ISOTOPIC DATA SHOW: CARBON METABOLISM IN A PHOTOSYNTHESIZING CELL IS AN OSCILLATING PROCESS

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The hypophesis on photosynthetic oscillations

The analysis of the literature on photosynthesis shows that practically all the researchers who take up with carbon metabolism consider photosynthesis to be a steady-state process where all the including reactions proceed in the light continuously and simultaneously in a steady-state regime. In this way some endeavors to describe photosynthesis were undertaken [1-3]. In the present work basing on carbon isotopic data ($^{13}C/^{12}C$) wc argue the assertion that photosynthesis is the oscillating process consisting of two phases: $C0_2$ assimilation and photorespiration. The phases correspond to two different carbon fluxes moving in Calvin cycle. One is the $C0_2$ transformation into glucose-$_6$-phosphate (G_6P) in the assimilation phase, the other is reverse oxidation of G_6P into $C0_2$ in the photorespiration phase. It is the key photosynthetic enzyme Rubisco (ribulose-bisphosphate carboxylase/oxygenase) that regulates the switches in carbon substrate movement due to its double ability to function as carboxylase either oxygenase depending on the CO_2 / O_2 concentration ratio that periodically changes in the cell [4].

The discovery of carbon isotope effect in photorespiration having opposite sign in respect to that in photosynthetic assimilation [5] in 1993 was the first step to prove the oscillating nature of the photosynthesis. So far it was widely adopted that photosynthesis is the effect resulting in ^{12}C-enrichment of the assimilated carbon (biomass) relative to the atmospheric $C0_2$. The phenomenon was called ^{13}C isotope discrimination. Some data that were inconsistent with this hypothesis were regarded as artifacts. They include ^{13}C accumulation in primary assimilates [6], the advantageous assimilation of ^{13}C (not ^{12}C) in gas exchange chambers [7]. The cases have been usually observed when $C0_2$ assimilated was enriched in ^{13}C. In addition the gradual enrichment of the nutrient $C0_2$ in ^{13}C relative to the natural values first decreases ^{13}C discrimination, then results in the isotope effect sign inversion and the effect of ^{12}C discrimination begins to grow. The "heavy" isotope C has been predominantly accumulated in biomass [8]. These facts combined with the impact of such environmental factors like salinity, water availability, light intensity, $C0_2/0_2$ ratio, etc., on isotope ratio of biomass drew us to the conclusion on the existence of the unknown carbon isotope effect of photosynthesis additional to the known effect of $C0_2$ assimilation. The effect was supposed to be bound to photorespiration. Isotope effect of $C0_2$ assimilation, being stronger, concealed photorespiration effect and that is why the latter wasn't disclosed so long. Bearing this in mind we have analyzed the glycolate photorespiration pathway in search of possible points where carbon isotope fractionation could be. The analysis enabled us to conclude that the key point of carbon isotope fractionation in photorespiration is the enzymatic glycine decarboxylation [5].

In accordance with the assumption, in cooperation with prof. Igamberdiev we began to study the reaction *in vitro,* and found the above reaction, indeed, resulted in carbon isotope fractionation. However the observed isotope effects turned out to be of the opposite signs [9,10]. To clarify the real situation in a living cell the carbon isotope fractionation in wild type barley and that in GDC-deficient barley were examined. The mutant bar-

ley plants were obtained by introducing into plastids deficient glycine decarboxylase complex with the reduced function of the enzyme using gene engineering technique [11]. The comparison of two types of plants showed that *in vivo* all mutant plants proved to be enriched in ^{12}C. It meant that carbon isotope effect in photorespiration is of opposite sign relative to that in $C0_2$ assimilation (Tab.I)[1]. The same results were obtained on *Arabidopsis thaliana* and *Solanium tuberosum* (potato) [13].

Table 1

Carbon isotope ratio of barley leaf in wild plant and in GDC-mutant [11]. $\delta^{13}C$ Values are given relative to PDB standard, n is the number of experiments

Plant	Age	$\delta^{13}C$‰
Barley, wild type	1 week	-39.0±0.7 (n=4)
	6 weeks	-31.3±0.6 (n=2)
Barley, GDC-mutant	1 week	-45.5±0.6 (n=5)
	6 weeks	-37.5±0.3 (n=4)

[1] It is adopted to express carbon isotope composition of samples via differential notation: $\delta^{13}C = \frac{(\frac{^{13}C}{^{12}C})_{sample} - (\frac{^{13}C}{^{12}C})_{standard}}{(\frac{^{13}C}{^{12}C})_{standard}} 10^3 \cdot$ The ratio of isotopic concentrations in a sample is normalized to that in a standard. The PDB standard is carbon dioxide obtained from belemnite limestone (Pee Dee formation in South Carolina). It has the $^{13}C/C^{12}$ value of $1.1237 \cdot 10^{-5}$[12]

Thus the predicted point of carbon isotope effect emergence in photorespiration and its sign were proved experimentally.

Why the imagination on two isotope effects in photosynthesis having opposite signs proves the oscillating nature of the process? To answer the question, let's see first the way how the periodic change in CO_2/O_2 concentration ratio switches over the Rubisco function from carboxylase to oxygenase one and back.

The probable switching mechanism bases on the assumption that the rates of enzymic reactions including RuBP carboxylation and glycine decarboxylation are much greater than the rate of $C0_2$ diffusion from the atmosphere into a cell and back. The low activation energy of mesophyll cells conductance in respect to $C0_2$ (10.2 - 12.5 kJ/mole at 25°C [14]) showed that rate-limiting stage in CC_2 assimilation is diffusion. It supports the assumption and enables to describe the switching mechanism as follows [15].

