Thermal inhibition and recovery of the maximum quantum yield of photosystem II and the maximum electron transport rate in zooxanthellae of a reef-building coral

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Abstract: To examine whether damage to zooxanthellar photosystem II (PSII) is the primary step of thermally induced coral bleaching, we first assessed the relationship between the maximum quantum yield of PSII (F_v/F_m) or active PSII centers (F_v/F_o) and maximum electron transport rate (ETR_{max}), which represent the PSII activity and electron flow beyond the PSII, respectively, in the symbiotic algae of the coral *Pachyseris rugosa*. Next, the photo-physiological responses of the symbionts to stress treatments of 1 h high temperature (33.5°C) and/or high light (1030 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$) stress and 6 h recovery were investigated. The 1 h high temperature treatment in darkness produced a significant, but reversible (6 h recovery) decline in ETR_{max} without any change in F_v/F_m . The 1 h high light treatment reduced F_v/F_m , but not ETR_{max} . High temperature in combination with high light resulted in a more pronounced reduction in F_v/F_m , along with a significant decline in ETR_{max} . Neither F_v/F_m nor ETR_{max} recovered fully within 6 h. Reversible increases in non-photochemical quenching (NPQ), energy dissipation in PSII, were also recorded. Chloramphenicol (CAP), an inhibitor of synthesis of D1 protein of PSII reaction center, revealed that a minimum of ~60% of the F_v/F_m or ~30% of active PSII centers (F_v/F_o) is required to maintain ETR_{max} in the *in hospite* symbionts. These results suggest that the primary step of heat-induced damage in the symbiont's photosynthetic apparatus involves a component beyond the PSII, probably at the level of the dark reaction as indicated by reduced ETR_{max} and the PSII damage is secondary.

Key words: coral, bleaching, PAM fluorometry, thermal stress, PSII

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INTRODUCTION

Coral bleaching, characterized by loss of endosymbiotic dinoflagellates (= zooxanthellae) and/or their pigments, has been linked to global climate change. Elevated seawater temperatures and solar radiation have received considerable attention as plausible large-scale causes (Glynn 1993; Hoegh-Guldberg 1999; Wilkinson 2000). Bleaching related to high irradiation is widespread in corals on shallow intertidal reef flats throughout the tropics, where levels of light

above saturation are potentially damaging to the photosynthetic machinery of the zooxanthellae (Brown *et al.* 1999; Hoegh-Guldberg and Jones 1999; Lesser and Gorbunov 2001). Exposure to elevated temperatures can also reduce the photosynthetic rate of zooxanthellae (Hoegh-Guldberg and Smith 1989; Warner *et al.* 1996) and might predispose their photosynthetic apparatus to further damage by excess light (Fitt and Warner 1995; Lesser 1996; Jones *et al.* 2000; Bhagooli and Hidaka 2004). In most cases, wherever coral reef bleaching has been reported,

it occurred during the summer season when conditions favor localized heating in combination with higher intensities of solar irradiance than usual (Hoegh-Guldberg 1999). The key to understanding the underlying photo-physiological mechanism of coral bleaching lies in the explanation of how elevated temperature enhances the effect of incident irradiance. In order to investigate such a phenomenon, it is necessary to identify (1) which component of the photosynthetic machinery fails when zooxanthellae are exposed to thermal stress, (2) what is the definitive sequence of events leading to the destabilization of their photosynthetic function, and (3) the dynamics of recovery of the photochemical efficiency in corals following heat damage.

Several studies on the mechanisms of coral bleaching, from the zooxanthellae photo-physiological perspective, have been undertaken over the last decade. Using the oxygen-evolution method with/without DCMU (3-(3,4-dichlorophenyl)-1, 1-dimethylurea, a herbicide inhibiting photosynthesis) fluorescence, evidence for damage to cultured zooxanthellar photosynthesis at temperatures above 30°C has been reported (Iglesias-Prieto *et al.* 1992). Damage to both photosystem II (PSII) (Jones *et al.* 1998; Warner *et al.* 1999; Bhagooli and Hidaka 2002, 2003, 2004) and the activity of the primary carboxylating enzyme, Rubisco, resulting in a decrease in productivity of thermally stressed cultured zooxanthellae have been described (Lesser 1996). Use of chlorophyll fluorescence fluorometry has led to the suggestion of a photo-physiological mechanism for coral bleaching (Jones *et al.* 1998; Bhagooli and Hidaka 2004) emphasizing that carboxylation within the Calvin cycle is the first site of heat damage, as has been observed in higher plants under thermal stress (Feller *et al.* 1998; Crafts-Brandner and Law 2000). On the other hand, PSII as the heat-stress target, particularly damage to the D1 protein of the PSII reaction center has been reported to occur in *in hospite* zooxanthellae at elevated temperatures and is thus regarded as the determinant of coral bleaching (Warner *et al.* 1999). Consequently, the primary site of heat damage and its secondary effect on the photosynthetic machinery by light are still subject to debate.

