ISOTOPE FRACTIONATION IN THE BIOSPHERE

INTRODUCTION

As we noted, biological processes often involve large isotopic fractionations. Indeed, biological processes are the most important cause of variations in the isotope composition of carbon, nitrogen, and sulfur. For the most part, the largest fractionations occur during the initial production of organic matter by the so-called primary producers, or autotrophs. These include all plants and many kinds of bacteria. The most important means of production of organic matter is photosynthesis, but organic matter may also be produced by chemosynthesis, for example at mid-ocean ridge hydrothermal vents. Large fractions of both carbon and nitrogen occur during primary production. Additional fractionations also occur in subsequent reactions and up through the food chain as heterotrophs consume primary producers, but these are generally smaller.

CARBON ISOTOPE FRACTIONATION DURING PHOTOSYNTHESIS

The most important means of producing isotopic fractionation of carbon is photosynthesis. As we earlier noted, photosynthetic fractionation of carbon isotopes is primarily kinetic. The early work of Park and Epstein (1960) suggested fractionation occurred in several steps. Subsequent work has elucidated the fractionations involved in these steps, which we will consider in more detail here.

For terrestrial plants (those utilizing atmospheric CO$_2$), the first step is diffusion of CO$_2$ into the boundary layer surrounding the leaf, through the stomata, and internally in the leaf. The average $\delta^{13}$C of various species of plants has been correlated with the stomatal conductance (Delucia et al., 1988), indicating that diffusion into the plant is indeed important in fractionating carbon isotopes. On theoretical grounds, a 4.4‰ difference in the diffusion coefficients is predicted ($^{13}$CO$_2$ will diffuse more rapidly; see Lecture 27) so a fractionation of $\sim$4.4‰ is expected. Marine algae and aquatic plants can utilize either dissolved CO$_2$ or HCO$_3^-$ for photosynthesis:

$$\text{CO}_2(g) \rightarrow \text{CO}_2(aq) + \text{H}_2\text{O} \rightarrow \text{H}_2\text{CO}_3 \rightarrow \text{H}^+ + \text{HCO}_3^-$$

An equilibrium fractionation of $\sim$0.9 per mil is associated with dissolution ($^{13}$CO$_2$ will dissolve more readily), and an equilibrium $\pm 7$ to $\pm 12$‰ fractionation (depending on temperature) occurs during hydration and dissociation of CO$_2$. Thus, we expect dissolved HCO$_3^-$ to be about 8 to 12 per mil heavier than atmospheric CO$_2$.

At this point, there is a divergence in the chemical pathways. Most plants use an enzyme called ribulose bisphosphate carboxylase oxygenase (RUBISCO) to catalyze a reaction in which ribulose bisphosphate reacts with one molecule of CO$_2$ to produce 2 molecules of 3-phosphoglyceric acid, a compound containing 3 carbon atoms, in a process called carboxylation (Figure 29.1).

Energy to drive this reaction is provided by another reaction, called photophosphorylation, in which electromagnetic energy is used to dissociate water, producing oxygen. The carbon is subsequently reduced, carbohydrate formed, and the ribulose bisphosphate regenerated. Such plants are called C$_3$ plants, and this process is called the Benson-Calvin, or Calvin, cycle. C$_3$ plants constitute about 90% of all plants and include

$$\text{CH}_2\text{OPO}_3\text{H}_2$$

$$\text{C}=\text{O}$$

$$\text{H} \text{C} \text{OH} + \text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{H} \text{C} \text{OH} + \text{H} \text{C} \text{OH}$$

$$\text{CH}_2\text{OPO}_3\text{H}_2$$

$$\text{C}=\text{O}$$

$$\text{OH}$$

$$\text{Ribulose biphosphate}$$

$$\text{Two molecules of 3-phosphoglycerate}$$

Figure 29.1. Ribulose bisphosphate (RuBP) carboxylation, the reaction by which C$_3$ plants fix carbon during photosynthesis.
algae and autotrophic bacteria and comprise the majority of cultivated plants, including wheat, rice, and nuts. There is a kinetic fractionation associated with carboxylation of ribulose bisphosphate that has been determined by several methods to be $-29.4\%$ in higher terrestrial plants. Bacterial carboxylation has different reaction mechanisms and a smaller fractionation of about $-20\%$. Thus for terrestrial plants a fractionation of about $-34\%$ is expected from the sum of the fraction. The actual observed total fractionation is in the range of $-20$ to $-30\%$.

