Carbon Isotopic Analysis of Four Ancient Herbivores

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Abstract

After an animal's burial, its unaltered tissue can be studied to gain valuable evidence about its paleo-environment through chemical isotopic analysis. In particular, the study of carbon isotopes in tooth enamel is extremely useful since enamel is resistant to alteration. Four teeth, each from a different ancient herbivore from a different time period, were chemically treated and then studied in this experiment. Using certain chemicals to purify the enamel and a vacuum line to extract the carbon in the form of CO_2 gas, numerical data was obtained about each tooth's carbon isotopic composition via a mass spectrometer. From this, conclusions were drawn and inferences were made about each animal's paleo-diet. Done on a much larger scale, this same technique can be used to gain more information about factors such as the availability of certain plants, the selectivity of the herbivores' diets, and perhaps some of their internal biochemical pathways.

Introduction

Fossil teeth are used as a primary source of evidence in reconstructing the environment of ancient animals. If left unaltered, an organism's tooth, bone, and tissue composition will directly reflect its diet. Because fossils, rare in themselves, are vastly

more common than preserved tissue matter, the study of teeth and bones has been a historical staple in studying paleo-environments (Koch et al., 63).

Teeth and bones both contain biogenic phosphate (i.e., hydroxyapatite), the target substance most readily available for study. (Tooth dentin is more easily altered and therefore less useful for study.) Bone phosphate, however, is highly porous and therefore allows infiltration of water and other impurities. Subsequent alteration leads to difficulty in direct study of such matter. Tooth enamel, on the other hand, is dense and has low porosity. Such properties bolster resistance to change, making tooth enamel a strong candidate for study (Koch et al., 63).

The analysis of carbon and oxygen isotopes in tooth enamel samples is particularly useful and popular. Isotopes are different forms of the same element; they have the same number of protons and possess very similar chemical properties. Their masses, however, are distinct. A mass spectrometer is able to detect isotopic ratios of carbon and oxygen given a closed sample of gaseous carbon dioxide.

Three oxygen isotopes exist in the natural environment:

Table 1: Introduction to Oxygen Isotopes

Isotope	Natural Abundance
^{16}O	99.63%
^{17}O	0.04%
^{18}O	0.20%

A mass spectrometer is able to detect the ratio of ^{18}O to ^{16}O. ^{17}O is disregarded because its natural abundance is virtually negligible (0.04%). Furthermore, the mass difference between ^{16}O and ^{18}O is larger than the difference between any two other

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oxygen isotopes, leading to a greater (and therefore more easily measured) fractionation value. Oxygen isotopic ratios are used primarily in determining drinking habits and physiological processes and can yield valuable information, for example, about local rainwater (Wang et al., 241). In this experiment, however, since all four samples were derived from different species, oxygen isotopic analysis is of little use. Animals of different species will naturally fractionate oxygen isotopes differently.

When dealing with only one sample of each species, carbon isotopic analysis is far more practical. Three carbon isotopes exist:

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Table 2: Introduction to Carbon Isotopes

Isotope	Natural Abundance	Naturally Occurring?
^{12}C	98.89%	Yes
^{13}C	1.11%	Yes
^{14}C	N/A	Yes

Since ^{14}C is radioactive and therefore unstable, attention is directed to ^{12}C and ^{13}C. A mass spectrometer is able to determine the ratio of ^{13}C to ^{12}C for isotopic analysis. For pragmatic results, the objective of this experiment is to convert this ratio into a useable form and compare it to a standard value. A simple formula is used to integrate this methodology:

$$\delta^{13}C\ (‰) = [(R_{sample} - R_{standard})/R_{standard}] \times 1000, \text{ where } \mathbf{R = {}^{13}C/{}^{12}C}$$

In addition to calculating the carbon isotopic ratios, the mass spectrometer automatically inserts the values into the formula to yield an appropriate $\delta^{13}C$ value. A positive $\delta^{13}C$ value with respect to the standard corresponds to ^{13}C enrichment in the

sample relative to the standard, whereas a negative value indicates ^{13}C depletion in the sample relative to the standard.

Methodology

Fossil teeth were obtained from four ancient herbivores, yielding four samples. Information regarding the samples is given below:

Table 3: Introduction to Tooth Samples

Sample #	Sample Name	Animal Type	Age (years)
1	TB-1	Deer	18,000
2	TB-2	Pig	17,000
3	TB-3	Rhinoceros	3,000,000
4	TB-4	Horse	7,500,000

Table 4: Tooth Sample Sources

Sample Name	Source (Province)	Source (Mtn. Chain)
TB-1	Gyirong	Hengduan
TB-2	Sichuan	Hengduan
TB-3	Sichuan	Hengduan
TB-4	Gyirong	Himalayas