Recent investigations have focused on the recovery of maximum quantum yield and/or maximum electron transport rate using chlorophyll fluorescence techniques in *in hospite* zooxanthellae of several coral species, with special attention given to explaining bleaching susceptibilities

(Takahashi *et al.* 2004; Yakovleva and Hidaka 2004). Takahashi *et al.* (2004) have suggested the repair machinery of PSII as the primary target of heat stress among four scleractinian corals. In contrast, Yakovleva and Hidaka (2004) have put forward the 'differential recovery hypothesis' based on differential recovery of the PSII and the electron transport rate (ETR) in one bleaching-susceptible and one bleaching-resistant coral. This controversy can be resolved more appropriately by understanding the relationship between PSII and the maximum electron transport rate (ETR_{max}). We further need to comprehend how damage to and recovery of both PSII and ETR_{max} affect a given scleractinian coral following exposure to elevated temperature.

Simultaneous comparison of the dynamic changes in chlorophyll fluorescence parameters such as the maximum quantum yield of PSII (F_v/F_m) and ETR (electron flow beyond PSII) allows us to conduct a rapid assessment of the effects of temperature and/or light stresses on the PSII photochemical efficiency and electron flow beyond the PSII in *in hospite* zooxanthellae. Therefore, the present study attempts to clarify the primary step of photo-physiological damage and associated phenomena under heat and light stresses in the zooxanthellae of the scleractinian coral *Pachyseris rugosa* using pulse-amplitude modulated (PAM) fluorometry. Recovery of thermally stressed corals was measured to assess how reversible effects were. Non-photochemical quenching (NPQ), a measure of dissipation of excitation energy in PSII as heat mainly via the xanthophyll cycle, was also monitored. Furthermore, the relationships between the maximum quantum yield of PSII or active PSII centers and ETR_{max} were evaluated in the presence of chloramphenicol (CAP), a chloroplast-encoded protein synthesis inhibitor.

MATERIALS AND METHODS

Collection and maintenance of specimens

Four colonies of the scleractinian coral, *Pachyseris rugosa*, were collected from a depth of 1-2 m at Sesoko Island, Okinawa, Japan. Colonies were maintained in shaded, aerated and flow-through outdoor tanks for 24 h ($< 400 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$) before they were fragmented ($\sim 1 \times 1.5 \text{ cm}^2$). Fragments were allowed to recover for 9-12 days. The temperature in the tanks was $21.4 \pm 0.73^\circ\text{C}$ (range, 20.5-23.3°C, Tidbit temperature data logger).

Experimental protocols

Before treatments, coral fragments were dark-adapted, and F_v/F_m and rapid light curves (RLC) were measured. Fragments were then exposed to either 25 or 33.5°C in darkness or high light (1030 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$) for 1 h, followed by a 6 h recovery period in dim light ($\sim 3 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$) in an indoor flow-through seawater tank. Photosynthetic parameters were again monitored after treatment and recovery. A 1 h treatment period was chosen to detect early changes in photo-physiological characteristics, using the chlorophyll fluorescence parameters, which usually precede the bleaching process via decrease in chlorophyll content or loss of zooxanthellae from the coral. A 1 h treatment to high temperature and/or light might not be mimicking the actual field situation, but it will definitely provide valuable insights into the early alteration in the photosynthetic parameters when corals are exposed to elevated temperatures. Previous research showed that recovery periods of 6 and 8 h are sufficient to have a significant (*Porites cylindrica*) and complete (*Stylophora pistillata*) restoration of dark-adapted F_v/F_m , respectively (Hoegh-Guldberg and Jones 1999).

