Marine Biology 103, 381–385

Marine Biology
International Journal on Life in Oceans and Coastal Waters

Light harvesting in the green alga *Ostreobium* sp., a coral symbiont adapted to extreme shade*, **

D.C. Fork and A.W.D. Larkum

School of Biological Sciences, The University of Sydney, Sydney, New South Wales 2006, Australia

Abstract

Ostreobium sp. (Chlorophyta: Siphonales) can be found as green bands within the skeletal material of a number of stony corals in the Indo-Pacific and Caribbean regions. Many of these corals also contain symbiotic dinoflagellates in the overlaying coral polyps that effectively screen out all the typical photosynthetically active radiation from the algae in the green bands below. *Ostreobium* sp., nevertheless, grows photosynthetically. Its action spectrum and absorption spectrum have been shown to extend much further into the near infra-red compared to other green algae. In the present study, carried out in 1987, fluorescence excitation and emission spectra were measured in *Ostreobium* sp. and compared to spectra obtained from the green alga *Ulva* sp. and the brown alga *Endarachne* sp. Xanthophylls, probably siphonein and an unidentified xanthophyll probably related to siphonaxanthin, are photosynthetically active in *Ostreobium* sp., and can sensitize Photosystem II fluorescence at 688 nm and Photosystem I (PS I) fluorescence at 718 nm. The fluorescence emission spectra of *Ostreobium* sp. measured at 25 °C and 77 K were not remarkably different from those of the green alga *Ulva* sp. Absorbance changes induced by light were measured in *Ostreobium* sp. from 670 to 750 nm and were like those normally seen in green plants except that, in addition to the minimum expected for the reaction-center chlorophyll of PS I (P700) at 703 nm, another minimum was seen at 730 nm. It is possible that this spectrum reflects the functioning of a reaction center of Photosystem I that has adapted to function in light highly enriched in far-red wavelengths.

Introduction

The siphonaceous green algae of the genus *Ostreobium* sp. inhabit the calcareous skeleton of stony corals in the Indo-Pacific and Caribbean regions. Many of these corals also contain zooxanthellae, brown colored dinoflagellates, as symbionts within the living tissues of the polyps. In large brain corals such as the genus *Favia* sp., the dinoflagellates found in the overlaying animal tissues provide a dark brown covering that absorbs almost all the incident photosynthetically active radiation (PAR). It has been estimated by Halldal (1968) that, at most, 0.1% of the PAR between 340 and 680 nm is transmitted by this dinoflagellate layer. At 700 and 720 nm, the screening dinoflagellate layer transmits only 1 and 2%, respectively. Halldal made a preliminary study of the light-intensity response of photosynthesis as well as action spectra for photosynthetic oxygen production by *Ostreobium* sp. A comparison of absorption and action spectra for *Ostreobium* sp. with those measured by Haxo and Blinks (1950) for the green alga *Ulva taeniata* reveals striking differences, especially in the red regions of the spectrum. *U. taeniata* has its main red absorption and action peak near 672 nm, while *Ostreobium* sp. has its absorption and action maxima near 690 nm and a very strong shoulder of activity around 720 nm. The broad shoulder in the action spectrum of *U. taeniata* around 650 nm reflects the participation of chlorophyll (chl) *b*. The action spectrum of *Ostreobium* sp. has an even stronger and wider shoulder, produced by the participation of chl *b* which is known to be abundant in this alga. Perhaps even more striking in *Ostreobium* sp. is the contribution to photosynthetic-oxygen evolution of pigments absorbing at wavelengths as long as 750 nm. It is clear from a comparison of measurements made on these two green algae that *Ostreobium* sp. has an unusual complement of light-harvesting pigments that permit it to survive in a light environment that, for most plants, would be extremely unfavorable or impossible. This study represents an initial investigation into the pigments responsible for light-harvesting in photosynthesis of *Ostreobium* sp.

