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An active Mehler-peroxidase reaction sequence can prevent cyclic PS I electron transport in the presence of dioxygen in intact spinach chloroplasts

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Abstract

Simultaneous measurements of 9-aminoacridine (9-AA) fluorescence quenching, O_2-uptake and chlorophyll fluorescence of intact spinach chloroplasts were carried out to assess the relationship between the transthylakoidal ΔpH and linear electron flux passing through Photosystem II. Three different types of O_2-dependent electron flow were investigated: (1) Catalysed by methyl viologen; (2) in the absence of a catalyst and presence of an active ascorbate peroxidase (Mehler-peroxidase reaction); (3) in the absence of a catalyst and with the ascorbate peroxidase being inhibited by KCN (Mehler reaction). The aim of this study was to assess the relative contribution of ΔpH-formation which is not associated with electron flow through Photosystem II and, which should reflect Photosystem I cyclic flow under the different conditions. The relationship between the extent of 9-AA fluorescence quenching and O_2-uptake rate was found to be almost linear when methyl viologen was present. In the absence of methyl viologen (Mehler reaction) an increase of 9-AA fluorescence quenching to a value of 20% at low light intensities was associated with considerably less O_2-uptake than in the presence of methyl viologen, indicating the involvement of cyclic flow. These findings are in agreement with a preceding study of Kobayashi and Heber (1994). However, when no KCN was added, such that the complete Mehler-peroxidase reaction sequence was operative, the relationship between 9-AA fluorescence quenching and the flux through PS II, as measured via the chlorophyll fluorescence parameter $\Delta F/Fm' \times PAR$, was identical to that observed in the presence of methyl viologen. Under the assumption that methyl viologen prevents cyclic flow, it is concluded that there is no significant contribution of cyclic electron flow to ΔpH-generation in intact spinach chloroplasts.

Abbreviations: AsA – ascorbate; 9-AA – 9-aminoacridine; DCMU, – 3-(3,4-dichlorophenyl)-1,1-dimethylurea; MDA – monodehydroascorbate; MV – methyl viologen; PAR – photosynthetically active radiation

Introduction

Whenever environmental stress causes limitation in Calvin cycle activity, excessive light bears the risk of photodamage, in particular at the PS II reaction centre level when the acceptor side becomes strongly reduced (see reviews by Demmig-Adams 1990; Foyer et al. 1990; Walker 1992). In recent years it has become clear that the transthylakoidal ΔpH plays a decisive role in the protection of the photosynthetic apparatus from such photodamage (Krause and Behrend 1986). Much effort has been devoted to the elucidation of the mechanisms by which the ΔpH provides protection. There is general agreement that the turnover of PS II is lowered with respect to that of PS I, whereas it is still not settled whether this 'down-regulation' of PS II is based on increased heat-dissipation at the reaction centres (Weis and Berry 1987; Schreiber and Neubauer 1987, 1990; Krieger et al. 1992) or at the antenna level, with the participation of zeaxanthin as dissipative trap

(Demmig et al. 1988; Demmig-Adams 1990). Alternatively, also a mechanism linked to the aggregation of LHCP II has been proposed (Horton et al. 1992). Another question, which is of primary importance in connection with the suggested protection against photodamage, relates to the mechanism by which the regulatory ΔpH is created. In a series of publications, we have shown that O_2-dependent electron flow has the potential of creating a large ΔpH which induces strong energy-dependent quenching of chlorophyll fluorescence, indicative of increased heat-dissipation and PS II down-regulation (Neubauer and Schreiber 1989; Schreiber and Neubauer 1990; Schreiber et al. 1991; Neubauer and Yamamoto 1992; Hormann et al. 1993; Neubauer 1993). On the other hand, arguments have been put forward, that cyclic electron flow at PS I is mainly responsible for ΔpH-formation when assimilatory electron flow becomes limited (Wu et al. 1991; Heber et al. 1992; Katona et al. 1992; Heber and Walker 1992, Kobayashi and Heber 1994). Also in the latter work a decisive role of molecular oxygen has been recognized, but with O_2 reduction only being considered to 'poise' the redox state of the electron transport chain, such that 'over-reduction' is prevented and cyclic PS I flux can become effective. Hence, the question has to be settled, how large the 'poising' O_2 reducing flux is with respect to cyclic flux and what are the relative contributions of these two types of electron flow to the overall ΔpH formed.

