Proc. Estonian Acad. Sci. Chem., 1993, 42, 4, 185—197
https://doi.org/10.3176/chem.1993.4.04

INFLUENCE OF OXYGEN CONCENTRATION ON THE RATES OF CARBON FLUXES IN THE BIOCHEMICAL SYSTEM OF CO_2 ASSIMILATION

Hiie IVANOVA, Olav KEERBERG, and Tiit PÄRNIK

Eesti Teaduste Akadeemia Eksperimentaalbioloogia Instituut (Institute of Experimental Biology, Estonian Academy of Sciences), Instituudi tee 11, EE-3051 Harku, Harjumaa, Eesti (Estonia)

Presented by A. Aaviksaar

Received July 7, 1993; accepted July 12, 1993

Abstract. Kinetics of the incorporation of ^{14}C into the products of steady-state photosynthesis and decarboxylation of early photosynthates in tobacco (*Nicotiana tabacum*) leaves at different oxygen concentrations (21 and 1.5%) have been studied. From the kinetic data the pool sizes of intermediates and rates of carbon fluxes in the biochemical system of CO_2 assimilation were calculated. In 1.5% O_2, the rate of decarboxylation of early photosynthates was significantly higher than could be predicted by the linear oxygen-dependence of RuBP oxygenation in the glycolate cycle which suggested that in addition to the metabolites of the glycolate cycle some other photosynthates, possibly C_3- and C_4-acids, were decarboxylated in the reactions saturated at low oxygen concentrations. A 14-fold decrease in O_2 concentration brought about only a 3-fold decrease in the rate of carbon flux through the glycolate cycle. This fact points to the possibility that under low oxygen a portion of glycolate was synthesized by a non-oxygenase mechanism, most probably in the transketolase reaction of the reductive pentose phosphate cycle. The reduction of the O_2 concentration resulted in a significant acceleration of the syntheses of sucrose and C_3-acids, particularly alanine, in the cytosol while the rate of starch synthesis in the chloroplasts remained unchanged. These results were interpreted as indicating an indirect effect of oxygen on the intracellular concentration gradient of orthophosphate and on the cytosolic ATP/ADP ratio.

Key words: photosynthetic carbon metabolism, photorespiration, oxygen concentration, *Nicotiana tabacum*, decarboxylation, primary products of photosynthesis, glycolate cycle, starch synthesis, sucrose synthesis.

The main source of photorespiratory CO_2 in C_3 plants is considered to be decarboxylation of glycine in the glycolate cycle. The rate of this reaction is determined by its supply with the substrate via the oxygenation of RuBP*, the reaction linearly dependent on oxygen concentration. If the decarboxylation of glycine were the sole reaction of CO_2 production, the evolution of CO_2 from the leaf should exhibit the oxygen dependence identical with that of the oxygenase reaction. However, in some C_3 species unproportionally high rates of light-dependent CO_2 evolution have been observed under the conditions where oxygenation of RuBP

* Abbreviations used: PhAR — photosynthetically active radiation; PEP — phosphoenolpyruvate; RPPC — reductive pentose phosphate cycle; RuBP — ribulose-1,5-bisphosphate, TP — triose phosphates.

is suppressed by low O_2 or high CO_2 concentrations [1, 2]. Two explanations of this phenomenon may be proposed. First, a portion of glycolate metabolized in the glycolate cycle originates from some other reaction, beyond oxygenation of RuBP, e.g. from C_2-units in the transketolase reaction of RPPC [3]. Second, in addition to glycine, some other early metabolite(s) of the photosynthetic carbon assimilation may be decarboxylated in the light.

In this work the oxygen dependence of the decarboxylation of primary products of photosynthesis in tobacco leaves has been studied in detail. Kinetics of the incorporation of ^{14}C into the products of steady-state photosynthesis at different O_2 concentrations has been determined. From the kinetic data the rates of carbon fluxes in the biochemical system of CO_2 assimilation were calculated with the aim of estimating the contribution of different metabolic processes to the CO_2 evolution from the newly synthesized products of photosynthesis.

MATERIAL AND METHODS

Tobacco (*Nicotiana tabacum*) was grown on soil under fluorescent lamps with a 16 h light/8 h dark cycle and PhAR intensity of 50 $W \cdot m^{-2}$. Mature leaves of the 2nd and the 3rd insertion levels were used in the experiments. Pieces of 2.4×2.5 cm were cut out of leaves and placed with their lower edges into small polyethylene bags containing water.

