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Regulation of Photosynthetic Electron Transport and Photophosphorylation in Intact Chloroplasts and Leaves of *Spinacia oleracea* L.

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Abstract. Oxygen ist reduced by the electron transport chain of chloroplasts during CO_2 reduction. The rate of electron flow to oxygen is low. Since antimycin A inhibited CO_2-dependent oxygen evolution, it is concluded that cyclic photophosphorylation contributes ATP to photosynthesis in chloroplasts which cannot satisfy the ATP requirement of CO_2 reduction by electron flow to NADP and to oxygen. Inhibition of photosynthesis by antimycin A was more significant at high than at low light intensities suggesting that cyclic photophosphorylation contributes to photosynthesis particularly at high intensities. Cyclic electron flow in intact chloroplasts is under the control of electron acceptors. At low light intensities or under far-red illumination it is decreased by substrates which accept electrons from photosystem I such as oxaloacetate, nitrite or oxygen. Obviously, the cyclic electron transport pathway is sensitive to electron drainage. In the absence of electron acceptors, cyclic electron flow is supported by far-red illumination and inhibited by red light. The inhibition by light exciting photosystem II demonstrated that the cyclic electron transport pathway is accessible to electrons from photosystem II. Inhibition can be relieved by oxygen which appears to prevent over-reduction of electron carriers of the cyclic pathway and thus has an important regulatory function. The data show that cyclic electron transport is under delicate redox control. Inhibition is caused both by excessive oxidation and by over-reduction of electron carriers of the pathway.

Key words: Cyclic photophosphorylation – Electron transport – Light scattering – Photosystem I/II – Regulation of electron flow.

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Introduction

It is still a matter of debate, how much ATP is synthesized in photosynthesis, when two electrons are transferred from water to an acceptor molecule such as NADP. The corresponding number is called the ATP/2 e ratio. Most published values vary between 0,9 and 2 (Hall, 1976). Robinson and Wiskich (1976) observed recently ATP/2 e ratios approaching 2, while Chain and Arnon (1977) reported values close to 1. These data were obtained with broken chloroplasts which had lost the capability to photoreduce CO_2. In photosynthetically competent chloroplasts, calculated ATP/2 e ratios ranged between 1.1–1.4 (Heber and Kirk, 1975). CO_2 reduction needs somewhat more ATP than that. The ATP/2 e requirement of the Calvin cycle is 1.5. Intact chloroplasts can also photoreduce glycerate (Heber et al., 1974). Its reduction to dihydroxyacetone phosphate needs 2 molecules of ATP per 2 electrons, but since phosphoglycerate (PGA) is an intermediate which is easily lost from the chloroplasts by counterexchange with phosphate, the actual ATP/2 e requirement is higher than 2. It is obvious that, when the ATP requirement of a chloroplast reaction cannot be met by the ATP synthesized when 2 electrons travel from water to NADP, extra ATP must be produced by another photoreaction. We have shown previously (Egneus et al., 1975) that intact spinach chloroplasts can reduce oxygen to hydrogen peroxide during CO_2 reduction. During PGA reduction, no hydrogen peroxide formation was observed. As CO_2 reduction has a higher ATP requirement than PGA reduction (the ATP/2 e requirement is 1.0 for PGA reduction), we proposed that electron transport to oxygen supplied extra ATP needed for CO_2 reduction at least at low light intensities.

Similar results obtained with different types of chloroplasts and intact plants have later been

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presented by others (Huber and Edwards, 1975; Glidewell and Raven, 1975; Givan, 1976; Forti and Gerola, 1977; Jennings and Forti, 1975). The question of how much ATP can be supplied by electron transport to oxygen in intact chloroplasts during CO_2 reduction remained unanswered. The rate of oxygen uptake in the Mehler-reaction (Mehler, 1951) is slow in the absence of an added electron acceptor (Forti and Jagendorf, 1961).

In this paper we present evidence that in CO_2-reducing intact spinach chloroplasts which are not sufficiently well coupled to satisfy the ATP-demand by linear electron transport, cyclic electron transport takes part in ATP production. The affinity of electrons to different electron carriers and redox control (poising) regulate the distribution of electrons between NADP, oxygen and the primary acceptor of the cyclic electron transport pathway.