The coral fragments were placed in 300 ml containers filled with 70 ml FSW. These containers were placed in water baths with temperature control units (RACOMACE, Model HT-10D and EYELA, Thermister Tempet T-80). The light source was provided by optical fibers from either Coldspot or Nikon light systems and light intensities were measured by a LICOR lightmeter (Model, LI-250). One coral fragment out of four fragments per colony was used for each treatment (25°C in darkness, 25°C under high light, 33.5°C in darkness and 33.5°C under high light). To provide four replicates, the experiment was carried out four times using healthy-looking coral fragments from the four respective colonies, except for 33.5°C treatment in darkness, where $n = 3$.

To examine the relationship between the maximum quantum yield of PSII (F_v/F_m) and the maximum electron transport rate (ETR_{max}), chloramphenicol (CAP), an inhibitor of chloroplast-encoded protein synthesis was employed. CAP prevents the synthesis of PSII reaction center D1 proteins (Schnettker *et al.* 1994). When the number of functional PSII centers decreases beyond a certain level, then the ETR_{max} will start to decrease too. Thus, we can estimate the level of maximum quantum yield of PSII (F_v/F_m) or the number of functional PSII centers (F_v/F_o)

required to maintain the maximum electron transport rate (Behrenfeld *et al.* 1998; Crofts *et al.* 1993). Coral fragments were incubated in 70 ml of either FSW (controls) or 400 $\mu\text{g ml}^{-1}$ CAP (prepared with FSW) and exposed to 250 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ at 25°C for 15, 45, 60, 120, 180, 240, 360 and 480 min. Since CAP at a concentration of 200 $\mu\text{g ml}^{-1}$ for 120 min had no significant effect on F_v/F_m of cultured symbiotic algae (Warner *et al.* 1999), we employed higher concentration in order to obtain significant effect of CAP on F_v/F_m within one-hour treatments. Corals were pre-incubated in CAP for 15 min prior to exposure to light treatment. Measurements of chlorophyll fluorescence were done at 25°C in FSW before and after exposure to light treatment at various time intervals. Both F_v/F_m and RLC were recorded in FSW prior to pre-incubation in CAP but for CAP-treated samples measurements were done in CAP solution after treatment.

Chlorophyll a fluorescence measurements

In this study, chlorophyll fluorescence was determined using a pulse-amplitude modulated (PAM) fluorometer (MINI-PAM: Walz, Germany), and the following parameters were measured: minimum fluorescence (F_o), maximum fluorescence (F_m), maximum quantum yield (F_v/F_m), electron transport rate (ETR) and non-photochemical quenching (NPQ). After dark-adaptation for 20 min., F_v/F_m was determined prior to and after 1 h treatments to high temperature and/or high light stresses and after 6 h recovery. The ratio of variable to maximal fluorescence in a darkened sample (dark-adapted F_v/F_m) is correlated to the maximum quantum yield of PSII (Genty *et al.* 1989). Under normal conditions, a linear relationship between the fraction of PSII reaction centers and F_v/F_o has been reported (Crofts *et al.* 1993), and F_v/F_o can be approximately estimated from its hyperbolic relation with F_v/F_m as $F_v/F_m / (1 - F_v/F_m)$.

Rapid light curves for ETR and NPQ were measured after a five minutes light adaptation at 85 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ followed by a 30 s dark period. The internal light source provided light intensities ranging from 0 - 460 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ at intervals of 20 s to produce the rapid light curves (RLCs). The range 0 - 460 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$ was used to prevent any damage due to high light to the photosynthetic apparatus and thus allowed reliable repeated measurements to be possible. Maximum electron transport rate (ETR_{max}) entails both the assimilatory and non-assimilatory electron flow, which

thus can be taken as electron flow beyond PSII. Linear relationships between gross photosynthesis rate measured by O_2 evolution and ETR were found under moderate irradiances (Yakovleva and Hidaka 2004; Wu *et al.* 2005), suggesting that ETR measurements provide an estimate of carbon fixation rate. The F_m values determined just before treatment exposures were used to determine the NPQ of the respective samples which were exposed to different treatments to allow reliable comparisons to be made among treatments (Li *et al.* 2003).

The distance between the specimens and the optic fiber head of PAM was kept constant (13 mm) for each sample so that accurate measurement of F_o and F_m could be achieved. PAM measurements after stress and recovery were done at the same position on the coral samples as before stress to avoid spatial heterogeneity in active chlorophyll signals of coral tissues (Ralph *et al.* 2002). All measurements were made at 25 °C. All corals except CAP-treated samples were immersed under filtered (0.45 μ m) seawater (FSW) during measurement.