In the present investigation we have attempted to provide an answer to this question for isolated, intact spinach chloroplasts by combined measurements of O_2-dependent flux, assessed by polarographic O_2-uptake, and chlorophyll fluorescence quenching analysis (Schreiber et al. 1986; Genty et al. 1989), and of the transthylakoidal ΔpH via 9-aminoacridine (9-AA) fluorescence quenching (Schuldiner et al. 1972; Tillberg et al. 1977; Kobayashi and Heber 1994). In these experiments the integrity of the ascorbate peroxidase scavenging system of the chloroplasts was essential. Only recently it has been fully realized that under in vivo conditions the Mehler reaction (Mehler 1951) (light-driven O_2-reduction to superoxide and consequent dark-dismutation to H_2O_2 and O_2) and the ascorbate peroxidase reaction (Nakano and Asada 1981) (ascorbate oxidation by H_2O_2 forming monodehydroascorbate) are closely linked, as not only the peroxidase but also the site of light-driven monodehydroascorbate reduction are membrane-bound, presumably in the vicinity of PS I where O_2 is reduced (Neubauer and Schreiber 1989; Miyake and Asada 1992; Hormann et al. 1993). Very recently Kobayashi and Heber (1994) assessed proton transport coupled to cyclic electron flow in the presence of KCN by comparative measurements of 9-AA fluorescence quenching in the presence and absence of methyl viologen, based on the rationale that there is no cyclic flow in the presence of this catalyst. In the present communication it will be shown that the monodehydroascorbate, which is formed by ascorbate peroxidase activity in the absence of KCN, can be as effective as methyl viologen in preventing ΔpH formation by cyclic electron flow.

Materials and methods

Intact chloroplasts were isolated from freshly harvested, green-house grown spinach essentially as previously described (Asada et al. 1990). To ensure a high degree of intactness, the isolation procedure included a step of centrifugation through a layer of 40% (w/v) Percoll (Sigma). In order to obtain highly active ascorbate peroxidase, 5 mM ascorbate was present in all isolation media. Chloroplast intactness was higher than 90%, as judged by the ferricyanide method (Heber and Santarius 1970). The reaction medium was supplemented with 15 mM ascorbate for measurements of the Mehler-peroxidase reaction and the pH was adjusted to pH 8. Experiments were carried out at 20 °C and at 30 μg Chl·ml^{-1}. When KCN was used for inhibition of the ascorbate peroxidase, samples were preincubated for 10 min to assure a saturating effect, as controlled by testing for fluorescence quenching by 0.1 mM H_2O_2 (Neubauer and Schreiber 1989).

9-aminoacridine fluorescence was measured with a laboratory-built pulse modulation fluorometer based on 1 μsec xenon-flashes applied at a frequency of 16 Hz (Schreiber et al. 1993). The excitation pathway contained a broadband interference filter passing between 370 and 440 nm (Type K1, Balzers, Liechtenstein). The photodiode detector was protected by a combination of 3 mm BG 39 (Schott, Mainz, FRG) and Kodak gelatine filter type Wratten 58. The 9-AA concentration was 2 μM, by which the chloroplasts were not yet uncoupled. 9-AA fluorescence quenching was calculated from the ratio ΔF/F and expressed in percent.

Chlorophyll fluorescence was measured with a PAM-101 Chlorophyll Fluorometer including the accessory modules PAM-102 and 103 for actinic light control and saturation pulse control, respectively (Walz, Effeltrich, FRG). Red actinic light up to

50 μmol quanta $m^{-2}s^{-1}$ was obtained from an LED-source (102-L, Walz) with an emission peak at 655 nm. For intensities up to 300 μmol quanta $m^{-2}s^{-1}$ a laboratory-built fiberilluminator based on seven H-3000 LEDs (Stanley) was used. Still higher intensities were obtained with a halogen lamp fiberilluminator (Type KL 1500, Schott, FRG) equipped with a red dichroic filter (Type R61, Balzers) and a short pass filter (Type DT Cyan special, Balzers), passing light below 690 nm. Photosynthetically active radiation (PAR) was measured as incident photon flux density in μmol quanta $m^{-2}s^{-1}$ with a micro quantum sensor (LI-COR, Model LI-189, USA). Pulses of saturating white light were applied with the help of a relais-controlled fiberilluminator (F1 103, triggered by PAM-103, Walz).