The disparity between the observed total fractionation and that expected from the sum of the steps presented something of a conundrum. The solution appears to be a model that assumes the amount of carbon isotope fractionation expressed in the tissues of plants depends on ratio the concentration of $\text{CO}_2$ inside plants to that in the external environment. The model may be described by the equation:

$$\Delta = a + \left(\frac{c_i}{c_o}\right)(b-a) \quad 29.1$$

where $a$ is the isotopic fractionation due to diffusion into the plant, $c_i$ is the interior $\text{CO}_2$ concentration, $c_o$ is the ambient or exterior $\text{CO}_2$ concentration, and $b$ is the fractionation occurring during carboxylation. According to this model, where an unlimited amount of $\text{CO}_2$ is available (i.e., when $c_i/c_o = 1$), carboxylation alone causes fractionation. At the other extreme, if the concentration of $\text{CO}_2$ in the cell is limiting (i.e., when $c_i/c_o = 0$), essentially all carbon in the cell will be fixed and therefore there will be little fractionation during this step and the total fractionation is essentially just that due to diffusion alone. Both laboratory experiments and field observations provide strong support for this model.

More recent studies have shown that Rubisco enzyme exists in at least 2 different forms and that these two different forms fractionate carbon isotopes to differing degrees. Form I, which is by far the most common, typically produces the fractionation mentioned above; fractionation produced by Form II, which appears to be restricted to a few autotrophic bacteria and some dinoflagellates, can be as small as $17.8\%$.

The other photosynthetic pathway is the Hatch-Slack cycle, used by the $C_4$ plants, which include hot-region grasses and related crops such as maize and sugarcane. These plants use $\text{phosphoenol pyruvate carboxylase (PEP)}$ to initially fix the carbon and form oxaloacetate, a compound that contains 4 carbons (Fig. 29.2). A much smaller fractionation, about $-2.0$ to $-2.5\%$ occurs during this step. In phosphoenol pyruvate carboxylation, the $\text{CO}_2$ is fixed in outer mesophyll cells as oxaloacetate and carried as part of a $\text{C}_4$ acid, either malate or asparate, to inner bundle sheath cells where it is decarboxylated and refixed by RuBP (Fig. 29.3). The environment in the bundle sheath cells is almost a closed system, so that virtually all the carbon carried there is refixed by RuBP, so there is little fractionation during this step. $C_4$ plants have average $\delta^{13}C$ of $-13\%$. As in the case of RuBP photosynthesis, the fractionation appears to depend on the ambient concentration of $\text{CO}_2$. This dependence can be modeled as:

$$\Delta = a + (b_1 + b_2\phi - a)\left(\frac{c_i}{c_o}\right) \quad 29.2$$

where $a$ is the fractionation due to diffusion of $\text{CO}_2$ into the plant as above, $b_i$ is the fractionation during transport into bundle-sheath cells, $b_2$ is the fractionation during carboxylation ($\sim -3\%$), $\phi$ is the fraction $\text{CO}_2$ leaked from the plant.
A third group of plants, the CAM plants, have a unique metabolism called the ‘Crassulacean acid metabolism’. These plates generally use the C₄ pathway, but can use the C₃ pathway under certain conditions. These plants are generally adapted to arid environments and include pineapple and many cacti, they have δ¹³C intermediate between C₃ and C₄ plants.

