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WAVELENGTH-DEPENDENT QUANTUM YIELD OF ATP SYNTHESIS AND NADP REDUCTION IN NORMAL AND DICHLORODIMETHYLPHENYLUREA-POISONED CHLOROPLAST

MARTIN SCHWARTZ

Research Institute for Advanced Studies, Baltimore, Md. (U.S.A.)

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SUMMARY

At short wavelengths (525–690 mμ) the direct measurement of the quantum yield of the photoreduction of $NADP^+$ in normal O_2-evolving spinach chloroplasts is constant (ϕ approx. 0.3 equiv/*hν*). At short wavelengths (< 690 mμ) the quantum yield for $NADP^+$ reduction in 3(3,4-dichlorophenyl)-1,1-dimethylurea-poisoned chloroplasts supplied with the ascorbate–2,6-dichlorophenolindophenol couple (donor system) is approx. half as efficient as the normal system. At long wavelengths the quantum yield of $NADP^+$ reduction in the donor system increases by a factor of 2 (ϕ approx. 0.3 equiv/*hν*) when compared with the corresponding yield for the donor system at short wavelengths (ϕ approx. 0.15 equiv/*hν*).

Between 525 and 690 mμ, the phosphorylation yield for the normal system is constant ($\phi = 0.15$ ATP/*hν*), maintaining a constant P/2e ratio of unity. The P/2e ratios indicate a tight coupling between phosphorylation and electron transport encompassing a single phosphorylation site for the transfer of two electrons.

Between 525 and 680 mμ, the phosphorylation yield for the donor system is constant (ϕ approx. 0.04 ATP/*hν*), maintaining a P/2e ratio of approx. 0.5. At longer wavelengths (>690 mμ) the phosphorylation yield of the donor system rises (ϕ approx. 0.07–0.08 ATP/*hν*) concomitant with the rise in the yield of electron flow.

These experiments suggest the possibility that two types of phosphorylation processes operate in chloroplasts, (1) a short-wavelength process coupled to the normal O_2-evolving activity, and (2) a long-wavelength process coupled to the electron-donor activity of reagents such as DCIP.

INTRODUCTION

Photosynthetic electron transport (O_2 evolution and $NADP^+$ reduction) in higher plant chloroplasts is currently thought to involve two photoacts operating in series. Concomitant phosphorylation (ATP synthesis) presumably occurs at a single site in the electron-transport sequence responsible for the oxidation of the weak reductant of photosystem II by the weak oxidant of photosystem I (see refs. 1–3).

Abbreviations: DCMU, 3(3,4-dichlorophenyl)-1,1-dimethylurea; DCIP, 2,6-dichlorophenolindophenol; PMS, phenazine methosulphate; TMPD, *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine.

Inhibition of O_2 evolution and $NADP^+$ reduction by the inhibitor dichlorodimethylphenylurea (DCMU) was shown by VERNON AND ZAUGG[4] to be relieved in part by the ascorbate–dichlorophenolindophenol (DCIP) couple acting as an electron donor for $NADP^+$ reduction. LOSADA, WHATLEY AND ARNON[5] showed that this reaction system, where reduced DCIP replaces water as the donor, encompassed a site of phosphorylation. They interpreted their results as indicating that the ascorbate–DCIP system in the presence of DCMU (the donor system) represents the isolated activity of electron transport and phosphorylation associated with photosystem I.

HOCH AND MARTIN[6] and SAUER AND BIGGINS[7] compared the quantum requirement for $NADP^+$ reduction in the normal, O_2-evolving system and the DCMU-poisoned donor system. They reported that these two reactions are sensitized by different pigment systems in higher plant chloroplasts, the donor system showing more efficient electron-transport activity than the normal system in long-wavelength light (>700 mμ). HOCH AND MARTIN reported a minimum quantum requirement for $NADP^+$ reduction approaching 2 quanta per electron equivalent ($1/\phi = 2\ h\nu$/equiv) for the normal system (both photoacts) in short wavelengths (<680 mμ), and a minimum quantum requirement for $NADP^+$ reduction approaching 1 quantum per electron equivalent ($1/\phi = h\nu$/equiv) for the donor system (one photoact) in long-wavelength light (>700 mμ).

