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MEASUREMENT OF THE RELATIVE ELECTRON-TRANSPORT CAPACITY OF PHOTOSYSTEM I AND PHOTOSYSTEM II IN SPINACH CHLOROPLASTS

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The ratio of Photosystem (PS) II to PS I electron-transport capacity in spinach chloroplasts was compared from reaction-center and steady-state rate measurements. The reaction-center electron-transport capacity was based upon both the relative concentrations of the PS II_α, PS II_β and PS I centers, and the number of chlorophyll molecules associated with each type of center. The reaction-center ratio of total PS II to PS I electron-transport capacity was about 1.8:1. Steady-state electron-transport capacity data were obtained from the rate of light-induced absorbance-change measurements in the presence of ferredoxin-$NADP^+$, potassium ferricyanide and 2,5-dimethylbenzoquinone (DMQ). A new method was developed for determining the partition of reduced DMQ between the thylakoid membrane and the surrounding aqueous phase. The ratio of membrane-bound to aqueous $DMQH_2$ was experimentally determined to be 1.3:1. When used at low concentrations (200 μM), potassium ferricyanide is shown to be strictly a PS I electron acceptor. At concentrations higher than 200 μM, ferricyanide intercepted electrons from the reducing side of PS II as well. The experimental rates of electron flow through PS II and PS I defined a PS II/PS I electron-transport capacity ratio of 1.6:1.

Introduction

Numerous studies have shown that photosynthetic plants do not have a rigid PS II/PS I stoichiometry of 1:1 [1–5]. Conversely, the functional light-harvesting antenna of PS I is different from that of PS II [5–7]. Under light-limiting conditions, the measured rate of PS I and PS II electron transport in chloroplasts (electron-transport capacity) depends on the wavelength and intensity of the actinic light, the size and composition of the antenna associated with each type of reaction center as well as the concentration of each type of reaction center present. Recent measurements by Melis and Anderson [7] have shown that in spinach chloroplasts the PS II/PS I reaction-center ratio is greater than 1 and that the physical light-harvesting antenna is different for PS II and PS I complexes. Thus, the measurements of Melis and Anderson predicted a wavelength-dependent electron-transport capacity for PS II and PS I. With green actinic light, when PS I and PS II receive excitation in proportion to the physical size of their light-harvesting antenna, the estimated capacity ratio, C(PS II/PS I), was about 1.8:1.

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Abbreviations: Chl, chlorophyll; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; PS, Photosystem; P-700, reaction center of PS I; Q, primary electron acceptor of PS II; DMQ, 2,5-dimethylbenzoquinone; $NADP^+$, nicotinamide adenine dinucleotide phosphate; DCIP, 2,4-dichlorophenolindophenol; C_f, flattening correction factor; Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid.

In the present work we have used the techniques of Melis and Anderson to verify the PS II/PS I electron-transport capacity ratio based upon measurements of the relative reaction-center concentrations and of the relative antenna sizes. In addition, we have measured directly the photosynthetic electron-transport capacity ratio from steady-state rates of linear electron flow through each photosystem. Rates of PS I electron flow were determined from the rate of ferredoxin-$NADP^+$ and potassium ferricyanide reduction. The rate of PS II electron transport was determined from the rate of 2,5-dimethylbenzoquinone reduction under the same apparatus geometry and green light-limiting conditions. The accumulation of NADPH and of ferrocyanide was used as a direct assay of PS I activity. The accumulation of $DMQH_2$ was used as a measure of PS II activity. In order to accurately define the rate of the absorbance change due to $DMQH_2$ accumulation, we determined the partition of $DMQH_2$ between the aqueous and membrane-bound phases so that a correction for particle flattening could be made for the absorbance change associated with the fraction of $DMQH_2$ confined to the membrane. The PS II/PS I electron-transport capacity ratio determined from the steady-state absorbance rate measurements is in good agreement with the predicted reaction-center ratio.

Materials and Methods

Spinach plants (*Spinacea oleracea* L.) were grown in the greenhouse on half-strength Hoagland nutrient solution. Chloroplasts were isolated by grinding the leaves for 10 s in a Waring blender in 50 mM Tricine (pH 7.8) containing 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 1 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 [8]. Just before measurements took place, the sample was diluted to a concentration of 0.2 mg/ml by adding an appropriate amount of reaction buffer. The reaction buffer consisted of 50 mM Hepes (pH 7.2)/5 mM $MgCl_2$/10 mM NaCl/0.4 M sorbitol/50 μM gramicidin D.