Curve fitting of light curves and statistical analyses

ETR curves were fitted with the following equation (Platt *et al.* 1980):

$$P = P_s[1 - \exp(-\alpha E_d/P_s)]\exp(-\beta E_d/P_s)$$

P_s represents the maximum potential ETR in the absence of photoinhibition. α is the initial slope of light curve before the onset of saturation and represents the efficiency of light utilization. E_d is the downwelling irradiance (400-700 nm). β is the parameter characterizing photoinhibition. In the absence of photoinhibition in the light curves, where $\beta = 0$, the function becomes:

$$P = P_m[1 - \exp(-\alpha E_d/P_m)]$$

P_m is the maximum ETR (ETR_{max}) at light saturation and thus represents the photosynthetic capacity.

The ETR curves were compared among the initial, after stress and after recovery measurements at each temperature and/or light treatment by repeated measures analysis of variance (rm ANOVA). Since F_v/F_m , ETR_{max} and NPQ did not change significantly in corals that were kept at 25°C in darkness for 1 h and after 6 h recovery under dim light, it was used as control treatment. F_v/F_m , ETR_{max} and NPQ of the rest of the treatments were compared to the control. These comparisons were tested non-parametrically by Kruskal-Wallis test for analysis of variance by ranks. Relative values for F_v/F_m , ETR_{max} and NPQ, that is, values normalized to the initial

(before experimentation) ones, are independent of the topology of the coral fragments and thus were used for reliable comparison among treatments.

RESULTS

Maximum electron transport rate (ETR_{max}) and the maximum quantum yield of PSII (F_v/F_m) after stress treatments and recovery

Since the ETR_{max} and F_v/F_m represent the maximum electron transport rate and the maximum quantum yield of PSII, respectively, these two parameters were used in order to examine whether or not the primary site of thermal damage is the PSII in the photosynthetic apparatus of the zooxanthellae in *Pachyseris rugosa* exposed to light and heat stress (Fig. 1, 2). Comparison of rapid light curves for the electron transport rate between initial and either dark (Fig. 1A) or illuminated (Fig. 1B) 25°C treated corals did not reveal any significant deviations in ETR curves (rm ANOVA, $P > 0.05$), but exposure to 33.5°C did cause a significant decline in ETR_{max} both in darkness and under illumination (rm ANOVA, $P < 0.001$) (Fig. 1C, D). The 1 h light stress (1030 μ mol quanta $m^{-2} s^{-1}$) was sufficient to significantly affect the maximum quantum yield of PSII in *P. rugosa* under the control temperature conditions (25°C, Kruskal-Wallis test, $P < 0.05$, Fig. 2A). The combined effect of the light and temperature stresses (33.5°C) resulted in more pronounced decline in relative F_v/F_m (Kruskal-Wallis test, $P < 0.05$). In both cases, the disruption of photosynthesis was not restored to normal activity after the 6 h recovery under dim light. After exposure to 33.5°C in darkness, only the relative ETR_{max} suffered a pronounced but completely reversible decrease (Fig. 2). Although the relative ETR_{max} of *P. rugosa* exposed to 33.5°C decreased significantly to the same extent both in darkness and under illumination (Kruskal-Wallis test, $P < 0.05$), the recovery was incomplete after 6 h only in the light-treated samples.

Non-photochemical quenching upon stress treatments and recovery

The non-photochemical quenching (NPQ), a thermal dissipation process, was determined for the *in hospite* zooxanthellae before, after 1 h treatments and after 6 h recovery (Fig. 3). NPQ monitoring revealed reversible but significantly higher energy dissipation in PSII under high temperature and/or light levels used in this

Fig. 1. Rapid light curves for the electron transport rate of *in hospite* zooxanthellae of *Pachyseris rugosa* initially ($\diamond$), after 1 h treatment ($\square$) at 25°C (A, dark and B, illuminated at 1030 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$) and 33.5°C (C, dark and D, illuminated at 1030 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$), and after 6 h recovery ($\blacktriangle$) in dim light. Number of replicates was 4, except in graph C, where $n = 3$. Solid, semi-broken and broken lines represent curve fitting for initial, after 1 h treatment and after 6 h recovery, respectively. Means + SD.

