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Photosynthetic response to elevated temperature in the symbiotic dinoflagellate *Symbiodinium microadriaticum* in culture

(bleaching/coral reefs)

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ABSTRACT **Elevated temperature (28–34°C) has been hypothesized as the primary cause of the loss of algal endosymbionts in coral reef-associated invertebrates, a phenomenon observed on a world-wide scale over the last decade. In past studies of this "bleaching" phenomenon, there has been an underlying assumption that temperature adversely affects the animal hosts, the algae thereby being relegated to a more passive role. Because photosynthesis is a sensitive indicator of thermal stress in plants and has a central role in the nutrition of symbiotic invertebrates, we have tested the hypothesis that elevated temperature adversely affects photosynthesis in the symbiotic dinoflagellate *Symbiodinium microadriaticum*. The results, based on analyses of light-mediated O_2 evolution and *in vivo* fluorescence, indicate that photosynthesis is impaired at temperatures above 30°C and ceases completely at 34–36°C. These observations are discussed in the context of possible mechanisms that may function in the disassociation of algal-invertebrate symbioses in response to elevated temperature.**

Over the past several years, there have been numerous reports of the phenomenon termed "coral bleaching" (1–8). This phenomenon manifests itself by the loss of color in symbiotic invertebrates characteristically associated with coral reefs and is often the result of the loss of their endosymbiotic algae, though reduction or loss of algal pigments, without changes in algal population density, may produce an apparently similar result (6, 9). In the field, symbiotic reef-dwelling invertebrates bleach in response to lowered salinity (10), and bleaching is correlated with episodic increases in temperature (28–34°C), an example being the severe El Niño Southern Oscillation ocean warming event of 1982–1983 in the Pacific Ocean (1, 2, 5, 11–13). However, extremes of several environmental factors (temperature, salinity, irradiance, O_2 tension) may produce a similar response under laboratory conditions (14–16). It has also been hypothesized that elevated temperature, acting in synergy with other factors (elevated pO_2, ultraviolet radiation) may be the causative agent in bleaching (17, 18).

In the context of symbiosis, bleaching can be viewed as a disassociation of the algae and their respective hosts. Hence, in understanding how these symbioses respond to environmental stimuli, it is necessary to determine which partner in the association perceives the stimulus and how it is transduced into the observed response. In many discussions of the phenomenon of bleaching, an implicit assumption has been that high temperature (or some other environmental stimulus) adversely affects the hosts; the algal symbionts by implication are passive participants (5, 8). Alternatively, others (6, 12, 17) have hypothesized that the stimulus may be perceived by the algae, leading to metabolic changes which ultimately result in a disassociation of the symbiosis. In resolving how a two-component system such as a symbiosis responds to environmental stimuli, it is necessary to analyze the two components separately, as well as the intact association. To our knowledge, no studies taking this approach have been reported.

To initiate a systematic analysis of the response of a symbiosis to elevated temperature, we have analyzed the photosynthetic response to elevated temperature of the symbiotic dinoflagellate *Symbiodinium microadriaticum* in culture, under rigorously controlled laboratory conditions. Photosynthesis is a sensitive indicator of thermal stress in plants (19). The results indicate that photosynthesis is impaired at temperatures above 30°C and completely ceases at about 34°C, suggesting that the algae themselves are adversely affected by elevated temperatures.

METHODS

Maintenance of Dinoflagellate Cultures. Populations of the symbiotic dinoflagellate *S. microadriaticum*, originally isolated from the jellyfish *Cassiopeia xamachana*, were cultured axenically in 1 liter of ASP-8A medium (20) in 2.8-liter Fernbach flasks at 26°C, with illumination provided by fluorescent tubes delivering 80 μmol of quanta·m^{-2}·s^{-1} photosynthetically active radiation (PAR, 400–700 nm), on a photoperiod of 14:10 hr (light:dark).

For experimental purposes, algal cells were separately grown under hyperoxic (63%, vol/vol), normoxic (21%), and hypoxic (1–3%) pO_2 as described previously (21). To standardize the influence of culture age on physiological responses, all measurements were made using cultures in exponential growth phase. In addition, because some periodicity was observed in photosynthetic rates, all measurements were made at the same time of day (apparent noon).

Measurement of Photosynthesis and Respiration. Photosynthesis was measured by assessing the light-saturated rate of net photosynthesis (net P_{max}) based on O_2 evolution. Respiration was measured on the same cell suspensions by assessing O_2 consumption in the dark.