Chloroplast fluorescence and absorbance difference measurements were performed with a laboratory-constructed modulated split-beam spectrophotometer previously described [9]. The optical path length of the cuvette in the direction of the measuring beam was 0.18 cm. Actinic light was provided in the green region of the spectrum by a combination of Corning CS 4-96 and CS 3-69 filters. This combination of filters was selected because we wished to define a spectral region in which the differences in absorption of actinic light by Chl *a* and Chl *b* in the photosystem antennae would be minimal. As discussed elsewhere [7], for ether-solubilized pigments, the integrated absorption of light by Chl *b* in the wavelength region transmitted by the Corning CS 4-96 and CS 3-69 filters exceeds that of Chl *a* by 22–28%. At the photosystem level, if about 14% of the PS I antenna chlorophyll is Chl *b* [7,10], and about 36% of the PS II antenna chlorophyll is Chl *b* [7,11], then PS II would absorb 5–6% more excitation than if PS II and PS I contained equal amounts of Chl *b* in their light-harvesting pigment. While these calculations are based on the extinction coefficient of Chl *a* and Chl *b* in ether [12], it is reasonable to assume that the values in vivo are similar. It is likely, in fact, that the multiple association of chlorophylls with protein would lessen the sharp spectral differences between Chl *a* and Chl *b* in ether. As a consequence, the small difference in the rate of excitation of PS I and PS II by green light, arising because of their difference in Chl *a* and Chl *b* composition, would be minimized to less than 5%. Thus, any systematic error, introduced in our measurement because of our assumption that PS I and PS II would receive green excitation in proportion to their antenna size *N*, would be negligibly small.

We used differential extinction coefficients of 19.6 $mM^{-1} \cdot cm^{-1}$ at 257 nm for DMQ, 6.2 $mM^{-1} \cdot cm^{-1}$ at 340 nm for $NADP^+$, and of 1.02 $mM^{-1} \cdot cm^{-1}$ at 420 for ferricyanide. The DMQ differential extinction coefficient was the same in H_2O and ethanol solution, and we assumed the same value in the membrane. The DMQ concentration in the reaction mixture was 250 μM. The ferri-

cyanide concentration was varied from 50 μM up to 1.5 mM. The $NADP^+$ concentration was 4 mM, with 21 μM ferredoxin also added. For some of the absorbance measurements with ferredoxin-$NADP^+$, 10 mM sodium ascorbate and 200 μM DCIP were also present.

Results

Under green excitation of light-limiting intensity, the measured electron turnover by a particular photosystem (i.e., photosystem electron-transport capacity) will depend on the concentration of that photosystem, [PS], in the thylakoid membrane and on the number, N, of pigment molecules associated with that type of reaction center. Both [PS] and N vary from species to species and also vary with different growth conditions for a single species [1,2]. Using the spectrophotometric and kinetic procedure described in a previous publication from this laboratory [7], we measured the following relative concentrations of the spinach chloroplast photosystems: [PS] = 1, [PS II_α] = 1.4, and [PS II_β] = 0.5. The number of chlorophyll molecules associated with each type of center were measured to be: $N_{P\text{-}700} = 210$, $N_\alpha = 230$ and $N_\beta = 100$ *. On the basis of these results, the ratio of PS II to PS I electron-transport capacity is then given by:

$$\frac{C(PS\ II)}{C(PS\ I)} = \frac{[PS\ II_\alpha]N_\alpha + [PS\ II_\beta]N_\beta}{[PS\ I]N_{P\text{-}700}} = 1.77 \qquad (1)$$

Eq. 1 predicts that under green light-limiting conditions PS II would turn over electrons at about 1.8 times the rate of PS I turnover. In the absence of artificial PS II electron acceptors, the excess electrons would accumulate between PS II and PS I. The extra electrons would lead to a steady-state situation in which many of the PS II centers remained in the Q^- state, resulting in an elevated variable fluorescence yield [7]. At steady-state, the diminished rate of electron flow through open PS II centers should equal the rate of electron flow through PS I centers. We sought to verify this prediction directly by measuring the rates of PS II and PS I electron transport under green light-limiting conditions.

Rate of PS I electron flow to ferredoxin-$NADP^+$ and potassium ferricyanide

The principal physiological electron acceptor for PS I is $NADP^+$ via ferredoxin and the enzyme ferredoxin-$NADP^+$ reductase. We monitored the rate of the PS I electron flow from the absorbance change at 340 nm due to NADPH accumulation. To verify that our actinic illumination was non-saturating, we measured the rate of NADPH accumulation as a function of actinic light intensity. Fig. 1 shows a linear dependence of the ΔA_{340} rate with light intensity (triangles), verifying the above

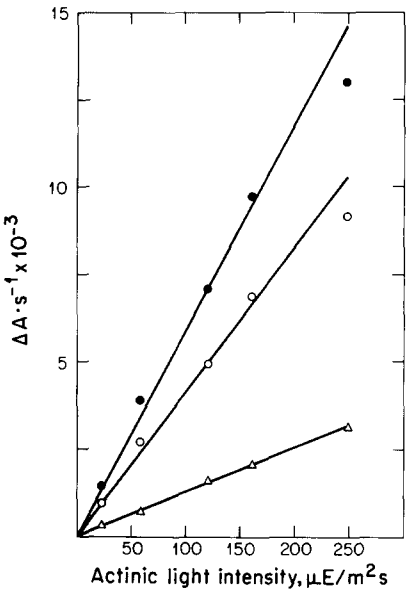

Fig. 1. Rates of absorbance change associated with electron transfer to $NADP^+$ (△) and DMQ (○) as a function of actinic-light intensity. The chlorophyll concentration was about 200 μM. The optical path-length of the cuvette in the direction of measuring beam was 0.18 cm. Rates of absorbance change at 340 nm in the presence of 4 mM $NADP^+$, 21 μM ferredoxin, 10 mM sodium ascorbate, and 200 μM DCIP (△). Rates of absorbance change at 257 nm in the presence of 250 μM DMQ (○). The ΔA_{257} rates corrected for the particle flattening effect (●). For the fraction of DMQ in the thylakoid membrane, the average flattening correction factor at 257 nm was 2.15. The ratio of $DMQH_2$ accumulating in the thylakoid membrane versus that in the solution was about 1.3 (see Results).

