Role of pyridine nucleotides in controlling the respiration of the dinoflagellate *Oxyrrhis marina*

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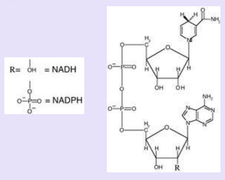
INTRODUCTION

Respiration in eukaryotes is a physiological process that occurs wherever the oxygen and organic matter are present. Respiration is oxygen consumption (**Ro_2**) catalyzed by the respiratory electron transport system (**ETS**) enzymes. The physiological Ro_2 rate depends directly on the enzymatic control of the respiratory ETS activity. According to Packard et al. (1996), we argue that substrate availability is the most probable regulatory mechanism controlling this activity. The major ETS substrates are the pyridine nucleotides (PNs) and they occur as nicotine adenine dinucleotide (**NAD**) and as nicotine adenine dinucleotide phosphate (**NADP**). Up to now, no studies have quantified their intracellular concentrations in marine dinoflagellates. *Oxyrrhis marina* is a microheterotrophic marine dinoflagellate which has been widely investigated. Nevertheless, its respiratory metabolism has never been characterized from measurements of oxygen consumption (Ro_2) and ETS activity.

Therefore, our main **objectives** were 1) to measure Ro_2 and ETS activity in *O. marina* and to determine their behavior during starvation, and 2) to quantify the PN levels during the same period and compare them with the respiration rates.



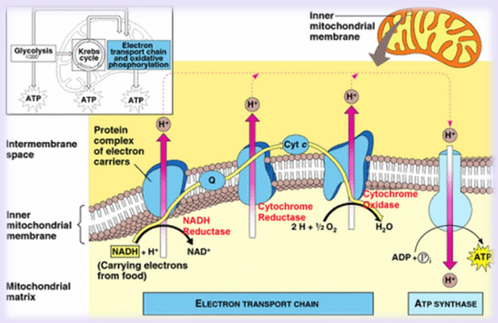
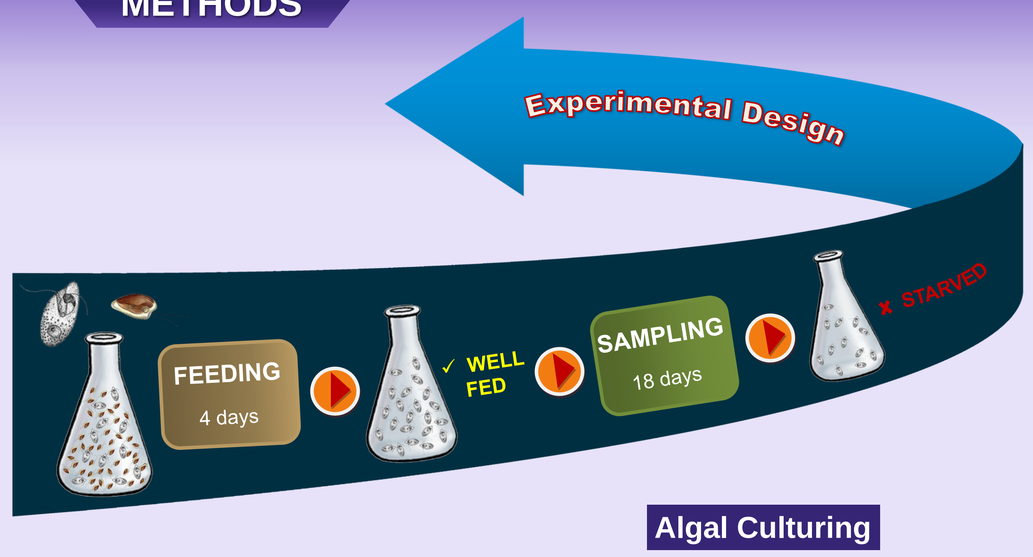


Figure 1. Structure of the Electron Transport System (ETS) coupled with energy (ATP) formation.

METHODS



Algal Culturing

Triplicate cultures of *O. marina* were fed *Rhodomonas salina* at the beginning of the experiment; after that, no more food was added. The sampling began when the amount of *Rhodomonas* in the culture was negligible.

Hereinafter, samples every 2 days were taken for 18 days, until *O. marina* was completely starved.


Rhodomonas salina


Oxyrrhis marina

Figure 2. Cultures were maintained at constant temperature and light conditions during the entire experiment.

Analytical Methods

Respiration
Oxygen electrodes

Protein
(Lowry et al., 1951)

ETS activity
(Packard et al.,1996)

Pyridine nucleotides
(Wagner and Scott, 1994)

Figure 3. Temperature controlled incubation system . A 6-channel oxygen sensor was used to record changes in the dissolved O_2 concentration during the experiment.

RESULTS & DISCUSSION

Time Profiles of Respiration, ETS activity and Protein in Batch Cultures of *O. marina*

