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Mitochondrial electron transport activity and metabolism of experimentally bleached hermatypic corals

Sylvain Agostini [a,*], Hiroyuki Fujimura [b], Hiroyuki Hayashi [b], Kazuhiko Fujita [c]

[a] Shimoda Marine Research Center, University of Tsukuba, 5-10-1 Shimoda, Shizuoka, Japan
[b] Department of Chemistry, Biology and Marine Science, University of the Ryukyu, 1 Senbaru, Nishihara-cho, Okinawa 903-0213, Japan
[c] Department of Physics and Earth Science, University of the Ryukyu, 1 Senbaru, Nishihara-cho, Okinawa 903-0213, Japan

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ABSTRACT

Bleached corals (*Porites cylindrica* and *Galaxea fascicularis*) were obtained through extended incubation (over 45 days) under light depletion and privation: low light and dark conditions, and heat stress (32 °C). The colonies in the different treatments became bleached and had reduced metabolic rates, photosynthesis, calcification and respiration; reduced biomass, zooxanthellae density, and chlorophyll *a* concentrations; and reduced mitochondrial electron transport system activity, which represent potential respiration rates. The most important reduction in mitochondrial electron transport activity was shown when the activities were normalized by the unit of surface and not by the unit of host protein. This result indicates that the reduction in activity could be mainly explained by the reduction of biomass and tissue thickness. However, increased Manganese Superoxide dismutase (Mn SOD) activity, a mitochondrial SOD, suggests that ROS production occurs in the mitochondria under heat stress with the consequence of potentially damaging the electron transport system. The reduced calcification rates observed are hypothesized to be the results of a decrease in the energy available for calcification due to the reduced photosynthetic rates, limiting the availability of substrates for respiration and therefore the energy production, and the decreased in the number of active mitochondrial electron transport system. Electron transport system activity associated with respiration is the basis of all metabolic processes and is not biased by incubation like traditional measurements of respiration in an aquarium. Therefore, ETSA could be used as an overall indicator of coral health, especially for host animal health.

1. Introduction

Respiration is the basis of metabolism in animals, including corals. It provides the energy required for all of the subsequent metabolic processes in the form of ATP. Aerobic respiration is divided in two phases: the oxidation of an organic substrate through glycolysis and the citric acid cycle, and the reduction of the terminal electron acceptor, oxygen. The transfer of electrons from the reduced co-factors, NADH and FADH2 produced during the oxidation phase is accomplished through a chain of enzymatic transporters embedded in the internal membrane of the mitochondria. Impairment of the mitochondrial machinery, especially the electron transport chain, will have strong repercussions on the respiration process and, subsequently, the metabolism of the organism.

Elevated temperature and other environmental factors can cause coral bleaching: the loss of their photosynthetic symbionts or their pigments (Glynn et al., 1992; Hoeghguldberg and Smith, 1989). The most accepted model for the mechanism of bleaching proposes that it starts with the impairment of the zooxanthellae photosystems (Warner et al., 1999), which leads to the production of reactive oxygen species (ROS) (Higuchi et al., 2010; Jones et al., 1998). However, mitochondria and their electron transport system are also an important source of ROS in animals, and the mitochondrial production of ROS may not be negligible in corals (Downs et al., 2002). This suggests that the importance of the host in the bleaching process may have been underestimated. Recently, Dunn et al. (2012) showed degradation of the host mitochondria in anemone under heat stress. This degradation was associated with the decreased expression of the gene coding for cytochrome c and complex IV, both of which are proteins that are important parts of the mitochondrial electron transport system. Corals incubated in the dark for a long period have also been shown to bleach (Hoegh-Guldberg and Smith, 1989; Tolleter et al., 2013; Yonge et al., 1930). It has been shown that bleached corals have lower calcification rates independently of the cause of bleaching, whether due to heat stress, bacterial factors (Higuchi et al., 2013) or depletion of light. Corals incubated in total darkness for a long period have highly reduced calcification rates with only 10% of the light calcification rate after 7 h (Al-Horani et al., 2007).

Photosynthesis and calcification show an intimate relationship at both the community level (Barnes and Chalker, 1990; Gattuso et al., 1996; Kinsey, 1985) and organism level (Allemand et al., 2004; Furla et al., 2000; Moya et al., 2006). The increase in the calcification rates under light for photosynthetic organisms is called light-enhanced

* Corresponding author.
E-mail address: agostini.sylvain@shimoda.tsukuba.ac.jp (S. Agostini).

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calcification. On average, corals show three times higher calcification rates in the light than in the dark. Several hypotheses have been raised to explain this enhancement (these are reviewed in Allemand et al., 2004). Among them, one proposal is that OH^- resulting from photosynthesis titrate H^+ that is formed during the calcification process. For corals, photosynthesis is generally the main source of reduced carbon and is used as combustible for respiration and therefore for the production of the energy required for calcification, which could represent 30% of the coral's total energy budget (Cohen and Holcomb, 2009). Inhibition of respiration (oxidative phosphorylation) has been shown to inhibit calcification in the light, which then falls to the same level as calcification in the dark (Chalker and Taylor, 1975). In a recent paper (Agostini et al., 2013), the respiration limited calcification model, which supports the models by Chalker and Taylor (1975) and, more generally, the Trans-calcification model of McConnaughey (1997) was introduced. In this model the hypothesis made is that calcification in symbiotic coral is limited by the energy production through host respiration, itself limited by the ETSA and the amount of substrate for respiration produced by the photosynthetic activity of the symbiont.