Simultaneously with 9-AA fluorescence, electron transport was measured either polarographically or via chlorophyll fluorescence quenching analysis. A miniature O_2-electrode (Pt/AgCl, Bachofer, FRG) was used reaching from the top of a standard 10 × 10 mm quarz cuvette into the stirred suspension. The O_2-concentration signal was electronically differentiated to obtain the uptake rate. The assembly with light-guiding quartz rods and fiberoptics was essentially as reported before (Schreiber et al. 1993).

Fluorescence quenching analysis by the saturation pulse method was carried out as described by Schreiber et al. (1986). The fluorescence nomenclature proposed by van Kooten and Snel (1990) was applied. The effective Photosystem II quantum yield was determined under steady-state conditions from the parameter $\Delta F/Fm'$ according to Genty et al. (1989). The applicability of this parameter for assessment of electron flow in isolated chloroplasts was recently confirmed by Hormann et al. (1994).

Results and discussion

Figure 1 shows the light-intensity dependence of 9-AA quenching for intact chloroplasts under three different conditions of O_2-dependent electron flow. In the presence of the catalyst methyl viologen (MV-reaction), the relationship between 9-AA fluorescence quenching and light intensity is characterized by a steep rise curve, showing early saturation around 400 μmol quanta $m^{-2}s^{-1}$. On the other hand, in the absence of this catalyst, when the Mehler-peroxidase reaction is effective (MP-reaction), there are two well separated phases, with an initial, steep rise to approxi-

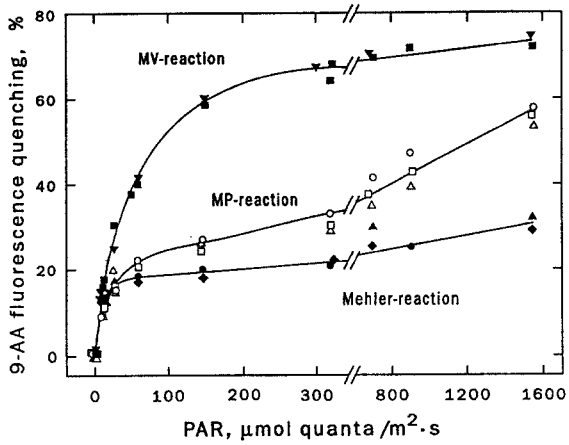

Fig. 1. Light intensity dependence of 9-AA fluorescence quenching in intact spinach chloroplasts. Three different conditions of O_2-dependent electron flow were studied: Catalysed by 0.4 mM methyl viologen (MV-reaction); in the absence of an artificial acceptor and with the ascorbate peroxidase being active, i.e. Mehler reaction combined with ascorbate peroxidase and monodehydroascorbate reduction (MP-reaction); in the absence of an artificial acceptor and the ascorbate peroxidase being inhibited by 3 mM KCN (Mehler reaction). In all cases, 3 mM glycolaldehyde was present to prevent ribulosebisphosphate carboxylase/oxygenase activity. The different symbols relate to independent measurements performed on different days. Measurements were done 1 min after the establishment of a given photon flux density of red light, indicated in units of photosynthetically active radiation, PAR. In the case of MV-reaction and Mehler-reaction the same sample was used for measurement of one set of data, whereas for the MP-reaction each data point corresponds to a fresh sample. For further conditions see 'Materials and methods'.

mately 20% quenching at 30 μmol quanta $m^{-2}s^{-1}$ and a relatively flat further rise, which does not saturate up to 1600 μmol quanta $m^{-2}s^{-1}$. When the peroxidase reaction is prevented by KCN, such that only O_2 reduction is allowed (Mehler reaction), the second rise phase is mostly suppressed. Removal of O_2 by the glucose/glucose oxidase trap with the given preparation of intact chloroplasts led to full suppression of 9-AA quenching (data not shown). Therefore, reduction of internal substrates such as nitrite or oxaloacetate does not appear to contribute to ΔpH-formation during steady-state illumination.

