PHOSPHATE-DEFICIENCY REDUCES THE ELECTRON TRANSPORT CAPACITIES OF THYLAKOID MEMBRANES THROUGH LIMITING PHOTOSYSTEM II IN LEAVES OF CHINESE CABBAGE

YOUN-IL PARK and YOUNG-NAM HONG*
Department of Biology, Seoul National University, Seoul 151-742, Korea

Abstract − Experiments were carried out to investigate whether P_i deficiency in detached 25 mM mannose-feeding led to a decline of the photosynthetic electron transport rates through acidification of the thylakoid lumen. With increasing mannose-feeding time, the maximal CO_2 exchange rates and the maximal quantum yields of photosynthesis decreased rapidly up to 6 h by 73% then with little decrease up to 12 h. The ATP/ADP ratio declined by 54% 6 h after the treatment and then recovered to the control level at 12 h. However, the NADPH/NADP$^+$ ratio was not significantly altered by mannose treatment. Electron transport rates of thylakoid membranes isolated from 6 h treated leaves did not change, but they decreased by 30% in 12 h treated leaves. The quenching analysis of Chl fluorescence in mannose-treated leaves revealed that both the fraction of reduced plastoquinone and the degree of acidification of thylakoid lumen remained higher than those of the control. The reduction of PSI in mannose fed leaves was inhibited due to acidification of thylakoid lumen (high qE). The reduction of primary quinone acceptor of PSII was inhibited by mannose feeding. Mannose treatment decreased the efficiency of excitation energy capture by PSII. Fo quenching was induced when treated with mannose more than 6 h, and had a reverse linear correlation with (Fv)m/Fm ratio. These results suggest that P_i deficiency in Chinese cabbage leaves reduce photosynthetic electron transport rates by diminishing both PSII function and electron transfer from PSII to PSI through acidification of thylakoid lumen, which in turn induce the modification of photosynthetic apparatus probably through protein (de)phosphorylation.

INTRODUCTION

Ample evidence shows that photosynthesis can be P_i-limited *in vivo* when a sudden or large inhibition of end-product synthesis (due to low temperature, chemical inhibitors or removal of enzyme) or a large stimulation of CO_2 fixation (due to saturating CO_2) leads to an imbalance between CO_2 fixation and synthesis of sucrose or starch.[1] While low P_i inhibits the rate of photosynthesis, the precise mechanism remains unclear, even in isolated chloroplasts. P_i deficiency in chloroplasts could inhibit photosynthesis through limitation of either RuBP regeneration or Rubisco activity. Possible limitation by RuBP regeneraton is mainly based on the findings that the ATP/ADP ratio and RuBP content are decreased in P_i-deficient chloroplasts, accompanied by increased energization of thylakoid membranes.[2,3] On the other hand, possible limitation by Rubisco activity has been favoured by the findings

that the ATP/ADP ratio and RuBP pools are increased, but thylakoid membrane energization is decreased in chloroplasts suspended in low-P_i media.[4] Under *in vivo* conditions, RuBP limitation is favoured by recent findings that thylakoid membrane energization increases as the RuBP content declines in leaves of spinach,[5] sugar cane,[6] maize and sunflower[7] grown in P_i-deficient media. Since P_i deficiency could affect carbon, lipid and nucleic acid metabolisms as well as photosynthesis,[8] the interpretation of long-term *in vivo* studies using leaves grown in P_i-deficient media seem to be problematic. Therefore, short-term (minutes to hours) changes of P_i level in the cytosol have been manipulated by feeding glucose analogues, such as deoxyglucose and mannose (MA), to detached leaves for the study of carbon partitioning of newly-fixed CO_2 between sucrose and starch.[9,10,11]

Despite intense interest in triose P_i utilization mentioned above, however, there has been few report on photosynthetic control of thylakoid electron transport by P_i deficiency *in vivo*.[9,11,12,13] Our

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* To whom correspondence should be addressed.

previous work, using P_i-deficient leaves of Chinese cabbage fed with MA, shows that most excitation energy falling upon PSII is chiefly dissipated non-photochemically, and suggests that the photosynthetic apparatus may be tuned to the state most likely to protect it from over-excitation, probably through protein phosphorylation.[14] In the present study, we show that P_i deficiency limits the photosynthesis by the decrease of ATP, which then or simultaneously induces more acidification of the thylakoid lumen, resulting in the inhibition of PSII function. In addition, indirect results for protein phosphorylation / dephosphorylation of PSII complexes in rather longer P_i deficient leaves were obtained and discussed in terms of protection of the photosynthetic apparatus from over-excitation.

MATERIALS AND METHODS

Plant materials. Primary leaves were excised from 15-day old Chinese cabbage (*Brassica campestris* L. cv Seoul) grown in a controlled environment growth chamber with a 15 h light/9h dark cycle at 25°C and then floated on either distilled water or 25 mM mannose for various times with white light at 80 μmol $\cdot$ m^{-2} $\cdot$ s^{-1} as previously described.[14]

Determinations of CO_2 exchange rates and quantum yields of photosynthesis. Rates of CO_2 exchange from detached leaves were measured using an open-gas exchange system with an infrared gas analyzer (LCA2, ADC CO., U.K.) as previously described.[14] Maximal quantum yields of photosynthesis (ΦCO_2) were calculated by dividing CO_2 uptake rates by incident photon fluence rates (PFR) according to Nie *et al.*.[15]

Determinations of adenylate and nicotinamide adenine dinucleotide phosphate contents. Leaf adenylates were isolated according to Fader and Koller[16] with a slight modification as follows. Four leaves were boiled in 4 mL of 20 mM Tris-HCl buffer (pH 7.8) containing 2 mM EDTA for 2 min and then macerated with a cold mortar and pestle after incubation at 4°C for 5 min. After mixing with 5 mL of chloroform, the supernatant was collected after centrifugation at 5,000 g for 20 min, and then used for the estimation of adenylates. ATP, ADP and AMP contents were measured luminometrically in the dark with luciferin-luciferase (Sigma FLE50) according to Strehler.[17]

Leaf NADP$^+$ and NADPH were isolated according to Macijewska and Kacperska.[18] Each of four leaves was cut into halves with a razor blade along the main vein. One half was filled with 2 mL of 50% ethanol containing 0.1 N HCl and the other with an equal volume of 50% ethanol containing 0.1N NaOH. After boiling for 3 min, they were macerated with a cold mortar and pestle and then mixed with 3 mL of chloroform. The supernatant was collected by centrifugation at 5,000 g for 20 min and used for measurements of NADP$^+$ and NADPH. Contents of NADP$^+$ and NADPH were determined by the enzyme cycling assay of Matsumura and Miyachi.[19]

