OSCILLATORY PATTERN OF PHOTOSYNTHETIC $C0_2$ ASSIMILATION AFFECTS ^{13}C DISTRIBUTION IN CARBOHYDRATES

A.A. IVLEV[1]*, A.YU. DUBINSKY[2], A.U. IGAMBERDIEV[3]

(' Department of inorganic and analytical chemistry of Russian State Agrarian University - MTAA named after K.A. Timiryazev, Moscow 127550 Russia;[2] Keldysh Institute of Applied Mathematics, Russian Academy of Sciencies, 4 Miusskaya Sq., Moscow 125047 Russia; [3] Department of Plant Science, University of Manitoba, Winnipeg, Manitoba, R3T 2N2 Canada)

Abstract. ^{13}C distribution in carbohydrates is closely related to the mechanism of photosynthetic oscillations. According to the oscillation hypothesis, carbon metabolism in photosynthesis may exhibit non-damped oscillations consisting of two phases — $C0_2$ assimilation and photorespiration. The former corresponds to the carboxylase phase of Rubisco, the latter corresponds to the oxygenase phase. We argue that carbohydrates formed in the carboxylase phase have approximately uniform ^{13}C distribution along the carbon skeleton of hexose units, whereas ^{13}C distribution in hexoses formed in the oxygenase phase, are characterized by a specific heterogeneous isotopic pattern. The above hypothesis explains well the available data on ^{13}C distributions in carbohydrates from C_3 and C_4 plants. It also explains that isotopic differences of total carbon in most carbohydrates are determined by the changes in depletion of substrate pool consumed in the course of photorespiration**.**

Key words: carbon isotope fractionation, photorespiration, Rubisco, carbohydrates, metabolic modeling.

Introduction

The ,^{3}C distribution in carbohydrates has drawn attention of the increasing number of the researchers (Gleixner *et al.,* 1997; Duranceau *et al,* 1999, 2001; Ghashghaie *et al,* 2003; Schmidt, 2003; Tcherkez *et al.,* 2004; Ivlev, 2005) during last years. The interest stems from the expectation that isotopic data on carbohydrates, which are primary assimilates, can give new information on photosynthesis mechanism and the Calvin cycle operation. Another reason for the interest is in the fact that carbohydrates provide the carbon for the synthesis of many metabolites and their isotope ratio and isotopic pattern might be helpfUl for establishing metabolic links and pools origin. In addition, direct participation of carbohydrates in many metabolic processes ($C0_2$ assimilation and photorespiration, glycolysis and dark respiration) by means of isotopic data helps to better realize the role of carbohydrates in them and in cell metabolism as a whole.

Carbon isotope ratio of carbohydrates is poorly studied. As compared with the other biochemical fractions they are enriched in ^{13}C in most cases. There are some differences in $5^{13}C$ values between individual carbohydrate components, but no distinct regularities have been established. For example, in some cases labile sugars and starch are enriched in ^{13}C as compared to other carbohydrate components while in the others they are depleted.

* Author for correspondence: Alexander A. Ivlev, Dr. E-mail: aa.ivlev@list.ru

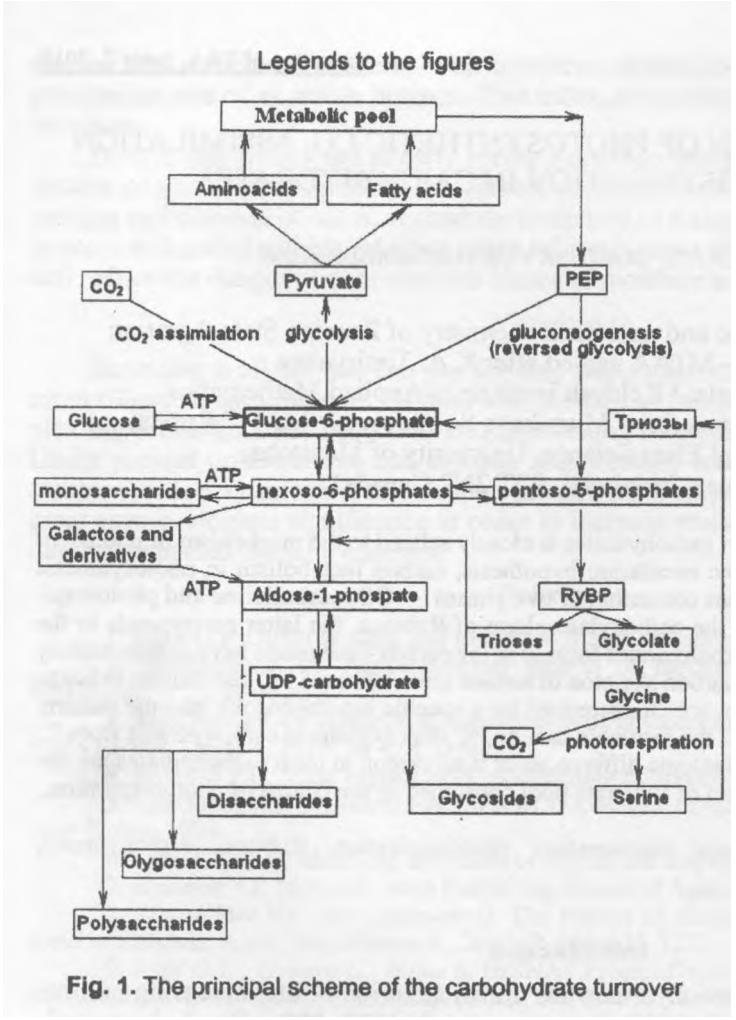

Fig. 1. The principal scheme of the carbohydrate turnover

Isotope distribution in carbohydrates may be quite different depending on the plant organ (leaf, stem, root or seeds) (Hobbie & Werner, 2004), on habitat conditions, or plant type (Borland *et al.,* 1994). To find out the cause for carbohydrate isotopic variations in plants it is necessary to determine where and how carbon isotope effects emerge in their synthesis as well as what factors affect the carbohydrate carbon isotope ratio. In this work we attempt to fill this gap.

The scheme on fig.l demonstrates the links between different carbohydrate pools. As it follows, the synthesis of glucose-6-phosphate (G6P) is the key point of the whole carbohydrate metabolism. The latter is derived in the Calvin cycle. The syntheses of the other hexoses and pentoses as well as related polysaccharides occur via G6P. To understand what factors determine carbon isotope ratio of various carbohydrates one needs to know first the factors that influence carbon isotope ratio of G6P. Such formulation of the problem requires modeling of carbon isotope fractionation in photosynthesis.

Steady state and oscillatory models describing carbon isotope fractionation in photosynthesis

Two different approaches to carbon isotope fractionation description in photosynthesis are known from the literature. The main postulate of the steady-state model asserts that under invariable conditions all processes in photosynthetic cells proceed simultaneously and continuously (Vogel, 1980, 1993; O'Leary, 1981; Farquhar, 1980; Farquhar *et al.,* 1982; Brugnoli & Farquhar, 2000). The oscillation model assumes a periodic and discrete nature of metabolic processes, definite temporal sequence of them (Ivlev, 1989,1993, 2008; Ivlev *et al.,* 2004). The discreteness of the processes, according to the last model, is a result of periodic exhaustion of metabolic pools alternating with their filling.

Steady-state model. The basic equation of the steady-state model in notations of Farquhar and colleagues (Farquhar *et al.,* 1982; Brugnoli & Farquhar, 2000) looks like:

$$\Delta = a + (b - a)\, p_i / p_a, \qquad (1)$$

where A is a value of ^{13}C discrimination in photosynthesis, a is a carbon isotope effect at diffusion, *b* is an isotope effect at the ribulose-l,5-bisphosphate (RuBP) carboxylation site. The expression (1) links the value of carbon isotope discrimination with partial pressures of $C0_2$ inside a leaf p_i and that in the atmosphere p_a. The impact of the environmental factors like temperature, water availability, salinity of irrigating waters, concentration of $C0_2$ and 0_2 in the atmosphere, etc. on carbon isotope ratio of plants, according to the model, is transmitted via $C0_2$ partial pressure ratio. Accounting of the experimental values of *a*-- 4,4%o, and *b* = -29%o, (Farquhar *et al.,* 1982), the model describes the range of natural Д variations and explains their relation to the environmental factors.

In practice the application of the equation (1) results in a considerable number of violations of the linear correlation postulated by the model. To fit the facts more adequately a complicated form of equation (1) taking into account all the processes that might result in carbon isotope discrimination and capable to explain the violations was introduced. One of its versions looks like:

$$\Delta = a_b (p_a - p_i)/p_a + a\,(p_s - p_i)/p_a + (e_s + a_l)(p_i - p_c)/p_a + b \cdot p_c/p_a - \\ -1/p_a \cdot (eR_d/k + f\Gamma^*), \qquad (2)$$

where p_a, p_s, p, and p_c are the $C0_2$ partial pressures in the atmosphere, at leaf surface, inside the leaf prior to $C0_2$ dissolution in cytoplasm, and at carboxylation sites correspondingly; a_b is a ^{13}C discrimination at $C0_2$ diffusion through a boundaiy layer, *a* is an isotope effect of $C0_2$ diffusion in the air, e_s is a carbon isotope fractionation at $C0_2$ dissolution in cytoplasm, a, is a carbon isotope fractionation at $C0_2$ diffusion through liquid, *b* is a carbon isotope fractionation in an enzymatic RuBP carboxylation in C_3 plants; *e* and *f* are possible carbon isotope fractionation values at dark respiration R_d and photorespiration correspondingly; A: is a carboxylation efficiency and Γ^* is a compensation point defined in the absence of dark respiration. All isotope fractionation factors are determined experimentally either in model processes or *in vitro.* The following values were obtained: a_b = -2.9%o (Farquhar, 1980), *a*= -4.4%o (Craig, 1954), e_s = -l.l%o at 25°C (Vogel, 1980) *a,=-0.7%o* (O'Leary, 1984).

The isotope discrimination *b* in RuBP carboxylation appeared to be gently dependent on pH. At pH 9.0 it was equal to 1.029, and at pH 7.0 - 1,030 (Roeske, O'Leary, 1984). As to isotope fractionation factors at dark respiration *e* and at photorespiration *f* they are usually neglected and taken into consideration only in cases, when deviations from simplified model (1) become significant. Accepting, that the above deviations are related to the discussed effects, they are used to calculate the mentioned isotope fractionation factors. Some researchers have confirmed their small value (von Caemmerer & Evans, 1991; Lin & Ehleringer, 1997), the others (Duranceau *et al.,* 1999) inferred, that *e* is significant and is equal to 6%o. The estimates of the photorespiration isotope fractionation factor /appeared to be even more arguable because of the complexity of its determination. They were studied by measuring $C0_2$ carbon isotope ratio nearby the compensation point. Rooney (1988) estimated *f* to be 7%o, while von Caemmerer and Evans (1991), found *f* equal to 5%o. Indirect estimates of *f* amounted to 3.3%o for wheat and 0.5%o for bean (Gillon & Griffiths, 1997) but it was mentioned by Brugnoli and Farquhar (2000) that Gillon and Griffiths obtained later the values closer to those reported by Rooney (1988). In the paper of Igamberdiev *et al.* (2004) the authors compared isotope discrimination of wild-type and photorespiratory

mutants. The calculated effect of photorespiration was in the range of 10-13‰ for potato, barley and Arabidopsis.

