Oxidative Stress, Small Heat-shock Proteins and Coral Bleaching

Craig A. Downs[1], Phillip Dustan[2], Erich Mueller[3], Aaron G. Downs[4], Susan Phillips[5], Cheryl M. Woodley[1]

[1] Center for Coastal Environmental Health and Biomolecular Research, U.S. NOAA, NOS Charleston, South Carolina, U.S.A.
[2] Department of Biology, University of Charleston, Charleston, South Carolina, U.S.A.
[3] Mote Marine Laboratory, Summerland Key, Florida, USA
[4] Department of Biology, Kenyon College, Gambier, Ohio, U.S.A. [5] Department of Biology, Brevard College, Palm Bay, Florida, USA

Introduction

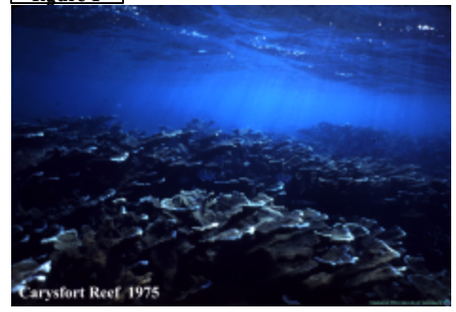
Corals are symbiotic organisms composed of a photosynthetic dinoflagellate (zooxanthellae) and an anthazoan animal and are responsible for the massive calcium carbonate structures of coral reefs. Coral reefs constitute some of the largest ecological structures on earth and provide an estimated 10% of the world's fisheries. Over the past two decades, coral reefs have experienced extensive degradation due in part to extensive coral bleaching (Figure 1). Coral bleaching involves either the mass expulsion of the zooxanthellae or the loss of photosynthetic pigments within the dinoflagellate (Figure 2). Strong correlations between high water temperature and coral bleaching suggest that the cause of coral bleaching may be related to heat stress. Corals may recover and regain their zooxanthellae after several months of bleaching as water temperature decreases due to seasonal changes (Figure 2). If water temperatures remain high through most of the growing season (e.g., global warming, major climatic changes such El Niño), corals may not recover their zooxanthellae and die (Figure 1) (Dustan, 1999).

The mechanism of bleaching is suggested to be heat stress-associated thermolability of certain components of the photosynthetic machinery of the dinoflagellate. Photosystem II is one of the most thermolabile components of photosynthesis. Heat stress can alter electron flow within Photosystem II from its normal pathway from chlorophyll to quinone and instead to oxygen, forming superoxide. Hydrogen peroxide formed from the Oxygen Evolving Complex of Photosystem II may react with superoxide to form hydroxyl radicals. These hydroxyl radicals may be destructive to Photosystem II if it overwhelms the anti-oxidant defenses of the chloroplast. Evidence from several labs indicates that coral bleaching is caused by increased oxidative stress originating from within the chloroplast and that this can cause destruction of a number of different photosynthetic components (Lesser, 1995; Warner 1999).

One adaptation of vascular plants is the production of the chloroplast small heat-shock protein (chlpsHsp). The chlpsHsp is known to specifically protect Photosystem II from both heat stress and oxidative stress (via ultraviolet radiation or chemically induced hydroxyl radicals) (Heckathorn et al., 1998; Downs et al., 1999b). The chlpsHsp has a unique methionine-rich domain and is known to bind and protect the Oxygen Evolving Complex of Photosystem II (Downs et al., 1999a; Heckathorn et al., in press). Preliminary evidence indicates that the chlpsHsp acts as a target-specific anti-oxidant (Downs et al., 1999a).

It is unknown if dinoflagellates possess a chlpsHsp or whether this protein acts in a homologous fashion by protecting Photosystem II. The objectives of this study are to determine: (1) whether there exists a sHsp in the chloroplasts of dinoflagellates; (2) if this chlpsHsp has an antigenically similar methionine-rich domain; (3) the induction pattern of the chlpsHsp in corals during coral bleaching.

