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Rate-Limiting Steps of Electron Transport in Chloroplasts during Ontogeny and Senescence of Barley[1]

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PETER J. HOLLOWAY, DONALD J. MACLEAN, AND KENNETH J. SCOTT
Department of Biochemistry, University of Queensland, St. Lucia, Queensland 4067 Australia

ABSTRACT

Partial photochemical activities and concentrations of electron carriers were measured relative to chlorophyll in barley (*Hordeum vulgare* L.) thylakoids, isolated from primary leaves during ontogeny and senescence. Thylakoids from mature leaves generated somewhat higher quantum efficiencies than thylakoids from premature or senescing leaves; this phenomenon did not appear to be caused by any deficiency of water-splitting enzyme. Under conditions of saturating light, the noncyclic electron flux from water to the reducing side of photosystem I increased during leaf ontogeny, peaked at maturity, and declined during senescence. However, electron fluxes appeared to be limited at different steps before and after leaf maturity. Before leaf maturity, the rate-limiting step was located prior to the reoxidation of plastohydroquinone. After leaf maturity, the decline in noncyclic electron flux correlated with a decrease in the concentration of cytochromes *f* and b_6. This correlation, together with a consideration of mechanisms of entry and exit of electrons in 3-(3,4-dichlorophenyl)-1,1-dimethylurea-treated thylakoids, suggests that the cytochrome *f*/b_6-containing complex, and not plastocyanin or P700, is the site of entry of electrons from the reduced forms of 2,6-dichlorophenolindophenol and diaminodurene. It is therefore proposed that in senescing leaves the cytochrome *f*/b_6-containing complex limited electron transport by constraining the rate of reduction of cytochrome *f* by plastohydroquinone.

It has been well established that maximum rates of net photosynthesis, as measured by O_2 evolution or CO_2 assimilation, are achieved at or just before leaf maturity, followed by an extended period of decline (7, 24, 26). Previous research on changes in chloroplast electron transport during foliar ontogeny and senescence (for a review, see Sestak [25]), have shown that rates of chloroplast electron transport in isolated thylakoids, expressed on a Chl basis, generally follow similar patterns of rise and decline to net photosynthesis in whole leaves.

Most methods for measuring rates of electron transport *in vitro* rely on artificial electron donors, acceptors and inhibitors. Recently, the sites of action of many of these compounds have been clarified (13, 29–31). The purpose of the present work was to use some of the more thoroughly characterized compounds to determine rates of partial photochemical activities in thylakoids isolated from barley leaves of different ages, and concurrently, to measure the concentrations of natural electron carriers. Changes in the concentration of carriers directly involved in rate-limiting steps of electron transport, could be expected to correlate directly with changes in photochemical activities. Thus, changes in thylakoid components and activities which occur naturally during leaf ontogeny and senescence, were used to probe and identify rate-limiting steps of electron transport.

MATERIALS AND METHODS

Chemicals. MV,[2] PD, DCPIP, DPC, ascorbic acid, superoxide dismutase type 1, Tricine, and EDTA, were obtained from Sigma Chemical Co.; DBMIB, MDBQ, and DAD, were kind gifts from Professors A. Trebst and N. Good.

Plant Material. Barley seeds (*Hordeum vulgare* L. cv Prior), were purchased from a local seed merchant, planted in vermiculite at a density of 15 g/20-cm-diameter pot, and maintained in a growth cabinet (fluorescent lights with tungsten supplements) at a light intensity of 200 μE m^{-2} s^{-1} at 22°C for 18 h, followed by 6 h dark at 18°C. Plants were irrigated with water and Aquasol (Hortico Australia) mineral solution (1 g/l) on alternate days.

Isolation of Thylakoids. Thylakoids were isolated by a method modified from Neilsen and Smillie (21). Barley segments (3 g, apical 5 cm of primary leaves) were cut into 5 mm^2 pieces and immersed in 25 ml of ice-cold homogenization buffer (50 mM Sörenson's phosphate [pH 7.5], 30 mM NaCl, 1 mM EDTA, and 0.5% w/v BSA). This mixture was homogenized in an Ultra-Turrax homogenizer (Janke and Kunkle, Breisgau, Federal Republic of Germany) operating at 75% line voltage for two 5-s bursts. The resulting brei was squeezed through two layers of Miracloth (Calbiochem), centrifuged at 800*g* for 30 s to remove cell debris, and spun again at 2000*g* for 5 min to sediment thylakoids. This pellet was resuspended in 10 ml of resuspension buffer (5 mM Na-Tricine [pH 7.5], 30 mM NaCl), centrifuged at 2000*g* for 5 min, and the pellet resuspended in fresh buffer at a concentration equivalent to 250 μg Chl ml^{-1}, stored on ice, and used within 2 h.

Stromal enzymes appeared to be absent from thylakoid preparations, as evidenced by the absence of bands corresponding to the mol wt of both large and small subunits of ribulose bisP carboxylase-oxygenase when thylakoid preparations were subjected to SDS-polyacrylamide gel electrophoresis (6). Estimations of Chl *a* and *b* (28) were compared with total Chl (5), to show that degradation products were not significant (8).

Photochemical Activities. Relationships between electron donors, carriers, and inhibitors used during this investigation are shown in Figure 1.