* CIW-DPB Publication No. 1021
** Please address all correspondence and requests for reprints to Dr. Fork at his permanent address: Department of Plant Biology, Carnegie Institution of Washington, 290 Panama Street, Stanford, California 94305, USA

Materials and methods

The coral used in this study (*Favia* sp.) was obtained from the north-eastern portion of the Great Barrier Reef near the Lizard Island Research Station and from collection stations from reefs near the Australian Institute for Marine Science in Townsville, Australia, in 1987. Coral samples were kept in aerated seawater in the laboratory in dim light of about 25 $\mu Em^{-2} s^{-1}$ at 30 °C. A number of attempts were made to free the filaments of *Ostreobium* sp. from the stony coral skeleton, using different techniques such as maceration of the stony coral and differential centrifugation, density-gradient separation, and detergent solubilization, but without success. As a consequence, the spectroscopic measurements reported here were done using flat slices about 2 mm thick, containing embedded green algal filaments that had been cut out of the coral with a diamond saw whose blade was cooled during operation with flowing seawater. Thalli of *Ulva* sp. and *Endarachne* sp. were collected from the rocks at Coogie, New South Wales, and were maintained in aerated seawater at 23 °C in the laboratory.

An arrangement of fiber optics permitted the excitation and measurement of fluorescence from the same surface of the coral slice or algal thallus. The output of one branch of the fiber optic was positioned with the aid of a lens so as to illuminate the entrance slit of a Perkin Elmer fluorescence spectrophotometer (MPF-44B). For absorbance-change measurements in the red region, a laboratory-constructed single-beam apparatus was used which relied upon red cut-off and interference filters and a separation of 32 cm between the sample and the photomultiplier to selectively reduce fluorescence contamination of the signal. To further remove the fluorescence artifact, another photomultiplier was placed out of the measuring beam but positioned to measure only scattered fluorescence. The output of this photomultiplier served as one input of a differential amplifier (Hewlett-Packard 2470A). A second photomultiplier, which recorded absorbance changes and the fluorescence contamination, provided the other input of the differential amplifier. This arrangement allowed almost all the fluorescence artifact to be eliminated. The grating monochromator (Bausch and Lomb) that defined the measuring beam had a half-band pass of 3 nm. The blue actinic light was defined by using Corning CS 4-96 and CS 5-60 glass filters and a Balzers Calflex C heat-reflecting filter and a 21 V DC tungsten filament lamp. The output of the differential amplifier was fed to a storage oscilloscope, digitized and plotted. The resulting points could be resolved into a rapid and a slow phase. The initial amplitude of the rapid phase was used for plotting.

Results

Fluorescence-emission spectra measured at 25 °C are compared in Fig. 1 for *Ostreobium* sp., the green alga *Ulva* sp., and the brown alga *Endarachne* sp. The peak for *Ostreobium* sp. occurs at 688 nm, those for *Ulva* sp. and *Endarachne* sp.