These data confirm earlier findings, in which neutral red was used to monitor lumen acidification (Neubauer and Yamamoto 1992; Neubauer 1993), which indicated that a functional ascorbate peroxidase can cause a substantial increase of the ΔpH beyond that observed when only O_2 reduction takes place. This is in agreement with our previous suggestion that the light-driven reduction of H_2O_2 (via monodehydroascorbate) is mainly responsible for the

energy-dependent quenching of chlorophyll fluorescence (Schreiber et al. 1991). Relatively high photon flux densities are required for stimulation of 9-AA quenching beyond the 20% already observed with the Mehler reaction alone. This can be understood on the basis of the fact, that consecutive reactions are involved (O_2 reduction, H_2O_2 reduction, monodehydroascorbate reduction). The overall rate is limited by the slowest partial reaction which in this case is O_2 reduction. The relative stimulation of ΔpH formation by the Mehler-peroxidase reaction at high light-intensities with respect to that by the Mehler reaction alone may reflect the autocatalytic stimulation of the Mehler-peroxidase reaction by the ΔpH which it is forming (Schreiber et al. 1991; Hormann et al. 1993). In the cited work it has been shown that O_2 reduction is favored by low pH, with an optimum around pH 5. This has been explained by superoxide formation and protonation within the thylakoid membrane, the latter being favored by acidification of the thylakoid lumen. An argument put forward in favor of this explanation has been the unusual response of the Mehler-peroxidase-dependent flux towards uncoupling by the protonophore nigericin, which causes inhibition instead of the stimulation of flux found in presence of methyl viologen (Hormann et al. 1993).

For a full evaluation of this argument it should be considered that the fluorescence parameter ΔF/Fm′, which is taken as an indicator of the effective PS II quantum yield (Genty et al. 1989), is composed of two factors (ΔF/Fm′ = qp × Fv′/Fm′), which may be influenced in different ways by the uncoupling: First, the quantum capture efficiency of open PS II centres will be increased, as reflected by an increase in Fv′/Fm′ (Kitajima and Butler 1975; Genty et al. 1989), which is equivalent to a rapid rise in light intensity causing an increase in the reduction rate of PS II acceptors. Second, the potential rate of electron transfer from PS II to PS I is speeded up as the half-time of the limiting step between plastoquinone to cyt b/f is lowered. While the first change will tend to decrease the extent of PS II centre 'openness', reflected by a decrease of the photochemical quenching coefficient, qp, the second change will cause an opposing effect. As the second effect should be most pronounced at high rates, when the turnover of cyt b/f becomes limiting, it could be argued that a flux stimulation by nigericin is characteristic only for electron transport involving an efficient acceptor, like methyl viologen, whereas with any slow acceptor a relative inhibition of flux might be the rule rather than the exception. This argument,

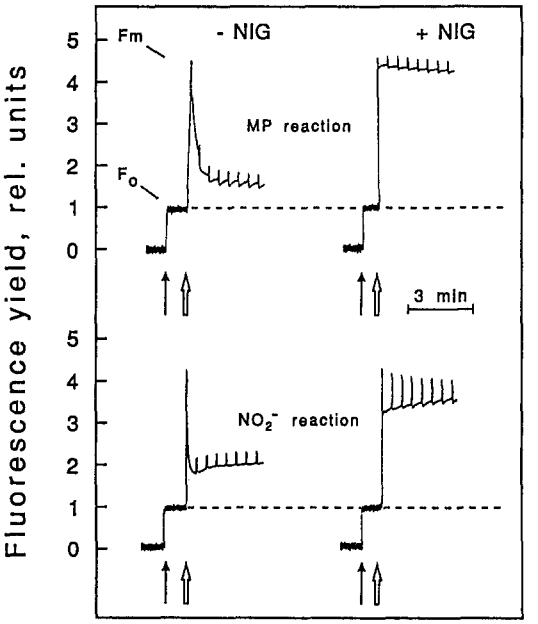

Fig. 2. Assessment of the effect of uncoupling by nigericin on the effective quantum yield of intact chloroplasts showing similar coupled fluxes of the Mehler-peroxidase reaction and of nitrite-dependent electron flow as determined by chlorophyll fluorescence quenching analysis. Calvin cycle was inhibited by 3 mM glycolaldehyde. Nitrite-concentration 50 μM. Nigericin, where present, 0.5 μM. In the nitrite experiment, the sample was made anaerobic by βD-glucose/glucose oxidase (3 mM/ 30 units per ml) and catalase (3000 units /ml). Light intensity, 200 μmol quanta m^{-2} s^{-1}. The effective quantum yield, ΔF/Fm′ (see text), was determined with the fourth saturation pulse applied after onset of continuous illumination. Determination of ΔF = (Fm′-F) was based on the fluorescence yield, F, observed before saturation pulse application.