Our recent investigation of the phenazine methosulfate (PMS)-catalyzed phosphorylation activity of spinach chloroplasts[8] indicated that phosphorylation sensitized by long-wavelength light may involve a different site of phosphorylation and an alternate electron-transport sequence from phosphorylation and electron transport sensitized by short-wavelength light. However, the catalytic activity of PMS on electron transport cannot be studied directly. For this reason, a concurrent study was undertaken of the wavelength-dependent quantum yield of electron transport and concomitant phosphorylation in the normal system and the DCIP donor system in the presence and absence of DCMU. This report presents wavelength-dependent quantum yield profiles for electron flow and concomitant phosphorylation in spinach chloroplasts for the normal system, the normal system supplied with catalytic quantities of the ascorbate–DCIP couple, and the DCMU-poisoned chloroplast system supplied with the ascorbate–DCIP couple.

METHODS

The methods employed for the preparation for spinach chloroplasts, the determination of total chlorophyll, the measurement of ATP synthesis, the determination of chloroplast absorption at particular wavelengths, and the measurement of actinic intensities have been described previously[8,9].

The concentration of DCIP employed in these experiments was determined in 0.05 mM Tris–HCl (pH 8) at 600 mμ, employing an $\varepsilon_{mM} = 21.8$ (see ref. 10).

The reduction of $NADP^+$ and the reduction of ferricytochrome *c* were monitored with the KOK split-beam spectrophotometer which allowed for actinic illumination of the sample while recording absorbance changes.

The standard reaction mixture contained the following components in μmoles per 2 ml; Tris–HCl (pH 8), 50; $MgCl_2$, 15; NaCl, 35; sucrose, 40; ^{32}P-labeled potassium

phosphate (containing approx. $3 \cdot 10^6$ counts/min), 3; ADP, 2.5; spinach chloroplast and other components as indicated in the figures and tables.

The unit of intensity employed in this report is the mμEinstein (mμmole quanta) per min.

The quantum efficiency (yield) of phosphorylation (ϕATP) and the quantum yield of $NADP^+$ reduction (ϕNADP) are defined as follows:

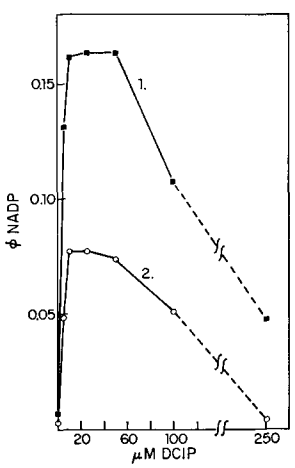
$$\phi\text{ATP} = \frac{\text{ATP molecules formed}}{\text{light quanta absorbed}}$$

and

$$\phi\text{NADP} = \frac{\text{NADP}^+ \text{ molecules reduced}}{\text{light quanta absorbed}}$$

RESULTS

Table I shows observed quantum yields of $NADP^+$ reduction for the normal O_2-evolving system and for the DCMU-poisoned donor system in 640- and 720-mμ actinic light. The data illustrate that under our experimental conditions within the range of intensities utilized the ϕNADP values are independent of light intensity. This important aspect of these experiments was confirmed repeatedly, since all yield



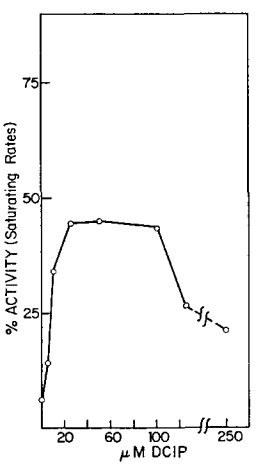


Fig. 1. Concentration effect of DCIP on the quantum yield of $NADP^+$ reduction in DCMU-poisoned chloroplasts. The experimental system contained the standard reaction mixture described in the METHODS section. The absorbed actinic light intensities were approx. 18 mμEinstein/min at 710 mμ (Curve 1) and 640 mμ (Curve 2). The concentration of the additional reactants were: 1 mM $NADP^+$ (*plus* ferredoxin); 10 mM sodium ascorbate; 2 μM DCMU; and 5 μg total chlorophyll per ml. The concentration of DCIP is indicated in the figure. The quantum yield of $NADP^+$ reduction for the normal O_2-evolving system in 640-mμ light with the same batch of chloroplasts was, ϕNADP = 0.146. The temperature was 20° and the atmosphere was argon.