Materials and Methods

Intact chloroplasts capable of high rates of CO_2-dependent oxygen evolution were isolated from greenhouse – or field-grown spinach as described previously (Egneus et al., 1975; Heber, 1973). Preparations used in this investigation contained more than 80% (up to 98%) chloroplasts which had retained their envelopes during isolation as measured routinely by the ferricyanide method (Heber and Santarius, 1970). CO_2-dependent oxygen evolution was measured by a Clark electrode, the quenching of 9-aminoacridine fluorescence (concentration 5 μM) by a photomultiplier. Rates of CO_2-reduction ranged between 130 and 280 μmoles (mg chlorophyll)$^{-1}$ h^{-1}. Light source and filter arrangements were described by Tillberg, Giersch and Heber (1977). Chloroplast suspensions were made and kept anaerobic by adding 10 mM glucose and some glucose oxidase. H_2O_2 formed during glucose oxidation was decomposed by an excess of catalase (usually about 1,300 international enzyme units ml^{-1}). Transient oxygenation of anaerobic chloroplast suspensions was brought about by injecting known amounts of H_2O_2. Light absorption by chloroplasts was determined in an Ulbricht sphere. Scattering of a measuring beam of 535 nm light by leaves or isolated chloroplasts was measured usually in transmission (Heber, 1969), but occasionally also as 70° backscattering. Leaves were kept in a stream of CO_2-free air or nitrogen during the measurements. The flow rate was between 40 and 60 l h^{-1}. Mass spectrometric experiments were performed as described by Egneus et al. (1975).

Results

1. Electron Flow to Oxygen and Cyclic Electron Transport during CO_2 Reduction of Isolated Spinach Chloroplasts

Table 1 shows photosynthetic oxygen evolution during CO_2 reduction by intact chloroplasts and simultaneous oxygen uptake at different light intensities as measured in a mass spectrometer. Oxygen uptake during glycolate production in the ribulose bisphosphate oxygenase reaction was minimized by using a saturating bicarbonate concentration. Glycolate synthesis is competitively inhibited by CO_2. Most of the $^{18}O_2$ uptake measured is therefore caused by oxygen reduction by the electron transport chain which is coupled to ATP formation (Egneus et al., 1975). The results presented in Table 1 show that, as the light intensity is increased, the reduction of oxygen does not increase proportionally to the CO_2-dependent oxygen evolution, i.e. oxygen reduction during photosynthesis appears to be saturated at rather low light intensities. This suggests that at high light intensities electron transport to oxygen cannot contribute all the ATP needed for photosynthesis in insufficiently well-coupled chloroplasts. We wanted to know whether cyclic photophosphorylation also provides ATP for photosynthesis. As cyclic photophosphorylation is known to be sensitive to antimycin A (Tagawa et al., 1963;

Table 1. Oxygen exchange by intact spinach chloroplasts under different light intensities as measured with a mass spectrometer. Substrate: 2 or 4 mM HCO_3^-

Incident intensity of red light (Wm^{-2})	CO_2-dependent oxygen exchange, [in μmol (mg chl)$^{-1}$ h^{-1}]		% intact chloroplasts in preparation
	net O_2 evolution	O_2 uptake	
9	16	6	93
12	34	4	87
84	67	7	93
120	70	11	72

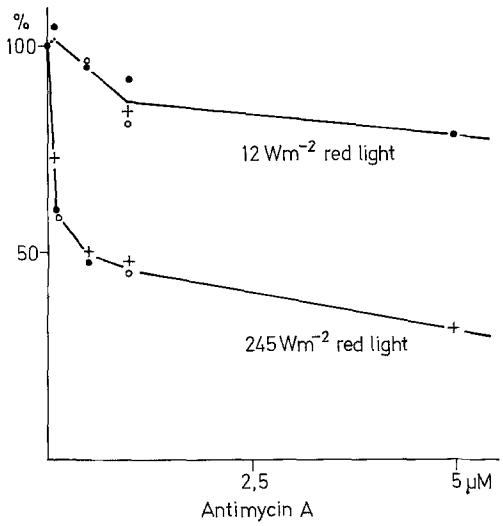

Fig. 1. CO_2-dependent oxygen evolution by intact chloroplasts as a function of the concentration of antimycin A. Photosynthetic rates are expressed as percent of control rates observed in the absence of antimycin A. In 3 different experiments, control rates at 245 Wm^{-2} were 131,210 and 258 μmol (mg chl)$^{-1}$h^{-1}, at 12 Wm^{-2} 17, 36 and 42 μmol (mg chl)$^{-1}$h^{-1}

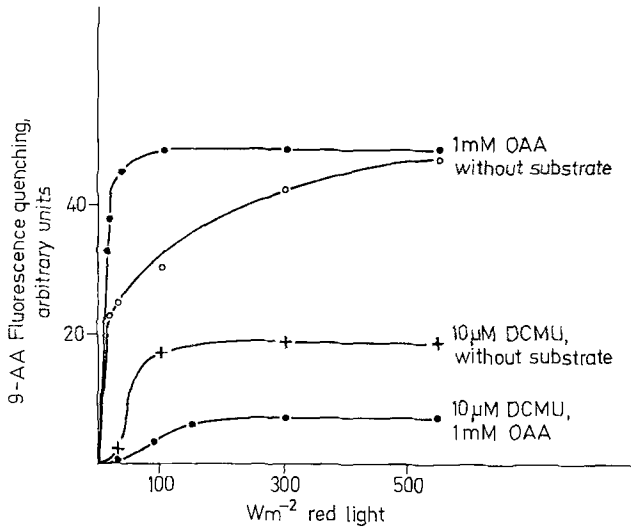

Fig. 2. Quenching of 9-aminoacridine fluorescence by intact spinach chloroplasts as a function of the intensity of a broad band of red light (half bandwidth from 630 to 800 nm). Oxygen was present at a concentration close to 0.28 mM. *DCMU* = 3-(3,4-dichlorophenyl)-1,1-dimethylurea; *OAA* = oxaloacetate