Let us imagine that at certain moment (point 1 in Fig.l) corresponding to the carboxylase phase of Rubisco operation (when the $C0_2$ concentration in the cell is equal to the $C0_2$ concentration in the medium) carbon dioxide enters the cell due to the concentration gradient between medium and the sites of carboxylation, generated by CO_2 fixation at the active site of the enzyme. Since the rate of carboxylation is higher than the rate of diffusion, the $C0_2$ concentration (more precisely the $C0_2/0_2$ ratio, because the 0_2 concentration is assumed to be constant) drops to the critical level (point 2 in Fig.l) which corresponds to the switching of Rubisco to the oxygenase function. At these steps (1 - 2), $C0_2$ diffusion elevates its concentration in the cell, while carboxylation decreases it, i.e. their influences are opposite (shown by arrows in Fig.l). We assume that in order to avoid the wasteful recirculation of substrates, the switching of Rubisco should occur rapidly, in a trigger-like regime. After switching to the oxygenase phase of Rubisco, the $C0_2$ concentration in the cell starts to rise (according to the model assumption), but in this case the diffusion rate is slower than the rate of the GDC reaction. It reaches the $C0_2$ level in the medium (point 3 in Fig.l) and then exceeds it. At this time interval, corresponding to the interval 2-3 on the curve describing concentration changes, the GDC reaction and the diffusion act in the same direction by increasing the $C0_2$ concentration in the cell. At the time interval 3-4, the $C0_2$ concentration increases until it reaches the upper critical level corresponding to switching of the enzyme to the carboxylase function (point 4 in Fig.l). At this moment the impact of diffusion and glycine decarboxylation

reaction on $C0_2$ concentration in the cell are opposite. Diffusion causes $C0_2$ to escape from the cell into the medium, decreasing its concentration, while the GDC reaction causes $C0_2$ accumulation in the cell. This proceeds until the $C0_2$ concentration in the cell becomes equal to that in the medium (point 1' in Fig.l). It follows from the mechanism that photorespiration contributing to the $C0_2$ removal from the cell occupies only a part of the oxygenase phase (3-4) and a part of carboxylase phase (4-1') when $C0_2$ concentration in the cell is higher than that in the medium.

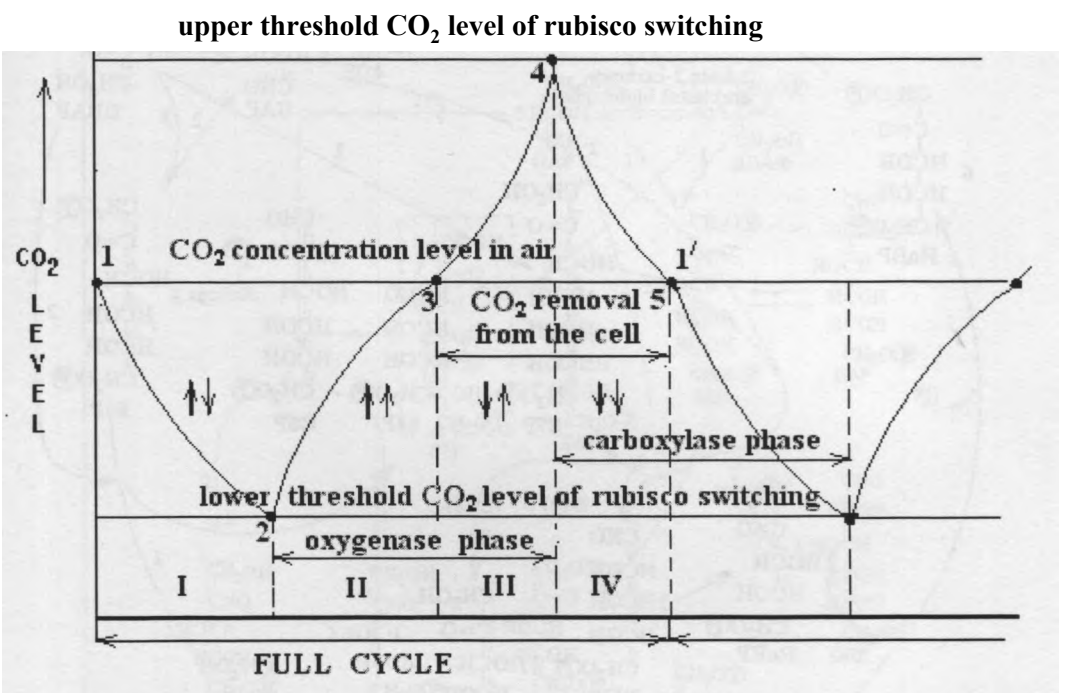

Fig. 1. The hypothetical mechanism of Rubisco switchings from carboxylase phase to oxygenase one depending on CO_2/O_2 concentration ratio

In the above mechanism the critical level concentration points 2 and 4 corresponding to the change in Rubisco function play the special role. Essentially they determine the carboxylase and oxygenase phase duration which in turn defines their contribution to carbon metabolism of photosynthesis. The critical $C0_2/0_2$ concentration ratios depend on some factors, including external ones, which bound to adaptive ability of the photosynthesizing organism.

Thus the sequence of metabolic events in photosynthesis is the following.

