Effects of $pCO_2$ on production of CaCO$_3$ by skeletal organic matrix in coral cell cultures

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I. Research Context

We examined the production of new CaCO$_3$ and five skeleton organic matrix (SOM) proteins in primary cell cultures of the stony coral, Stylophora pistillata. Cell cultures of S. pistillata were incubated in four CO$_2$ treatments (400, 700, 1000 and 2000 ppm CO$_2$). Cultures were evaluated for photosynthetic efficiency, calcification and SOM protein expression rates, and mineralogical, elemental and isotopic composition. The primary cell cultures assembled into organic “proto-polyps” precipitating aragonite crystals, which formed on the external face of the proto-polyps and were identified by their distinctive elongated crystallography and X-ray diffraction pattern. The preliminary data show an apparent link between the protein expression and CaCO$_3$ mass accumulation. CaCO$_3$ mass decreased substantially at pCO$_2$ levels above 700 ppm, which could be related to the apparently inhibited protein expression at high pCO$_2$ or to re-dissolution of mineral following initial formation in more acidic media. Boron isotope ratios, a proxy for pH, of culture-precipitated aragonite follow the inorganic precipitation line with a relatively constant positive offset, consistent with observations from nubbins cultures, providing evidence for pH increase at the calcification site occurring at the molecular or cellular level.

II. Methods

1. Medium & Culture Preparation
   • Artificial seawater was equilibrated in a controlled CO$_2$ growth chamber to the desired pCO$_2$ before addition of growth medium (DMEM to 12.5% final volume). Growth medium + seawater was then equilibrated.
   • S. pistillata rubbins were treated in Ca-free seawater followed by equilibrated growth medium for 1-2 days.
   • Dissociated S. pistillata cells were filtered to 20 mm and grown in Primaria culture dishes in equilibrated medium for 1-2 days.
   • Artifical seawater was equilibrated in a controlled CO$_2$ growth chamber to the desired pCO$_2$ before addition of growth medium (DMEM to 12.5% final volume). Growth medium + seawater was then equilibrated.

2. SOM Protein Expression
   • Expression rates of a cathepsin (GenBank AGG36357.1) were measured by quantitative western blot.
   • Standardized total protein content. Expression fold change was calculated relative to T$_0$.
   • Expression rates of CARP4 and STPCA2 (GenBank AGG36361.1 and AGG36361.1) were measured by qPCR.
   • 18S as housekeeping gene and ΔΔCt converted to expression fold change from 400 ppm pCO$_2$ T$_0$.

3. Sr-based Calcification Rates
   • Total Sr content of dissolved CaCO$_3$ measured by ICP-MS.
   • The distribution coefficient for Sr incorporation into aragonite is used to calculate total mass of carbonate mineral precipitated per culture well.

4. Boron Isotope ratios
   • Each cell culture was bleached of organic matrix, and treated with NaOH, with repeated rinses between each step. CaCO$_3$ was preserved by extended centrifugation. Residual organics were floated off with Tetraethylammonium.
   • Carbonate was dissolved in 2 N HNO$_3$ and micro-sublimated at 120°C for 12 hours.
   • Boron isotope ratios of the sublimate were measured on a Thermo Neptune multi-collector ICP-MS.

III. Preliminary Results

IV. Discussion and Future Work

• CARP4 and STPCA2 expression is not significantly different at pCO$_2$ levels of 400 and 700 ppm. At both levels we see an increased precipitation of aragonite to near-maximum mass in the first 5 days coincident with increasing protein expression. The decrease in CaCO$_3$ between 5 and 9 days at 400 ppm, is coincident with the decrease in protein expression.

• In the 1000 and 2000 ppm conditions significantly less aragonite was measured, which could be related to the apparently inhibited protein expression at high pCO$_2$ (as manifested by the flat line of CARP4 in the 1000ppm experiment) or to re-dissolution of mineral following initial formation in more acidic media.

• A$^{11}$B is consistent with measurements of rubbins cultures, indicating that chemical modification of the calcification site occurs at a cellular level. The offset from the inorganic precipitation line suggests that precipitation occurs at pH levels significantly higher than in the ambient medium, indicating that CARP4s are active in modifying the calcification site.

V. Acknowledgements

This research is funded by the National Science Foundation Grant 43262 to RR, YR, & RS. We are grateful to Athena Fx. and Christine Lee for assisting with sample preparation and analysis.