Determination of in vitro whole chain electron transport in thylakoid membranes. Thylakoids were isolated as described by Critchley[20] by homogenizing 10 leaves in a Waring blender for 4 sec in an isolation medium consisting of 50 mM NaCl, 1 mM EDTA, 0.5% BSA (w/v), and 50 mM K-phosphate buffer (pH 7.5). Whole chain electron transport rates of thylakoids were measured in a Clark-type oxygen monitor (YSI, USA) in the presence of 0.1 mM MgCl$_2$, 0.1 mM methylviologen, 2.5 mM NH$_4$Cl, 0.5 mM NaN$_3$, and 50 mM K-phosphate (pH 7.5) at 25°C. The Chl content in the reaction medium, measured according to Arnon,[21] was 20 μg/mL. The incident PFR from a slide projector was 1,500 μmol$\cdot$m$^{-2}\cdot$s^{-1}.

Determinations of room temperature Chl fluorescence. Modulated Chl fluorescence emission from the upper surface of detached leaves was measured with a PAM fluorometer (PAM 101, 102 and 103, Walz Co., F.R.G.). Prior to measurements, detached leaves were dark-treated at room temperature for at least 1 h, which is sufficient to relax light-induced, energy-dependent quenching of Chl fluorescence. Data for fast induction kinetics and fluorescence relaxation kinetics following a single-turnover saturating flash (XST 103, Walz Co., F.R.G.) were collected with an IBM compatible computer fitted with a data acquisition board. Slow induction kinetics and quenching analyses were conducted as previously described by Park *et al.*[14] The PFR of actinic light for photosynthesis was 80 μmol $\cdot$ m^{-2} $\cdot$ s^{-1}. Saturating flashes of 3,800 μmol $\cdot$ m^{-2} $\cdot$ s^{-1}. were given every 30 s with duration of 0.8 s. Dark level fluorescence (Fo) and maximal fluorescence (Fm) were measured prior to actinic light illumination, and the ratio of maximal variable [(Fv)m = Fm-Fo] to maximal fluorescence (Fm), (Fv)m/Fm, was used as a measure of the efficiency of excitation energy capture by open PSII reaction centres.[22] Quenching coefficients of photochemical (qU) and nonphoto-chemical (qN) during steady state photosynthesis, corrected for Fo quenching (Fo'), were determined as described in Schreiber et al[23], and "energy"-dependent quenching (qE) and residual quenching (qR) coefficients of qN as described in Walters and Horton.[24] The Fo' level was determined immediately after turning off actinic light and then Fo quenching coefficient (qO = 1 - Fo'/Fo) was calculated according to Bilger and Schreiber.[25] Photochemical efficiency of PSII during steady state photosynthesis (ΦPSII) was expressed as CO_2/qQ according to Weis and Lechtenberg.[26]

Determinations of the oxidized proportion of PSI (a820) and its efficiency (%P700°). The proportion of oxidized P700 in the reaction center of PSI (a820) during steady state assimilation was determined according to Weis and Lechtenberg[26] by using a modified emitter-detector unit of the PAM Chl fluorometer system as described by Schreiber *et al.*[27] a820 coefficient (ΔAs/ ΔAmax) during steady state photosynthesis was calculated according to the equation of Weis and Lechtenberg.[26] ΔAs is the absorbance change at 820 nm during steady state induced by actine light and ΔAmax maximal absorbance change at 820 nm driven by a saturating far red pulse (> 715 nm, 180 μmol $\cdot$ m$^{-2}\cdot$s^{-1}.) for 1 sec

followed by 1 min of darkness after actinic light off. Quantum efficiency of PSI (%P700°) during steady state photosynthesis was expressed as (1 - a820) x 100 according to those of Harbinson et al.[28]

As all fluorescence parameters and a820 values in control leaves did not change significantly throughout the experiments, they were not represented in figures.

RESULTS AND DISCUSSION

Effects on CO_2 exchange rates and quantum yields of photosynthesis

The decrease in maximal photosynthetic CO_2 exchange rates in Chinese cabbage leaves treated with mannose (MA) for 12 h under light was considered to be due to the decline of stromal P_i content.[14] Thus, in order to investigate the changes of quanum yield of photosynthesis and of maximal photosynthetic rates caused by the development of P_i deficiency, MA-fed leaves were monitored as a function of MA feeding time. In Figure 1, maximal photosynthetic CO_2 exchange rates based on leaf area
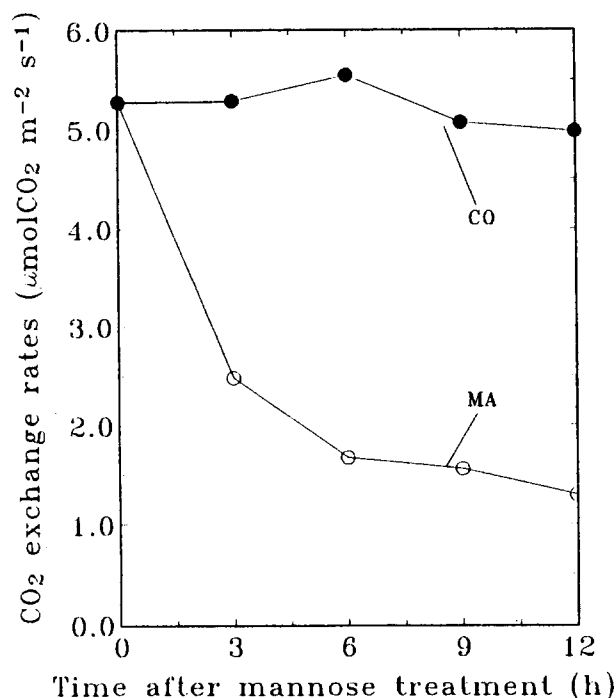

Figure 1. Changes in CO_2 exchange rates (μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$) in detached 15-day old primary leaves of Chinese cabbage as a function of mannose feeding time (h) under the light condition. CO, distilled water treated; MA, 25 mM mannose treated. The PFR for the measurement of CO_2 exchange rates was 1,200 μmol $\cdot m^{-2} \cdot s^{-1}$.

did not change significantly in contol leaves during the experiment period, but those in MA-treated

leaves rapidly declined by 73% after 6 h of treatment and thereafter, surprisingly, remained almost unchanged. Change of maximal quantum yield of photosynthesis (CO_2 fixation), obtained by dividing maximal CO_2 exchange rates by incident photon fluence rates (PFR), was very similar to that of photosynthetic rates in MA-treated leaves (Fig. 2).

