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TRIBUTE

Novel effects of methyl viologen on photosystem II function in spinach leaves

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Abstract Methyl viologen (MV) is a well-known electron mediator that works on the acceptor side of photosystem I. We investigated the little-known, MV-induced inhibition of linear electron flow through photosystem II (PS II) in spinach-leaf discs. Even a low [MV] decreased the (1) average, light-adapted photochemical efficiency of PS II traps, (2) oxidation state of the primary quinone acceptor Q_A in PS II during illumination, (3) photochemical efficiency of light-adapted open PS II traps, (4) fraction of absorbed light energy dissipated constitutively in a light-independent manner or as chlorophyll (Chl) *a* fluorescence emission, (5) Chl *a* fluorescence yield corresponding to dark-adapted open reaction-center traps (F_o) and closed reaction-center traps (F_m), and (6) half-time for re-oxidation of Q_A^- in PS II after a single-turnover flash. These effects suggest that the presence of MV accelerates various "downhill" electron-transfer steps in PS II. Therefore, when using the MV to quantify cyclic electron flow, the inhibitory effect of MV on PS II should be taken into account.

Keywords Cyclic electron flow · Exciton-radical pair equilibrium · Linear electron flow · Methyl viologen · Photosystem II

Abbreviations

ATP	Adenosine triphosphate
CEF	Cyclic electron flow
Chl	Chlorophyll
Cyt	Cytochrome
D1, D2 protein	psbA, B gene product, respectively
F_o, F_m	Chl fluorescence corresponding to open and closed PS II traps in the dark-adapted state, respectively
F_o', F_m'	Chl fluorescence corresponding to open and closed PS II traps in the light-adapted state, respectively
F_v'	Variable Chl *a* fluorescence in the light-adapted state ($=F_m' - F_o'$)
LEF	Linear electron flow
MV	Methyl viologen
$NADP^+$	Oxidized nicotinamide adenine dinucleotide phosphate
$\Phi_{f,D}$	The fraction of absorbed light either dissipated constitutively as heat in a light-independent manner or emitted as Chl *a* fluorescence
Φ_{NPQ}	The fraction of absorbed light partitioned as heat dissipation in a light-dependent manner
$\Phi_{PS\ II}$	The average quantum yield of PS II photochemistry in the light

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P_{680}, P_{700}	Special Chl pair in the PS II, I reaction centers, respectively
PC	Plastocyanin
PQ	Plastoquinone
Ph	Pheophytin
PS I, II	Photosystem I, II, respectively
Q_A, Q_B	Primary, secondary quinone acceptor in PS II, respectively
qP	Oxidation state of Q_A

Introduction

Photosynthesis begins with absorption of light by photosystem II (PS II) and PS I complexes spanning photosynthetic membranes (thylakoids or membrane sacs) that separate an outer aqueous phase (the stroma) from an inner aqueous space (the lumen). The structure and organization of photosynthetic membranes is closely related to function, as reviewed succinctly by Ort and Yocum (1996). Each photosystem is made up of many protein subunits, pigments, and redox cofactors. Light induces the oxidation of water at a catalytic site within the PS II complex, in a unique reaction that occurs at an optimal thermodynamic driving force:

$$2H_2O \rightarrow O_2 + 4H^+ + 4e^-$$

The oxygen is a by-product. The protons are deposited in the lumen of thylakoids, while the electrons are transferred uphill and then downhill via plastoquinone (PQ) to the membrane-spanning cytochrome (Cyt) *bf* complex. The Cyt *bf* complex oxidizes PQH_2, depositing additional protons in the lumen. Half of the electrons are transferred onwards to plastocyanin (PC), while the other electrons go through a Q cycle that includes two Cyt *b* haems within the complex, and which functions to reduce PQ and translocate more protons into the lumen. The reduced PC delivers electrons to P_{700}^+ (oxidized special Chl pair) in PS I, in which light drives an uphill electron transfer, followed by downhill transfer to reduce ferredoxin that in turn reduces $NADP^+$ (oxidized nicotinamide adenine dinucleotide phosphate) to NADPH. Some of the electrons carried by ferredoxin, instead of reducing $NADP^+$, are channelled back to the Cyt *bf* complex in cyclic electron flow (CEF) around PS I, translocating more protons into the lumen in the process. The protons in the lumen diffuse through the membrane-spanning ATP synthase down a proton electrochemical potential gradient, forming ATP from ADP and inorganic phosphate. NADPH and ATP then drive the reduction of carbon dioxide to sugars in the stroma of the chloroplast.

Cyclic electron flow around PS I (reviewed by Bendall and Manasse 1995; Joliot and Joliot 2002) is important in that it supplements the deposition of protons in the lumen, thereby producing extra ATP needed to maintain a ratio of three ATP per pair of NADPH required for carbon assimilation. Quantification of the rate of CEF has been hampered by the absence of a net product formed. Fan et al. (2007) and Jia et al. (2008) quantified CEF in leaves by utilizing the inhibition of CEF by methyl viologen (MV, paraquat), a herbicide that rapidly mediates electron flow to oxygen at the expense of ferredoxin reduction. In the absence of MV, both CEF and linear electron flow (LEF) from PS II merge at the Cyf *bf* complex, the combined flux going through P_{700} in PS I, together with a negligible but measurable electron flux originating from other reductants in the stroma (Fan et al. 2007). In the presence of adequate MV, only LEF remains, together with a negligible stromal electron flux. Thus, in principle, the difference between the combined CEF + LEF_1 in the absence of MV and the LEF_2 in the presence of MV would give the CEF if $LEF_1 = LEF_2$. Unfortunately, MV decreased LEF, so that $LEF_2 < LEF_1$.

