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## **The photosynthetic electron transport in thylakoids of the facultative CAM plant *Mesembryanthemum crystallinum* (L.)**

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### **Introduction**

Crassulacean acid metabolism (CAM) is characterized by a circadianly varying demand for ATP relative to NADPH. In addition, the absolute demand for electrons could fluctuate with the availability of CO<sub>2</sub>.

In CAM phases II and IV (nomenclature according to Osmond 1978) CO<sub>2</sub> is fixed in a C<sub>3</sub> like manner and the whole chain electron transport in thylakoids is considered to be sufficient to deliver ATP and NADPH in the required stoichiometric ratio of 1.2 to 1.5. In the malate decarboxylation phase (CAM phase III) one pyruvate per reduced CO<sub>2</sub> is regenerated to hexoses by gluconeogenesis (Holtum and Osmond 1981, Winter and Smith 1996). In this cycle, the pyruvate-P-dikinase (PPDK) requires 2 extra ATP. As in *M. crystallinum* the PPDK is located in the chloroplasts (Kondo et al. 1998), the plastidic demand for ATP, relative to NADPH, transiently increases to a value of 1.6 to 2.0. This extra demand for ATP cannot be covered by linear electron flow. In this phase, chloroplasts need an additional source of ATP, otherwise, the photosynthetic carbon cycle and related reactions would become limited by ATP availability. This would diminish the efficiency of photosynthetic metabolism and provide the danger of photodynamic damage in high light.

One possible mechanism to produce the required extra ATP could be the induction of cyclic electron flux around PS I. As cyclic flux requires Cyt.-bf and PS I otherwise involved in linear flux (Albertsson 1995), its induction could also serve to reduce linear electron flux and thus prevent photooxidative reactions like the Mehler reaction.

In this study, we investigated the photosynthetic electron transport in the facultative CAM-plant *M. crystallinum*. Our results provide evidence for a substantial circadian reorganization of the photosynthetic electron transport chain, which could serve to adjust the electron fluxes to the variable demand for NADPH and ATP during the CAM cycle. In CAM phases II and III the intrinsic capacity of the electron transport from H<sub>2</sub>O to PS I was diminished, but completely recovered during the C<sub>3</sub>-like phase IV. Obviously, in phases II and III one fraction each of PS I and cyt.-bf was deprived from whole chain electron transport.

### **Materials and methods**

*M. crystallinum* was grown hydroponically (10h light, 350µmole quanta m<sup>-2</sup> s<sup>-1</sup>; 20°C day, 14°C night temperature). CAM was induced in 8 to 10 week old plants by step-wise addition of NaCl to a final concentration of 500mM in the nutrient solution. Measurements were

performed 14 to 28 days after NaCl application. Thylakoids were isolated according to Jensen and Bassham (1966, modified).