Fig. 2. Maximum quantum yield of PSII, F_v/F_m , (empty bars) and maximum electron transport rate, ETR_{max} , (filled bars) relative to their respective initial values after 1 h treatment (A) and 6 h recovery (B) for *in hospite* zooxanthellae of the coral *Pachyseris rugosa*. Mean $\pm$ SD. $N = 4$, except for 33.5°C treatment in darkness, where $n = 3$. Different letters indicate significant differences at $P < 0.05$ (Kruskal-Wallis test) when the different treatments are compared to the control ones. Capital and small letters represent comparisons for F_v/F_m and ETR_{max} , respectively. 25+D, 25°C in dark; 25+L, 25°C under 1030 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$; 33.5+D, 33.5°C in dark; 33.5+L, 33.5°C under 1030 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$.

Fig. 3. Non-photochemical quenching (NPQ) relative to their respective initial values after 1 h treatment (filled bars) and 6 h recovery (empty bars) for *in hospite* zooxanthellae of the coral *Pachyseris rugosa*. Mean $\pm$ SD. N = 4, except for 33.5°C treatment in darkness, where n = 3. Significant differences between the controls and treatments are indicated by asterisk (Kruskal-Wallis test, $P < 0.05$). 25+D, 25°C in dark; 25+L, 25°C under 1030 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$; 33.5+D, 33.5°C in dark; 33.5+L, 33.5°C under 1030 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$.

study. Both high temperature in darkness and control temperature under high light treated samples exhibited two-fold higher NPQ (Kruskal-Wallis test, $P < 0.05$), while the combined high temperature and light treated samples showed three-fold higher NPQ compared to the control treatment (Kruskal-Wallis test, $P < 0.05$). NPQ for the recovered samples treated under any conditions were not significantly different when compared to control ones (Kruskal-Wallis test, $P > 0.05$).

Relationship between maximum quantum yield of PSII or active PSII centers and maximum electron transport rate

In order to examine the extent to which the maximum quantum yield of PSII and the active PSII centers are in excess of the maximum electron transport rate in zooxanthellae of *P. rugosa*, the relationships between the maximum quantum yield of PSII (F_v/F_m) or active PSII centers (F_v/F_o) and maximum electron transport rate (ETR_{max}) were studied with or without CAP, which prevents the repair of PSII reaction center D1 protein (Fig. 4). In the absence of CAP, the F_v/F_m decreased approximately linearly for the first 3 h to 60% of its initial value. Then it maintained the reduced level for the following 5 h, while the ETR_{max} was not affected during the 8 h experimental period (Fig. 4A). In the presence of CAP, the ETR_{max} was not affected significantly for the first 15 min, while F_v/F_m decreased to about 70%. The ETR_{max} value started

to decrease when F_v/F_m value decreased beyond 60% of its initial value (Fig. 4B). These findings indicate that more than 40% inhibition of the maximum quantum yield of PSII (Fig. 4B, inset) or 70% inactivation of PSII centers (Fig. 5) in symbiotic dinoflagellates limited their capacity for transporting electrons, i.e. relative ETR_{max} , and further inactivation resulted in a linear decrease of ETR_{max} in proportion to the maximum quantum yield of PSII or active PSII centers (Fig. 4B - inset, Fig. 5).

DISCUSSION

Coral bleaching due to elevated temperature has been subject of photo-physiological studies, which have led to a better understanding of the mechanism of this phenomenon. Most of the investigations including this study showed that PSII damage occurs under combined heat and light stress conditions (Jones *et al.* 1998; Warner *et al.* 1999; Bhagooli and Hidaka 2003, 2004). Our detailed investigation indicates that a component beyond PSII, possibly at the level of the dark reaction, might be the primary step of heat-induced damage in the algal photosynthesis and PSII damage is secondary. This is also consistent with Jones *et al.* (1998), who suggested that the first target of heat stress was the Calvin cycle, based on the use of induction curves which showed a decrease in fluorescence yield when non-photochemical quenching is enhanced at high

Fig. 4. Relative maximum quantum yield of PSII (F_v/F_m , empty squares) and relative maximum electron transport rate (ETR_{max} , empty circles) of in hospite zooxanthellae of the coral *Pachyseris rugosa* exposed to $250 \mu\text{mol quanta m}^{-2} \text{s}^{-1}$ light intensity at ambient temperature (25°C) for various lengths of time, in absence (A) and presence (B) of chloramphenicol. Mean $\pm$ SD. $N = 4$. Inset depicts the relationship between the relative maximum quantum yield of PSII and relative maximum electron transport rate, using pooled data ($n = 52$) from curve A and B. Curve was fitted with a third order polynomial ($r^2 = 0.88$).