It is well known that MV mediates electron transfer to oxygen at the acceptor side of PS I. In addition, we observed an inhibition of LEF by MV in spinach leaves in using MV to abolish CEF (Jia et al. 2008). However, to our knowledge no specific study of the mechanism of inhibition of PS II LEF by MV seems to have been reported apart from two remotely related papers by (1) Yruela et al. (2001) on the effects of MV on light-induced absorption spectra of the D1–D2–Cyt *b*559 complex of PS II in anaerobic conditions and (2) Schansker et al. (2005) on the effects of MV and dibromothymoquinone in relation to the role of PS I in the Chl *a* fluorescence rise OJIP. In this paper, we report on an investigation of the mechanism of action of MV on PS II electron transfers and photochemical efficiency. Our results suggest that the effects on PS II parameters arise from MV-mediated acceleration of "downhill" electron transfers between redox components within PS II.

Materials and methods

Growth of plants

Spinacea oleracea L. (cv. Yates hybrid 102) plants were grown in a polycarbonate greenhouse at approximately 28/15°C (day/night) under natural light during autumn and winter (maximum ~1,000 μmol photons m^{-2} s^{-1}). The potting mixture was supplemented by a slow-release fertilizer (Osmocote, Scotts Australia, Castle Hill).

Vacuum infiltration of leaf discs

Leaf discs (~1.5 cm diameter) were floated on water under fluorescent light (100 μmol photons m^{-2} s^{-1}) for 1 h. They were then immersed in water or a solution of MV at a selected concentration, vacuum infiltrated, blotted with absorbent paper, and allowed to evaporate off excess intercellular water in darkness. The total dark time before measurement was ~1 h. Vacuum infiltration with water, followed by loss of excess inter-cellular water, decreased the electron flux to P_{700} by about 18% compared with no infiltration. Therefore, the control for MV infiltration was infiltration with water.

Measurement of electron flow to P_{700}^+ by post-illumination re-reduction kinetics of P_{700}^+

Redox changes of P_{700} in spinach-leaf discs were observed with a dual wavelength (820/870 nm) unit (ED-P700DW) attached to a pulse amplitude modulation (PAM) fluorometer (Walz, Effeltrich, Germany) and used in the reflectance mode (Chow and Hope 2004; Fan et al. 2007). Each leaf disc was placed inside a chamber with a transparent lid. A piece of matting moistened with a mixture of 1 M $NaHCO_3$ and 1 M Na_2CO_3 at pH 9 was placed inside the closed chamber to supply ca. 1% CO_2. The light guide was positioned at an angle of about 60° to the plane of the leaf disc, while an actinic light was directed at an angle of about 45° but on the other side of the normal to the leaf surface.

Actinic light was provided by an array of light-emitting diodes (LED 700-66-60, Roithner LaserTechnik, Vienna) fitted with a focusing lens, with a peak emission at 697 nm (full width at half peak height = 24 nm). The spectral irradiance was measured by an LI1800 spectroradiometer (Licor, USA), the integrated irradiance at the leaf surface being ca. 500 μmol photons m^{-2} s^{-1} (650–740 nm) at maximum. The actinic light was transmitted by an electronic shutter triggered open by a pulse/delay generator (Model 565, Berkeley Nucleonics, USA) for a 120-s interval and then turned off. Each leaf segment was illuminated only once, after a standard total dark treatment time of 1 h.

All post-illumination P_{700}^+ signals were normalized to the maximum signal corresponding to maximum photo-oxidizable P_{700} ($P_{700}^+{}_{max}$) to give the fraction of oxidized P_{700} at any instant (Fan et al. 2007; Jia et al. 2008). The re-reduction kinetics were well fitted by a sum of three negative exponentials, with normalized amplitudes A_1, A_2, and A_3 (=1 − A_1 − A_2), and rate coefficients k_1, k_2, and k_3. The initial rate of post-illumination re-reduction of P_{700}^+ is $A_1k_1 + A_2k_2 + A_3k_3$, equal to the total electron flux to P_{700}^+ immediately before cessation of illumination, having units e^- s^{-1} P_{700}^{-1}.

The photochemical efficiency of PS II in the dark-adapted state measured by Chl *a* fluorescence

The relative chlorophyll (Chl) *a* fluorescence yield (F_o) corresponding to open PS II reaction-center traps was measured using a Pulse Amplitude Fluorometer (PAM 101, H. Walz, Effeltrich, Germany) fitted with an ED101 BL emitter/detector that excited fluorescence with modulated blue light (~0.1 μmol photons m^{-2} s^{-1}). Chl *a* fluorescence above 660 nm was detected, with relatively little contamination by PS I fluorescence. The maximum relative Chl *a* fluorescence yield (F_m) was measured with a 1-s saturating light pulse (~10,000 μmol photons m^{-2} s^{-1}).