In leaves fed with MA for 6 h, the response of photosynthetic rates to increasing PFR was investigated to check whether these declines of photosynthesis are casued by P_i deficiency. As shown in Figure 3, the maximal photosynthetic rates are inhibited by 73% at saturating PFR, but have little or no effect on the rate of photosynthesis at low PFR. It is generally considered that MA feeding to leaves without mannose phosphate isomerase inhibits photophosphorylation of chloroplasts because it lowers stromal P_i content by limiting recycling of P_i between the cytosol and chloroplasts.[29,30,13,11] By using a non-aqueous fractionation technique, Sharkey and Vanderveer[31] reported that stromal P_i content in spinach leaves declined by MA feeding, accompanied by a decline of maximal photosynthetic rates at saturating PFR. Accordingly, decreases of photosynthetic CO_2 exchange rates and maximal quantum yields of photosynthesis in MA fed leaves are attributable to the decline of stromal P_i.
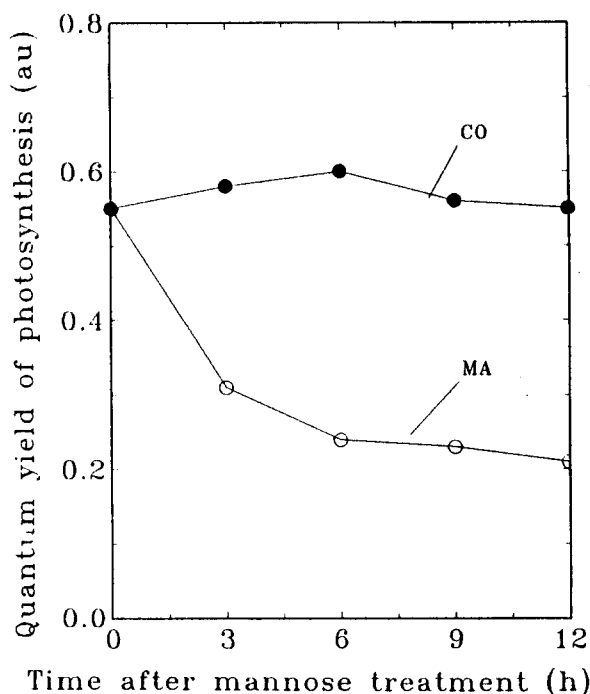

Figure. 2. Changes in the normalized apparant quantum yield of CO_2 assimilation (ΦCO_2 = CO_2 uptake rate/PFR) in detached 15-day old primary leaves of Chinese cabbage as a function of mannose feeding time (h) under light conditions. CO, distilled water treated; MA, 25 mM mannose treated. The PFR for measurements of CO_2 exchange rates was 1200 μmol$\cdot m^{-2} \cdot s^{-1}$.
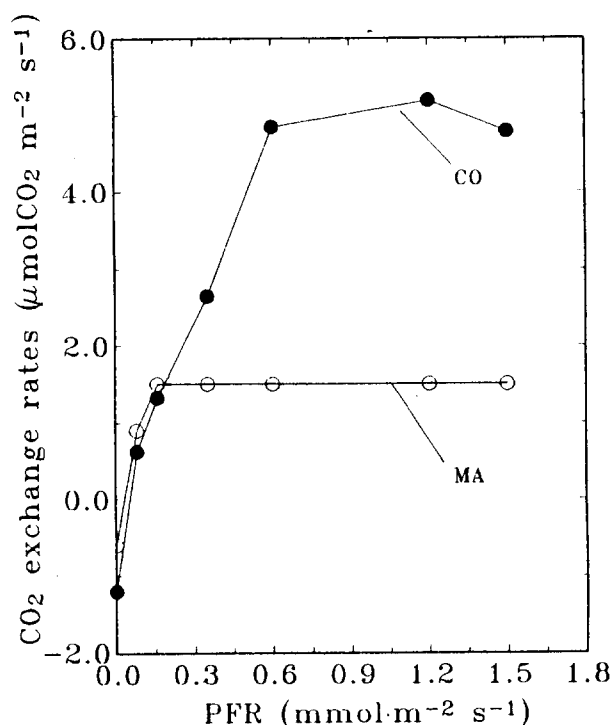
Figure 3. Changes in CO_2 exchange rates (μmol $CO_2 \cdot$ m$^{-2} \cdot$s^{-1}), as a function of photon flux density, in 15-day old detached primary leaves of Chinese cabbage treated with (MA) or without (CO) 25 mM mannose for 6 h under light conditions.

Effects on energy metabolites

Decrease of stromal P$_i$ by MA should be revealed as the declines of adenylate and NADP(H) contents, especially the ATP/ADP ratio, so we checked them in MA-fed leaves. As expected, ATP, ADP and AMP levels in MA-fed leaves declined as compared to controls (Table 1).

Table 1. Changes of adenylate content (nmol/leaf) and ratio of ATP/ADP in detached 15-day old primary leaves of Chinese cabbage (CO-0 h) incubated for 6 h and 12 h with 25 mM mannose (MA-6 h, MA-12 h) or without (CO-6 h, CO-12 h) under the light condition.

Treatment	CO-0h	CO-6h	MA-6h	CO-12h	MA-12h
ATP	0.44±0.06	0.43±0.08	0.15±0.03	0.32± 0.06	0.14±0.02
ADP	1.89±0.44	1.26±0.41	0.94±0.17	1.25±0.09	0.57±0.11
AMP	2.23±0.29	1.14±0.07	0.55±0.19	0.94±0.14	0.42±0.12
SUM	4.29±0.81	2.58±0.33	1.76±0.66	2.51±0.22	1.15±0.16
ATP/ADP	0.23	0.34	0.16	0.26	0.25

* means ± SD (n= 4 - 6)

While the ATP/ADP ratio declined by 54% in leaves treated for 6 h, interestingly, this ratio recovered to that of controls in 12 h treated leaves. This decline in the ATP/ADP ratio was reported for spinach chloroplasts with insufficient P$_i$ in media[2] and MA-treated corn leaves.[9] Assuming that changes in the ATP/ADP ratio in leaves in the light are mainly due to those of chloroplasts,[32] the decrease of this ratio after 6 h of treatment is likely to reflect inhibiton of photophosphorylation in chloroplasts through the lack of stromal P$_i$. However, recovery of the ATP/ADP ratio in 12 h MA-treated leaf implies the presence of the regulatory mechanisms which nullify the P$_i$ deficiencyinduced perturbations of photosynthetic electron transport.