Schuermann et al., 1971), we measured the effect of antimycin A on CO_2 reduction. Results are presented in Figure 1. At a light intensity, which appeared sufficient to saturate electron transport to oxygen, CO_2-dependent oxygen evolution was much more inhibited than at a low light intensity. Even in the latter case, antimycin A caused significant inhibition of photosynthesis. The effect of antimycin A on photosynthesis suggests that cyclic electron transport participates in the synthesis of auxiliary ATP more at high than at low light intensities.

2. *Acceptor Control of Electron Flow in Chloroplasts*

Qualitatively, the occurence of cyclic electron flow can directly be demonstrated in intact chloroplasts. During coupled electron flow, protons are transferred from the stroma into the intrathylakoid space. A proton gradient is formed which can be used to indicate electron transport. Permeable amines with a suitable pK follow the proton gradient (Pick et al., 1975; Schuldiner et al., 1972). A permeable fluorescent amine is 9-aminoacridine, whose fluorescence is quenched, when it is trapped in the acidified intrathylakoid space. Figure 2 shows fluorescence quenching as an indicator of the magnitude of the proton gradient in intact chloroplasts at different light intensities. In the presence of oxygen as the sole electron acceptor (=without substrate; endogenous CO_2 and bicarbonate had been carefully removed) much more light was needed to saturate the proton gradient than in the presence of oxaloacetate, whose reduction to malate stimulates electron flow and, thereby, proton transport. The results support the conclusion drawn from Table 1, that oxygen is not a very efficient electron acceptor in intact chloroplasts. In the presence of the herbicide DCMU, linear electron transport is inhibited at a site close to photosystem II (Good and Izawa, 1973). As should be expected, no proton gradient is seen in the presence of DCMU under low light intensity illumination. Obviously under these conditions neither linear electron transport nor cyclic electron flow are possible to support the formation of a significant pH gradient. However, as the light intensity is increased, a proton gradient appears in a sigmoid response curve. DCMU inhibition is light-dependent. It decreases with increasing light intensity. As electron flow to oxygen would be expected to increase linearly with light intensity in DCMU inhibited chloroplasts, the sudden formation of a significant proton gradient suggests that cyclic electron flow became possible under the redox conditions produced by illumination with about 50 Wm^{-2} red light in the DCMU-inhibited chloroplasts. If this were correct, draining of electrons to an acceptor with a higher electron affinity than that of the acceptor of the cyclic pathway should decrease the proton gradient, as the cyclic pathway would be expected to be depleted of electrons. Indeed, when oxaloacetate (OAA) was added to the DCMU-inhibited chloroplasts, the proton gradient decreased. This is in striking contrast to the stimulation of the proton gradient by oxaloacetate in the absence of DCMU.

The effect of different concentrations of DCMU on the sigmoid response curve of 9-aminoacridine fluorescence quenching to light is shown in Figure 3. There is a shift toward higher light intensities as the DCMU concentration is increased and linear electron transport consequently decreased. Also, the maximum level of fluorescence quenching seen in the absence of DCMU with oxygen as the only electron acceptor present is not reached in the presence of DCMU. This indicates that the rate of cyclic electron flow which supports the proton gradient in DCMU-poisoned chloroplasts is low even under high intensity illumination.

The sigmoid response curve of 9-aminoacridine fluorescence quenching by intact DCMU-poisoned chloroplasts seen in Figures 2 and 3 is not observed in chloroplasts, which had been osmotically shocked before the experiment. In such chloroplasts, even high-intensity illumination does not lead to the formation of a significant proton gradient when DCMU is present.

Figure 4 shows data from a simultaneous recording of 9-aminoacridine fluorescence quenching and

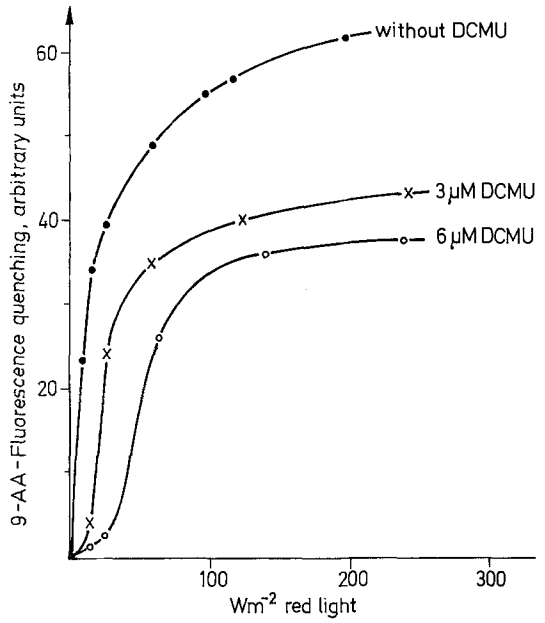

Fig. 3. Quenching at 9-aminoacridine fluorescence by intact chloroplasts as a function of the intensity of a broad band of red light (half bandwidth from 630 to 800 nm). Oxygen was present at a concentration close to 0.28 mM. Substrate: 2 mM HCO_3^-