PS II in the light-adapted state measured by Chl *a* fluorescence

A leaf disc was placed inside the same chamber, and Chl fluorescence was measured in the same geometry as for investigating P_{700}^+ kinetics using a PAM fluorometer. The same LED actinic light with a peak wavelength at 697 nm was also used to determine the relative Chl *a* fluorescence yield (F) at 120 s illumination and the maximum relative Chl *a* fluorescence yield in the light-acclimated state (F_m'). The average quantum yield of PS II photochemistry (Genty et al. 1989), $\Phi_{PS\ II} = (1 - F/F_m')$, was taken as a directly proportional measure of linear electron transport rate through PS II (ETR), since it is often used to calculate ETR as $(1 - F/F_m') \times I \times$ absorptance $\times\ \alpha$ where I is the irradiance and α the fraction of absorbed light partitioned to PS II (Schreiber 2004).

The oxidation state of the primary quinone acceptor (Q_A) in PS II was calculated as $qP = (F_m' - F)/(F_m' - F_o')$, where the Chl *a* florescence yield F_o' is for open PS II traps in the light-adapted state (Schreiber 2004). F_o' was calculated from F_o, F_m, and F_m' (Oxborough and Baker 1997).

The photochemical efficiency of open PS II traps was calculated as F_v'/F_m', where $F_v' = F_m' - F_o'$. The fraction of absorbed light partitioned as heat dissipation in a light-dependent manner was calculated as $\Phi_{NPQ} = F/F_m' - F/F_m$; the fraction of absorbed light either dissipated constitutively as heat in a light-independent manner or emitted as Chl *a* fluorescence was calculated as $\Phi_{f,D} = F/F_m$ (Hendrickson et al. 2004). Note that $\Phi_{PS\ II} + \Phi_{NPQ} + \Phi_{f,D} = 1$.

Decay of the Chl *a* fluorescence yield after a flash

The decay of the flash-induced increase in Chl *a* fluorescence yield in a leaf disc was measured at room temperature using a pulse-modulated fluorometer (PAM101 and 103, Walz, Effeltrich, Germany). A single-turnover flash was given by an XE-STC xenon flash lamp unit (model

XF-103, Walz). Weak monitoring light (650 nm) was applied at 1.6 kHz and automatically changed to 100 kHz when a single actinic flash was given. Data acquisition (time constant 15 μs) was achieved by home-built equipment and a computer program (Chow and Hope 2004). Twenty successive flashes were given every 5 s, and the signals were averaged.

Results

Decrease in the electron flux through PS I with increase in [MV] and associated changes in Chl fluorescence parameters of PS II

The electron flux through P_{700} decreased by a factor of ~2 on increasing [MV], reaching a steady electron flux at ≥50 μM (Fig. 1a). This decrease was partly due to an inhibition of ferredoxin-dependent CEF by MV which outcompeted ferredoxin for reducing equivalents. However, the decrease was also partly caused by inhibition of PS II by MV. This is shown in Fig. 2a, where infiltration with even 2 μM MV decreased $\Phi_{PS\ II}$ considerably. Since LEF is directly proportional to $\Phi_{PS\ II}$, we conclude that MV inhibited LEF.

In Fig. 1a, the electron flux at each [MV] in general consisted of an LEF component and any residual CEF that was not yet completely abolished by MV. The maximum extent of the CEF abolishable by MV, after correction for inhibition of LEF by MV (see Jia et al. 2008), can be equated with the true CEF. At a nonsaturating [MV], however, we obtained only the apparent CEF (Fig. 1b). It is seen that as [MV] increased, the apparent CEF increased; above about 50 μM MV, which was apparently needed to abolish the entire CEF, the saturated CEF was taken as the true CEF value (~28 e^- P_{700}^{-1} s^{-1} or ~40% of the total electron flux through P_{700} in the selected light regime).

$\Phi_{PS\ II}$ is equal to the product of qP and F_v'/F_m'. Figure 2b shows that qP decreased substantially at low [MV], rising somewhat at higher [MV]. F_v'/F_m' also decreased to some extent in the presence of MV (Fig. 2c). Thus, the decrease in $\Phi_{PS\ II}$ was due to both a more reduced state of Q_A (restricting the onward linear flow of electrons) and lower photochemical efficiency of light-adapted open PS II traps.

Partitioning of absorbed light energy in PS II

While $\Phi_{PS\ II}$ is the fraction of absorbed light energy utilized photochemically, the fraction $\Phi_{f,D}$ is constitutively lost either as heat in a light-independent manner or as fluorescence emission. $\Phi_{f,D}$ is normally roughly constant, perhaps increasing marginally with irradiance (Hendrickson et al. 2004). Interestingly, $\Phi_{f,D}$ *decreased* slightly in the presence of MV (Fig. 3a). The sum of $\Phi_{PS\ II}$, $\Phi_{f,D}$, and Φ_{NPQ} is unity. With the decreases in $\Phi_{PS\ II}$ and $\Phi_{f,D}$, the remainder, Φ_{NPQ}, increased markedly at low [MV], decreasing slightly on further increasing [MV] (Fig. 3b).