The operation of the Calvin cycle in the carboxylase phase is well known and according to the generally accepted scheme is shown on Fig. 2. Because of carbon isotope fractionation in RuBP carboxylation proceeding at the Calvin cycle entry, the G6P and other substrates produced in the cycle get enriched in ^{12}C relative to the environmental $C0_2$ by the value of ^{13}C isotope discrimination. Furthermore carbon atoms of G6P skeleton have approximately the same carbon isotope composition due to transketolase and transaldolase reactions randomizing atoms in the cycle reactions. A part of the synthesized G_6P is stored in carbohydrate pools as polysaccharides to be used as a source of

carbon for the dark synthesis of metabolites via glycolitic chain [15]. Their carbon isotope composition and intramolecular patterns to a great extent are determined by corresponding characteristics of G6P and by the specificity of enzymatic interactions.

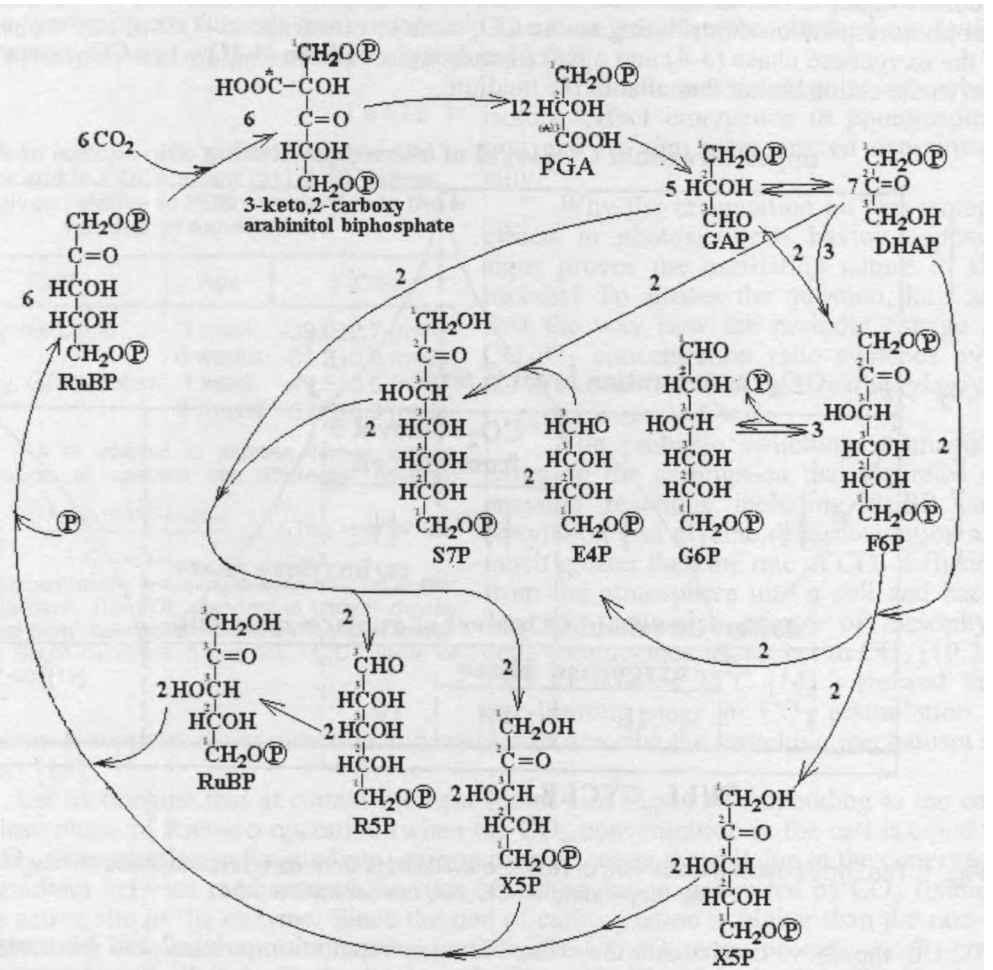

Fig. 2. Scheme of the Calvin cycle operation in the carboxylase phase showing the transfer and mixing of carbon atoms at each turn

When Rubisco switches over to oxygenase function (Fig.3) the rest of labile carbohydrates from the pool rushes into glycolate chain of photorespiration loop where photorespiration products such as oxalates, glycolic acid, some aminoacids (glycine, serine, proline) are synthesized. A portion of labile carbohydrates are reserved as sucrose which is used in the dark for organic acid synthesis.

Owing to carbon isotope fractionation in glycine decarboxylation proceeding in photorespiration loop ^{13}C is accumulated in G6P pool. The more intense photorespiration is (more turns carbon flux performs in the loop), the more ^{13}C is concentrated in G6P

pool. The kinetic nature of the isotope effect following glycine decarboxylation (what means that isotopic shifts appear at the points where C-C bonds are cleaved) causes the appearance of isotope heterogeneity in carbon skeleton of G_6P. The latter is inherited by descendants synthesized in metabolic pathways. That is the metabolic sequence that follows from the oscillation idea and from the coupling of isotope effects arising in $C0_2$ assimilation and photorespiration.

