Plant Physiol. (1993) 102: 1287–1290

Correlations between the ^{13}C Content of Primary and Secondary Plant Products in Different Cell Compartments and That in Decomposing Basidiomycetes

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Relative carbon isotope ratio ($\delta^{13}C$ values) of primary and secondary products from different compartments of annual plants, pine needles, wood, and decomposing Basidiomycetes have been determined. An enrichment in ^{13}C was found for storage tissues of annual plants, because of the high level of the primary storage products sucrose and starch; however, the enrichment was even greater in leaf starch. All of these compounds had the same relative ^{13}C enrichment in positions 3 and 4 of glucose. Secondary products in conifer needles (lignin, lipids) were depleted in ^{13}C by 1 to 2 ‰ relative to carbohydrates from the same origin. Air pollution caused a small decrease in $\delta^{13}C$ values; however, the relative content of plant products, especially of the soluble polar compounds, was also affected. Decomposing fungi showed a global accumulation of ^{13}C by 4‰ relative to their substrates in wood. Their chitin was enriched by 2‰ relative to the cellulose of the wood. Hence, Basidiomycetes preferentially metabolize "light" molecules, whereas "heavy" molecules are preferentially polymerized. Our results are discussed on the basis of a kinetic isotope effect on the fructose-1,6-bisphosphate aldolase reaction and of metabolic branching on the level of the triose phosphates with varying substrate fluxes.

The biomass of plants is globally depleted in ^{13}C relative to the atmospheric CO_2 pool; isotope effects of different reactions are responsible for this fact (O'Leary, 1981). The $\delta^{13}C$ values of the primary products of photosynthesis and of immediately descending carbohydrates are directly correlated with the kinetic isotope effects on the CO_2-fixing reactions, the Rubisco and the phospho*enol*pyruvate carboxylase reactions, respectively. Secondary products are additionally depleted, e.g. due to the kinetic isotope effect of the pyruvate dehydrogenase reaction (Melzer and Schmidt, 1987, and refs. therein), initiating pathways to fatty acids, isoprenoids, and acetogenins. Depletion is also observed for other groups of secondary plant products originating from the shikimic acid pathway, e.g. lignin, aromatic compounds, and flavonoids. The level of this depletion depends on the metabolic distance from the branching point (Schmidt et al., 1993). Any of these metabolites also have characteristic nonstatistical isotope patterns within their molecules (Schmidt et al., 1993), as earlier reported by us for Glc (Roßmann et al., 1991).

Additional reasons for the observed ^{13}C depletions may be due to isotope effects on short- and long-distance transport, as shown for CO_2 (Farquhar et al., 1982a) or for carbohydrates in corn seedlings (Deléens-Provent and Schwebel-Dugué, 1987). The $\delta^{13}C$ values are also influenced by changes of growth conditions, e.g. light intensity and quality (Ehleringer et al., 1986; Evans et al., 1986), salinity (Downton et al., 1985), transpiration rates (Farquhar et al., 1982b), yields of crops (Condon et al., 1987), air pollutants (Freyer, 1979; Martin et al., 1988), and the physiological state of the organ (Araus et al., 1992). The observed relative depletions can help assign the origin of plant products (Schmidt et al., 1993).

However, any interpretation of $\delta^{13}C$ values determined with whole plants has to consider that changes in external conditions influence the relative amounts of primary and secondary products as well. These effects may thus indirectly contribute to the isotopic shifts observed. Therefore, our contribution will deal with both relative amounts and isotope abundances of main representatives of primary and secondary products in different plant compartments. In this context, we have also determined $\delta^{13}C$ values of components of wood-degrading fungi to learn whether the "light" or the "heavy" intermediate products are preferentially used for catabolism or anabolism by the saprophytes.

MATERIALS AND METHODS

Plant Material

Fully developed, whole plants of sugar beet (*Beta vulgaris*), potato (*Solanum tuberosum*), and corn (*Zea mays*), grown in ordinary fields near Freising, Germany, were harvested and separated into C-source and C-sink organs. The plant material was cut into slices, immediately frozen in liquid nitrogen, lyophilized, ground, and stored until use. Beet plants were harvested at different times during the same day.

Needles of *Picea abies* from two different years and two different natural habitats were kindly provided by Prof. A. Kettrup, Technische Universität München. The Velmerstot habitat (400 m NN, Eggegebirge, Germany) is exposed to polluted air from Eastern Europe, whereas Medebach (650 m NN, Sauerland, Germany) is not exposed to these polluting emissions. The needles were lyophilized, ground to a fine powder, and stored in a vacuum desiccator.