Fig. 2. Concentration effect of DCIP on the saturation rate of $NADP^+$ reduction in DCMU-poisoned chloroplasts. Reaction conditions are described in Fig. 1. The chloroplast concentration employed in these experiments contained either 2.5 or 5 μg total chlorophyll per ml. The saturating light intensity contained a broad band of wavelengths longer than 550 mμ. The incident light beam could be decreased by 50% without affecting the rate of $NADP^+$ reduction. The saturating rate of $NADP^+$ reduction for the normal, O_2-evolving system with the same batch of chloroplasts was 181 μmoles $NADP^+$ reduced per mg total chlorophyll per h.

determinations reported in this communication were made at least with two different actinic intensities. The data show that at short wavelengths (640 mμ) the quantum yield of $NADP^+$ reduction with the DCIP donor system is approx. half as efficient as the normal system. At long wavelengths ϕNADP for the donor system increases by a factor of 2 when compared with ϕNADP for the donor system at short wavelengths. At 720 mμ the ϕNADP for the donor system is approximately equivalent to ϕNADP for the normal system at short wavelengths (640 mμ). Variation of the ascorbate concentration between 2 and 20 mM and the DCIP concentration between 10 and 40 μM does not effect ϕNADP in the donor system reaction at any wavelength.

Fig. 1 shows the range of DCIP concentration satisfying the requirement for maximum electron flow in the donor system at rate-limiting intensities. Essentially the same concentration range is optimal (10–50 μM) for maximum quantum yields in short-wavelength light (640 mμ) or long-wavelength light (710 mμ). As shown in Fig. 2 the range of DCIP concentrations sustaining saturation rates of $NADP^+$ reduction for the donor system is somewhat broader. Whereas 0.1 mM DCIP still maintains the maximum rate of $NADP^+$ reduction, 0.1 mM DCIP suppresses ϕNADP by approx. one-third in weak light. The data show that the observed saturation rate of $NADP^+$ reduction in the donor system does not exceed 50 % of the saturation rate of $NADP^+$ reduction in the normal, O_2-evolving system. With fresh chloroplast preparations the saturation rate of electron flow in the donor system is always suppressed by at least 50 % when compared with the rate of electron flow in the normal system.

Upon the addition of the ascorbate–DCIP couple in the absence of the inhibitor DCMU (Fig. 3) the ϕNADP values are increased (Curve 1) at long wavelengths (710 mμ) and suppressed (Curve 2) at short wavelengths (640 mμ). Whether the

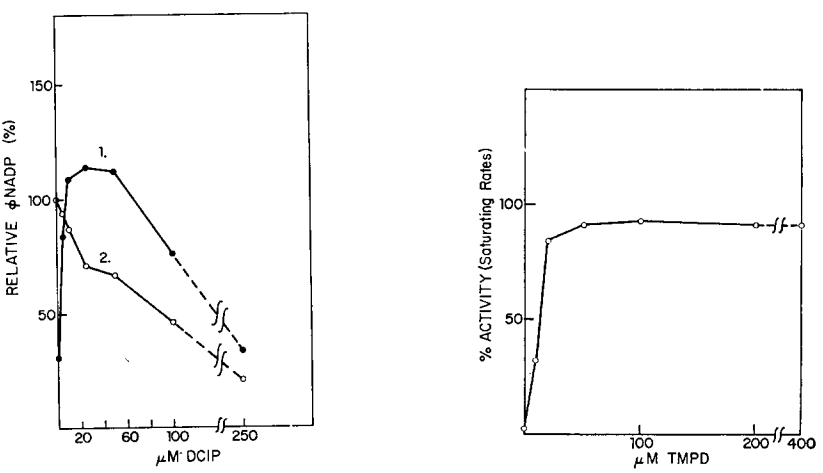


Fig. 3. Concentration effect of reduced DCIP on the quantum yield of $NADP^+$ reduction in normal chloroplasts. The experimental conditions are the same as those described in Fig. 1 with the exception that DCMU was not added to the reaction mixture. Curve 1 was obtained in 710-mμ light, and Curve 2 in 640-mμ light. For this chloroplast preparation a relative ϕNADP of 100 % equals an absolute ϕNADP = 0.141.

Fig. 4. Concentration effect of TMPD on the saturation rate of $NADP^+$ reduction in DCMU-poisoned chloroplasts. Reaction conditions are described in Figs. 1 and 2.

suppression of ϕNADP at short wavelengths represents an inhibition of the normal electron flow or represents a competition between oxidized DCIP and ferredoxin as an oxidant for the strong reductant of photosystem I is not resolved.