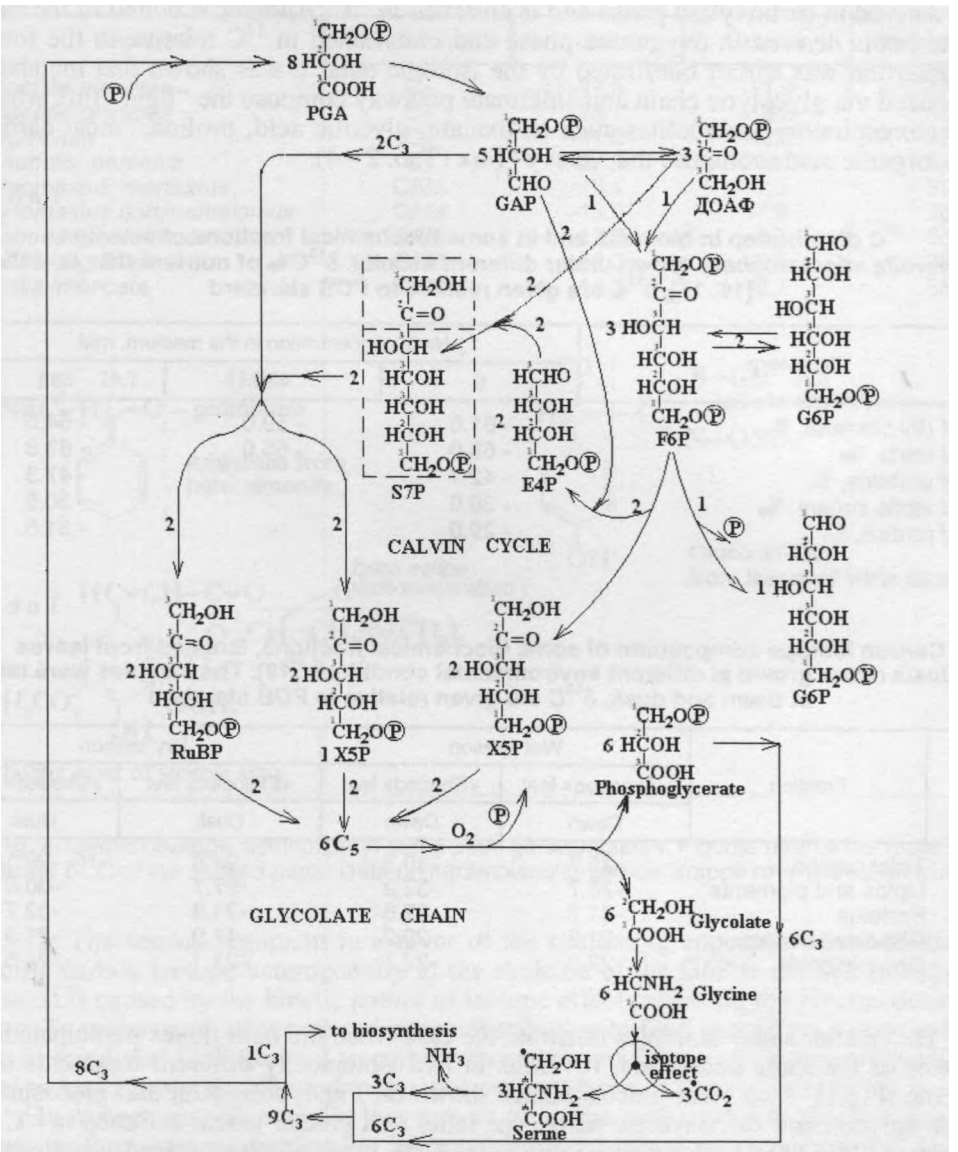

Fig. 3. Scheme of the Calvin cycle operation in the oxygenase phase showing the transfer and mixing of carbon atoms at each turn. Carbon isotope fractionation unit related to GDC reaction is denoted on the scheme by dotted line

Facts and the theoretical basis supporting oscilation idea

Let's consider the arguments that validate the described sequence of metabolic events.

1. According to the oscillation hypothesis two isotopically different carbon fluxes should exist in the photosynthesizing cells. One of them is bound to the carbohydrate pools derived in carboxylase phase and is enriched in ^{12}C. Another is bound to the carbohydrate pools derived in oxygenase phase and is enriched in ^{13}C relative to the former. This assertion was earlier confirmed by the isotopic data. It was shown that metabolites synthesized via glycolytic chain and shikimate pathway compose the "light" flux whereas the photorespiratory metabolites such as oxalate, glycolic acid, proline, most carbohydrates, organic acids compose the "heavy" flux (Tab. 2 - 4).

Table 2

^{13}C distribution in biomass and in some biochemical fractions of sea alga *Chorella stigmatophora* grown under different salinity. $\delta^{13}C$‰ of nutrient $C0_2$ is -21‰ [16,17]. $\delta^{13}C$ are given relative to PDB standard

Параметр	NaCl concentration in the medium, mM		
	0	425	595
$\delta^{13}C$ of dry biomass, ‰	-61.6	-59.0	-64.5
$\delta^{13}C$ of lipids, ‰	-66.0	-65.0	-63.8
$\delta^{13}C$ of proteins, ‰	-42.1		-47.3
$\delta^{13}C$ of labile sugars, ‰	-30.0	-	-30.5
$\delta^{13}C$ of proline, ‰	-29.0	-	-31.5

Table 3

Carbon isotope composition of some biochemical fractions, isolated from leaves of *Clusia minor* grown at different environmental conditions [18]. The samples were taken at dawn and dusk. $5^{13}C$ are given relative to PDB standard

N	Fraction	Wet season		Dry season	
		«Exposed» leaf	«Shaded» leaf	«Exposed» leaf	«Shaded» leaf
		Dawn	Dawn	Dusk	Dusk
1	Total carbon	-25.7	-30.3	-24.6	-29.1
2	Lipids and pigments	-28.7	-32.2	-27.7	-30.8
3	Proteins	-31.7	-32.6	-31.3	-32.7
4	Dissolved sugars	-21.2	-29.2	-17.9	-21.9
5	Organic acids	-22.3	-27.7	-21.1	-24.5