* The solution of the appropriate equations yielding N_α, N_β and $N_{P\text{-}700}$ is based on the assumption of approximately equal quantum yield of photochemistry at the three photosystems, i.e., $\phi_\alpha = \phi_\beta = \phi_{P\text{-}700}$ [7].

TABLE I

RATES OF PS I AND PS II ELECTRON TRANSPORT IN μM (ELECTRON)·mM^{-1} Chl·s^{-1}

Experimental rates are the averages of three to six experiments. Experimental variability was approx. 10%. The rate of electron turnover by PS I was measured with ferredoxin (21 μM) and $NADP^+$ (4 mM), both in the presence and in the absence of ascorbate (10 mM) and DCIP (200 μM). Electron turnover by PS I was also measured with 100 μM potassium ferricyanide. The rate of electron turnover by PS II was measured in the presence of 250 μM DMQ. The fraction of closed PS II centers was estimated from variable fluorescence yield increase measurements of chloroplasts illuminated in the presence of 21 μM ferredoxin and 4 mM $NADP^+$, 100 μM potassium ferricyanide, and 250 μM DMQ, respectively. The fraction of closed PS II centers was obtained from the non-linear relationship between F_v and Q^- given in Ref. 14 (and in parentheses from Ref. 13).

Artificial electron acceptor	Experimental rate	$\frac{F_v \text{ (acceptor)}}{F_v \text{ (total)}}$	Fraction of closed PS II centers	Rate of electron turnover		Ratio of PS II/PS I electron-transport capacities
				PS II	PS I	
Ferredoxin, $NADP^+$ (340 nm)	11.5	0.38	52 (58)%	–	–	–
Ferricyanide (420 nm)	12	0.24	40 (44)%	20.0 (21.4)	12	1.67 (1.78)
DMQ (257 nm)	16.5	0.03	11 (7)%	18.5 (17.7)	–	1.54 (1.48)

contention. For the rest of our rate measurements, the actinic light irradiance was set at 100 $\mu E \cdot m^{-2} \cdot s^{-1}$ (about half of the maximum value shown in Fig. 1). Measurements of NADPH accumulation with the actinic light intensity set at 100 $\mu E\ m^{-2} \cdot s^{-1}$ yielded a PS I electron transport rate of about 11.5 $\mu Me^- \cdot mM^{-1}$ Chl $\cdot s^{-1}$ (Table I). The measurement of $NADP^+$ reduction is a direct assay of PS I activity. We obtained nearly the same rates with and without the presence of the added donor pair, ascorbate and DCIP, suggesting that under our conditions all PS I reaction centers were functional and that the rate of electron transfer through PS II is sufficient to sustain the operation of all the PS I reaction centers. An indirect estimate of the fraction of PS II centers active in supplying electrons to the PS I centers was obtained from the measurement of the steady-state variable fluorescence yield (Fig. 2). Under the same light conditions as the absorbance measurements, but without any added ascorbate and DCIP, we measured the level of the variable fluorescence yield in the presence of ferredoxin-$NADP^+$ relative to that obtained with saturating illumination (Table I). With saturating illumination, the primary electron acceptor Q of PS II becomes fully reduced and the variable fluorescence yield reaches a maximum level. This maximum corresponds to a state in which all of the PS II centers are closed due to the reduction of Q. In the presence of ferredoxin-$NADP^+$, the level of variable fluorescence yield was 38% of that detected with saturating illumination (Fig. 2). The fraction of the variable fluorescence yield increase, F_v, is a complex function of the fraction of closed PS II reaction centers, Q^-. Experimental evidence has accumulated suggesting a non-linear relationship between F_v and Q^- [13,14]. Determined independently in the absence [13] and presence [14] of the electron transport inhibitor DCMU, the non-linear relationship between F_v and Q^- predicted a greater fraction of closed PS II centers for any given fraction of variable fluorescence yield increase. Using the non-linear relationship, we estimated that a 38% increase in variable fluorescence corresponds to a state with about 52% [14] or 55% [13] of Q in the Q^- state, indicating that about half of the PS II centers were sufficient to provide electrons to all of the PS I centers.