Many tools are available to assess the state of the symbionts in corals, such as PAM fluorescence, zooxanthellae density, and pigment concentration. However, only a few are available for the host and are rather general, such as the lipid or protein content, or require incubation, such as respiration and calcification. Moreover, traditional respiration measurements also include symbiont respiration, which may bias the interpretation of the results. Therefore, coral biology is in need of tools to assess host health, and ETSA could be one of these tools.

The long-term effect of the decrease in photosynthetic activity on the respiratory activity and calcification rates was tested on two different coral species: *Galaxea fascicularis* and *Porites cylindrica*. Reduced photosynthetic rates were obtained in two ways: corals were maintained under reduced light or exposed to a gradual elevation of temperature. The aim of this experiment is to first better understand the relationship of photosynthesis, respiration and calcification in corals, and second, to investigate the possibility of a direct effect of temperature on mitochondrial ETS, which leads to decreased growth rates. Two different hypotheses were made regarding the mechanisms that lead to a decrease in the respiration potential (ETSA) which both lead to reduced host metabolism (calcification and respiration). The first mechanism would be a decrease in the biomass based on tissue per unit of surface and tissue thickness, resulting in a reduction in the number of active ETS (expressed per unit of surface). The second, caused by damage due to heat stress, would be a decrease in the number of active ETS (expressed in per mg protein, representing the host biomass). Overall, the possibility of using ETSA as an indicator of coral health was tested.

2. Materials and methods

2.1. Coral specimens

Colonies of *Galaxea fascicularis* and *Porites cylindrica* were collected from a coastal region off Okinawa Island, Japan, with permission from the Okinawa Prefecture government (No. 23-7). The *P. cylindrica* colonies were fractionated to obtain small fragments, and the colony of *G. fascicularis* was fractionated into single polyps. The fragments were suspended on a nylon thread and maintained for several months in an outdoor aquarium with running seawater at the Sesoko Station, Tropical Biosphere Research Center, University of the Ryukyus, Okinawa, Japan. Micro-colonies, of which the skeleton was entirely covered by tissue, were selected for the experiments.

2.2. Experimental design

Colonies were moved into indoor aquariums with running seawater and maintained under controlled conditions for a minimum of 45 days. Four different conditions were tested, and the different treatments were applied after a minimum of one week to let the corals acclimatize to the indoor conditions. The control treatments corresponded to the natural seawater temperature (24 to 27 °C for *P. cylindrica* and 22 to 26 °C for *G. fascicularis*), and an illumination of 300 μmol of photon s^{-1} m^{-2} (measured with a 2pi quantum sensor, JFE Advantech, Japan) provided a 12 h cycle by metal halide lamps. The heat treatment (high temperature stress) was conducted under the same illumination as the control treatment, and the temperature was gradually elevated to reach 32 °C in the last week of the experiment over the total incubation period at a maximum rate of 1 °C per week. In the dim treatment (dimmed light), the colonies were incubated under ambient light with a maximum recorded light of 50 μmol photon s^{-1} m^{-2} and an average of 2.5 μmol photon s^{-1} m^{-2} in the daytime, and the temperature was the natural seawater temperature (the same as the control). The dark treatment was obtained by covering the aquarium with dark cloth; no measurable light occurred, and the temperature was same as the control. Four micro-colonies of each species were used for each treatment and suspended on a nylon thread. Aeration was continuously provided in all treatments.

2.3. Metabolism measurement

The colonies were enclosed in individual 300-ml vessels directly in their respective aquariums to avoid stress due to manipulation and light regime changes. The water was continuously stirred in the vessels. The incubation was conducted under the appropriate treatment light levels and temperature. The colonies were first incubated under light for 3 h and then under dark conditions for 2 h. For the dark treatment, only a dark incubation was conducted. The dissolved oxygen was measured at the beginning and end of each incubation using an Orion 4-Star pH-DO sensor equipped with an RDO probe (Thermo Scientific). Subsamples of the incubation water were sampled at the beginning and end of each incubation period and filtered through a 0.45-μm membrane filter to measure the total alkalinity. The total alkalinity was determined via titration with HCl at 0.1 mol l^{-1} with a Metrohm titrator (785 DMP titrino). The calcification rates were calculated using the alkalinity anomaly method (Gattuso et al., 1996), and net photosynthesis and respiration were calculated based on the variation in the dissolved oxygen during the light and dark incubation, respectively.

2.4. Separation of the host fraction

The coral tissues were removed using an airbrush with phosphate buffer saline and then homogenized using a Teflon potter homogenizer to break the host cells and release the zooxanthellae. The host and zooxanthellae fractions were separated by centrifugation at 1500 rpm for 20 min.

2.5. ETSA measurement

Electron transport system acitivity was measure as described in Agostini et al. (2013). Five milliliters of the supernatant (the host fraction) was collected and homogenized using a sonicator (Smurt 155 NR-50 M, Microtec Co., Ltd., Funabashi, Japan) at 25% for 5 min in the presence of polyvinylpirrolidone k30 at 1.5 mg l^{-1}, MgSO4 at 75 μmol l^{-1}, Triton X-100 at 0.20% and EDTA 2Na at 10 mmol l^{-1}. The extract was then cleared by centrifugation at 10,000 rpm for 5 min. All the steps were conducted on ice or in a cooling centrifuge at 3 °C; 300 μl of the cell free extract was incubated in the presence of NADH (1.5 mmol l^{-1}), NADPH (0.15 mmol l^{-1}) and tetrazolium salt 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyl tetrazolium chloride (INT) (0.4 mg l^{-1}). The reaction was stopped using 50% formalin after 20 min, and the absorbance was immediately read at 490 nm. The absorbances were corrected against a turbidity blank (containing 300 μl of the samples, but the reagents were replaced by phosphate buffer saline) and a chemical blank (containing all