Hereinafter some examples illustrate the case when the both fluxes participate in the synthesis of the same compound. It results in two isotopically different fragments of the molecule (Fig.4). Two plant glucosilonates shown on Fig.4, amigdalin and glucosinalbin, include aglycon and carbohydrate parts. The latter to a greater extent enriched in ^{13}C than the former [21]. That's why the aglycon part of the molecule synthesized via glycolithic chain and fed through the pool accumulated in carboxylase phase is enriched in C more than the carbohydrate part which is supposedly formed from the pool accumulated in oxygenase phase. The third glucosilonate on Fig.4 is choline ether of sinapic acid [22]. Choline part of it inherits carbon from serine formed in glycolate chain of photorespiratory loop. It

again explains the observed enrichment in ^{13}C of the part inherited from serine relative to the rest of the molecule primarily synthesized via shikimate pathway.

Table 4

Carbon isotope composition of leaf and oxalates of some oxalate accumulating oxalate accumulating C_3 - plants [19, 20]. $\delta^{13}C$ are given relative to PDB standard

Plant type	Assimilation type	Leaf	Oxalates	Referen
Spinaceae oleracea	C_3	-27.5	-11.9	37
Spinaceae oleracea	C_3	-25.7	-19.9	38
Pelagronium	C_3	-31.0	-12.4	37
Mereurialis perennis	C_3	-27.9	-13.7	37
Echinomastus intertextus	CAM	-13.4	-7.3	38
Echinomastus horizonthalomus	CAM	-13.0	-7.8	38
Escobaria ruberoulosa	CAM	-12.3	-8.3	38
Opuntia euglemannii	CAM	-13.3	-8,5	38
Opuntia imbricata	CAM	-14.1	-8.7	38

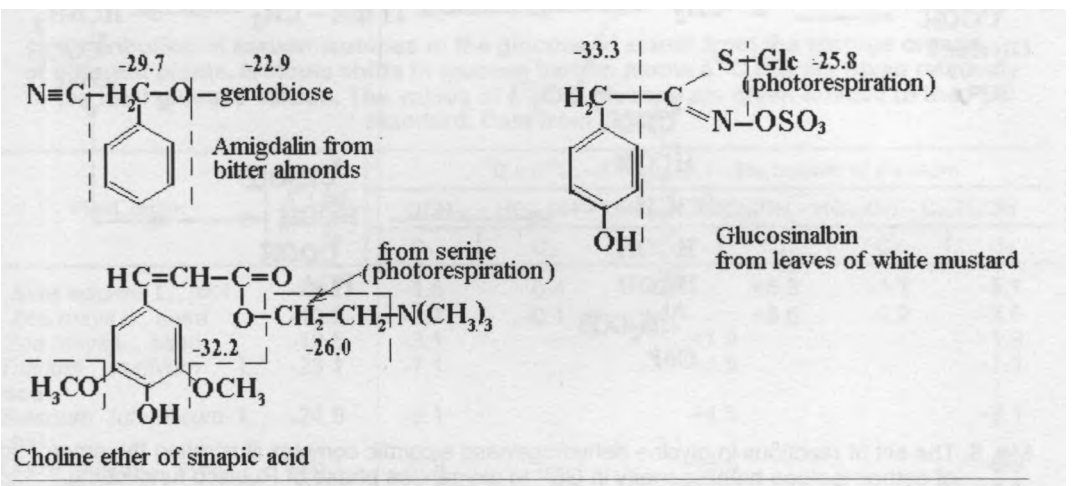

Fig. 4. Carbon isotope distribution in some plant glucosinolates. Figures nearby the molecules indicate $\delta^{13}C$ of the studied parts. Data on intramolecular carbon isotope ratio are taken from [21]

2. The second argument in a favor of the oscillating concept was the finding that specific carbon isotope heterogeneity in the skeleton of the G_6P is derived in oxygenase phase. It is caused by the kinetic nature of isotope effect following the glycine decarboxylation. To understand what carbon isotope distribution of G_6P should be, it is necessary to bear in mind that G_6P derived in carboxylase phase of Calvin cycle has practically uniform carbon isotope distribution in the molecule skeleton as shown before [23].

In oxygenase phase on the first turn of glycolate cycle, prior to glycine decarboxylation, uniform isotope distribution inherited from the initial substrate remains. However it becomes heterogeneous as soon as two glycine molecules transform into serine with $C0_2$ splitting in mitochondria. The isotope heterogeneity acquired by serine in the following transformations into G_6P extends to the latter.

To estimate isotopie pattern of serine, and hence that of G_6P, one should investigate isotope fractionation occurring in the enzymatic glycine dehydrogenase complex (Fig.5). Having been decarboxylated glycine gets ^{13}C enrichment at the particular carbon positions corresponding to the broken bond (denoted by asterisks on Fig.5) owing to kinetic carbon isotope effect accompanying the C - C bond cleavage. Simultaneously carbon atoms in CO_2 and that in methylene group which is linked with tetrahydrofolic acid (THFA), C_1-fragment carrier, get enriched in ^{12}C (denoted by filled circles on Fig.5). THFA carries methylene fragment and attaches it to the undestructed glycine converting it to serine [24]. Thus the carboxylic and neighbor to him carbon atoms get enriched in ^{13}C whereas the end carbon atom of methoxylic group is enriched in ^{12}C.