Oxygen flux was measured by using a Clark-type O_2 electrode (Yellow Springs Instruments 57). All experiments were conducted using the same stirring speed, algal cell densities of $2.4 \cdot 10^5$·ml^{-1}, and a photon flux density of 500 μmol of quanta·m^{-2}·s^{-1} PAR. Temperature within the jacketed chamber was controlled to ±1°C with an external recirculating water bath. Samples were preincubated in the dark for 30 min at experimental temperature, and $NaHCO_3$ was added to a final concentration of 5 mM to prevent CO_2 limitation. In all cases, initial O_2 tension in the experimental chamber was 20% saturation (22). The solubility of O_2 in sea water was calculated from published values (23). Data were collected directly by means of a personal computer equipped

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Abbreviations: PAR, photosynthetically active radiation (400–700 nm); DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea.

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with an analog/digital converter and corresponding software (Datacan IV, Sable Systems, Salt Lake City, Utah).

Whole-Cell Fluorescence Measurements. Whole-cell fluorescence emission, measured in the absence and presence of 50 μM 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) (24–26), was assayed with a Perkin–Elmer spectrofluorometer (LS-50) equipped with a red-sensitive photomultiplier tube (model R-928) and a temperature-controlled cuvette holder. With excitation at 435 nm (4-nm slit width), fluorescence emission, detected at 680 nm (20 nm slit width), was integrated over 30 s. Low cell densities ($5 \cdot 10^4 \cdot ml^{-1}$) were employed to minimize reabsorption. The cells were exposed, in the dark, to the experimental temperatures for 45 min, with DCMU added to the controls after 5 min. The value F_{+DCMU}/F_{-DCMU} is an indication of the efficiency of coupling of energy absorption and photochemistry (26).

Estimation of Chlorophyll and Cell Numbers. Chlorophyll was estimated spectrophotometrically after extracting it from algal cells three or four times in acetone/dimethyl sulfoxide (90:10, vol/vol), using the equations of Jeffrey and Humphrey (27). Cell numbers were estimated by the method described by Schoen (28).

RESULTS

Effect of Temperature on Algal Photosynthesis. To study the effects of short-term exposure to elevated temperature in *S. microadriaticum*, cells grown at 26°C were exposed to temperatures ranging from 20°C to 35°C (intervals of 5°C) for 45 min.

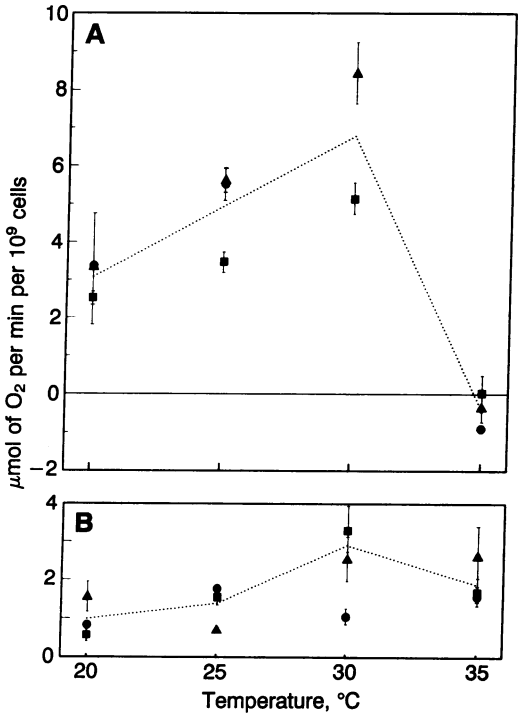

FIG. 1. (*A*) Effect of temperature on photosynthesis (net P_{max}) in *S. microadriaticum*. ■, cells grown under hyperoxic conditions; ●, cells grown under normoxic conditions; ▲, cells grown under hypoxic conditions. Each symbol represents the mean of three replicate analyses, and the bars represent SEM. The three-replicate data set for normoxic cells at 30°C was lost. All data from each O_2 treatment (in *A* and in *B*) were generated from the same culture. The data for the three growth conditions at each temperature were pooled, and the means are connected by the broken line, with no statistical significance implied. (*B*) Effect of temperature on respiration in *S. microadriaticum*. Symbols as in *A*. In each case, the symbol represents the mean of three replicate analyses, and the bars represent ±1 SEM. The data for the three growth treatments were pooled, and means are connected by the broken line. No statistical significance is implied.