Terrestrial plants, which utilize CO₂ from the atmosphere, generally produce greater fractionations than marine and aquatic plants, which utilize dissolved CO₂ and HCO₃⁻, together referred to as dissolved inorganic carbon or DIC. As we noted above, there is about a +8‰ equilibrium fractionation between dissolved CO₂ and HCO₃⁻. Since HCO₃⁻ is about 2 orders of magnitude more abundant in seawater than dissolved CO₂, many marine algae utilize this species, and hence tend to show a lower net fractionation during photosynthesis. Diffusion is slower in water than in air, so diffusion is often the rate-limiting step. Most aquatic plants have some membrane-bound mechanism to pump DIC, which can be turned on when DIC is low. When DIC concentrations are high, fractionation in aquatic and marine plants is generally similar to that in terrestrial plants. When it is low and the plants are actively pumping DIC, the fractionation is less because most of the carbon pumped into cells is fixed. Thus carbon isotope fractionations can be as low as 5‰ in algae. The model describing this fractionation is:

\[ \Delta = d + b_3 + F_3 / F_1 \]  \hspace{1cm} (29.3)

where \( d \) is the equilibrium effect between CO₂ and HCO₃⁻, \( b_3 \) is the fractionation associated with carboxylation, and \((F_3/F_1)\) is the fraction of CO₂ leaked out of the cell. Though the net fractionation varies between species and depends on factors such as light intensity and moisture stress, higher C₃ plants have average bulk δ¹³C values of -27‰; algae and lichens are typically -12 to 23‰.

In aquatic systems where the pH is lower than in seawater, CO₂ becomes a more important species and algae can in some cases utilize this rather than HCO₃⁻. In those cases, the total fractionation will be greater. An interesting illustration of this, and the effect of the CO₂ concentration on net fractionation is shown in Figure 29.4, which shows data on the isotopic composition of algae and bacteria in Yellowstone hot springs.

Some fractionation is also associated with respiration (the oxidation of carbohydrate to CO₂), but the net effect is uncertain.

Not surprisingly, the carbon isotope fractionation in C fixation is also temperature dependent. Thus higher fractionations are observed in cold water phytoplankton than in warm water species. However, this observation also reflects a kinetic effect: there is generally less dissolved CO₂ available in warm wa-
ters because of the decreasing solubility at higher temperature. As a result, a larger fraction of the CO₂ is utilized and there is consequently less fractionation. Surface waters of the ocean are generally enriched in $^{13}$C because of uptake of $^{12}$C during photosynthesis (Figure 29.5). The degree of enrichment depends on the productivity: biologically productive areas show greater enrichment. Deep water, on the other hand, is depleted in $^{13}$C (perhaps it would be more accurate to say it is enriched in $^{12}$C). Organic matter falls through the water column and is decomposed and "remineralized", i.e., converted in inorganic carbon, by the action of bacteria, enriching deep water in $^{12}$C. Thus biological activity acts to "pump" carbon, and particularly $^{12}$C from surface to deep waters.

Essentially all organic matter originates through photosynthesis. Subsequent reactions convert the photosynthetically produced carbohydrates to the variety of other organic compounds utilized by organisms. Further fractionations occur in these reactions. These fractionations are thought to be kinetic in origin and may partly arise from organic C-H bonds being enriched in $^{12}$C and organic C-O bonds are enriched in $^{13}$C. $^{15}$C is preferentially consumed in respiration (again, because bonds are weaker and it reacts faster), which would tend to enrich residual organic matter in $^{13}$C. Thus the carbon isotopic composition of organisms becomes more positive moving up the food chain.

Interestingly, although the energy source for chemosynthesis is dramatically different than for photosynthesis, the carbon-fixation process is similar and still involves the Calvin cycle. Not surprisingly then, carbon fractionation during chemosynthesis is similar to that during photosynthesis. Some chemosynthetic bacteria, notably some of the symbionts of hydrothermal vent organisms, have Rubisco Form II, and hence show smaller fractionations.

### Nitrogen Isotope Fractionation in Biological Processes

Nitrogen is another important element in biological processes, being an essential component of all amino acids and proteins. As in the case of carbon, most of terrestrial nitrogen isotopic variation results from biological processes. These processes, however, are considerably more complex because nitrogen exists in more forms and more oxidation states. There are five important forms of inorganic nitrogen: molecular nitrogen (N₂), nitrate (NO₃⁻), nitrite (NO₂⁻), ammonia (NH₃), and ammonium (NH₄⁺). Equilibrium isotope fractionation that can be quite large occur between these five forms. Except for the ammonium-ammonia reaction, the reactions between these forms are all redox reactions and they are predominantly biologically mediated. Significant kinetic fractionations occur during these biological mediated reactions.