Samples of dead wood, infected with cellulose-decompos-

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Abbreviation: $\delta^{13}C(‰)_{PDB}$, relative carbon isotope ratio = $[(R_{sample} - R_{PDB}) / R_{PDB}] \times 1000$, where R is the isotope ratio; PDB, Pee Dee Belemnite international standard.

ing (soft rot) or lignin- and cellulose-decomposing (white rot) Basidiomycetes, were collected in forests near Freising (Germany) and separated into noninfected wood, infected wood without mycelia, and intact fungi. The samples were dried and ground subsequently.

Fractionation and Isolation of Main Components

Leaves and reserve organs of sugar beets, potatoes, and corn were fractionated into starch, cellulose, and soluble sugars according to the methods of Brugnoli et al. (1988) and Deléens and Garnier-Dardart (1977). Cellulose, lignin, and chitin from wood and fungi samples were isolated as described by Göring and van Soest (1970). The products of *P. abies* needles were fractionated by a modified method described by Epstein et al. (1976): lyophilized and ground plant material (about 3 g) was extracted five to six times with 100 mL of benzene:chloroform (1:1, v/v) for 3 to 4 h. The combined extracts were evaporated to dryness, yielding the lipid fraction. The remaining insoluble plant material was extracted in a similar way with water:acetone (1:1, v/v) yielding the polar substance fraction. The remaining plant material was digested with sodium chlorite (Green, 1963), yielding holocellulose. The amount of the lignin/protein fraction was calculated from the difference between holocellulose and the material from which it had been extracted. The holocellulose was digested in 17% (w/w) NaOH, yielding α-cellulose.

Hydrolysis of starch and Suc and the fermentation of hexoses for positional isotope analysis was performed as described earlier (Roßmann et al., 1991).

Determination of $\delta^{13}C$ Values

Isotope ratios were determined as described by Winkler and Schmidt (1980) on a Sira 24 and a MM 903 (VG Isogas, Middlewich, UK). The sample oxidation was performed in an automated sample converter (Roboprep CN; Europa Scientific, Crewe, UK) or with an elemental analyzer (Micro U; Heraeus, Hanau, Germany).

RESULTS AND DISSCUSSION

$\delta^{13}C$ Values of Primary Metabolites from Different Organs of Annual Plants

The global $\delta^{13}C$ values of the different organs from corn, potatoes, and sugar beets indicate a relative enrichment of ^{13}C in the storage tissues as compared to the leaves (Table I), because of the preponderance of starch or Suc over cellulose in these organs. In sugar beet leaves, starch is much heavier then Suc, as previously described (Deléens and Garnier-Dardart, 1977; Brugnoli et al., 1988). The $\delta^{13}C$ values for cellulose are nearly identical for vegetative and storage organs but lower than the $\delta^{13}C$ values for starch and Suc. Whereas the "sugars" in the leaf are transitory intermediates originating immediately from the first isotope discrimination by Rubisco, Suc in the root comes from a first branching reaction path.

Of further interest is the increase of ^{13}C enrichment in leaf starch during the day. This effect is pronounced even if the $\delta^{13}C$ values are calculated for the additional amount of starch synthesized during the course of the afternoon (−21.9‰). This ^{13}C enrichment is obviously an expression of the decreased stomatal conductance (Farquhar et al., 1989).

Table I. *$\delta^{13}C$-values of different organs and substances ([‰]$_{PDB}$)*

Plant	Organ/Metabolite	$\delta^{13}C$-Value ([‰]$_{PDB}$)			
		At harvesting time			In position C_3, C_4
		10:00	14:00	17:30	
Corn	Leaf		−13.1		
	Grain		−11.7		
Potato	Leaf		−27.4		
	Tuber		−25.9		
Beet	Leaf	−28.9	−28.2	−27.0	
	Leaf/sugar	−28.9	−28.0	−27.5	−22.7
	Leaf/starch	−24.8	−24.3	−23.9	−19.3
	Leaf/cellulose	−26.8	−27.4	−27.0	
	Root	−27.8	−26.9	−25.8	
	Root/sugar	−26.1	−26.4	−25.7	−20.9
	Root/cellulose	−28.4	−27.5	−27.1	

To evaluate the cause of the ^{13}C discriminations observed (kinetic isotope effects, metabolic branching, transport), we have measured the relative isotope enrichment in positions 3 and 4 of the carbohydrates in sugar beet. The ^{13}C enrichment found was nearly identical in leaf sugars (5.4‰), in root sugars (5.2‰), and in leaf starch (5.0‰). This indicates that all of these carbohydrates originate from the same reaction (aldolase reaction) and that their isotopic differences are probably due to a different use of isotopomer triose phosphates for recycling of the pentose phosphate cycle and for export to the cytosol.

