*Biochimica et Biophysica Acta* 849 (1986) 175–182
Elsevier



BBA 41984

# Quantitation of Photosystem II in spinach chloroplasts

Steven W. McCauley and Anastasios Melis

*Division of Molecular Plant Biology, 313 Hilgard Hall, University of California, Berkeley, CA 94720 (U.S.A.)*

(Received August 22nd, 1985)
(Revised manuscript received December 30th, 1985)

Key words: Photosystem II; Reaction center; Primary quinone acceptor; Semiquinone anion; Absorbance change; (Spinach thylakoid)

**The concentration of photochemical reaction centers in spinach chloroplasts was measured with direct spectrophotometric techniques. Quantitation of Photosystem II (PS II) was implemented (a) from the light-induced absorbance change in the near-ultraviolet ($\Delta A_{320}$), monitoring the primary quinone acceptor $Q_A$ of PS II; and (b) from the light-induced absorbance change in the green region ($\Delta A_{540-550}$), monitoring the band-shift C550 of the PS II reaction center pheophytin molecule. Both measurements yielded identical chlorophyll (Chl) per PS II ratios (Chl/PS II = 380:1). Hydroxylamine treatment and the loss of Mn from the oxygen-evolving enzyme of photosynthesis had no effect on the quantitation of $Q_A$ by $\Delta A_{320}$. It is concluded that, under the experimental conditions employed, measurements of $Q_A$ photoreduction are not overlapped by state transitions of the water-splitting enzyme. Quantitation of Photosystem I (PS I) was implemented from the light-induced absorbance change in the red region of the spectrum ($\Delta A_{700}$), monitoring photooxidation of the PS I reaction center P-700. A Chl/PS I = 650:1 was derived. Thus, a PS II/PS I stoichiometric ratio of 1.7 was estimated in spinach chloroplasts.**

## Introduction

Quantitation of Photosystem II (PS II) from the light-induced ultraviolet absorbance change ($\Delta A_{320}$) in the presence of 3-(3′,4′-dichlorophenyl)-2,2-dimethyl urea (DCMU) and potassium ferricyanide (measuring the concentration of the semiquinone primary electron acceptor $Q_A$ of PS II) yielded chlorophyll (Chl)/PS II ratios mostly in the range of 300–400 and reaction center (RC) ratios RC II/RC I of 1.4–2.0 in several higher plant chloroplasts [1–3]. The significance of the observation that RC II/RC I > 1 in a variety of higher plant chloroplasts is that it is contrary to the conventional prejudice (from an extremely literal interpretation of the Z-scheme) that the ratio of the two photosystems should be equal to 1.0. A controversy has developed, however, since a flash-induced $H^+$ release from $H_2O$ oxidation, measured with isolated chloroplasts in the presence of the artificial electron acceptors ferricyanide and 2,5-dimethylbenzoquinone, yielded Chl/PS II = 630 and RC II/RC I = 1, invariable in several higher plant chloroplast preparations [4,5]. In order to explain the discrepancy of the results from the two experimental approaches, Whitmash and Ort [4] suggested that quantitation of PS II from the semiquinone anion formation grossly overestimates the concentration of PS II because of uncertainty in the differential extinction coefficient of $Q_A^-$ minus $Q_A$ and also because

Abbreviations: PS II, Photosystem II; PS I, Photosystem I; Chl, Chlorophyll; RC, reaction center; P-680, reaction center of PS II; P-700, reaction center of PS I; $Q_A$, primary quinone electron acceptor of PS II; Ph, pheophytin; Z, secondary PS II donor; DCMU, 3-(3′,4′-dichlorophenyl)-2,2-dimethyl urea; $C_f$, differential flattening correction factor; Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine.

of possible overlapping absorbance changes at 320 nm due to the advancement of the S states associated with the function of the water-splitting enzyme.

An independent method for the quantitation of PS II in higher plant chloroplasts is from the measurement of the absorbance change due to the indicator pigment C550. It has been shown that the storage of a negative charge on the primary electron quinone-acceptor $Q_A$ of PS II causes, through the influence of a localized electric field, the electrochromic band shift of the reaction center pheophytin (Ph) molecule, thus giving rise to a typical absorbance-difference change in the green region of the spectrum, termed C550 [6–10]. The C550 signal emanates from all functional PS II reaction centers [8]. However, this property has not been used for PS II quantitation in chloroplasts, primarily because of the lack of reliable differential extinction coefficients.