As it follows from the equation (2), isotope fractionation processes (except diffusion and RuBP carboxylation) contribute in constant increments to A making the above relationship deviate from linearity, but for all that contribution the RuBP carboxylation isotope effect remains to be basic. Due to subsidiary parameters, the equation (2) allows to explain the observed deviations from the linearity, but the relationship of the isotope discrimination with the environmental factors becomes vague. Besides, a number of the facts stay behind the frameworks of the model. For example, it remains unclear, why in some gas exchange experiments on $C0_2$ assimilation the preferential fixation of ^{13}C was observed (Sanadze *et al.,* 1978; Ivanov *et al.,* 1978), why in some cases primary assimilates were enriched in the "heavy" carbon relative to ambient $C0_2$ (Voznesenskii *et al.,* 1982). ^{U}C enrichment of photorespiratory metabolites was also unexplainable (Rivera & Smith, 1979; Raven *et al.,* 1982; Kalinkina & Udelnova, 1990, 1991). Some conclusions resulting from the model turned to be incompatible with the facts (Ivlev, 2002).

The point is that the steady-state model describing satisfactory the dependence of isotope ratio of the bulk carbon and major fractions of plant tissue on the environmental factors fails to explain isotopic discrepancies when it is necessary to describe intramolecular ^{13}C distribution of the metabolites. The only attempt to explain isotope heterogeneity of G6P by introducing into the model carbon isotope fractionation factors for different enzymatic reactions of the Calvin cycle was entertained recently by Tcherkez *et al.* (2004) and brought about the results that seem controversial and not fully substantiated.

The steady-state model operates with the averaged fluxes of carbon substrates in a cell whereas description of isotopic patterns of the molecules demands to take into consideration kinetic isotope effects of the enzymatic reactions, conditions and mechanisms of cell processes determining isotope shifts of individual atoms.

Oscillation model. Alternative oscillation model (Ivlev, 1993; Ivlev *et al.,* 2004) asserts, that carbon isotope fractionation in photosynthesis emerges in two conjugate processes — $C0_2$ assimilation and photorespiration. Two isotope effect-producing reactions occur in the processes. In $C0_2$ assimilation it is RuBP carboxylation, in photorespiration it is glycine decarboxylation. Isotope effect in $C0_2$ assimilation results in the enrichment of a biomass in ^{12}C, vice versa isotope effect in photorespiration reduces the above enrichment, i.e. the processes are accompanied by isotope effects with inverse signs. The analysis of the mechanism of carbon isotope effects coupling draws to the conclusion on the existence of non-damped photosynthetic oscillations (Ivlev *et al.,* 2004) one phase of which is $C0_2$ assimilation while another phase is photorespiration. This means both processes are separated in time switching over from one to another and back. Such oscillations can arise due to the capability of Rubisco to work as carboxylase and oxygenase following the periodical changes in $C0_2/0_2$ ratio in photosynthetic cells. As a result $C0_2$ enters and leaves the cell by separate batches.

On contrary to the steady-state model, the oscillation model assuming filling/ depletion substrate pools results in Raleigh type isotope fractionation in the course of pool depletion. The latter in turn determines a specific intra- and intermolecular carbon isotope distribution in metabolites, reflecting temporal organization of metabolic processes. As it will be shown later the oscillation model enables to explain isotope patterns and differences of carbohydrate molecules.

These oscillations have been verified recently by measuring variations of the internal $C0_2$ concentration in tobacco leaves exposed to the atmosphere with low $C0_2$ concentration (near the compensation point) (Roussel *et al.,* 2007).

The data of Satoh and Katoh (1983) who observed a peak of delayed luminescence at the fifth second after the excitation impulse in the experiments with green alga *Bryopsis maxima* also may be regarded as the confirmation of the photosynthetic oscillations since it proves that the feedback of photorespiration and light reactions of photosynthesis are separated in time. Although the authors worked with isolated chloroplasts, the synthesis of photorespiratory intermediates (phosphoglycolate and glycolate) may result in the observed feedback via pH changes and the oxidation of glycolate that in part is associated with chloroplast and can directly transfer electrons to the chloroplastic electron transport chain causing the feedback effects (Goyal and Tolbert, 1996). The feedback peak of delayed luminescence is of the same duration as post-illumination burst, the phenomenon caused by the oxidation of photorespiratory glycine after light is turned off.

The validity of the explanation is proven by the theoretical modeling of the $C0_2$ assimilation and photorespiration feedback to photosynthetic electron transport resulting after excitation impulse in two peaks of delayed luminescence of chlorophyll separated by 1.6 s (Kukushkin and Soldatova, 1996). The authors showed when they removed the impact of photorespiration one of two peaks in luminescence spectra disappeared.

Discreteness of $C0_2$ assimilation generates discrete periodic pattern of metabolic processes. This explains the fact why isotope effects in enzymatic reaction are often accompanied with the Raleigh effect of substrate pool depletion. The combination of both effects resulting in the temporal organization of cell processes was demonstrated earlier for glycolysis (Ivlev, 1992).

The ^{12}C enrichment of the assimilated carbon relative to ambient $C0_2$ emerging in RuBP carboxylation during $C0_2$ assimilation phase can be described as

$$(\delta^{13}C_{\text{biomass}} \cdot 10^{-3} + 1) = (\delta^{13}C_{\text{co2}} \cdot 10^{-3} + 1)\,[1 - (1 - F)^{1/\alpha}], \qquad (3)$$

where $\delta^{13}C_{\text{biomass}}$ and $\delta^{13}C_{co2}$ are the carbon isotope ratios of the assimilated carbon (biomass) and feeding $C0_2$ respectively. F is the extent of $C0_2$ batch depletion, $a = k^{12}/k^{13}$ is the effective isotope fractionation coefficient at RuBP carboxylation.

The ^{12}C enrichment of $C0_2$ relative to carbohydrate pool derived in $C0_2$ assimilation emerging in glycine decarboxylation during photorespiration phase can be expressed in a similar way

$$(\delta^{13}C_{\text{co2}} \cdot 10^{-3} + 1) = (\delta^{13}C_{G6P} \cdot 10^{-3} + 1)\,[1 - (1 - F)^{1/\alpha'}], \qquad (4)$$

where $\delta^{13}C_{G6P}$ and $\delta^{13}C_{co2}$ are the carbon isotope ratios of the G6P and photorespiratory $C0_2$ correspondingly. F is the extent of G6P pool depletion used to feed glycolate chain, $a' = {}^{12}k'/{}^{13}k'$ is the effective isotope fractionation coefficient in the enzymatic decarboxylation of glycine.

How these general assertions can be applied to describe ^{13}C distribution in G6P?

Intramolecular carbon isotope distribution in G6P according to the oscillation model

First of all we should analyze the sequence of metabolic events in carbon metabolism of photosynthesis, according to the oscillation model, and the place of the Calvin cycle where the synthesis of G6P occurs.

In the carboxylase phase of Rubisco operation when a $C0_2$ batch enters the cell, a portion of it is transformed into carbohydrates via the Calvin cycle. At the entrance of the

cycle in RuBP carboxylation all the assimilated carbon, including G6P, becomes enriched in ^{12}C relative to $C0_2$. Thus carbohydrates formed in the carboxylase phase become enriched in ^{12}C too. The extent of the ^{12}C enrichment is determined by the degree of $C0_2$ batch depletion. Some carbohydrates supposedly in the form of starch are stored to provide the glycolytic chain with substrates in the dark. Some others are used to derive lignins. The rest part of carbohydrates stays in a labile dissolved form and when Rubisco switches over to oxygenase function the flux of carbohydrate substrates via the Calvin cycle is directed to the photorespiratory loop which includes the glycolate cycle coupled with the Calvin cycle. Having passed the glycolate cycle, the carbon flux after resynthesis of G6P in the Calvin cycle returns to the carbohydrate pool.

In the oxygenase phase, carbon isotope effect emerges in the glycine decarboxylation reaction. $C0_2$ evolved in the course of decarboxylation is enriched in ^{13}C relative to the carbohydrate carbon accumulated in the carboxylase phase while the rest of the carbohydrate pool accumulates ^{13}C. Photorespiratory metabolites derived in the course of carbon flux spinning in the loop are enriched in ^{13}C too. And again the extent of ^{13}C enrichment of the metabolites depends on the degree of carbohydrate pool depletion.

Now let's see in detail how the isotopic pattern of G6P is formed in the Calvin cycle in the carboxylase and oxygenase phases.

The analysis of carbon isotope distribution of G6P in the carboxylase phase. The Calvin cycle operation in the carboxylase phase is well examined and described in many monographs on photosynthesis (Edwards & Walker, 1983). Using the scheme of the cycle (fig. 2), carbon isotope distribution in the cycle product — G6P can be described in the following way. Denoting atoms of $C0_2$ and RuBP acceptor entering the cycle, as shown on fig. 2, one can get material and isotopic balance equations for each atom of any cycle substrate (Ivlev, 2005) (see Appendix). It enables to evaluate carbon isotope distribution in G6P at each turn of the Calvin cycle in the carboxylase phase. The relation of isotope distribution depending on the number of cycle turns is given by simple recurrent equations taking into account the mixing of atoms in transketolase and transaldolase reactions and the specificity of enzymic interactions (Ivlev, 2005). Contrary to Tcherkez *et al.* (2004), we accepted the absence

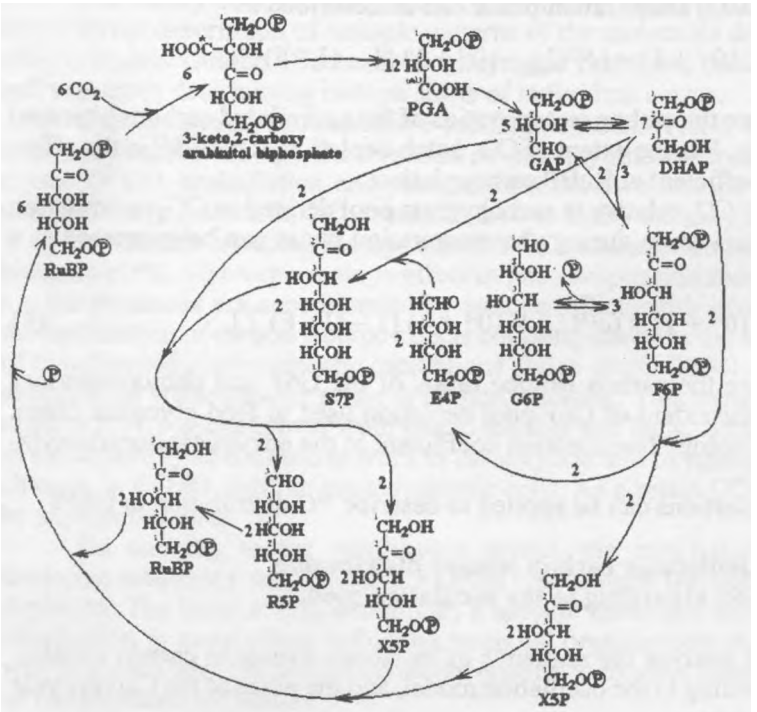

Fig. 2. Scheme of the Calvin cycle operation in the carboxylase phase showing the transfer and mixing of carbon atoms at each turn

of carbon isotope fractionation in the Calvin cycle since no direct indications of its presence were noticed. As to indirect facts including carbon isotope fractionation in the *in vitro* reactions they cannot be regarded as the arguments in favor of the same isotope fractionation *in vivo,* since it is impossible to simulate cell conditions *in vitro.* As shown before real cell conditions can change not only the value but even the sign of isotope effect observed *in vitro* (Ivlev *et al.,* 1996, 2004, Igamberdiev *et al.,* 2001).