Fig. 5. Relationship between relative active PSII centers (F_v/F_o) and relative maximum electron transport rate (ETR_{max}) from pooled data of CAP and non-CAP treated samples from Fig. 4. Data points represent relative values to the initial ones for respective samples ($n = 52$). Curve was fitted with a third order polynomial ($r^2 = 0.89$).

temperature.

Effect of high light and elevated temperature on the maximum quantum yield of PSII and maximum electron transport rate

The maximum quantum yield of PSII in the symbiotic dinoflagellates of *Pachyseris rugosa* was more sensitive to high light than to high temperature treatment, while the maximum electron transport rate was more sensitive to high temperature than to high light (Fig. 2). Photoinactivation of PSII and repair via de-novo protein synthesis occurs during normal photosynthesis, even under

normal growth conditions (Sundby *et al.* 1993; Melis 1999). Under high light exposure the repair might not completely keep pace with photoinactivation/damage of the photosystem II. It thus results in net loss of functionality of PSII, but this does not necessarily affect the rest of the electron transport chain and the carbon fixation cycle. Such an inactivation or slow repair of the PSII, which is still within the range of maintaining the ETR_{max} , might be an advantageous feature, since it protects the photosynthetic apparatus of the *in hospite* zooxanthellae by reducing the entry of excess electrons into

the photosynthetic transport chain due to excess light energy. In other words, the slow rate of re-synthesis of D1 protein of PSII can act as a safety mechanism to protect excessive electron transport downstream PSII.

The high sensitivity of ETR_{max} to high temperature could possibly be result from the sensitivity of the Rubisco activase enzyme to high temperatures as has been shown in higher plants (Feller *et al.* 1998; Crafts-Brandner and Law 2000). Calvin cycle inhibition is characterized by a moderate drop in electron transport rate along with a strong enhancement of energy-dependent non-photochemical quenching (Jones *et al.* 1998). Our results indicate a significant decrease in ETR_{max} (Fig. 1C) concurrent with a significant increase in non-photochemical quenching (Fig. 3) under high temperature stress conditions. Thus, it is likely that the observed decrease in ETR_{max} in this study is due to the inhibition of Calvin cycle.

In hospite zooxanthellae exposed to high temperature showed no damage to the maximum quantum yield of PSII (F_v/F_m), but a decreased ability to process absorbed light energy (ETR_{max}). Normal PSII activity accompanied by a reduced processing capability leads to over-reduction of the electron transport chain and production of active oxygen species. When high temperature and high light were used in combination, severe damage to PSII, reflected by decreased F_v/F_m , was observed. This can be explained by oxidative stress (Lesser 1997) generated as a consequence of blockage of electron flow downstream the PSII and the electrons reacting with oxygen molecules to form active oxygen species.

Recovery of the maximum quantum yield of PSII and maximum electron transport rate from high temperature and/or high light treatments

Differential restoration of the equally damaged ETR_{max} after high temperature treatment in darkness and after high temperature and high light treatments following the 6 h recovery period under dim light indicates possible damage to the recovery mechanism of the affected components contributing to ETR_{max} by light. Parallel recovery of the maximum quantum yield of PSII and the maximum electron transport rate were observed in corals exposed to combined high temperature and high light treatment (Fig. 2B). This implies that the repair of the PSII reaction center and the components of ETR_{max} occurred at a comparably equal rate. After the 6 h recovery period, the maximum quantum yield of PSII was

restored to a mean of about 60%, which normally should not limit the normal electron flow process (Fig. 4). The incomplete recovery of ETR_{max} , along with the recovery of functional PSII to a level that can sustain the ETR_{max} after 6 h recovery, in the corals exposed to combined high temperature and high light will most likely make the photosynthetic machinery more vulnerable to normal irradiance, as more electrons will be flowing through the PSII than could be processed as ETR_{max} . Thus the incomplete recovery of the damaged ETR components could be a more important determinant of further photodamage than PSII if corals are further exposed to either normal or high light.