Figure 1



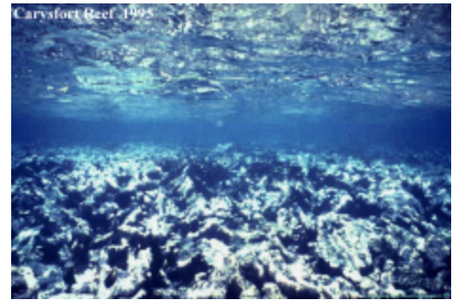
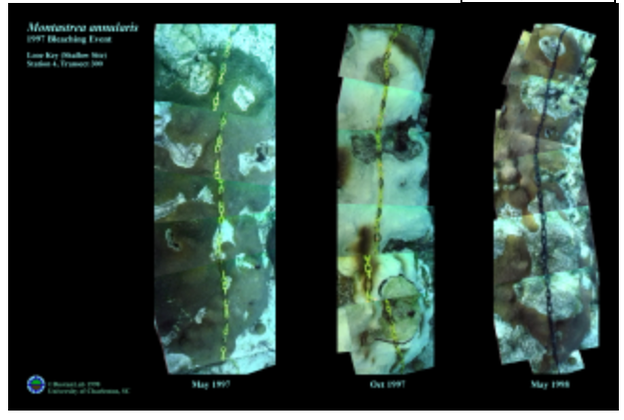


Figure 2



Materials & Methods

Dinoflagellate *Symbiodinium* spp. were kind gifts from Drs. Mark Warner and William Fitt (Univ. of Georgia, Athens, Georgia, USA). Dinoflagellates were grown under conditions of 25°C temperature and 50 µM m^{-2} s^{-1} PAR during a 14 hour photoperiod. Dinoflagellates were heat stressed by ramping the temperature from 25C to 31C over a three hour period, held at 31C for two hours, ramped again from 31C to 35C over a three hour period, held at 35C for one hour and then ramped down from 35C to 25C over four hours. Dinoflagellates were then harvested at the end of the heat stress regime. Chloroplasts from both control and heat-stressed dinoflagellates were isolated according to a modified methods of Downs et al., 1998 by breaking open cells using a modified "bead-beater" method.

Coral Field Collection: *Montastrea annularis* corals were collected from Carysfort Reef, Key Largo, Florida. All corals samples were collected around 20 ft. of depth. Five types of corals were collected: healthy, lightly bleaching, intermediate bleaching; advanced bleaching, and bleached. Corals were water picked using a SDS-homogenization buffer, placed in Falcon tubes, and frozen immediately upon harvesting.

Coral Laboratory Collection: *Monstatrea annularis* corals were raised at Mote Marine Research Laboratory in Pigeon Key, Florida, USA under 25°C-28°C temperature and 400 µM m^{-2} s^{-1} PAR. Corals were subjected to heat stress by ramping the temperature from 25°C to 33°C and held for eight hours. Corals were frozen after heat stress regime.

Both corals and dinoflagellate tissue were homogenized in an SDS-homogenization buffer. Protein concentration of samples were determined and samples were subjected to SDS-PAGE. Gels were then subjected to western blotting, and membranes were assayed with a number of different antibodies: antibody against Hsp60; antibody against the "methionine-rich" domain of the chlpsHsp; antibody against chloroplast Mn-superoxide dismutase and antibody against Cu/Zn superoxide dismutase.

Results

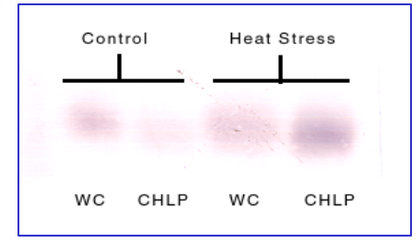


Figure 3. Western blot of control and heat-stressed dinoflagellates. Dinoflagellates were subjected to control and heat-stress temperatures. Chloroplasts were isolated from dinoflagellates under both treatments. Whole cell and purified chloroplasts were subjected to SDS-PAGE (50 µg/per lane), Western blotted, and assayed using an antibody specific to the "methionine-rich" domain of the chloroplast small heat-shock proteins of terrestrial plants.

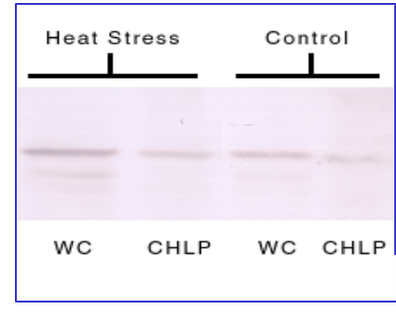


Figure 4. Hsp60. Western blot of control and heat-stressed dinoflagellates. Dinoflagellates were subjected to control and heat-stress temperatures. Chloroplasts were isolated from dinoflagellates under both treatments. Whole cell and purified chloroplasts were subjected to SDS-PAGE (50 µg/per lane), Western blotted, and assayed using an antibody to Hsp60, a protein found only in the mitochondrion and chloroplast.