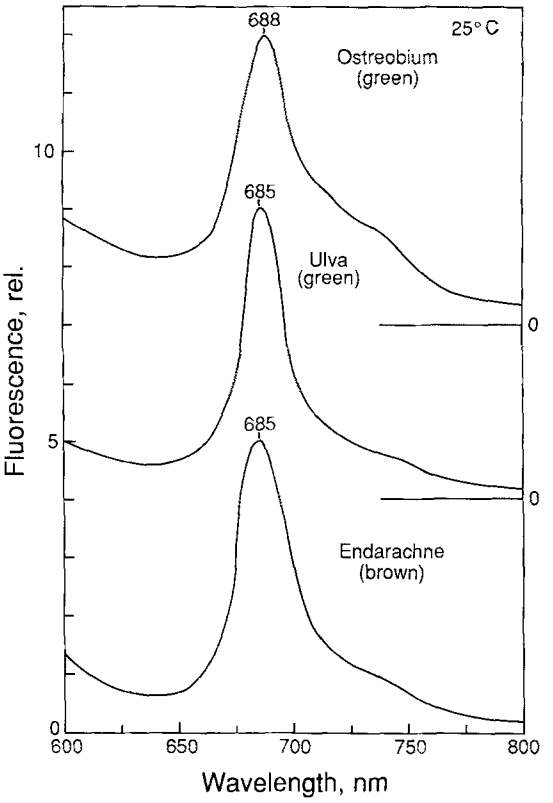

Fig. 1. *Ostreobium* sp., *Ulva* sp., *Endarachne* sp. Fluorescence emission spectra measured at 25 °C in a 2 mm-thick coral slice containing *Ostreobium* sp. and in thalli of *Ulva* sp. and *Endarachne* sp. Excitation wavelength was 440 nm for *Ulva* sp. and *Endarachne* sp. Emission monochromator had half bandwidth of 4 nm. For *Ostreobium* sp., 450 nm was used for excitation and emission measured with half bandwidth of 7 nm

at 685 nm. *Ostreobium* sp. has higher fluorescence in the 700 to 800 nm region than *Ulva* sp. and *Endarachne* sp. Fluorescence emission spectra measured at 77 K are presented in Fig. 2. The spectra of *Ostreobium* sp. and *Ulva* sp. are similar, but *Ulva* sp. has a higher fluorescence peak at 688 nm compared to 720 nm than *Ostreobium* sp. *Endarachne* sp. has its main low temperature fluorescence peaks at 692 and 706 nm, typical of those observed for brown algae (Sugahara et al. 1971, Govindjee and Satoh 1986). For comparison, fluorescence-excitation spectra were measured at 77 K in *Ostreobium* sp. and *Endarachne* sp. (Fig. 3). In *Ostreobium* sp., the excitation of F688 and F718 (where, e.g. F688 = fluorescence at 688 nm, etc.) was sensitized by chl *a* absorbing near 445 nm, chl *b* absorbing near 484 nm, and by a pigment having a broad absorption in the green with a maximum at 546 nm. In the case of *Endarachne* sp. F690 was excited by absorption of chl *a*, represented by a small inflection around 440 nm; by chl *c* and carotenoids, represented by peaks at 467, 483 and 494 nm; and by fucoxanthin, at 547 nm, which is known to be a photosynthetically-active carotenoid in this alga (Fork 1963). The fluorescence excitation spectrum for F740 in *Ostreobium* sp. is given in Fig. 4 and shows peaks at 623, 684 and 702 nm.

Absorbance changes were measured in the region from 670 to 750 nm in *Ostreobium* sp. treated with 50 μM 3-(3,4-

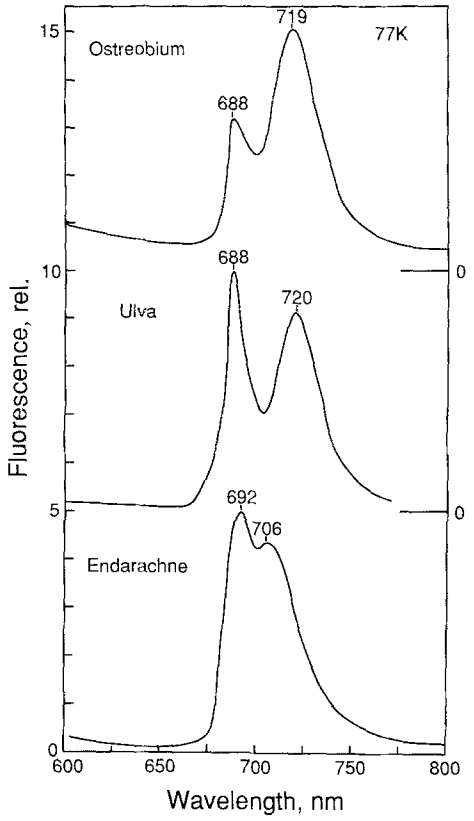

Fig. 2. *Ostreobium* sp., *Ulva* sp., *Endarachne* sp. Fluorescence-emission spectra measured at 77 K in a 2 mm-thick coral slice containing *Ostreobium* sp. and in thalli of *Ulva* sp. and *Endarachne* sp. Excitation wavelengths were 480, 470, and 465 nm, for *Ostreobium* sp., *Ulva* sp. and *Endarachne* sp., respectively. The half bandwidth of emission monochromator was set for 4 nm in each case