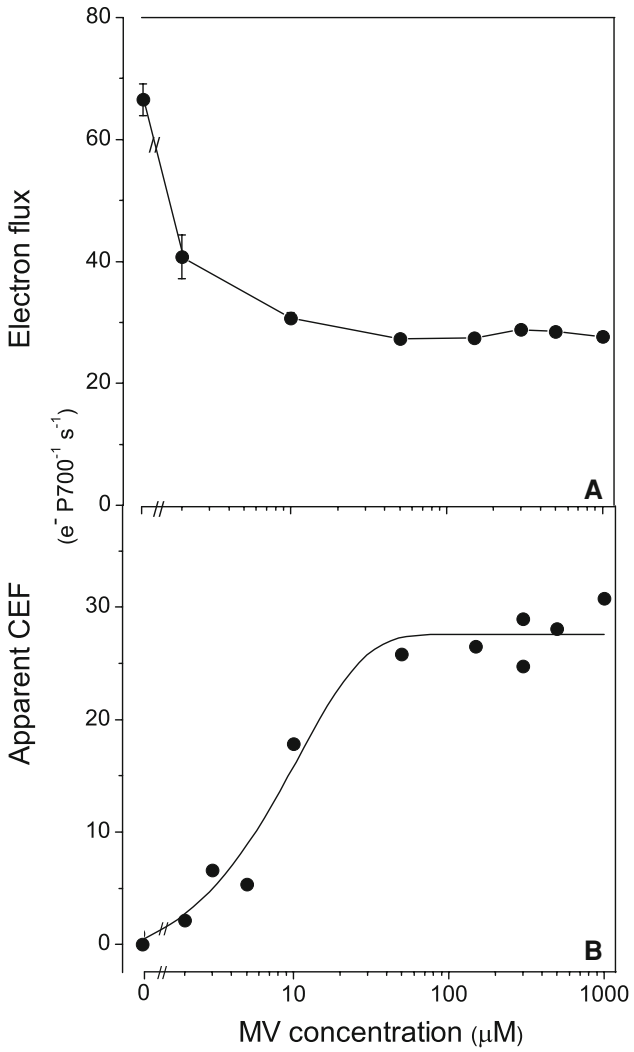
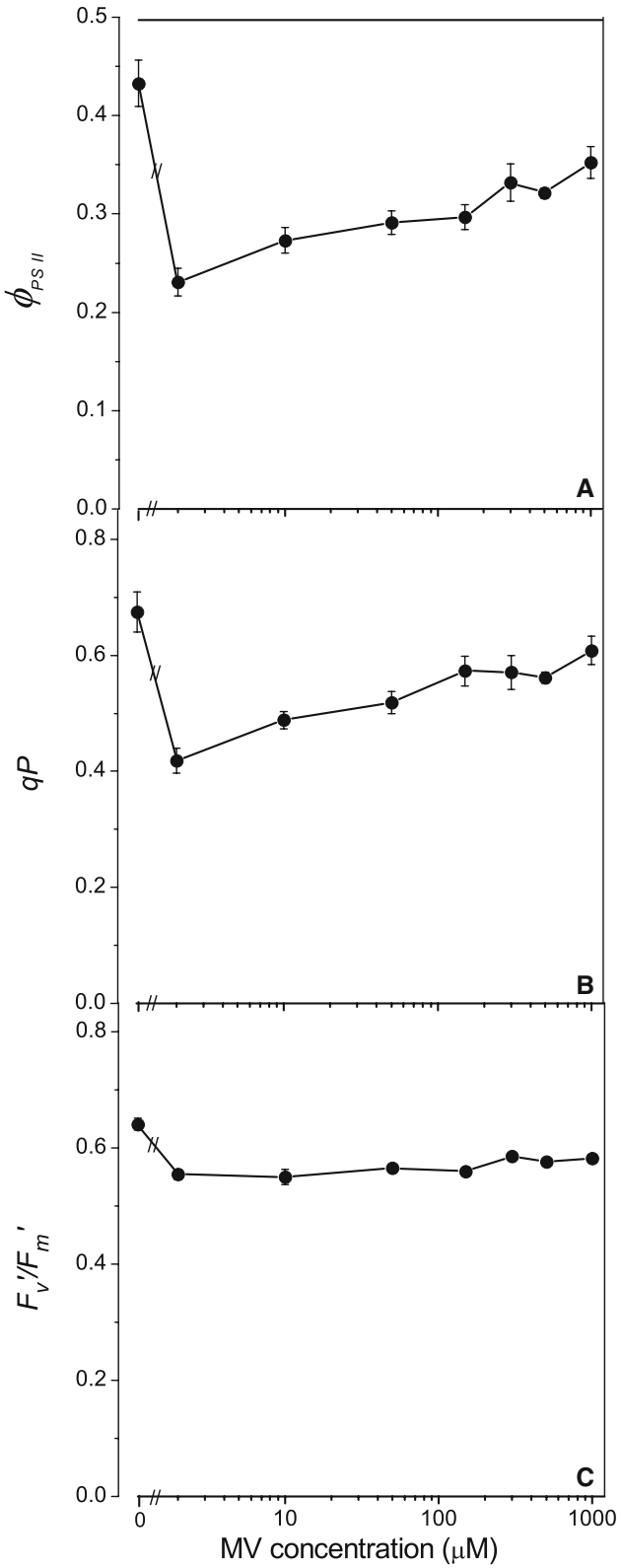