In the present work, we measured the concentration of PS II in spinach chloroplasts by a variety of experimental techniques. We used the absorbance-change measurement at 320 nm ($\Delta A_{320}$) with intact spinach thylakoids, with hydroxylamine-treated thylakoids in which the S states and the oxygen-evolving enzyme was destroyed, and with resolved membranes from the grana partition region in which the concentrations of other PS II integral components are known. We used the latter to provide a calibration of the differential extinction coefficients of C550 and then applied the C550 quantitation method to unfractionated spinach thylakoids. With unfractionated spinach thylakoids, all of the above methods yielded Chl/PS II ratios in the range between 350–400.

We conclude that flash yield measurements with isolated chloroplasts [4,5] may not yield reliable estimates of total PS II concentration in thylakoid membranes because the maximum yield of one electron equivalent per flash per PS II reaction center cannot be assured under in vitro conditions.

## Materials and Methods

Chloroplast thylakoid membranes were isolated from freshly-harvested, hydroponically grown spinach (*Spinacia oleracea* L.) by grinding the leaves in a blender in a buffer containing 50 mM Tricine-NaOH (pH 8.0), 0.4 M sucrose/10 mM NaCl/5 mM $MgCl_2$. The slurry was filtered through miracloth and chloroplasts were precipitated by centrifugation at $5000 \times g$ for 5 min. The pellet was resuspended in a small amount of the isolation buffer to a Chl concentration of about 2 mg/ml using a Wheaton homogenizer. The sample was then kept in the dark on ice until use. Chlorophyll concentrations were determined in 80% acetone using the procedure of Arnon [11].

Hydroxylamine treated thylakoids were obtained upon incubation of chloroplasts for 20 min at a Chl concentration of 500 $\mu$M in the presence of 20 mM $NH_2OH$. Control and $NH_2OH$-treated membranes were washed twice and finally resuspended in a small volume of the chloroplast isolation buffer. All operations were carried out in dim light at 0°C. Triton X-100 treated chloroplasts were obtained by a modification of the procedure of Berthold et al. [12]. Thylakoids (2 mg Chl/ml) suspended in 50 mM Tricine-NaOH (pH 7.8)/0.4 M sucrose/10 mM NaCl/5 mM $MgCl_2$/5 mM $CaCl_2$ were incubated with Triton X-100 (Triton/Chl = 25 : 1, w/w) at 0°C for 30 min. The membranes of the grana partition region were precipitated by centrifugation at $40\,000 \times g$ for 30 min and then resuspended in the original chloroplast isolation buffer.

Absorbance-difference measurements in the ultraviolet and red regions of the spectrum were performed with a laboratory-constructed difference spectrophotometer [13]. The optical pathlength of the cuvette for the measuring beam was 2.06 mm and for the actinic beam it was 1.46 mm. Actinic excitation was provided in the green region by a combination of Corning CS 4-96 and CS 3-69 filters. The concentration of $Q_A$ was measured from the light-induced absorbance change at 320 nm ($\Delta A_{320}$) using a differential extinction coefficient of 13 $mM^{-1} \cdot cm^{-1}$ [14]. The P-700 concentration was determined from the light-induced absorbance change at 700 nm ($\Delta A_{700}$) using a differential extinction coefficient of 64 $mM^{-1} \cdot cm^{-1}$ [15]. Measurements of the system II indicator pigment C550 were obtained with an Aminco DW-2a instrument operated in the dual-beam mode. Thylakoids were suspended in the presence

of 20 μM gramicidin, 15 μM DCMU and 2 mM potassium ferricyanide as described in Ref. 8. The half-band width of the measuring beam was set at 1 nm and the optical pathlength of the cuvette was 1.0 cm.

Quantitation of the functional PS II reaction centers was obtained from the amplitude of the absorbance change $\Delta A_{320}$ ($Q_A$) and $\Delta A_{540-550}$ (C550). In both measurements, thylakoid membranes and resolved membranes from the grana partition region were suspended in the presence of 20 μM DCMU and 2 mM potassium ferricyanide. The experimental protocol for $Q_A$ and C550 quantitation involved one preillumination for the complete oxidation of cytochrome *f* and of P-700. During subsequent illuminations of the sample, these electron transport components remained oxidized (presence of DCMU and ferricyanide). Following the preillumination, each sample was illuminated four times at a rate of one per min for the registration of $\Delta A_{320}$ and $\Delta A_{540-550}$. We verified that a 60 s dark interval between illuminations was adequate for the complete relaxation of $Q_A$ and C550. Signal recovery and processing was implemented by a Hewlett-Packard 3437A digital voltmeter interfaced with an on-line HP 86B computer. The results were plotted on an HP 7475A plotter.