however, can be refuted by the experiment of Fig. 2. The rationale of this experiment is as follows: Fluorescence quenching is measured with intact chloroplasts, which after inhibition of carboxylation/ oxygenation by glycolaldehyde are performing either the Mehler-peroxidase reaction or nitrite-reduction, both in the absence and presence of nigericin. The nitrite concentration was adjusted such that in the absence of nigericin the observed flux, as judged by the parameter ΔF/Fm′, was similar to that observed with the Mehler-peroxidase reaction. The sample was depleted of O_2 to measure specifically nitrite dependent electron flow. Hence, the conditions were chosen such that in both cases before nigericin addition approximately the same overall electron flow rate was given. The fundamental difference in these two types of electron flow becomes apparent when comparing the traces in the presence of nigericin. With the Mehler-peroxidase reaction the effective PS II quantum yield, ΔF/Fm′, is lowered by

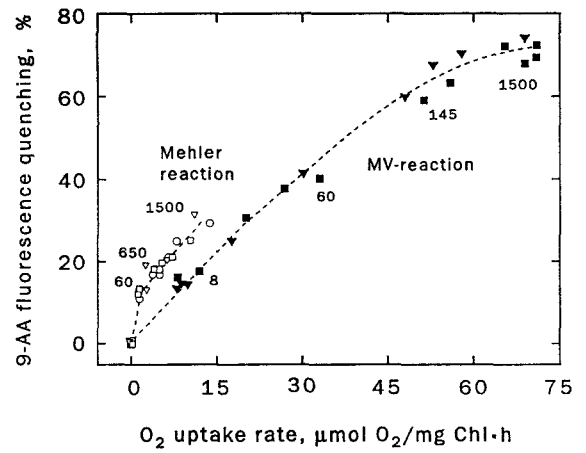

Fig. 3. Relationship between 9-AA fluorescence quenching and polarographically measured O_2-uptake rate in intact chloroplasts. Measurements were done under the conditions of Fig. 1 in the presence and absence of 0.4 mM methyl viologen. In the latter case 4 mM KCN was present to inhibit the ascorbate peroxidase. The varied parameter is photon flux density of red light, indicated in µmol/m^2s for some characteristic points of the relationship. For the complete relationship between 9-AA fluorescence quenching and light intensity see Fig. 1.

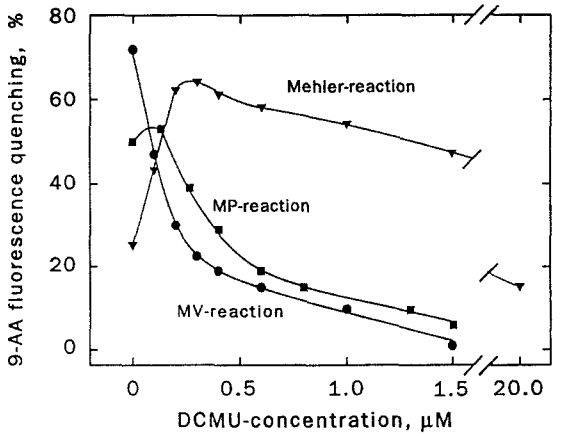

Fig. 4. DCMU-concentration dependence of 9-AA fluorescence quenching during steady-state illumination of intact chloroplasts performing three different types of light-driven O_2-dependent flow. The conditions for methyl viologen dependent flow (MV-reaction), Mehler-peroxidase dependent flow (MP-reaction) and Mehler reaction were as described for Figs. 1 and 3. In all cases the same photon flux density of 650 µmol/m^2s red light was applied.

nigericin (from 0.12 to 0.05) whereas it is increased in the case of nitrite reduction (from 0.13 to 0.18). This confirms our previous finding that the Mehler-peroxidase reaction is unique in being stimulated by the proton gradient (Hormann et al. 1993).