Assays for net P_{max} expressed on a chlorophyll-specific basis (not shown) or a cell-specific basis (Fig. 1*A*) produced identical results. Algal chlorophyll *a* content remained unchanged under our experimental conditions. The data indicate an increase in photosynthesis between 20°C and 30°C. At 35°C, the net P_{max} value was either zero or negative. Should the respiration values (Fig. 1*B*) be added to the net P_{max} values to calculate gross P_{max}, then at 35°C gross P_{max} would range between 0.5 and 1.5 μmol of O_2 per min per 10^9 cells, much less than the values obtained at 20°C. The Q_{10} ratio for net photosynthesis (the mean over all treatments) over the temperature range 20–30°C was 2.2. In Fig. 1*A*, the data points suggest that cells grown under hypoxic and normoxic conditions demonstrated higher net P_{max} values than those grown under hyperoxic conditions, but the temperature-related decline in photosynthesis between 30°C and 35°C was the same in all cases.

Effect of Temperature on Algal Respiration. Changes in respiration rates were very small (Fig. 1*B*), as has been observed by others (29). Although it is possible to construe a general increase in respiration rates between 20°C and 30°C, and perhaps a leveling off or small decline between 30°C and 35°C, the variation between treatments is too high to warrant any conclusion. The significant point is that in no treatment did respiration completely cease at 35°C, indicating that the cells were not dead.

Effect of Temperature on Whole-Cell Fluorescence. To ascertain a mechanism for the sharp decline observed in photosynthetic rates above 30°C, and to more accurately assess the temperature at which the decline in photosynthesis occurred, we employed the sensitive assay of whole-cell fluorescence at 2°C intervals over the temperature range 20–36°C. The ratios of F_{+DCMU}/F_{-DCMU} (Fig. 2) in cells grown under normoxic conditions were higher than those grown under hyperoxic conditions, but in both cases the ratio decreased rapidly above 30°C, and at 36°C was 1.0, indicating complete uncoupling of energy absorption and photochemistry. Hence the *in vivo* fluorescence data corroborate the data based on O_2 evolution.

DISCUSSION

Our results indicate that elevated temperatures (32–36°C) adversely affect photosynthesis in *S. microadriaticum* in

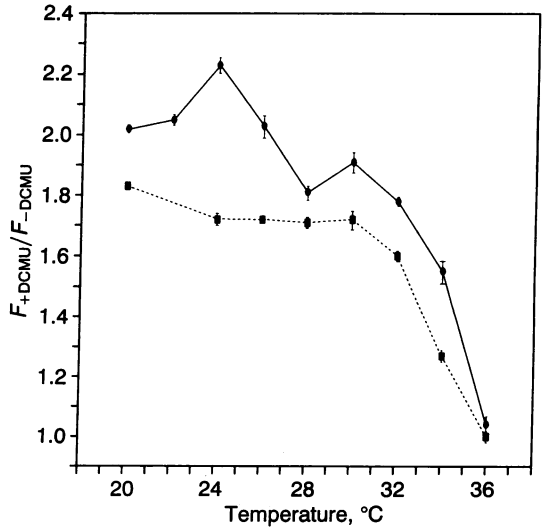

FIG. 2. Effect of temperature on whole-cell fluorescence (F_{+DCMU}/F_{-DCMU}) in *S. microadriaticum*. Symbols as in Fig. 1. Bars represent ±1 SEM for nine replicates. In some instances the error bars are contained within the symbol.

culture. These temperatures coincide with those reported as correlative with bleaching in symbiotic coral reef-associated invertebrates (5, 18). Growth of the cells at different oxygen tensions did not appear to have any direct influence on the temperature-related decrease in photosynthesis. Although we did not specifically test the hypothesis that elevated temperature and oxygen hypertension act synergistically to effect bleaching (17), our results do not indicate any direct influence of oxygen tension on the temperature-related decline in photosynthesis in the algae. It may be significant, however, that although the light-saturated rates of photosynthesis decreased above 30°C, and photosynthesis ceased completely at 34–36°C, algal cell respiration did not, indicating that the cells were not dead. The data indicate that the algae themselves perceive and respond to elevated temperature. Therefore, any mechanistic model for thermal bleaching in symbiotic reef-dwelling invertebrates must include the algae as an integral component.