In unfractionated thylakoids, potassium ferricyanide acts as a PS I acceptor [7,15,16]. We obtained an independent measure of the rate of electron flow through PS I by using low concentrations of ferricyanide. Under our actinic light conditions, we monitored the rate of the absorbance change at 420 nm in the presence of 50–200 μM

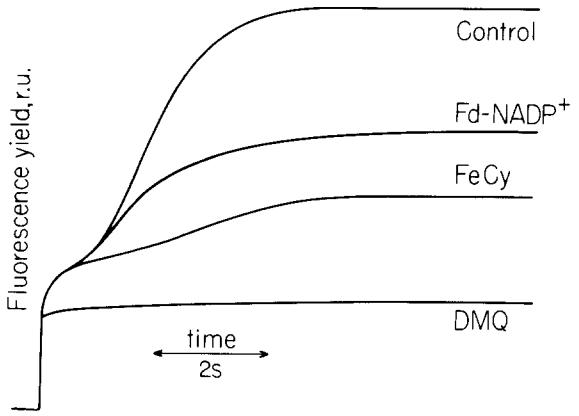

Fig. 2. Fluorescence induction curves of isolated spinach chloroplasts suspended in the absence of any artificial electron acceptors (Control), in the presence of 21 μM ferredoxin and 4 mM $NADP^+$ (Fd-$NADP^+$), in the presence of 100 μM potassium ferricyanide (FeCy), or in the presence of 250 μM 2,5-dimethylbenzoquinone (DMQ). The amplitude of the variable portion of the fluorescence yield was calibrated versus that with saturating illumination and found equal to 64% (Control), 38% (Fd-$NADP^+$), 24% (FeCy) and 4% (DMQ) of the total variable fluorescence yield. Chlorophyll concentration, 220 μM; gramicidin, 50 μM.

ferricyanide and measured a rate of electron transport of about 12 $\mu Me^- \cdot mM^{-1}$ Chl $\cdot s^{-1}$, as shown in Table I. This rate was found to be invariable in the 50–200 μM ferricyanide range and is similar to that obtained with ferredoxin-$NADP^+$, suggesting that under our experimental conditions PS I has the capacity to transport approximately 12 $\mu Me^- \cdot mM^{-1}$ Chl $\cdot s^{-1}$. We tested to verify that under these experimental conditions PS I reaction centers were fully functional (open) and that the supply of electrons from PS II was sufficient to sustain the operation of PS I. This was implemented by monitoring the oxidation-reduction state of P-700 and Q during illumination. Fig. 3 (FeCy) shows that upon the onset of illumination, a short-lived transient oxidation of P-700 was detected in the presence of 1 mM potassium ferricyanide. Within less than 1 s, however, the signal crossed the baseline showing an overall positive absorbance change at steady-state (an overall light-induced reduction of P-700). We confirmed that the addition of 1 mM ferricyanide caused the oxidation of only a small portion of P-700 in the dark. This chemical oxidation of P-700 was fully reversed in the light, in spite of the relatively high ferricyanide concentration used in this measurement, suggesting that under these conditions PS I reaction centers were fully operational. This conclusion was corroborated by the observation that, under the same steady-state conditions, a portion of PS II reaction centers were in the reduced state, suggesting an overall greater turnover of electrons by PS II.

To estimate the full PS II capacity for electron turnover, we measured the steady-state fluorescence yield increase in the presence of ferricyanide. Fig. 2 shows that upon illumination in the presence of 100 μM ferricyanide, a variable fluorescence yield increase occurs of about 24% of the total (Table I). Assuming the non-linear function of Ref. 14 for the relationship between F_v and Q^-, we conclude that about 40% of all PS II reaction centers were in the reduced state (see Table I). From the non-linear function of Ref. 13, we estimated that about 44% of the PS II centers were in the reduced state. By the same reasoning as applied to the ferredoxin-$NADP^+$ measurements, we argued that either 60% or 56% (depending on the F_v versus Q^- relationship assumed) of the PS II centers were sufficient to balance the activity of all of the PS I centers. Therefore, on the basis of this measurement, it appeared that under our actinic light conditions the PS II electron-

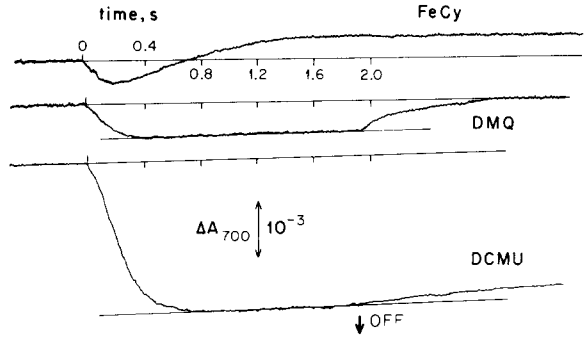

Fig. 3. Light-induced absorbance change kinetics at 700 nm (ΔA_{700}) attributed to oxidation-reduction changes of the reaction center P-700 of PS I. Isolated chloroplasts suspended in the presence of 25 μM DCMU and 200 μM methylviologen (DCMU), in the presence of 250 μM dimethylbenzoquinone (DMQ), or in the presence of 1 mM potassium ferricyanide (FeCy). The samples contained about 210 μM Chl and 50 μM gramicidin. The green actinic light came 'ON' at zero time and went 'OFF' after 2 s.

transport-capacity exceeded that of PS I either 1.7 : 1 or by 1.8 : 1 (see Table I).