Figure 3 shows the relationship of 9-AA fluorescence quenching versus O_2-uptake rate for the methyl viologen catalyzed reaction and the Mehler reaction. No equivalent information can be obtained for the Mehler-peroxidase reaction, as in this reaction sequence as much O_2 is evolved as taken up, such that there is no net O_2-exchange. For the catalyzed reaction, the relationship is almost linear up to about 50% quenching of 9-AA fluorescence. This phenomenological feature can be useful for the evaluation of linear and cyclic contributions to overall 9-AA fluorescence quenching (Kobayashi and Heber 1994). In the case of the Mehler reaction (peroxidase inhibited by KCN), the rise to approximately 20% 9-AA fluorescence quenching correlates with only very low O_2-uptake rates. Very similar observations were made by Kobayashi and Heber (1994). At higher values the curve proceeds in parallel to that observed in the presence of methyl viologen, which should reflect exclusively linear electron flow from H_2O to O_2. On the basis of this rationale, the initial rise to 20% 9-AA fluorescence quenching could reflect ΔpH-formation by cyclic flow. In agreement with the interpretation of the data in Figs. 1 and 2, it appears that effective O_2-reduction requires a certain ΔpH, a feature reflecting the decisive role of superoxide protonation (Hormann et al. 1993). Hence, cyclic electron flow may prime the Mehler reaction by providing the necessary membrane acidification. Actually, in view of the logarithmic relationship between ΔpH and 9-AA quenching, the 20% quenching reached at low intensity corresponds to a substantial ΔpH (approximately ΔpH = 2). On the other hand, once O_2-reduction is primed, with further increases of light intensity it appears to become mainly responsible for further increases in ΔpH, whereas the cyclic contribution remains limited to the value built-up at low light intensities. This suggests that in the presence of O_2, even when the peroxidase reaction is inhibited, the relative contribution of cyclic flow in competition with O_2-dependent flow decreases with light intensity. Presumably, at high light intensities, excessive reduction of the electron transport chain prevents cyclic flow, which is known to require delicate redox poising (Arnon and Chain 1975; Heber et al. 1978; Ziem-Hanck and Heber 1980).

When excessive reduction of the electron transport chain is prevented by blocking part of the PS II reaction centres with DCMU, cyclic electron flow can be substantially stimulated (Arnon and Chain 1975; Kaiser and Urbach 1975; Ziem-Hanck and Heber 1980; Kobayashi and Heber 1994). This is confirmed in Fig. 4, which shows that addition of DCMU leads to an increase of 9-AA fluorescence quenching from 25%

to 65% when only O_2 is available as electron acceptor (Mehler reaction). At the given photon flux density of 650 μmol quanta $m^{-2}s^{-1}$ red light, maximal stimulation is observed at 0.3 μM DCMU. Much higher concentrations are required to suppress 9-AA quenching (75% suppression of maximal value at 20 μM DCMU). This shows that in the absence of the peroxidase reaction, cyclic flux in principle can compete with O_2-reduction also at higher quantum flux densities, provided that 'over-reduction' of the electron transport chain is prevented.

When the peroxidase reaction is functional, the combined Mehler-peroxidase reaction can generate relatively strong 9-AA fluorescence quenching at high light intensities, as was already shown in Fig. 1. This quenching, however, is not significantly stimulated by DCMU-addition (Fig. 4, MP-reaction). On the contrary, it is suppressed by DCMU with a half-maximal effect at 0.4 μM. The same is true for the 9-AA fluorescence quenching caused by methyl viologen dependent electron flow (Fig. 4, MV reaction), with a half-maximal inhibition at 0.2 μM DCMU.

