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An Overview of Alkenone Biomarker Analysis for Paleothermometry

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Overview

Source: Laboratory of Jeff Salacup - University of Massachusetts Amherst

Throughout this series of videos, natural samples were extracted and purified in search of organic compounds, called biomarkers, that can relate information on climates and environments of the past. One of the samples analyzed was sediment. Sediments accumulate over geologic time in basins, depressions in the Earth into which sediment flows through the action of fluid (water or air), movement, and gravity. Two main types of basins exist, marine (oceans and seas) and lacustrine (lakes). As one might guess, very different types of life live in these settings, driven in large part by the difference in salinity between them. Over the last few decades, organic geochemists discovered a toolbox of biomarker proxies, or compounds that can be used to describe climate or environment, some of which work in marine environments and some of which work in lacustrine. We turn our attention here to the marine realm and alkenone paleothermometry using the $U^{k'}_{37}$ sea surface temperature proxy.

The most well-established and widely applied open-ocean biomarker sea surface temperature (SST) proxy is $U^{k'}_{37}$.

$U^{k'}_{37}$ = (C37:2) / (C37:2 + C37:3) (see Herbert[1] for a review)

The index is based on the ratio of two polyunsaturated long-chain alkyl ketones, called alkenones, produced by some classes of haptophyte algae[2,3]. Culture[4,5] and core-top sediment[6] calibration studies led to the development of the $U^{k'}_{37}$ Index as a quantitative SST proxy. Amazingly, the culture-based calibration of Prahl *et al.*[4]:

$U^{k'}_{37}$ = 0.034(SST) + 0.039,

And the core-top calibration of Müller *et al.*[6],

$U^{k'}_{37}$ = 0.033(SST) + 0.044,

are statistically identical.

Reconstructed $U^{k'}_{37}$ temperatures correlate best with mean annual SST for a variety of climate and haptophyte production regimes in the global ocean[7]. Alkenones are detected in marine sediment cores of early Eocene to modern age[8], and in exposed outcrops of uplifted marine sediment[9] suggesting they are very stable over geologic time, and thus useful as a paleoclimate tool. $U^{k'}_{37}$ has been used to document paleo sea surface temperature changes at decadal[10] to orbital[11,12] timescales and are therefore very versatile.

In the open ocean, the coccolithophores *Emiliania huxleyi* and *Gephyrocapsa oceanica* are responsible for most alkenone production. It is not yet known why these haptophytes alter the unsaturation ratio of alkenones based on growth temperature. It was initially thought that alkenones were components of haptophyte cell walls and that their unsaturation was adjusted in order to keep the membrane fluid, much like saturated fats are solid at room temperature, while unsaturated fats are fluid. However, experiments aimed at this question found that instead of being associated with the cell membranes, alkenones were associated with energy storage structures inside the cell. Thus, their use inside the cell remains an open question.

Recently, alkenones have been found in lacustrine environments. However, their utility has so far been limited. Different alkenone producers than those in the marine realm dwell in lakes and thus the calibration between water temperature and unsaturation ($U^{k'}_{37}$) is different. Moreover, this calibration is different between lakes, making the creation of a 'global' calibration unlikely. Unfortunately, the creation of local calibrations is expensive and time consuming and so the future for $U^{k'}_{37}$ in lakes is also currently limited.

Alkenones are usually extracted from marine sediments. Very often the same organisms that produce alkenones produce fatty acid methyl esters of those alkenones called alkenoates. These compounds co-elute with the alkenones on a gas chromatograph and complicate their quantification. Therefore, these extracts will often undergo a saponification to remove alkenoates. Because the saponification produces carboxylic acids that are not gas chromatograph amenable, a silica gel column must be performed after the saponification to remove the carboxylic acids from the extract. The alkenones come out in the middle polarity ketone fraction that elutes in dichloromethane while the acids are left on the column. Lastly, in extreme cases, such as in sediments acquired from highly polluted areas, like estuaries near industrial centers, a urea adduction may also be required to remove unknown compounds that coelute with the alkenones on the gas chromatograph.

Once the total lipid extract is purified, the extracted and purified sample is run on a gas chromatograph coupled to a flame ionizing detector. The relative concentration of the two alkenones is determined by obtaining the area under the curve for each of the compounds on computer software designed for just this purpose (such as Agilent Chemstation). These areas are then put into the $U^{k'}_{37}$ ratio equation shown above to get a $U^{k'}_{37}$ value that ranges between 0 and 1. These $U^{k'}_{37}$ values are then mapped to sea surface temperature value using a calibration such as those described above.

Paleothermometry is the calculation of past temperatures by analysis of specific chemicals in natural samples, like those left behind by prehistoric algae.

Algae are a diverse group of organisms that have been abundant in Earth's oceans and lakes for millennia. Certain chemical compounds, which are deposited in sediment by ancient algae, act as biomarkers – organic compounds that can provide researchers with valuable insight into Earth's

history. In fact, analysis of algal biomarker content in sediment allows researchers to determine the Earth's temperature hundreds of millions of years ago.

One such record comes from some species of coccolithophores. These algae produce varying amounts of alkenones, a class of robust biomarkers, based on the temperature of their environment. Alkenone analysis is primarily used to calculate the sea surface temperature of Earth's oceans eons and eons ago.

This video will illustrate the use of alkenones in paleoclimatology and describe the process of isolating, purifying, and analyzing alkenones to calculate past sea surface temperature.