The fact that NADP$^+$ in chloroplasts is made from NAD$^+$ and P$_i$ by the action of NAD$^+$ kinase prompted us to investigate both the NADP (H) contents and the NADPH/NADP$^+$ ratio since they may decline in parallel with stromal P$_i$ levels. NADP (H) content, as expected, decreased in leaves fed with MA for 6 and 12 hs, without significant change in the ratio of NADPH/NADP$^+$ (Table 2). Considering that photoperiodic changes of NADPH/NADP$^+$ in sugarcane leaves chiefly represent the changes in chloroplasts[32] and that in spinach chloroplasts photosynthetic electron transport is inhibited through over-reduction the acceptor side of PSI by decline of NADP$^+$ content,[33] the decreased photosynthetic rates of MA-fed leaves (Fig. 1) are probably due to decreased NADP(H) contents.

Effect on in vitro whole chain electron transport activity

If decreases *in vivo* maximal CO_2 exchange rates in MA-fed leaves (Figs. 1 and 3) were caused by the decline of photophosphorylation due to lack of stromal P$_i$, *in vitro* electron transport rates of thylakoid membrane isolated from MA-fed leaves should remain unchanged. We hence investigated the whole chain electron transport activities of thylakoids isolated from MA-fed leaves (Table 3).

As expected, electron transport rates of 6 h MA-treated leaves did not change, but those of the leaves from MA-treated for 12 h decreased by 30%. This decline is rather surprising since little or no further decline of photosynthetic rates occurred in leaves over 6 h (Fig. 1). These results strongly suggest that decreases in CO_2 exchange rates of 6 h MA-treated leaves resulted from limitations in photophosphorylation, while electron transport remained unchanged as in the case of spinach leaves.[5] However, the fact *in vitro* thylakoid electron transport capacity

declined by 30% for leaves treated with MA for 12 h (Table 3) with no change of photophosphorylation (Table 1) leads us to propose that some changes must have been induced in photosynthetic thylakoid membranes in case of rather prolonged P_i deficiency.

Table 2. Changes of NADP⁺ and MADPH contents (nmol/leaf) and ratio of NADPH/NADP⁺ in detached 15-day old primary leaves of Chinese cabbage (CO-0 h) incubated for 6 h and 12 h with 25 mM mannose (MA-6 h, MA-12 h) or without (CO-6 h, CO-12 h) under the light condition.

Treatment	CO-0h	CO-6h	MA-6h	CO-12h	MA-12h
NADP⁺	0.90±0.15	1.27±0.18	0.78±0.08	1.11±0.18	0.52±0.09
NADPH	0.70±0.07	0.58±0.09	0.37±0.05	0.59±0.12	0.28±0.05
SUM	1.60	1.85	1.15	1.70	0.80
NADPH/NADP⁺	0.78	0.45	0.48	0.53	0.54

* means ± SD (n= 4 - 6)

Table 3. Changes in the whole chain electron transport rates ($\mu molO_2 \cdot mgChl^{-1} \cdot h^{-1}$) of thylalkoid membrantes isolated from detached 15-day old primary leaves of Chinese cabbage (CO-0 h) incubated for 6 h and 12 h with 25 mM mannose (MA-6 h, MA-12 h) or without (CO-6 h, CO-12 h) under the light condition.

Treatment	CO-0 h	CO-6 h	MA-6 h	CO-12 h	MA-12 h
Electron transport	230.32	224.94	215.98	228.56	159.44
($\mu molO_2 \cdot mgChl^{-1} \cdot h^{-1}$)	±3.05	±19.63	±20.65	±20.65	±18.47

* means ± SD (n= 8 - 9)

The acidification of the thylakoid lumen inhibits thylakoid electron transport through not only inhibition of PSII function but also the decrease of excitation energy reaching PSII reaction centres.[34,35,36,37] Further, inhibition of PSII activity at acidic pH can be abolished when thylakoids are returned to alkaline conditions.[38] Since there were no changes in in vitro electron transprot rates in 6 h MA-treated leaves at alkaline condition, it seems natural that decreases of in vivo CO_2 exchange rates in 6 h MA-treated leaves resulted from the inhibition of PSII activities through acidification of thylakoid lumen on account of limited photophosphorylation. However, the decline of in vitro electron transport rates in 12 h MA-treated leaves could be the result of longer-term acidification of the thylakoid lumen. To evaluate the above assumptions, we first checked whether MA-fed leaves show the acidification of thylakoid lumen.

Effect on quenching coefficients during steady state photosynthesis

Quenching analysis of Chl fluorescnece allows us to monitor changes in the thylakoid lumen pH in intact leaves.[37] In order to check the acidification and its maintenance in the MA-fed leaves, quenching analysis of Chl fluorescence were conducted (Fig. 4). As the time of MA feeding increased, the fraction of reduced PSII (1 - qQ) and nonphotochemical quenching (qN) gradually increased. The energy-dependent quenching (qE), regarded as an indicator of thylakoid lumen pH, promptly increased from 0.06 to 0.19 at 3 h of treatment and then remained higher than the control during experimental period. The increase of qE implies that the thylakoid lumen of MA-fed leaves was more acidified than the control,though it is still uncertain whether it results from dissipation of excitation energy by PSII reaction centers[39,40] or de-excitation in the antenna Chl of PSII related to thylakoid zeaxanthin formed from violaxanthin at a low intrathylakoid pH.[41] Anyway, the increases of (1 - qQ) and qE suggest the above assumptions that P_i-deficiency may induce the acidification of thy-
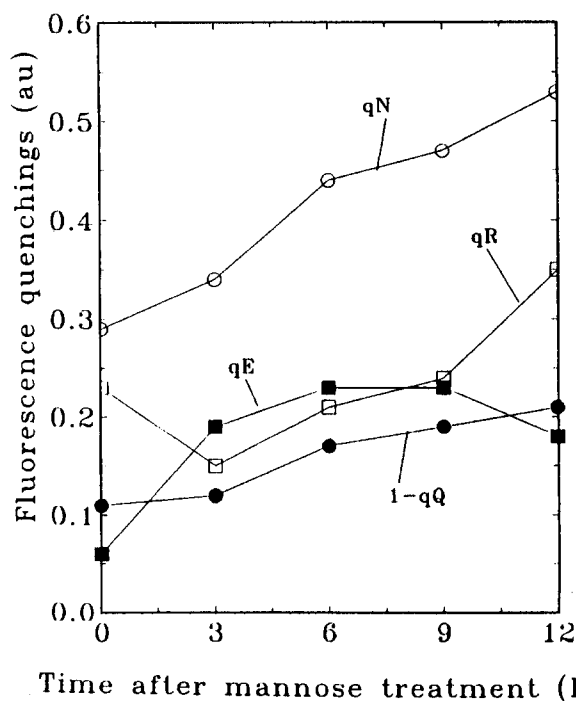