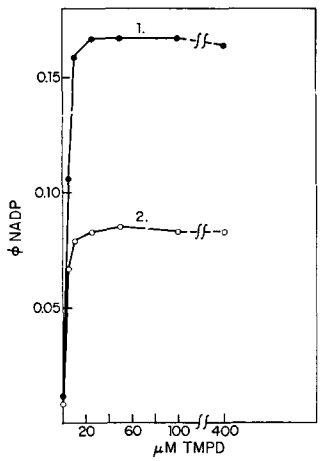


Fig. 5. Concentration effect of TMPD on the quantum yield of $NADP^+$ reduction in DCMU-poisoned chloroplasts. Reaction conditions are described in Fig. 1. Curve 1 was obtained in 710-mμ light and Curve 2, in 640-mμ light.

TABLE I

INVARIANCE OF THE QUANTUM YIELD OF $NADP^+$ REDUCTION AT RATE-LIMITING LIGHT INTENSITIES

Reaction mixture as described in the METHODS section. The concentration of the various additional reactants were: normal system: 1 mM $NADP^+$ (*plus* ferredoxin); 10 mM sodium ascorbate; donor system: 1 mM $NADP^+$ (*plus* ferredoxin); 10 mM sodium ascorbate; 25 μM DCIP; 2 μM DCMU; the total chlorophyll concentration was 5 μg/ml. The temperature was 20° and the atmosphere was argon. The table of chloroplast absorption values at the various wavelengths are tabulated in Table I of ref. 8.

Wavelength (mμ)	*System*	*$NADP^+$ formed (mμmoles/min)*	*$I_{abs.}$ (mμEinstein per min)*	*φNADP ($NADP^+$/quanta)*
640	Normal	1.18	7.8	0.151
		2.66	17.8	0.149
		5.72	37.6	0.155
640	Donor	1.36	17.8	0.076
		2.93	37.6	0.078
680	Normal	1.46	10.6	0.138
		2.85	20.0	0.143
		5.05	37.7	0.134
680	Donor	1.42	20.0	0.071
		2.79	37.7	0.074
720	Donor	0.84	5.3	0.159
		1.80	11.1	0.162
		3.27	21.0	0.156

The substitution of *N*-tetramethyl-*p*-phenylenediamine (TMPD) for DCIP in the donor system[11] allows saturation rates of $NADP^+$ reduction approximately equivalent to those seen in the normal system (Fig. 4). However, ϕNADP for DCMU-poisoned chloroplasts supplied with the ascorbate–TMPD couple is equivalent to ϕNADP for the ascorbate–DCIP couple. Fig. 5 shows ϕNADP measured at 640 and 710 mμ for the ascorbate–TMPD system, yielding results equivalent to the ascorbate–DCIP system (compare with Table I and Fig. 1). Figs. 4 and 5 show the relatively large range of concentrations of TMPD (as compared with DCIP) yielding high saturation rates of $NADP^+$ reduction and maximum quantum yields. Earlier experiments reported elsewhere[12] revealed that these concentrations of TMPD do not support significant rates of phosphorylation concomitant with $NADP^+$ reduction.

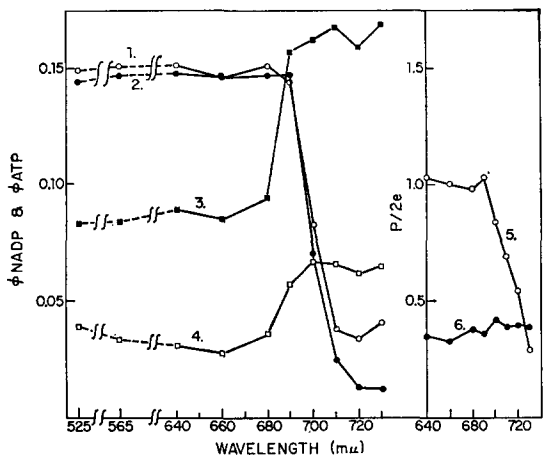


Fig. 6. Wavelength profile of the quantum yield of $NADP^+$ reduction and ATP synthesis in normal chloroplasts and DCMU-poisoned chloroplasts. All cuvettes contained the standard reaction mixture as described in the METHODS section. The absorbed light intensities were approx. 22 mμEinstein/min at all wavelengths. All experiments contained chloroplasts with a total chlorophyll concentration of 5 μg/ml. The concentrations of the various additional reactants were: normal system: Curve 1 (ϕNADP) and Curve 2 (ϕATP), 1 mM $NADP^+$ (*plus* ferredoxin); donor system: Curve 3 (ϕNADP) and Curve 4 (ϕATP), 1 mM $NADP^+$ (*plus* ferredoxin); 10 μM DCIP; 10 mM sodium ascorbate; 2 μM DCMU. Curves 5 and 6 represent the P/2e ratios calculated for the normal system and donor system respectively.