Heterotrophs get their nitrogen from what they eat and there is only a slight difference, generally around 1 or 2‰, between animals and what they eat. Autotrophs, including algae, plants and bacteria, must assimilate nitrogen from the environment. Plants and algae cannot assimilate and utilize N₂ they must used some type of fixed nitrogen, which can be any of the remaining 4 forms listed above. The dirty work of converting nitrogen to ammonia (and from there to other forms of nitrogen), a process called fixation, is done by bacteria, including photosynthetic ones. This involves only a small fractionation of -3 to +1‰.

Reduced nitrogen (e.g., ammonia) is the form of nitrogen that is ultimately incorporated into organic matter by autotrophs (it is ultimately incorporated into organic molecules as NH₃ amine groups). There is a fractionation of up to 20‰ when autotrophs uptake of ammonium (¹⁴N is taken up preferentially), the extent depending on the ammonium abundance.

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Fractionation</th>
</tr>
</thead>
<tbody>
<tr>
<td>N₂ fixation</td>
<td>N₂ → 2NH₃</td>
</tr>
<tr>
<td></td>
<td>NH₃ → NH₄⁺</td>
</tr>
<tr>
<td>Nitrification</td>
<td>NH₄⁺ → NO₃⁻</td>
</tr>
<tr>
<td>Denitrification</td>
<td>NO₃⁻ → N₂</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>NO₃⁻ → NH₄⁺</td>
</tr>
</tbody>
</table>

Table 29.1 Nitrogen Isotopic Fractionation in the Exogenic Nitrogen Cycle.
When ammonium is abundant, fractionation tends to be large, when it is not, most available ammonium is taken up and there is little fractionation (Figure 29.6). Most plants, including many marine algae, can utilize oxidized nitrogen, NO\textsubscript{3} and NO\textsubscript{2}, as well as reduced nitrogen. In these cases, nitrogen must first be reduced by the action of reductase enzymes. δ\textsubscript{15}N fractionations of 0 to −24‰ have been measured for the assimilation of NO\textsubscript{3}. There is a small fractionation when that ammonium is subsequently incorporated into organic molecules. There are two principle reactions by which ammonia is incorporated into organic matter: formation of glutamate from α-ketoglutarate via the glutamate dehydrogenase reaction, and formation of glutamine from glutamate via the enzyme glutamine synthetase. A positive fractionation (i.e., the product is enriched in 15N) of +2 to +4‰ has been measured for the glutamate dehydrogenase reaction, and the fractionation for the glutamine synthetase reaction is also expected to be positive, because N is bound more strongly in the product than in ammonia.

The net result of these various fractionations is that organic nitrogen is usually heavier than atmospheric nitrogen. The isotopic compositions of marine particulate nitrogen and non-nitrogen-fixing plankton are typically −3‰ to +12‰ δ\textsuperscript{15}N. Terrestrial plants unaffected by artificial fertilizers generally have a narrower range of +6‰ to +13 per mil. Legumes (and a few other kinds of plants) are a special case. While they cannot fix nitrogen, they have symbiotic bacteria in their root nodules that can. As a result, legumes have distinctly lower δ\textsuperscript{15}N than other terrestrial, in the range of −2 to +4‰. Marine blue-green algae range from -4 to +2, with most in the range of -4 to -2‰.

A caveat to all this is fixed nitrogen in ecosystems now derived from artificial fertilizers. These fertilizers contain ammonia derived from atmospheric N\textsubscript{2} through the Haber process, in which there is little isotopic fractionation. Consequently, modern plants, particularly those raised on artificial fertilizers, have lower δ\textsuperscript{15}N.

