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"Super-quenching" state protects *Symbiodinium* from thermal stress — Implications for coral bleaching

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ABSTRACT

The global rise in sea surface temperatures causes regular exposure of corals to high temperature and high light stress, leading to worldwide disastrous coral bleaching events (loss of symbiotic dinoflagellates (*Symbiodinium*) from reef-building corals). Our picosecond chlorophyll fluorescence experiments on cultured *Symbiodinium* clade C cells exposed to coral bleaching conditions uncovered the transformations of the alga's photosynthetic apparatus (PSA) that activate an extremely efficient non-photochemical "super-quenching" mechanism. The mechanism is associated with a transition from an initially heterogeneous photosystem II (PSII) pool to a homogeneous "spillover" pool, where nearly all excitation energy is transferred to photosystem I (PSI). There, the inherently higher stability of PSI and high quenching efficiency of P_{700}^{+} allow dumping of PSII excess excitation energy into heat, resulting in almost complete cessation of photosynthetic electron transport (PET). This potentially reversible "super-quenching" mechanism protects the PSA against destruction at the cost of a loss of photosynthetic activity. We suggest that the inhibition of PET and the consequent inhibition of organic carbon production (e.g. sugars) in the symbiotic *Symbiodinium* provide a trigger for the symbiont expulsion, i.e. bleaching.

1. Introduction

Coral reefs provide the scaffolding and the habitat for some of the largest and most diverse ecosystems on the planet. Corals consist of a complex and fragile symbiosis between an animal host (phylum Cnidaria) and endosymbiotic microalgae (dinoflagellates from the genus *Symbiodinium*). Dinoflagellate photopigments (chlorophylls (Chl) *a*, c_2; and carotenoids) give the coral an overall brown coloration, but a range of host pigments combine to produce the colorful array of species we see across a coral reef. Prolonged exposure of corals to high irradiance and an elevated seawater temperature, ~1–2 °C above the summer average, induces pigment degradation and/or triggers the release of the *Symbiodinium* photosymbiont from the host [1], which in turn causes uncovering of the white skeleton underlying the animal tissue (*coral bleaching*). The bleaching phenomenon is often followed by death of the coral [1,2]. In this respect, the health of the coral holobiont is critical for the survival of corals and therefore of coral reefs and their denizens. However, global climate changes (especially the rising ocean temperature and ocean acidification) are threatening the health of this symbiotic relationship worldwide [2–4]. Current predictions indicate that coral reefs may face extinction by 2050 [3,4]. Therefore, it is critical to understand the fundamental processes in the algal–host association, especially the photosynthetic processes of the algae, which appear to be a crucial trigger point of coral bleaching [5,6].

The general consensus is that mass coral bleaching is due to the dysfunction of photosynthetic processes (such as pronounced reduction in the activity of photosystem (PS) II and in linear electron transport) in the algal symbiont as a result of the combined action of elevated temperature and light-stress [7]. This initial impact is followed by activation of the Mehler Ascorbate Peroxidase (MAP) pathway, which at low stress levels may have a protective effect [8]. However, at high stress levels it is

Abbreviations: Chl, chlorophyll; DAS, decay-associated spectra; F_B, stress conditions equivalent to coral bleaching conditions; F_C, control condition; F_{HL}, high light stress condition; F_{HT}, elevated temperature stress conditions; FWHM, full width at half maximum; LHCs, light-harvesting complexes; NPQ, non-photochemical quenching; PSA, photosynthetic apparatus; PSI, photosystem I; PSII, photosystem II; PSU, photosynthetic unit; RC, reaction center; RP, radical pair; SAES, species-associated emission spectra; SI, Supporting information; TEM, transmission electron microscopy.

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likely that generation of reactive oxygen species in this pathway will actually stimulate bleaching [9].

Over the last decade, a variety of *Symbiodinium* clades with differing photosynthetic properties and tolerances to elevated water temperatures have been studied [2,10,11]. However, scarce knowledge on the ultrastructure and the light-dependent reaction of the photosynthetic apparatus (PSA) in dinoflagellates has hindered the understanding of coral bleaching. It is known that the structure of the thylakoid membrane, that houses the light reactions, differs substantially from that in higher plants [12]. In higher plants, the thylakoid membranes form tightly appressed, stacked regions (grana) connected by single stroma thylakoids, due to the inter-membrane interaction of the PSII peripheral light-harvesting complexes (LHCs). This thylakoid ultrastructure enforces strict lateral separation of PSII and PSI between the grana and the stroma membrane regions respectively, which does not allow for intersystem excitation energy transfer (known as "spillover" in the literature [13]). In contrast, the thylakoids of dinoflagellates, as in many microalgae [14], are loosely grouped in threes which allows mixing of PSII and PSI units and thus spillover.

High levels of irradiance exceeding the utilization capacity of the PSA can be damaging for the photosynthetic complexes. In this respect, photosynthetic organisms have developed an adaptive feature, known as non-photochemical quenching (NPQ), where excess harvested light energy is converted into heat [15]. Many studies have demonstrated that symbiotic algae in corals have similarly developed adaptive processes to cope with conditions of high solar irradiation [2,16] to protect the photosystems [17] and ultimately the whole cell against oxidative damage [7]. However, the details of NPQ and the mechanisms of redistribution of excitation energy operating in dinoflagellates and especially in *Symbiodinium* sp. are largely unknown.