Figure 5. Mn superoxide dismutase. Coral cultures were grown in the laboratory for over six months. Corals were heat stressed both in the light and in the dark. Coral tissue was homogenized in an SDS buffer, subjected to SDS-PAGE, Western blotting, and the blot was assayed with an antibody specific to manganese superoxide dismutase (localized to only the mitochondrion and chloroplast) as an indicator of oxidative-stress response.

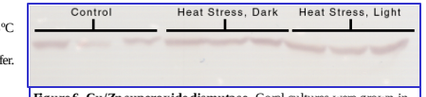


Figure 6. Cu/Zn superoxide dismutase. Coral cultures were grown in the laboratory for over six months. Corals were heat stressed both in the light and in the dark. Coral tissue was homogenized in an SDS buffer, subjected to SDS-PAGE, Western blotting, and the blot was assayed with an antibody specific to Cu/Zn superoxide dismutase (localized to only the mitochondrion and chloroplast) as an indicator of oxidative-stress response.

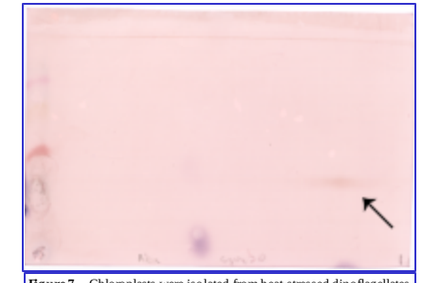


Figure 7. Chloroplasts were isolated from heat-stressed dinoflagellates and solubilized in a 2D-PAGE buffer consisting of 8 M urea and 1% triton X-100. Sample was subjected to pH 3-10 isoelectric focusing and then 1D SDS-PAGE, Western blotted, and the blot was assayed with an antibody specific to the "methionine-rich" domain of the chloroplast small heat-shock protein of terrestrial plants (Abmet).

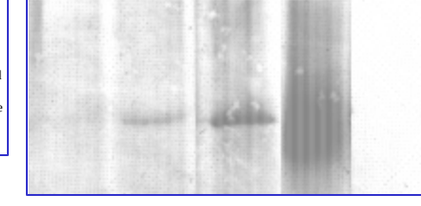


Figure 8. Separate coral colonies from Carysfort Reef were collected and subjected to SDS-PAGE, Western blotting and assayed with Abmet. Lane 1 = healthy; lane 2 = slightly bleaching; lane 3 = intermediate bleaching; lane 4 = severe bleaching; ; lane 5 = completely bleached (no zooxanthallae)

Discussion

In summary, we present evidence for the existence of a heat-inducible chloroplast small heat-shock protein (chlpsHsp) that has antigenic homology to the methionine-rich chloroplast sHsps of terrestrial plants. Further, there is a positive relationship between the quantitative accumulation of this protein and coral bleaching. If the dinoflagellate chlpsHsp has a homologous function to that of terrestrial chlpsHsp, then this would indicate that: (1) during bleaching, Photosystem II is being damaged, either by heat stress or oxidative-stress mediated heat stress; (2) progression of coral bleaching would indicate a gradual increase of oxidative/heat stress; (3) the chlpsHsp is not only a diagnostic indicator of coral stress, but could possibly be used as a prognostic indicator for the pathology of coral bleaching.

Evidence from our own lab and that of our collaborators at the University of Georgia (Athens, Georgia, USA) suggests that Photosystem II may be central for the generation of oxidative stress within corals. Observations from the scientific coral community indicate that there exists clades of dinoflagellates that are more likely to incur bleaching. These observations, coupled with this data, is now being used to further research in alleviating coral bleaching: either via transgenic approaches or by classical selection culturing. Such dinoflagellate strains could be hypothetically used to seed bleaching coral to such an end that may either prevent further bleaching and/or aid in the recovery of bleaching.

It has been suggested that a prime cause of bleaching is increasing regional or global temperatures. Other factors such as eutrophication, pesticides, herbicides, and other pollutants may be contributing factors to coral bleaching, and may be primary factors for bleaching in the absence of high temperatures. Our labs, in collaboration with many other laboratories have begun the elucidation of both the function and mechanism of protection for the chlpsHsp, as well as other sHsps, in corals and determining if toxin-mediated oxidative stress is a relevant factor for many regions where coral bleaching exists.

References

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