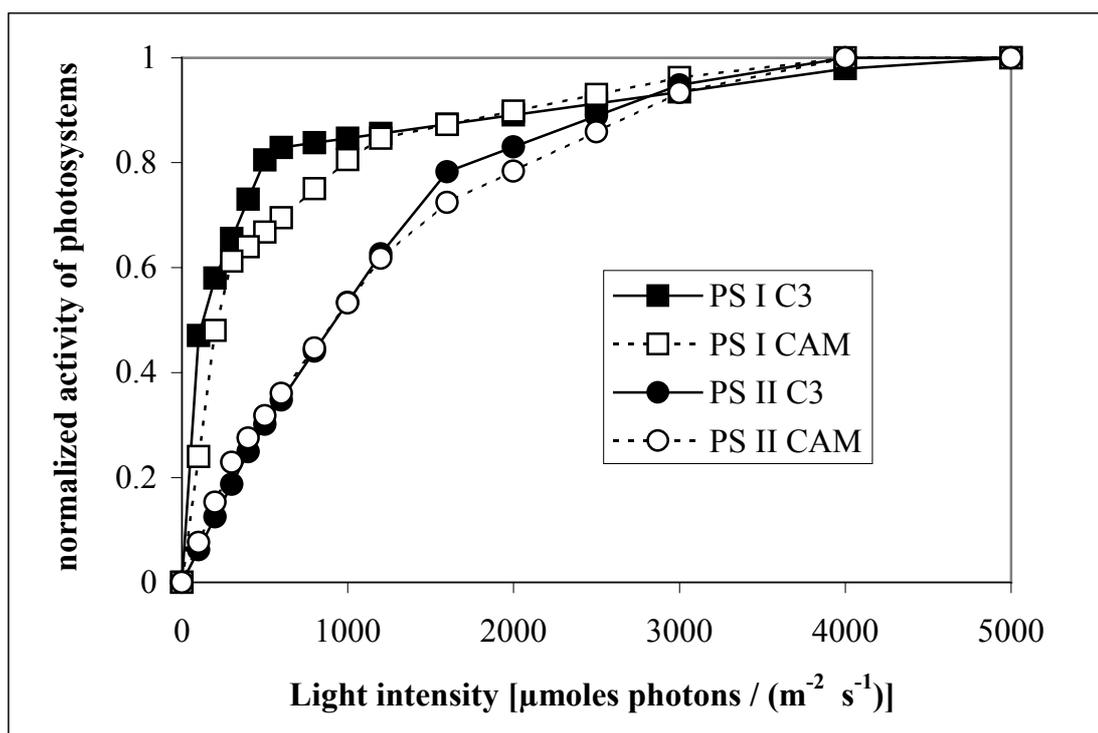
Electron transport was measured at 20°C in saturating light in presence of 10µM nigerizin with a Clark-type oxygen electrode (Hansatech). Linear electron flux was measured in presence of 1mM NaN<sub>3</sub> and 100µM methylviologen (H<sub>2</sub>O → MV). PS II activity was determined with 1mM DMBQ as acceptor, PS I activity in the presence of 100µM DCMU and with 10mM ascorbat/100µM TMPD as donorsystem. Cyt.-b<sub>6</sub>f activity was measured in presence of DCMU and MV with 2mM duroquinole as donor, and corrected for “electron leakage” to plastocyanin by measuring electron flow in the presence of the cyt.-b<sub>6</sub>f inhibitor DBMIB. The plastoquinone pool size was determined from chl.-*a*-fluorescence induction as described by McCauley and Melis (1986). The number of PS I centers was determined by the light induced absorption change at 702nm (P<sub>700</sub>) using an extinction coefficient  $\epsilon = 64\text{cm}^2 / \mu\text{mol}$ . PSII centers were determined from the absorption band shift (C550,  $\epsilon = 2,53\text{cm}^2 / \mu\text{mol}$ ). The spectroscopic set-up was essentially as described by Kirchhoff et al. 1998. The concentration of cyt.-bf was determined by chemical difference-spectroscopy.

Redox kinetics in leaf discs (infiltrated with 10 mM MV) were determined using a PAM 101 spectroscop (Walz Effeltrich). Photoreduction of Q<sub>A</sub> and photooxidation of P<sub>700</sub> were induced by saturating light pulses [200ms, 5000µmoles quanta m<sup>-2</sup> s<sup>-1</sup>]. Reduction of photooxidized P<sub>700</sub> was recorded using the emitter-detector unit (ED) P700DW (810 minus 870nm), oxidation of photoreduced Q<sub>A</sub> using ED101.

## Results

Absorption cross sections of PSI and PSII: Light response curves, recorded on thylakoids isolated at predawn, were mathematically defolded into three components (Blackman transformation), called  $\alpha$ -,  $\beta$ - and  $\gamma$ -components (Fig. 1). In a first approximation, the initial slopes of these components represent their relative absorption cross section. They may represent different fractions of photosystems connected to either large ( $\alpha$  – components) or small ( $\beta$ -,  $\gamma$ - components) antennae.

The data revealed an unusual small absorption cross section for PS II, compared to “standard” C<sub>3</sub> plants like spinach. Only a small fraction of PS II (about 15% of total) saturated at “usual” light intensities of about 400 µmoles quanta m<sup>-2</sup> s<sup>-1</sup>. The two major components saturated at about 2000 and 4000 µmoles quanta m<sup>-2</sup> s<sup>-1</sup>. The small number of PS II with high absorption cross section (“PSII- $\alpha$ ”) was confirmed by fluorescence induction studies (not shown). The absorption cross section of PS I was considerably higher than that of PS II. The  $\alpha$  – phases made up for about 50% of total PS I and saturated at about 100 to 150 µmoles quanta m<sup>-2</sup> s<sup>-1</sup>. So, in both the C<sub>3</sub> and CAM state, the rate of PS I driven electron transport clearly exceeded PS II activity and thus linear electron flux at growth irradiances of 350µmole quanta m<sup>-2</sup> s<sup>-1</sup>. This suggests that a huge fraction of PS I does not have to contribute to the linear electron flux. The functional significance of this extreme imbalance of excitation energy distribution between PS I and PS II in both metabolic states of *M. crystallinum* is not clear.



**Figure 1.** Light saturation curves of PS I and PS II dependent electron transport in thylakoids from C<sub>3</sub>- and CAM-plants. Thylakoids were isolated at predawn.