Rate of PS II electron flow to 2,5-dimethylbenzoquinone

To obtain a more direct assay of PS II activity, we monitored the rate of ΔA_{257}, the absorbance change at 257 nm in the presence of DMQ as an artificial electron acceptor. DMQ is a moderately lipophilic molecule capable of penetrating into the thylakoid membrane and of accepting electrons between PS II and PS I [15]. We tested for the extent of DMQ accumulation in the thylakoid membrane by separating chloroplasts (200 μM Chl) from the (250 μM) DMQ-containing medium. With this method we determined that approx. 60% of the total DMQ (the equivalent of about 150 μM DMQ) was taken up from the solution by the thylakoid membrane. Fig. 2 (Control) shows that in the absence of an artificial electron acceptor, electrons accumulate in the PQ pool, and ultimately on Q, with the concomitant inhibition of PS II activity and fluorescence yield increase. Fig. 2 (DMQ) shows that in the presence of DMQ the variable fluorescence yield (F_v) remained almost fully suppressed, whereas the non-variable emission (F_0) was practically unaffected. Based on the interpretation that static quenching affects the variable (F_v) and non-variable (F_0) components in proportion to each other [17], fluorescence quenching by DMQ is not static but the result of efficient electron removal from the PQH_2 pool by DMQ. It was corroborated further by the observation that DCMU prevented the DMQ-induced variable fluorescence yield quenching (results not shown). It is implied that DMQ prevents most electron accumulation on the primary electron acceptor Q and, therefore, it may be used as an artificial electron acceptor with only minor correction for closed PS II centers in PS II electron-transport rate measurements. In the presence of DMQ, the variable fluorescence yield increase is reduced to only about 3% of its maximum value, while the F_0 level is relatively unaffected. A 3% variable fluorescence yield increase indicated that either 7% [13] or about 11% [14] of the PS II are in the reduced state (Table I). Thus, in contrast to ferredoxin-$NADP^+$ and ferricyanide, DMQ reduction is not limited by PS I. From the linearity of ΔA_{257} as a function of actinic light intensity (Fig. 1, circles), we verified that under our experimental conditions the rate of PS II electron transport is light-intensity-limited. Under actinic light conditions identical to those employed for $NADP^+$ and ferricyanide, the experimental rate of ΔA_{257}, due to DMQ reduction, is distorted by the particle flattening effect. The experimental rate of ΔA_{257} must be corrected for this effect before yielding meaningful information on the rate of electron transport through PS II. Flattening corrections are needed whenever absorbance or absorbance change measurements are made with particles in suspension rather than in a uniformly dispersed solution [18,19]. In the particle, mutual shading effects occur which are not encountered when the absorbing species are homogeneously dispersed in solution. In photosynthetic studies, flattening effects are present in absorbance change measurements of lipophilic molecules such as DMQ or plastoquinone. Flattening corrections are not necessary for the absorbance change measurements of molecules that are dispersed uniformly in aqueous solution such as $NADP^+$, or ferricyanide at low concentrations. In order to correct the ΔA_{257} signal for flattening effects, we needed to know the proportion of the observed signal due to $DMQH_2$ in the membrane, versus that originating in aqueous solution. The ΔA_{257} signal from the fraction of $DMQH_2$ in the membrane will be less than its true value due to the flattening effect. The ΔA_{257} signal from the aqueous $DMQH_2$ will not need this correction. In the following section we provide a precise determination of the partition of reduced DMQ in the thylakoid membrane and aqueous phases.

The results of Fig. 3 (DCMU) show that in the presence of the electron transport inhibitor DCMU, when PS II electrons are prevented from reaching PS I, illumination of chloroplasts causes a large decrease in the absorbance (ΔA_{700}) attributed to the light-induced photooxidation of P-700. To investigate whether any PS II electrons reach PS I in the presence of DMQ, we measured the steady-state oxidation level of P-700, under the same excitation conditions, in the presence of DMQ but in the absence of DCMU. Fig. 3 (DMQ) shows that upon the onset of the actinic illumination, there was a negative absorbance change which

was promptly restored in the dark. Compared to the amplitude of the ΔA_{700} signal in the presence of DCMU, we estimated that about 25% of all P-700 became oxidized in the light in the presence of DMQ. Since this light-induced oxidation of P-700 was readily reversed in the dark, it may be assumed that $DMQH_2$, the reduced form of DMQ, donated electrons directly or indirectly to P-700, keeping a significant portion of it (about 75%) reduced at all times and, therefore, functional in terms of electron transport through PS I. The latter observation represents a complication in the determination of electron flow to DMQ, since it raises the question of $DMQH_2$ partitioning between the thylakoid membrane and surrounding aqueous phase and the possibility that some of the electrons were transferred from $DMQH_2$ in the membrane via PS I to DMQ in the solution or to molecular oxygen. Although quinone molecules have been reported as efficient electron acceptors from the reducing side of PS I [15,16], it was not known whether DMQ reduction is competitive with the reduction of molecular oxygen in the Mehler reaction. We have determined that the rate of dimethylbenzoquinone reduction was increased by about 5–10% when working under anaerobic conditions. This suggests that in the presence of DMQ only a small portion of the electrons from PS I were transferred to molecular oxygen, a process inhibited in N_2-saturated media. PS I may thus act as a pump to move electrons from membrane-bound $DMQH_2$ and other electron transport intermediates to aqueous DMQ.

