Chaperone molecules: a radioresistance lead for photosynthetics organisms?

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***Abstract*- Acute or chronic exposure to ionizing radiation (UVC, gamma rays, X rays…) leads to the formation of reactive oxygen species (ROS) which affect the genome and the proteome of cells. Thus, although photosynthetic microorganisms such as *Chlamydomonas reinhardtii* constitute one of the main hopes for developing loop systems during long-term space travel, their use is called into question by the decrease of efficiency of the photochemistry and by the growth arrest cause by ROS. Our project aims to make *Chlamydomonas reinhardtii* produce a peptide complexing with the Mn2+ ion inspired by a metabolite found in the radioresistant organism *Deinococcus radiodurans* and acting as a ROS scavenger. This study requires to demonstrate a decrease in ROS within the cell during production of the peptide and verifying the growth of microalgae cultivated in minimum medium (photosynthesis dependent growth).**

***Index Terms*-** *Chlamydomonas reinhardtii*, transgene, radioresistance, ROS scavengers.

I. INTRODUCTION

A space trip such as an Earth-Mars/Mars-Earth journey could last between one and three years. The type and quantity of radiation absorbed by the crew would then be very different from those encountered in the shelter of the Earth's magnetic field: UVB, UVC but also X-rays and gamma rays, would reach doses close to 0.210 +/- 0.040 mGy/day (Hassler et al., 2014). The availability of food and breathable air resources are other limitations to long-duration space flights. To address these challenges, scientists are currently attempting to build loop systems that mimic the functioning of terrestrial ecosystems to convert crew waste into assimilable resources, including the use of photosynthetic organisms such as *Chlamydomonas reinhardtii* (Häder, 2020). However, the photochemistry and growth of this organism seem to be altered in several experiments, depending on the strain studied and the intensity and duration of exposure, primarily due to the action of ROS (*Reactive Oxygen Species*) on its photosystem (Gomes et al., 2017).

ROS refer to chemical species that contain one or more unpaired electrons, such as hydroxyl radicals () or superoxide radicals (), as well as species that do not possess a radical nature but can be converted radicals, such as hydrogen peroxide () (Munteanu et al., 2015). Depending on the environment or situation, these radicals can behave as oxidants or as reductants and generate significant cellular damage (Munteanu et al., 2015): it is estimated that only 20% of DNA damage during radiation exposure would be directly due to radiation, while 80% would be due to indirect consequences of radiation such as ROS (Ghosal et al., 2005).

Radiation-resistant organisms typically have a significant manganese (II) oxide content: the cellular content of the *Deinococcus radiodurans*, for example, reaches up to 30mM of this compound, which is 15-150 times the total content of a radiosensitive organism (Peana et al., 2018). An Mn-Proteome performed in-silico reveals that many Mn(II)-interacting biomolecules within *Deinococcus* play a more or less direct role in the defense against ROS. However, the action of Mn(II) would also be direct: manganese-phosphate or manganese-carbonate complexes would protect proteins by catalyzing the dismutation of superoxide ions via the formation of two intermediates during a second-order reaction (1).

$$Mn^{2+}\text{-L'} + O_2^{\cdot-} \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} MnOO^{+}\text{-L'} \qquad \text{(inter 1) [3]}$$

$$Anion^{n-} + MnOO^{+}\text{-L'} \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} [MnOO\text{-L'-Anion}]^{n-1} \qquad \text{(inter 2) [4]}$$

$$MnOO^{+}\text{-L'} + MnOO^{+}\text{-L'} \xrightarrow[H^+]{k_5} 2\,Mn^{2+}\text{-L'} + H_2O_2 + O_2 \qquad [5]$$

(1) Catalytic mechanism for dismutation of a superoxide ion proposed by Barnese et al. The L represents phosphate and/or carbonate binding to manganese to form MnHPO4 and/or MnHCO3+ and the n- anion an additional bond between the molecule and these compounds (Barnese et al., 2012).