*Photosynthetic electron transport chain in isolated thylakoids of M. crystallinum.* Moderate changes in the number of PS I, PS II and cyt.-bf were found after CAM induction (*table I*). As both PS I and cyt.-bf increased, while PS II slightly decreased, the “high potential chain” (cyt. f → P700) became overexpressed, relative to the electron generating “low potential chain” (PSII → PQ). However, in comparison to spinach or tobacco, already *M. crystallinum* in the C<sub>3</sub> state contained relatively high concentrations of the components of the “high potential chain”, especially cyt.-bf, relative to PS II.

**Table I.** Concentrations of photosystems, cyt.-bf and plastoquinone in mmol per mol chlorophyll. Concentrations were determined in thylakoids isolated at predawn.

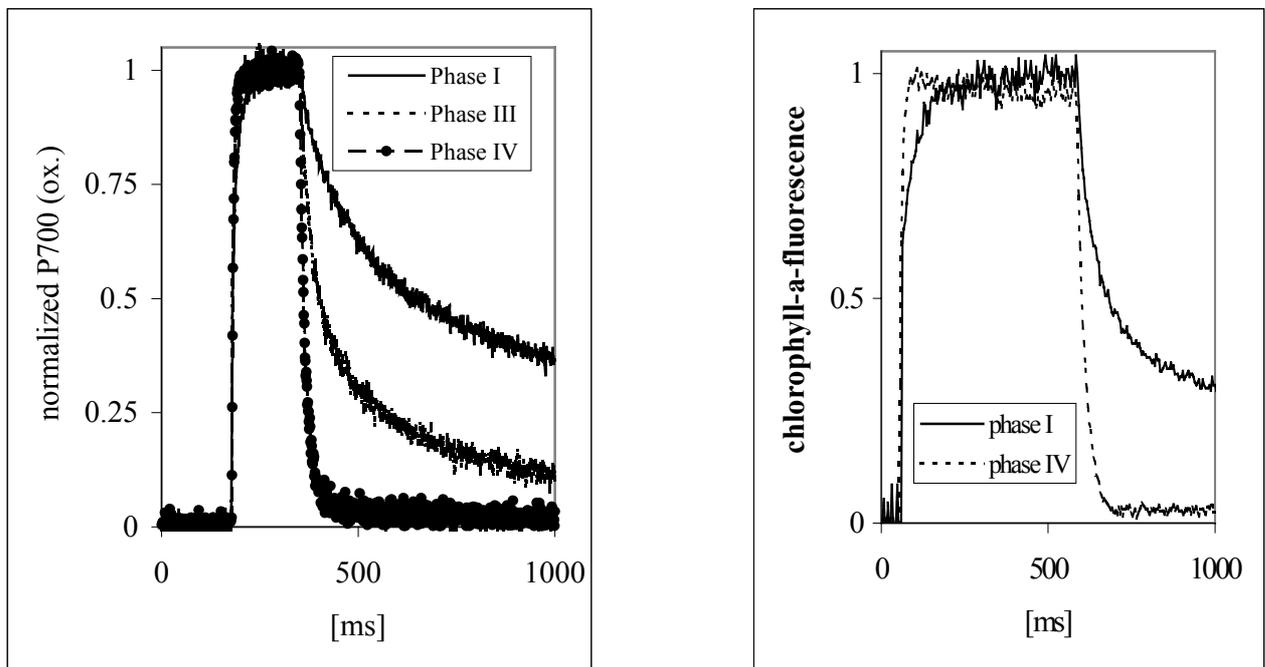
Component of electron transport chain:	C <sub>3</sub> thylakoids [mmol / mol chlorophyll]:	CAM-induced thylakoids [mmol / mol chlorophyll]:
<b>PS II:</b>	3.08	2.91
PS I:	1.74	1.84
Cyt.-bf:	1.13	1.38
Plastoquinone:	14.8	14.9
Chlorophyll a/b ratio:	3.45	3.50

Changes in the concentrations were reflected in the activities of these complexes (*table II*). Interestingly, after CAM induction, the whole chain flux ( $\text{H}_2\text{O} \rightarrow \text{MV}$ ) decreased while the overall capacity of cyt.-bf even increased. Electron flow through the cyt.-bf is considered as slowest step in electron transport and, hence, should considerably contribute to the overall flux control. In  $\text{C}_3$  plants the whole chain flux nearly matched the overall cyt.-bf activity. In CAM plants, however, there was a considerable surplus of cyt.-bf activity not involved in whole chain flux (about 20% of total cyt.-bf activity): One fraction of cyt.-bf seemed to be deprived from the linear electron transport chain.