## Results

### *Quantitation of $Q_A$*

A criticism of the spectroscopic $\Delta A_{320}$ method for the quantitation of PS II was the uncertainty of the in situ differential extinction coefficient $\Delta\varepsilon$ of $Q_A$ and the question of absorbance change contributions from the oxidizing side of PS II [4,5]. The question of the in situ $\Delta\varepsilon$ of $Q_A$ has already been addressed by several investigators using different experimental calibrations: Bensasson and Land [16] measured the difference spectrum of plastosemiquinone-9 anion minus plastoquinone in ethanol. They derived a $\Delta\varepsilon_{320} = 13$ $mM^{-1} \cdot cm^{-1}$. Van Gorkom [7] estimated in situ $\Delta\varepsilon_{320} = 11.6$ $mM^{-1} \cdot cm^{-1}$ for $Q_A^-$ minus $Q_A$ in deoxycholate-derived PS II particles. Ke et al. [17], working with an oxygen-evolving PS II particle from a blue-green alga, reported a reaction center component stoichiometry of P-680/Ph/$Q_A$/Mn as 1:1:1:4. Their optical-difference measurements in the ultraviolet were consistent with a difference extinction coefficient for $Q_A$ in the range of 11–13 $mM^{-1} \cdot cm^{-1}$ at 320 nm [17]. In summary, there is agreement for a $\Delta\varepsilon$ value in the range of 11–13 $mM^{-1} \cdot cm^{-1}$ for $Q_A^-$ minus $Q_A$ at 320 nm [7,16,17].

Absorbance change contributions in the ultraviolet from the oxidizing side of PS II have been reported from several laboratories. The secondary donor Z of PS II undergoes transient changes with a maximum of 255 nm [18,19]. At 320 nm the contribution of $Z^+$ minus Z is negligible. Moreover, under continuous illumination of low intensity employed in our measurements, $Z^+$ will not accumulate in any detectable amount. For the reasons stated, we will not concern ourselves with absorbance changes due to Z in the results shown below.

The charge-storing and oxygen-evolving enzyme of PS II was also reported to undergo light-induced absorbance changes in the ultraviolet. At present, there is no agreement as to the precise difference spectrum and the nature of the chemical species underlying it. Renger and Weiss [20] attributed a large flash-induced absorbance change at 320 nm to the advancement of the S states. Dekker et al. [21] reported identical difference spectra for each of the S transitions ($S_0 \rightarrow S_1$, $S_1 \rightarrow S_2$ and $S_2 \rightarrow S_3$) with a broad band peaking at 295 nm. According to these authors [18,21], the contribution at 320 nm from the transition of the S states occurred with a differential extinction coefficient of 5–6 $mM^{-1} \cdot cm^{-1}$. A 3-times larger absorbance change of opposite amplitude was implied for the relaxation of the enzyme during the $S_3 \rightarrow S_4$ ($S_0$) transition.

The question we addressed in this portion of our work is whether the light-induced absorbance change contributions due to the S state transitions manifest themselves under our experimental conditions at 320 nm, i.e., in the presence of DCMU and a high (2–3 mM) concentration of potassium ferricyanide. We approached this point directly by comparing the electron transport and ultraviolet absorbance change properties of control (untreated) and hydroxylamine-treated spinach chloroplasts in which the oxygen-evolving enzyme was fully impaired. Fig. 1 shows fluorescence induc-

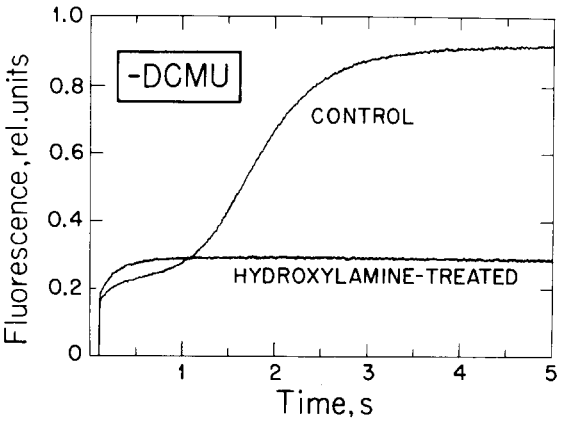


Fig. 1. Fluorescence-induction measurements in control (untreated) and hydroxylamine-treated spinach thylakoids. The hydroxylamine-treatment inhibited the $O_2$-evolving capacity of PS II.