Previous studies by Berlett et al. (1990) already indicated the formation of such a complex but added a peptide component (Berlett et al., 1990). These peptides would act as ligands to activate Mn2+ and would have a synergistic action with it. In 2016, a synthetic decapeptide (DEHGTAVMLK) was made by assembling some amino acids found in *Deinococcus radiodurans* lysate and was inoculated with Mn2+ and phosphate into mice. The mice survived an average of 30 days of 9.5 Gy radiation (LD70/30), while the control group recorded a lethality of nearly 63% at the end of this period. The team also demonstrated that their synthetic peptide was able to protect the activity of a T4 DNA ligase exposed to 60,000 Gy (Gupta et al., 2016).
Here we aim to produce this synthetic peptide in our photosynthetic model organism *Chlamydomonas reinhardtii* and verify its radioprotective action.

II. MATERIALS AND METHODS

A. *Transformation of Chlamydomonas reinhardtii with undecapeptide (MDEHGTAVMLK)*

The assembly method chosen during these experiments is the Golden Gate cloning method (Engler & Marillonnet, 2014). In

addition, we used the MoClo Toolkit adapted to *Chlamydomonas reinhardtii* made by Pierre Crozet's team (Crozet et al., 2018).

The double-stranded DNA sequence corresponding to the antioxidant peptide was previously suspended from reaching a final concentration of 1ng/µL before ligation into the plasmid pAMG9121 (level 0 plasmid). Details of the ligation protocol are available in the appendix (appendix 1). Ligations were always performed using the restriction enzymes BbsI, BbsI-HF or BsaI-HF (NEB), CutSmart buffer (NEB) and a T4 ligase (NEB/Fisher). The transformation steps were performed on *E. coli* DH10β bacteria (New England Biolabs) rendered chemically competent by heat shock. Bacteria were grown on solid medium (Lysogeny Broth medium supplemented with 20% m/V agar, spectinomycin 50 µg/mL, X-gal 40 µg/mL from Sigma-Aldrich) overnight at 37°C. Colonies containing the plasmid of interest pL0-294 were re-grown overnight in a liquid medium. Plasmid DNA extraction was performed using the Macherey-Nagel "Nucleospin" kit, following the manufacturer's instructions. The different ligation protocols for the L1 and LM levels are available in the appendix (appendix 1). Quality controls were performed at each step by restriction followed by 1% agarose gel electrophoresis. The L0 level was also checked by sequencing. A transformation of *Chlamydomonas reinhardtii* by electroporation was set up following the Onishi Lab electroporation protocol (Onishi & Pringle, 2016).

B. *Proof of concept of undecapeptide activity*

The antioxidant activity of undecapeptide was tested on three oxidants: methyl viologen (1,1′-Dimethyl-4,4′-bipyridinium dichloride), hydrogen peroxide H2O2, and rose bengal (4,5,6,7-tetrachloro-3',6'-dihydroxy-2',4',5',7'-tetraiodo-3H-spiro-3-one). For this purpose, the LD50 of *Chlamydomonas reinhardtii* was determined for these three compounds: increasing ranges of each oxidant were performed (appendix 2), and their activity on cells was monitored by UV-vis spectrometry and by cell counting after addition of 1% Evans blue by fluorescence microscopy. A range is performed by testing for each oxidant three conditions, namely a control, a first solution with manganese alone, and a solution with decapeptide or undecapeptide, for a total volume within each well of 50 µL (appendix 3).

III. PRELIMINARY RESULTS

A. *Transformation of Chlamydomonas reinhardtii with undecapeptide (MDEHGTAVMLK)*

All quality checks (sequencing of the level 0 plasmid, digestion and electrophoretic migration of the resulting plasmids) indicated that the intermediate plasmids pL0-294, pL1-226 and pM-156 were correctly assembled (see FIGURE 1 and 2).

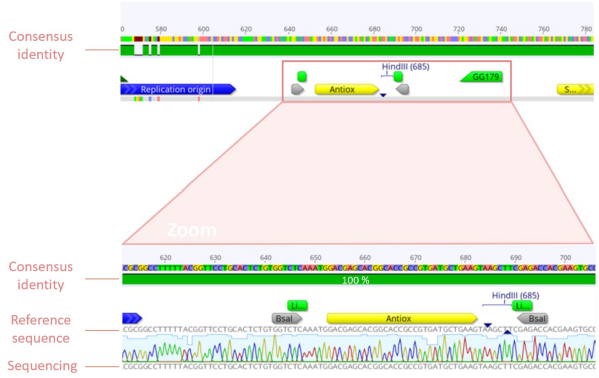

FIGURE 1: Sequencing results of the first L0-level plasmid obtained. The results indicate a consensus identity between the expected sequence for pL0-294 and the sequence obtained.

