On the relative contributions of the PSII-dependent and Nda2-dependent pathways to the hydrogen photoproduction in Sulfur-depleted cells of *Chlamydomonas reinhardtii*.

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Introduction

Previous studies on anaerobic H_2 photoevolution by *C. reinhardtii* under sulfur-deprivation (S-deprivation) have established that two pathways co-exist in this process (ref. 1) :

-the PSII-dependent pathway, in which water photolysis provides the electrons to the hydrogenase through the whole photosynthetic electron transport chain;

-The PSII-independent pathway, in which electrons are first donated to the PQ pool by non-photochemical reduction and are further energized through PSI. It was shown recently that a type II dehydrogenase, Nda2, catalyses this PQ reduction at the expense of NAD(P)H in *C. reinhardtii* (refs. 2 et 3). NAD(P)H is thought to arise from starch catabolism (ref. 1).

The relative contributions of these two pathways is matter of debate. On the one hand, it was shown that the inactivation of Nda2 leads to important decrease of H_2 photoevolution (ref. 2), suggesting a large contribution of the PSII-independent pathway. On the other hand, it was recently reported (ref.4) that H_2 photoevolution is not decreased in mutants that do not accumulate starch, whereas H_2 photoevolution by wild-type cells is strongly inhibited by the PSII-inhibitor DCMU. The latter results suggest that H_2 -photoevolution is essentially PSII-dependent.

We have used two RNAi cell lines (S1 and S2), which lack the chloroplastic, type II dehydrogenase Nda2 (ref.2) , in order to re-investigate the importance of non-photochemical PQ reduction for H_2 photoevolution in S-deprived cultures of *C. reinhardtii*. In the same experimental system, the effect of DCMU was also assayed in order to evaluate in the same conditions the dependence of hydrogen production on PSII activity.

The S1 and S2 lines are differently affected in the expression of Nda2. The protein is not detected in S2 while traces subsist in S1.

Protocol

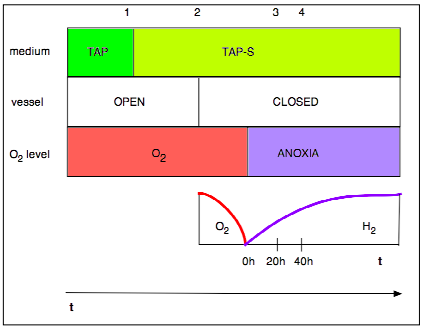

Fig 1. Schematic representation of H_2 production protocol. First the cultures were grown in TAP until they reach a concentration of 20 µg Chl.ml^{-1}. Then the algae were placed in sulfur starvation to reduce photosynthetic activity. Next, the cultures were put in air-tight photobioreactor with online monitoring of O_2 consumption and H_2 evolution. Anoxia was reached after about 12h and H_2 evolution started thereafter. Four timepoints were chosen to aliquot a part of the culture for further analyses. 1 : Cultures in TAP, 2 : culture in open TAP-S, and 3 and 4 : 20h and 40h after start of H_2 evolution, respectively.

Results

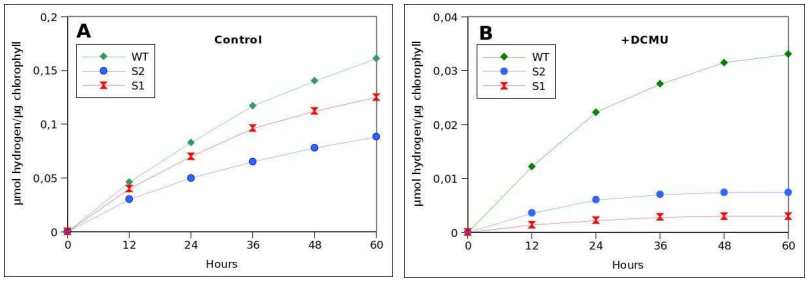

Fig 2. Time courses of H_2 photoproduction during the first 60 hours after establishment of anaerobiosis. Conditions were: light intensity 200 µmol/m^2/s, cell concentration 6-7.10^6 cells – chlorophyll concentration 20 µg.ml^{-1}, temperature 26°c, atmospheric pressure, initial pH 7,5, liquid phase 950 ml, gas phase 50 ml. Green squares : WT, blue circles : S2 (Nda2-RNAi(2)), red crosses: S1 (Nda2-RNAi(1)).
(A) incubation in sulfur deprived medium (TAP-S).
(B) incubation in sulfur deprived medium (TAP-S) with DCMU (10 µM).
Note that Y-scales are different for graphs A and B.

Fig. 2, A shows that, as previously reported, H_2 photoevolution is affected by the absence of Nda2. The S2 line, which completely lacks Nda2, is affected by 50 % in the amount of H_2 produced after 60 h. The S1 line, in which traces of Nda2 were found, has a behaviour intermediate between WT and S2. This strong effect of Nda2 deficiency is not due to a different adaptation to S-deficiency in terms of photosynthetic activity (data not shown).

In the same protocol, addition of DCMU caused a 80 % inhibition of H_2 photoevolution in WT. In both S1 and S2 lines, H_2 photoevolution with DCMU was very low (30 times less than in untreated WT) (Fig. 2,B).

Nda2 expression profile during a hydrogen production experiment

In WT, Nda2 can be detected all along the hydrogen production experiment. From the initial culture in TAP to the bioreactor until 48h of production. Nda2 seems not influenced by the presence of 10 µM DCMU (a PSII inhibitor), at 24h and 48h. The protein level seems to increase from 24 to 48h after the start of H_2 photoevolution.

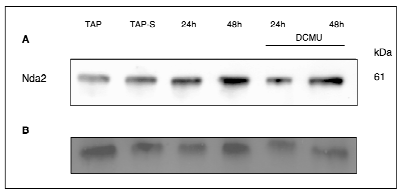

Fig 3. Western Blot analysis of purified membrane extracts of *C. reinhardtii* (2,5 µg per well) with a polyclonal antibody raised against Nda2: CrNda2. A : detection of Nda2 in a WT strain during a hydrogen production experiment, with or without addition of DCMU. B : Coomassie Blue staining of the blot as loading control.

Fig.4 show that, as expected, no Nda2 signal is visible for S2 strain, in TAP. The same result is true under sulfur depletion. During the hydrogen production phase, no signal is visible at the two timepoints 24h and 48h in the S2 strain while a signal is observed in WT strain. This signal is much increased between 24h and 48h of production, suggesting an induction of the non-photochemical pathway.

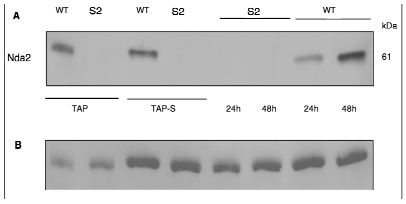

Fig 4. Western Blot analysis of purified membrane extracts of *C. reinhardtii* (2,5 µg per well) with a polyclonal antibody raised against Nda2: CrNda2. A : Detection of Nda2 in WT and S2 : Nda2-RNAi strains at differents checkpoints of hydrogen production phase : first in TAP, TAP-S and at 24 and 48 hours of production. B : Coomassie Blue staining of the blot as loading control.

Conclusions

-The PSII-independent pathway is Nda2-dependent.

- From our results, it appears that the sum of the apparent capacities of the PSII-dependent and Nda2-dependent makes more than the actuel H_2 photoevolution rate. This suggests either that the two pathways cannot operate independently from each other, or that there are indirect effects of the absence of one or the other pathway.

References

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