Fig. 6 shows the quantum yield profiles of $NADP^+$ reduction and the concomitant phosphorylation for the normal system and the ascorbate–DCIP donor system. The data were obtained from more than a dozen experiments in which light intensity, chloroplast concentration, and cofactor concentration were varied. Although the absolute ϕ values for both $NADP^+$ reduction and phosphorylation varied 15–20 % with different chloroplast preparations, the shape of the profiles always showed the pattern presented in Fig. 6. The ϕNADP values for the DCIP donor system rise sharply at 690 mμ attaining a relatively constant value beyond 700 mμ (Curve 3). This long-wavelength maximum for ϕNADP is only slightly greater than the short-wavelength maximum obtained with the normal system (Curve 1).

An examination of the quantum yield of the normal system (Curve 1) at wavelengths beyond 700 mμ shows a constant ϕNADP value of approx. 25 % of the yield at short wavelengths ($<$ 690 mμ). The existence of this DCMU-sensitive electron

TABLE II

QUANTUM YIELD OF ELECTRON FLOW IN CHLOROPLASTS IN SHORT- AND LONG-WAVELENGTH LIGHT

The standard components of the reaction mixture are described in the METHODS section. The concentration of the various additional reactants were: $NADP^+$ reduction: 1 mM $NADP^+$ (*plus* ferredoxin); 10 mM sodium ascorbate; ferricytochrome *c* reduction: 50 μM ferricytochrome *c*; 10 μM PMS; and 2 μM DCMU (where indicated); the total chlorophyll concentration (Chl_t) is indicated in the table. The temperature was 20° and the atmosphere was argon. The incident actinic intensity was not varied at either wavelength. The variation in the absorbed intensity ($I_{abs.}$) reflects the variation in per cent absorption caused by increasing total chlorophyll concentration (see Table I, also ref. 8).

Wavelength (*mμ*)	*System*	*Chl_t* (*μg/ml*)	*Rate* (*mμequiv per min*)	*$I_{abs.}$* (*mμEinstein per min*)	*ϕ* (*equiv/hv*)
640	$NADP^+$	5	2.31*	7.8	0.297
640	$NADP^+$	10	4.49	14.2	0.316
640	$NADP^+$ + DCMU	10	—	14.2	0
640	Ferricytochrome *c*	5	2.42**	7.8	0.311
640	Ferricytochrome *c*	10	4.78	14.2	0.337
640	Ferricytochrome *c* + DCMU	10	—	14.2	0
710	$NADP^+$	20	3.65	48.1	0.076
710	$NADP^+$ + DCMU	20	—	48.1	0
710	Ferricytochrome *c*	10	1.83	24.8	0.074
710	Ferricytochrome *c*	20	3.84	48.1	0.080
710	Ferricytochrome *c*	40	7.86	91.0	0.086
710	Ferricytochrome *c* + DCMU	40	—	91.0	0

* The rate of $NADP^+$ reduction is half this value (1 equiv = 0.5 $NADP^+$).
** The rate of ferricytochrome *c* reduction is equal to this value (1 equiv = ferricytochrome *c*).

flow at long wavelengths could be demonstrated also with other oxidants such as ferricytochrome *c* as shown in Table II. The results indicate that normal electron flow with concomitant O_2 evolution occurs at long wavelengths with a constant and relatively high efficiency of approx. 25 % of the maximum (*i.e.* ref. 6).

Fig. 6 also shows that P/2e ratios approaching unity are obtained at rate-limiting intensities for the normal system at all wavelengths shorter than 700 mμ (Curve 5). A marked decrease in the P/2e ratio at longer wavelengths (>700 mμ) is observed. This result was unexpected since phosphorylation concomitant with $NADP^+$ reduction appears tightly coupled at short wavelengths. In contrast, the P/2e wavelength profile of the DCIP donor system (Curve 6) is constant despite the long-wavelength rise of the yield of both phosphorylation and electron flow. However, even under our conditions for optimal quantum yields, the P/2e ratio for the donor system never exceeded 0.5.

