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

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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 AHP 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 (Rudner and Kok 1976). In *Chlamydomonas*, conflicting views were expressed on the amplitude of Mehler-type O$_2$ uptake at steady-state (Priller and Thübeck 1983, Sälhammer 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 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 oxidase/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 anerobiosis 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). Fo 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$_i$, increased with intensity to a maximum near state 2 during photochemical quenching. This F$_i$ yield was strongly increased by anoxia, indicating large O$_2$-dependent electron flow. Relative ETR (as the product $\Phi$PSII*PAR) was strongly affected by O$_2$-depletion over the whole light intensity range studied (Fig. 1, B). Relative ETR showed saturation at around 550 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 dam2 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$_2$ depletions 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 dam2 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 3/4 state 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 PSI, may also be due to a general decrease of linear electron transport at state 2.

**Figure 1.** Short-term effects of anoxia on the fluorescence parameters of wild-type *C. reinhardtii* grown on minimal medium as function of pre-induction intensity. Cells were dark-adapted for 2 hours. The F$_i$ (Fo) and F$_m$ (Fm') levels were measured (A) during a 1 s 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$_m$' and F$_o$: 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.