CARBON-CONCENTRATING MECHANISMS AND \( \beta \)-CARBOXYLATION: THEIR POTENTIAL CONTRIBUTION TO MARINE PHOTOSYNTHETIC CARBON ISOTOPE FRACTIONATION

A DISSERTATION SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF HAWAI'I IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

OCEANOGRAPHY

DECEMBER 2003

By

Nicolas Cassar

Dissertation Committee:

Edward A. Laws, Chairperson
Robert R. Bidigare
David M. Karl
Fred T. Mackenzie
Brian N. Popp
Paul G. Falkowski
ABSTRACT

The ability of the ocean to buffer the concentration of CO$_2$ in the atmosphere through the so-called biological pump depends on the extent to which the photosynthetic rate of marine phytoplankton is limited by the concentration of CO$_2$ in the water. If CO$_2$ becomes available to phytoplankton by passive diffusion through the boundary layer around the cell, then the growth of large cells, which are believed to contribute disproportionately to the biological pump, could be limited by CO$_2$ availability. However, many species appear to have the ability to circumvent diffusion control through the use of carbon-concentrating mechanisms (CCMs) such as active CO$_2$ uptake, bicarbonate (HCO$_3^-$) transport, and carbonic anhydrase activity. These mechanisms are likely adaptations to the fact that the main carbon fixing enzyme, ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco), is less than half saturated at normal seawater CO$_2$ concentrations.

Using short-term $^{14}$CO$_2$-disequilibrium experiments, a clone of the marine diatom *Phaeodactylum tricornutum* was shown to take up little or no HCO$_3^-$ even under conditions of severe CO$_2$ limitation. These results agree with predictions based on stable carbon isotopic fractionation data and demonstrate that combining isotopic disequilibrium experiments with continuous growth cultures and stable isotope fractionation experiments is a powerful tool for understanding the response of oceanic primary producers to anthropogenic CO$_2$ emissions as well as for interpreting paleoceanographic carbon isotope data.
Isotopic disequilibrium experiments were also performed in the field to estimate
the extent of photosynthetic bicarbonate (HCO$_3^-$) uptake in the oceans. The experiments
were conducted in the Southern Ocean during the Southern Ocean Iron Experiment
(SOFeX). In contrast to the results with *P. tricornutum*, approximately half of the
photosynthetic inorganic carbon uptake was direct HCO$_3^-$ uptake, the other half being
direct CO$_2$ uptake (passive and/or active uptake). A low-CO$_2$ treatment induced an
increase in uptake of CO$_2$ through increased enzymatically mediated extracellular
dehydration of HCO$_3^-$ (carbonic anhydrase activity), which was at the expense of direct
HCO$_3^-$ transport across the plasmalemma. Because of the presence of CCMs, biological
productivity in the Southern Ocean is unlikely to be directly regulated by natural or
anthropogenic variations in atmospheric CO$_2$ concentration. These results are consistent
with stable isotope fractionation models and could have important implications for the
global biogeochemical cycle of carbon.

It is generally believed that most of the variations in stable isotope fractionation
are associated with changes in CCM activity. A review and experimental study of the
various factors that influence CCM activity and therefore photosynthetic carbon isotope
fractionation revealed that, other than CCMs, several factors that have been essentially
ignored in the scientific literature may also contribute to the isotopic signature of
photosynthetic organic matter. In this study, photorespiration appeared to be of greater
magnitude than commonly reported in marine diatoms, although its contribution to
isotopic fractionation was negligible. Isotopic fractionation during photosynthesis in *P.
tricornutum* was found to be well correlated to changes in Rubisco enzyme kinetics and
to the molar organic carbon to nitrogen ratio (C/N). Contrary to the general scientific
belief, the C/N proved to be dependent on the CO₂ concentration, with the greatest dependency at lower growth rates, presumably because of luxury carbon uptake at lower growth rates. At higher growth rates, a tighter coupling of the organic nitrogen and carbon cycles may explain the lower responsiveness of C/N to changes in CO₂ concentration.

The contribution of carboxylases other than Rubisco to photosynthetic stable carbon isotope fractionation was also examined. Some β-carboxylation enzymes, such as phosphoenolpyruvate carboxylase (PEPC), have a carbon isotope discrimination factor different from Rubisco and may significantly contribute to carbon fixation. Changes in PEPC/Rubisco activity under various growth conditions may explain some of the variations in stable isotope fractionation. The β-carboxylase activity in *P. tricornutum* increased with decreasing growth rates and increasing CO₂ concentrations. PEPC activities larger than generally reported in the literature were observed. This difference may be attributable to variations in methodological approaches.

A multitude of factors may influence overall photosynthetic carbon isotope fractionation. Understanding these factors will be crucial to the use of isotopic analyses for paleo-CO₂ reconstruction.