These data suggest that the monodehydroascorbate formed by the intrinsic ascorbate peroxidase is an effective electron acceptor, interfering with cyclic electron flow at high as well as at low electron donation rates from PS II, essentially as found with methyl viologen as acceptor. This assessment is further substantiated by the data of Fig. 5. In this experiment, use of fluorescence quenching analysis is made in order to measure electron transport associated with the Mehler-peroxidase reaction which, as outlined above, cannot be determined by O_2-exchange measurements. In previous work we have shown that the parameter $\Delta F/Fm'$ is not only a reliable indicator for the effective PS II quantum yield in intact leaves (Genty et al. 1989) but in isolated chloroplasts as well (Hormann et al. 1993, 1994). When multiplied by the photon flux density of photosynthetically active radiation (PAR), this parameter provides a relative measure of the rate of electron transport passing through PS II. Fig. 5 shows that the relationship between 9-AA fluorescence quenching and $\Delta F/Fm' \times$ PAR is curvilinear, which had to be expected in view of the curvilinear relationship between $\Delta F/Fm'$ and the quantum yield of electron transport, measured via methyl viologen dependent O_2-uptake or ferricyanide reduction (Hormann et al. 1993, 1994). Irrespective of the nature and interpretation of this relationship, it is clear that it is identical for the methyl viologen reaction and the Mehler-peroxidase reaction. Therefore, if the assumption is correct that the ΔpH formed by methyl viologen dependent electron flow is exclusively due to linear electron flow, the same must be true for the Mehler-peroxidase reaction. This leads to the conclusion that in intact spinach chloroplasts with a functional ascorbate peroxidase and in the presence of O_2 the contribution of cyclic PS I flow to ΔpH formation is negligibly small.

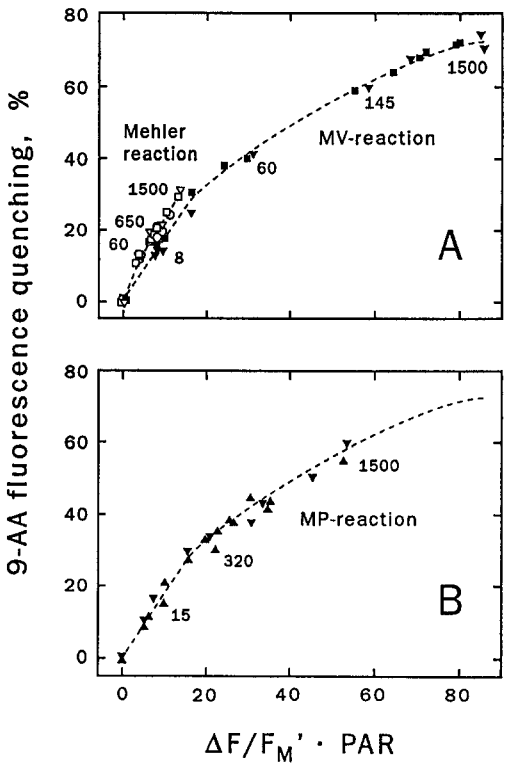

Fig. 5. Relationship between 9-AA fluorescence quenching and $\Delta F/Fm' \times$ PAR, the relative electron flow rate determined by fluorescence quenching analysis. (A) Data points obtained for methyl viologen catalysed O_2-reduction (MV-reaction) and for non- catalysed flow in presence of 4 mM KCN (Mehler reaction). (B) Data points obtained with an active ascorbate peroxidase (MP-reaction). The dotted line is identical to that in (A) representing methyl viologen catalysed flow. The varied parameter is photon flux density of red light, indicated in μmol/ m^2s at some characteristic points of the relationship. Conditions as described for Figs. 1 and 3.

In Fig. 5A also the corresponding relationship for the Mehler-reaction (only O_2-reduction, with peroxidase inhibited) is depicted. In this case, as was expected in view of the data in Fig. 1, the 9-AA fluorescence quenching is somewhat higher than can be accounted for by linear flux. The observed deviation is smaller than predicted from Fig. 1, which means that in the experiment of Fig. 1 the contribution of cyclic PS I flow may have been overestimated. It could be argued

that possibly the membrane bound ascorbate peroxidase cannot be fully inhibited by KCN, such that some monodehydroascorbate can still be formed. However, we consider it more likely that in the presence of KCN, with the superoxide dismutase being inhibited, there is non-enzymatic reduction of superoxide by ascorbate resulting in formation of monodehydroascorbate. This would be reduced by the photosynthetic electron transport chain, leading to an increase of $\Delta F/Fm' \times PAR$ whereas net O_2-uptake would be decreased due to O_2-evolution from H_2O-splitting.