In the presence of ferricyanide aqueous $DMQH_2$ should not accumulate, since ferricyanide would accept electrons efficiently either from PS I or from the aqueous $DMQH_2$. To elucidate the interaction of $DMQH_2$ with ferricyanide in the solution surrounding the thylakoid membrane, we measured the rate of ΔA_{257} of chloroplasts suspended in the presence of DMQ, ferricyanide, or both (Fig. 4). At this wavelength, upon illumination of chloroplasts with DMQ alone, there is a rapid negative absorbance change reflecting the transition of DMQ to $DMQH_2$. In the presence of ferricyanide alone, there is a slow positive absorbance change reflecting the photoreduction of ferricyanide to ferrocyanide [20]. In the presence of both electron acceptor molecules, we observed a slow negative absorbance change, i.e., in the same direction as with DMQ alone. The qualitative interpretation of the latter measurement is that upon illumination both electron acceptors ($DMQH_2$ and ferrocyanide) accumulate in the reduced form. This apparently reflects $DMQH_2$ accumulation in the thylakoid membrane phase and of ferrocyanide in the aqueous medium surrounding the thylakoids. The above qualitative interpretation of the results in Fig. 4 is clear; nevertheless, the results cannot be used readily for the quantification of the rates of $DMQH_2$ accumulation in the thylakoid membrane phase and of ferrocyanide accumulation in the solution phase. This is because both DMQ and ferricyanide contribute to the absorbance change at this wavelength (257 nm). We were able to overcome the above limitation by measuring the rate of DMQ reduction at 280 nm, where ferricyanide absorbance changes do not occur (isosbestic point for FeCy [20]).

We determined the relative partition of reduced DMQ between the thylakoid membrane and aqueous phases from measurements of the rate of ΔA_{280} in the presence and absence of ferricyanide. The

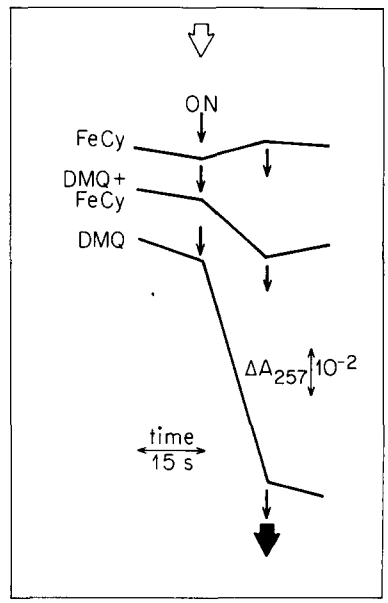

Fig. 4. The rate of the absorbance change at 257 nm in the presence of 200 μM ferricyanide (FeCy), 250 μM DMQ and 200 μM FeCy, or of 250 μM DMQ. The chlorophyll concentration was about 200 μM.

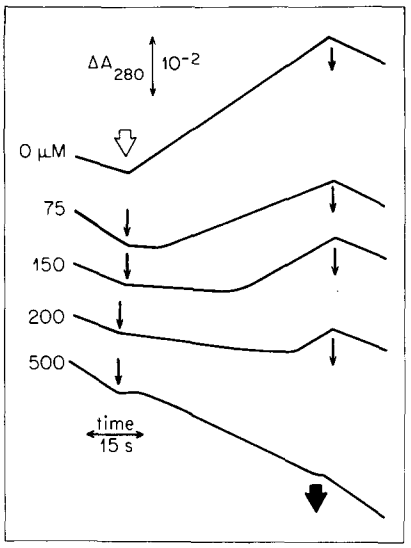

Fig. 5. The rate of the absorbance change at 280 nm in the presence of 250 μM DMQ and various ferricyanide concentrations as indicated. The chlorophyll concentration was about 200 μM.

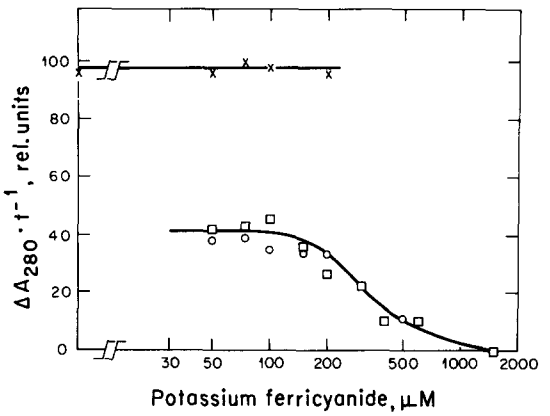

Fig. 6. The rate of the absorbance change at 280 nm as a function of potassium ferricyanide concentration. 250 μM DMQ was present in all samples. The chlorophyll concentration was about 200 μM. Average values of the initial rates with two separate chloroplast preparations are shown by the (○) and the (□). The rates after the complete reduction of ferricyanide, as well as the rate with no ferricyanide present, are shown by (×).