Fig. 1 **a** The electron flux through P_{700} as a function of [MV], measured as the initial rate of re-reduction of P_{700}^+ at the instant of cessation of illumination (2 min, ~500 μmol photons m^{-2} s^{-1}, peak wavelength 697 nm from an array of light-emitting diodes). This electron flux is assumed equal to that just before cessation of illumination. **b** The apparent CEF obtained as the difference between the electron flux for a water-treated sample and that of a sample treated with a selected [MV]. The latter flux (in the presence of MV) was corrected for an inhibitory effect of MV on the LEF (see Jia et al. 2008). Values are means of four to six leaf discs ± SE (pooled from two separate experiments)

Changes in F_o and F_m in dark-adapted leaf discs

To further probe the effects of MV on PS II, we measured F_o and F_m, the Chl *a* fluorescence yield corresponding to open and closed PS II traps in the dark-adapted state,



◀ **Fig. 2** Effects of increasing [MV] on **a** the photochemical efficiency $\Phi_{PS\ II}$ averaged over closed and open PS II traps; **b** the oxidation state *qP* of the primary quinone acceptor Q_A in PS II; and **c** the photochemical efficiency F_v'/F_m' of open, light-adapted PS II traps. Measurements were made at 2 min illumination time. Other conditions as in Fig. 1. Values are means of four to six leaf discs ± SE

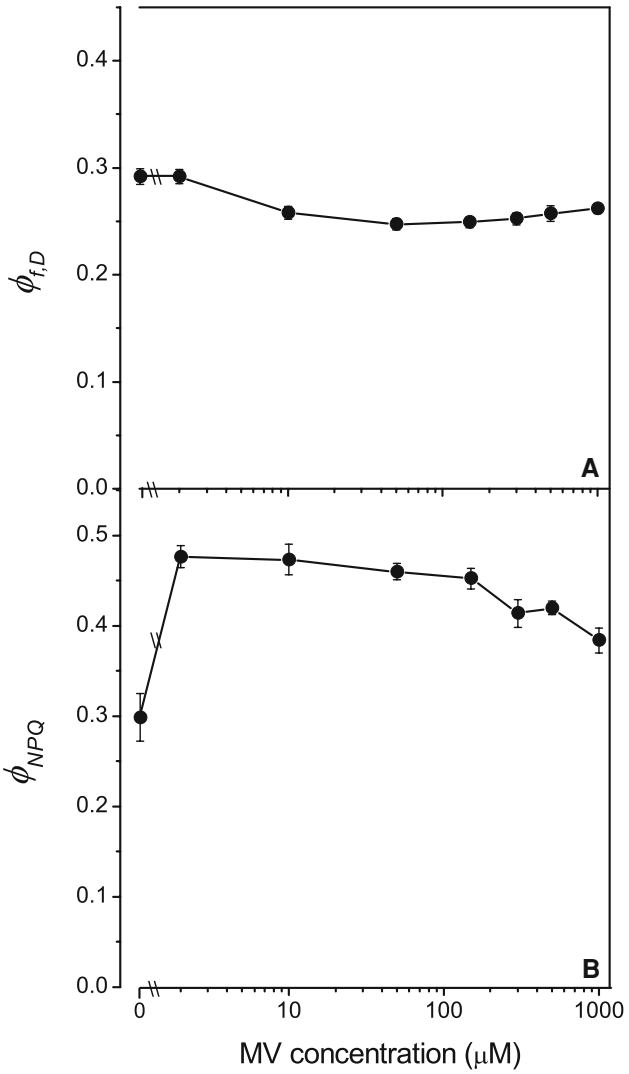


Fig. 3 The fraction of absorbed light energy lost nonphotochemically and constitutively in a light-independent manner and as Chl *a* fluorescence ($\Phi_{f,D}$, **a**), and the fraction dissipated nonphotochemically in a light-dependent manner (Φ_{NPQ}, **b**). Measurements were made at 2 min illumination time. Other conditions as in Fig. 1. Values are means of four to six leaf discs ± SE

respectively. F_o decreased slightly at low [MV] before increasing to some extent at high [MV] (Fig. 4a). F_m behaved similarly, suggesting that their changes had a common cause (Fig. 4b).

The kinetics of re-oxidation of Q_A^-

On reduction of the primary quinone acceptor Q_A in PS II after a flash, re-oxidation occurs when the electron is transferred onwards with multi-phasic kinetics to the secondary quinone acceptor or to other acceptors such as an electron hole on the donor side of PS II (Renger et al.