tion measurements taken with control and $NH_2OH$-treated thylakoid membranes. The variable fluorescence yield increase in control chloroplasts, occurring after about 1 s of illumination, is a manifestation of electron accumulation in the plastoquinone pool due to the activity of PS II. No such accumulation of electrons occurs in the $NH_2OH$-treated samples and the low-fluorescence yield, observed in the latter, is evidence of incapacitated $O_2$-evolving enzyme. Fig. 2 shows the amplitude of the absorbance change at 320 nm in control and $NH_2OH$-treated spinach chloroplasts, suspended in the presence of 25 $\mu$M DCMU and 2.5 mM potassium ferricyanide. Each sample was illuminated 4 times at the rate of 1 per min. A 60 s dark interval between illuminations was fully adequate for the relaxation of $Q_A$. The curves represent the average of 12 measurements with three samples. After correction for flattening ($C_{f\,320} = 1.35$) we estimated a Chl/$Q_A$ ratio of 380 in the control and a Chl/$Q_A$ ratio of 460 in the $NH_2OH$-treated sample. In the latter, however, not all PS II centers undergo a stable $Q_A \rightarrow Q_A^-$ transition upon illumination. The lower steady-state fluorescence yield obtained in $NH_2OH$-treated samples suspended in the presence of DCMU and ferricyanide (Fig. 3) indicates that a significant fraction of PS II cannot acquire the single electron needed to generate a stable charge separation ($Q_A^-$ state). Most PS II centers in the $NH_2OH$-treated samples are able to acquire one electron to form the stable $Q_A^-$ state. The electron donor in this case is probably residual hydroxylamine molecules lodged in the thylakoid lumen. Using the non-linear relationship between $Q_A^-$ and $F_v$ described in Refs. 8 and 22 we determined that in $NH_2OH$-treated chloroplasts only about 87% of PS II centers undergo a stable charge separation. Therefore, a Chl/$Q_A$ = 460 in $NH_2OH$-treated samples underestimates the true concentration of

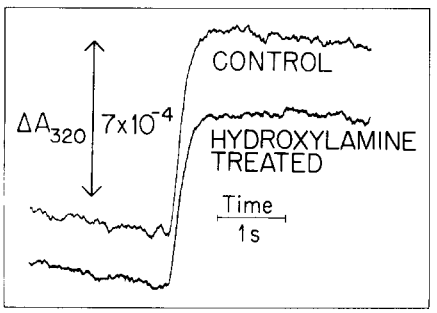


Fig. 2. The amplitude of the light-minus-dark absorbance change at 320 nm ($\Delta A_{320}$) in control and hydroxylamine-treated spinach thylakoids. Thylakoid membranes (Chl $a$/Chl $b$ = 2.53) were suspended at 160 $\mu$M Chl in the presence of 25 $\mu$M DCMU and 2.5 mM potassium ferricyanide. Optical pathlength of the cuvette was 2.06 nm. Differential flattening correction factor at 320 nm ($C_{f\,320}$) was 1.35. Each sample was illuminated 4 times at a rate of one per min. The data represent the average of 12 measurements with three separate samples. The molecular ratio of Chl to PS I in the same sample was equal to 650 ± 50.

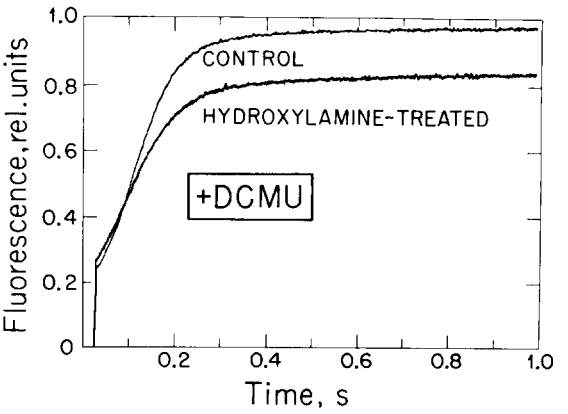


Fig. 3. Fluorescence induction measurements in control (untreated) and hydroxylamine-treated spinach thylakoids suspended in the presence of 25 $\mu$M DCMU and 2.5 mM potassium ferricyanide. The lower steady-state fluorescence yield of hydroxylamine-treated samples reflects the stable photoreduction of only about 87% of $Q_A$ [8]. For other conditions, see legend of Fig. 2.

