CARBON-CONCENTRATING MECHANISMS AND β-CARBOXYLATION: THEIR POTENTIAL CONTRIBUTION TO MARINE PHOTOSYNTHETIC CARBON ISOTOPE FRACTIONATION

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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 CO_2 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 CO_2 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 CO_2 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-CO_2 reconstruction.