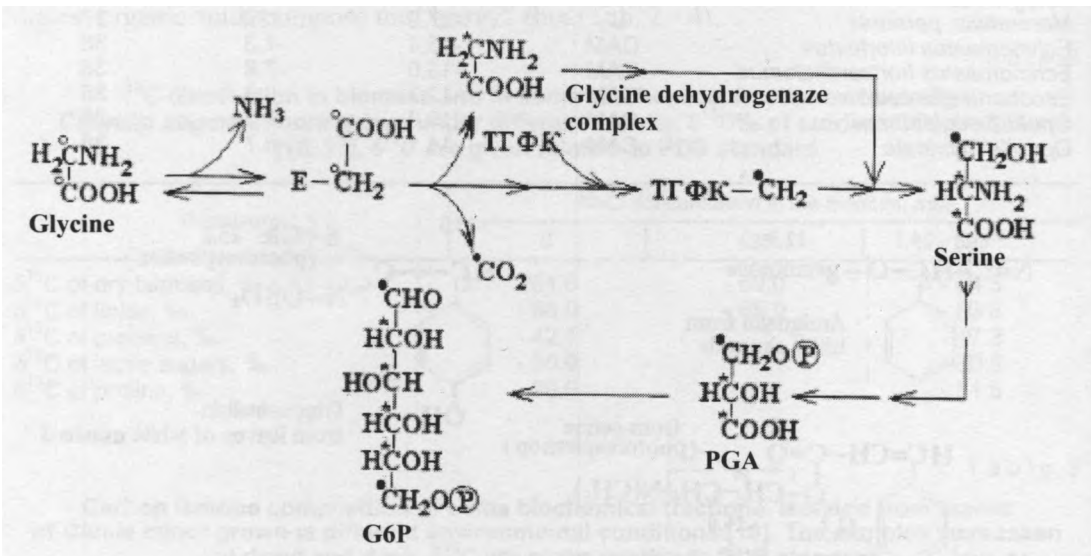

Fig. 5. The set of reactions in glycine dehydrogenase enzymic complex illustrating the emergence of carbon isotope heterogeneity in G6P in oxygenase phase of Rubisco functioning. See detail explanations in the text

In G6P synthesis from serine the glucose carbon skeleton is formed in such a way that "heavy" carbon atom of serine get to C-3 and C-4 positions of G_6P. Atoms in positions C-2 and C-5, because of mixing, appear to be rather enriched in ^{12}C relative to C-3 and C-4, but is "heavier" than total carbon of G_6P. In addition, atoms in positions C-l and C_{-6} of G_6P found to be enriched in ^{12}C relative to the initial substrate.

The results of the model computation [23], given in Tab.7, illustrate that at the 2-nd and at the following cycle turns the above isotope heterogeneity remains and strengthens. The more turns carbon flux performs spinning in photorespiration loop the "heavier" G_6P carbon becomes and isotopic discrepancies in G_6P carbon skeleton grow (Tab.5).

The analysis of the publications on carbon isotope distribution in carbohydrate fraction draws to the conclusion that starch glucose from storage organs of different plants has the similar pattern. It allows saying that starch from storage organs of plants is synthesized at the expense of G_6P formed in oxygenase phase (Tab.6).

Table 5

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 Rubisco functioning. Isotope composition of ambient G6P feeding glycolate chain was taken equal to -20‰ with the uniform isotope distribution along carbon skeleton (from the carboxylase phase). Carbon isotope fractionation coefficient in glycine decarboxylation reaction was taken equal to y = $^{12}k/^{13}k$ = 1.020 [23].

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

Table 6

Distribution of carbon isotopes in the glucose of starch from the storage organs of different plants. Isotopic shifts in glucose carbon atoms $\delta^{13}C$ (‰) are given relatively to the total glucose carbon. The values of $\delta^{13}C$ of glucose are given relative to the PDB standard. Data from [25-27]

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

The values of $\delta^{13}C$ of C-3 and C-4 atoms were calculated from the data assuming that the isotope composition of other atoms is equal to that of C-1 [25].

These results are consistent with the data obtained from the analysis of the destruction products of glucose fermentation [28]. As it follows from Fig.6 CO_2 derived in fermentation inherits atoms C-3 and C-4 of glucose and carbon atoms of ethanol are derived from atoms C-1, C-2, C-5 and C-6 atoms correspondingly. CO_2 found to be much more enriched in ^{13}C as compared with the ethanol carbon. By the other words all the researchers get very close results.

Hence it might be concluded that carbon isotope distribution of glucose from starch of storage organs corresponds to that predicted for G_6P synthesized in oxygenase phase and this in turn is an argument in favor of photosynthetic oscillations.

The conjecture is supported by the fact that plant storage organs are formed at the late stage of ontogenesis where the plants are characterized by the numerous facts evidencing in favor of the photorespiration increase. In fact, at the stage of flowering, ripen-

ing and bearing the accumulation of the oxidizing products in the old leaves, the increase of hydrogen peroxide concentration as well as peroxidase activity is observed [29, 30]. The enrichment of plant biomass in ^{13}C that is taking place at this stage [31] and some other signs [32, 33] also indicates the intensification of photorespiration.

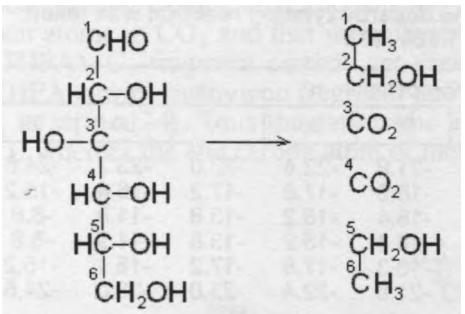

Fig. 6. Scheme illustrating carbon atoms inheritance in the glucose fermentation process.