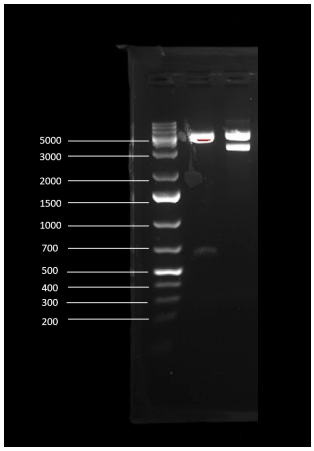

FIGURE 2: Restriction profiles of the LM level plasmid. From left to right: scale: Generuler 1Kb Plus, pM-156 control (600bp band corresponding to LacZ and 4600bp band corresponding to the backbone), pM-156 (3175 band corresponding to the insert and 4600bp band corresponding to the backbone).

B. *Proof of concept of undecapeptide activity*

The cell death caused by the three oxidants can be qualitatively perceived by the loss of colouration in the multi-well plates after several hours of exposure or quantitatively by cell count or absorbance measurement (appendix 5 and 6). However, we do not yet have preliminary results to establish the antioxidant activity of our undecapeptide

IV. DISCUSSION

This study made the photosynthetic model organism *Chlamydomonas reinhardtii* produce a peptide inspired by amino acids of the radioresistant organism *Deinococcus radiodurans.*

We are currently awaiting preliminary results on the antioxidant action of our peptide. These results, however, will only strengthen the theory that the accumulation of small peptides increases the radioresistance of an organism. To confirm whether the increase in radiation protection comes from the formation of a Mn-peptide complex or from a simple synergistic effect, we should test the action of the peptide in isolation and highlight the formation of a possible complex by NMR experiments coupled with X-ray crystallography. These experiments would bring a new light on the action of small metabolites in extremophilic organisms.

Finally, the demonstration of radioprotection at such scales (9.5 Gy) on photosynthetic organisms has not yet been studied to our knowledge. We want to test the efficiency of our undecapeptide in natural conditions, i.e. by exposing it to gamma and X-rays without having previously dehydrated the microalgae. We hope that understanding the mechanisms of action and the limitations associated with manganese could have many applications in space research, medicine (radiotherapy) or industry (nuclear).

V. CONCLUSION

Our preliminary results do not yet allow us to decide on a possible antioxidant activity of our peptide. However, even if our results were positive, further research will be necessary to understand this peptide's mechanism of action and investigate its genuine interest in inducing radioresistance in a photosynthetic organism. Additional in-situ studies on the metabolism of *Chlamydomonas reinhardtii* under oxidative stress following actual exposure to ionizing radiation are also planned.

APPENDIX

Appendix 1: Ligation protocols.
https://docs.google.com/document/d/19KdRFd03ux3vvEVoAXQpSSpm3D_1ULWeYPkdYTZ_CAw/edit?usp=sharing

Appendix 2: Detailed protocol of toxicity test (LD50) of Chlamydomonas reinhardtii exposed to several oxidants.
https://docs.google.com/document/d/1WYR5KfO38dJTpr-DuFz8fBQpiUyMoCx04A0rDbsEJgI/edit?usp=sharing

Appendix 3: Detailed protocol for proof of concept of antioxidant activity of the peptide and synthetic undecapeptide.
https://docs.google.com/document/d/1dDL3pTD3P2ndFZg91gAbgsgecuofEXV3UKxxbXIoXK4/edit?usp=sharing

Appendix 4: Absorbance measurements of the LD50 test of Chlamydomonas reinhardtii exposed to several oxidants.

Appendix 5: Results of toxicity tests and antioxidant effect of undecapeptide and synthetic peptide on three oxidants (cell life and death counts).
https://docs.google.com/spreadsheets/d/15i8tY2Kc5NDKBfj3KZpUybjQmmM51vwukUZ-TMUMD8M/edit?usp=sharing

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