The Mehler reaction in *Chlamydomonas reinhardtii* during photosynthetic induction and steady-state photosynthesis in wild-type and in a mitochondrial mutant.

Fabrice Franck and Pierre-Alain Houyoux

Lab. of Photobiology, University of Liege, Bvd du Rectorat 27, B-4000 Liège, Belgium

The occurrence, relative rate and physiological functions of O_2-dependent electron flow through Mehler reaction *in vivo* are matters of debate (Asada 1999, Badger et al. 2000). Among possible functions, initiation of ΔpH formation and of non-photochemical quenching, regulation of ATP supply and dissipation of excess light energy under stressful conditions have been suggested, and this is likely to vary from one group of photosynthetic organisms to another. The Mehler reaction is likely to occur at high rates during photosynthetic induction when enzymes of the Calvin cycle are poorly active. Its relative rate during steady-state photosynthesis in unstressed plants is, however, controversial. In green microalgae, large rates of light-dependent O_2 uptake during photosynthetic induction were reported (Radmer and Kok 1976). In *Chlamydomonas*, conflicting views were expressed on the amplitude of Mehler-type O_2-uptake at steady-state (Peltier and Thibault 1985, Sültemeyer et al. 1986). In this study we analyse in more detail the O_2-dependency of electron transport in *Chlamydomonas.*

O_2-dependent electron transport flow depends on culture conditions, respiratory activity and pre-illumination

O_2-dependency of electron transport rate (ETR) was investigated by measuring short-term effects of O_2-depletion on PSII photochemical efficiency in the light through fluorescence measurements. Measurements were performed rapidly (less than 1 min) after O_2-removal by the glucose/glucose oxydase/catalase system in order not to induce significant state 2 transition (as verified by fluorimetry).

Fig. 1 shows an example of the results obtained when such short-time effects of anaerobiosis were analysed in wild-type cells grown on minimal medium. Fluorescence parameters during a saturating light pulse were measured in control and in O_2-depleted cells after 15 s pre-illumination of varying intensities (Fig. 1A). Fm' slightly decreased with increasing intensities, which indicated a minor, O_2-independent, non-photochemical quenching at this time (15 s). The fluorescence yield at the start of the saturating light pulse, F, increased with light intensity due to decreasing photochemical quenching. This F yield was strongly increased by anoxia, indicating large O_2-dependent electron flow. Relative ETR (as the product ΦPSII*PAR) was strongly affected by O_2-depletion over the whole light intensity range studied (Fig. 1, B). Relative ETR showed saturation at around 500 µmol.m^{-2}.s^{-1}, which was essentially due to saturation of the O_2-dependent electron flow.

The table compares control and O_2-depleted ETR values obtained for wild-type in different conditions as well as for the dum22 mitochondrial mutant lacking mitochondrial complexes I and III (see Cardol et al. 2003). The presence of acetate in the culture medium significantly lowered the O_2-dependency of ETR during photosynthetic induction in dark-adapted cells. Experiments with KCN (2 mM) + BHAM (2 mM) showed that the O_2-dependency of ETR was not due to chloroplast-mitochondria interactions. The O_2-dependency of ETR during steady-state photosynthesis could be evaluated by performing short-term O_2-depletion after a 2 min pre-illumination with actinic light of 500 µmol.m^{-2}.s^{-1} in wild-type cells grown on minimal medium. Only a slight (10-15%) and poorly significant decrease in ETR was found in these conditions upon O_2-depletion. We conclude that the Mehler reaction is not quantitatively significant during steady-state photosynthesis at a light intensity close to photosynthetic saturation.

The effect of acetate on the O_2-dependency of ETR during photosynthetic induction is intriguing. Acetate strongly stimulates respiration and also induces a partial state 2 transition (Endo and Asada 2006, and data not shown). The dum22 mitochondrial mutant shows strongly impaired respiration and is completely shifted to state 2 due to non-photochemical PQ reduction (Cardol et al. 2003). In this mutant, ETR was low compared to wild-type during photosynthetic induction even in the presence of O_2. Altogether, the data suggest that O_2-dependent electron flow during photosynthetic induction is affected by state 1/state2 energy distribution. Since cyclic electron transport is favoured over linear electron flow in state 2 (Wollman 2001), this may indicate that cyclic electron flow effectively competes with O_2 reduction at PSI. Reduction of O_2-dependent electron transport, evaluated at PSII, may also be due to a general decrease of linear electron transport at state 2.

strain	medium and conditions	ETR (control)	ETR (anoxia)
Wt	TMP, dark-adapted	174 ± 9	36 ± 2
Wt	TAP, dark-adapted	162 ± 3	84 ± 11
Wt	TAP, dark-adapted (+KCN and BHAM)	150 ± 11	-
Dum 22	TAP, dark-adapted	52 ± 2	26 ± 3
Wt	TMP, light-adapted	199 ± 18	174 ± 16

(TAP: Tris-acetate medium / TMP: Tris-minimal medium)

Electron transfer to O_2 in saturating light leads to complete oxidation of PSI donor side in the s time range

In wild-type cells grown on minimal medium, the fluorescence induction curve from Fo ('O') to Fm ('P') was not affected by the removal of O_2 (Fig. 2A). However, the decline after 'P' was slower in the absence of O_2, which is consistent with earlier observations (Schreiber et al. 1995) and with the O_2-dependency of electron flow during photosynthetic induction, as shown above.