Photochemical Activities Measured Spectrophotometrically. The light-driven reduction of ferricyanide or DCPIP in both reactions a and b (below) was followed by an Aminco-DW2a

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[2] Abbreviations: MV, methyl viologen; PD, *p*-phenylenediamine; DCPIP, 2,6-dichlorophenolindophenol; DPC, 1,5-diphenylcarbazide; DBMIB, dibromothymoquinone; MDBQ, methylenedioxydimethylbenzoquinone; DAD, diaminodurene; PQ, plastoquinone; PC, plastocyanin; PD_{ox}, *p*-phenylenediamine (oxidized form); Cyt b_{559} H.P., cytochrome b_{559} high potential form; Cyt b_6, cytochrome b_6 (b_{563}).

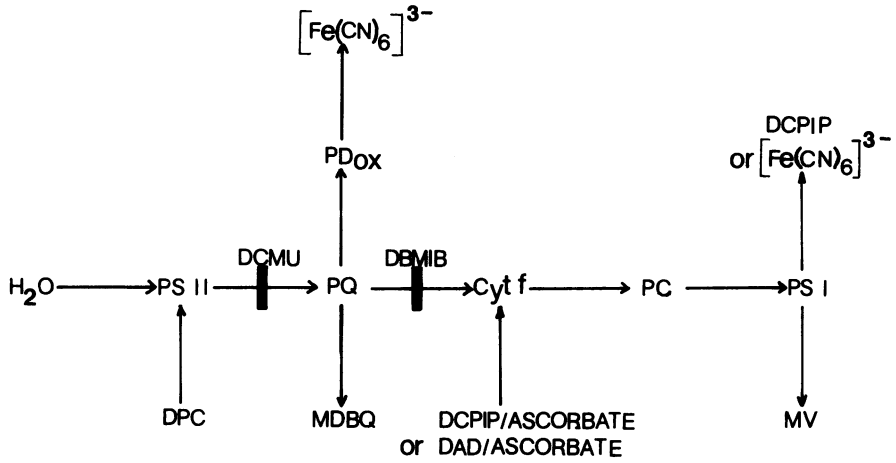

FIG. 1. Relationships between functional components of the chloroplast electron transport chain and compounds used in this investigation, showing sites of entry and exit of electrons, and inhibition of electron flow.

Reaction a: $H_2O \rightarrow PSII \rightarrow PQ \rightarrow Cyt\,f \rightarrow PC \rightarrow PSI \rightarrow [Fe(CN)_6]^{3-}$

Reaction b: $H_2O \rightarrow PSII \rightarrow PQ \rightarrow PD_{ox} \rightarrow [Fe(CN)_6]^{3-}$

Reaction c: $H_2O \rightarrow PSII \rightarrow PQ \rightarrow MDBQ$

Reaction d: $DCPIP \rightarrow Cyt\,f \rightarrow PC \rightarrow PSI \rightarrow MV$

Reaction e: $DAD \rightarrow Cyt\,f \rightarrow PC \rightarrow PSI \rightarrow MV$

Reaction f: $H_2O \rightarrow PSII \rightarrow PQ \rightarrow Cyt\,f \rightarrow PC \rightarrow PSI \rightarrow MV$

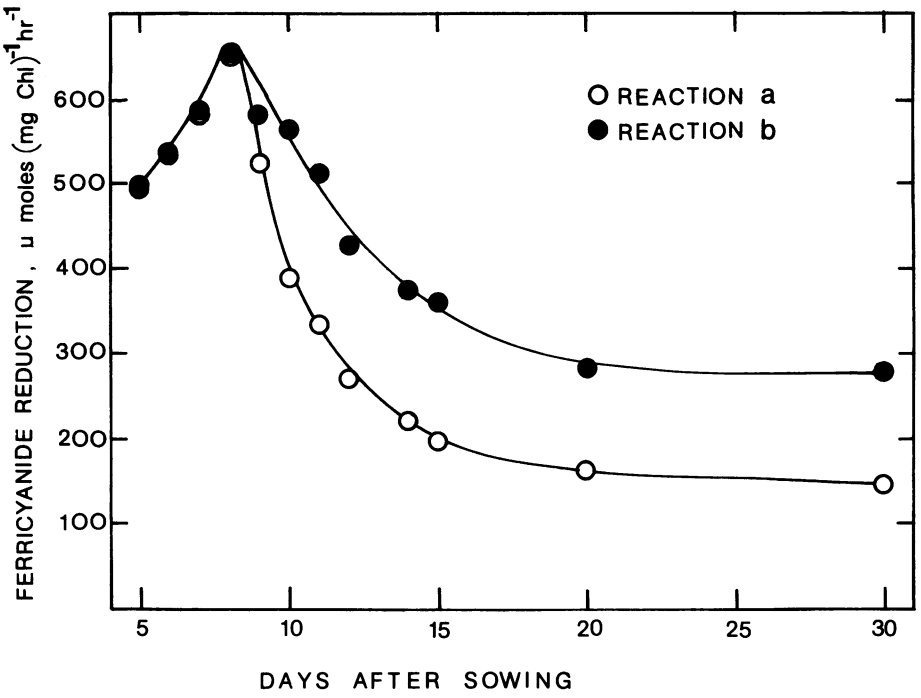

FIG. 2. Electron flux through the electron transport chain of thylakoids isolated from barley leaves of different ages, measured as rates of ferricyanide reduction in reactions a (○) and b (●). Each data point represents the mean value of two independent determinations using plants sown on different occasions; the average deviation always fell within ±5% of this mean.