The probable input of glucose derived in carboxylase phase cannot distort the above distribution pattern since, as said, the latter should have approximately uniform isotope distribution. The above argument not only proves the hypothesis on photosynthetic oscillations but also testify in favor of considerable biosynthetic role of photorespiration at the stage of storage organs formation in particular.

3. The comparison of the intramolecular patterns in glucose isolated from storage organs of C_3 and C_4 plants gives another evidence for the existence of G_6P derived in carboxylase and oxygenase phases of Rubisco functioning and hence more argument in favor of oscillating regime of Calvin cycle. Hobbie and Werner [28] having investigated isotope distribution of glucose from storage organs found that glucose from starch of C_3 plants has carbon isotope heterogeneity much greater than glucose from C_4 plants. Their results are given in Tab. 7.

Table 7

The comparison of carbon isotope distribution in starch glucose from C_3 and C_4 plants. The data were obtained in the experiments on glucose fermentation. The figures in columns 2 and 3 are the difference between carbon isotope ratio of atoms at i-th position of glucose and its total carbon ($\Delta_i = \delta^{13}C_i - C_{total}$)- The figures in the last column is the difference between the figures in two first columns [28]

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

The cause for the difference, illustrated by the Tab. 7, is the greater photorespiration in C_3 than in C_4 plants. As it was shown, the greater photorespiration is followed by the more considerable isotope heterogeneity.

4. The information obtained in the study of C_3 plant dark respiration supports the oscillation idea also. Recently [34] in gas exchange experiments it was found that plant leaves having been illuminated by high level light prior to the switching off strongly respired $C0_2$ within light-to-dark transition just about an hour. The phenomenon was called light enhanced dark respiration (LEDR).

Having applied tunable diode laser spectrometer allowing to measure concentration and $\delta^{13}C$ of CO_2 simultaneously, Barbour and colleagues [35] revealed that $C0_2$, respired by leaves of *Ricinus communis* L. during 15min just right after switching off the light, was up to 3 - 7‰ (sometimes up to 11‰) enriched in ^{13}Crelative to phloem sap sugars regarded as a substrate for respiration (Tab.8). Moreover they found that previous illumination of leaves prior to the switching off resulted in a greater extent of ^{13}C enrichment in the respired $C0_2$. The next hours the intensity of dark respiration was reduced and $C0_2$ grew "lighter". After 24 h and 48 h $5^{13}C$ of $C0_2$ became enriched in ^{12}C in respect to phloem sap sugars.

Table 8

Temporal dynamics of $\delta^{13}C$ values of CO_2, respired by leaves of *Ricinus communis* L. in the dark in gas exchange experiments. Experiment conditions: high humidity; exposition to high level light no less than 20 min prior to switching off the light [35]

Time after switching off the light	$\Delta = \delta^{13}C_{CO2} - \delta^{13}C_{substrate}$	The suggested substrate for dark respiraior
10 – 15 min after start of dark period	до +11‰	Sap sugars from phloem
30 – 60 min after start of dark period	от +3 до +7‰	Sap sugars from phloem
2 – 5 h after start of dark period	до +4.5‰	Sap sugars from phloem
24 h after start of dark period	– 1,1‰	Sap sugars from phloem + input of lipid destruction products
48 h after start of dark period	– 1,6‰	Sap sugars from phloem + input of lipid destruction products

The authors failed to explain this phenomenon whereas oscillation concept gives the reasonable explanation for the above facts.

According to the concept when Rubisco operates in carboxylase phase G6P accumulated in the pool enters partly the phloem and partly is stored as polymerized polysaccharides (starch, etc.) to provide the cells with carbon source in the dark synthesis in glycolytic chain and in coupled the pathways. When Rubisco switches over to oxygenase phase the rest of G6P pool as labile sugars feeds photorespiratory loop (glycolate chain and Calvin cycle) and is used for photorespiration products syntheses. Partly G6P is stored as labile sugars to make pool for organic acid synthesis in the dark, partly is used for some polysaccharide synthesis and for other cell needs. It is important to underline that G6P accumulated in the oxygenase phase is enriched in ^{13}C relative to G6P accumulated in carboxylase phase. As said before the extent of ^{13}C enrichment as well as carbon isotope heterogeneity of G6P depends on photorespiration intensity. Note that light intensity is one of the factors that strengthens photorespiration [36]. If so, the illumination of the leaves just before switching off the light should intensify photorespiration and thus results in more ^{13}C enrichment of sucrose. We assume, that just after the light being switched off, the sucrose, accumulated in the oxygenase phase, begins to destroy converting into organic acids. The data obtained by Borland et al. [18] supports this assertion. In fact, the authors found strong relation between $\delta^{13}C$ values of sucrose derived in the light and that of organic acids synthesized in the dark. The same idea was expressed in the work [35]. If the picture is true, then the cause for high ^{13}C enrichment of $C0_2$ at the beginning of dark period is explained by splitting carbon atoms in C-3 and C-4 positions of hexoses in the course of sucrose transformation into organic acids. Since the above atoms mostly enriched in ^{13}C, their evolution provides the above effect of dark respiration while the rest parts of hexose (C_2-fragments) are used to form organic acids. Note the extent of ^{13}C enrichment of atoms in C-3 and C-4 positions of G6P just corresponds to the ^{13}C enrichment of $C0_2$ observed at the start of dark respiration (Tab. 8).