Ultrafast time-resolved Chl fluorescence, in combination with kinetic modeling [18], has contributed significantly to our understanding of the light harvesting processes and their regulation in photosynthetic organisms. The combination of these two methods has provided a tool which gives much greater detail and much better reliability on the location and mechanisms of the photoprotection in the antennae than was possible with the widely used steady-state Chl fluorescence methods. This non-invasive approach has also been successfully applied to study NPQ in diatom algae [19] and lichens [20], but not yet in dinoflagellates.

We have employed the above-mentioned approach to characterize the largely unknown structural and functional changes that occur in the PSA of coral symbionts exposed to stress conditions relevant to coral bleaching. Our studies on the temperature induced transformation of cultured *Symbiodinium* cells from unstressed to photoprotective mode of operation reveal the molecular origins of a "super-quenching" state that plays a central role in both photoprotection and coral bleaching.

2. Materials and methods

2.1. Sample preparation

The *Symbiodinium* cultures used in the experiments were from the strain CS-156 (corresponding to clade C, originating from the Hawaiian scleractinian coral *Montipora verrucosa*; obtained from the Commonwealth Scientific and Industrial Research Organization (CSIRO), Hobart, Australia). The algae were grown in f/2 medium [21] at 24 °C under 40 μmol photons m^{-2} s^{-1} on a 12:12 h light:dark cycle. In preparation for the picosecond time-resolved fluorescence measurements, algae were concentrated using a centrifuge at 1000 *g* for 10 min; the supernatant was discarded and algae were resuspended in 1 l of fresh f/2 medium to a cell concentration of ~10,000–15,000 cells/ml.

During the experiments, the algae were continuously stirred to keep them in a homogenous suspension and monitored for aggregation on the reservoir walls. To prevent clumping of the algal culture, which created a problem with the flow-through cuvette, the sample reservoirs were treated for short periods of time in an ultrasound bath at 80 W (Sonorex Digitec DT 100/H, Bandelin, Germany) at random intervals during the experimental runs. The monitoring of the effective quantum yield of PSII ($\Delta F/F_m$) by a Water Pulse-Amplitude Modulated (water-PAM) fluorometer (Walz, Effeltrich, Germany) showed that the ultrasound had no effect on the physiological state of the algae.

2.2. Time-correlated single photon counting fluorescence set-up

Time-resolved fluorescence measurements were performed to examine the changes in the functional state of the *Symbiodinium* cells and relate these to structural changes in the PSA. The single-photon timing technique was used to record the picosecond fluorescence decays at 10 different wavelengths in the range 670–750 nm. The set-up consisted of a synchronously-pumped, cavity-dumped, mode-locked dye laser operating at 800 kHz or 4 MHz repetition frequency [22]. The pulse of the dye laser has a full width at half maximum (FWHM) of ~10 ps and the whole response of the system is ~30 ps FWHM, which after deconvolution results in a time resolution of ~2 ps. The laser intensity at the sample was <0.5 mW, ~0.8 mm spot diameter. The excitation wavelength to selectively excite the bulk antenna Chls was 662 nm. A flow-through cuvette system, as designed previously [19], was used for the measurements (Supporting information (SI), Fig. S4).

2.3. Measures for achieving homogeneous PSII redox state during the measurement

The control sample and the other measurement conditions were carefully designed to ensure that the kinetics under the various treatments could be easily compared. Different light and temperature conditions in the pretreatments would naturally lead to a heterogeneity in the redox state of the PSII RCs, which would complicate the analysis of the time-resolved fluorescence data or even make a meaningful kinetic modeling impossible [19]. We have thus chosen the measuring conditions to ensure that in all cases all of the PSII RCs were in a closed state at the time of the measurement (i.e. when the measured sample volume passes the measuring laser beam in the flow-through cuvette). Such a homogeneous state is easily achieved either by DCMU incubation (in the case of unquenched dark adapted samples) or by short additional illumination (~200 ms duration of 3000 μmol photons m^{-2} s^{-1}) applied by a high intensity LED right before the sample volume enters the measuring laser spot.

2.4. Experimental pretreatments and measurement conditions

The experimental treatments applied to the cultured *Symbiodinium* were designed to compare optimal growth conditions and conditions known to cause coral bleaching [23]. Four different treatments (incubation and measurement periods) were applied to the samples (Fig. 1): 1) control condition (F_C) — unquenched cells with closed (Q_A^-) PSII reaction centers (RCs) at growth temperature of 24 °C (immediately before the measurement the culture was treated with 10 μM DCMU and 10 μmol photons m^{-2} s^{-1} of illumination was applied to the sample reservoir for closing PSII reaction centers during the measurement); 2) high light stress (F_{HL}) — quenched high light-adapted cells during both incubation and measurement periods (600 μmol photons m^{-2} s^{-1} illumination on the sample reservoir at 24 °C); 3) elevated temperature stress (F_{HT}) — unquenched cells at high temperature (31 °C and 10 μmol photons m^{-2} s^{-1} illumination) during the incubation period, which were converted to quenched light-adapted cells with closed PSII RCs for the measurement periods (31 °C and 600 μmol photons m^{-2} s^{-1} illumination on the sample reservoir), and 4) quenched light-adapted cells (F_B) (31 °C and 600 μmol photons m^{-2} s^{-1} illumination on the sample reservoir) during both the incubation and the measurement periods and