**Table II.** Light saturated whole chain electron flux ( $\text{H}_2\text{O} \rightarrow \text{MV}$ ) and PS II-, PS I- and cyt.-bf dependent electron flux. All rates are given as  $\mu\text{moles electron pairs mg chl}^{-1} \text{h}^{-1}$  and were determined in thylakoids isolated at predawn (end of CAM phase I).

Metabolic state	$\text{H}_2\text{O} \rightarrow \text{MV}$	<b>PS II</b>	PS I	Cyt.-b <sub>6</sub> f	cyt.-bf <i>minus</i> $\text{H}_2\text{O} \rightarrow \text{MV}$
$\text{C}_3$ :	339.7	703.7	524.7	359.1	19.4
CAM:	310.5	645.8	557.7	386.3	75.8

*Photosynthetic electron transport in leaves.* The control of the linear electron flux “in situ” was investigated from chlorophyll-*a*-fluorescence relaxation (flux from photoreduced PQ through cyt.-bf) and reduction of photooxidized  $\text{P}_{700}$  (“high potential chain”,  $\text{PQH}_2 \rightarrow \text{PS I}$ ) after a saturating pulse. In  $\text{C}_3$  plants, more than 90% of photooxidized  $\text{P}_{700}$  were reduced

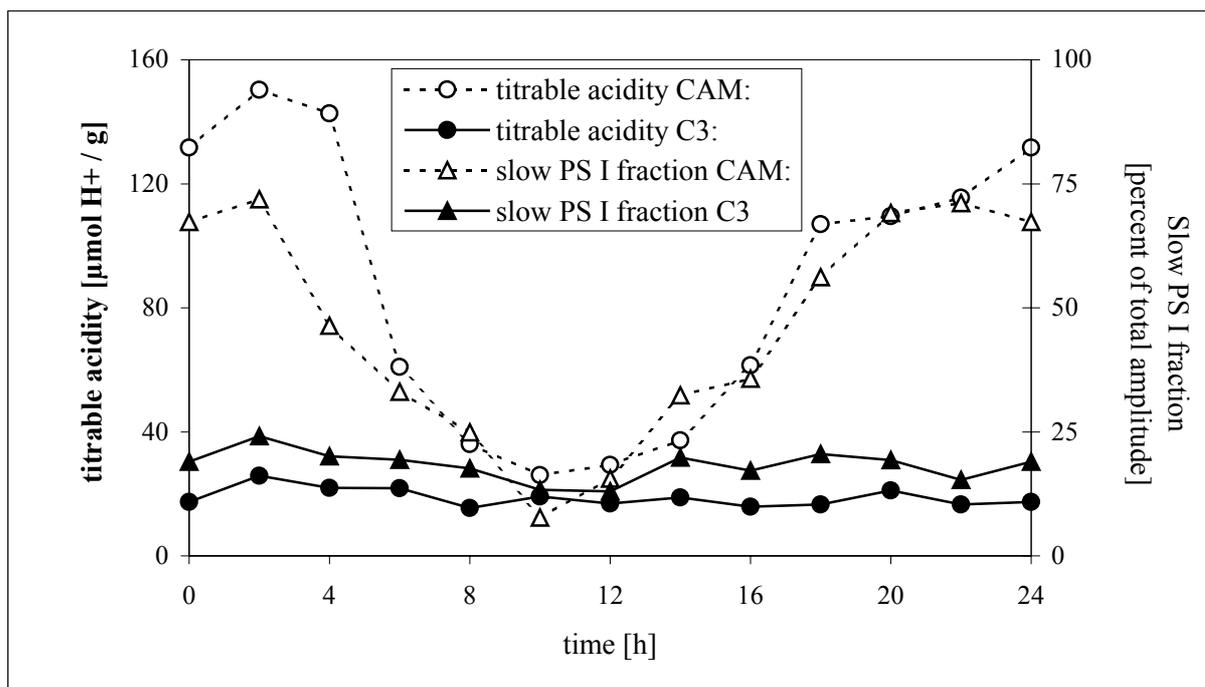


**Figure 2 (left).**  $\text{P}_{700}^+$  transients (P810) in leaf discs induced by saturating light pulses in CAM plants. Leaf discs were harvested from plants at the end of CAM phase I, at the end of phase III and in phase IV, and infiltrated with MV. The saturating light pulse was given between 150 and 350ms. Signal amplitudes were normalized to one.