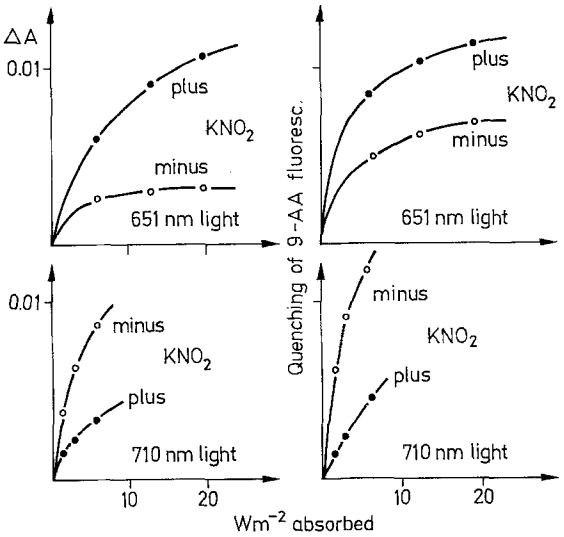

Fig. 4. Changes in light scattering at 535 nm (left part of figure) and the quenching of 9-aminoacridine fluorescence (right part) by intact chloroplasts under illumination with red (651 nm) and far-red (710 nm) light. The oxygen concentration was 0.28 mM, the nitrite concentration 1 mM. Note stimulation of light scattering and fluorescence quenching by nitrite under red and inhibition under far-red light

scattering of 535 nm light by intact chloroplasts illuminated with short-wavelength red or far-red light. As shown by Krause (1973), under certain conditions light sctattering is a useful indicator of the proton gradient. In order to compare the effects caused by red and far-red light, the chloroplast response was plotted against absorbed rather than incident light. In 651 nm light, the fluorescence quenching and the scattering response was smaller with oxygen as electron acceptor than with nitrite (KNO_2). KNO_2, which is reduced to NH_3 in intact chloroplast, stimulates electron flow from water through both photosystems. The effect of nitrite on the proton gradient is similar to the effect of oxaloacetate which is shown in Figure 2 (upper curve). Under 710 nm illumination, however, nitrite did not stimulate, but inhibited both the scattering response and 9-aminoacridine fluorescence quenching. 710 nm light excites predominantly photosystem I. Linear electron transport from water to oxygen is very slow under 710 nm illumination. It therefore appears that the proton gradient supported by far-red light is mainly caused by cyclic electron transport. The inhibitory effect of nitrite on the proton gradient under far-red light is explained by the interception of electrons by this effective electron acceptor, which depletes the cyclic pathway of electrons. The situation is similar to that described in Fig. 2 for DCMU-poisoned chloroplasts, which were illuminated with red light. In this case, oxaloacetate was used to drain electrons from the cyclic pathway. Oxaloacetate and nitrite are interchangeable as electron acceptors.

3. Redox Control of Electron Flow in Chloroplasts

In Figure 5 the formation of the proton gradient as revealed by 9-aminoacridine fluorescence quenching is compared under anaerobic and under aerobic conditions. In the absence of oxygen, short-wavelength red light did not produce a significant proton gradient. Obviously, little cyclic electron transport was possible under these conditions. After oxygen was admitted, illumination with red light caused the formation of a large proton gradient. As the oxygen concentration was decreased, the proton gradient decreased also (Fig. 5A) until only a small signal was left under anaerobic conditions. This could be repeated several times. In contrast to red light, far-red light was moderately effective in supporting a proton gradient under nitrogen. High concentrations of oxygen usually decreased the proton gradient produced by far-red light. As the oxygen concentration was lowered, the proton gradient increased (Fig. 5B). A maximum was seen at very low oxygen concentrations. Under completely anaerobic conditions, the proton gradient decreased again to some extent, when the intensity of far-red light was high.