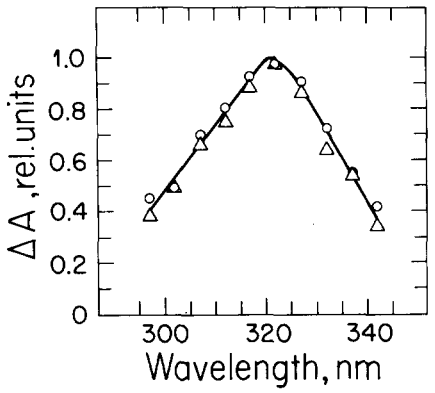


Fig. 4. Light-minus-dark absorbance difference spectra of control (○) and hydroxylamine-treated (△) spinach thylakoids. The conditions are the same as described in the legend to Fig. 2. The spectra are normalized to the same maximum value.

$Q_A$ in the thylakoid membranes. Corrected for this inefficiency a Chl/$Q_A$ ratio of $460 \times 0.87 \approx 400$ was derived. This value is in close agreement with the Chl/$Q_A$ ratio of 380 measured on control chloroplasts (see Fig. 2) and suggests that under our experimental conditions no substantial absorbance change contributions occur at 320 nm from the advancement of the S states.

To support our contention on the absence of absorbance change signals in the 320 nm region from the S states, we measured the light minus dark difference spectrum in the wavelength region between 290 and 350 nm. Fig. 4 shows the absorbance difference spectra obtained with untreated (circles) and $NH_2OH$-treated (triangles) chloroplasts, both normalized at 322 nm. The two difference spectra are identical. Since the $O_2$-evolving system is functional in the untreated samples, but fully incapacitated in $NH_2OH$-treated thylakoids, one may conclude that no contribution from S state transitions is manifested in either sample. It is evident that the in vivo PS II concentration is 380–400 Chl per $Q_A$. Independently, we measured a Chl/P-700 ratio of 650 translating into RC II/RC I ratio of approx. 1.7 in greenhouse-grown spinach.

*Quantitation of C550*

An independent method in the approach of PS II quantitation is from the amplitude of the absorbance change due to the indicator pigment C550 [6–10]. We performed the C550 measurements with samples suspended in the presence of 20 μM gramicidin/15 μM DCMU/2 mM FeCN. Under these conditions, the absorbance change due to the transmembrane electric field is eliminated [23]. The cytochromes of the photosynthetic apparatus and the reaction center P-700 remain in the oxidized state following preillumination [6,8,19]. With overlapping absorbance changes eliminated, the $\Delta A_{540-550}$ change is solely due to the electrochromic band-shift of Ph associated with the primary charge separation of PS II. We approached the question of C550 quantitation by determining the differential extinction coefficients from the absorbance difference spectrum of C550. This was implemented with oxygen-evolving resolved membrane preparations from the grana partition region, prepared according to Berthold et al. [12]. Such membranes are significantly enriched in PS $II_\alpha$ [24] have a low Chl *a*/Chl *b* ratio (less

TABLE I

QUANTITATION OF Chl AND OF REACTION CENTER CONTENT FROM $\Delta A_{700}$ (P-700), $\Delta A_{320}$ ($Q_A$) AND $\Delta A_{540-550}$ (C550) IN SPINACH CHLOROPLASTS

The differential flattening correction factor, $C_f$, is given at different wavelengths for unfractionated thylakoid membranes and for resolved grana partition regions. The latter were isolated upon Triton solubilization of isolated chloroplasts (see Materials and Methods). A value of Chl/C550 = 250:1 in resolved grana partition regions was based on the concentration of other integral PS II components (P-680/Z/Mn/Cyt *b*-559/Chl($a + b$) = 1:1:4:2:250) in similar preparations (see text).

| | $\frac{\text{Chl } a}{\text{Chl } b}$ | $\frac{\text{Chl}}{\text{P-700}}$ | $C_{f\,320}$ | $\frac{\text{Chl}}{Q_A}$ | $C_{f\,550}$ | $\frac{\text{Chl}}{\text{C550}}$ |
|---|---|---|---|---|---|---|
| Unfractionated thylakoids | 3.0 | 650 | 1.35 | 380 | 1.05 | 390 |
| Resolved grana partition regions | 1.9 | 3200 | 1.1 | 230 | 1.02 | 250 |

than 1.9) and a high Chl/P-700 ratio (at least 3200), i.e., they are substantially depleted in PS I, but enriched in PS II (see Table I). In work with such preparations from this laboratory [24], we determined the presence of two cytochrome *b*-559 molecules per 250 Chl($a+b$) molecules, i.e., Chl/PS II = 250 : 1 [24]. In work with similar preparations from several laboratories, the concentration of integral PS II components was determined to be in the range of 1 PS II per 220–270 Chl($a+b$) molecules. In particular, the following PS II component quantitation has been reported: P680/signal $II_f$/signal $II_s$/Cyt *b*-559/Mn/Chl($a+b$) molecules = 1 : 1 : 1 : 2 : 4 : 250 (see Table I, also [17,24–27]). Working with resolved membranes from the grana partition region we measured the amplitude of the light-induced absorbance change at 540 minus 550 nm ($\Delta A_{540-550}$, see Fig. 5B).