**Figure 3 (right).** Chlorophyll-a-fluorescence relaxation; data normalized to  $F_0 = 0$  and  $F_M = 1$ . Leaf discs were harvested from plants at the end of CAM-phase I and in CAM-phase IV and infiltrated with MV. The saturating light pulse was given between 50 and 550ms.

within a half time of about 20 ms, indicating that nearly all PS I were involved in the fast linear flux (turnover time 10 to 20 ms). In CAM plants,  $P_{700}$  kinetics were highly variable (figure 2). Rapid reduction was seen in the  $C_3$ -like CAM-phase IV. During CAM-phase I, however, in parallel to malate accumulation a fraction of PS I appeared which was no longer reduced by linear electron flux, as was suggested by the extremely slow half time ( $> 250$ ms) of the reduction kinetics. At the beginning of the decarboxylation phase less than 50% of  $P_{700}$  relaxed rapidly, the other  $P_{700}$  belonged to the slowly reduced fraction. The slow PS I fraction disappeared again in parallel to malate decarboxylation (figure 4). While PS I function oscillated with the circadian CAM cycle, the overall number of PS I centers (determined from the amplitude of the  $P_{810}$ -signal) remained constant throughout the day.

Similar to  $P_{700}$  reduction, chlorophyll-a-fluorescence relaxed rapidly in  $C_3$  plants and phase IV of the CAM cycle (half time about 20ms). At the end of CAM phase I, however, the relaxation half time increased to about 80ms, indicating that the flow of electrons from photoreduced PQ through *cyt.-bf* was considerably slowed down (figure 3). Like  $P_{700}$ , fluorescence relaxation oscillated in parallel with malate accumulation (data not shown). We observed similar kinetics in isolated thylakoids; however, flux control at the end of phase I was less pronounced compared to kinetics determined on leaf discs. Overall, these observations were in good agreement with the conclusion drawn from the electron transport activities (table II), that in CAM phases I to III a fraction of *cyt.-bf* complexes was reversibly deprived from linear electron transport chain.



**Figure 4.** Circadian changes of titrable acidity (given as  $\mu\text{mol H}^+ \text{g}^{-1}$  freshweight) and the amplitude of the slowly reduced  $P_{700}^+$  (given as percent of total signal amplitude);  $P_{810}$  measurements performed as in figure 2; plants were illuminated between 0<sup>th</sup> and 10<sup>th</sup> hour.

## Discussion.

The data reveal clear evidence for a reversible depression of the whole chain electron flux during the malate decarboxylation phase of the CAM cycle, when the demand for extra ATP is highest and  $\text{CO}_2$  availability may finally become limiting: (1) In  $C_3$  plants and during phase IV of the CAM cycle, nearly all *cyt.-bf* and PS I were involved in linear flux. In parallel to

malate accumulation, a distinct slow component appeared in the reduction kinetics of photooxidized P700, indicating that a fraction of PS I (> 50%) did no more equilibrate with the high potential electron transport chain. This fraction did not contribute to the fast linear electron flux. (2) Chl.-fluorescence relaxation kinetics indicated that electron flow from the plastoquinone pool through cyt.-bf was also slowed down when malate accumulated. As the number of cyt.-bf increased during CAM induction, the redox kinetics indicated that part of them was separated from linear electron flow. Taking these observations together, it seems that after CAM induction a variable fraction of the high potential chain – PS I and cyt.-bf – is reversibly deprived from the linear electron transport.

It is tempting to speculate that these changes reflect a redistribution of photosynthetic complexes between a linear and a cyclic electron transport system. Linear flux is thought to be restricted to grana stacks while PS I and cyt.-bf located in stroma lamellae shall take part in cyclic fluxes (Anderson, 1992, Albertsson 1995). Lateral separation and restriction in the long range plastoquinone diffusion are important factors in separating distinct pools of cyt.-bf engaged in either linear or cyclic flux (Kirchhoff et al. 2000). The slowly reducing P700 also provide evidence for a fraction of PS I, well separated from the linear electron flux.

As the total number of photosynthetic complexes remained rather constant during CAM induction and within the circadian cycle, a short term redistribution of these complexes (and not concentration changes) seems to be the adaptive strategy to adjust the electron transport system to circadian fluctuations in the demand for ATP and NADPH during the CAM cycle. In the decarboxylation phase the extra ATP could be provided from a transiently established cyclic transport system.

Additionally, the restriction of linear electron flux could serve to fit the availability of electrons to the demand of the Calvin cycle, which could be slowed down in CAM phase II (when PEP-carboxylase and Rubisco compete for CO<sub>2</sub>) and at the end of phase III (when CO<sub>2</sub> is depleted due to a shortage of the vacuolar malate pool). An appropriate adjustment of electron transport capacity to the electron demand of the Calvin cycle is important to avoid potentially dangerous side reactions like the Mehler reaction.

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