Almost all plants can be classified as C3 plants, C4 plants, or CAM plants, depending on their photosynthetic pathways. C3 plants include all trees, most shrubs, and the cool season grasses; C4 plants include the warm season grasses. CAM plants are not commonly found except in deserts. Both C3 and C4 plants have markedly distinct stable

carbon isotopic ratios. C3 plants possess a $\delta^{13}C$ value ranging from –20‰ to –35‰, with a middle value of approximately –27‰. C4 plants have a $\delta^{13}C$ value ranging from –9‰ to –17‰, with a middle value of approximately –13‰ (Wang et al., 241). The figure below outlines the fractionation pathways of atmospheric carbon dioxide to the C3 and C4 plants, and finally to the herbivores being studied:

Table 5: Two Pathways of Diet

Path 1:	**Atmospheric CO_2**	**-->**	**C3 plants**	**--> Herbivore tooth enamel**	
	($\delta^{13}C$ = -6‰)		($\delta^{13}C$ = -27‰)	($\delta^{13}C$ = -13.5‰)	
Path 2:	**Atmospheric CO_2**	**-->**	**C4 plants**	**--> Herbivore tooth enamel**	
	($\delta^{13}C$ = -6‰)		($\delta^{13}C$ = -13‰)	($\delta^{13}C$ = -1‰)	

By grinding the tooth enamel into powder and purifying it with various chemicals, we can ultimately extract the sample's desired carbon content in the form of gaseous carbon dioxide (Wang et al., 243). This CO_2 can be introduced to the mass spectrometer to yield a $\delta^{13}C$ value. After comparison of this value to a standard $\delta^{13}C$ value, conclusions can be made regarding the herbivore's diet. This same method is used by scientists on a much larger scale to reconstruct the paleo-environment of animals and to provide evidence supporting or refuting modern theories.

Sample TB-4, for example, can be used to shed light on when the massive uplift of the Himalayas occurred, a heavily debated topic. A widely accepted theory states that such a dramatic uplift of land plates would cause heightened rock to react with carbon dioxide and water. A dramatic decrease in atmospheric carbon dioxide would result,

leading to an expansion of C4 plants in many low latitude and low altitude environments around the world (Cerling et al., 155).

Materials & Methods

The first step in preparing the teeth was to manually separate, as accurately as possible, tooth enamel from dentin. A high-powered drill was used to accomplish this. Next, the enamel was ground into powder using a mortal and pestle. The powder was left overnight to react in 5% sodium hypochlorite in order to remove organic impurities such as humates, bacterial proteins, and even grease from human hands. The powder was then filtered and heavily rinsed via vacuum filtration to save only the apatite particles, and 1M acetic acid was added. The solution was allowed to react overnight under weak vacuum to remove diagenetic carbonates. Further washing, via vacuum filtration, and drying left our powder consisting mainly of structural carbonate. Finally, carbon dioxide gas was produced by treatment of 100% phosphoric acid at 25°C on a vacuum line. This gas was purified and introduced to a mass spectrometer for carbon and oxygen isotope measurements (Wang et al., 243).

Results

Table 6: Resulting δ^{13}C Values

Sample Name	δ^{13}C* (‰)
TB-1	-11.4
TB-2	-9.8
TB-3	-11.1
TB-4	-6.0

*Values obtained are averages of 6 runs. All values have a standard deviation of ±0.05 ‰ or better.

Discussion

With only four samples, one of each species, reconstructing the entire paleo-environment of the animal being studied is impossible. However, we can state with certainty that all four animals incorporated C3 plants into their diet. TB-1 and TB-3 possessed a similar $\delta^{13}C$ value (-11.4‰ and –11.1‰, respectively). According to Table 5, an herbivore with a diet consisting entirely of C3 plants would have a $\delta^{13}C$ value of approximately –13.5‰. We can therefore safely conclude that the paleo-deer and the paleo-rhinoceros predominantly ingested C3 plants.

The paleo-pig (TB-2) possessed a slightly less negative $\delta^{13}C$ value of –9.8‰. From this, we can draw a conclusion that both C3 and C4 plants were incorporated into the pig's diet, with the former being the more prominent component.

The paleo-horse (TB-4) deviates even further from the other samples. With a $\delta^{13}C$ value of –6.0‰, we can infer that both C3 and C4 plants were consumed in significant quantities. Moreover, we can infer that the uplift of the Gyirong basin to its present elevation of nearly 5 kilometers occurred after this horse's life, because no horse could survive at such high elevation. We can attribute the less negative $\delta^{13}C$ value at least in part to the animal's selectivity of diet. The degree of such selectivity, however, is unknown.

From only four samples, it is impossible to determine what the relative amounts of C3 and C4 plants were thousands to millions of years ago. Availability and selectivity of diet are both factors that could profoundly affect the $\delta^{13}C$ values found in their tooth

enamel. Using the procedure outlined above, the study of samples on a much larger scale could shed some light on the paleo-environment of the animals.

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