The very small proton gradient caused by red illumination in nitrogen was increased dramatically in

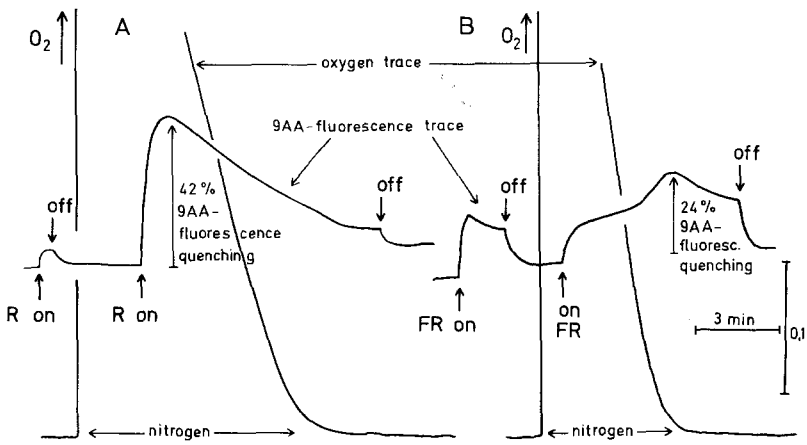

Fig. 5. Quenching of 9-aminoacridine fluorescence by intact chloroplasts (24 μg chlorophyll ml^{-1}) under illumination with 40 Wm^{-2}red (*A*) and far-red (*B*) light in the presence of nitrogen and oxygen. The oxygen concentration is recorded polarographically. Percent values indicate how much of the total 9-aminoacridine fluorescence was quenched. The red light (*R*) had a half bandwidth from 618 to 670 nm, the far-red (FR) light from 710 to 720 nm

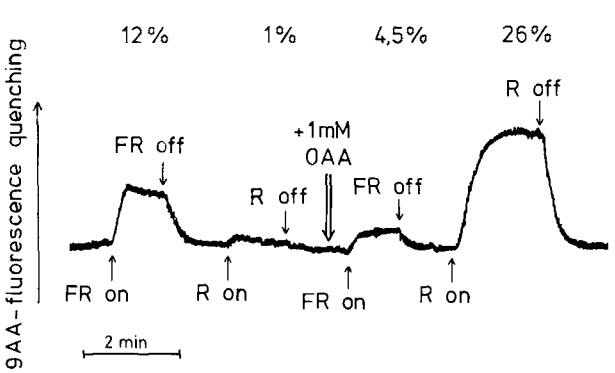

Fig. 6. Quenching of 9-aminoacridine fluorescence by intact chloroplasts (11 μg chlorophyll ml^{-1}) illuminated with 100 Wm^{-2} red (*R*) or far-red (*FR*) light (half bandwidth from 633 to 673 nm or from 710 to 729 nm) before and after the addition of 1 mM oxaloacetate (*OAA*). The chloroplast suspension was anaerobic. Percent values indicate how much of the total 9-aminoacridine fluorescence was quenched

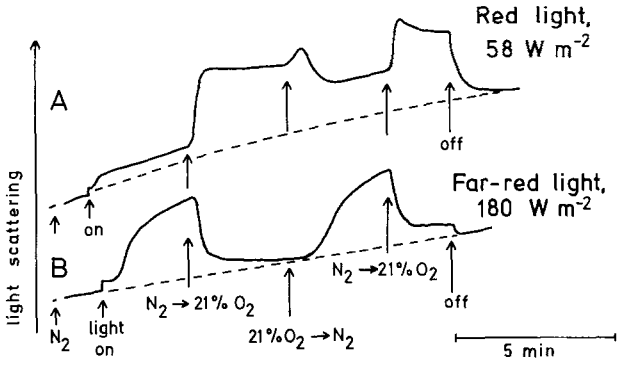

Fig. 7. Back scattering of a beam of low-intensity green light by a spinach leaf under nitrogen and in the presence of oxygen as influenced by red and far-red illumination. Note that the transition from 21% O_2 to nitrogen and vice versa was slow. About 3 min were required by a stream of nitrogen to remove oxygen completely from a cuvette holding the leaf in CO_2-free air. The red light had a half bandwidth from 618 to 670 nm, the far-red light from 695 to 805 nm

the presence of an electron acceptor such as oxaloacetate (Fig. 4) or nitrite, while that caused by far-red light was decreased (see also Fig. 4).

The data show that red light inhibits cyclic electron flow under anaerobic conditions. Linear electron flow is not possible when no electron acceptor is present. The inhibion of cyclic electron flow is not seen under far-red light. Still, the rate of cyclic electron transport produced by far-red apparently is not high. This is shown by the fact that the proton gradient produced by far-red is smaller than that established during linear electron transport to an acceptor such as oxaloacetate (Fig. 6). The observation that even a very low concentration of oxygen is sufficient to relieve th inhibition of electron transport by red light shows that oxygen plays an important role in regulating electron flow. Even though electron flow to oxygen is slow, it appears to be sufficient to prevent over-reduction of electron carriers of the electron transport chain which causes inhibition of cyclic electron flow under red light, when no electron acceptor is present.