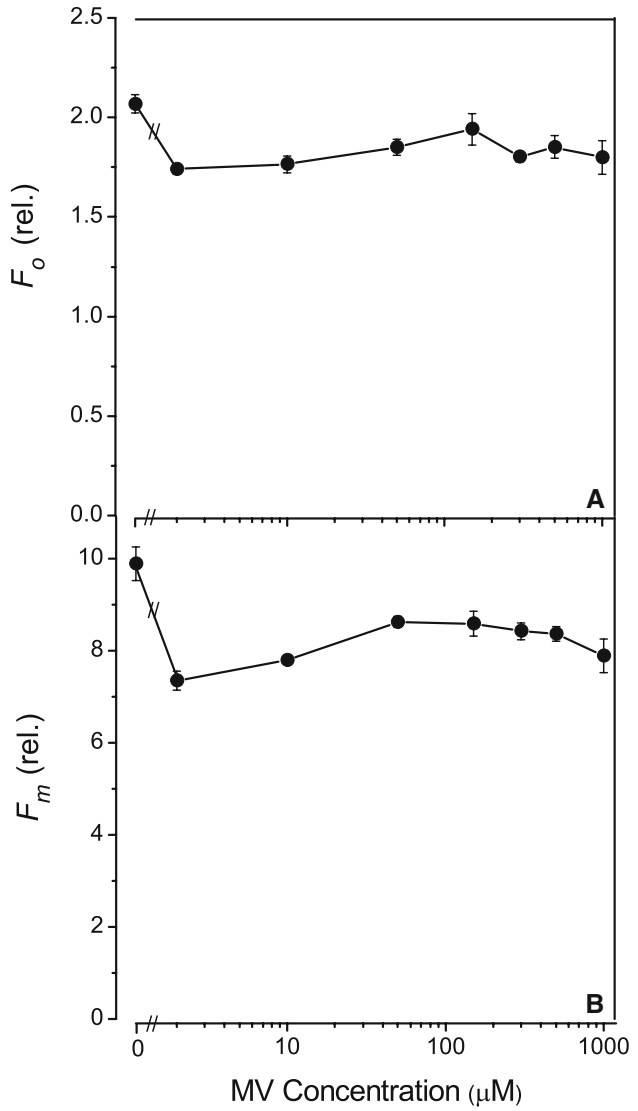


Fig. 4 Effects of increasing [MV] on **a** F_o and **b** F_m. Leaf discs were dark-adapted for about 60 min before measurement. Values are means of four to six leaf discs ± SE

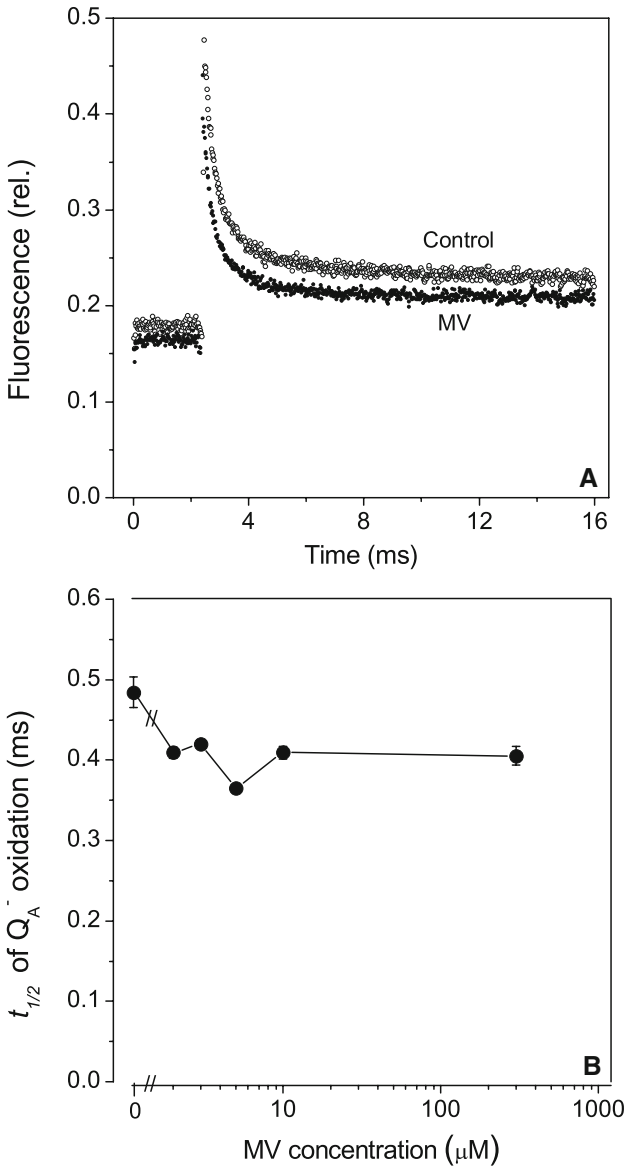


Fig. 5 **a** Kinetic traces showing the decrease in Chl *a* fluorescence yield after a single-turnover flash given to dark-adapted leaf discs, associated with Q_A^- re-oxidation, in water or leaf discs treated with 300 μM MV. A flash overload artefact obscured the initial rise of the signal. Each trace is the average of 20 signals. **b** The half time, $t_{1/2}$, for re-oxidation of Q_A^- after a flash. Values are means of four to six leaf discs ± SE

1995). Examples of such re-oxidation kinetics are shown in Fig. 5a. An overall inverse measure of the rate of electron transfer is the half-time, $t_{1/2}$, for Q_A^- re-oxidation. Figure 5b shows that $t_{1/2}$ decreased slightly at [MV] ≥2 μM, suggesting that Q_A^- re-oxidation was accelerated somewhat.