The increased photosynthetic rates observed between 20°C and 30°C can be explained by increased activity of enzymes of the reductive pentose cycle. The Q_{10} values for photosynthesis that we obtained are in agreement with those reported in algae and plants over the same temperature range (29, 30). The observed decrease in oxygen evolution above 30°C is also reflected in the fluorescence analyses, and it is probably the result of changes in the electron transport capacity caused by phase transition (increased fluidity) of polar lipids in the thylakoid membranes (26, 31). Similar responses probably occur in other taxa of symbiotic algae, the phytoplankton, and benthic macrophyte populations associated with coral reefs. Our results indicate the response of the algae to short-term exposure to elevated temperature and should therefore be regarded as conservative estimates of physiological "stress." In their natural environment, symbiotic dinoflagellates may be exposed to elevated temperatures for substantially longer periods (13, 32), and long exposure to temperatures of 27–30°C may have the same impact as short-term exposure to temperatures above 30°C (8). Physiological adjustments in response to changes in temperature may be short-term acclimatory responses or long-term genotypically fixed adaptations, *sensu* Berry and Raison (31). The extent to which *S. microadriaticum* may acclimate or adapt to elevated temperatures was not addressed in this study, but unpublished observations from our laboratory on *S. microadriaticum* and on the *Symbiodinium* sp. from *Aiptasia tagetes* (W. K. Fitt, personal communication) indicate that growth of these algae is markedly reduced above 30°C, suggesting a genetically fixed temperature limitation. As the algae exposed to the highest temperatures still respired, the possibility exists that they may recover, but recovery from thermal stress was not addressed in this study.

In any attempt to understand thermal bleaching, there are several observations that must be taken into consideration. Some pertinent observations include the following: In most recorded episodes of bleaching, some symbiotic invertebrate species populations lose their algae while others in close proximity, often presumed to be the same species, do not; bleaching may occur in response to several different environmental stimuli (temperature, salinity, O_2 tension, ultraviolet radiation); and many invertebrates in shallow lagoonal environments may be exposed daily to elevated temperatures at low tides, but do not bleach, while those on the forereef slopes do bleach when exposed to the same range of elevated temperatures.

It should be emphasized that the disassociation of algal-invertebrate symbioses appears to be in response to *extremes* in environmental conditions. The fact that these symbioses involve taxonomically diverse algae (33–39) and hosts would suggest that different associations possess different levels of tolerance to changes in environmental parameters (8). The literature contains very little information on aspects of physiological tolerance in coral reef-associated invertebrates and even less on symbiotic dinoflagellates. Understanding the bleaching response at the level of natural populations is also rendered difficult in the face of the paucity of secure taxonomy of both symbionts and hosts. Given the species diversity of the algae and the high degree of host–symbiont specificity demonstrated by these symbioses (40, 41), it is possible that the different sibling species of *Montastraea annularis* (42) harbor different species of dinoflagellates with different temperature tolerances. Hence, when exposed to the same temperature, one population may bleach and not the other. The patchy distribution of bleaching within species populations of corals may be a fundamental problem with coral taxonomy; the insecurity of coral taxonomy is probably more widespread than is currently appreciated (42).

The basic phenomenon underlying thermal bleaching is signal transduction. Components of the symbiosis perceive an environmental stimulus, and this stimulus is transduced into a response, the end result of which is the disassociation of the symbiosis. On the basis of the results of our study, it is evident that algal photosynthesis is inhibited at temperatures above 30°C, because of an uncoupling of energy absorption and photochemistry, probably resulting from changes in lipid characteristics of the thylakoid membranes. Algal photosynthesis is of major trophic significance to symbiotic invertebrates (43), and hence a reduction or cessation in the movement of metabolites from algae to hosts could potentially result in disruption of the interactions between them. The recent observations of Markell *et al.* (44) that symbiotic dinoflagellates in culture release proteins/glycoproteins, candidates for chemical recognition signals passing between symbionts and hosts, and that this phenomenon also occurs in the hosts (D. A. Markell and R.K.T., unpublished observations) raise the possibility that impairment or cessation of photosynthesis disrupts the flow of such signals, resulting in the dissolution of the association. Details of the cellular and molecular aspects of these events, from the perspective of the animal hosts and the symbionts, await further investigation.

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