As its name implies, "Alkenone paleothermometry" is based on the analysis if lipids, known as *alkenones* Alkenone paleothermometry is based on alkenones; long-chain, unsaturated alkyl ketones that contain 37 carbon atoms and 2 to 4 double bonds. Each double bond is a site of unsaturation. At low sea surface temperatures, alkenone producers generate more unsaturated alkenones than saturated. The ratio of saturation to unsaturation is known as the Alkenone Unsaturation Index.

The alkenones usually evaluated are $C_{37:2}$ and $C_{37:3}$, which have 37 carbons and two or three double bonds, respectively. The Unsaturation Index of these alkenones, or the $U^{K'}_{37}$, is positively related to sea surface temperature. The analytical method know as gas chromatography is generally sensitive enough to separate these alkenones from one another. However, alkenone-producing algae often also generate chemically-similar fatty acid methyl esters, or alkenoates, which cannot be distinguished from alkenones using this technique. Hydrocarbon contamination from pollution may also further muddy chromatographic analysis. To accurately determine relative alkenone concentration, alkenoates and unknown hydrocarbons must be removed before analysis by the methods of saponification and urea adduction.

Now that the relationship of sediment alkenone ratios to sea surface temperature has been reviewed, let's look at the techniques for their purification from a total lipid extract and analysis of the unsaturation ratio.

Once marine sediment has been collected and extracted, the total lipid extract, or TLE, must go through a multistep purification process, and analyzed. First, the extract undergoes saponification to convert alkenoates into carboxylate salts and methanol using a strong base and heat. Other fatty acid esters present in the TLE will be saponified into salts and glycerol.

After cooling the mixture to room temperature, an aqueous salt solution is added to form salts and glycerol. The mixture is then acidified to protonate the carboxylate anions, producing fatty acids. Finally, the alkenones and fatty acids are extracted from the mixture with hexane.

Silica gel chromatography is then performed to remove both apolar compounds and the polar fatty acids produced by saponification. The dried and saponified TLE is dissolved in hexane and then loaded onto a column. Silica retains polar compounds more strongly than apolar ones.

First, apolar compounds are removed with an apolar solvent, like hexane. Next, alkenones are eluted by a moderately polar solvent, such as dichloromethane, leaving the highly polar fatty acids and other unwanted polar compounds on the column.

If the original sediment sample was collected from a highly polluted area, urea adduction is performed to remove any remaining highly branched or cyclic hydrocarbons. The dried mid-polarity fraction is dissolved in a solvent mixture in which the strongly polar urea is minimally soluble, such as DCM and hexane. A concentrated solution of urea in methanol is then added to the TLE, causing urea crystals to precipitate.

Straight-chain molecules such as alkenones fit into the spaces between molecules in the urea crystal lattice, but highly branched and cyclic molecules do not, and are expelled.

Once crystal growth has finished, the urea crystals are dried and then washed with an apolar solvent to remove expelled compounds. Then, the crystals are dissolved in a small amount of water. The alkenones are extracted from the water with an apolar solvent for analysis.

While all previous purification steps did not differentiate between alkenone species, small differences in boiling point and molecular structure are sufficient for separation on a gas chromatography column. When paired with a flame-ionization detector, relative concentrations of the alkenones, can be determined.

Molecules are identified on the chromatogram by their retention time, or the time needed for the compound to be exit the column. The retention times of the desired compounds are ascertained with alkenone standards.

The relative concentrations of the alkenones are determined from analysis of the areas under the peaks of interest. The $U^{K'}_{37}$ value is then calculated from the concentrations of $C_{37:2}$and $C_{37:3}$ in the sample. With the sea surface temperature proxy relationship and the $U^{K'}_{37}$ value, the analyst can solve for sea surface temperature at the time of the sediment deposition.

Many different facets of Earth's history can be investigated by analysis of sediment and sedimentary rock.

Biostratigraphy is the study of determining the ages of layers, or strata, of rock by analysis of the fossils present. As there are many sources of sediment, sedimentary rocks from the same time period may have dramatically different compositions around the world. Certain sets of species throughout Earth's history, such as the ammonites, existed worldwide and underwent rapid evolution. If visually dissimilar rock strata both contain the same species of ammonite, then a temporal correlation between the strata can be drawn. When combined with techniques such as paleothermometry, extensive information about Earth's history can be determined from fossil records in natural samples.

Many species of foraminifera, or forams, are found in marine sediments worldwide. Forams have calcium carbonate shells and have existed throughout Earth's oceans for millions of years. Many species live on the ocean floor, and thus can provide temperature information about deeper parts of the ocean. The magnesium to calcium ratio of forams corresponds to temperature, as they incorporate more magnesium into their shells in warmer climates. The multitude of species and the abundance of forams makes their fossil record useful for tracking changes in ocean currents throughout Earth's history and for biostratigraphy.

As tectonic plates diverge, new rock forms between them. Correspondingly, the properties of the rock surrounding a divergent plate boundary provide information about plate movements over time. For instance, changes in Earth's magnetic field are preserved in some minerals found in fossils, rock, and sediment. The discovery of symmetric changes in magnetism about mid-ocean ridges significantly contributed to the current understanding of seafloor spreading and plate tectonics.

You've just watched JoVE's Overview of Alkenone Paleothermometry. You should now understand the principles of paleothermometry and the relationship of alkenone ratios in marine sediment to sea surface temperature. The following videos in this series will go into more detail about this complex process.

Thanks for watching!

References

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