Time after mannose treatment (h)

Figure 4. Changes in Chl fluorescence quenching coefficients during steady-state photosynthesis in detached 15-day old primary leaves of Chinese cabbage as a function of mannose feeding time (h) under light conditions. qQ, photochemical quenching coefficient; qN, nonphotochemical quenching coefficient; qE, energy- dependent quenching coefficient; qR, residual quenching coefficeint (qT + qI). PFR of actinic light was 80 $\mu mol \cdot m^{-2} \cdot s^{-1}$. All quenching coefficients of control leaves did not change throughout the experiment.

lakoid lumen due to the inhibiiton of photophosphory-lation. Similar results have been reported in spinach thylakoid membranes suspended in < 1 mM P$_i$[36] and in sugar cane leaves grown in P$_i$-deficient media.[42]

The residual quenching coefficient (qR) including both state-transition related quenching (qT) and photoinhibitory quenching (qI)[24] was slightly de-creased from 0.23 to 0.15 at 3 h treatment and then increased upto 0.30 after 12 h. Incidentally, the ac-tinic light used in this experiment for both induction of photosynthesis and MA treatment was only 80 μmol·m^{-2}·s^{-1}, which is probably too low to induce the qI components, so the greater part of qR is regarded as qT.[43,44] Since the leaves are unlikely to be photoinhibited,[14,5] considerable increases in qR in 12 h treated leaves might indirectly reveal the occurrence of state-transition in PSII complexes.

Effects on Chl fluorescence induction kinetics

Reduction of PSII has also been known to be inhibited by the acidification of thylakoid lumen.[38] Therefore, we investigated both the fast induction kinetics of Chl fluorescnece and dark decay kinetics. The kinetics of biphasic Chl fluorescence rise upon a dark-light transition in Chinese cabbage leaves are shown in Figure 5. Both the fluorescence rise from the constant fluorescence (Fo) to the intermediate fluorescence (Fi) and from Fi to peak fluorescence (Fp) decreased gradually for upto 9 h of MA-feeding and then remained unchanged, accompanied by an increase in Fo in 9 h - and 12 h - treated leaves. Since the fluorescence rise from Fo is dependent on the redox state of primary PSII electron acceptor (Q$_A$),[44] this decrease in variable fluorescence in MA-treated leaves might be caused by either the limited reducton of oxidized Q$_A$ or accelerated reoxidation of reduced Q$_A^-$ or both.

The kinetics of reoxidation of Q$_A^-$ is usually monitored by tracing the rapid dark decay kinetics using single turnover flashes.[45] The reoxidation kinetics of Q$_A^-$ in MA-fed leaves for various periods were measured using single turnover flash lamp (Fig.6). With increasing MA-feeding time the maximal intermediate fluorescence level (F$_{MI}$)
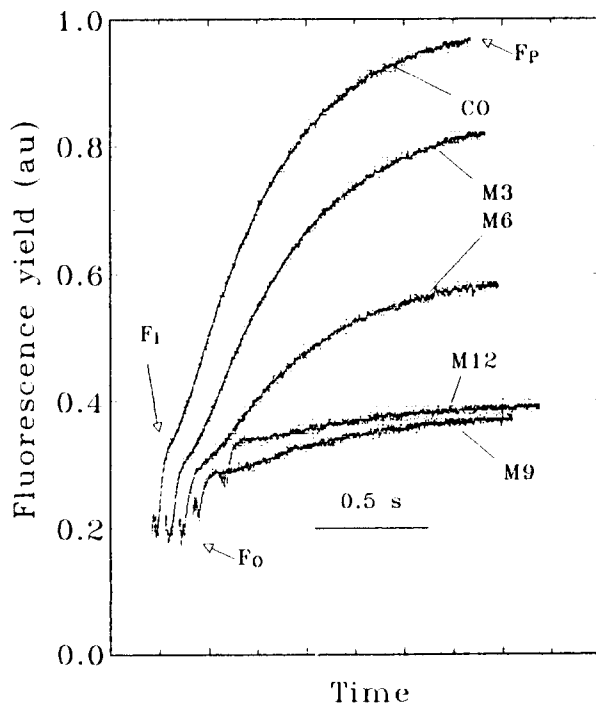

Figure 5. Changes in rapid induction kinetics of Chl fluorescence in detached 15-day old primary leaves of Chinese cabbage as a function of mannose feeding time (h) under light conditions. CO, distilled water treated; M, 25 mM mannose treated; Fo, dark level of fluorescence, Fi, intermediate fluorescence yield; Fp, peak fluorescence yield. Each number represents the mannose feeding time (h). The PFR of actinic light was 80 μmol ·m^{-2} ·s^{-1}. The rapid induction kinetics of Chl fluorescence in control leaves did not change throughout the experiment.
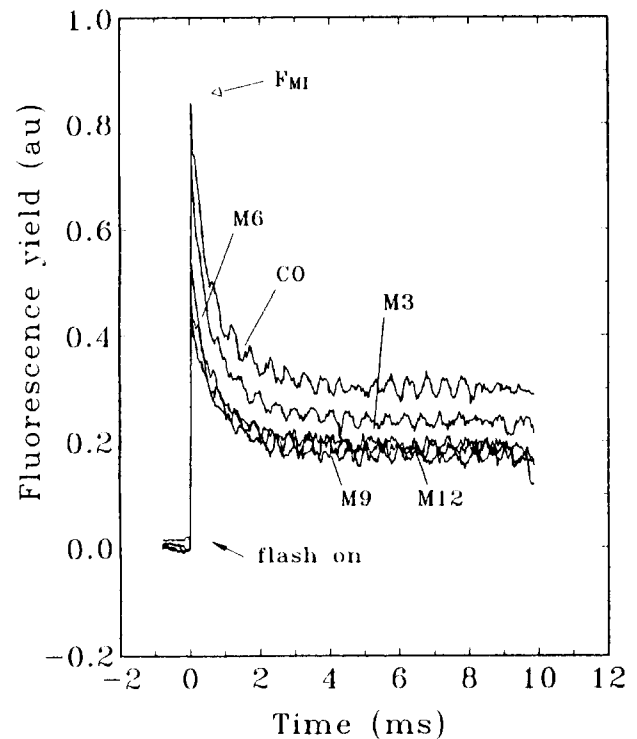