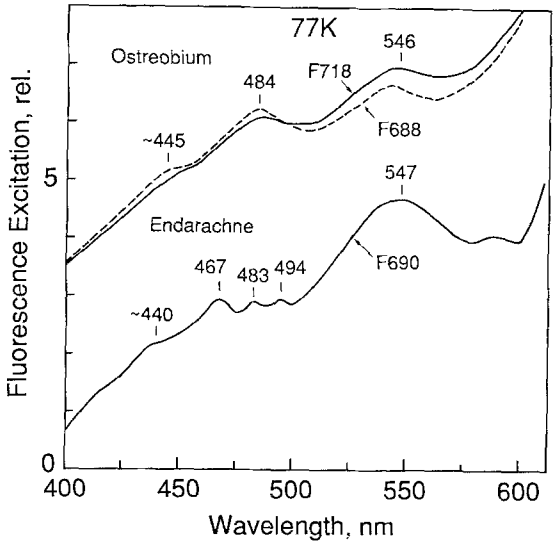

Fig. 3. *Ostreobium* sp., *Endarachne* sp. Fluorescence-excitation spectra measured at 77 K from 400 to 600 nm for fluorescence (F) at 688 nm and at 718 nm in *Ostreobium* sp. and fluorescence at 690 nm in *Endarachne* sp. The half bandwith of excitation monochromator was set at 10 nm and emission monochromator at 4 nm

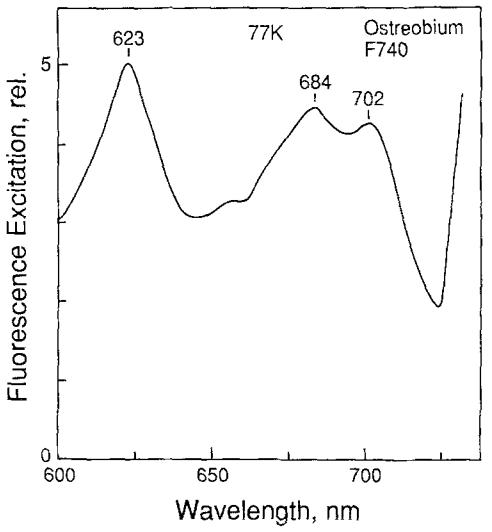

Fig. 4. *Ostreobium* sp. Excitation spectrum measured at 77 K from 600 to 725 nm for fluorescence at 740 nm. The half bandwidth of excitation monochromator was set at 10 nm and emission monochromator at 4 nm

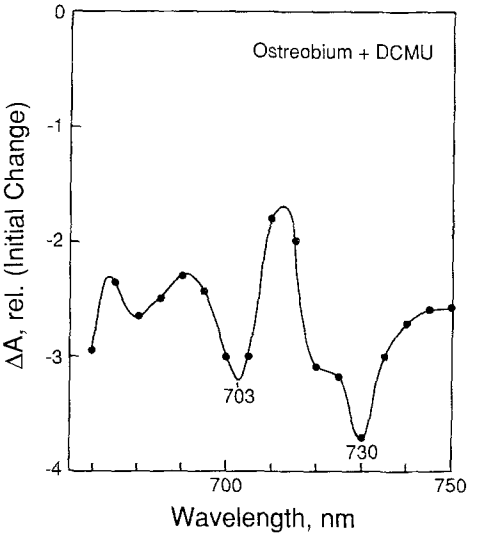

Fig. 5. *Ostreobium* sp. Difference spectrum for initial amount of rapidly decaying phase of light-induced absorbance changes measured in 50 μM DCMU at 27 °C with a 2 mm-thick coral slice containing *Ostreobium* sp. filaments, as described in "Materials and methods"

dichlorophenyl)-1,1-dimethylurea (DCMU) and exposed to 0.1 s flashes of blue actinic light. The dark decay of the absorbance changes was biphasic. The spectrum given in Fig. 5 was obtained by plotting the initial extent of the fast-decaying component. Peaks can be seen at 703 and 730 nm. The spectrum of the slowly-decaying component displayed no characteristic features.

