Photosynthetic electrons transfer in *Thalassiosira pseudonana* in anoxic condition

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Introduction

Diatoms are responsible for a large part of oceanic primary production. This ecological success suggests that they have developed a range of strategies to cope with various biotic and abiotic stress factors. In this respect, several diatoms can experience more or less prolonged periods in hypoxia or anoxia. Under these conditions, cells produce ATP through catabolic pathways, usually by the glycolysis activity. Glycolysis entails the accumulation of reduced cofactors and cells have to reoxidize reduced cofactors produce in a process that involves the transfer of electrons to suitable acceptors (other than O_2) to sustain metabolic flux and allow cell viability. In this condition, photosynthetic electron flow is theorically inhibited because of Calvin-Benson cycle (CBB) inhibition in the dark and the lack of oxygen prevents oxygen photoreduction (water to water cycle). Interestingly it was shown that in similar environmental conditions, several green algae express an hydrogenase that is able to oxidize reduced ferredoxin leading to photosynthetic electron flow in anoxia. Together with cyclic electron flow around PS1 (CEF), this hydrogenase allows the Calvin-Benson Cycle (CBB) reactivation by increasing the ATP/NADPH ratio (Godaux *et al,* 2015)

Objectives

In this work, we study **the availability of photosynthetic electron acceptors and photosynthetic reactivation during dark anoxic acclimation** in the centric diatom *Thalassiosira pseudonana (TP)*. In the light of the recent report on the putative presence of several fermentative pathways in *T. pseudonana* (Atteia *et al*, 2013) we investigate the possibility that some anaerobic pathways could oxidize photosynthetic electron acceptors in diatoms.

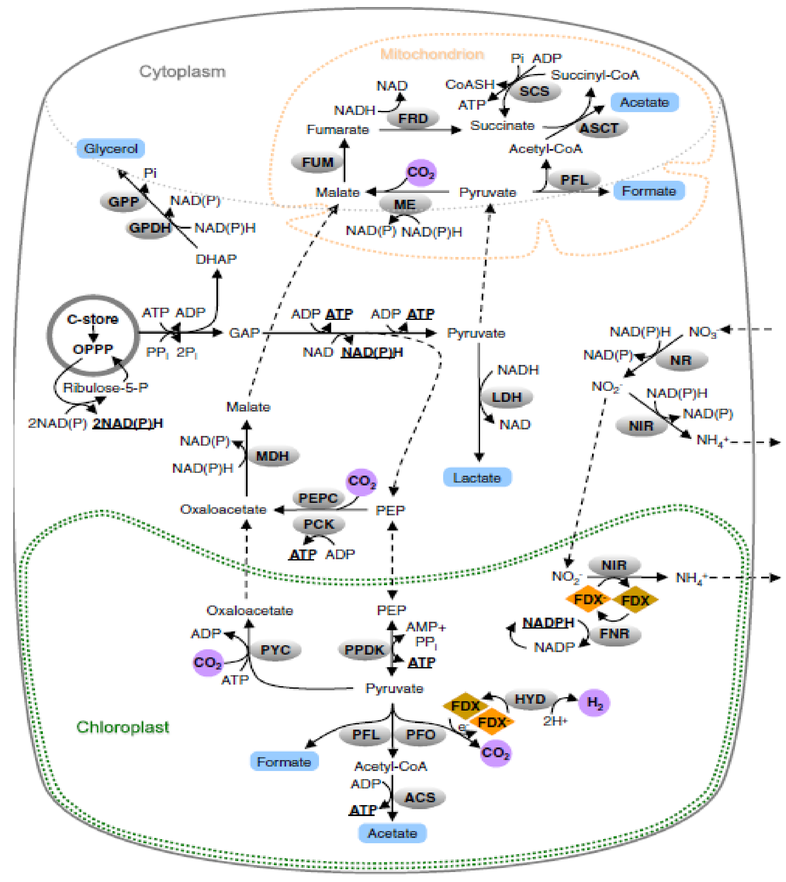
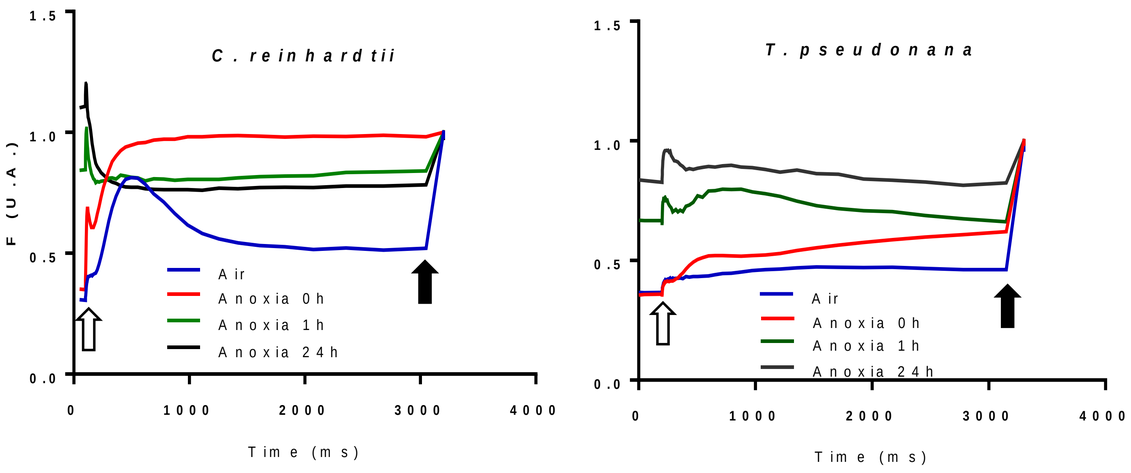