Figure 6. Changes in rapid decay kinetics of Chl fluorescence in detached 15-day old primary leaves of Chinese cabbage as a function of mannose feeding time (h) under light conditions. Saturating single turnover flash was given by a xenon flash lamp (XST 103, H. Walz). F$_{MI}$, maximal fluorescence yield induced by saturating single flash; CO, distilled water treated; M, 25 mM man-nose treated. Each number represents the mannose feed-ing time (h). The rapid decay kinetics of Chl fluo-rescence in control leaves did not change throughout the experiment.

declined significantly for upto 9 h and then decreased no further, with little change in decay kinetics from F_{MI} to steady sate level (F_S). F_{MI} level is consistent with the approximate fraction of reduced Q_A^-, and the decay kinetics from F_{MI} to F_S reveals the electron transfer from reduced Q_A^- to secondary electron acceptor (Q_B).[45] Accordingly, decreases in variable fluorescence shown in Figure 5 are due to the restriction of Q_A reduction, not because of the promotion of electron transport from Q_A^- to Q_B (and to the PQ pool). These MA effects on the fast induction kinetics and rapid dark decay kinetics over 6 h are similar to those of hydroxylamine and Tris, acidic pH, which act as inhibitors on the PSII acceptor sides in spinach chloroplasts.[46,40] Therefore, it is reasonable to think that Q_A reduction in MA-fed leaves is inhibited by that of PSII donor side owing to acidification of thylakoid lumen.

Effects on efficiency of excitation energy capture by PSII

The efficiency of excitation energy capture by open PSII reaction centres, expressed as the ratio of maximum variable (Fv)m to maximal Chl

fluorescence (F_m), declined from 0.82 to 0.65 during the 6 h of MA treatment due to decline of (Fv)m and then further decreased to 0.4 at 12 h through an increase in Fo in addition to a decrease of (Fv)m, as shown in Figure 7. Cleland *et al*[47] suggested that decreases in the (Fv)m/Fm ratio are caused by increases in Fo or decreases in (Fv)m, or both. According to them, decreases in (Fv)m reveal the increased thermal dissipation of excitation energy in PSII, while increases in Fo indicates damage in PSII due to photoinhibition. However, photoinhibition was seldom induced by 12 h treatment of MA,[14] so decrease in efficiency of excitation energy capture by PSII was primarily ascribed to an increase in nonphotochemical quenching, and then accelerated by the changes of PSII antenna sizes. Though photoinhibited chloroplasts or leaves usually show an increase in Fo, the Fo level can be also varied by phosphorylation and dephosphorylation of PSII complexes including LHCII.[45]

It has been reported that Fo quenching represents the phosphorylation of PSII complexes including LHCII.[25] Hence we analyzed the slow induction kinetics of Chl fluorecence to check whether phosphorylation in P_i-deficient Chinese cabbage leaves were induced (Fig. 8).
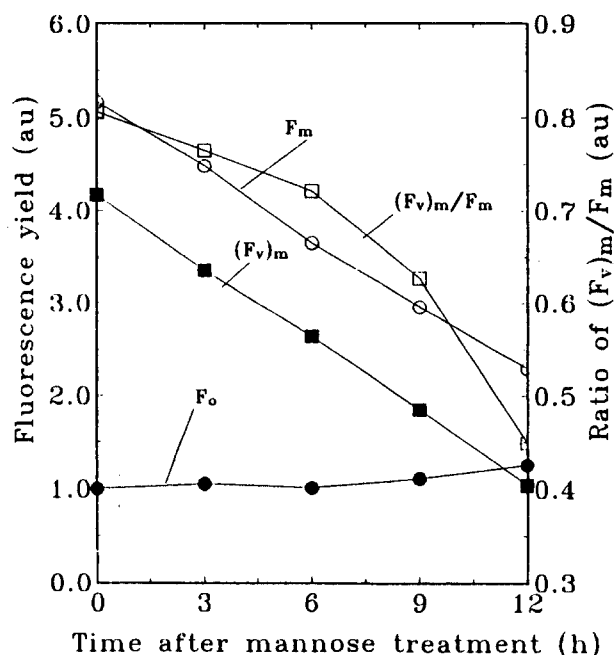
Slow induction kinetics of control leaves are

Figure 7. Changes of fluorescence parameters in detached 15-day old primary leaves of Chinese cabbage as a function of mannose feeding time (h) under the light condition. Fo, dark fluorescence yield; (Fv)m, maximal variable fluorescence yield; Fm, maximal fluorescence yield. The PFR of saturating light and pulse length were 3700 μmol·m^{-2}·s^{-1} and 800 ms, respectively. The fluorescence parameters in control leaves did not change throughout the experiment.
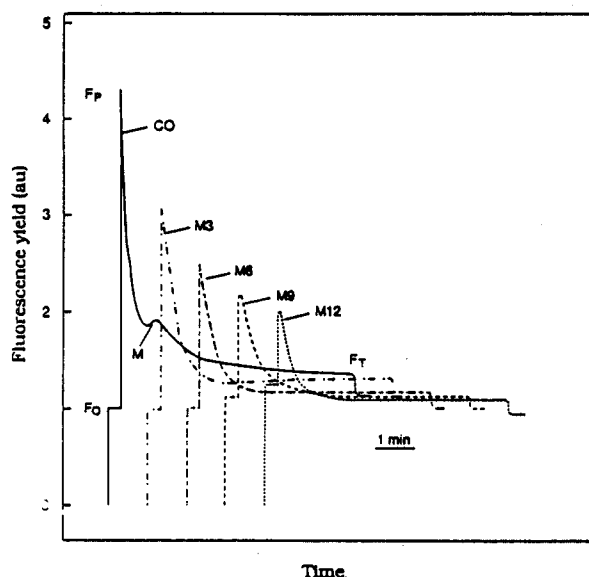

