Phycoerythrin Signatures In The Littoral Zone

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LONG-TERM GOALS

My long-term goal is to contribute to our understanding of factors which determine the distribution and productivity of individual phytoplankton taxa. I am particularly interested in understanding the way that the evolutionary history and genetic diversity of particular taxa constrain their modern distribution and their ability to adapt to environmental change.

OBJECTIVES

Phycoerythrin (PE) is the principal light harvesting pigment of a ubiquitous group of marine picocyanobacteria, the marine *Synechococcus*, and cryptomonads, a group of eucaryotic species occasionally found in high abundance in coastal waters. There are many different closely related pigment-proteins in the PE pigment family and they differ in chromophore composition and number. All PEs are relatively easy to distinguish from other photosynthetic pigments based on fluorescence excitation and emission properties, and the differing chromophore compositions of different PE types can also be distinguished using relatively straightforward fluorescence methodologies (Wood *et al.*, 1985; Wood *et al.*, 1998,1999).

The two chromophores found in the PEs of marine *Synechococcus* are phycourobilin (PUB), a chromophore which absorbs maximally in the blue-green region of the spectrum, and phycoerythrobilin (PEB), a chromophore which absorbs maximally at green wavelengths. All PEs described to date contain at least some PEB, but some organisms synthesize PEs in which the absorption of shorter wavelengths is enhanced by the addition of PUB to the overall protein-chromophore complex. The relative absorption of green and blue-green light is determined, therefore, by the relative abundance of PUB and PEB chromophores in the form(s) of PE synthesized by the cells. Differences in chromophore composition and relative abundance lead to characteristic differences in the *in vivo* fluorescence excitation and emission spectra produced by different PEs.

Marine *Synechococcus* are sufficiently abundant that PE fluorescence signatures can be obtained from bulk water samples in most environments. Recent work (Wood *et al.* 1998,1999) has shown that organisms making different spectral forms of PEs are associated with different optical or physical environments. In the Northwest Atlantic, it appears that PE-fluorescence signatures can be used to distinguish between “green” water [$K_d(440)>K_d(550)$] of oceanic origin and “green” water of coastal origin. This has implications for remote sensing since algorithms for deducing chlorophyll concentration and primary production are much better developed for Case 1 waters than “yellow substance” dominated coastal waters. If the evidence from along the continental shelf of

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North America can be generalized to other regions, then the fluorescence signature of PE may serve as an easily detected optical signal that can be used to determine when algorithms developed for Case 1 waters can be applied to data from continental shelf and shelf-edge environments.

The objectives of the proposed research are 1) to evaluate the relationship between PE fluorescence signature and water column optical properties in a wide range of water mass types and 2) to examine the biological mechanisms which underlie the differences in PE fluorescence signature of different water mass types.

APPROACH

This project has both a laboratory and field component. In the laboratory, I am exploring the idea that most strains of *Synechococcus* which make a PUB-lacking PE have only a single copy of the PE gene and are incapable of changing their spectral phenotype, but that strains which synthesize PEs containing PUB generally have multiple copies and may have the ability to physiologically adjust their spectral phenotype by altering the relative intracellular concentration of different PEs.

In the field experiments, the PE fluorescence signature and the abundance of cryptomonads and cyanobacteria is being determined across a wide range of water mass types found in continental shelf environments. Specific research sites include the Sea of Cortez (Gulf of California), the Oregon Coast, and the Gulf of Mexico. Sampling locations range from “blue water” environments to water masses rich in suspended sediments and dissolved organic material. The correlation of these properties with optical parameters and other factors influencing the optical environment at each station will be determined using in-water optical measurements made by other investigators.

WORK COMPLETED

About 30 strains of *Synechococcus* which synthesize a PUB-lacking PE have been isolated from a range of environments, including the Black Sea and Arabian Sea, for experiments on plasticity of the spectral phenotype. I have completed the Intensive Molecular Methods course taught by Kate Field and Walt Reams at Oregon State University and will begin work on copy number of the PE genes in these strains during the second year of this project.

We also have data from two cruises, an NRL cruise on the West Florida shelf and a cruise in the Sea of Cortez. Both cruises went very well; extreme weather on the West Florida shelf limited some aspects of data collection at a few stations, but a great deal of optical and biological data were collected. In the Sea of Cortez, we were able to sample extensively along the continental shelf of mainland Mexico and the Baja peninsula, as well as in both upwelling-influenced and oligotrophic environments offshore.

RESULTS

While we have not finished integrating optical data and PE data from the Gulf of Mexico cruise, our flow cytometry data and cell counts clearly show that there is an incredible range of size, morphology, and PE spectral form among picocyanobacteria in warm coastal waters. Many of these are cells which primarily (or exclusively) synthesize the PUB-lacking form of PE. There was also a dramatic alternation between dominance of the picocyanobacterial community by *Prochlorococcus* or PE-containing cells, depending on station location.

The most common form of PE in the samples we have analyzed from the Sea of Cortez appears to be a PE containing low amounts of PUB. Many of our samples were from upwelling-influenced water masses, including filaments observed in SeaWifs satellite imagery (Mueller, personal communication). The association of low PUB-PEs with upwelling-influenced water was observed in the Arabian Sea (Wood *et al.*, 1999), and these new data suggest that such environments are consistently dominated by *Synechococcus* synthesizing the low PUB form of PE.

IMPACT/APPLICATIONS

Our measurements are providing some of the first detailed data sets on the abundance and spectral forms of cyanobacteria in the littoral zone. The collaboration with optical oceanographers provides a novel opportunity to determine whether or not the fluorescence signature of PE from a sample provides other information about the in-water optical properties because of a close correlation between the occurrence of different PE types and the optical environment. Our culture studies will be the first to examine the possibility that there is a coastal "clade" of marine *Synechococcus* adapted to the unique physical/chemical/optical conditions of the nearshore environment. Further, if we can identify conditions when prochlorophytes are generally absent from the littoral zone, then cyanobacterial-specific molecular tools can be used to study *Synechococcus* spp. in coastal waters with little concern about contaminating DNA from prochlorophytes.

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RELATED PROJECTS

1. Optical measurements and pigment data for Sea of Cortez studies are being provided by Scott Pegau and Ron Zaneveld (OSU) and Chuck Trees and Jim Mueller (CHORS). They are funded through ONR and NASA SIMBIOS. Ship time has been provided by Helmut Maske (CICESE, Centro de Investigacion Cientifica y de Educacion Superior de Ensenada, Baja California, Mexico).

2. Optical measurements and data interpretation for the West Florida Shelf are being provided by Alan Weideman, Rick Gould, and Curt Davis (NRL) through Hyperspectral and SeaWifs projects funded by NRL and ONR.

3. Our Oregon Coast work, planned for 2000, is a collaboration with Tim Cowles, Ron Zaneveld, and Scott Pegau (OSU) who are funded by ONR and NSF for examination of fine structure in the biological and optical fields.

4. Work on the diversity and phenotypic plasticity of our coastal strains of *Synechococcus* is being coordinated with Brian Palenik's (SIO) work on his NSF-funded project on *Synechococcus* diversity.