spectrophotometer operating in the dual wavelength mode. Saturating actinic light, at right angles to the measuring beams, was filtered through 5 cm of water, resulting in an intensity at the cuvette of 1500 μE m^{-2} s^{-1}. The rate of ferricyanide or DCPIP reduction was determined by recording the absorbance difference between 430 and 450 nm, or 575 and 550 nm, respectively, immediately before and after a 5.0-s flash of actinic light. The rate of ferricyanide reduction at light intensities below 200 μE m^{-2} s^{-1} was determined by placing a Corning CS2-60 red cut-off filter between the actinic light and the cuvette, and continuously monitoring the absorbance difference between 430 and 450 nm. A CS4-96 filter screened the photo-multiplier from scattered actinic light.

Reaction a:

$$H_2O \rightarrow PSII \rightarrow PQ \rightarrow Cyt\,f \rightarrow PC \rightarrow PSI \rightarrow [Fe(CN)_6]^{3-}$$

The reaction mixture, modified from (21), final volume of 2.5 ml, contained 30 mM NaCl, 30 mM methylamine hydrochloride, 80 μM potassium ferricyanide, 5 mM Na-Tricine (pH 7.5), and

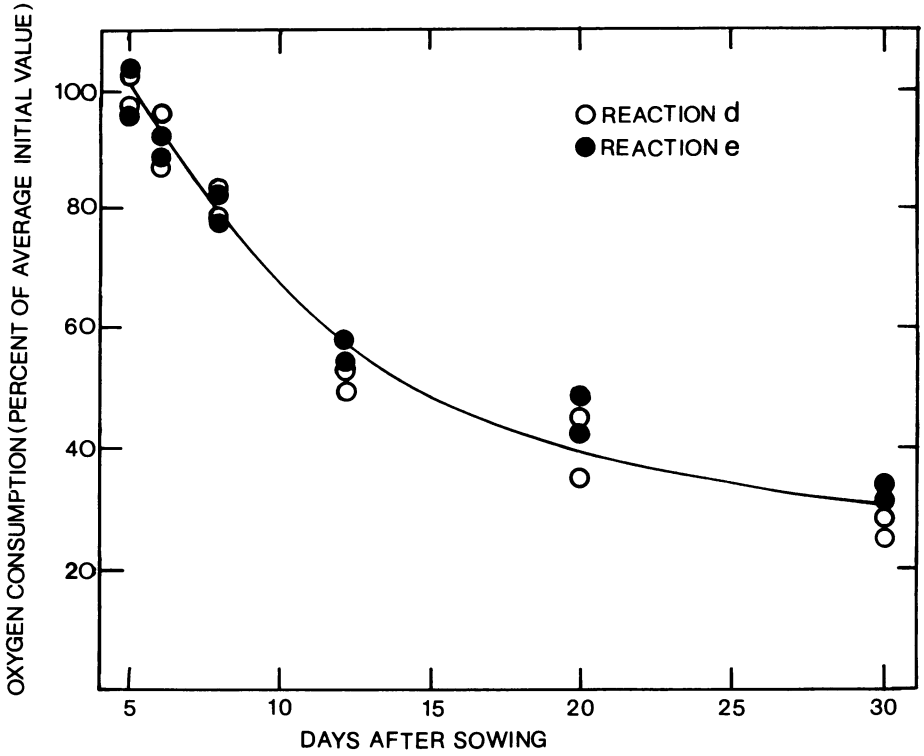

FIG. 3. Reduction of MV (measured as O_2 consumption) by thylakoids isolated from barley leaves of different ages. Rates of O_2 consumption are expressed as per cent of the mean of values determined in thylakoids isolated 5 d after sowing. The two electron donors used, DCPIP/ascorbate (○, reaction d), and DAD/ascorbate (●, reaction e), gave rates of 246 and 1,990 μmol O_2 h^{-1} (mg Chl)$^{-1}$, respectively. Two thylakoid preparations, from plants sown on separate occasions, were assayed for each reaction at each leaf age.

thylakoids equivalent to 5 μg Chl ml^{-1}. The reaction rate was inhibited by 82–86% when DBMIB (0.05–2.0 μM for thylakoids from 6-d-old plants, 0.05 μM for all other ages) was added to the reaction mixture. Because DBMIB inhibits electron transport between plastohydroquinone and Cyt *f* (31), it appears that at least 84% of the ferricyanide was reduced by PSI acting in series with PSII, with the remaining 16% being reduced prior to the site of plastohydroquinone oxidation. DCMU (3 μM) completely inhibited the reduction of ferricyanide. Reaction rates were decreased by 75% if the uncoupler methylamine was omitted from reaction mixtures. In some experiments, 4.0 μM DCPIP was substituted for ferricyanide as terminal electron acceptor, and 2.0 mM DPC was added as an electron donor to PSII (13).