4. *Control of Electron Flow in Leaves*

9-aminoacridine cannot be used to monitor the formation of a transthylakoid proton gradient in leaves. However, as shown in Fig. 4, light scattering is a useful indicator of the proton gradient (see also Krause et al., 1977). Figure 7 shows measurements of light scattering by a spinach leaf. When the leaf was gassed with a stream of nitrogen and far-red was turned on, scattering increased indicating formation of a proton gradient by photosystem I-dependent cyclic electron flow (Fig. 7B). On admission of oxygen, the scattering signal collapsed, apparently because oxygen drained electrons from the cyclic pathway. The situation is similar to that described in the experi-

ments of Figures 4 and 6, where far-red supported cyclic electron flow was inhibited by nitrite or oxaloacetate. Interestingly, in intact chloroplasts excited by far-red light oxygen did not inhibit cyclic electron flow as much as it did in leaves (Fig. 5). It appears that oxygen is a better interceptor of electrons in intact leaves than in intact chloroplasts (Krause and Heber, 1971). The reason for this discrepancy is not known. The inhibitory effect of oxygen on cyclic electron flow in far-red light was observed in all plant species investigated, including C_4 species such as *Amaranthus caudatus* and *Sorghum halepense.*

It has been suggested that stroma lamellae of chloroplasts contain only photosystem I which supports cyclic electron transport (Park and Sane, 1971). The results of Figure 7 clearly show that if photosystem I is not connected to an electron donor system, cyclic electron flow cannot function in the presence of oxygen which appears to oxidize carriers of the cyclic pathway. The donor system supplying electrons to photosystem I is photosystem II. This is shown in the experiment of Figure 7A. Illumination of a leaf kept under a stream of nitrogen with red light which excites both photosystem II and I did not produce a significant scattering response indicating formation of only a small proton gradient. In some experiments with *Hydrangea hortensia,* a transient scattering signal was observed when a leaf kept under anaerobiosis was illuminated with a moderate or high intensity of short-wavelength red light (not shown). The initial increase in scattering was in these cases soon followed by a decrease. However, as soon as oxygen was admitted to the leaf system, the formation of a large proton gradient was indicated by a large increase in light scattering (Fig. 7A). Oxygen appeared to act as an electron acceptor. It also facilitated cyclic electron transport. When oxygen was replaced by nitrogen, scattering decreased again after a transient increase, which was seen at low oxygen concentrations. Lower scattering levels at higher oxygen concentrations are caused by energy-consuming photorespiratory reactions (Krause et al., 1978).

The inhibition of light scattering in short-wavelength red light under anaerobic conditions indicates that the two photosystems of the electron transport chain interact, and that electrons from photosystem II reduce carriers of the cyclic electron transport pathway. Cyclic electron flow requires partially oxidized electron acceptors. Inhibition of cyclic electron flow will result, if acceptors become over-reduced. This inhibition was investigated more closely (Fig. 8). Photosystem I was excited under anaerobic conditions by far-red light. A proton gradient supported by cyclic electron transport was formed. The leaf was then irradiated with a second beam of red light. At

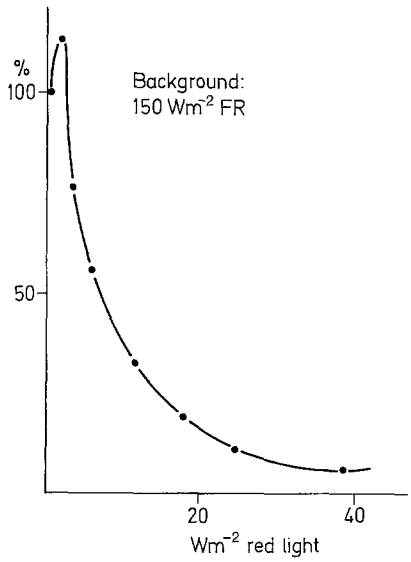

Fig. 8. Inhibition of light scattering by a spinach leaf which was illuminated with far-red light (*FR*) under nitrogen, by short-wavelenght red light. Light quality as in Figure 7

low intensities of red light the proton gradient increased. Intensities above 5 Wm^{-2} inhibited cyclic electron flow, and the steady state proton gradient decreased. Complete inhibition due to electron transport from photosystem II was not observed even at high light intensities. When oxygen was admitted to a leaf system, in which far-red supported cyclic electron flow was inhibited by a moderate intensity of red light, light scattering increased dramatically. Oxygen released the over-reduction of the electron transport chain, which caused inhibition of cyclic electron flow. A relevant light scattering experiment is shown in Figure 9. Red light could not induce a significant proton gradient in a spinach leaf gassed with nitrogen because over-reduction of the electron transport chain inhibited cyclic electron flow (A). Addition of a far-red beam to the red light did not change the situation much. When far-red was given first (B), cyclic electron transport was possible under nitrogen. It was inhibited by the subsequent addition of red light. Inhibition was relieved, after the red light was turned off. In contrast to the situation seen under anaerobiosis, red light supported formation of a proton gradient in the presence of 21% oxygen (C). Addition of a second beam containing far-red light increased the proton gradient, apparently by increasing the rate of cyclic electron flow. The proton gradient decreased again when far-red was turned off. Far-red alone was quite ineffective in the presence of oxygen to support a proton gradient (D). A large proton gradient could be formed only after red light was added. It collapsed after the red light was turned off. This experiment is a striking demonstration that a special redox condi-