Assessment of the relationship between the maximum quantum yield of PSII (F_v/F_m) or active PSII centers (F_v/F_o) and maximum photosynthetic rate (ETR_{max})

Chloramphenicol is a chloroplast-encoded protein inhibitor, more specifically employed to inhibit PSII D1 protein synthesis in plants (Schnetgger *et al.* 1994) and in zooxanthellae (Warner *et al.* 1999), and ensured the analysis of D1 protein degradation without re-synthesis. The maximum quantum yield of PSII decreased rapidly in the presence of CAP, suggesting that D1 protein re-synthesis was affected by this antibiotic (see Fig. 2A, B). The use of CAP in conjunction with light stresses for various lengths of exposure time allowed us to establish two phases in the relationships between the maximum quantum yield of PSII or the fraction of active PSII centers and the maximum electron transport rate measured by chlorophyll fluorescence technique (Fig. 4B - inset, Fig. 5). The initial linear phase (Fig. 5) might be interpreted as the maximum quantum yield of PSII or active PSII centers being a limiting factor in harnessing electrons from light and thus accounting for the linear decline of the ETR_{max} with decrease in F_v/F_m or F_v/F_o . Furthermore, the percentage of the maximum quantum yield of PSII and active PSII centers reducing the ETR_{max} to half its initial value was estimated to be 30 % (Fig. 4B inset) and 15% (Fig. 5), respectively. A reduction of the functional PSII to 19-36% has also been reported to reduce the maximum photosynthesis to half its normal value when using cultures of the marine diatom, *Thalassiosira weissflogii*, and the herbicide, 3-(3,4-dichlorophenyl)-1, 1-dimethylurea (DCMU), an inhibitor of PSII reaction centers functioning in conjunction with the oxygen evolution technique to determine maximum

photosynthesis (Behrenfeld *et al.* 1998).

The plateau phase occurring in the F_v/F_m range of 0.4 - 0.67 (~60% - 100%; Fig. 4B - inset), indicates that inactivation of PSII by up to 40% can still drive maximum ETR and hence maximum carbon fixation. Similar levels of partial inhibition of the maximum quantum yield of PSII that maintain ETR_{max} or maximum photosynthesis have been reported in higher plants as well as in zooxanthellae. A minimum of 52% of functional PSII in higher plants could maintain 100% of their maximum photosynthesis (Behrenfeld *et al.* 1998). Moreover, PSII of *Capsicum annuum* leaves does not limit the maximum rate of photosynthesis until more than about 40% of PSII have been photoinactivated (Lee *et al.* 1999). Decrease in the maximum quantum yield of PSII of up to 60 - 70% still maintains the maximum electron transport rate in endosymbionts of corals in the field where they experience natural high sunlight levels during midday (Hoegh-Guldberg and Jones 1999).

Damage and recovery of the maximum quantum yield of PSII and maximum electron transport rate

The insensitivity of ETR_{max} to significant declines in the maximum quantum yield of PSII (F_v/F_m) or active PSII centers (F_v/F_o) in the relationship evaluation experiment clearly suggests that the overall photosynthetic electron flow can remain practically unchanged, although substantial PSII photodamage has occurred. This uncoupling between the maximum quantum yield of PSII or functional PSII centers and rates of electron flow suggests that photoinhibition of PSII is not directly influencing the rate-limiting step of photosynthesis. The elevated temperature exposure experiments showed that high temperature alone can inhibit the rate-limiting step of photosynthesis, as indicated by the reduction in ETR_{max} . Furthermore, the restoration of PSII function up to a level that can maintain ETR_{max} , accompanied by an incomplete recovery of ETR_{max} , makes the photosynthetic machinery more vulnerable to even normal light levels and further heat damage. These findings indicate that damage in the process of ETR_{max} due to stress is more important than damage to PSII, though the incomplete recovery of some components of the photosynthetic machinery might add to that effect.

Takahashi *et al.* (2004) suggested that the repair system of D1 protein of PSII is the primary target of heat stress, as in their study the rate of recovery of F_v/F_m after light-induced photoinhibition

at 28 or 32°C was temperature-dependent. They also showed species-specific differences. If the repair system was inhibited by CAP, the temperature effect disappeared and species-specific differences were reduced. However, F_v/F_m of their samples recovered even in the presence of 600 μ M CAP indicating that inhibition of D1 protein repair system was incomplete in their experiments. Takahashi *et al.* (2004) also discussed that reactive oxygen species (ROS) might be involved in repair inhibition at high temperature. Since the production of ROS is enhanced through the inhibition of Calvin cycle, it is also possible that the primary target of heat stress is the Calvin cycle and the resulting production of ROS leads to increased damage of PSII and its repair inhibition as suggested by the present study.