The results of the calculation are presented in table 1. Since isotope effect in RuBP carboxylation arises at the entry of the Calvin cycle, isotope composition of each fixed carbon atom undergoes the same isotopic shift relative to the ambient $C0_2$ that is equal to the value of isotope discrimination. Bearing in mind the apparent absence of isotope fractionation in the cycle and rapid and effective mixing of carbon atoms in the cycle due to transketolase and transaldolase reactions one should assume that all the products of the cycle, including G6P, have uniform carbon isotope distributions in spite of the fact that RuBP molecules, serving as $C0_2$ acceptors, enter the cycle from outside and may be of heterogeneous isotope distribution (Ivlev, 1985). One can see from Table 1, that after the 20-th turn practically uniform carbon isotope distribution in all substrates is attained in spite of considerable initial discrepancies of $C0_2$ and RuBP adopted in the given example (-20‰ and -25‰ correspondingly). With the growth of number of cycle turnovers (the ratio of number $C0_2$ molecules to one RuBP molecule entering the cycle) the isotope ratio of each carbon atom in G6P approaches isotope ratio of the ambient $C0_2$ shifted on isotope discrimination value (-20‰). It should be noted that the final isotope distribution of G6P is practically independent of initial isotope distribution of RuBP molecules. Therefore we adopted that it should be uniform. It makes easier to trace isotopic shifts of atoms in cycle metabolites.

Table 1

Isotopic compositions of the carbon atoms in the G6P and RuBP, formed at the *n*-th turn in the Calvin cycle in the carboxylase phase of Rubisco functioning. Isotope discrimination $A^{13}C$ is taken equal to -20%*; the isotopic composition of the ambient CO_2 is 0‰. The isotopic composition of carbon atoms is expressed in per mille relative to the carbon of assimilated CO,

Number of Turns *n*		1		5		10		15		20	
Atom Position	Initial RuBP	G6P	RuBP	G6P	RuBP	G6P	RuBP	G6P	RuBP	G6P	RuBP
1	-25.0	-25.0	-25.0	-24.0	-22.9	-22.9	-21.2	-22.1	-20.5	-21.7	-20.2
2	-25.0	-25.0	-25.0	-24.0	-22.9	-22.9	-21.2	-22.1	-20.5	-21.7	-20.2
3	-25.0	-22.5	-22.5	-21.0	-20.1	-20.3	-20.0	-20.3	-20.0	-20.3	-20.0
4	-25.0	-22.5	-24.2	-21.0	-22.0	-20.3	-20.8	-20.3	-20.3	-20.3	-20.1
5	-25.0	-25.0	-24.2	-24.0	-22.0	-22.9	-20.8	-22.1	-20.3	-21.7	-20.1
6		-25.0		-24.0		-22.9		-22.1		-21.7	

Analysis of carbon isotope distribution of G6P in the oxygenase phase. G6P synthesized in the carboxylase phase supplies with carbon substrates both the glycolate cycle and the Calvin cycle composing a photorespiratory pathway (fig. 3). Isotope distribution of G6P was evaluated in the same manner as in the previous case. Taking into account the specificity of enzymic interactions, the mixing of carbon atoms in cycles as well as isotope fractionation in the glycine decarboxylase reaction of the glycolate cycle, material and isotopic balance equations were composed for each atom of the substrates taking part

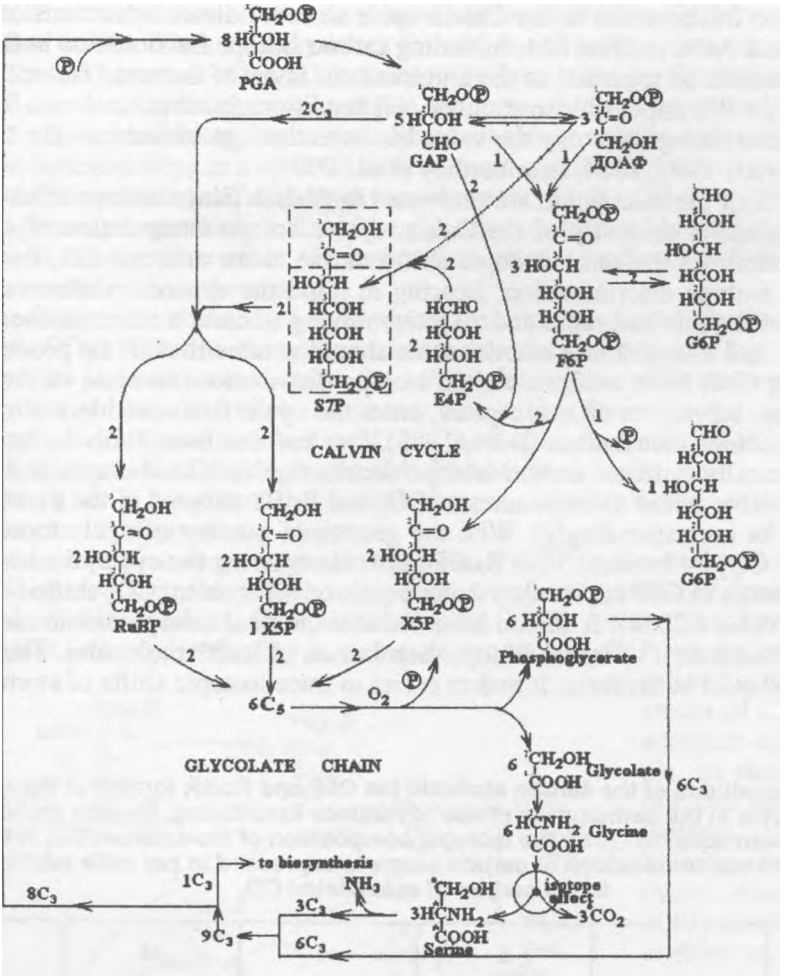

Fig. 3. Scheme of the Calvin cycle operation in the oxygenase phase showing the transfer and mixing of carbon atoms at each turn. Carbon isotope fractionation unit related to GDC reaction is denoted on the scheme by dotted line

in photorespiration (see Appendix). The relation of the carbon isotope composition of the substrate atoms with the increase of *n* turns of substrates spinning in the photorespiratory chain was described by the corresponding recurrent expressions. The result of the calculation is given in table 2.

The main difference in isotope distribution of G6P synthesized in the oxygenase phase from that synthesized in carboxylase phase is in the heterogeneity of isotope distribution of the first in respect to the uniform distribution of the latter. It is characterized by the ^{13}C enrichment of carbon atoms in position C-3 and C-4, by the reduction of the ^{13}C enrichment of the atoms in C-2 and C-5 positions and slight ^{12}C enrichment of the atoms in C-l and C-6 positions. The higher is the number of substrate flux turns *n* in photorespiratory chain, the more G6P carbon gets enriched in ^{13}C and the greater is the difference in carbon isotope composition of atoms in the mentioned positions.

Table 2

Isotopic compositions of the carbon atoms in the G6P and RuBP, formed at the n-th turn in the photorespiratory chain in the oxygenase phase of Rublsco functioning. Isotope composition of ambient G6P feeding glycolate chain was taken equal to -20%» with the uniform isotope distribution along carbon skeleton (from the above calculation). Carbon isotope fractionation coefficient in glycine decarboxylation reaction was taken equal to y = $^{n}k/^{a}k$ = 1.020. It was adopted that in the pool of G6P after carboxylase phase 100 molecule left. After each 10 turns of cycle the extent of pool depletion is 10%

Atom position	Ambient G6P	Number of turns *n*								
		10	20	30	40	50	60	70	80	90
1	-20.0	-20.3	-20.7	-21.1	-21.4	-21.9	-22.4	-23.0	-23.7	-24.6
2	-20.0	-19.7	-19.4	-19.1	-18.7	-18.3	-17.8	-17.2	-16.4	-15.2
3	-20.0	-19.5	-18.8	-18.1	-17.3	-16.4	-15.2	-13.8	-11.9	-8.8
4	-20.0	-19.5	-18.8	-18.1	-17.3	-16.4	-15.2	-13.8	-11.9	-8.8
5	-20.0	-19.7	-19.4	-19.1	-18.7	-18.3	-17.8	-17.2	-16.4	-15.2
6	-20.0	-20.0	-20.7	-21.1	-21.4	-21.9	-22.4	-23.0	-23.0	-24.6

The distinctions in isotope distributions of G6P produced in different phases of Rubisco operation are easily explained by the fact that in each turn of the Calvin cycle in the carboxylase phase, the initial isotope differences of $C0_2$ and RuBP substrates are eroded by newly coming batches of $C0_2$, whereas in oxygenase phase at each turn of substrate flux in photorespiration loop, carbon isotope effect of glycine decarboxylation is reproduced again and again.

>^{3}C distribution in starch glucose from storing organs of plants

It was the isotope distribution of glucose in starch of storing organs of some plants that was studied experimentally. For its interpretation one can use the above isotope distribution of G6P, since, as shown before (fig. 1), the latter inherits entirely isotope distribution of the former. Data of Abelson and Hoering (1961) enables to assume that polymerization/ depolymerization processes in starch formation and degradation into glucose are not followed by significant isotope effects. Hence ^{13}C distribution in starch glucose is determined by the distribution of G6P.

Isotopic patterns of starch glucose from storing organs of some plants were studied by different independent researchers (table 3). They appeared to be very close to each other. The specific feature of the intramolecular carbon isotope distribution is that carbon atoms of glucose in C-3 and C-4 positions are enriched in ^{13}C relative to the total carbon of the molecule and gradual depletion in ^{13}C takes place from the central atoms to the terminal ones.