absorbance difference signals measured at this wavelength originate from DMQ reduction only. Fig. 5 shows typical data from an experiment with 250 μM DMQ and various concentrations of ferricyanide. With DMQ in the absence of ferricyanide, there is a positive ΔA_{280} rate, reflecting $DMQH_2$ accumulation. In the presence of DMQ and low concentrations of ferricyanide (in the range of 20–200 μM), the ΔA_{280} rate was lowered to about 40% of that with DMQ alone. The constant residual rate of ΔA_{280} over this wide ferricyanide concentration range indicated that at such low concentrations a constant fraction of $DMQH_2$ is inaccessible to ferricyanide. It apparently reflects the fraction of $DMQH_2$ accumulating in the thylakoid membrane. The constant residual rate of ΔA_{280} lasted in proportion to the amount of ferricyanide present. After this initial period of time, ferricyanide was fully reduced to ferrocyanide. The rate of the ΔA_{280} then returned to the higher level observed in the control, suggesting the reduction of DMQ only (Fig. 5). For ferricyanide concentrations higher than 200 μM, the ΔA_{280} rate was lowered further, progressive with the ferricyanide concentration increase. Fig. 6 shows the measured rates over a wide range of ferricyanide concentrations. At 1.5 mM ferricyanide, the ΔA_{280} rate is nearly zero, suggesting that no accumulation of $DMQH_2$ occurs. The constant 60% inhibition of the ΔA_{280} rate observed at low concentrations of ferricyanide clearly reflects a direct reduction of this artificial electron acceptor by PS I or by $DMQH_2$ in the solution. At low concentrations (20–200 μM) ferricyanide is restricted to the aqueous phase. At higher concentrations it may penetrate into the thylakoid area where it could intercept electrons either directly from PS II or from any of the intermediates between PS II and PS I.

For our present purposes, the important observation is the constant 60% inhibition of the experimental ΔA_{280} rate at low ferricyanide concentrations. It represents the aqueous $DMQH_2$ that does not require a flattening correction at 280 nm. We determined a flattening correction factor ($C_{f\,280}$) of 1.9 at 280 nm for the remaining 40% of the ΔA_{280} [19]. The corrected ratio of the ΔA_{280} from membrane-bound to aqueous $DMQH_2$ is then:

$$\frac{(\Delta A_{280}(m))(C_{f\,280})}{\Delta A_{280}(a)} = \frac{(40)(1.9)}{60} = \frac{1.27}{1} \qquad (2)$$

This ratio of 1.27:1 represents the true ratio of

membrane-bound to aqueous $DMQH_2$ and will be valid at all wavelengths.

At 257 nm the flattening correction factor ($C_{f\,257}$) was determined to be about 2.15. Our measured rate of ΔA_{257} with DMQ alone in Fig. 4 ($\Delta A_{257\,(experimental)}$) then consists of a contribution from the rate of the DMQ being reduced in the aqueous phase ($\Delta A_{257}(a)$) and a contribution from the rate of the DMQ being reduced in the membrane ($\Delta A_{257}(m)$). The absorbance difference signal from DMQ reduction in the membrane is subject to flattening correction at 257 nm ($C_{f\,257} = 2.15$). Hence, our measured rate of $4.1 \cdot 10^{-3}$ s^{-1} ($\Delta A_{257\,(experimental)}$) contained a contribution of $2.6 \cdot 10^{-3}$ s^{-1} from the aqueous $DMQH_2$ and of $3.3 \cdot 10^{-3}$ s^{-1} from the membrane-bound $DMQH_2$. The latter had been 'flattened' to look like only $1.5 \cdot 10^{-3}$ s^{-1}. Thus, the true rate ΔA_{257} of DMQ reduction was $5.9 \cdot 10^{-3}$ s^{-1} (see Fig. 1).

An estimate of the rate of PS II electron transport was obtained from the true ΔA_{257} rate after a small correction for the fraction of closed PS II centers (see Table I). The PS II electron-transport capacity, under our light intensity conditions, was about 17.7–18.5 $\mu Me^- \cdot mM^{-1}$ Chl $\cdot s^{-1}$. The ratio of PS II/PS I electron-transport capacity was calculated from the rates of electron transport to DMQ (PS II) and to potassium ferricyanide (PS I) and found it to be in the range of 1.5 : 1 (Table I).

Discussion

Our results demonstrated that under green light-limiting conditions at which PS I and PS II receive excitation energy in proportion to their light-harvesting antenna size, PS II has an electron-transport capacity of 1.5–1.8 times that of PS I. Thus, the steady-state rate ratios (Table I) were close to the reaction-center rate ratio of 1.8, suggesting that the values used for the reaction-center concentrations and relative antenna sizes are meaningful. The experimental rate data of PS I obtained with ferredoxin and $NADP^+$ could not be used to estimate the rate of PS I turnover because of cyclic electron transport from reduced ferredoxin to the intermediate components between PS II and PS I instead of to $NADP^+$ [21]. Such a cyclic flow would lead to a slight decrease in the rate of $NADP^+$ reduction and to a small increase in the variable fluorescence yield from PS II. The underestimation of PS I capacity and simultaneous overestimation of PS II capacity would lead to a greater apparent PS II/PS I electron-transport capacity ratio. In the measurements with ferricyanide as the acceptor, however, cyclic electron transport would not occur. The electron transport rates measured in the presence of ferricyanide were consistently greater (about 5%) than those measured in the presence of ferredoxin-$NADP^+$ (Table I). It may be argued that, under our conditions, cyclic electron transport activity in the presence of ferredoxin accounts for only about 5% of the electrons generated by PS I.