$\delta^{13}C$ Differences of Primary and Secondary Polymers in *Pinus* Needles

In our early studies (Butzenlechner, 1990; Schmidt et al., 1993), we measured ^{13}C depletion between 3 and 6‰ for aromatic compounds relative to carbohydrates from the same source. This was coincident with results from other laboratories (Galimov, 1981; Benner et al., 1987). In the conifer needles in question, the depletion of aromatic compounds relative to carbohydrates was only 1.5‰ for needles from healthy trees and 1.0‰ for needles from polluted trees (Table II). We suppose that the rather small ^{13}C differences in conifer needles are due to the contamination of this fraction with protein (see "Materials and Methods"). In needles, this contamination is probably far more relevant than in wood.

To evaluate the influence of stress caused by air pollution on the $\delta^{13}C$ values, we must consider also the relative amounts of the needle fractions. In stressed plants, a dramatic ^{13}C depletion and also a mass decrease are observed for the soluble polar fraction (free sugars, organic acids, and other low mol wt intermediates; Table II). The polar fraction, however, does not appreciably contribute to the mean $\delta^{13}C$ value of the total plant material. The $\delta^{13}C$ values for the cellulose and lignin fractions are slightly more positive in polluted plants, and the lipids are more negative. However, none of these compounds are subjected to a substantial mass

Table II. *Relative amounts and $\delta^{13}C$-values ([‰]$_{PDB}$) of different products in P. abies needles from two habitats of different ages*

The "increment" is the product of relative amount (yield) and the $\delta^{13}C$ value.

Fraction	Polluted (Velmerstot)			Control (Medebach)		
	Yield	$\delta^{13}C$	Increment	Yield	$\delta^{13}C_{PDB}$	Increment
	[%]	[‰]$_{PDB}$		[%]	[‰]$_{PDB}$	
			1988			
Bulk	100.0	−27.4	−27.37	100.0	−27.0	−27.01
Lipids	37.8	−28.8	−10.89	45.2	−27.6	−12.48
Polar fraction	8.4	−31.3	−2.63	13.9	−26.8	−3.73
Holocellulose	18.7	−25.1	−4.69	18.8	−25.6	−4.81
α-Cellulose	7.7	−24.7	−1.90	7.7	−24.7	−1.90
Lignin (+ protein)	35.1	−26.1	−9.16	22.1	−27.1	−5.99
			1987			
Bulk	100.0	−27.6	−27.68	100.0	−26.8	−26.86
Lipids	38.5	−28.3	−10.90	35.8	−27.6	−9.88
Polar fraction	7.9	−37.8	−2.99	8.7	−29.2	−2.54
Holocellulose	19.5	−24.9	−4.86	22.5	−25.0	−5.63
α-Cellulose	9.8	−24.7	−2.42	13.3	−25.2	−3.35
Lignin (+ protein)	34.1	−26.0	−8.87	33.0	−26.7	−8.81

shift. Thus, a definite explanation for the $\delta^{13}C$ value shift cannot be given; however, it is certainly due to the change in the relative amounts of material in the fractions and to $\delta^{13}C$ value changes.

$\delta^{13}C$ Values of Wood-Degrading Fungi and of Their Substrates

The plant polymers cellulose and lignin are substrates of wood degradation by fungi. Several saprophytes utilize only cellulose, and others utilize cellulose and lignin. To meet their demand for nitrogen, such saprophytes must decompose much more biomass than they need for their energy requirement and for the synthesis of their own biopolymers.

The mean difference in ^{13}C abundance found between cellulose and lignin from wood was 3.7‰ (Table III). In any case, none of the structural polymers (cellulose, lignin, and chitin) showed a marked deviation from the mean values given in Table III. The chitin of the fungi was enriched by 1.5 to 2‰ relative to cellulose, independent of whether cellulose or cellulose and lignin were substrates. The enrichment of the hexose units of chitin must be even higher, because the polymer has substitution by acetyl groups, which should be depleted (Melzer and Schmidt, 1987).