Discussion

An explanation of the inhibition of PS II parameters by low concentrations of MV

A simple explanation that is consistent with many of the effects of MV on PS II is that electron mediation by even low concentrations of MV accelerated the "downhill" electron transfers within PS II, as depicted by thicker arrows in Fig. 6. Simple viologens such as MV are redox active species that can facilitate electron-transfer processes (Monk 1998). On reduction to the radical cation, the MV molecule, ~1.1 nm from end to end (Russell and Wallwork 1972), has an unpaired electron delocalized throughout the π-framework of the bipyridyl nucleus. Even the *N* and *N*′ substituents at either end of the molecule bear some of the charge (Evans et al. 1977). The delocalized charge of $MV^{+\bullet}$ may enhance the rate of electron transfer in PS II if

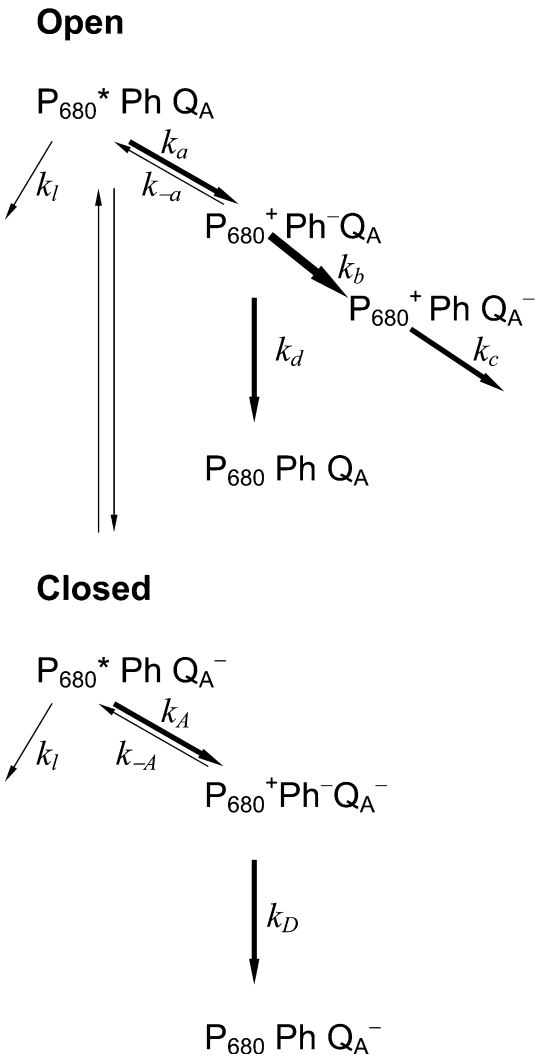


Fig. 6 A diagram showing an open and a closed PS II reaction-center trap, with energy transfer between them indicated by *thin vertical arrows*. "Downhill" electron transfers hypothesized to be accelerated by MV are indicated by *thick arrows*. In an open trap, k_a is the rate coefficient for charge separation, k_b for charge stabilization, k_c for re-oxidation of Q_A^-, k_d for dissipative charge recombination to the ground state of P_{680}, and k_{-a} for charge recombination to the excited state of P_{680}. In a closed trap, k_A is the rate coefficient for charge separation, k_D for dissipative charge recombination to the ground state of P_{680}, and k_{-A} for charge recombination to the excited state of P_{680}. In each trap, excitation energy may also be lost in the antenna with rate coefficient k_l

the radical is inserted into the protein matrix between two redox cofactors. Such an effect may be expected to occur even when [MV] is low. Indeed, low [MV] sufficient to achieve a minimum $\Phi_{PS\ II}$ ($\sim$2 μM) is consistent with this explanation. [PS II] is estimated to be about 10 nM in leaf tissue, two orders of magnitude smaller than 2 μM. However, as the PS II protein matrix has to compete with numerous other Donnan indiffusible negative charges in the cells for the dicationic MV, it seems likely that it adsorbs only a small portion of the MV infiltrated into the tissue.

The mechanism of action of MV in this explanation requires an elongated, redox-active molecule that is also a divalent cation, with delocalization of the unpaired electron on reduction of MV to a radical cation. These special properties of MV cannot be mimicked by Mg^{2+}, despite the abundance of Mg^{2+} in the chloroplast.

Acceleration of Q_A^- re-oxidation and Q_A reduction by MV in open PS II traps

The half-time, $t_{1/2}$, for re-oxidation of Q_A^- after a flash was shortened by μM concentrations of MV (Fig. 5). This is taken as direct evidence of electron mediation by MV, the electron going to (1) a specific site in PS II, presumably the Q_B pocket where PQ is bound or about to bind, or (2) the S-states on the donor side of PS II (Renger et al. 1995). Despite the faster loss of an electron from Q_A^-, however, Q_A was kept more reduced during illumination, as indicated by the lower *qP* at low [MV] (Fig. 2b). This suggests that MV accelerated reduction of Q_A (k_b in Fig. 6) even more than it accelerated the oxidation of Q_A^- (k_c in Fig. 6).

Possible acceleration of charge separation by MV in open PS II traps

In a PS II complex with an intact antenna, as opposed to an isolated reaction center, the rate coefficient for charge separation in an open-trap k_a is proportionally smaller, being inversely influenced by the number of pigment molecules (Schatz et al. 1988); indeed k_a is only slightly larger than k_b (Trissl and Lavergne 1995). Therefore, any increase in k_a due to the presence of MV would also help to keep Q_A more reduced (i.e., *qP* would be smaller, as observed).