Yakovleva and Hidaka (2004) compared the rate of recovery between PSII function and the dark reaction of zooxanthellae of two corals with different bleaching susceptibilities. They observed that the recovery of ETR_{max} after high temperature and high light stress treatment was slower than that of F_v/F_m in a bleaching susceptible coral, *Montipora digitata*, and this delay of ETR_{max} recovery increased with increasing temperature during the recovery period. In a bleaching tolerant coral, *Pavona divaricata*, the recovery of F_v/F_m and ETR_{max} was almost complete within 24 h after stress treatment. They suggested that the difference in the rate of recovery between PSII function and the dark reaction (measured as ETR_{max}) of zooxanthellae determines susceptibility of corals to bleaching.

The present study demonstrated that the electron transport beyond the PSII, possibly the dark reaction, might be the initial target of heat stress in zooxanthellae of the coral *Pachyseris rugosa* as evidenced by the combined measurements of ETR_{max} , NPQ and F_v/F_m . Decreases in electron transport rate accompanied by increases in non-photochemical quenching under heat stress in our study indicate inhibition of the Calvin cycle enzymes as initial target of heat damage. This study further showed that, even when the PSII function and electron transport rate recovered to a similar extent, partial (around 60%) recovery of PSII enabled the photosystem to generate enough electrons to drive the maximum electron transport rate, while these electrons cannot be fully consumed by an incompletely recovered electron transport system. Thus the recovery of the PSII up to a level that normally can maintain the maximum electron transport rate

accompanied by an incomplete recovery of the latter might make the photosynthetic machinery of zooxanthellae more susceptible to oxidative stress under normal irradiance conditions.

ACKNOWLEDGEMENTS

The authors would like to thank I.M. Yakovleva, M.P. Lesser, J.-F. Flot, G. Winters, A.H. Baird and anonymous reviewers for their critical and insightful suggestions on the manuscript. The authors also wish to thank the staff of Sesoko Station, Tropical Biosphere Research Center, University of the Ryukyus, for allowing us to use the facilities. R.B. is thankful to the Ministry of Education, Culture, Sports, Science and Technology, Japan for a scholarship.

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(Received: 6 Sept. 2005/Accepted: 17 Jan. 2006)

造礁サンゴ褐虫藻の光化学系Ⅱ最大量子収率と最大電子伝達速度の高温による阻害と回復について

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サンゴの白化を引き起こす最初のステップは褐虫藻の光化学系Ⅱ (PSⅡ) の損傷であるかを調べるために、シワリュウモンサンゴの褐虫藻において、PSⅡの活性を示すPSⅡ最大量子効率 (F_v/F_m) またはアクティブPSⅡ中心 (F_v/F_o) とPSⅡの下流での電子の流れの指標である最大電子伝達速度 (ETR_{max}) の関係を調べた。次に、高温 (33.5℃) または強光 (1030 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$) 1時間処理後および6時間回復後のこれらパラメータの変化を調べた。暗黒下で1時間高温処理すると、 F_v/F_m は変化しなかったが、 ETR_{max} は有意な、しかし回復可能な低下を示した。1時間の強光処理は、 F_v/F_m を低下させたが、 ETR_{max} には影響しなかった。強光+高温処理は、 F_v/F_m をより顕著に低下させ、 ETR_{max} も有意に低下させた。同時にPSⅡにおける熱消散を示す非光化学的消光 (NPQ) が一時的に上昇した。 F_v/F_m も ETR_{max} も6時間では完全に回復しなかった。PSⅡ反応中心のD1タンパクの合成を阻害するクロラムフェニコールを用いた実験の結果、 F_v/F_m の少なくとも60%またはアクティブPSⅡ中心の少なくとも30%が機能していれば最大電子伝達速度は維持された。褐虫藻光合成系の高温による損傷は、最初にPSⅡ以降の要素、おそらく炭素固定回路、に生じ、PSⅡの損傷は2次的なものであることが示唆された。