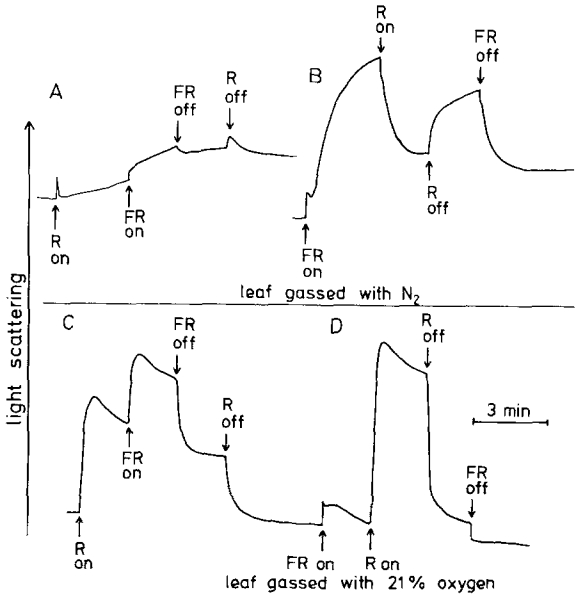

Fig. 9A–D. Light scattering by a spinach leaf produced by red (*R*) and far-red (*FR*) illumination given either alone or together under a stream of nitrogen or under 21% oxygen. Light scattering was measured in transmission. Fast responses seen immediately after turnung the light on are caused by absorption changes of the leaf and not by light scattering. The intensities of the red and far-red beams were 55 and 170 Wm^{-2}, respectively. The half bandwidths ranged from 622 to 677 (red) and from 688 to 734 nm (far-red). **A** and **C**: Red light was given first and far-red was superimposed on red. **B** and **D**: The above order of light additions was reversed

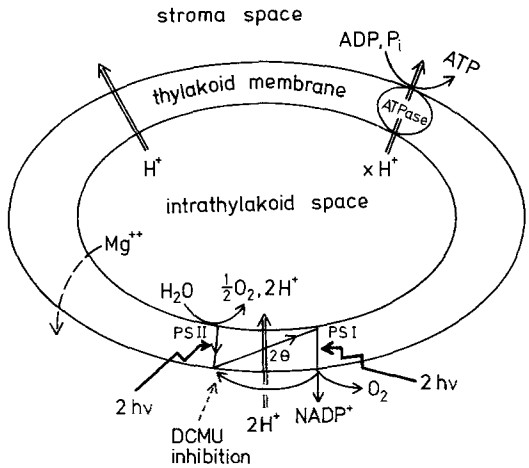

Fig. 10. Scheme showing the sequential arrangement of 2 photosystems (PS II and PS I) and vectorial electron and proton transport across the thylakoid membrane. Proton influx during gradient formation is thought to be electrically compensated by Mg^{2+} efflux (Krause, 1977). The proton gradient is assumed to be involved in phosphorylation (Mitchell, 1966). There is also a slow proton loss due to leakage

tion of the electron transport chain is required for cyclic electron flow. Electrons lost to oxygen must be resupplied by photosystem II in order to enable cyclic electron flow to occur.

Discussion

The results of this work can best be explained within the framework of the scheme shown in Figure 10. The transthylakoid proton gradient seen in light scattering and 9-aminoacridine fluorescence experiments is formed by light-dependent vectorial electron transport (Trebst, 1974). Two photosystems are arranged in sequence. Electrons can be donated by the electron transport chain to NADP, to oxygen or into the cyclic pathway. As long as NADP is available in the oxidized form, it traps electrons and thereby inhibits oxygen reduction or cyclic electron transport. When NADP becomes reduced, electrons are diverted to oxygen and into the cyclic pathway. The rate of cyclic electron transport is a function of the electron "pressure" between the two photosystems, of the excitation of photosystem I, and of the oxidation state of carriers on the acceptor side of photosystem I. In the absence of oxygen, the cyclic pathway becomes inhibited by over-reduction under red illumination. This shows that it is linked to photosystem II. When oxygen is added, it drains electrons from the cyclic pathway relieving inhibition by over-reduction. When photosystem II is not excited, oxygen causes inhibition of cyclic electron flow by oxidizing electron carriers. Obviously, cyclic electron transport chains must be linked to an electron donor system such as photosystem II to be functional in the presence of oxygen.