Discussion

The most striking feature of *Ostreobium* sp. obtained from the deeply shaded internal layers of corals is its strong ab-

sorption of light with wavelengths beyond 700 nm (Halldal 1968). By comparison, the absorption of *Ulva taeniata* becomes almost zero by 700 nm (Haxo and Blinks 1950). Another feature of the absorption spectrum of *Ostreobium* sp. compared to *U. taeniata* is the constant high absorption at all wavelengths less than about 690 nm. No doubt some of the high, apparent absorption measured by Halldal can be attributed to a scattering artifact, since the spectrum was measured using slices of coral containing the *Ostreobium* sp. filaments.

Ostreobium sp., like other green algae and higher plants, contains both chl *a* and *b*. As is the case with other members of the Siphonales group, *Ostreobium* sp. is rich in chl *b*, which constitutes two-thirds to three-quarters the amount of chl *a* (Jeffrey 1968). Although not members of the order Siphonales, certain benthic species of *Ulva* sp. are also rich in chl *b* (Yokohama and Misonou 1980). Some of the high absorption in the 670 nm region in *Ostreobium* sp. can no doubt be attributed to chl *b*. *Ostreobium* sp. has as its major carotene α-carotene, with minor amounts of β-carotene (Jeffrey 1968). The major xanthophylls found in the Siphonales are siphonein and siphonaxanthin, but *Ostreobium* sp. only contains significant amounts of siphonein (Jeffrey 1968). In addition, *Ostreobium* sp. has a major amount of an unidentified xanthophyll. It would appear from the results in Fig. 3 that one or both of these xanthophylls in *Ostreobium* sp. are photosynthetically active and can absorb and transfer green light similar to the photosynthetically active fucoxanthin in the brown alga *Endarachne* sp. An efficient excitation-energy transfer from siphonaxanthin to chl *a* was observed by Kageyama et al. (1977) in *Ulva japonica*, a species inhabiting deep water. Even though *Ostreobium* sp. can apparently utilize green light for photosynthesis, it would appear that only a very small amount of light of these wavelengths is transmitted by the overlying layer of dinoflagellates, which contain peridinin, a xanthophyll that absorbs strongly in the green region of the spectrum.

The peak at 484 nm in action spectrum for F688 and F718 in *Ostreobium* sp. can be attributed to absorption by chl *b*. The low chl *a* activity seen in the spectra in Fig. 3 is probably caused by the strong absorption of blue light by the highly scattering coral slices and *Endarachne* sp. thalli that were used for these measurements.

The fluorescence emission spectrum performed at room temperature for *Ostreobium* sp. is not markedly different from that of *Ulva* sp., except for a slightly red shifted peak to 688 nm and enhanced fluorescence emission in the region from 720 to 760 nm. The emission spectra at 77 K differ only in the relative heights of the 688 and the 720 nm peak. These differences may be more apparent than real, since reabsorption may be enhanced in the coral samples containing *Ostreobium* sp. filaments compared to thalli of *Ulva* sp. The low-temperature spectra of *Endarachne* sp. differ from both *Ostreobium* sp. and *Ulva* sp. in having peaks at 692 and 706 nm.

The action spectrum for long wavelength chlorophyll fluorescence at 740 nm in *Ostreobium* sp. is unusual in having a peak at 623 nm that is higher than the 684 nm chl *a* peak. It is not possible at present to give a satisfactory explanation for the 623 nm peak, but it may in part be related to the highly scattering nature of the coral samples used for the fluorescence excitation measurements; however, Kramer et al. (1985) demonstrated that scattering artifacts were virtually absent in excitation spectra obtained using polycrystalline and highly scattering samples of intact algae.