Figure 1 : Scheme of anaerobic energy metabolism of *T. pseudonana* based on genomic data study. Atteia *et al* , 2013

Results

1. Electron transfer in anoxia



We study and compare the fluorescence kinetics (3 seconds of light) in function of time in anoxia in *C. reinhardtii* (**Fig 1A**) and *T. pseudonana* (**Fig 1B**). We observe for T. *pseudonana* that at the beginning of anoxia (0h) an efficient electron transfer through PS2 occurs (**Fig 1B**) . This situation contrasts with what we observe in *C. reinhardtii* (**Fig 1A**) where the electron transfer is completely blocked in the absence of hydrogenase (0h). Then this electron transfer in *T. pseudonana* is still active after 24 hours (**Fig 1B**), but is strongly decreased. In C. reinhardtii electron transfer is sustain thanks to hydrogenase expression (**Fig 1A**). These observations suggest that there at least one pathways in anoxia that is able to reoxidize photosynthetic electron acceptors. Since this electron transfer occurs from the beginning of anoxia, we conclude that such a pathway is already expressed in oxic condition.

Fig 1 : Chlorophyll fluorescence induction curves upon 3s illumination at ~185 photons (λ = 640 nm) nm^{-2} s^{-1} after acclimation to dark aerobic and anaerobic conditions. Black arrows indicate the moment when the saturating light pulse was given. White arrows indicate the moment when the actinic light is turned on **A)** *C. reinhardtii* **B)** *T. pseudonana* . Maximal fluorescence (F_M) was normalized to 1. Anoxic condition is obtained by bubbling N_2 into the cells suspension for 15-20 min. Oxygen level is measured all along to confirm the shift in anoxic condition.