Why do plants such as spinach have the possibility to synthesize ATP in a cyclic pathway? If the coupling ratio of their electron transport chain (ATP/2 e ratio) would be higher than 1.5 as suggested by some measurements (Hall, 1976; Robinson and Wiskich, 1976) sufficient ATP would be avaible for CO_2 reduction and neither electron flow to oxygen nor cyclic electron flow would be necessary. NADP is a highly efficient electron sink and would utilize the total electron flow for CO_2-reduction. Only 8 light quanta should be required for the transfer of 4 electrons necessary for the reduction of a CO_2 molecule from water through 2 photosystems. In fact, the quantum requirement of CO_2 reduction is usually higher (Egneus et al., 1975). In vitro, the ATP/2 e ratio can easily be

lowered by partial uncoupling. It is significant that even in isolated intact chloroplasts having ATP/2 e ratios well below 1.5 the photoreduction of CO_2 can be observed at high rates. Obviously, these chloroplasts can synthesize extra ATP. In vivo, the exposure of plants to stress situations is a very common condition. It is known that freezing stress, drought stress, heat stress and salt stress cause uncoupling of phosphorylation from electron flow (Santarius, 1969; Heber and Santarius, 1976; Emmet and Walker, 1973). It is thus possible that ATP/2 e ratios vary even in vivo. Leaves of C_4 plants, which are believed to require 2.5 mol of ATP per mole of NADPH for photosynthesis, should always be expected to need additional ATP, as the very best experimentally observed ATP/2 e ratios barely approached values of 2 (Hall, 1976; Robinson and Wiskich, 1976). Electron transport to oxygen and cyclic electron transport cooperate to supply extra ATP at the least expenditure of light quanta. This cooperation avoids over-reduction of the electron transport chain which would lead to the collapse of photosynthesis. The problem is exemplified as follows: when in a C_3 plant less than 1.5 molecules of ATP are synthesized during reduction of 1 molecule of NADP, continuous oxidation of the NADPH by the Calvin cycle is not possible owing to ATP shortage. NADPH will accumulate. Such accumulation has indeed been seen in isolated chloroplasts during CO_2 reduction (Heber, 1973). Unavailability of NADP as electron acceptor must, in the absence of another electron sink, cause over-reduction of the electron transport chain under high intensity red light. This inhibits cyclic electron flow, as has been shown in Figures 5 to 9 for intact chloroplasts and leaves. Electron drainage to oxygen plays the role of the regulation device necessary for preventing over-reduction. One function of oxygen appears to be to "poise" electron carriers of the cyclic pathway. The importance of "poising" has been previously emphasized by Arnon's group (Chain and Arnon, 1977; Schuermann et al., 1971). On the other hand, electron flow to oxygen itself is a coupled process and leads to ATP synthesis (Egneus et al., 1975; Forti and Jagendorf, 1961). At low hight intensities and a slow rate of CO_2 reduction this electron transport appears to be largely sufficient to supply the extra ATP needed for CO_2 reduction (Fig. 1; cf. Egneus et al., 1975). At higher rates of photosynthesis, oxygen reduction is too slow at least in isolated spinach chloroplasts to satisfy the ATP demand. In contrast, in intact cells of *Anacystis* high rates of oxygen reduction have been observed (Patterson and Myers, 1973). When NADP is reduced and the electron flow to oxygen is largely saturated, electrons will spill over into the cyclic pathway, as the experiment of Figure 1 shows. Cyclic phosphorylation then contributes to the ATP needed for photosynthesis and other processes. Other recent work has also shown that cyclic electron transport can be important for supplying ATP to photosynthetic reactions (Arnon and Chain, 1975; Kaiser and Urbach, 1976: Slovacek and Hind, 1977).

Two points require brief comment. Our conclusion that cyclic photophosphorylation contributes ATP to photosynthesis mainly at moderate and high light intensities is in conflict with many of the early reports according to which cyclic photophosphorylation saturates at low light intensities (Simonis and Urbach, 1973; Gimmler, 1977). This discrepancy is due to mainly two factors. Under the experimental conditions often used to measure cyclic photophosphorylation (e.g. nitrogen atmosphere, red or white light), the cyclic pathway becomes over-reduced and electron transport is inhibited as the light intensity is increased (Figs. 8 and 9). Also, since cyclic electron flow cannot be measured directly, indicator reactions such as phosphate uptake had to be used to indicate photophosphorylation in intact cells (Simonis and Urbach, 1973; Gimmler, 1977). This introduced rate limitations different from those governing the turnover of electrons in the cyclic electron transport pathway.

The other point is that antimycin A has been found by us to inhibit photosynthesis. There are, however, reports according to which antimycin A stimulates CO_2 reduction by isolated chloroplasts (Schacter et al., 1971; Schacter and Bassham, 1972; Miginiac-Maslow and Champigny, 1974; Miginiac-Maslow and Hoarau, 1975). In these investigations the rate of CO_2 reduction was low compared to our rates. We have never observed stimulation of photosynthesis by antimycin A under optimal conditions and conclude that, when stimulation occurs, it is due to the removal of a rate-limiting step which is not present in chloroplasts reducing CO_2 at rates comparable to photosynthetic rates in vivo.

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