

Carbon Isotopic Analysis of Selected Nucleic Acids from Environmental Samples

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We are developing analytical methods for the determination of carbon isotope ratios of selected nucleic acids. This will connect molecular phylogeny (genetic sequence) with metabolic function. Our current scientific goals focus on processes occurring in marine sediments. For example, DNA extracted from anaerobic methane-oxidizing microbes from Eel River Basin, CA has a $\delta^{13}\text{C}$ value of -55% , consistent with methane being the major carbon source. Further purification of ribosomal RNA of known genetic sequence will enable the nucleic acids of the Archaea and Bacteria in this consortium to be analyzed separately. Data for preliminary tests of cultured organisms, as well as initial attempts at measuring carbon isotope ratios in natural samples, will be reported. The approach consists of three discrete steps. (1) To prepare clean RNA from natural and cultured samples, we utilize magnetic bead capture technology. The small-subunit (SSU) and/or large-subunit (LSU) rRNA targets are hybridized to complementary biotinylated oligonucleotide probes. Streptavidin magnetic beads are added and bind with the biotin end of the RNA-probe complex. The beads are then captured using a magnetic particle concentrator, and the RNA is eluted. Yield ranges from 1-10 μg RNA/sample. (2) These samples contain significant amounts of minor organic contaminants, fragments of cross-hybridized RNA, and degraded oligonucleotide probe. High-performance liquid chromatographic (HPLC) methods are used to clean up the samples and yield the SSU/LSU rRNA molecule in carbon-free buffer. (3) Carbon isotopic analysis is performed using a moving-wire interface to connect the HPLC with an isotope-ratio-monitoring mass spectrometer. The interface consists of a Ni wire travelling at 2 – 4 cm/s on which the samples are coated, dried, and combusted to yield CO_2 for isotopic analysis. This system can measure $^{13}\text{C}/^{12}\text{C}$ ratios of nonvolatile organic compounds containing <10 nmolC with a precision and accuracy better than $\pm 0.2\%$.

Eocene meridional temperature gradients from oxygen isotopes

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The Eocene is generally regarded as an interval of warm climate with no substantial polar ice and "tropical"-type organisms such as crocodiles, turtles and palms extended as far north as the Arctic circle. The leading theory to explain this is an enhanced greenhouse effect, but most oxygen isotope sea-surface temperature (SST) estimates from the tropics are much lower ($15\text{-}23^\circ\text{C}$) than are predicted by climate models and the distributions of fossil organisms ($\geq 30^\circ\text{C}$). This has led to an alternative theory for polar warmth, namely that ocean currents and winds were more efficient at distributing heat.

However, most oxygen isotope paleo-temperature results are "suspect" because plankton shells from ancient oozes and chalks are generally recrystallized on a microscopic scale. In effect, material may have been added to them in the cold conditions of the sea floor, biasing the results, especially in the tropics. Therefore, it is desirable to find and analyse exceptionally well-preserved foraminifer shells, which generally means extracting them from impermeable hemipelagic mudstones rather than deep-sea carbonates.

Previously published results from the tropics (Pearson et al. 2001) are augmented by new high-latitude data from the middle Eocene of New Zealand. The data suggest much warmer temperatures in the tropics and stronger meridional temperature gradients than previously thought. The general picture that emerges from analysis of well-preserved foraminifera is consistent with climate model simulations that have CO_2 levels several times modern. Comparison of the well-preserved assemblages with recrystallized material from deep-sea carbonates suggests that the latter may consist of at least 50% diagenetic calcite added on the sea floor, biasing the isotope ratios to apparently "cold" values. This has implications for the use of foraminifer $\delta^{18}\text{O}$ as a paleoceanographic proxy in ancient recrystallized material.

References

Pearson, P.N. et al., (2001). *Nature* **413**, 481-487.