Analogously to the formation of products in annual plants and in pine needles, the ^{13}C enrichment of chitin must be a consequence of the branching in anabolic and catabolic reactions of Glc. The light molecules are preferentially catabolized, and the heavy molecules are preferentially repolymerized. Assuming that Fru-1,6-bisP aldolase "prefers," for the bond fission, molecules with ^{12}C in positions 3 and 4, these positions must be additionally enriched in ^{13}C in chitin relative to cellulose.

Table III. *Mean $\delta^{13}C$-values ([‰]$_{PDB}$) of structural polymers from 12 different decomposer Basidomycetes, collected from different foliar trees (each in duplicate)*

	$\delta^{13}C$-Value ([‰]$_{PDB}$)	
	Soft rot	White rot
Wood		
Healthy	−26.2	−26.6
Cellulose	−24.5	−24.8
Lignin	−28.0	−28.7
Wood		
Infected	−25.3	−25.5
Cellulose	−24.5	−24.6
Lignin	−28.2	−28.3
Fungus	−22.7	−23.0
Chitin	−23.0	−22.5

CONCLUSIONS

Isotope discrimination between primary and secondary products of plants and between different representatives of the same substance group is the consequence of superimposed enzyme kinetic isotope effects and substrate fluxes at metabolic branching points. Most of the results reported here can be explained on the basis of isotope effects on the aldolase and/or the triose phosphate isomerase reactions (Brugnoli et al., 1988). These effects and the branching at the level of the triose phosphates induce ^{13}C enrichment in positions 3 and 4 of the hexoses (Fig. 1). During the branching of the triose phosphates, the aldolase of the chloroplast preferentially uses the heavy monomers, and the light monomers are exported to the cytosol. As a result, the starch of the chloroplast is markedly enriched in ^{13}C. Consequently, the triose phosphates exported to the cytosol are enriched in ^{12}C. Therefore, Suc synthesized in the cytosol is composed of lighter hexose monomers than the starch in the chloroplast. We assume that

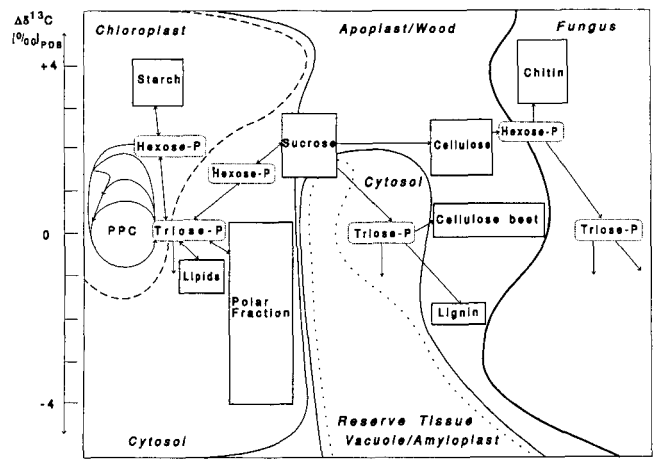

Figure 1. $\delta^{13}C$-values ([‰]$_{PDB}$) of primary and secondary plant products in different cell compartments and in decomposing Basidomycetes. The ^{13}C ranges of products are shown by the vertical extension of the solid boxes (for details, see text).

the magnitude of the difference in $\delta^{13}C$ values between Suc and leaf starch depends on metabolic effects on the translocation of triose phosphates out of the chloroplast (Neuhaus et al., 1989).

The negative $\delta^{13}C$ values of photosynthetic intermediates directly reflect the availability of CO_2 for photosynthesis (Farquhar et al., 1982b). Hence, the metabolic flux rates of the photosynthetic intermediates affect both the partitioning of triose phosphates (Heldt et al., 1986; Okita, 1992) and the ^{13}C enrichment in starch and Suc (Brugnoli et al., 1988). Reduced metabolic flux rates, e.g. in stressed conifer needles, will, therefore, result in the full impact of the kinetic isotope effect of the Rubisco reaction being expressed in lighter products.

In conclusion (Fig. 1), products that come directly from hexoses, e.g. cellulose, Suc, and starch, will be relatively rich in ^{13}C, compared to products synthesized from triose phosphates, formed via glycolytic breakdown. An exception is cellulose in beet roots, which possibly is synthesized from the cytosolic triose phosphates of the storage tissue and not, as in wood, from hexoses originating from Suc.

There are certainly multiple effects influencing the differences in $\delta^{13}C$ values of primary and secondary plant products in different compartments; however, the isotope effect on the aldolase reaction undoubtedly is most important (Schmidt et al., 1993).

Received January 13, 1993; accepted April 10, 1993.

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