Our results with ferricyanide and DMQ indicated that the former is restricted primarily in the aqueous phase at low concentrations. Significant amounts of ferricyanide may penetrate into the membrane at higher concentrations. The permeability of ferricyanide would depend, in addition to the ferricyanide concentration, on factors such as the membrane integrity, sample concentration and the thylakoid surface charge density. Ferricyanide molecules penetrating into the thylakoid lumen would intercept electrons efficiently from electron carriers between the two photosystems. An increase in the rate of electron transport to ferricyanide, however, may be accompanied by a decrease in the ΔA_{420} signal because of the large flattening effect at 420 nm and also because of undesirable inhibitory effects of high concentrations of potassium ferricyanide [22–24]. The need for a flattening correction would complicate interpretation of measurements of ΔA_{420} in the presence of high concentrations of ferricyanide.

A recent method for the measurement of PS II activity involved the counting of protons released by flash excitation of chloroplasts in the presence of DMQ and ferricyanide [25]. Our work has shown that a substantial fraction of reduced $DMQH_2$ in the membrane phase would be inaccessible to ferricyanide in the surrounding medium because, on the time-scale of our experiment, $DMQH_2$ diffusion from the thylakoid membrane to the surrounding solution is negligible. A simple calculation shows that, in the presence of DMQ, the activity of about 75% of PS I (Fig. 3) is fully adequate to account for all the reducing equivalents reaching ferricyanide (20–200 μM

range, Fig. 6). Therefore, the protons associated with $DMQH_2$ in the membrane phase would not be released into the aqueous phase. The lack of equilibrium between $DMQH_2$ and ferricyanide would inevitably result in underestimation of the PS II activity [25].

The significantly greater PS II than PS I electron-transport capacity is meaningful considering the differences between our actinic light conditions and those that a plant encounters when growing under natural conditions. A key factor in our experiment was selecting a spectral region where differences in the absorbance between Chl *a* and Chl *b* would be minimal. Plants in their physiological habitat, however, absorb light mostly in the blue and red spectral regions. In the blue spectral region, light absorption by Chl will be moderated because of the non-photosynthetic absorption by the carotenoids. In the red spectral region, PS II will benefit from the increased content in Chl *b* which extends the useful spectral window into the 650 nm region while PS I will benefit from the long wavelength forms of Chl *a* which extend the useful spectral window into the 690–700 nm region. Because of the lower extinction coefficient of Chl *b* than Chl *a* in the red region, the integrated absorption of light by Chl *b* is likely to be less than that of Chl *a* in vivo. This would tend to make the operational PS II/PS I electron-transport capacity ratio in vivo closer to unity, and thus to ensure an overall-balanced electron flow between the two photosystems.

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References

1 Melis, A. and Brown, J.S. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 4712–4716
2 Melis, A. and Harvey, G.W. (1981) Biochim. Biophys. Acta 637, 138–145
3 Falkowski, P.G., Owens, T.G., Ley, A.C. and Mauzerall, D. (1981) Plant Physiol. 68, 969–973
4 Myers, J. and Graham, J. (1983) Plant Physiol. 71, 440–442
5 Thielen, A.P.G.M. and Van Gorkom, H.J. (1981) Biochim. Biophys. Acta 635, 111–120
6 Melis, A. and Thielen, A.P.G.M. (1980) Biochim. Biophys. Acta 589, 275–286
7 Melis, A. and Anderson, J.M. (1983) Biochim. Biophys. Acta 724, 473–484
8 Arnon, D.I. (1949) Plant Physiol. 24, 1–15
9 Melis, A. (1982) Arch. Biochem. Biophys. 217, 536–545
10 Anderson, J.M., Brown, J.S., Lam, E. and Malkin, R. (1983) Photochem. Photobiol. 38, 205–210
11 Lam, E., Baltimore, B., Ortiz, W., Chollar, S., Melis, A. and Malkin, R. (1983) Biochim. Biophys. Acta 724, 201–211
12 Zscheile, F.P. and Comar, C.L. (1941) Bot. Gaz. 102, 463–481
13 Joliot, A. and Joliot, P. (1964) C.R. Acad. Sci. Paris 258, 4622–4625
14 Melis, A. and Schreiber, U. (1979) Biochim. Biophys. Acta 547, 47–57
15 Izawa, S. (1980) Methods Enzymol. 69, 414–434
16 Bishop, D.G. (1974) Photochem. Photobiol. 20, 281–299
17 Kitajima, M. and Butler, W.L. (1975) Biochim. Biophys. Acta 376, 105–115
18 Duysens, L.N.M. (1956) Biochim. Biophys. Acta 19, 1–12
19 Pulles, M.P.J., Van Gorkom, H.J. and Verschor, G.A.M. (1976) Biochim. Biophys. Acta 440, 98–106
20 Van Gorkom, H.J. (1976) Dissertation, Rijksuniversiteit Leiden, The Netherlands
21 Arnon, D.I. and Chain, R.K. (1975) Proc. Natl. Acad. Sci. U.S.A. 72, 4961–4965
22 Dreschler, A. and Neumann, J. (1982) Plant Physiol. 70, 840–843
23 Brewer, J.M. and Jagendorf, A.T. (1965) Plant Physiol. 40, 303–311
24 Ikegami, I. and Katoh, S. (1973) Plant Cell Physiol. 14, 829–836
25 Ort, D.R., Ahrens, W.H., Martin, B. and Stoller, E.W. (1983) Plant Physiol. 72, 925–930