Practically the same results were obtained in the experiments on fermentation of glucose and sucrose, where alcohol and carbon dioxide are formed as final products. One can see from fig. 4 that alcohol inherits carbon atoms from C-l, C-6, C-2 and C-5 positions of glucose while $C0_2$ inherits carbon atoms from C-3 and C-4 positions of glucose.

It provides the observed isotopic difference between glucose carbon and that of its products (table 4). In all cases $C0_2$ evolved in fermentation is proven to be enriched in ^{13}C as compared with ethanol carbon (table 4).

The observed pattern in its main features resembles the pattern of G6P produced in the oxygenase phase (table 3), but differs in some details. The ^{13}C enrichment of carbon

Table 3

Distribution of carbon isotopes in the glucose of starch from the storing organs of different *plants,* **isotopic shifts in glucose carbon atoms 5[11]C (%•) are given relatively to the whole glucose carbon. The values of 8[1J]C of glucose are given relative to the PDB standard. Data from the works of 'Galimov** *eta/.* **(1977); "Ivlev** *eta/.* **(1987); ~Rossman** *etal.* **(1991)**

Plant, organ	$\delta^{13}C$ of glucose	$\Delta^{13}C = \delta^{13}C_i - \delta^{13}C_{glucose}$, *i* — the number of the atom					
		$OCH_{(1)} - HC_{(2)}OH - OHC_{(3)}H - HC_{(4)}OH - HC_{(5)}OH - C_{(6)}H_2OH$					
		C_1	C_2	C_3	C_4	C_5	C_6
***Beta vulgaris* L., root	-25.2	-1.6	-0.4	+2.1	+6.3	-1.7	-5.1
***Zea mays* L., seed	-10.8	-1.7	-0.1	+1.1	+3.6	-0.2	-3.6
**Zea mays* L., seed	-12.5	-3.1		+1.9			-1.9
**Triticum aestivum* L., seed	-23.1	-7.1		+3.5			-7.1
**Solanum tuberosum* L., tuber	-24.9	-9.1		+4.5			-9.1
**Oryza sativa* L., seed	-26.1	-6.9		+3.5			-6.9
**Pisum sativum* L., seed	-24.9	-4.1		+2.1			-4.1

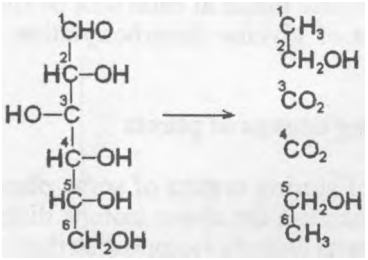

Fig. 4. Scheme illustrating carbon atoms inheritance in the glucose fermentation process

atoms in C-3 and C-4 positions is asymmetric. In C-4 position carbon atom is more enriched in ^{13}C than in position C-3. The slight asymmetry in ^{13}C enrichment was traced also in positions C-2 and C-5. Position C-2 found to be more enriched in ^{13}C. As to the observed difference in carbon isotope composition in C-l and C-6 positions, in two cases C-l atom prefers ^{13}C, in one case it is opposite.

Bearing in mind that model calculations for G6P derived in carboxylase phase bring about practically uniform carbon isotope distribution along the molecule skeleton, one should conclude that the observed isotope heterogeneity is associated with G6P derived in the oxygenase phase.

Table 4

Carbon isotope shifts of saccharides and their fermentation products. Numbering shows carbon atom source from parent glucose for ethanol and $C0_2$

Object	Isotopic shifts	Δ values	References
Sugar beet	Δ (glucose — ethanol)	2.0	Weber *et al.* (1997)
	Δ (glucose — CO_2)	-4.6	
Grape must	Δ (glucose — ethanol)	1.6	Scrimgeour *et al.* (1988)
	Δ (sucrose — CO_2)	-7.4	Rossmann *et al.* (1996)
Potato	Δ (glucose — ethanol)	1.6	Zhang *et al.* (1996)
Potato	Δ (starch — CO_2)	1.7	Rauschenbach *et al.* (1979)
Corn	Δ (starch — CO_2)	0.3	

As to the mentioned isotopic differences within the pairs of carbon atoms in G6P the following explanation is possible. The differences can emerge due to the intermolecular isotope exchange between corresponding pairs of atoms in PGA and DHAP (fig. 5) occurring in the reversible reactions of keto-enolic isomerization of trioses. It is not the isotope exchange between atoms of G6P and of trioses, as supposed by Gleixner and Schmidt (1997) and Tcherkez *et al.* (2004), but it is a result of isotope exchange between carbon atoms in positions 1, 2 and 3 of trioses in the course of reverse transformation.

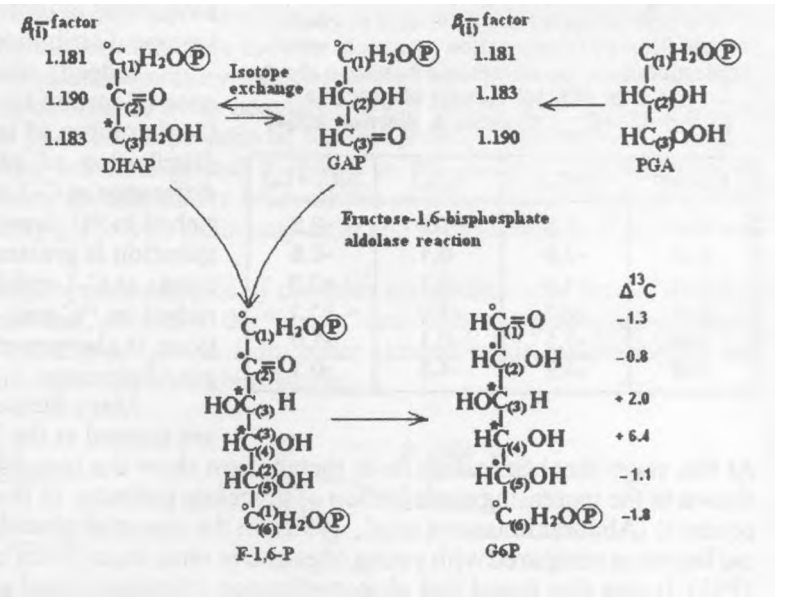

Fig. 5. Intermolecular carbon isotope exchange between trioses in the Calvin cycle. Light and dark dots and asterics denote the PGA and DHAP atoms involved in pairwise exchange; the positions in G6P to which these atoms are introduced during subsequent synthesis are also indicated. The column to the right of G6P shows the experimental values of isotope shifts for atoms relative to total carbon of the molecule

In case of absence of the exchange, the isotope composition within corresponding atoms should be identical. C-C bonds in these transformations are not cleaved. The assumption is supported by same signs of the isotopic shifts of carbon atoms observed in C-3 and C-4 positions and in C-2 and C-5 positions of G6P correspondingly and by the shifts that result from the assumption of isotope exchange between trioses. The latter was estimated by the calculation of theoretical equilibrium isotopic effects via β-factors (thermodynamic functions characterizing equilibrium isotope effects) (Ivlev, 2005). Since the isotope exchange is far from the equilibrium, the estimation can give only the trend in the isotope enrichment (the signs of the effects).

The above estimation gives the identical isotope composition of carbon atoms in C-l and C-6 positions. We have no explanation for the observed isotopic differences in these positions neither for ^{12}C, nor for ^{13}C enrichment. Having conflicting experimental results and the lack of data it is reasonable to get more information before the final assertion. It is also interesting to note that the analysis of radioactive label distribution in glucose for various plants (tobacco, sunflower, Chlorella) found in the gas exchange experiments with the use of $^{14}C0_2$discovered the identity in label activity in positions C-l and C-6 and very close correlation between the distribution of ^{14}C and natural distribution of ^{13}C in other positions (r^2 = 0.81- 0.87) (Hobbie & Werner, 2004).

More indirect evidence confirming the assumption that isotope heterogeneity of G6P is bound to photorespiration is the differences in carbon isotope ratio of skeleton atoms in G6P for C_3 and C_4 plants (table 5). It is known (Edwards & Walker, 1983) that C_4 plants,

Table 5

The comparison of carbon isotope distribution in starch glucose from C, and C_4 plants. The data were obtained in the experiments on glucose fermentation. Carbon isotope ratio of atoms at every position of fermentation products was calculated as the difference between them and that of total carbon of glucose ($\Delta_1 = \delta^{13}C$, - C_{total} (Hobbie & Werner, 2004)

Position	$\Delta(C_3)$	$\Delta(C_4)$	$\Delta(C_3 - C_4)$
C-1	-1.3	+0.9	-2.2
C-2	-0.9	-0.1	-0.8
C-3	+1.9	-0.7	+2.6
C-4	+6.3	+5.2	+1.1
C-5	-1.1	-0.1	-1.0
C-6	-4.9	-4.8	-0.1

opposite to C_3 plants, due to their anatomical peculiarities have a reduced photorespiration. Hence their carbohydrate pool should be mainly formed in the carboxylase phase and have close to uniform intramolecular carbon isotope distribution.

Indeed, one can see that the difference of carbon isotope ratio at each position (3-rd column of table 5) reproduces isotope distribution of photorespiratory G6P. The difference at C-3 and C-4 positions most enriched in ^{13}C shows that in C_3 plants photorespiration is greater than in C_4 plants. Carbon atoms at C-l and C-6 positions are most enriched in ^{12}C and less at C-2 and C-5 positions. It also proves the photorespiratory origin of glucose.

Many facts evidence for storage organs are formed at the late stages of ontogenesis. At this stage biochemical shifts in metabolism show the increase in photorespiration. It is shown in the increasing contribution of glycolate pathway, in the accumulation of oxidized products (Abdurakhmanova *et al.,* 1990), in the essential growth of the oxidase activity in old leaves as compared with young ones and in other traits given in the review (Igamberdiev, 1991). It was also found that photorespiration inhibitors retard aging (Rhodes, 1980).

These metabolic shifts correspond also to the observed ^{13}C accumulation in plant biomass with aging. Old leaves were found more enriched in ^{13}C as compared with young ones (Lerman *et al.,* 1974; Ivlev *et al.,* 2001). The same relation is confirmed by the ^{13}C gradient observed along the stem of wheat, by isotopic discrepancies of plant organs formed at different stages (Ivlev *et al.,* 2001). Strong reduction of ^{13}C discrimination was observed in wheat at the stage of ripening (Ivlev *et al.,* 2001). The same changes were found in bean (White, 1993) and cotton (Saranga, 1998). All the above facts allow to assert that the observed isotope heterogeneity of carbohydrate units of hexoses from starch of storage organs as well as their "heavy" carbon isotope composition, are bound to G6P produced in the oxygenase phase of Rubisco functioning.

