or whether the $\alpha$-centers of a domain of communicating reaction centers contain either $Q_H$ or $Q_L$ as an electron acceptor. The electron acceptor of $\beta$-centers also remains undetermined.

Such an assignment of electron acceptors to $\alpha$- and $\beta$-centers has been attempted [7,8,27] by correlating the sigmoidicity of the fluorescence induction curve with the relative proportions of $Q_H$ and $Q_L$ observed in redox titrations of chloroplast membranes under a variety of conditions. The sigmoidicity of the fluorescence induction curve can be explained either by energy transfer between the antenna of a domain of reaction centers or by a requirement for two successive photoreactions to close the reaction center [4,7,8,19,27]. Recent experiments on PS II particles from the blue-green alga *Phormidium laminosum* [28] favor the former explanation. Studies of the fluorescence decay kinetics of a variety of chloroplast membrane systems provide a means to distinguish between these two explanations for sigmoidal induction. Our ongoing work involves monitoring the fluorescence decay kinetics during potentiometric titrations of Q in chloroplast systems under conditions where the probability for energy transfer between reaction centers can be varied and in systems of variable relative proportions of $Q_H$ and $Q_L$.

A number of features of this study are comparable to recent investigations in this laboratory on the state of the PS II reaction center as determined by illumination conditions and the presence of various inhibitors [20,21]. That the yield of only the slow phase of the fluorescence decay is affected by reduction potential is consistent with the interpretation of the decay components [20,21]; the fast and middle components of the fluorescence decay arise from excitation lost prior to reaching the PS II reaction center and, therefore, should be largely independent of the redox state of the reaction center. The increase in the lifetime of the middle phase has previously been tentatively attributed to changes in energy-distribution regulation mechanisms as a consequence of changes in the redox state of Q [20,21]. However, some differences are apparent in the effect of reduction potential and of illumination conditions on the state of the PS II reaction center. For example, we find that chemical reduction of Q induces as much as an 8-fold increase in total fluorescence yield in chloroplasts at pH 8.0 (Fig. 2), whereas closing the PS II reaction center by high-intensity excitation effected only a 4-fold increase in total fluorescence yield [20,21]. Furthermore, we measure the lifetime of the slow component of fluorescence decay to be significantly greater when the closure of the PS II reaction centers is achieved by low reduction potential than by illumination [20,21]. Our present understanding of the PS II photosynthetic unit and the associated fluorescence do not adequately account for these differences. Future experiments will focus attention on these details.

Despite the similarities in the results of the potentiometric titrations of fluorescence induction and of the fluorescence decay kinetics, a conclusive interpretation of the observed PS II electron acceptor heterogeneity is not possible at this point. Potentiometric titrations using chloroplasts with compositional and structural differences, such as intermittent light-grown chloroplasts which do not contain the light-harvesting Chl *a/b*-protein complex, may provide insights into the question of PS II electron acceptor heterogeneity.

## Conclusion

Analysis of the potentiometric titration of the PS II fluorescence decay kinetics in spinach chloroplasts at room temperature in the presence and absence of DCMU indicates two distinct redox transitions in the total fluorescence yield. The yield of the slow phase of the fluorescence decay is also sensitive to the state of the PS II reaction center, but the fast and middle lifetime components of the fluorescence decay are not appreciably affected by the reduction potential of Q. The midpoint reduction potentials for the two fluorescence quenchers, $Q_H$ and $Q_L$, correlate well with those measured by Thielen and Van Gorkom [9] for $Q_\beta$ and $Q_\alpha$, respectively; however, changes in the lifetime of the slow component of fluorescence decay suggest the possible assignment of $Q_H$ and $Q_L$ as the electron acceptors in $\alpha$-centers. A significant difference in the $E_m$ value measured for $Q_H$ from other previous reports is attributable to quenching of fluorescence in the presence of the oxidized form of the mediator 2-hydroxy-1,4-naphthoquinone.

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