Reaction b:

$$H_2O \rightarrow PSII \rightarrow PQ \rightarrow PD_{ox} \rightarrow [Fe(CN)_6]^{3-}$$

The reaction mixture for reaction a above was modified by substituting 0.1 mM *p*-phenylenediamine for the methylamine (21), and by increasing the ferricyanide concentration to 280 μM. DBMIB (0.05 μM) inhibited this reaction by 16%, and 3 μM DCMU inhibited the reaction completely, indicating that at least 84% of the electron flow followed a pathway consistent with that designated reaction b.

Photochemical Activities Using Autoxidisable Electron Acceptors. Reactions c, d, e, and f (below) were assayed in a Clark O_2 electrode (Rank Bros.; Bottisham, Cambs., U.K.) illuminated with a 150-w tungsten light source (Phillips photoflood). Saturating light (1200 μE m^{-2} s^{-1} at the exterior surface of the electrode) was used in all experiments. Reaction rates were determined by monitoring O_2 consumption during the autoxidation of reduced terminal electron acceptors as specified below. For reasons which will be outlined later, we assume that the reduced forms of DCPIP and DAD, the electron donors to reactions d and e, respectively, directly reduce Cyt *f* rather than PC or P_{700}.

Reaction c: $H_2O \rightarrow PSII \rightarrow PQ \rightarrow MDBQ$

The 3.0-ml reaction mixture modified from Trebst and Reimer (32), contained 30 mM NaCl, 0.2 mM MDBQ, 0.05 μM DBMIB, 50 mM Na-Tricine (pH 8.0), and thylakoids equivalent to a concentration of 10 μg Chl ml^{-1}. This reaction was completely inhibited by 3 μM DCMU.

Reaction d: DCPIP → Cyt *f* → PC → PSI → MV

DCPIP/ascorbate was used as electron donor. The 3.0-ml reaction mixture modified from Izawa (13), contained 30 mM NaCl, 3 μM DCMU, 2.5 mM ascorbate, 0.4 mg superoxide dismutase, 30 mM methylamine hydrochloride, 20 μM DCPIP, 100 μM MV, 1 mM KCN, 50 mM Na-Tricine (pH 8.0), and thylakoids equivalent to 10 or 22.5 μg Chl ml^{-1}.

Reaction e: DAD → Cyt *f* → PC → PSI → MV

This reaction was identical to reaction d except that 2.5 mM DAD was substituted for DCPIP. Both reactions d and e were completely insensitive to 0.05 μM DBMIB.

Reaction f:

$$H_2O \rightarrow PSII \rightarrow PQ \rightarrow Cyt\,f \rightarrow PC \rightarrow PSI \rightarrow MV$$

The 3.0-ml reaction mixture contained 30 mM NaCl, 30 mM methylamine hydrochloride, 100 μM MV, 1 mM KCN, 0.4 mg superoxide dismutase, 50 mM Na-Tricine (pH 8.0), and thylakoids equivalent to 10 μg Chl ml^{-1}. This reaction was completely inhibited by both 0.05 μM DBMIB and 3 μM DCMU.

Estimation of Electron Carriers. All Cyt were determined in thylakoid preparations adjusted to a concentration of 100 μg Chl ml^{-1}, using an Aminco DW-2a spectrophotometer operating in