Other cases of carbon isotope composition of different saccharides and their interpretation in the frames of oscillation hypothesis

The above data demonstrate the existence of two isotopically different carbon fluxes of metabolites, arising in the cell in carboxylase and oxygenase phases of Rubisco operation as a result of photosynthetic oscillations. The latter proves the presence of two isotopically different carbohydrate pools that feed these fluxes (Ivlev *et al.,* 2004). The question is: how two carbon fluxes passing through the Calvin cycle in carboxylase and oxygenase phases, where G6P is produced, can result in two isotopically different carbohydrate pools and do not mix? To answer the question one should bear in mind that the pools are active in different time. In carboxylase phase G6P formed in the Calvin cycle is immediately utilized (in the limits of $C0_2$ batch depletion) for synthesis of the reserved carbohydrate pool that feeds glycolytic chain in dark and for lignin synthesis. They are stored in a polymerized form and can't participate in the subsequent reactions of oxygenase phase.

The "light" pool, formed in the carboxylase phase, feeds the glycolytic chain and coupled synthesis of fatty acids and amino acids with substrates as well as lignin synthesis. That is the reason for their ^{12}C enrichment. It also provides substrates for dark respiration via pyruvate decarboxylation and the Krebs cycle operation (Ivlev, 1985).

In oxygenase phase labile G6P pool feeding photorespiratory loop gets enriched in ^{13}C at each turn of spinning carbon flux. It is used in the same manner to derive different pools of carbohydrates and photorespiratory substrates, such as serine, glycine, proline, oxalates, etc. The synthesis of organic acids occurring in the dark also uses carbohydrate pool derived in oxygenase phase. Isotope ratio of the metabolites depends on the extent of pool depletion when they are formed and it results in the isotopic discrepancies of them. Note that in some plants the alternative biosynthetic pathways of the above metabolites using carbon flux passing glycolytic chain may exist and their carbon isotope ratio will be other.

Here (fig. 6) we present some examples of complex metabolites one part of which is bound to the "light" substrate flux, another to the "heavy" one. They are plant glucosinolates studied by Schmidt *et al.* (1995), amigdalin from bitter almond seeds, glucosinalbin and choline ester of sinapic acid from white mustard leaves.

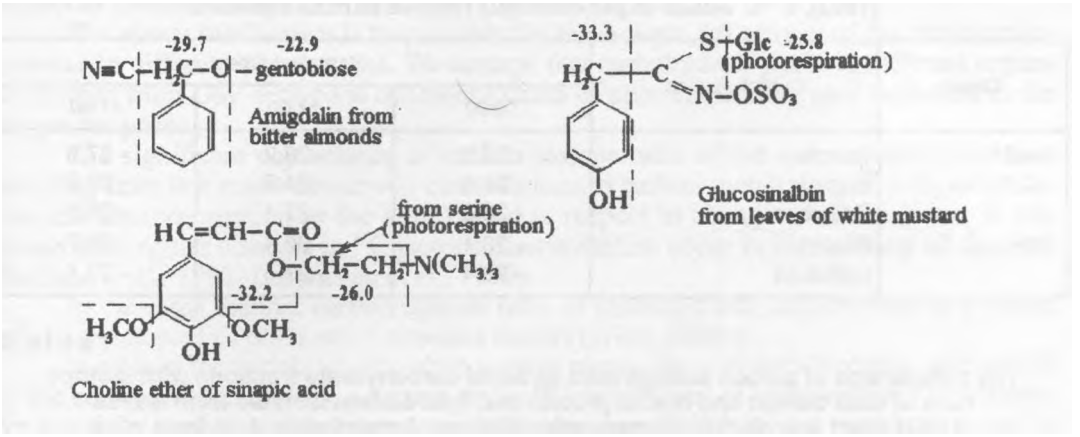

Fig. 6. Carbon isotope distribution in some plant glucosinolates. Figures nearby the molecules indicate $\delta^{13}C$ of the studied parts. Data on intramolecular carbon isotope ratio are taken from the paper of Schmidt *et al.* (1995)

The first two molecules have the two parts differing in the carbon isotope ratio. One of them is aglycon (non-carbohydrate part) synthesized via glycolytic chain and bound to the "light" carbohydrate pool formed in the carboxylase phase. Another part is of carbohydrate origin supposed to be synthesized in the oxygenase phase and enriched in ^{13}C as compared with previous one. The third glucosinolate (fig. 6) has no carbohydrate part, but has the fragment inheriting serine skeleton enriched in ^{13}C. It reflects that serine formation occurs in the oxygenase phase.

Let us see how isotope data on total carbon of carbohydrates correspond to the hypothesis. The experimental material presented in tables 6-9 draws to the following conclusions.

1. Among biochemical fractions, carbohydrates are most enriched in ^{13}C.
2. Individual carbohydrates are different in the carbon isotope ratio.

Table 6

Carbon isotope variations of some carbohydrate components isolated from leaves of C,-CAM plant *Clusia minor.* According to Borland *etal.* (1994). $6^{13}C$ Values in per mille (%•) relative to POB standard

Biomass fractions and components	Wet season		Dry season	
	Exposed leaf	Shaded leaf	Exposed leaf	Shaded leaf
Structural components	-26.6 + -27.2	-31.2 + -31.4	-27.1 + -27.5	-31.0 + -31.8
Lipids, pigments	-28.2 + -28.7	-32.2 + -33.3	-27.1 + -27.7	- 30.8
Amino acids	-30.2 + -31.7	-32. 0 + -32.6	-31.2 + -31.3	-32.4 + -32.7
Dissolved sugars	-21.2 + -23.6	-29.2 + -30.5	-17.9 + -20.4	-21.4 + -24.3
Glucans	-22.3 + -22.6	-27.6 + -27.5	-23.6 + -26.8	-23.6 + -26.8
Starch	-22.3 + -22.1	-27.2 + -27.8	-23.1 + -23.6	-26.6 + -27.9
Organic acids	-20.6 + -22.3	-27.6 + -27.7	-17.6 + -21.1	-21.8 + -24.5

Table 7

$6^{13}C$ values of different organs and substances of beet According to data Gleixner *et al.* (1993). 6 ^{13}C Values in per mille (%•) relative to PDB standard

Organ	Carbohydrates	Sampling time		
		10:00	*14:00*	*17:00*
Leaf	Starch	- 28.9	- 28.2	- 27.5
	Sugars	- 24.8	- 24.3	- 23.9
	Cellulose	- 26.8	- 27.4	- 26.8
Root	Sugars	- 26.1	- 26.4	- 25.7
	Cellulose	- 28.4	- 27.5	- 27.1

Table 8

The comparison of carbon isotope ratio of some carbohydrate fractions with isotope ratio of total carbon and that of protein and lipid carbon isolated from leaves of CAM plant *Bryophyllum daigremontiana* Berger. According to data from work (Deleens & Garnier-Dardart, 1977). 6 ^{13}C values in per mille (%•) relative to PDB standard

Fraction		Morning	Evening
Carbohydrates	Cellulose	-16.8+ -17.7	-16.2+ -16.8
	Starch	-13.3+ -13.8	-12.5+ -13.4
Proteins, total carbon		-22.1+ -22.6	-22.6+ -22.7
Lipids, total carbon		-25.8 + -27.3	-24.6 +-27.3

3. Carbohydrate pools from different plant organs have different carbon isotope composition.

4. Depending on sampling time the same carbohydrate fractions have different carbon isotope composition.

According to the above hypothesis, the ^{13}C enrichment of carbohydrates relative to the other fractions is explained by the fact that most of carbohydrate pools are synthesized in the oxygenase phase of Rubisco functioning.

Table 9

Carbon isotope composition of biomass fractions of marine alga *Chlorella stigmatophora* under different water salinity. According to Ivlev & Kalinkina (2001)

Index	Concentration of NaCl in the medium, mM		
	0	425	595
$\delta^{13}C$ of lipids, ‰	- 66.0	- 65.0	- 63.8
$\delta^{13}C$ of proteins, ‰	- 42.1	- 49.0	- 47.3
$\delta^{13}C$ of proline, ‰	- 29.0	-	- 30.5
$\delta^{13}C$ of labile sugars, ‰	- 30.0	-	- 31.5

$\delta^{13}C$ of ambient CO_2 = -21‰.

Discrepancies in the carbon isotope ratio of individual carbohydrates emerge in the oxygenase phase due to different saccharides emerge at different state of G6P pool depletion. Earlier the same mechanism of isotope discrepancies origin was proven in respect to metabolites synthesized in glycolysis (Ivlev, 2004a).

The above mechanism is responsible for the isotope differences of the same carbohydrates in different plant organs. We assume that carbohydrate pools in different organs are formed from G6P derived at different extents of photorespiratory pool depletion in the oxygenase phase.

The significant dependence of carbon isotope ratio of the same carbohydrates on sampling time is a result of varying contributions to carbon metabolism of $C0_2$ assimilation and photorespiration in the light period in respect to change of illumination. It was shown that regular changes in ^{13}C isotope discrimination occur in plant during all daytime (Borland *et al.,* 1993; Duranceau et al., 1999).

In the same manner carbon isotope ratio of carbohydrates and biomass as a whole reflects the impact of other environmental factors (Ivlev, 2004b)

In spite of the variability of carbon isotope composition of carbohydrates determined by the environmental factors, a certain stable ^{13}C distribution in carbohydrates is retained. For example, the structural components (cellulose, hemicellulose) are mostly enriched in ^{12}C, and have very close values of the carbon isotope ratio (it means they are derived practically at the same time), while the labile sugars, in opposite, are usually enriched in ^{13}C. Starch and glucans have intermediate carbon isotope composition. Sometimes this order is disturbed under the action of some environmental factors. In wet season in shaded leaves dissolved sugars turned to be sharply enriched in ^{12}C as well as starch carbon shows the same value in dry season in shaded leaves (Borland *et al.,* 1994). It means that both evolution and adaptation to the immediate environment determine ^{13}C distribution in carbohydrates by the change in the temporal sequence of the biosyntheses.

Carbon isotope ratios of carbohydrates are often compared with that of lignins since their formations have much in common (fig. 7). The lignin synthesis is coupled with the Calvin cycle where erythrose is derived. The erythrose interacts further with two molecules of pyruvate to form phenyl propanoic structure (C_6 - C_3). These structures undergo polymerization what, in turn, results in lignin formation. It was found that lignins independent of plant type (C_3 or C_4) are always enriched in ^{12}C relative to cellulose (table 10).