The ϕ profiles for $NADP^+$ reduction and phosphorylation in Fig. 7 were obtained by the addition of reduced DCIP to the normal system in the absence of the inhibitor DCMU (see Fig. 3 also). The ϕATP profile (Curve 1) for the normal system (reproduced from Fig. 6, Curve 2) is included for reference. Curves 2 and 3 in Fig. 7 show the respective profiles for ϕNADP and ϕATP obtained upon the addition of 10 μM DCIP (reduced by excess ascorbate). In this system the ϕNADP profile (Curve 2) is nearly constant over the entire wavelength region (640 mμ to 730 mμ). This constancy of the quantum yield (Curve 2) is probably a combination of the electron flow reflecting the activity of the normal system at short wavelengths

and the donor system at long wavelengths. At short wavelengths (< 680 mμ) the corresponding ϕATP profile (Curve 3) for this system yields P/2e ratios approaching unity, indicating that the slight decrease in ϕNADP and ϕATP (compared with the normal system) is most probably the result of a slight inhibition of the normal

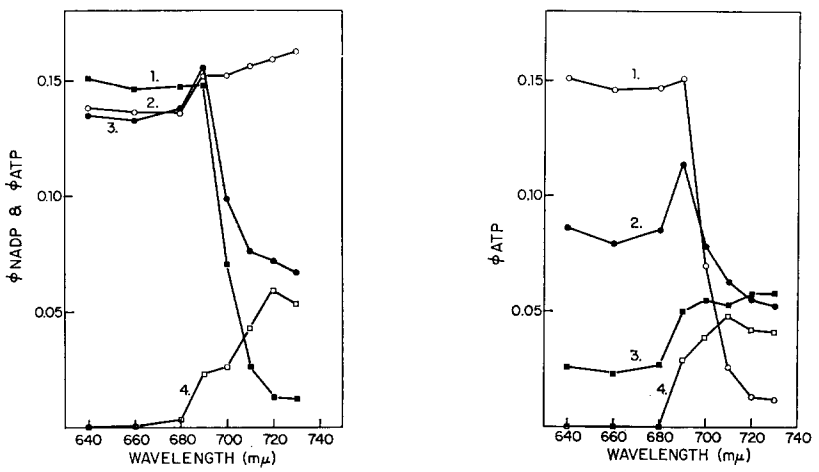


Fig. 7. Wavelength profile of the quantum yield of $NADP^+$ reduction and ATP synthesis in normal chloroplasts supplied with reduced DCIP. All cuvettes contained the standard reaction mixture as described in the METHODS section. The absorbed light intensities were approx. 22 mμEinstein per min at all wavelengths. All experiments contained chloroplasts with a total chlorophyll concentration of 5 μg/ml. Curve 1 represents ϕATP in the normal O_2-evolving system which is reproduced from Fig. 6, Curve 2, as a profile of reference. The concentration of the additional reactants were: Curve 2 (ϕNADP) and Curve 3 (ϕATP), 1 mM $NADP^+$ (*plus* ferredoxin); 10 μM DCIP; 10 mM sodium ascorbate. Curve 4 represents a derived ϕATP profile as explained in the text of this report.

Fig. 8. Wavelength profile of the quantum yield of ATP synthesis in normal chloroplasts and DCMU-poisoned chloroplasts supplied with the ascorbate–DCIP couple in the absence of added ferredoxin and $NADP^+$. All cuvettes contained the standard reaction mixture as described in the METHODS section. The absorbed light intensity and chloroplasts concentration are given in Figs. 6 and 7. Curve 1 represents ϕATP reproduced from Fig. 6, Curve 2. Curve 2 (ϕATP) is the wavelength profile obtained with normal chloroplasts supplied only with 10 μM DCIP and 10 mM ascorbate. Curve 3 (ϕATP), same conditions as Curve 2, *plus* 2 μM DCMU. Curve 4 (ϕATP), a derived profile as explained in text.

system by reduced DCIP (Fig. 3). However, at long wavelengths (>680 mμ) a different effectiveness in respect to ATP formation is indicated by a comparison of the ϕATP profile for this system (Curve 3) and the ϕNADP profile (Curve 2). At long wavelengths the P/2e ratio drops to 0.4. In Fig. 7 we plotted the difference between Curves 3 and 1 (corrected by 10%), which shows a long-wavelength rise of ϕATP (Curve 4). This difference curve (Curve 4) is strikingly similar to the observed long-wavelength ϕATP profile obtained with the ascorbate–DCIP donor system (Fig. 6, Curve 4).