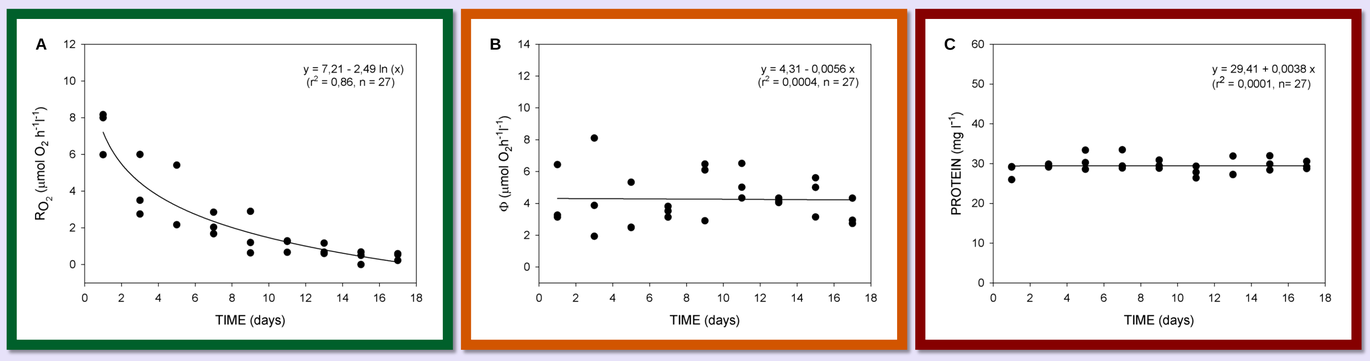


Figure 4. (**A**) Experimentally determined **Ro_2** showed an exponential decrease with increasing starvation time. (**B** and **C**) By sharp contrast, the potential respiration (**Φ,** measured as ETS activity) and biomass (**protein**) stayed stable during this period. The parallel behavior of these two parameters was expected as the ETS activity has a long history of being a good index of the living biomass.

Protein-Specific Pyridine Nucleotide Time Profiles during Food Starvation

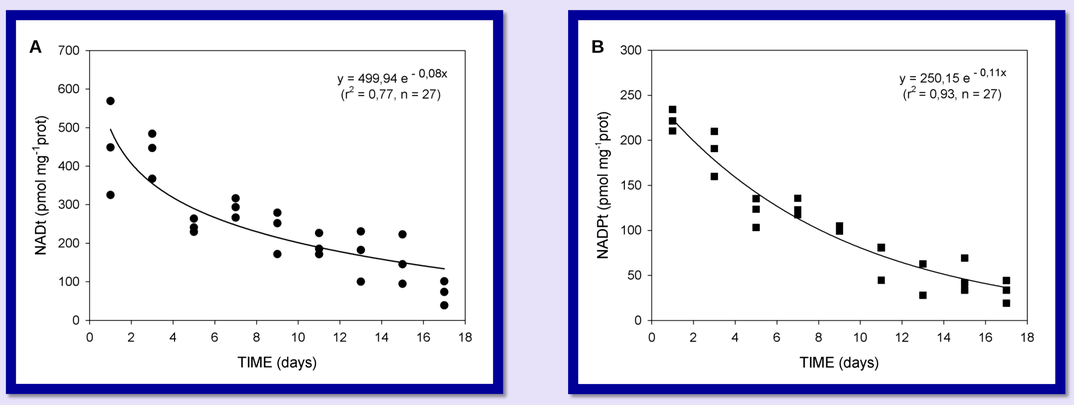


Figure 5. Exponential decrease of protein-specific PN levels with the increase in time of starvation. The general features of (**A**) the total NAD-NADH pool (**NADt**) and (**B**) the total NADP-NADPH pool (**NADPt)** time courses were similar, although the concentration of NADt was two-fold higher than that of NADPt. This is the **first determination** of intracellular PN concentrations in a marine dinoflagellate.

Relationship of Pyridine Nucleotide Levels and Respiration Rates

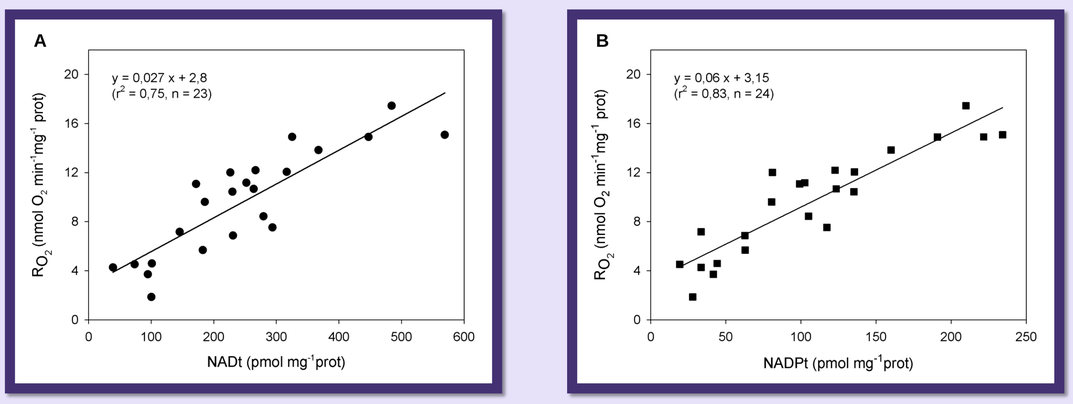


Figure 6. Correlations of Ro_2 time course with (**A**) NADt and (**B**) NADPt time courses. The Spearman correlation coefficients were 0,87 and 0,91 (n = 23, p < 0,001) for NADt and NADPt, respectively. This results supports the respiration model based on substrate limitation described by Packard et al. (1996).

CONCLUSSIONS

I. The parallelism between the behavior of potential respiration and biomass over a long period of starvation argues that the ETS enzyme complexes in *O. marina*'s mitochondria are constitutive.

II. Divergence of respiration and potential respiration during food deprivation in the marine dinoflagellate *O. marina* has been demonstrated.

III. The fall in the respiration during the onset of starvation suggests that respiration is substrate limited during this period.

IV. Total PNs and respiration are well correlated during starvation in this marine dinoflagellate. This observation supports the use of a respiration model based on substrate limitation.

References

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