The reason for this difference should be searched in isotope effects accompanying their synthesis. The “light” carbon isotope composition of lignins, which is close to that of

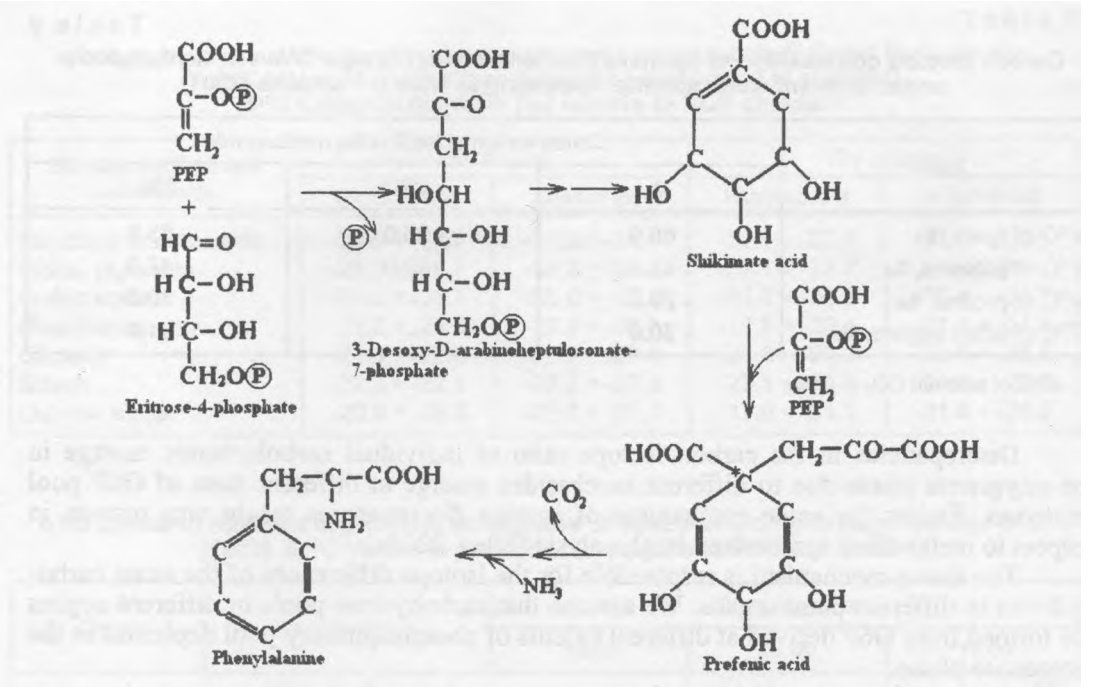

Fig. 7. Shikimate biosynthesis pathway and synthesis of phenylpyruvic acid which carbon skeleton is used for lignin biosynthesis in plants

lipids, enables to assume that the formation of erythrose occurs in the carboxylase phase of Rubisco operation. It is supported by the fact that two phosphoenolpyruvate (PEP) molecules joining the erythrose further belong to the carbohydrate flux arising in the carboxylase phase. Thus the stable difference between $5^{13}C$ of cellulose and lignins is explained by the fact that the first is derived in the carboxylase phase while the second in the oxygenase phase.

Table 10

The comparison of the difference in carbon isotope ratio (Д= $5^{13}C_{Ml)<JIQM}$ - $5^{13}C_{Ilgnln}$> of Ilgnin and cellulose for different C, and C_4 plant tissues. According to Werner & Hobbie (2004

The compared biological objects	C_3 ‰	C_4 ‰	Tissue type
Cellulose — lignin	3.5	-	Wood
Cellulose — lignin	4.1	6.2	Different tissues
Cellulose — lignin	4.6	5.1	Leaves
Cellulose — lignin	2.5	4.6	Roots

One can see from the table 10 that the difference between carbon isotope ratio of cellulose and lignins is greater for C_3 plants as compared with C_4. In our opinion, the reason for that is outside of isotope fractionation in carbohydrate metabolism and is associated with isotope fractionation occuring in the glycolytic chain in the dark. The key effect generating reaction in the processes is pyruvate decarboxylation. Carbon isotope ratio of PEP is determined by that of pyruvate (Ivlev, 2004b). This effect as well as the effect of glycine decarboxylation is combined with the effect of substrate pool depletion. In the cases where PEP is needed for lignin synthesis (assumed more in C_3 plants if compared with those in C_4 plants), the greater depletion of the pyruvate pool is observed. It means that

in the above cases the PEP used for the synthesis should be more enriched in ^{13}C and cause corresponding ^{13}C enrichment of lignins as a whole. That is the reason for the reduction of isotopic shifts in cellulose-1 ignin pairs in C_3 plants as compared with C_4.

This explanation is supported by fig. 8 where the correlation of the isotopic shifts between lignin and cellulose ($A = 5,^3C_{celhllosc}$ - o Cugnm) and lignin concentrations in the tissue for trees, C_4 grasses and C_3 herbaceous vegetation is presented (Hobbie & Werner, 2004). The more lignin concentration (the greater the extent of pyruvate pool depletion) is, the "heavier" lignins and less isotopic shifts between cellulose and lignins are (fig. 8). However we consider this explanation as preliminary one because of the lack of the experimental data.

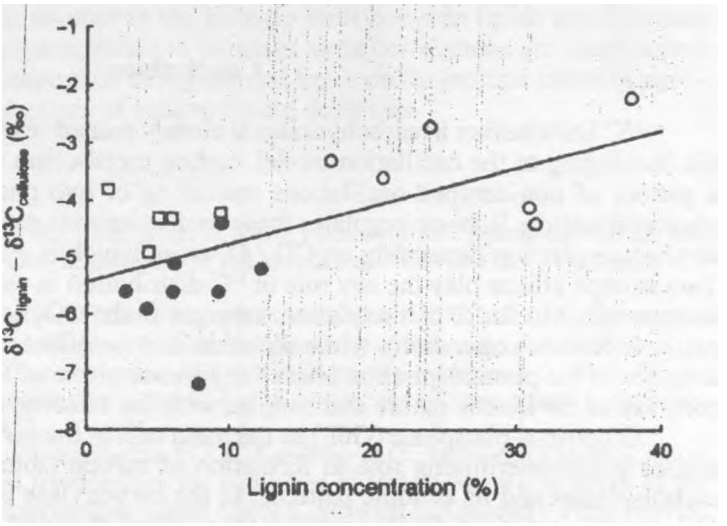

Fig. 8. The correlation of lignin concentration in biomass vs isotopic shift of lignin relative to cellulose from Hobbie & Werner (2004). Open circles, trees; filled circles, C_4 grasses; open squares, herbaceous vegetation

The explanation connecting carbon isotope ratio of lignins with the isotope fractionation in the glycolytic chain is indirectly confirmed by the similar type of the correlation of the isotopic shifts between lipids and bulk carbon and lipid concentration in biomass. Lipids synthesis is also bound to the "light" flux passing via glycolytic chain in the dark. Their carbon isotope ratio is determined by the isotope effect of pyruvate decarboxylation and depends on the extent of pyruvate pool depletion (Ivlev, 1985). On fig. 9 one can see

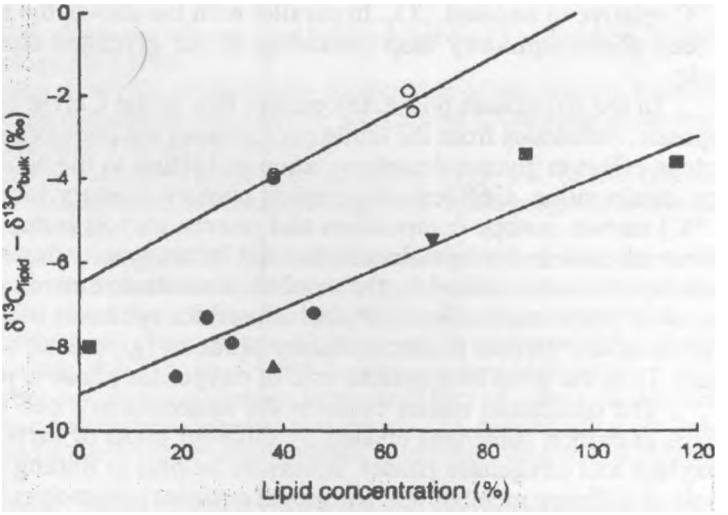

Fig. 9. The correlation of lipid concentration in biomass vs isotopic shift of lipids relative to bulk carbon from Hobbie & Werner (2004). Open circles, trees; filled circles, C_4 grasses; open squares, herbaceous vegetation; upward-pointing triangles, fern; downward-pointing triangles, shrubs

practically the same character of the correlations as on the fig. 8. One for tree, another for non-tree vegetation. The correlation was first disclosed by Park and Epstein (1961) and later was affirmed by Chikaraishi and Naraoka (2001). Both correlations have simple explanation. The same reason generates the same correlation.

Conclusions

^{13}C Distribution in carbohydrates is closely related to the mechanism of photosynthesis. According to the oscillation model, carbon metabolism in photosynthesis may follow a pattern of non-damped oscillations consisting of two phases — $C0_2$ assimilation and photorespiration. Rubisco regulates these oscillations via switching over from assimilation to photorespiration depending on $C0_2/ 0_2$ ratio, which is changed in the cell periodically. Two isotope effects play the key role in ^{13}C distribution in carbohydrates. One of them, the isotope effect in RuBP carboxylation, emerges in the $C0_2$ assimilation phase (carboxylase phase of Rubisco operation), while the other isotope effect in the glycine decarboxylation, emerges in the photorespiration phase (oxygenase phase of Rubisco functioning). Both effects are of the kinetic nature and coupled with the effect of substrate pool depletion.

Glucose-6-phosphate (G6P) as the main unit in the carbohydrate metabolism in both phases plays determining role in formation of carbon isotope composition of individual carbohydrates and its isotopic patterns. In the carboxylase phase, the main product of the Calvin cycle activity G6P, due to the isotope effect of RuBP carboxylation and randomization of atoms in transaldolase and transketolase reactions acquires “light” carbon isotope ratio (enriched in ^{12}C) and close to uniform carbon isotope distribution. The extent of ^{12}C enrichment depends on the extent of $C0_2$ batch depletion entered into the cell. The assimilated carbon is utilized to reserve carbohydrate pool which in the dark feeds the glycolytic chain and coupled synthesis most lipids, proteins, lignins, etc. All the products get enriched in ^{12}C relative to ambient $C0_2$. In parallel with the above, the labile pool of G6P is derived to feed photorespiratory loop consisting of the glycolate chain coupled with the Calvin cycle.

In the oxygenase phase, the carbon flux in the Calvin cycle changes its direction to opposite. Substrates from the labile pool passing via photorespiratory loop undergo carbon isotope effect in glycine decarboxylation and return to the pool with a different carbon isotope composition. G6P resynthesized in photorespiratory loop acquire “heavy” (enriched in ^{13}C) carbon isotope composition and uneven carbon isotope distribution. The extent of ^{13}C enrichment and intramolecular isotope heterogeneity depend on the extent of substrate pool depletion determined by the number of substrate turns in photorespiratory loop. In the course of photorespiration, G6P pool is used for synthesis of different carbohydrate pools, organic acids, various photorespiratory products (glycolate, oxalates, proline, serine, glycine). Thus the great biosynthetic role of oxygenase phase is predicted.