Curve 2 in Fig. 8 shows the ϕATP profile observed with the ascorbate–DCIP couple without added $NADP^+$, ferredoxin or DCMU. The normal ϕATP profile (Fig. 6, Curve 2) is included for reference (Curve 1). Curve 2 shows a maximum at 690 mμ and falls gradually at longer wavelengths to what appears to be a constant

ϕATP plateau (ϕATP approx. 0.06) of the magnitude of the phosphorylation capacity demonstrated by the donor system (compare with Fig. 6, Curve 4). Upon the addition of DCMU to the ascorbate–DCIP system (in the absence of added $NADP^+$ *plus* ferredoxin) one observes a ϕATP profile (Curve 3) very similar to that obtained with the donor system (Fig. 6, Curve 4). By assuming that at short wavelengths the phosphorylation activity of the ascorbate–DCIP couple (Curve 2) represents the normal open system phosphorylation operating at a reduced efficiency (approx. 56 %), a long-wavelength ϕATP profile (Curve 4) can be obtained by subtraction of 56 % of the normal phosphorylation profile (Curve 1) from that obtained with the ascorbate–DCIP couple (Curve 2). Again, this derived ϕATP profile (Curves 1–2) is suggestive of the operation of a long-wavelength phosphorylation process observed in DCMU-poisoned chloroplasts with the donor system (Fig. 6, Curve 4 and Fig. 8, Curve 3) and also observed in DCMU-poisoned chloroplasts with the ascorbate–PMS couple[8].

DISCUSSION

The ϕNADP and ϕATP profiles (Fig. 6, Curves 1 and 2) obtained at short wavelengths (< 690 mμ), and the corresponding P/2e ratio of unity for the normal, O_2-evolving chloroplast reaction (Fig. 6, Curve 5) indicate the involvement of a single site of phosphorylation concomitant with the transfer of 2 electron equivalents in photosynthesis. The discrepancy in the normal system between ϕNADP and ϕATP at long wavelengths (>700 mμ), yielding decreased P/2e ratios (Fig. 6, Curve 5), can be accounted for by the non-linearity in phosphorylation rates as a function of light intensity when the phosphorylation rate is extremely low. This type of "lag" in phosphorylation has been reported by SHEN AND SHEN[13]. The constant long-wavelength (710–730 mμ) ϕNADP value of the normal system (approx. 25 % of the maximum) implies that a constant proportion (approx. 12.5 %) of long-wavelength light is always absorbed by photosystem II (see refs. 2, 3).

The decrease in ϕNADP obtained with the donor system at 640 mμ (Table I, Figs. 1 and 6) suggests the possibility of a competition between oxidized DCIP and ferredoxin for the strong reductant of photosystem I. Such a competition would mask the real rate of electron transport since the monitoring technique employed in these experiments measures only the net rate of $NADP^+$ reduction. The low saturation rates of $NADP^+$ reduction in the donor system (Fig. 2) might also reflect a competition between DCIP and ferredoxin. HOCH AND MARTIN[6] and SAUER AND BIGGINS[7] also observed that in short-wavelength light the ϕNADP values obtained with the donor system are significantly lower than those obtained with the normal system. No explanation of this observation was offered by them.

The low P/2e ratios (0.4–0.5) obtained with the donor system (Fig. 6, Curve 6) are unexpected. Possibly they result from the action of reduced DCIP as a reductant at more than one site in the electron-transport sequence, one site encompassing phosphorylation and another site bypassing phosphorylation. TREBST[11] has proposed multiple sites of entry for explaining the extremely low P/2e ratios obtained with the ascorbate–TMPD donor system under conditions similar to those employed for obtaining the data for Figs. 4 and 5. However, such a mechanism still leaves unexplained the low ϕNADP values.

At long wavelengths (>700 mμ) increased values of ϕNADP are obtained for the donor system (Table I, Figs. 1 and 6). In our experiments the maximum long-wavelength ϕNADP values do not approach a doubling of the maximum ϕNADP values obtained with the normal system as reported by others[6,7]. HOCH AND MARTIN[6] reported that the DCIP donor system in 710-mμ actinic light shows a long-wavelength rise in the absolute quantum yield of electron flow approaching 80% of the theoretical maximum of 1 equiv/*hv*. Their value, ϕ approx. 0.8 equiv/*hv* was calculated by the Hofstee extrapolation method of the Michaelis–Menten equation[14] employing data in which the quantum yield of electron flow varied with light intensity. According to their report (ref. 6, Fig. 5) the highest ϕ value actually measured at 710 mμ was approx. 0.5 equiv/*hv* (ϕNADP approx. 0.25) which is comparable to our measured value (ϕNADP = 0.17) in long-wavelength light. The data of this report show that the Hofstee plot is inappropriate for these experiments since all ϕNADP values were obtained in an intensity range where ϕ is invariant with increasing or decreasing light intensity. Naturally, in experiments at much higher light intensities where ϕNADP decreased with increasing intensities, ϕ values obtained by the Hofstee extrapolation were greater than those obtained by direct determinations.