Decrease in F_v'/F_m' due to low [MV]

The lower *qP* was partly responsible for the lower $\Phi_{PS\ II}$ (Fig. 2a). Since $\Phi_{PS\ II} = qP \times (F_v'/F_m')$, a decrease in F_v'/F_m' (the efficiency of open PS II traps) in the presence of low [MV] was also responsible for the lower $\Phi_{PS\ II}$. Presumably the decrease in F_v'/F_m' was due to an acceleration of dissipative charge recombination of the radical pair $P_{680}^+Ph^-$ to the ground state directly, i.e., increased k_d in Fig. 6, where P_{680} is the primary donor in PS II and Ph (pheophytin) is the primary acceptor in PS II. Normally k_d in open traps is smaller than that in closed traps by three orders of magnitude (Trissl and Lavergne 1995). Perhaps MV increased k_d even in open traps.

Partitioning of absorbed light energy in PS II

While $\Phi_{PS\ II}$ is the fraction of absorbed energy utilized in photochemical conversion by PS II, the fraction $\Phi_{f,D}$ is lost

as heat in a constitutive, light-independent manner or as Chl *a* fluorescence. $\Phi_{f,D}$ is practically an inevitable loss, which explains the observations that (1) the best photochemical efficiency of PS II is about 80–85% in a healthy leaf in a dark-adapted state or in low light and (2) the maximum quantum yield of oxygen evolution of diverse C3 plants in nonstress conditions is 0.106 as compared with a theoretical 0.125 mol O_2 (mol absorbed photons)$^{-1}$ (Björkman and Demmig 1987). Hendrickson et al. (2004) suggested that this constitutive loss is due to the equilibrium between excitons and the radical pair $P_{680}{}^{+}Ph^{-}$, which acts as a shallow trap of the excitation energy. Although the quantum yield of charge separation (formation of the radical pair) is very high (ca. 0.96), the shallowness of the PS II reaction-center trap means a high likelihood of charge recombination to yield an excited state that will lead to charge separation again. If each primary charge separation has an efficiency of 0.96, then four successive charge separations will bring the overall efficiency down to 0.85. In our present hypothesis, an increase in k_b due to the presence of MV would decrease the probability of charge recombination, thereby decreasing $\Phi_{f,D}$ as observed (Fig. 3a).

With the decreases in both $\Phi_{f,D}$ and $\Phi_{PS\ II}$ in the presence of low [MV], it is expected that Φ_{NPQ} would increase as observed in Fig. 3b since their sum is equal to unity.

Decreases in F_o and F_m in the presence of low [MV]

A low [MV] brought about a small but significant decrease in both F_o and F_m. These changes in Chl fluorescence yield can also be accommodated in our present hypothesis. In the exciton-radical pair equilibrium model, Trissl and Lavergne (1995) obtained the following expressions for the two quantities (symbols in Fig. 6). For an open PS II trap,

$$F_o = \frac{k_f}{\frac{k_a(k_b+k_d)}{k_{-a}+k_b+k_d} + k_l}$$

where k_l is the loss at the antenna and k_f the radiative decay rate coefficient of an antenna pigment. It can be shown that an increase in either k_b or k_d will decrease F_o. Further, any increase in k_a due to MV (hinted at above in relation to possible acceleration of charge separation by MV in open PS II traps) obviously decreases F_o. For a closed PS II trap,

$$F_m = \frac{k_f}{\frac{k_A k_D}{k_{-A}+k_D} + k_l}$$

It can be shown that an increase in k_D will decrease F_m. Further, any increase in k_A due to MV obviously decreases F_m. Therefore, we suggest that the observed decreases in F_o and F_m may be due to enhancement of charge separation and/or charge recombination to the ground state of P_{680} in the respective open and closed PS II traps.

Effects of high [MV]

qP in Fig. 2b decreased to a minimum at a low [MV], showing that Q_A was maximally reduced; it recovered to a considerable extent at a high [MV]. It is likely that a high [MV] $\geq$50 μM was needed to efficiently transfer electrons out of the acceptor side of PS I, notably to oxygen, thereby keeping the PQ pool more oxidized. $\Phi_{PS\ II}$ (Fig. 2a) behaved in a similar manner as did *qP*. Thus, LEF through PS II decreased to a minimum at a low [MV], but recovered to a considerable extent at a higher [MV] because of the enhanced electron transfer from the acceptor side of PS I. Therefore, high concentrations of MV partially improved LEF through PS II despite an inhibition at a low [MV]. Consistent with the requirement of high concentrations of MV to transfer electrons out of the acceptor side of PS I, maximum apparent CEF was achieved at $\geq$50 μM MV (Fig. 1b). At lower concentrations, MV could not completely abolish CEF, so that the apparent CEF was small.

In conclusion, the various effects of MV on PS II function are likely to be due to the acceleration of downhill electron transfers mediated by MV molecules adsorbed to PS II. It appears that excessive enhancement of charge separation led to closing of PS II traps, resulting in inhibition of LEF. This inhibitory effect justifies, indeed necessitates, the correction of LEF when MV is used to quantify CEF (Jia et al. 2008).

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