Figure 8. Changes in slow induction kinetics of Chl fluorescence in detached 15-day old primary leaves of Chinese cabbage as a function of mannose feeding time (h) under light conditions. CO, distilled water treated; M, mannose treated; Fo, dark level fluorecence yield; Fp, peak level fluorecence yield; F_T, terminal fluorescence yield. Each number represents the mannose feeding time (h). The PFR of actinic light was 80 μmol·m^{-2}·s^{-1}. The slow induction kinetics of Chl fluorescence in control leaves did not change throughout the experiment.

typical of Kautsky curves, but MA-treated leaves showed decreased peak level fluorescence (Fp) and a slower decline from Fp to the steady state level (Fs) without an intermediate peak (M). Further, MA-treated leaves over 9 h showed the decline of Fs below the original Fo level, resulting in the occurrence of Fo quenching after actinic light was turned off. Similar results as shown in MA-fed leaves upto 6 h were also reported in spinach leaf discs[12] and corn leaves[9] fed with MA. They can be explained in terms of the decrease in the ATP/ADP ratio (Table 1). In 6 h treated leaves photophosphorylation declined by 54% as compared to control, so it is expected that under these conditions the lowered pH of the thylakoid lumen results in inhibition of electron transport from PSII to PSI, representing slower decline of Chl fluorescence from Fp to Fs and lowered photosynthetic rates (Fig. 1).

Fo quenching, as in our experiments, has also been reported in leaves subjected to high actinic light intensity, CO_2 depletion and high temperature,[25,48] being explained as the decline of PSII antenna size due to detachment of LHCII.[45] Phosphorylation of PSII complexes is usually induced in the state of the over-reduction of the acceptor side of PSII and by energization of thylakoid membranes.[49] Further, thylakoid membranes with phosphorylated PSII complexes show lower electron transport rates due to the decline of PSII activity.[50,51] Therefore, we expect that the decline of in vitro whole chain electron transport rates in 12 h-MA treated leaves resulted from the phosphorylation of PSII complexes, as shown by increased Fo quenching.

In MA-fed leaves over 6 h, the efficiency of excitation energy capture was greatly decreased (Fig. 7), assuming that there are close relations between decrease of (Fv)m/Fm ratio and Ft - Fo, and Fo quenching (qO). The plot of qO against Fvm/Fm and against Ft - Fo, respectively, revealed clearly that qO coefficients had an inverse linear correlation with (Fv)m/Fm (r^2 = 0.75) and Ft - Fo (r^2 = 0.88), as shown in Figure 9. This means that Fo quenching was greater as the efficiency of excitation energy capture decreased. These relations were also reported in photoinhibited spinach leaves with no plausible explanation.[52] Though rapid decline of the (Fv)m/Fm ratio, accompanied by increased Fo, is usually regarded as the photoinhibtion of PSII, which involved the damage of D1 protein,[53] we cannot expect that photoinhibition in MA-fed leaves was induced.[14] Similar results were reported in P_i-deficient spinach leaves.[5] Accordingly, Fo increase in these experiment might be ascribed to an increase of PSII antenna size, probably due to increased dephosphoylation of phosphorylated LHCII induced
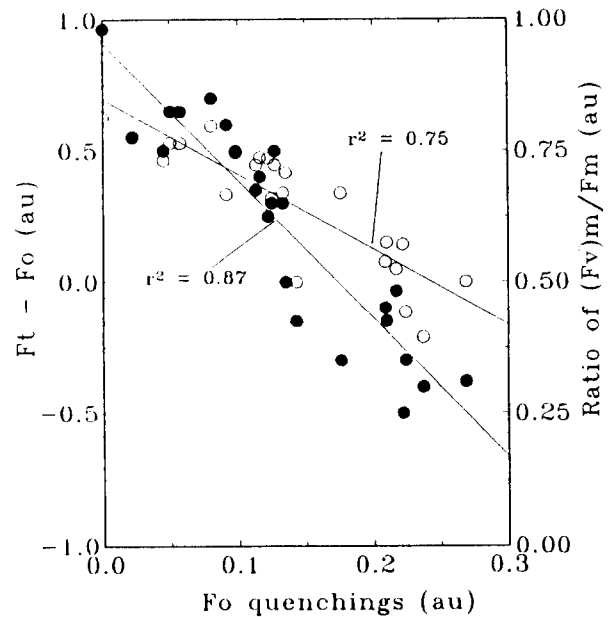

Figure 9. Correlative changes of Ft - Fo (- ● -) and (Fv)m/Fm (- O -) ratio with dark level fluorescence quenching in detached 15-day old primary leaves of Chinese cabbage treated with 25 mM mannose under light conditions. Fo quenching values were calculated after actinic light off according to Bilger and Schreiber (1986).

by P_i deficiency under light conditions. Assuming that dephosphoylation of LHCII in MA-treated leaves is faster than in control leaves during dark adaptation for 1 h, NaF, a potent inhibitor of protein phosphatase responsible for thylakoid membrane protein dephosphorylaiton[57] would enbale Fo, Fv, and (Fv)m/Fm ratio to recover faster than is the case in the control. As suspected, fluorescence parameters of 12 h MA-treated leaves nearly recovered to control levels by NaF treatment for 1 h at dark condition (Table 4).

Table 4. Effects of 25 mM NaF on fluorescence parameters of detached 15-day old primary leaves of Chinese cabbage incubated for 12 h with 25 mM mannose (MA-L, MA-D) or without (CO-L, CO-D) under the light (CO-L, MA-L) or dark (CO-D, MA-D) conditions. NaF were pretreated for 1 h in dark condition before measurements of fluorescence parameters.

Treatment	CO-L	CO-D	MA-L	MA-D	CO-L + NaF	MA-L + NaF
Fo	1.00	0.94±0.08	1.25±0.13	1.08±0.10	0.86±0.05	1.03±0.02
(Fv)m	4.90±0.40	3.35±0.31	1.35±0.36	3.10±0.38	2.64±0.18	3.26±0.14
Fm	5.90±0.44	4.30±0.38	3.00±0.42	4.18±0.06	3.50±0.11	4.33±0.14
(Fv)m/Fm	0.82±0.01	0.78±0.01	0.45±0.06	0.74±0.02	0.76±0.12	0.73±0.12

* means ± SD (n = 9 - 11)

Hence, we noticed that the degree of phosphorylation/ dephosphorylation of PSII complexes are increased in P_i-deficient conditions.