The prolongation of dark respiration for more than 24 h or 48 h was followed by the drop in the ^{13}C enrichment up which finally leads to the change in the sign (^{12}C enrichment of $C0_2$) of the effect (Tab.8). This phenomenon however doesn't link with the photosynthetic oscillations but with the vibrations occurring in glycolytic chain. The vibrations are caused by energy metabolism requirements with period of about 24 h [37]. They compose of two phases - glycolysis and gluconeogenesis. One phase corresponds to dark period, another to the light one. In case of dark period prolongation, when Calvin

cycle doesn't work, the lipids become the main contributor to carbohydrate pool in the course of lipid-carbohydrate exchange in gluconeogenesis phase. The latter provide glycolitic chain and Krebs cycle by means of [38]. "Light" carbon isotope composition of lipids causes the ^{12}C enrichment of $C0_2$ in the prolonged dark respiration.

5. The oxygen isotope effect arising in photosynthesis gives one more argument in favor of photosynthetic oscillations. As early as 1941 Vinogradov and Teiss [39] in study of atmospheric oxygen origin by means of labeled water H_2O^{18} have established that oxygen evolving in photosynthesis reaction

$$nCO_2 + n\,H_2O^{18} \rightarrow (\,CH_2O\,)_n + O_2{}^{18}$$

originates from water. They expected that it should be of the same oxygen isotope ratio as the water but were greatly puzzled when found that molecular oxygen was always enriched in ^{18}O as compared with water. It took them and others [40-42] a lot of time before they found that the reason was the participation of the evolving oxygen in light respiration. The pool of oxygen formed in the photosynthetic reaction is used then in the following respiration process. The observed ^{16}O enrichment is caused by oxygen pool depletion in $^{16}0$ in the course of the advantageous consumption of ^{16}O in the light respiration (Releigh effect). It was proved by the fact that the extent of ^{18}O enrichment depended greatly on light intensity, $C0_2$ concentration in the medium and by some other factors known to be parameters that impact the intensity of photorespiration [43]. Different $^{18}0$ enrichment observed for photosynthesizing organisms was explained by different intensity of photorespiration characteristic of them.

Taking this explanation we can assert that oxygen pool depletion can occur only in the case if the above photosynthetic reaction forms the oxygen as separate batches. Following this logic one can conclude that $C0_2$ participating in the reaction enters the cell in photosynthesis as separate batches. It is the idea that we put forward in 1989 [44] and it brought us to oscillatory hypothesis. Discreteness of $C0_2$ assimilation resulted from the alternating assimilation and photorespiration processes.

6. The oscillation concept is in a good accordance not only with isotopic data but with the other facts as well. There is well known phenomenon of post illumination burst (P1B) [45]. It is the burst of $C0_2$ observed just in the first seconds after light is switched off. The burst of $C0_2$ appears because the $C0_2$ assimilation ceases whereas oxidation of substrates accumulated in carboxylase phase is going on. Supposedly the lag in peak emergence is the time required to glycine to achieve mitochondria where decarboxylation occurs [15]. Additional argument for that is the increase of PIB peak in conditions of high glycolate production (low $C0_2$ concentration, high light intensity) corresponding to intense photorespiration [34].

7. Time diversity of carboxylase and oxygenase phases, a sign of photosynthetic oscillations is also supported by data on delayed luminescence of chlorophyll [46]. Previously it was shown that $C0_2$ assimilation in Calvin cycle and photorespiration impact on electron transport in photosystem II [47, 48]. In the experiments with intact chloroplasts isolated from green alga *Brvoopsis maxima* Satoh and Katoh [49] have investigated the induction curves of delayed luminescence and found two peaks on the curve. Kukushkin and Soldatova [46] having performed theoretical calculation have obtained very similar picture. They supposed that the peaks were result of interaction of the above processes with photosystem II. It was confirmed by the disappearance of one of them when parameters responsible for the photorespiration were removed.

8. Recently the endeavor to disclose photosynthetic oscillations experimentally has been undertaken by Roussel and colleagues [50]. Measurements of internal $C0_2$ concentrations in substomatal cavities in tobacco leaves using fast response $C0_2$ exchange sys-

tern showed that in the light switching from 350 pL/L to a low $C0_2$ concentration of 36.5 pL/L (promoting high photorespiration) resulted in the C_i oscillating near the $C0_2$ compensation point. The oscillations were highly irregular, with the range of C_i varying by 2-4 p.L/L and a period of a few seconds. The amplitudes of the signals were well above the 0.01 pL/L $C0_2$ the precision of individual measurements in the applied system. The analysis of the frequency spectrum after the transient using the fast Fourier transformation shows the dynamical system approaches the attractor. Attractor reconstruction indicates that the observed oscillations are not chaotic but exhibit stochastic behavior. The period of oscillations is consistent with the duration of photorespiratory post-illumination burst (PIB). Thus the reconstruction of the attractor evidences in favor of the undamped oscillations existence. Since the oscillations occurred near the $C0_2$ compensation point it was suggested that the observed oscillations may be due to a similar mechanism to that which leads to PIB, and may play a role in switching mitochondrial operation between oxidation of the photorespiratory glycine and of the tricarboxylic acid cycle substrates.

The conclusion

The above factual material received independently by different authors is in good consistence with the oscillation hypothesis and makes a solid ground for its use. It allows in a new fashion to view fundamental problems of temporal organization and regulation of a photosynthesis and open new opportunities in further study of cell mechanism. It also open new perspectives in the application the theory to the solution of the many applied problems, in particular, to the increase of plant productivity, to working out of new techniques for genetic selection, for raising new kinds of plant resistant to unfavorable external factors, etc.

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