**Oxygen and Hydrogen Isotope Fractionation by Plants**

Oxygen is incorporated into biological material from CO\textsubscript{2}, H\textsubscript{2}O, and O\textsubscript{2}. However, both CO\textsubscript{2} and O\textsubscript{2} are in oxygen isotopic equilibrium with water during photosynthesis, and water is the dominant form. Therefore, the isotopic composition of plant water determines the oxygen isotopic composition of plant material. The oxygen isotopic composition of plant material seems to be controlled by exchange reactions between water and carbonyl oxygens (oxygens doubly bound to carbon):

\[
\text{C}^{16}\text{O} + \text{H}_2^{18}\text{O} \leftrightarrow \text{C}^{18}\text{O} + \text{H}_2^{16}\text{O}
\]

Fractionations of +16 to +27‰ (i.e., the organically bound oxygen is heavier) have been measured for these reactions. Consistent with this, cellulose from most plants has δ\textsuperscript{18}O of +27±3‰. Other factors, however, play a role in the oxygen isotopic composition of plant material. First, the isotopic composition of water varies from δ\textsuperscript{18}O = −55‰ in Arctic regions to δ\textsuperscript{18}O = 0‰ in the oceans. Second, less than complete equilibrium may be achieved if photosynthesis is occurring at a rapid pace, resulting in less fractionation. Finally, some fractionation of water may occur during transpiration, with residual water in the plant becoming heavier.

---

Figure 29.6. Dependence of nitrogen isotope fractionation by bacteria and diatoms on dissolved ammonium concentration.
Hydrogen isotope fractionation during photosynthesis occurs such that the light isotope is enriched in organic material. In marine algae, isotope fractionations of $-100$ to $-150\%$ have been observed, which is similar to that observed in terrestrial plants: $-86$ to $-120\%$. Among terrestrial plants, there appears to be a difference between C$_3$ and C$_4$ plants. The former fractionations of $-117$ to $-121\%$, while fractionations $-86$ to $-109\%$ have been observed in C$_4$ plants. However, little is known in detail about the exact mechanisms of fractionation.

As is the case for oxygen, variations in the isotopic composition of available water and fractionation during transpiration are important in controlling the hydrogen isotopic composition of plants. This is illustrated in Figure 29.7.

**BIOLOGICAL FRACTIONATION OF SULFUR ISOTOPES**

Though essential to life, sulfur is a minor component in living tissue (C:S atomic ratio is about 200). Plants take up sulfur as sulfate and subsequently reduce it to sulfide and incorporate into cysteine. There is apparently no fractionation of sulfur isotopes in transport across cell membranes and incorporation, but there is a fractionation of $+0.5$ to $-4.5\%$ in reduction process, referred to as *assimilatory sulfate reduction*. This is substantially less than the expected fractionation of about $-20\%$, suggesting that nearly all the sulfur taken up by primary producers is reduced and incorporated into tissue.

Sulfur, however, plays two other important roles in biological processes. First, sulfur in the form of sulfate can act as an electron acceptor or oxidant, and is utilized as such by sulfur-reducing bacteria. This process, in which H$_2$S is liberated, is called *dissimilatory sulfate reduction* and plays an important role in biogeochemical cycles, both as a sink for sulfur and source for atmospheric oxygen. A large fractionation of $+5$ to $-46\%$ is associated with this process. This process produces by far the most significant fractionation of sulfur isotopes, and thus governs the isotopic composition of sulfur in the exogene. Sedimentary sulfate typically has $\delta^{34}S$ of about $+17$, which is similar to the isotopic composition of sulfate in the oceans ($+20$), while sedimentary sulfide has a $\delta^{34}S$ of $-18$. The living biomass has a $\delta^{34}S$ of $\approx 0$.

The final important role of sulfur is a reductant. Sulfide is an electron acceptor used by some types of photosynthetic bacteria as well as other bacteria in the reduction of CO$_2$ to organic carbon. Most unique among these perhaps are the chemosynthetic bacteria of submarine hydrothermal vents. They utilize H$_2$S emanating from the vents as an energy source and form the base of the food chain in these unique ecosystems. A fractionation of $+2$ to $-18\%$ is associated with this process.

**REFERENCES AND SUGGESTIONS FOR FURTHER READING**