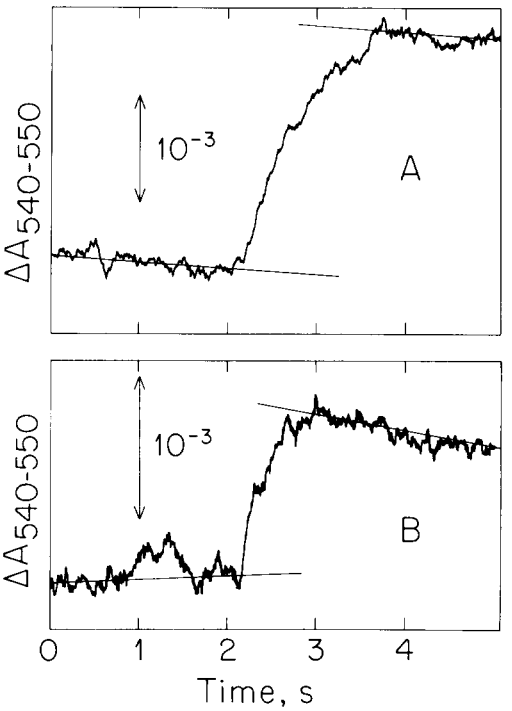


Fig. 5. The amplitude of the light-induced 540–550 nm absorbance change in control (A) and Triton X-100 treated (B) thylakoids. Both samples were suspended in a 1-cm pathlength cuvette in the presence of 20 μM gramicidin/15 μM DCMU/2 mM FeCN. The final concentration of the control (Chl *a*/Chl *b* = 3.0) was 174 μM, with a flattening correction factor ($C_f$) of 1.05 at this wavelength. The Triton-treated samples (Chl *a*/Chl *b* = 1.9) final concentration was 50 μM, with a $C_f$ value of 1.03.

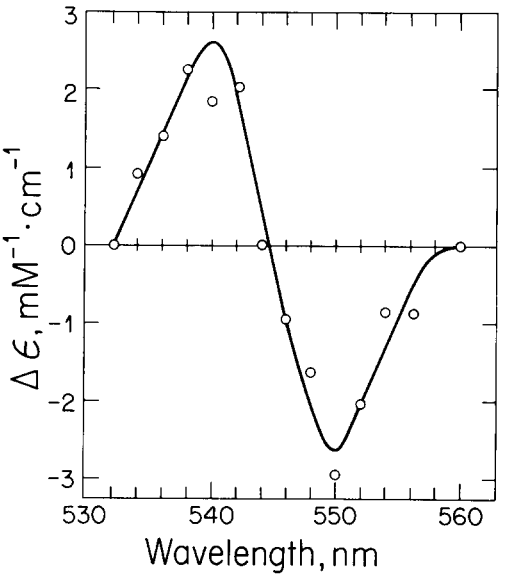


Fig. 6. Light-minus-dark absorbance difference spectrum of Triton X-100 treated thylakoids. The conditions are the same as described in the legend to Fig. 5.

Assuming a Chl/C550 ratio of 250 in this preparation, we calculated a differential molar extinction coefficient $\Delta\varepsilon_{540-550} = 5.2\ mM^{-1} \cdot cm^{-1}$ for C550. Fig. 6 shows the differential extinction coefficient spectrum for C550 in the wavelength region between 530 and 560 nm. The shape of this spectrum is identical to that reported by other investigators [6–10]. However, the absolute values of the differential extinction coefficients are substantially higher than those given by Van Gorkom [7]. We applied the newly derived differential extinction coefficients $\Delta\varepsilon_{540-550} = 5.2\ mM^{-1} \cdot cm^{-1}$ in the determination of C550 concentration in unfractionated spinach thylakoids. Fig. 5A shows the amplitude of the light-induced absorbance change at 540–550 nm ($\Delta A_{540-550}$) in unfractionated thylakoids suspended in the presence of DCMU and FeCN. Using Beer's law we estimated a Chl/C550 ratio of 390 in unfractionated thylakoids (see Table I). This value is in good agreement with our previously determined value of Chl/$Q_A$ = 380, using the absorbance change at 320 nm (see Table I). In summary, quantitation of PS II in unfractionated thylakoids both from the measurement of $Q_A$ and from the amplitude of C550, resulted in Chl/PS II values around 390, in agreement with previous estimates from this laboratory [1–3].