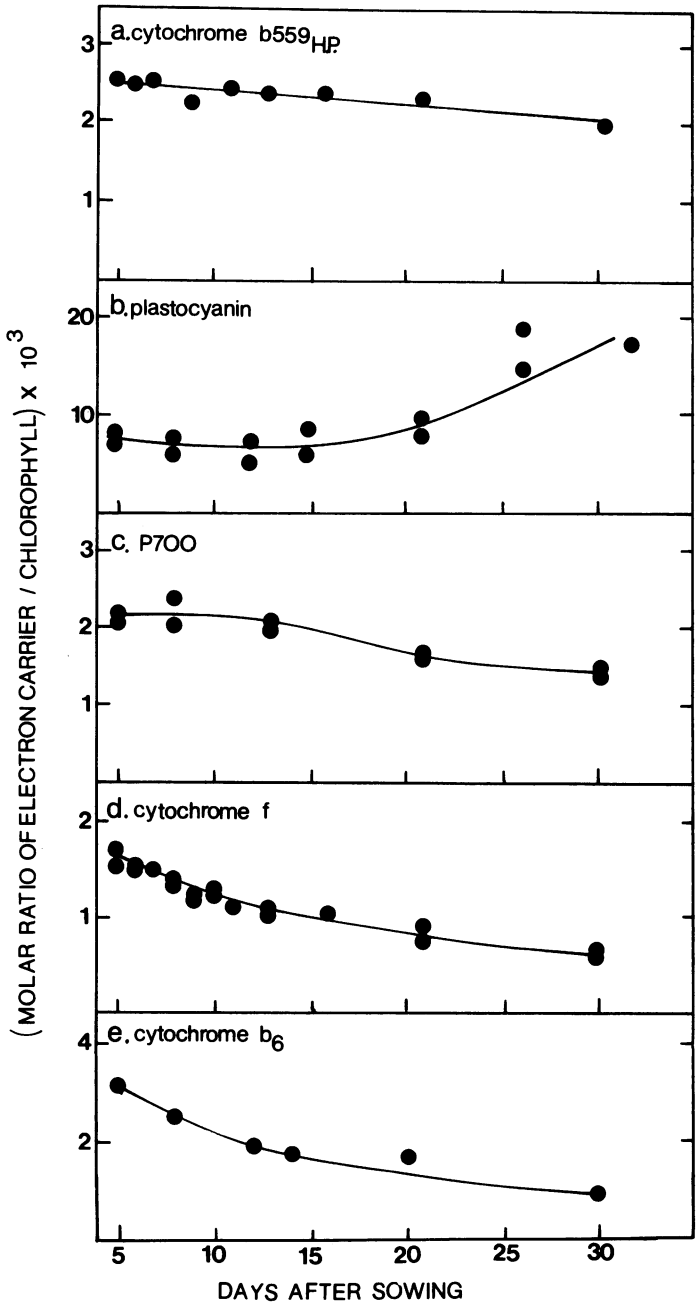

FIG. 4. Concentrations of five electron carriers in thylakoids isolated from barley leaves of different ages. Data points, each representing an individual determination, are expressed as the molar ratio of the carrier to Chl.

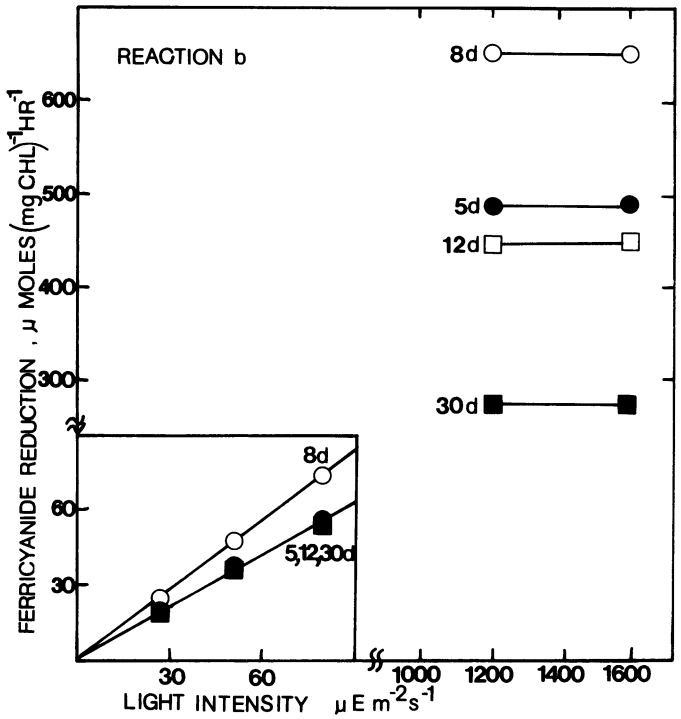

FIG. 5. Increase in reaction b activity with light intensity, in thylakoids isolated from barley leaves of different ages (age indicated as days after sowing). Each data point represents the mean of two independent determinations on plants sown on different occasions; the average deviation always fell within ±5% of this mean.

the split beam mode. Cyt b_{559} H.P. was measured by the method of Henningsen and Boardman (11), and Cyt b_6 by the method of Bendall *et al.* (4); estimation of Cyt *f* by both of these methods gave identical results.

PC was estimated by measuring total thylakoid copper, assuming a PC:copper molar ratio of 1:1 in barley thylakoids (22). Thylakoid preparations were adjusted to 1 mg Chl ml^{-1}, 1-ml aliquots were digested with 0.5 ml concentrated HNO_3 for 30 min at room temperature, and centrifuged at 2000*g* for 5 min. Copper was estimated in the digest supernatant using a Varian AA-6 atomic absorption spectrophotometer.

P_{700} was estimated by measuring the absorbance change at 700 nm relative to its isosbestic point at 725 nm (*cf.* 20) using an Aminco DW-2a spectrophotometer operating in the dual wavelength mode. Thylakoid preparations were put in the dark for 5 min, the absorbance was measured, after which potassium ferricyanide was added (10 mM final concentration) and the absorbance monitored until stable. Complete oxidation of P_{700} took about 5 min. The concentration of P_{700} was calculated using a difference molar absorption coefficient of 7.0×10^4 M^{-1} cm^{-1} (19).

RESULTS AND DISCUSSION

Measurements made during the course of this investigation showed that dry weight, surface area, and total Chl of first leaves of barley reached a maximum 8 d after sowing. The total Chl content per leaf slowly decreased after this time, while dry weight and surface area remained relatively constant, showing that leaves reached maturity 8 d after sowing. Chl *a*/*b* ratios (2.4–2.6) remained unchanged during the experiment.

Light-Dependent Reduction of Ferricyanide. The locations of rate limiting reactions of electron transport at various stages of leaf ontogeny and senescence were explored by measuring electron fluxes through different portions of the chloroplast electron transport chain. Our first experiment measured the rate of electron flow from water to the reducing side of PSI in uncoupled thylakoid preparations, using ferricyanide as terminal electron acceptor (Fig. 2, reaction a). Expressed on a unit Chl basis, it is clear from Figure 2 that this flux reached a maximum 8 d after sowing and thereafter sharply declined. Similar rates of electron flow were obtained by substituting MV for ferricyanide as electron acceptor (reaction f, data not shown), indicating that the mechanism of exit of electrons from PSI to the artificial acceptor is unlikely to limit rates of electron transport.

