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Partitioning between cytochrome *c* oxidase and alternative oxidase studied by oxygen kinetics of dark respiration in *Chlamydomonas reinhardtii*: a microalgae model organism

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Introduction

Bioenergetics is the study of how living organisms acquire and transform energy to perform biological work. Energetic coupling between chloroplasts and mitochondria has been described in algae, demonstrating that a good functionality and interaction between both organelles is necessary to maintain metabolic integrity. High-resolution respirometry (HRR) is widely used to assess mitochondrial respiration and other bioenergetics parameters in the biomedical field of mitochondrial research and its clinical applications [1]. In our interdisciplinary study, we adapted the multimodal approach of the Oroboros O2k high-resolution respirometer to investigate algal bioenergetics for biotechnological purposes [2].

In contrast to mammalian cells, algal mitochondria possess alternative oxidases (AOX), which bypass electron transfer from the Q-junction through Complexes CIII and CIV [3]. Therefore, in algae we can distinguish between respiration through the Q-AOX and Q-CIV branches.

Material and methods

The microalgal model organism *Chlamydomonas reinhardtii* wild-type strain *wt12* was grown at RT in Tris-Acetate-Phosphate (TAP) medium in a 16:8 h light:dark cycle. Oxygen flux, J_{O2}, was monitored in *wt12* living cells in the exponential growth phase at 25 °C in Oroboros O2k high-resolution respirometers excluding any light in the chambers. Substrate-uncoupler-inhibitor titration (SUIT) protocols were specifically developed to characterise activities of the Q-AOX and Q-CIV branch (SUIT-022 [4] and SUIT-023 O2 [5], respectively). To quantify the contribution of the Q-AOX branch to algal dark respiration, we studied the oxygen kinetics of (*1*) ROUTINE-respiration in TAP medium, (*2*) Q-AOX dependent respiration after inhibition of CIV with 1 mM potassium cyanide (KCN), and (*3*) Q-CIV dependent respiration after inhibition of AOX with 1 mM salicylhydroxamic acid (SHAM). Oxygen kinetics was obtained from aerobic-anaerobic transitions with high time resolution at a data sampling interval of 0.2 s. p_{50} is the O_2 partial pressure, p_{O2}, at 50% of maximal respiration, J_{max} [6]. The p_{50} was calculated from hyperbolic fits using the Oroboros O2Kinetics software for automatic O_2 calibration, correction for zero O_2 signal drift, instrumental background O_2 flux and exponential time constant of the polarographic oxygen sensor [7]. A single shifted hyperbolic fit was used to fit J_{O2} as a function of p_{O2} in each aerobic-anaerobic transition.

Results and conclusions

p_{50} ranged from 0.06 to 0.08 kPa for ROUTINE-respiration with an excellent fit by a first-order hyperbolic function. This oxygen affinity is comparable to that in small mammalian cells [8]. Upon inhibition of CIV with KCN, J_{O2} was significantly impaired (Fig. 1A) and p_{50} increased three-fold up to 0.35 kPa (Fig. 2). No decline of J_{O2} and p_{50} was observed relative to ROUTINE-respiration after inhibition of AOX with SHAM (Fig. 1B). In all cases, excellent fits of respiration as a function of oxygen pressure were obtained by a first-order hyperbolic function.