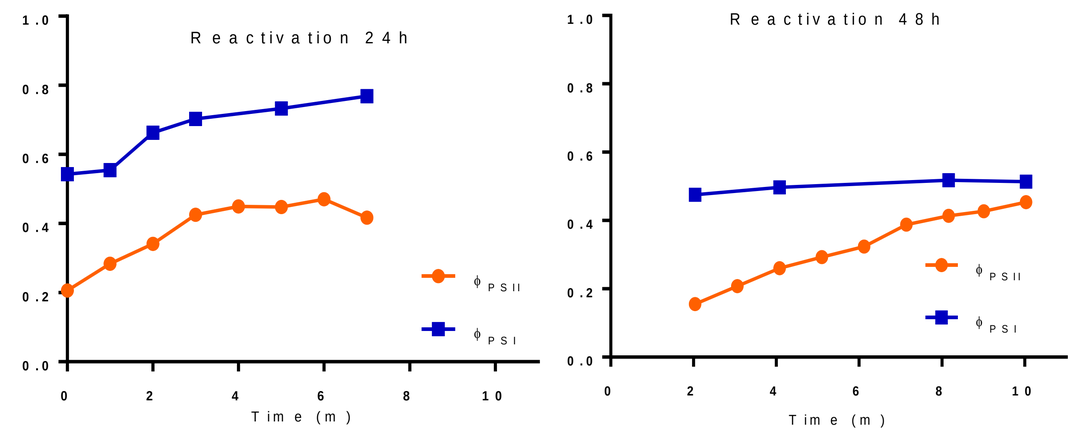
2. Identification of active fermentation pathways

In order to test if putative fermentation pathways previously identified (Atteia *et al*, 2013) are active in anoxia we made a qualitative dosage of putative fermentative products. In this analysis we specifically detect NH_4^+ and acetate after 24h in dark anoxia. In contrast no hydrogen or lactate was observed . These results show that some of proposed fermentative pathways are active in *T. pseudonana*.

Putative fermentative pathways in *T. pseudonana* (Atteia *et al*, 2013)	Fermentation Product	Dosage methods	Product detection
DNRA	NH_4	Amonnium megazyme® kit	+
ASCT, ACS	Acetate	HPLC	+
PFL	Formate	HPLC	-
HYD	H_2	Modify Clark electrode	-
LDH	Lactate	HPLC	-

Table 1. Putative fermentation pathways, associated products based on the publication of Atteia *et al,* 2013. Detection method used for highlight the presence of fermentation products after 24h of anoxia. +, product was found – product not found, ? Not tested. **DNRA** , Dissimilatory nitrate reduction to ammonium. **ASCT**, acetate:succinate CoA-transferase. **ACS**, acetyl-CoA synthase. **PFL**, pyruvate formate lyase. **Hyd**, hydrogenase. **LDH**, lactate dehydrogenase.

3. Photosynthetic reactivation



We finally monitored the activity of PS2 (ϕPS2) and PS1 (ϕPS1) during 10 minutes of illumination after 24h or 48h of anoxia. In both cases we observe that the reactivation of PS2 activity increase more than PS1 activity. This suggests an important contribution of CEF to PS1 activity during the first minutes of illumination. We also notice that PS2 reactivation is faster after 24 hours of anoxia than after 48 hours, suggesting that a fermentative pathway oxidizes reduced photosynthetic electron acceptors.

Figure 2. Activity of PS2 and PS1 (quantum yield) during a shift from dark anoxic to continuous light (~185 photons (λ = 640 nm) nm^{-2} s^{-1}) A. After 24h of anoxia, B. After 48h; C) relationship between photosystem activity during reactivation.

Conclusions

In this work, we show that in the centric diatom *Thalassiosira pseudonana* the availability of photosynthetic electron acceptors decreases during anoxic acclimation and reaches a steady state (up to 48h). We also show that some of the previously proposed fermentation pathways leading to acetate (acetate:succinate CoA-transferase and /or acetyl-CoA synthase) and NH_4^+ (dissimilatory nitrate reduction to ammonium) (Atteia et al, 2013) are active and might contribute to oxidize photosynthetic electron acceptors in anoxia. We finally show that photosynthetic reactivation after prolonged periods of dark anoxic acclimation relies on high and transient CEF. This last result contrasts with the absence of high CEF in oxic condition in diatoms (Bailleul et al, 2015). Altogether our results point to peculiar regulatory mechanisms (CEF and fermentation) that might contribute to sustain photosynthetic capacity in diatoms in hypoxic / anoxic environments.

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