## Discussion

The results presented in this work support the notion of a Chl/PS II ratio in the range of 350–400 and of RC II/RC I ratio significantly greater than 1 in spinach chloroplasts. The recent criticism directed against the use of the light-induced ultraviolet absorbance-change ($\Delta A_{320}$) measurement in the quantitation of $Q_A$ [4,5] apparently lacks merit: as evidenced by the results with $NH_2OH$-treated chloroplasts, the contribution from the advancement of the S states in our $\Delta A_{320}$ measurement is either negligible or nil. Thus, $\Delta A_{320}$ signal distortion from light-induced transition of the S states is not apparent under our sample illumination conditions in the presence of DCMU and ferricyanide. The reason for this discrepancy is not clear at present and further work is currently in progress in this laboratory in order to elucidate this question. A similar conclusion is reached upon quantitation of the PS II indicator pigment C550 from light-induced absorbance change measurements in the green region of the spectrum ($\Delta A_{540-550}$). Using highly resolved oxygen-evolving membranes from the grana partition region, we derived a differential extinction coefficient $\Delta\varepsilon_{540-550} = 5.2\ mM^{-1} \cdot cm^{-1}$ for C550. This derivation was based on the measured amplitude of $\Delta A_{540-550}$ and on the measurement of a Chl/PS II = 250 : 1 in these preparations [24–28].

Using the value of $\Delta\varepsilon_{540-550} = 5.2\ mM^{-1} \cdot cm^{-1}$, we measured about 390 Chl per C550 in control (unfractionated) thylakoids. Our measurements demonstrate that the amplitude of the visible absorbance change due to C550 and the amplitude of the ultraviolet absorbance change due to $Q_A$ reduction yield the same value for the PS II reaction center concentration in spinach chloroplasts.

Quantitation of PS II in higher plant chloroplasts can be obtained either from measurements of the concentration of integral components of PS II (Mn/Cyt *b*-559/Z/P-680/Ph/$Q_A$/herbicide-binding protein = 4 : 2 : 1 : 1 : 1 : 1 : 1 [12,24–33]) or from Emerson and Arnold type $O_2$ flash yield and $H^+$ flash yield measurements [4,5,34,35]. The advantage of measuring the amount of 'integral' PS II components is that such quantitation is not subject to limitations imposed by the structural integrity of the thylakoid membrane and the functional integrity of the linear electron transport intermediates. Shortcomings of the flash yield method are particularly acute with isolated chloroplasts in which problems due to inactivated PS II complexes are compounded by artifacts introduced because of the use of non-physiological electron acceptors. The Chl/PS II = $630 \pm 60$ reported from $H^+$ flash yield measurements in isolated chloroplasts from marked spinach [4] may then represent the active in vitro rather than the total in vivo PS II of the chloroplast.

Recently, an attempt has been made at reconciling the discrepancy in PS II quantitation between the $\Delta A_{320}$ and flash-yield methods. Thus, Jursinic and Dennenberg [34] proposed that about 50% of all PS II reaction centers evolve oxygen (have $Q_A$ as primary quinone acceptor), whereas the remaining 50% have Q-400 as primary quinone acceptor and are inactive both in terms of linear electron transport and oxygen evolution [34,35]. We find it unnecessary to postulate that under physiological conditions about 50% of all PS II is inactive in electron transport. If such a high fraction of non-functional PS II is evident with isolated chloroplasts, it may be due to the above-mentioned problems with inactivated PS II complexes. It is worth pointing out that inactivation of $H_2O$ oxidation results in microsecond reduction phases of the PS II reaction center P-680$^+$. The amplitude of such microsecond reduction phases, hence the extent of damage to the $O_2$-evolving enzyme of PS II, depends on the method of chloroplast isolation, and it could account for up to 50% of the total PS II [36].

In conclusion, quantitation of $Q_A$ from the light-induced ultraviolet absorbance change is in excellent agreement with the reported concentration of thylakoid membrane complexes intimately associated with PS II and supports the notion of a PS II/PS I stoichiometry ratio greater than 1 in higher plant chloroplasts. Conversely, flash yield measurements with isolated chloroplasts may not yield reliable estimates of PS II concentration in thylakoid membranes, since the maximum yield of one electron equivalent per flash per PS II reaction center cannot be assured under in vitro conditions.

## Acknowledgements

The work was supported by a USDA Competitive Research Grant. Computer software was developed with the expert assistance of Mr. Flemming Larsen.