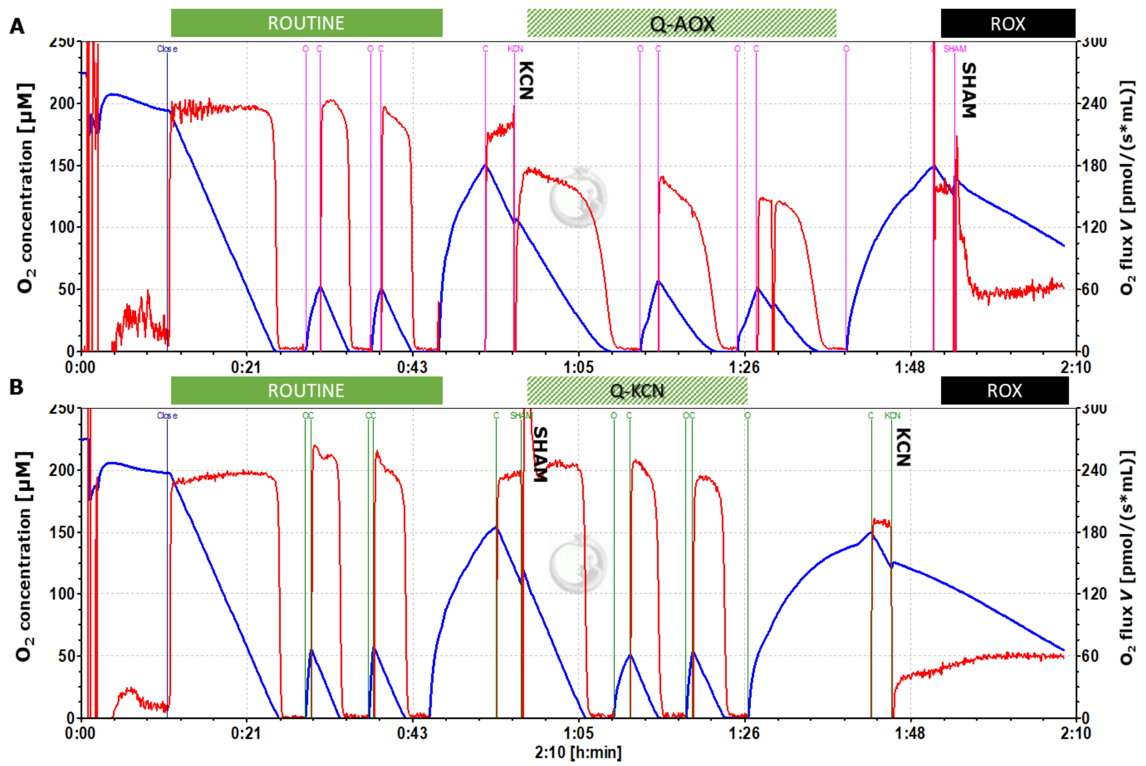

Figure 1. High-resolution respirometry for the study of dark respiration and O_2 kinetics with *C. reinhardtii wt12.* Representative O2k traces showing O_2 concentration and O_2 flux per chamber volume with repeated aerobic-anoxic transitions (O_2 kinetics) and re-oxygenations. **A:** Protocol SUIT-022: AOX-ce CN+SHAM. **B:** Protocol SUIT-023: AOX-ce SHAM+CN. Note the high technical reproducibility of ROUTINE-respiration in both protocols, and the identical and relatively high residual oxygen consumption, *Rox*, after titration of both inhibitors in both protocols.

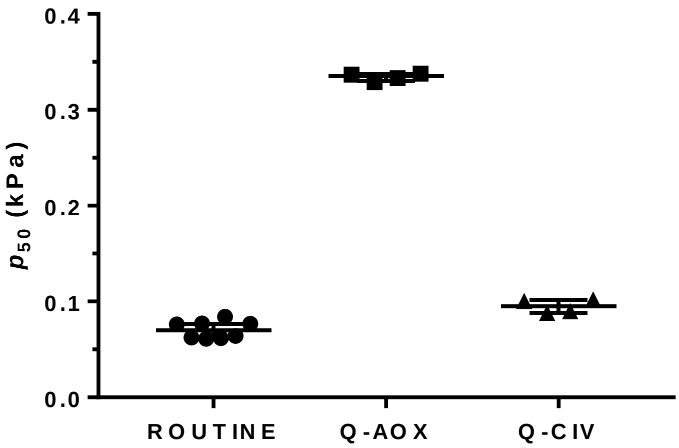

Figure 2. p_{50} in living cells of *C. reinhardtii* in the ROUTINE-state of respiration, and metabolic pathways restricted to the Q-AOX or Q-CIV branch. O_2 kinetic experiments were run in presence of the cytochrome *c* oxidase inhibitor potassium cyanide (AOX group) or the alternative oxidase inhibitor salicylhydroxamic acid (CIV group). The data represents *n*=8 technical replicates, *N*=2, median ± IR.

If the potential contribution of the Q-AOX branch in the ROUTINE-state would be compensated for by increased Q-CIV flux after addition of SHAM, then the mixed Q-AOX and Q-CIV fluxes would give rise to biphasic hyperbolic oxygen kinetics, with a contribution of the high-affinity Q-CIV branch and the low-affinity Q-AOX branch. Taken together, our results provide evidence against a contribution of AOX to ROUTINE-dark respiration in *wt12* cells under the presently applied culture conditions. Oxygen kinetics provides a sensitive and fast method for detection of Q-AOX and Q-CIV contributions to dark respiration in living cells. This kinetic approach is based on the difference of O_2 affinities of the two pathway branches, which will extend our understanding of the bioenergetics and physiology of all types of cells harbouring AOX and CIV.

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