Quantitating Core Biochemical Complexes in Natural Samples - Relating macromolecular ratios to taxonomy and physiology.

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Environmental Proteomics

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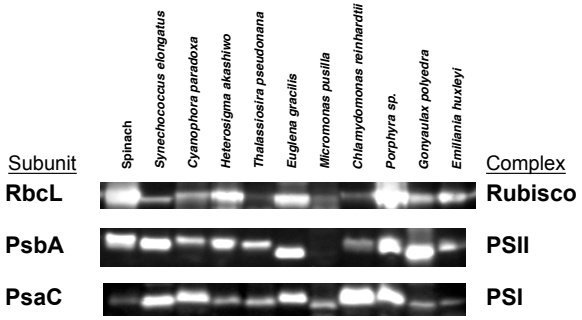
The proteomes of phytoplankton are quantitatively dominated by large pools of the key photosynthetic enzymes and biochemical complexes that carry out light capture, conversion of excitation energy to biosynthetic reductant and ATP, carbon fixation and nitrogen assimilation. Within a given catalytic complex, certain peptide sequences of some subunits are well conserved over wide ranges of taxa. We have raised antibodies with high specificity toward such conserved sequences of protein subunits, using bioinformatics to select conserved peptide sequence tags, rather than whole proteins, to elicit an antibody response (Campbell, *et al*, 2003). These global antibodies therefore recognize all members of their target protein family equally, and we can thus use a single quantitation standard for each target protein family, across taxonomic ranges.

Objectives

- To demonstrate the feasibility of cross taxa quantitation of core complexes.
- To compare intraspecific plasticity under environmental variation to interspecific differences in levels of key biochemical complexes across a range of photoautotroph taxa.

Taxonomic Group	Species examined	Size (μm)	Origin of Plastid	Major Pigments	Habitat
Cyanobacteria	*Synechococcus elongatus*	2		Chl a, PC	Freshwater mesophilic,eutrophilic
Green Algae - Prasinophytes	*Micromonas pusilla*	2	Primary	Chl a, b	Marine
Green Algae - Chlorophytes	*Chlamydomonas reinhardtii*	10	Primary	Chl a, b	Freshwater / soil
Euglenoids	*Euglena gracilis*	50	Secondary (Green)	Chl a, b	Freshwater
Glaucocystophytes	*Cyanophora paradoxa*	15-20	Primary	Chl a, PC	Freshwater
Red Algae - Bangiophytes	*Porphyra sp.*	macro	Primary	Chl a, PC, PE	Marine-Coastal
Ochrophytes - Raphidophytes	*Heterosigma akashiwo*	20-30	Secondary (Red)	Chl a, c	Marine - coastal
Ochrophtes - Diatoms	*Thalassiosira pseudonana*	30-40	Secondary (Red)	Chl a, c	Marine phytoplankton
Haptophytes	*Emiliania huxleyi*	5-7	Secondary (Red)	Chl a, c	Marine phytoplankton
Alveolates - Dinoflagellates	*Gonyaulax polyedra*	40	Secondary (Red)	Chl a, c	Marine - coastal



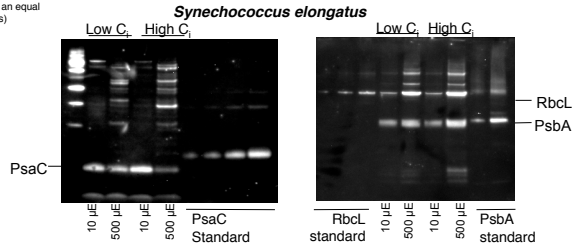
(Lanes loaded on an equal chlorophyll *a* basis)

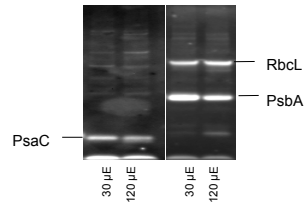
Using global antibodies to detect PsbA, PsaC and RbcL in a broad range of taxa, we found differences in the abundances and ratios of these complexes across taxa.

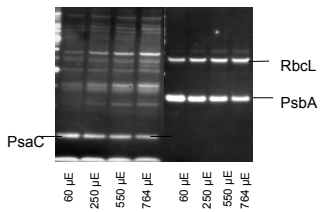
We are growing phytoplankton from this range of taxa under high and low light regimes. Comparing levels of Rubisco, PSI and PSII, we find extensive plasticity in patterns of resource allocations within taxa. In those taxa tested to date, the magnitude of this Intraspecific variation in response to different environments is comparable to any taxonomic differences in the levels of core complexes.

Nevertheless, different taxa do show diverse response patterns to light. We will determine whether functional groupings of phytoplankton light responses correlate with taxonomy, pigment composition, habitat, or a combination of these criteria.

(Lanes loaded on an equal chlorophyll *a* basis)







*preliminary data

Molecules/cell	Low C_i Low light	Low C_i High light	High C_i Low light	High C_i High light
Chl a	$1.4X10^7$	$1.4X10^7$	$1.4X10^7$	$1.3X10^7$
Phycocyanin	$9.9X10^6$	$7.9X10^6$	$1.1X10^7$	$1.4X10^7$
Rubisco LSU	$1.6X10^5$	$9.5X10^5$	$1.6X10^5$	$12.3X10^5$
PSI	$1.2X10^5$	$0.8X10^5$	$2.0X10^5$	$0.3X10^5$
PSII	$0.6X10^5$	$1.0X10^5$	$0.5X10^5$	$1.4X10^5$
PSI:PSII	2.0	0.9	3.7	0.2

Molecules/cell	Low light	Moderate light	Low light	Moderate light	High light 1	High light 2
Chl a	$2.9X10^7$	$1.8X10^7$	$107 X10^8$	$71 X10^8$	$41 X10^8$	$28 X10^8$
Chl b	$2.7X10^7$	$1.5X10^7$	$8.0 X10^8$	$5.6 X10^8$	$2.9 X10^8$	$2.8 X10^8$
Rubisco LSU	$4.5X10^5$	$3.4X10^5$	$16 X10^7$	$13 X10^7$	$8.5 X10^7$	$5.9 X10^7$
PSI	$3.2X10^5$	$1.4X10^5$	$22 X10^7$	$11 X10^7$	$5.9 X10^7$	$3.4 X10^7$
PSII	$1.2X10^5$	$0.4X10^5$	$13 X10^7$	$5.4X10^7$	$3.1 X10^7$	$1.6 X10^7$
PSI:PSII	2.6	3.2	1.7	2.1	1.9	2.2

Future work

- Our approach now allows quantitative comparisons of resource allocations to core complexes, across taxa and among environmental conditions.
- We are now launching studies of natural phytoplankton communities, to analyze the roles of intraspecific plasticity and interspecific variation under environmental change.
- In parallel, we are expanding our quantitation systems to Glutamine synthetase, ATP Synthase, and Cytochrome b6f, for which global antibodies are also available (www.agrisera.se).

Materials and methods

Protein complexes were detected in total protein extracts by immunoblotting using Global antibodies (www.agrisera.se) and quantitated using recombinant protein standards (www.environmentalproteomics.ca)

Literature cited

Campbell DA, *et.al.* 2003 Analyzing Photosynthetic Complexes in Uncharacterized Species or Mixed Communities using Global Antibodies. Physiolog Plantarum 119: 322–27.