## References

1 Melis, A. and Anderson, J.M. (1983) Biochim. Biophys. Acta 724, 473–484
2 Melis, A. (1984) J. Cell. Biochem. 24, 271–285
3 Glick, R.E., McCauley, S.W. and Melis, A. (1985) Planta 164, 487–494
4 Whitmarsh, J. and Ort, D.R. (1984) Arch. Biochem. Biophys. 231, 378–389
5 Whitmarsh, J. and Ort, D.R. (1984) in Advances in Photosynthesis Research (Sybesma, c., ed.), Vol. III, p. 231–234, Martinus Nijhoff/Dr. W. Junk Publishers, Dordrecht, The Netherlands
6 Knaff, D.B. and Arnon, D.I. (1969) Proc. Natl. Acad. Sci. USA 63, 963–969
7 Van Gorkom, H.J. (1974) Biochim. Biophys. Acta 347, 439–442
8 Melis, A. and Schreiber, U. (1979) Biochim. Biophys. Acta 547, 47–57
9 Erixon, K. and Butler, W.L. (1971) Biochim. Biophys. Acta 234, 381–389
10 Butler, W.L. and Okayama, S. (1971) Biochim. Biophys. Acta 245, 231–239
11 Arnon, D.I. (1949) Plant Physiol. 24, 1–15
12 Berthold, D.A., Babcock, G.T. and Yocum, C.F. (1981) FEBS Lett. 134, 231–234
13 Melis, A. and Hart, R.W. (1980) Carnegie Inst. 79, 170–172
14 Melis, A. and Brown, J.S. (1980) Proc. Natl. Acad. Sci. USA 77, 4712–4716
15 Hiyama, T. and Ke, B. (1972) Biochim. Biophys. Acta 267, 160–171
16 Bensasson, R. and Land, E.J. (1973) Biochim. Biophys. Acta 325, 175–181
17 Ke, B., Inoue, H., Babcock, G., Fang, Z.-X. and Dolan, E. (1982) Biochim. Biophys. Acta 682, 297–306
18 Dekker, J.P., Van Gorkom, H.J., Brok, M. and Ouwehand, L. (1984) Biochim. Biophys. Acta 764, 301–309
19 Diner, B.A. and DeVitry, C. (1984) in Advances in Photosynthesis Research (Sybesma, C., ed.), Vol. I, pp. 407–411, Martinus Nijhoff/Dr. W. Junk Publishers, Dordrecht, The Netherlands
20 Renger, G. and Weiss, W. (1983) Biochim. Biophys. Acta 722, 1–11
21 Dekker, J.P., Van Gorkom, H.J., Wensink, J. and Ouwehand, L. (1984) Biochim. Biophys. Acta 767, 1–9
22 Joliot, P. and Joliot, A. (1964) C.R. Acad. Sci. Paris 258, 4622–4625
23 Junge, W. and Witt, H.T. (1968) Z. Naturforsch. 23B, 244–254
24 Lam, E., Baltimore, B., Ortiz, W., Chollar, S., Melis, A. and Malkin, R. (1983) Biochim. Biophys. Acta 724, 201–211
25 Golbeck, J.H. and Warden, J.T. (1985) Biochim. Biophys. Acta 806, 116–123
26 Ghanotakis, D.F., Babcock, G.T. and Yocum, C.F. (1984) 765, 388–398
27 Murata, N., Miyao, M., Omata, T., Matsunami, H. and Kuwabara, T. (1984) Biochim. Biophys. Acta 765, 363–369
28 Babcock, G.T., Ghanotakis, D.F., Ke, B. and Diner, B.A. (1983) Biochim. Biophys. Acta 723, 276–286
29 Van Best, J.A. and Mathis, P. (1978) Biochim. Biophys. Acta 503, 178–188
30 Eckert, H.-J., Renger, G. and Witt, H.T. (1984) FEBS Lett. 167, 316–320
31 Yocum, C.F., Yerkes, C.T., Blankenship, R.E., Sharp, R.R. and Babcock, G.T. (1981) Proc. Natl. Acad. Sci. USA 78, 7507–7511
32 Vermaas, W.F.J. and Arntzen, C.J. (1983) Biochim. Biophys. Acta 725, 483–491
33 Jursinic, P. and Stembler, A. (1983) Plant Physiol. 73, 703–708
34 Jursinic, P. and Dennenberg, R. (1985) Arch. Biochem. Biophys. 241, 540–549
35 Dennenberg, R.J. and Jursinic, P.A. (1985) Biochim. Biophys. Acta 808, 192–200
36 Schlodder, E., Brettel, K. and Witt, H.T. (1985) Biochim. Biophys. Acta 808, 123–131