Oxygen dependence of photosynthesis and light-enhanced dark respiration studied by High-Resolution PhotoRespirometry

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Abstract

The bioenergetic crosstalk between mitochondria and chloroplasts plays a key role in maintaining metabolic integrity and controlling metabolite production for growth and regulation of cell concentration. Dark respiration and photosynthesis were measured in the green alga *Chlamydomonas reinhardtii* at varying oxygen concentrations and three cell concentrations using the NextGen-O2k with the PhotoBiology Module. Maximum net photosynthesis at a light intensity of 350 μmol·s⁻¹·m⁻² (blue light) was inhibited at hyperoxia by 40% at oxygen concentrations of 550 to 650 μM. Light-enhanced dark respiration reached a (negative) maximum within 30 to 60 s after light-dark transitions and was 3.5- to 4-fold higher than steady-state dark respiration independent of O₂ concentration in the range of 200 to 650 μM.

Keywords – high-resolution respirometry, photosynthesis, dark respiration, *Chlamydomonas reinhardtii*

High-Resolution PhotoRespirometry and cell culture

High-resolution respirometry based on the Oroboros O2k is extensively applied to the study of mitochondrial physiology in the biomedical field [1,2]. Real-time oxygen flux was measured using the NextGen-O2k, a two-chamber instrument, in growth medium TRIS at 25 ºC. Light intensities (blue) were controlled with the PhotoBiology-Module in the range from 0 to 350 μmol·s⁻¹·m⁻² (Figure 1). Data were recorded by DatLab 7.4.

Algae were grown photoautotrophically in growth medium TRIS (N- and P-nutrient replete) at 25 ºC and a light intensity of 100 μmol·s⁻¹·m⁻² (16:8 h L:D) [3]. Six cultures (N=6) were harvested by centrifugation at 1000 g (10 min) and diluted in TRIS.
1. O₂ flow as a function of the light regime and O₂ concentration

The net O₂ production rate (net photosynthesis NP) was stimulated from dark respiration DR at normoxia to a maximum by stepwise increments of light intensity (blue light; 10 to 350 µmol·s⁻¹·m⁻²). The compensation point at zero NP was between 10 and 20 µmol·s⁻¹·m⁻². Light-enhanced dark respiration LEDR was a sharp (negative) maximum of respiration immediately after switching off the light (Figure 1).

1a. The O₂ concentration was prevented from reaching severe hyperoxia by intermittently opening the chambers (arrows, air) and continuing the record of O₂ flow per cell I₀₂ [amol·s⁻¹·x⁻¹] [4].

1b. The O₂ concentration increased in the closed chamber due to NP. The decline in maximum NP was reversed by lowering the O₂ concentration.

1c. Light-enhanced dark respiration LEDR was a sharp (negative) maximum respiratory flux per volume j₀₂ [pmol·s⁻¹·mL⁻¹] at 30-60 s after light-dark transitions. Instrumental background BG indicated a small transient disturbance of the O₂ signal by switching off the light, which was accounted for in the background correction for O₂ flux.

Figure 1. O₂ flow I₀₂ as a function of the light regime and O₂ concentration cO₂. Superimposed traces of cO₂ and I₀₂ in two O₂k-chambers. Maximum net photosynthesis NP was obtained at light intensities of 300 to 350 µmol·s⁻¹·m⁻² (vertical numbers).

2. Dark respiration

In each of five culture harvests (experimental replica; N=5), dilution group G3 was diluted to G2. G2 was diluted further to G1. Cell concentration Cce of G3 was approximately 9·10⁶ x·mL⁻¹. Dark respiration DR expressed as O₂ flow per cell [amol·s⁻¹·x⁻¹] was independent of Cce. DR is shown relative to DR of G3 (Figure 2). DR was measured initially at normoxia simultaneously in two technical repeats of three cell dilutions (n=2 repeats × 3 dilution groups; Figure 3).

Figure 2. Dark respiration DR measured simultaneously in three cell dilutions, expressed relative to dilution group G3.
3. Maximum net photosynthesis as a function of cell concentration and O$_2$ concentration

A stepwise increase of light intensity (Figure 3; vertical numbers, 10 to 350 µmol·s$^{-1}$·m$^{-2}$) stimulated net photosynthesis $NP$ to a maximum while O$_2$ concentration increased from 220 µM to 400, 520, and 550 µM depending on cell count per volume in the closed reaction chamber (Figure 3; dilution groups G1 to G3).

The lower $NP$ capacity at higher cell concentration was caused by hyperoxic inhibition of photosynthesis (Figure 4).

Figure 3. O$_2$ flow at different cell concentrations (G1 to G3) determines O$_2$ concentrations at increasing light intensities in the closed chamber. Superimposed traces of oxygen concentration cO$_2$ and O$_2$ flow per cell lO$_2$ in two O2k-chambers. Maximum net photosynthesis $NP$ was obtained at light intensities of 300 to 350 µmol·s$^{-1}$·m$^{-2}$ (vertical numbers). DR returned to initial levels 2 h after the LEDR peak.

4. Oxygen dependence of net photosynthesis and light-enhanced dark respiration

Independent of cell concentration, $NP$ was inhibited gradually from normoxia to severe hyperoxia by up to 40 %. There were no consistent differences between measurements in the morning (am) or afternoon (pm; Figure 4).

Light-enhanced dark respiration LEDR measured at normoxia and hyperoxia was 3.5- to 4-fold higher than DR. LEDR did not significantly depend on O$_2$ concentration (Figure 4).

Figure 4. Oxygen dependence of net photosynthesis $NP$ and light-enhanced dark respiration LEDR. O$_2$ flux ratios normalized for DR. Red and green circles: data from Figure 1a and 1b.
Conclusions

The decline of net O₂ production under hyperoxia was not caused by compensatory light-enhanced photorespiration LEPR, if LEDR is proportional to LEPR [5,6], but by inhibition of photosynthesis at high oxygen concentrations. LEDR was 3.5- to 4-fold higher than steady-state dark respiration DR. DR returned to initial levels 2 h after the LEDR peak.

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Author contributions

NW and MDM conducted and EG designed the experiment. NW and EG carried out the data analysis and co-wrote the manuscript. All authors commented on and approved the manuscript.

Conflicts of interest

EG is founder and CEO of Oroboros Instruments, Innsbruck, Austria.

Data availability

Original files are available Open Access at Zenodo repository: 10.5281/zenodo.4729616

Abbreviations

C_ce count concentration of cells [Mx·mL⁻¹]; c_o2 amount concentration of oxygen [µM]; DR dark respiration; J_o2 oxygen flux per volume [pmol·s⁻¹·mL⁻¹]; I_o2 oxygen flow per cell [amol·s⁻¹·x⁻¹]; LEDR light-enhanced dark respiration; NP net photosynthesis

References


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