The oscillation model explains the existence in a cell of two isotopically different fluxes of carbon substrates created by different pools of carbohydrates derived in the carboxylase and oxygenase phases. It may be helpful in finding metabolic links between the pools of different metabolites. The model explains intramolecular isotopic patterns of starch glucose in storing organs and the difference in the intramolecular distributions of glucose in C_3 and C_4 plants. The isotope heterogeneity of some complex plant glucosinolates is explained by the participation in the synthesis of the particular parts of the molecules of two isotopically different carbohydrate pools derived in different phases of photosynthetic oscillations.

It is shown that the substrates for lignin synthesis emerge in the carboxylase phase of photosynthetic oscillations but their carbon isotope ratio depends also on the isotope effect in pyruvate decarboxylation. The model explains the same nature of the correlations, one of which is the isotopic shift between cellulose and lignins ($5^{13}C_{cellulose}$ - $8^{13}C_{Цр,к}$) and lignin concentration in biomass, another is the isotopic shift between lipids and biomass ($6^{13}C_{biomass}$ - $§^{13}C_{lipi<is}$) and lipid concentration in biomass. Both correlations are conditioned by the dependence of carbon isotope ratio of lignins and lipids on the isotope effect in pyruvate decarboxylation and on the extent of substrate pool depletion.

References

Abdurakhmanova Z.N., Aliev R.A., Abdullaev A. 1990. Photosynthetic metabolism of carbon and conversion of [1-^{14}C] glycolic acid in ontogeny of the cotton leaf// Soviet Plant Physiology 37: 513-518.

Abelson PH., Hoering T.C. 1961. Carbon isotope fractionation in formation of amino acids by photosynthetic organisms // Proceedings of the National Academy of Sciences USA 47: 623-631.

Borland A.M., Griffiths H., Broadmeadow Fordham M.C., Maxwell C. 1993. Short-term changes carbon isotope discrimination in the C_3-CAM intermediate Clusia minor L. growing in Trinidad // Oecologia 95: 444-453.

Borland A.M., Griffiths H,, Broadmeadow M.S. J., Fordham M.C., Maxwell C. 1994. Carbon isotope composition of biochemical fractions and the regulation of carbon balance in leaves of the Cj-Crassulacean acid metabolism intermediate Clusia minor L. growing in Trinidad // Plant Physiology 105:493-501.

Brugnoli E., Farquhar G.D. 2000. Photosynthetic fractionation of carbon isotopes In: Leegood RC, Sharkey TD and von Caemmerer eds. // Photosynthesis: Physiology and Metabolism, Kluwer, the Netherlands, 399-434.

Chikaraishi K, *Naraoka H.* 2001. Organic hydrogen — carbon isotope signature of terrestrial higher plants during biosynthesis for distinctive photosynthetic pathways // Geochemical Journal 35:451-458.

CraigH. 1954. Carbon-13 in plants and relationships between carbon-13 and carbon-14 variations in nature // Journal of Geology 62: 53-92.

Deleens E,, Garnier-Dardart J. 1977. Carbon isotope composition of biochemical fractions isolated from leaves of Bryophyllum daigremontianum Berger, a plant with CAM some physiological aspects related to $C0_2$ dark fixation // Planta 135: 241-248.

Duranceau M., Ghashghaie J., Badeck F., Deleens E., Comic G. 1999. $5^{13}C$ of $C0_2$ respired in the dark in relation $5^{IJ}C$ of leaf carbohydrates in Phaseolus vulgaris L. under progressive drought // Plant Cell & Environment 22: 515-523.

Duranceau M., Ghashghaie J., Brugnoli E. 2001. Carbon isotope discrimination during photosynthesis and dark respiration in intact leaves of *Nicotiana sylvestris* comparisons between wild type and mitochondrial mutant plants // Australian Journal of Plant Physiology 28: 65-71.

Edwards G., Walker D. 1983. C_3, C<: Mechanisms, Cellular, and Environmental Regulation of Photosynthesis. Blackwell Scientific Publications, Oxford, London, Boston, Melbourne.

Farquhar G.D. 1980. Carbon isotope discrimination by plants: Effect of carbon dioxide concentration and temperature via the ratio of intercellular and atmospheric CO, concentration. In: Pearman G.I. (ed.) Carbon Dioxide and Climate: Australian Research. Australian Academy of Science, Canberra, 105-111.

Farquhar G.D., O'Leary M.H., Berry J.A. 1982. On the relationship between carbon isotope discrimination and intercellular carbon dioxide concentration in leaves // Australian Journal of Plant Physiology 9: 121-137.

Galimov E.M., Kodina L.A., Generalova V.N., Bogachova M. V. 1977. A study of carbon isotope distribution in biogenic compounds. In: A.V. Sidorenko, ed. The 8th International Congress on Organic Geochemistry. Abstracts, Vol. 2, Moscow, 156.

Ghashghaie J,, Badeck F.-W, Lanigan G.. Nogues S., Tcherkez G., Deleens E., Comic G., Griffiths H. 2003. Carbon isotope fractionation during dark respiration and photorespiration in C_3-plants. Phytochemistry Reviews 2: 145-161.

Gillon J.S., Griffiths H. 1997. The influence of (photo) respiration on carbon isotope discrimination in plants // Plant, Cell & Environment 20: 1217-1230.

Gleixner G., Schmidt H.-L. 1997. Carbon isotope effects on fructose-1,6-bisphosphate aldolase reaction, origin for non-statistical ^{13}C distribution in carbohydrates // Journal of Biological Chemistry 272: 5382-5387.

Goyal A., Tolbert N.E. 1996. Association of glycolate oxidation with photosynthetic electron transport in plant and algal chloroplasts. Proceedings of the National Academy of Sciences USA 93: 3319-3324

Hobbie E.A., Werner R.A. 2004. Intramolecular, compound-specific, and bulk carbon isotope patterns in C_3 and C< plants: a review and synthesis // New Phytologist 161: 371-385.

Igamberdiev A.U. 1991. Peroxisomal oxidation in plants // Soviet Plant Physiology 38: 569-579.

Igamberdiev A.U., Ivlev A.A., Bykova N. V., Threlkeld C.N., Lea P.J., Gardestrom P. 2001. Decarboxylation of glycine contributes to carbon isotope fractionation in photosynthetic organisms // Photosynthesis Research 67: 177-184.

Igamberdiev A.U., Mikkelsen T.N., Ambus P., Bauwe H., LeaP.J., Gardestrom P. 2004. Photorespiration contributes to stomatal regulation and carbon isotope fractionation: a study with barley, potato and Arabidopsis plants deficient in glycine decarboxylase // Photosynthesis Research 8i: 139-152.

Ivanov M. V., Zyakun A.M., Gogotova G.I., Bondar' V.A. 1978. Fractionation of carbon isotopes by photosynthesizing bacteria grown on bicarbonate enriched in carbon-13. Doklady Akademii Nauk SSSR 242: 1417-1420.

Ivlev A.A. 1985. The nature of carbon isotope effects in the living cell // Biofizika 30: 506-516.

Ivlev A.A. 1989. On discreteness of $C0_2$ assimilation by C_3 plants in the light // Biofizika 34: 887-891.

Ivlev A.A. 1992. Carbon isotope effects and coupled mechanism of photosynthesis and photorespiration // Soviet Plant Physiology 39: 545-552.

Ivlev A.A. 1993. On the flows of light and heavy carbon during photosynthesis and photorespiration coupling // Russian Journal of Plant Physiology 40: 752-758.

Ivlev A.A. 2002. Carbon isotope ($^{13}C/^{12}C$) effect of photorespiration in photosynthesizing organisms. Evidence in favor of the existence and a possible mechanism // Biofizika 47: 55-70.

Ivlev A.A. 2003. Carbon isotope effect as a tool to study photosynthesis // Chemical Probes in Biology, ed. Schneider MP. Kluwer Academic Publishers, Netherlands, p.269-285.

Ivlev A.A. 2004a. Contribution of photorespiration to changes of carbon isotope characteristics in plants affected by stress factors // Russian Journal of Plant Physiology 51: 271-280.

Ivlev A.A. 2004b. Intramolecular isotope distribution of metabolites in the glycolytic chain// Biofizika 49: 436-452.

Ivlev A.A. 2005. The isotope effect in the glycine dehydrogenase reaction is the cause of the intramolecular isotope inhomogeneity of glucose carbon of starch synthesized during photorespiration//Biofizika 50: 1079-1087.

Ivlev A.A. 2008. Isotope fractionation and cell mechanisms of carbon metabolism in photosynthesizing cell. Moscow: RGAY-MCKhA of Timiryazev. 74 p.

IvlevA.A., Bykova N.V., Igamberdiev A.U. 1996. Fractionation of carbon ($^{13}C/^{12}C$) isotopes in glycine decarboxylase reaction. FEBS Letters 386: 174-176.

IvlevA.A., Kalinkina L.G. 2001. Experimental evidence for the isotope effect in photorespiration // Russian Journal of Plant Physiology 48: 400-412.

IvlevA.A., Pichuzhkin V.I., Knyazev D.A. 2001. Developmental changes in the carbon isotope composition of wheat organs in relation to photorespiration // Russian Journal of Plant Physiology 46:443-451.

Ivlev A. A., Igamberdiev A.U, Dubinsky A. Yu. 2004. Isotopic composition of carbon metabolites and metabolic oscillations in the course of photosynthesis // Biofizika 49 (Suppl. 1): 3-16.

Kalinkina L.G., Udelnova T.M. 1990. Effect of photorespiration on fractionation of stable isotopes of carbon in a marine Chlorella // Soviet Plant Physiology 37: 72-78.

Kalinkina L.G., Udelnova T.M. 1991. Mechanism of involvement of the glycolate pathway in accumulation of free proline in a marine chlorella under conditions of salinity // Soviet Plant Physiology 38: 687-696.

Kukushkin A.K., Soldatova E.A. 1996. The effect of photorespiration on the induction of delayed millisecond luminescence of higher plants photosystem II. Theoretical investigation // Biofizika 41: 440-444.

Lerman J.C., Deleens E., NatoA., MoyseA. 1974. Variations in the carbon isotope composition of a plant with Crassulacean Acid Metabolism // Plant Physiology 53: 581-584.

Lin G.H., Ehleringer J.R. 1997. Carbon isotope fractionation does not occur during dark respiration in C_3 and C_4 // Plant Physiology 114: 391-394.

O 'Leary M.H. 1981. Carbon isotope fractionation in plants // Phytochemistry 20: 553-567.

O'Leary M.H. 1984. Measurement of the isotope fractionation associated with diffusion of carbon dioxide in aqueous solution // Journal of Physical Chemistry 88: 823-825.

ParkR., Epstein S. 1961. Metabolic fractionation of $^{13}C/^{12}C$ in plants //Plant Physiology 36: 133-139.

Rauschenbach P., Simon H., Stickler W., Moser H. 1979. Comparison of the deuterium and carbon-13 contents of ethanol obtained by fermentation and chemical synthesis // Zeitschrift filr Naturforschung 34c: 1-4.