By extrapolation, SAUER AND BIGGINS[7] also obtained high quantum yields for $NADP^+$ reduction, $\phi = 0.5$ equiv/*hv* for the normal system, and $\phi = 1$ equiv/*hv* for the donor system. The data in their report (Figs. 6 and 7, ref. 7) show that the maximum observed yield for the normal system in 703-mμ light was 0.2 equiv/*hv*, and for the donor system, 0.3 equiv/*hv*. Our observed values in 700-mμ light are 0.15 equiv/*hv* for the normal system and 0.34 equiv/*hv* for the donor system. The essential point is that no observed yields for $NADP^+$ reduction in the normal chloroplast system exceed approx. 0.3 equiv/*hv*. As far as we can ascertain from the literature, the observed quantum yields for the donor system with normal chloroplasts do not exceed 0.5 equiv/*hv* and certainly do not approach 1 equiv/*hv* even in long-wavelength light.

A single exception which shows a high quantum yield for the normal system is found in the report of SAUER AND PARK[15] where, in 650-mμ light, the reduction of DCIP by normal chloroplasts attains an observed maximum yield of 0.5 equiv/*hv*. This observed yield was constant over the 10-fold intensity range. However, at 680 mμ the quantum yield of DCIP reduction decreased to approx. 0.3 equiv/*hv*, indicating that the yield varied very significantly with wavelength. This variation of ϕ between 650 and 680 mμ differs from our observations of the yield of $NADP^+$ reduction and those reported by others[6,7]. Moreover, our quantum yield determinations of ferricytochrome *c* reduction catalyzed by 1 μM DCIP (unpublished) showed that the maximum yield was constant at short wavelengths (ϕ approx. 0.3 equiv/*hv*). The quantum yield and its wavelength profile were equivalent to those obtained with PMS as catalyst for ferricytochrome *c* reduction[16]. Whether an experimental discrepancy exists, or whether the 30-fold higher DCIP concentration employed by SAUER AND PARK[15] causes both an increase in the absolute quantum yield at 650 mμ and a change in the shape of the quantum yield profile remains to be resolved.

The addition of reduced DCIP to the normal system in the absence of DCMU (Fig. 7, Curve 2) demonstrates a possibility of a competition between water and DCIP as electron donors. Under these conditions, the ϕNADP profile indicates that the electron-donor activity of DCIP is suppressed in short wavelengths (< 690 mμ)

by the electron-donor activity of water. At longer wavelengths (>690 mμ) the electron-donor activity of DCIP increases as the electron-donor activity of water decreases. The corresponding ϕATP profile (Fig. 7, Curve 3) therefore reflects the phosphorylation associated with the normal O_2-evolving system at short wavelengths (< 690 mμ) with water as electron donor, and at longer wavelengths reflects the combined phosphorylation associated with water and DCIP as electron donors. The derived ϕATP profile (Curve 4) calculated from the difference between Curves 1 and 3, shows the long-wavelength phosphorylation, characteristic of the DCIP donor system in DCMU-poisoned chloroplasts (compare with Fig. 6, Curve 4).

The ϕATP profile (Fig. 8, Curve 2) obtained with the ascorbate–DCIP couple without added ferredoxin or $NADP^+$ can also be interpreted as the combined activity of water and DCIP as electron donors. That is, the ϕATP profile (Curve 2) is the result of phosphorylation at short wavelengths due to (1) the normal O_2-evolving activity of the ascorbate–DCIP couple (operating at approx. 56 % efficiency) with DCIP serving as a Hill oxidant, and at long wavelengths (2) the electron-donor activity of reduced DCIP. A subtraction of 56 % of the normal ϕATP profile (Curve 1) from Curve 2 shows a ϕATP profile (Curve 4) reflecting the long-wavelength donor system phosphorylating activity. The similarity can be seen between this profile (Curve 4), the profile of the ascorbate–DCIP couple *plus* DCMU (Curve 3), and the profile of the donor system (Fig. 6, Curve 4).

In effect, the data indicate the possibility of two types of phosphorylation processes operating in chloroplasts, (1) a short-wavelength process tightly coupled with the normal O_2-evolving activity of chloroplasts, and (2) a long-wavelength process operating at a reduced efficiency and associated with the electron-donor activity of reagents such as DCIP and PMS (ref. 8). Whether the long-wavelength phosphorylation process and the long-wavelength electron-transport process involve a different phosphorylation site and a different electron-transport sequence, is still unresolved.

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