The above results confirm work on a wide range of other plant species, in which chloroplasts isolated after leaf maturity generally show a reduced ability to transport electrons from H_2O to the reducing side of PSI (14, 25). A study of the transient fluorescent changes that occur upon illumination of dark pretreated bean

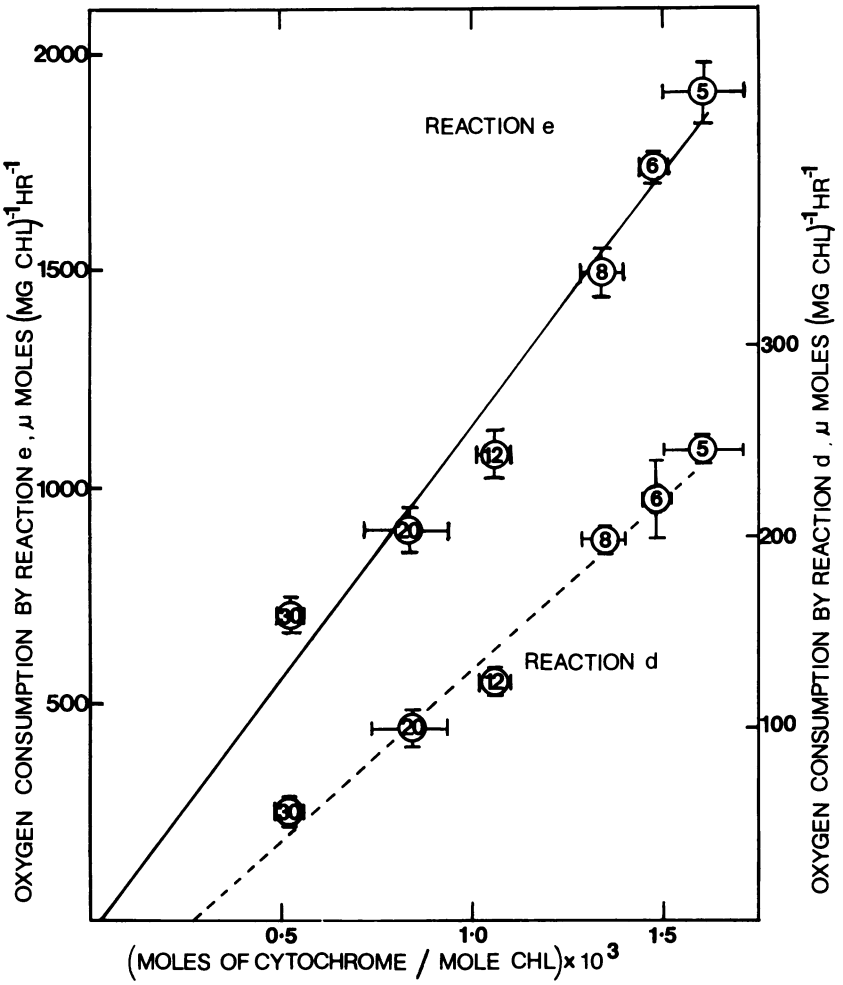

FIG. 6. Correlations between Cyt *f* concentration, and rates of electron flow to MV from DCPIP (reaction d) and DAD (reaction e). The age of plants (d) from which thylakoids were isolated is indicated; error bars indicate the range of duplicate determinations on plants sown on different occasions. See text for determination of correlation lines.

(*Phaseolus vulgaris*) leaves (16) srongly suggests that this reduced electron transport capability occurs *in vivo* and is not an artifact caused by poor techniques for isolating thylakoids.

To examine changes in the electron transport chain prior to Cyt *f*, ferricyanide reduction was measured in the presence of PD (Fig. 2, reaction b). Electron flow was identical to that of reaction a until a maximum flux was reached 8 d after sowing; thereafter, the rate fell, albeit much more slowly than reaction a. When MDBQ was substituted for PD, and electron transport was measured by O_2 consumption rather than spectrophotometrically (reaction c, data not shown), an almost identical pattern of rise and decline to that found for reaction b was obtained. It is noted that Jenkins and Woolhouse (15), using coupled thylakoid preparations from bean in contrast to our uncoupled preparations from barley, also found that reaction f declined more rapidly than reaction b in senescing leaves.

From our data, it can be deduced that prior to leaf maturity at 8 d after sowing, component(s) of the chloroplast electron transport chain common to both reactions a and b, for example PQ or an earlier component, must limit the rate of electron flow from water to ferricyanide. It can also be deduced that, after leaf maturity, component(s) on the PSI side of PQ become rate limiting.

Electron Transport on the PSI Side of PQ. To confirm that the rate limiting step in electron transport after leaf maturity was located after PQ, we carried out further experiments to measure rates of electron flow from Cyt *f* to PSI. The reduction of MV by PSI in the presence of DCMU using reduced DCPIP as electron donor, showed a progressive decline from the 1st d of measurement to only 30% of the initial level by the end of the experiment (Fig. 3, reaction d). When reduced DAD was substituted for DCPIP as electron donor, a very similar pattern of decline was obtained (Fig. 3, reaction e).

The above results suggest that some steps of electron transport located after PQ, decrease in activity relative to Chl from 5 d after sowing, rather than from 8 d as might otherwise be inferred from reactions a and b (Fig. 2). However, this decrease did not limit overall chloroplast electron transport (reaction a) until after day 8.