Raven J.A., Griffiths H., Glidewell S.M., Preston T. 1982. The mechanism oxalate biosynthesis in higher plants: investigations with the stable isotopes oxygen-18 and carbon-13. Proceedings of the Royal Society (London), Series B. 216: 87 -101.

Rivera E.R., Smith B.N. 1979. Crystal morphology and ^{13}Carbon/^{12}Carbon composition of solid oxalate in Cacti // Plant Physiology 64: 966-970.

Rhodes MJ.C. 1980. In: Biochemistry of Plants. A Comprehensive Treatise. V.2. StumpfPK, Conn EE, eds., N.Y., Academic Press, 419-434.

Roeske C.A., O'Leary M.H. 1984. Carbon isotope effect on enzyme catalysed carboxylation of ribulose bisphosphate // Biochemistry 23: 6275-6284.

Rooney M.A. 1988. Short-term Carbon Isotope Fractionation in Plants. Ph.D. Thesis, University of Wisconsin, Madison, USA.

Rossmann A., Schmidt H-L., Reniero F., Verzini G., Moussa I., Merle M.H. 1996. Stable carbon isotope contents in ethanol of EC data bank wines from Italy, France and Germany. Zeitschrift fiir Lebensmitteluntersuchung und Forschung A 203: 293-301.

Rossmann A., Butzenlechner M., Schmidt H-L. 1991. Evidence for non-statistical carbon isotope distribution in natural glucose // Plant Physiology 96: 609-614.

Roussel M.R., Ivlev A. A., Igamberdiev A. U. 2007. Oscillations of the internal $C0_2$ concentration in tobacco leaves transferred to low $C0_2$ // Journal of Plant Physiology. 164: 1182-1196.

Sanadze G.A., BlackC.C., TevzadzeI.T., Tarkhnishvili G.M. 1978. A change in the ^CO^COj isotope ratio during photosynthesis by C_3 and C_4-plants // Soviet Plant Physiology 25: 171-172.

Saranga Y., Flash I., Yakir D. 1998. Carbon isotope ratio in cotton varies with growth stage and plant organ // Crop Science 38: 782-787.

Satoh K., Katoh S. 1983. Induction kinetics of millisecond-delayed luminescence in intact Bryopsis chloroplasts // Plant and Cell Physiology 24: 953-962

Schmidt H-L. 2003. Fundamentals and systematics of the non-statistical distributions of isotopes in natural compounds // Naturwissenschaften 90: 537-552.

Schmidt H-L., KexelH., Butzenlechner M., Schwarz S., Gleixner G,, ThimetS., Werner R.A., Gensler M. 1995. Non-statistical isotope distribution in natural compounds: mirror of their biosynthesis and key for their origin assignment. In Stable Isotopes in the Biosphere Wada E, Yoneyama T, Minagawa M, Ando T & Fry BD, eds., Kyoto University Press, Kyoto, 17-35.

Scrimgeour C.M., Bennet W.M., Connacher A.A. 1988. A convenient method of screening glucose for ^{13}C: ^{12}C ratio for use in stable isotope tracer studies // Biomedical and Environmental Mass Spectrometry 17: 265-266.

Tcherkez G., Farquhar G.D., Badeck F.-W., Ghashghaie J. 2004. Theoretical consideration about carbon isotope distribution in glucose of C_3 plants // Functional Plant Biology 131: 857-877.

Vogel J.C. 1993. Variability of carbon isotope fractionation. In: Stable Isotopes and Plant Carbon — Water Relations. Ehleringer JR, Hall AE, Farquhar GD (Eds), Academic Press, San Diego, 29-45.

Vogel J. C. 1980. Fractionation of carbon isotopes during photosynthesis. In: Sitzungsberichte der Heidelberger Akademie der Wissenschaften, Mathematisch-naturwissenschaftliche Klasse Jahrgang. 3 Abhandlung. Springer-Verlag, Berlin, 111-135.

Von Caemmerer S., Evans J.R. 1991. Determination of the average partial pressure of $C0_2$ in chloroplasts from leaves of several C_3 species // Australian Journal of Plant Physiology 18: 287-305.

Voznesenskii V.L., Glagoleva T.A., Zubkova E.K., Mamushina N.S., Filippova L.A., Chulanovskaya M. V. 1982. Metabolism of ^{11}C during prolonged rearing of Chlorella in the presence of ^{14}C-$C0_2$*11* Soviet Plant Physiology 29: 444-450.

Zhang B.I., Emeriau L., Martin G.J. 1991. Comparison of the isotopic behaviour of Leguminosae constituents. Characterization of lentils// Sciences des Aliments 11: 291-304.

White J.W. 1993. Implications of carbon isotope discrimination studies for breeding common bean under water deficits. In: Stable Isotopes and Plant Carbon — Water Relations. Ehleringer JR, Hall AE, Farquhar GD (editors), Academic Press, San Diego, 387-398.

Weber D., Kexel H., Schmidt L-H. 1997.^{13}C-pattem of natural glycerol: origin and practical importance // Journal of Agricultural and Food Chemistry 45: 2042-2046.

Appendix

Computing algorithm of carbon isotope distribution in G6P (glucose) derived in the Calvin cycle in the carboxylase phase of Rubisco functioning (fig.2).

Carbon isotopic patterns of the cycle's substrates are calculated using recurrent formulas allowing determination of the following values based on the previous ones. Let's take the initial carbon isotope distribution of ribulose-l,5-bisphosphate as follows:

RuBP (1), RuBP (2), RuBP (3), RuBP (4), RuBP (5)

By fixing $C0_2$ it splits into two molecules of PGA with the following isotope distribution

PGA (1) = 1/2 * (RuBP (1) + RuBP (5))

PGA (2) = 1/2 * (RuBP (2) + RuBP (4))

PGA (3) = 1/2 * (RuBP (3) + 5COj)

where $8C0_2$ is a carbon isotope ratio of the fixed carbon.

Then in the course of cycle functioning six molecules of FBP with the following carbon isotope distribution are formed:

FBP (1) = FBP (6) =PGA (1); FBP (2) = FBP (5) =PGA (2); FBP (3) = FBP (4) =PGA (3);

These F6P molecules turn into G6P molecules, enter the pool mixing there with the other molecules of the pool thus averaging carbon isotope distribution.

G6P (i) = (FBP (i) +G6P (i) *N_0) / (N_0 + 1)

where N_G is a number of glucose molecules in the pool. Next the "averaged" glucose moves transforming into RuBP.

RuBP (1) = PGA (1); RuBP (2) = PGA (2); RuBP (3) = PGA (3);

RuBP (4) = (PGA (3) +2*PGA (2)) /3; RuBP (5) = (PGA (3) +2*PGA (1)) /3;

Then the cycle is repeated.

Computing algorithm of carbon isotope distribution in G6P (glucose) derived in the Calvin cycle in the oxygenase phase of Rubisco functioning (Fig.3)

RuBP splits into PGA and glycolate, so that

PGA, (1) = RuBP (1), PGA, (2) = RuBP (2), PGA,(3) = RuBP (3).

The digits in parethesis denote carbon atoms numbers in the corresponding molecules

The molecule of the glycolate cycle — glycine, derives from RuBP, so that

Gly(l)= RuBP (4), Gly(2)= RuBP (5).

Denoting q, = Gly(l), q_2 = Gly(2), q_0 = 1 — q, — q_2, and y = k/k,,

where y is kinetic isotopic effect, к is the rate constant of glycine decarboxylase reaction in case of ^{12}C-^{12}C bond cracking, k, is the rate constant of glycine decarboxylase reaction in case of ^{12}C-^{13}C bond cracking, carbon isotope composition of PGA atoms after splitting of $C0_2$ will be:

PGA_b (1) = q_2 / (yq_0 + q, + q_2), PGA,, (2) = q, (2 (yq_0 + q, + qj -1) / (yq_0 + q, + q_2),

PGA_b (3) = q_2 (2 (yq_0 + q, + q^) -1) / (yq_0 + q, + q,)

Then PGA, and PGA_b are mixed: PGA (i) = (2*PGA, (i) + PGA_b (i)) /3

Then FBP molecules are derived just as in the Calvin cycle

FBP (1) = FBP (6) =PGA (1); FBP (2) = FBP (5) =PGA (2); FBP (3) = FBP (4) =PGA (3);

Then FBP molecule is mixed up with two G6P molecules

FBP (i) = (FBP (i) +2G6P (i)) /3

One of three produced FBP molecules transforming into G6P returned back into the G6P pool averaging carbon isotope distribution of the pool molecules:

G6P (i) = (FBP (i) + G6P (i) (N_G -2)) / (N_c -1)

One triose phosphate molecule and a piece of FBP molecule (C-3 and C-4 atoms) via erithrose phosphate formation are used to derive sedoheptulose-7-phosphate (S7P), so that

S7P (1) = PGA (1); S7P (2) = PGA (2); S7P (3) = PGA (3); S7P (4) = FBP (4); S7P (5) = FBP (3); S7P (6) = FBP (2); S7P (7) = FBP (1);

The rest part of FBP molecule (C-l and C-2 atoms) and triose phosphate molecule form xylulose phosphate molecule (XP J with carbon isotope distribution such as

XP, (1) = PGA (1); XP, (2) = PGA (2); XP, (3) = PGA (3); XP, (4) = PGA (2); XP, (5) = PGA(l);

Two first atoms of sedoheptulose-7- phosphate molecule and triose phosphate molecule form one more xylulose phosphate molecule (XP_b) with carbon isotope distribution analogous to the first (XP,).

The rest five atoms are used to form RuBP.

RuBP (1) = PGA (3); RuBP (2) = PGA (3); RuBP (3) = PGA (3); RuBP (4) = PGA (2); RuBP(5) = PGA (1);

Finally, all three molecules are mixed up giving an averaged molecule with the isotope distribution such as

RuBP (i) = (RuBP (i) + XPa (i) + XPb (i)) /3

Then the cycle is repeated.

Аннотация. Согласно осцилляционной гипотезе, углеродный метаболизм при фотосинтезе реализуется в виде колебательного процесса. Колебания являются незатухающими и состоят из двух фаз - ассимиляции $C0_2$ и фотодыхания. Первая соответствует карбоксилазной фазе функционирования Рубиско, вторая - оксигеназной. Распределение изотопа ^{13}C в углеводах тесно связано с фотосинтетическими осцилляциями, возникающими при фотосинтезе. Углеводы образуются в каждой из фаз. Мы утверждаем, что углеводы, образующиеся в карбоксилазной фазе, состоят из гексоз с относительно равномерным распределением ^{13}C вдоль углеродного скелета молекул, тогда как гексозы в углеводах, синтезированных в оксигеназной фазе, обладают специфическим неравномерным изотопным рисунком. Упомянутая гипотеза хорошо объясняет особенности распределения изотопов углерода и изотопный состав углеводов C_3 и C_4-растений, а также изотопные различия общего углерода углеводов, обусловленные разной степенью исчерпывания фонда субстрата, используемого при фотодыхании.