Of particular interest in the action spectra for long wavelength fluorescence is the appearance of a strong, long wavelength chl *a* species with a peak near 702 nm. This peak was also observed by Kramer et al. (1985) in the action spectrum for PS I fluorescence (at 733 nm) in *Chlorella vulgaris*, but was much less pronounced than in *Ostreobium* sp., where its prominence suggests that this coral alga contains large amounts of this long wavelength-absorbing chl *a*.

Ostreobium sp. is unusual in that the difference spectrum for P700 has peaks at 703 and 730 nm. Such a spectrum is unique for green photosynthetic organisms, and needs to be re-investigated using algal samples separated from the coral substrate. However, this difference spectrum is unlikely to be caused by fluorescence contamination produced during measurement, since fluorescence would be expected to be highest at shorter red wavelengths and to decrease gradually as the wavelengths increased to 750 nm. A fluorescence artifact would, furthermore, not result in a structure such as that in Fig. 5.

The absorption spectrum measured by Halldal (1968) clearly shows a dramatic enrichment of long wavelength-absorbing forms of chlorophyll in *Ostreobium* sp., and may represent an adaptation by this alga to life in an extremely unfavorable environment with mainly only the far-red wavelengths transmitted by the overlying dinoflagellate layer. The difference spectrum measured here may reflect a specific adaptation of the P700 of the reaction center of PS I in *Ostreobium* sp. to function in a light environment enriched in far-red wavelengths.

Acknowledgements. The authors are indebted to Dr E. Drew, Australian Institute for Marine Science, who kindly provided some coral samples that were used in these experiments. The authors thank the Natural Science Foundation for a grant (INT-8611470) from the U.S.-Australia Cooperative Research Program that partially supported this research.

Literature cited

Fork, D. C. (1963). Observations on the function of chlorophyll *a* and accessory pigments in photosynthesis. In: Kok, B., Jagendorf, A. T. (eds.) Photosynthetic mechanisms of green plants. Publs natn. Res. Counc., Wash. 1145: 352–361

Govindjee, Satoh, K. (1986). Fluorescence properties of chlorophyll *b*- and chlorophyll *c*-containing algae. In: Govindjee, Amesz, J, Ford, D.C. (eds.) Light emission by plants and bacteria. Academic Press, Orlando, p. 497–537

Halldal, P. (1968). Photosynthetic capacities and photosynthetic action spectra of endozoic algae of the massive coral *Favia*. Biol. Bull. mar. biol. Lab., Woods Hole 134: 411–424

Haxo, F. T., Blinks, L. R. (1950). Photosynthetic action spectra of marine algae. J. gen. Physiol. 33: 389–422

Jeffrey, S. W. (1968). Pigment composition of Siphonales algae in the brain coral *Favia*. Biol. Bull. mar. biol. Lab., Woods Hole 135: 141–148

Kageyama, A., Yokohama, Y., Shimura, S., Ikawa, T. (1977). An efficient excitation energy transfer from a carotenoid, siphonaxanthin to chlorophyll *a*, observed in a deep-water species of a chlorophycean seaweed. Pl. Cell Physiol., Tokyo 18: 477–480

Kramer, H. J. M., Westerhuis, W. H. J., Amesz, J. (1985). Low temperature spectroscopy of intact algae. Physiologie vég. 23: 535–543

Sugahara, K., Murata, N., Takamiya, A. (1971). Fluorescence of chlorophyll in brown algae and diatoms. Pl. Cell Physiol., Tokyo 12: 377–385

Yokohama, Y., Misonou, T. (1980). Chlorophyll *a*: *b* ratios in marine benthic green algae. Jap. J. Phycol. 28: 219–233

Date of final manuscript acceptance: August 4, 1989.
Communicated by M. G. Hadfield, Honolulu