Concentration of Electron Carriers in Thylakoids. The following electron carriers have been reported to change in concentration relative to Chl during greening of etiolated leaves of a variety of plant species: Cyt *f*, Cyt b_{559} H.P., Cyt b_{559} L.P., Cyt b_6, P_{700} (*cf.* 11, 23, 25). On the few occasions that these electron carriers have been measured during the full range of ontogeny and senescence, large changes were reported for Cyt *f* in pumpkin (9), and Cyt b_6 in barley (25). To investigate further the constraints to chloroplast electron transport during the ontogeny and senescence of barley leaves, we assayed the following electron carriers to determine whether or not their concentrations relative to Chl rose or declined similarly to photochemical activities.

(a) Cyt b_{559} H.P. showed a small, continuous decrease during

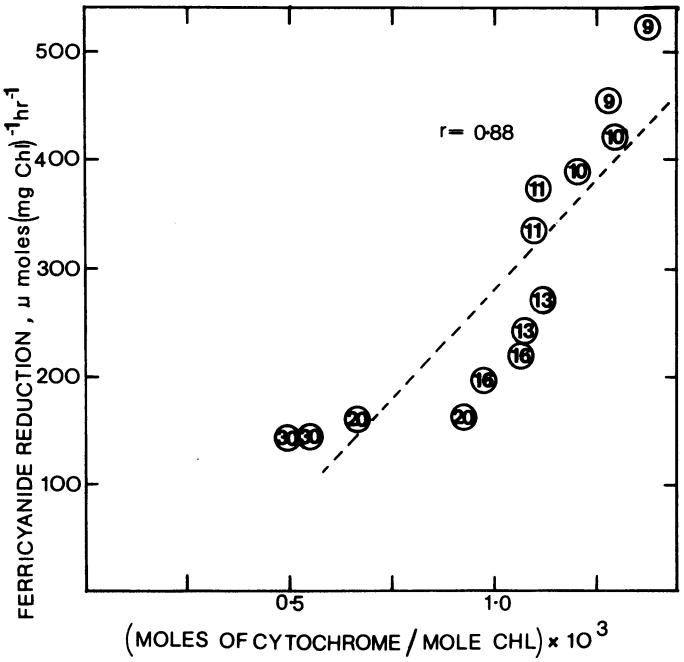

FIG. 7. Correlation between Cyt *f* concentration, and the rate of ferricyanide reduction by PSI (reaction a). Each point represents data from a different thylakoid preparation isolated from plants of the age shown, (r = 0.88, P > 0.001).

the experiment, dropping to 80% of initial values at 30 d after sowing (Fig. 4a). This Cyt is believed to be intimately associated with PSII *in vivo* (2, 10). Facilities were not available for measurement of the PSII reaction center directly.

(b) PC measured as bound thylakoid copper, remained constant relative to Chl for about 15 d after sowing, and thereafter increased markedly to over 200% of initial values (Fig. 4b). On a fresh weight basis, PC remained virtually constant after leaf maturity (data not shown). This apparent stability of PC during senescence has not been noted previously.

(c) P_{700}, the reaction center of PSI, gradually fell to 75% of initial values relative to Chl during the experiment (Fig. 4c).

(d) Cyt *f* and b_6 showed by far the largest changes in concentration of any electron carriers, and fell continuously relative to Chl, reaching 25% of their initial values by the end of the experiment (Fig. 4, d and e). Cyt b_6 was always about twice the concentration of Cyt *f*, suggesting that the Cyt *f*/b_6-containing complexes (2) are lost from thylakoids as complete units.

Rate-Limiting Step(s) of Electron Transport Prior to Leaf Maturity. As noted earlier, the rate-limiting step of electron transport before leaf maturity was located prior to the reoxidation of plastohydroquinone. This region of the electron transport chain is isolated by reaction b. At rate-limiting light intensities, the quantum efficiency of reaction b was slightly higher in thylakoids from 8- than 5-d-old plants. Although significant (Fig. 5), the cause of this small difference is not yet clear. However, as light intensities were increased to 'saturating' levels, a limiting flux of electrons through reaction b was reached, presumably controlled by slower steps than photon capture and charge separation in PSII. Such slower steps could be the passage of electrons either from water to the PSII trap, or from the PSII acceptor Q to ferricyanide. In this regard, Anderson (1) has suggested that the rate at which PQ diffuses between PSII and Cyt *f* might limit rates of electron transport in chloroplasts. Further experiments are necessary to identify conclusively the rate-limiting components and processes in immature leaves of barley.

Rate-Limiting Step(s) of Electron Transport after Leaf Maturity. Conversely to maturing leaves, in senescing leaves the rate-limiting step of chloroplast electron transport was located on the reducing side of plastohydroquinone. This region of the electron transport chain is isolated by reactions d and e. Using functional relationship analysis (18), plots of rates of these reactions against the concentration of electron carriers given in Figure 4 revealed a direct relationship with Cyt *f* (Fig. 6) and Cyt b_6, but not with P_{700} or PC.