Conclusions

The presented results for the first time give clear evidence that the light-generated ΔpH in intact spinach chloroplasts under close to in vivo condition does not involve a substantial contribution of cyclic PS I electron transport. It is shown that the Mehler-peroxidase reaction sequence can be as effective as methyl viologen catalysed O_2-reduction in preventing back cycling of electrons from the PS I acceptor side into the intersystem electron transport chain (Fig. 5). Notably, this is true at low as well as at high light intensities. Furthermore, partial closure of PS II centres by low concentrations of DCMU inhibits ΔpH formation when the Mehler-peroxidase reaction is active, whereas there is stimulation when the peroxidase reaction is prevented (Fig. 4). We conclude that the monodehydroascorbate formed from H_2O_2 at the PS I acceptor side is a very effective acceptor which can prevent cyclic electron flow. This confirms and extends previous measurements which suggest that ascorbate peroxidase catalysed reduction of externally added H_2O_2 can be as efficient as methyl viologen catalysed O_2-reduction (Neubauer and Schreiber 1989). The data show clearly that in intact spinach chloroplasts the role of O_2-dependent electron flow is not merely to serve for 'poising' cyclic PS I flow. This does not question the well established observation of such a function under less natural conditions, when the peroxidase is inactive (Arnon and Chain 1975). Also it cannot be excluded that different conditions are created in the intact leaves such that cyclic pathways then can more effectively compete for electrons at the PS I acceptor side.

In particular, as pointed out by Heber and coworkers (Heber et al. 1990, 1992; Heber and Walker 1992, Kobayashi and Heber 1994), stomata closure and the ensueing CO_2-deficiency in water stressed leaves will lead to the stimulation of photorespiration, the rate of which is likely to exceed that of the Mehler-peroxidase reaction. It is not easy to predict the consequences for cyclic PS I flow. On one hand, with the photorespiratory pathway another effective linear acceptor system is given which, from all we know about cyclic flow in isolated chloroplasts, should suppress electron cycling. On the other hand, photorespiratory flux would contribute to keeping the electron transport chain, and particularly the PS I donor side, relatively oxidized, which may favor cyclic flow. Which of the two contrasting aspects is dominating can only be decided by experiments with intact leaves under photorespiratory conditions. In this context, it may be mentioned that Foyer et al. (1990) reported on 'evidence for cyclic electron transport' on the basis of comparative measurements of PS I and PS II quantum yields. This assessments, however, was not confirmed by Klughammer and Schreiber (1994), who developed a refined method of PS I quantum yield determination and obtained a linear correlation between PS I and PS II quantum yields, also under photorespiratory conditions.

Since the discovery of cyclic and 'pseudocyclic' photophosphorylation by Arnon and co-workers (Arnon et al. 1961; Tagawa et al. 1963) the evaluation of the relative importance of these two processes for in vivo photosynthesis has undergone periodic changes (see reviews by Heber 1973; Simonis and Urbach 1973; Allen 1975; Gimmler 1977; Arnon 1977; Walker 1992; Heber and Walker 1992; Fork and Herbert 1993; Kobayashi and Heber 1994). The most recent reviews have been creating the impression that there is increasing evidence for cyclic PS I playing the major role in ΔpH-formation under stress conditions, when Calvin cycle is limited. Such a role, with all its implications for down-regulation of PS II and protection against photodamage had been first explicitly proposed for the Mehler-peroxidase reaction (Schreiber and Neubauer 1990; Schreiber et al. 1991). The latter view is strengthened by the data of the present contribution, which has shown once more that the physiological function of O_2-dependent electron flow strongly depends on an active ascorbate peroxidase system. The decisive aspect, as it appears, is the stimulation of O_2-reduction by the internal acidification of the thylakoids brought about by the light-driven reduction of monodehydroascorbate (Hormann et al. 1993). As has been concluded by Fork and Herbert (1993), "the initial discovery and characterization of cyclic photophosphorylation was necessarily made with simplified, in vitro preparations of photo-

synthetic membrane". Indeed, practically all demonstrations of significant cyclic fluxes in C_3-chloroplasts involved one or several of the following 'unnatural' conditions: absence or inhibition of ascorbate peroxidase, low O_2-concentrations, partial inhibition of PS II by DCMU, preferential excitation of PS I by far-red light. In agreement with Fork and Herbert (1993), we think that "with respect to the biological functions of cyclic photophosphorylation ... better understanding will be dependent upon experiments performed in vivo with intact organisms". This calls for unbiased experiments with intact leaves, designed on the basis of our knowledge obtained with intact chloroplasts.

Acknowledgements

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