In aerated cells, T820 first showed a rapid fluctuation (labeled T0-T1-T2 here) (Fig. 2B). This is due to a sequence of partial oxidation (T0-T1) and re-reduction (T1-T2) of P700 and PC, the oxidized forms of which both absorb light at 820 nm. The T1 minimum marks the time at which electrons arising from PSII reach the PSI donor side (Schansker et al. 2003). This fluctuation was not significantly affected by the removal of O_2. Aerated cells showed a large and slower decline of T820 (T2-T3) after the initial fluctuation. This decline had a half-time of 0.6 s in our conditions. It was completely abolished after short-term anaerobiosis. The T3 minimum in the presence of O_2 corresponds to complete oxidation of the PSI donor side, as indicated by experiments with 20 µM DCMU (PSII inhibitor) or 0.5 µM DBMIB (cyt b6/f inhibitor). With these inhibitors, the transmission decrease was accelerated but the T3 level was unchanged (data not shown).

This demonstrates that in saturating light the Mehler reaction leads to complete oxidation of the PSI donor side.

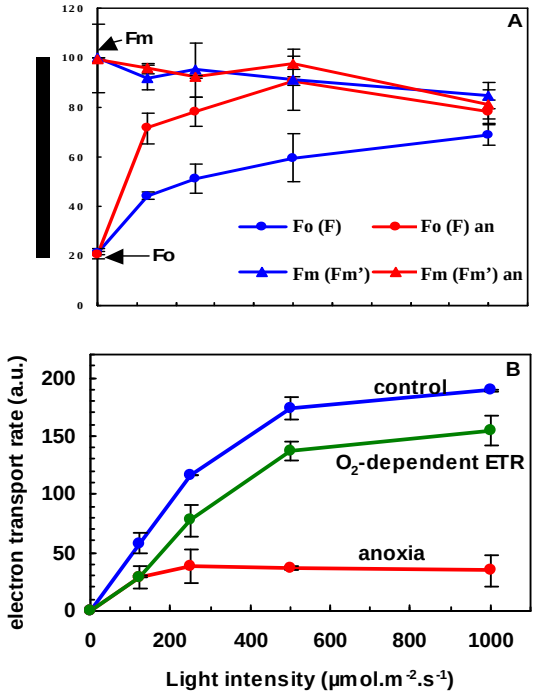

Figure 1. Short-term effects of anoxia on the fluorescence parameters of wild-type C. reinhardtii grown on minimal medium as function of pre-illumination intensity. Cells were dark-adapted for 2 hours. The F (Fo) and Fm'(Fm) levels were measured (A) during a 1s saturating light pulse (3500 µmol.m^{-2}.s^{-1}) after a 15 s pre-illumination of varying intensity. Relative electron transport rates (B) calculated from F and Fm'; O_2-dependent ETR is the difference between ETR values in presence or absence of O_2.

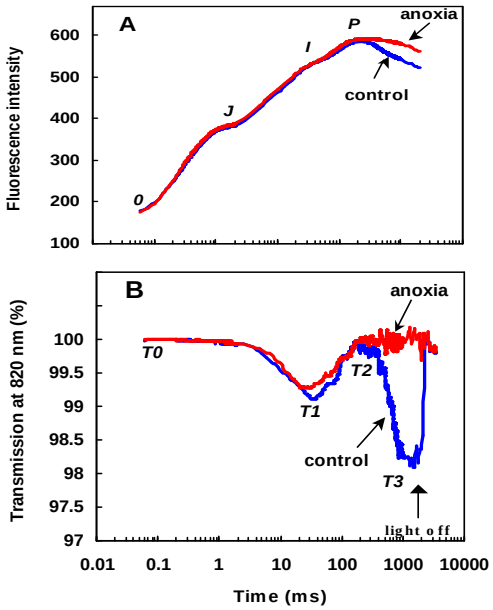

Figure 2. Short-term effects of anoxia on fluorescence induction (A) and of 820 nm transmission (B) recorded simultaneously during a saturating light pulse of 2000 µmol.m^{-2}.s^{-1} in dark-adapted wild-type cells grown on minimal medium.

References

- Asada K (1999) An. Rev. Plant Physiol. Plant Mol. Biol. 50:601-639
- Badger MR, von Caemmerrer S, Ruuska S, Nakano H (2000) Phil. Trans. R. Soc. Lond. B 355 :1433-1446
- Cardol, P, Gloire, G, Havaux, M, Remacle, C, Matagne, R, Franck, F (2003) Plant Physiol. 133:2010-2020
- Endo T, Asada K (1996) Plant Cell Physiol. 37:551-555
- Peltier G, Thibault P (1985) Plant Physiol. 79: 225-230
- Radmer JR, Kok B (1976) Plant Physiol. 58: 336-340
- Schansker G, Srivastava A, Govindjee, Strasser RJ (2003) Func. Plant Biol. 30:785-796
- Schreiber U, Endo T, Mi H, Asada K (1995) Plant Cell Physiol. 36:873-882
- Sültemeyer DF, Klug K, Fock HP (1986) Plant Physiol. 81:372-375
- Wollman F-A (2001) EMBO J 20 : 3623-3630