Effects on the quantum yields of PSII and PSI

The quantum yield of photosynthetic CO_2 exchange rates (ΦCO_2) during steady-state photosynthesis is dependent both upon the intrinsic quantum yield of open PSII ($\Phi PSII$) and the fraction of open PSII (qQ).[33,39] Since qQ coefficients in MA fed leaves gradually declined with time (Fig. 4), it might be assumed that the oxidation state of PSII during steady-state photosynthesis should be decreased in a similar pattern as that of CO_2 fixation. This was shown in Figure 10. Photochemical efficiencies of PSII ($\Phi PSII = \Phi CO_2/qQ$) during steady-state photosynthesis rapidly declined upto 6 h after MA treatment and then remained unchanged. As the decline pattern of $\Phi PSII$ was nearly the same as that of ΦCO_2 fixation, it might be concluded that P_i deficiency reduced the photosynthetic capacity due to the loss of quantum efficiency of open PSII reaction centres. This decrease in $\Phi PSII$ occurs in leaves or chloroplasts subjected to high PFR conditions, and is regarded as due to the development of non-photochemical quenchings, especially high-energy state quenchings.[34] Therefore,

we suggest that the decrease of $\Phi PSII$ in MA-fed leaves should be attributable to the acidification of thylakoid lumen.

Over-reduction of the PSII acceptor side in MA fed leaves could arise from either the inhibition of electron transport from PSII to PSI and/or back-pressure by over-reduction of PSI[54,27] due to the decline of ferredoxin-NADP$^+$ reductase activity or the NADP$^+$ pools[33]. In MA-fed leaves NADP$^+$ content was decreased, so it might be expected that during steady-state photosynthesis, PSI is more reduced by comparison with controls, and this condition results in the over-reduction of PSII. To check this, we investigated the changes of oxidized fraction of PSI (a820) in MA-fed leaves as a function of treatment period, as shown in Figure 11. a820 in MA-fed leaves slowly increased. Consequently, it is evident that the increase of reduced PSII resulted from the inhibition of electron transport from PSII to PSI, not because of the back-pressure from over-reduced PSI due to the decline of NADP$^+$ content. Incidentally, the oxidized form of PSI, P700$^+$, is an active nonphotochemical quencher of excitation energy, so there is a reverse correlation between a820 and the quantum efficiency of PSI during steady state photosynthesis (%P700°).[55] Consequently, %P700° declined in MA-fed leaves due to the increase of a820, as shown in Figure 11. The a820 values in many plants, except the Chl b-less barley mutant, increased in case that acidification of
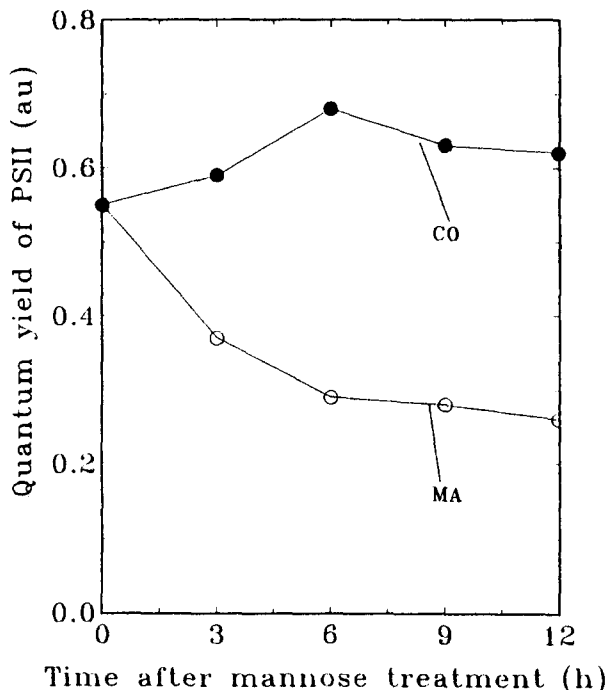

Figure 10. Changes in the normalized intrinsic quantum yield of open PSII ($\Phi PSII = \Phi CO_2/qQ$) in detached 15-day old primary leaves of Chinese cabbage as a function of mannose feeding time (h) under light conditions. CO, distilled water treated; MA, 25 mM mannose treated.
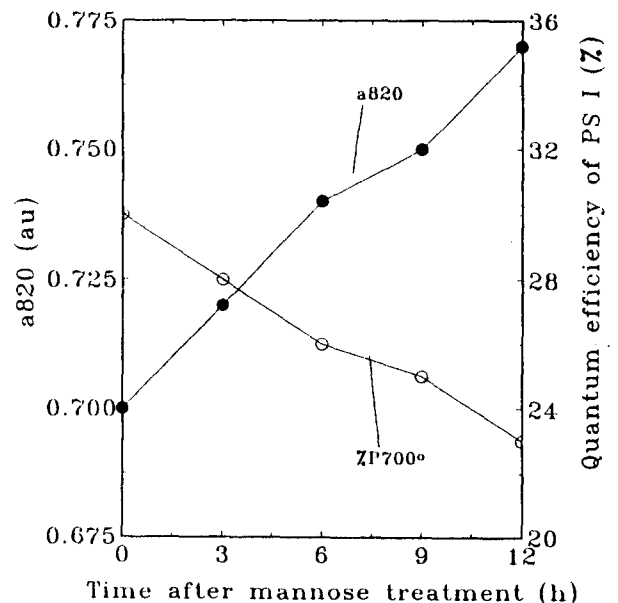

Figure 11. Changes in oxidized fraction of PSI (a820) and quantum efficeincy of PS I (%P 700°) in detached 15-day old primary leaves of Chinese cabbage as a function of mannose feeding time (h) under light conditions. Both values of a820 and %P700° in control leaves did not change throughout the experimental period.

thylakoid lumen were induced under high PFR or by methylviologen, with inhibition of PQH_2 reoxidation by Cyt b_6/f, resulting in both over-reduction of PSII and overoxidation of PSI.[56,54,33,27] From the above, it is reasonable to think that decreases of quantum efficiency of PSII and PSI in MA-fed leaves are ascribed to the acidification of thylakoid lumen (Fig. 4), resulting in the decrease of photosynthetic capacity in P_i deficient leaves.

CONCLUSION

The decreases of maximal photosynthetic rate in P_i-deficient Chinese cabbage leaves resulted from the inhibition of electron transport from PSII to PSI due to a low pH in the thylakoid lumen. When the acidification condition was maintained for a long period, the photosynthetic apparatus was tuned to a state which dissipated excitation energy in the PSII antenna, providing protection mechanism for the PSII reaction centre, probably *via* protein phosphorylation.

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