The joint 95% confidence limits for the slopes and intercepts of the lines obtained for both reaction d and reaction e showed that the intercepts were not significantly different from zero. Linear regression analysis was not used because photochemical activities were measured with different thylakoid preparations from those used for Cyt *f* measurements. Although Cyt b_6 is not generally believed to participate in noncyclic electron transport (30, 33), there is strong evidence that it forms part of a complex (containing one molecule of Cyt *f*, two molecules of Cyt b_6, and one Rieske iron-sulfur protein) which exists as a discrete unit within the thylakoid membrane (1, 2, 12). Our data therefore suggest that whole Cyt *f*/b_6-containing complexes decrease relative to Chl during the maturation and senescence of barley leaves, and are thus responsible for limiting electron flow through reactions d and e.

Some authors (reviewed by Izawa [13]) note that the site of electron donation by reduced forms of DCPIP or DAD have not yet been identified clearly. However, the close correlations we observed between the concentration of Cyt *f* or b_6, and the rate of reaction d or e (both reaction mixtures containing DCMU), are consistent with Cyt *f* (or some other component of the Cyt *f*/b_6-containing complex), and not PC or P_{700}, being the immediate recipient of electrons from these artificial electron donors.

Because rates of reaction a are limited after leaf maturity (from 9 d onwards) by an electron transfer step after plastohydroquinone, and because the rate-limiting step after plastohydroquinone (reactions d and e from 5 d) appears to be constrained by the concentration of Cyt *f*, it can be predicted that reaction a should correlate positively with Cyt *f* concentration from 9 d onwards. Such a prediction is consistent with the data presented in Figure 7. However, because the correlation between reaction a and Cyt *f* appears to be nonlinear, the rate of electron transport through reaction a after leaf maturity appears to be modulated by factors additional to limiting concentrations of the Cyt *f*/b_6-containing complex. Further investigation is necessary to identify these modulating factors. Jenkins and Woolhouse (15) determined the activities of various partial photochemical reactions in thylakoids isolated from senescing bean leaves, and concluded that noncyclic electron flow may be limited by Cyt *f*, although no direct measurements were made of electron carriers.

We will now consider the rate-limiting step of electron transport associated with the Cyt *f*/b_6-containing complex. Although the patterns of decline in rates of reactions d and e were very similar during ontogeny and senescence (Fig. 3), the absolute rates varied considerably, with reaction d (DCPIP as immediate electron donor) being about one-eighth the rate of reaction e (DAD as immediate electron donor). This shows that the mechanism of electron transfer from the artificial donor to the Cyt complex must be rate-limiting, rather than the mechanism of transfer of electrons from the complex to PC. Furthermore, the electron flux through reaction e in any particular thylakoid preparation was far greater than the flux through reaction a or reaction f (*e.g.* in thylakoids isolated from 5-d-old plants, reaction e = 3,980 μeq electrons h^{-1} [mg Chl]$^{-1}$; whereas, reaction a = 500 μeq electrons h^{-1} [mg Chl]$^{-1}$). Thus, steps in electron transport beyond the Cyt *f*/b_6-containing complex could not have limited reaction a. Presumably, in senescing leaves the rate-limiting step in reaction a is the transfer of electrons from plastohydroquinone to the Cyt *f*/b_6-containing complex.

The quantum efficiency of thylakoids reached a peak at day 8,

dropped to a plateau level by day 12, and thereafter remained constant to at least day 30 (Fig. 5). These data are consistent with PSII units losing functional activity during most of senescence, at the same rate as loss of Chl. One possible cause of lower quantum efficiency pre- and post-maturity, is a higher proportion of PSII complexes containing an inactive H_2O-splitting enzyme. This possibility was explored by measuring reaction a using DCPIP as electron acceptor, in the presence and absence of DPC, an artificial electron donor to PSII (13). Reaction a was not stimulated by DPC in thylakoids isolated from barley leaves of any age (data not presented). Under these conditions, leaves which have lost part of their H_2O-splitting activity as a result of chill, heat, or chemical treatment, show increased activity in the presence of DPC (27). Therefore, PSII complexes pre- and post-maturity do not appear to be deficient in the H_2O-splitting enzyme (*cf.* 17).

CONCLUSIONS

In this investigation, changes which occurred naturally in the relative concentration of functional components of the electron transport chain of barley thylakoids enabled us to probe the rate-limiting steps of electron transport at various stages of leaf ontogeny and senescence. Thus, measurement of partial photochemical activities and concentrations of electron carriers in isolated thylakoids have led to the following conclusions.

(a) At low light intensities, the quantum efficiency of the PSII complex is greatest in thylakoids from mature barley leaves (*i.e.* 8 d after sowing).

(b) At saturating light intensities with thylakoids isolated from premature leaves, the rate of noncyclic electron flow was limited prior to the oxidation of plastohydroquinone by the Cyt f/b_6-containing complex. The rate-limiting step was not clearly identified.

(c) At saturating light intensities with thylakoids isolated from postmature leaves, the rate of electron flow appeared to be limited by the concentration of the Cyt f/b_6-containing complex, which in turn limited the rate of oxidation of plastohydroquinone.

(d) Our data suggest that the Cyt f/b_6-containing complex, and not PC or P_{700}, is the immediate recipient of electrons from reduced forms of the artificial donors DCPIP or DAD.

(e) Present concepts of the structural organization of components which generate and transfer electrons in thylakoids, hold that there are three independent complexes (PSII, PSI, the Cyt f/b_6-containing complex), which are linked by mobile electron carriers (PQ, PC) (1, 3). By showing no fixed stoichiometry among these components, as judged by both concentration and activity assays, our study agrees with this concept.

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