Measurements of CO_2 exchange and labelling of metabolites were carried out under saturating light in the medium containing 0.03% CO_2 and 21 or 1.5% O_2 by means of the multichannel exposure chamber described earlier [4]. Release of CO_2 from early photosynthates was studied using a combined radio-gasometric method [5, 6]. By means of this method the rate of CO_2 evolution from the leaf, the rate of intracellular decarboxylation of early photosynthates, and the rate of reassimilation of respiratory CO_2 were determined under steady-state photosynthesis.

To study the kinetics of the incorporation of ^{14}C into the photosynthates, leaf pieces were placed into the chamber and illuminated in non-radioactive medium until the steady state of photosynthesis was achieved. Thereafter leaves were fed with $^{14}CO_2$ of the same concentration as at preillumination during 5, 20, 60, 120, 360, or 600 s. The exposed leaves were killed in boiling 80% ethanol. Soluble compounds were extracted and separated chromatographically [7]. Radioactivity of individual compounds and fractions was determined and plotted against the time of exposure to $^{14}CO_2$.

The experimental kinetic curves were analyzed by means of an interpretation procedure worked out in our laboratory in order to estimate intrinsic parameters of the photosynthetic carbon metabolism in the intact leaf *in vivo* [8, 9]. Using this procedure the pool sizes of metabolites and carbon fluxes in the biochemical system of photosynthetic CO_2 assimilation at different O_2 concentrations has been calculated.

RESULTS

Radio-gasometrically estimated values of the rates of true photosynthesis, evolution of CO_2 from primary products of photosynthesis and intracellular decarboxylation of these compounds at two O_2 concentrations are given in Table 1 as mean values of 3 replications. The rate of intracellular reassimilation of CO_2 (R_r) is calculated as the difference of the rates of decarboxylation (R_p) and CO_2 efflux from the leaf (R_l). The rate

of decarboxylation estimated at 1.5% O_2 is essentially higher than the value predicted by the linear oxygen dependence of decarboxylation (about 2 nmol $CO_2 \cdot dm^{-2} \cdot s^{-1}$). This suggests that beyond the glycolate cycle, other reactions, saturated at low O_2 concentration, should be involved in the decarboxylation of primary products of photosynthesis.

Table 1

Components of CO_2 exchange in the light

Carbon flux	Notation	nmol $CO_2 \cdot dm^{-2} \cdot s^{-1}$		Per cent true photosynthesis	
		21% O_2	1.5% O_2	21% O_2	1.5% O_2
True photosynthesis	P_t	119.8±6.0	130.3±6.5		
CO_2 evolution	R_l	20.6±0.1	6.6±0.3	17.2±1.7	5.1±0.5
Intracellular decarboxylation	R_p	30.1±0.6	9.7±0.7	25.2±1.8	7.4±0.9
Intracellular reassimilation	R_r	9.5±0.7	3.1±1.0	7.9±1.0	2.4±0.9

From the kinetics of the incorporation of ^{14}C into the main products of steady-state photosynthesis in 21 and 1.5% O_2 (Fig. 1 and 2), together with the data for other labelled compounds not shown in Figures, the rates of carbon flow and the pool sizes of metabolites were calculated. The results of calculations for the reactions of the glycolate cycle are given in Table 2**. The model [8] used in these calculations presumes that intermediates of the glycolate cycle have two metabolic pools with different kinetics of the incorporation of photosynthetic carbon: active pools with rapid turnover in organellae (peroxisomes and mitochondria) and less mobile pools in the cytosol. At the steady-state photosynthesis, these pools are in diffusional equilibrium. Using this model, the rate of carbon incorporation into RPPC (R_1) and the rate of carbon flux through the glycolate cycle (R_2) were calculated. From these data, the rate of glycine decarboxylation as $R_2/4$, and the rate of carboxylation of RuBP as the sum of R_1 and reassimilation (R_r in Table 1) has been estimated. The rate of carboxylation of RuBP (R_c) was almost equal at both concentrations of O_2. The rates of decarboxylation of glycine at 21 and 1.5% O_2 were significantly lower than the rates of decarboxylation of primary products measured radio-gasometrically (R_p in Table 1). This agrees with our assumption that concurrently with the glycolate cycle, some other reactions must be involved in the decarboxylation of early photosynthates. According to our data, about a third of CO_2 comes from other sources than glycolate cycle at both concentrations of O_2.

The pools of glycolate, glycine, and serine were saturated with the label during the first 2—3 minutes of exposure of leaves in $^{14}CO_2$ (Fig. 1). The pool sizes of glycolate and glycine were at 1.5% O_2 about 3 times lower than at 21% O_2 which is consistent with the lower rate of carbon flux through the glycolate cycle. Nevertheless, the active pools of serine were almost equal at the different O_2 concentrations.

** In Table 2, as well as in Tables 3, 4, and 6, mean values of the rates derived from the analysis of the curves of absolute and relative radioactivity are presented.

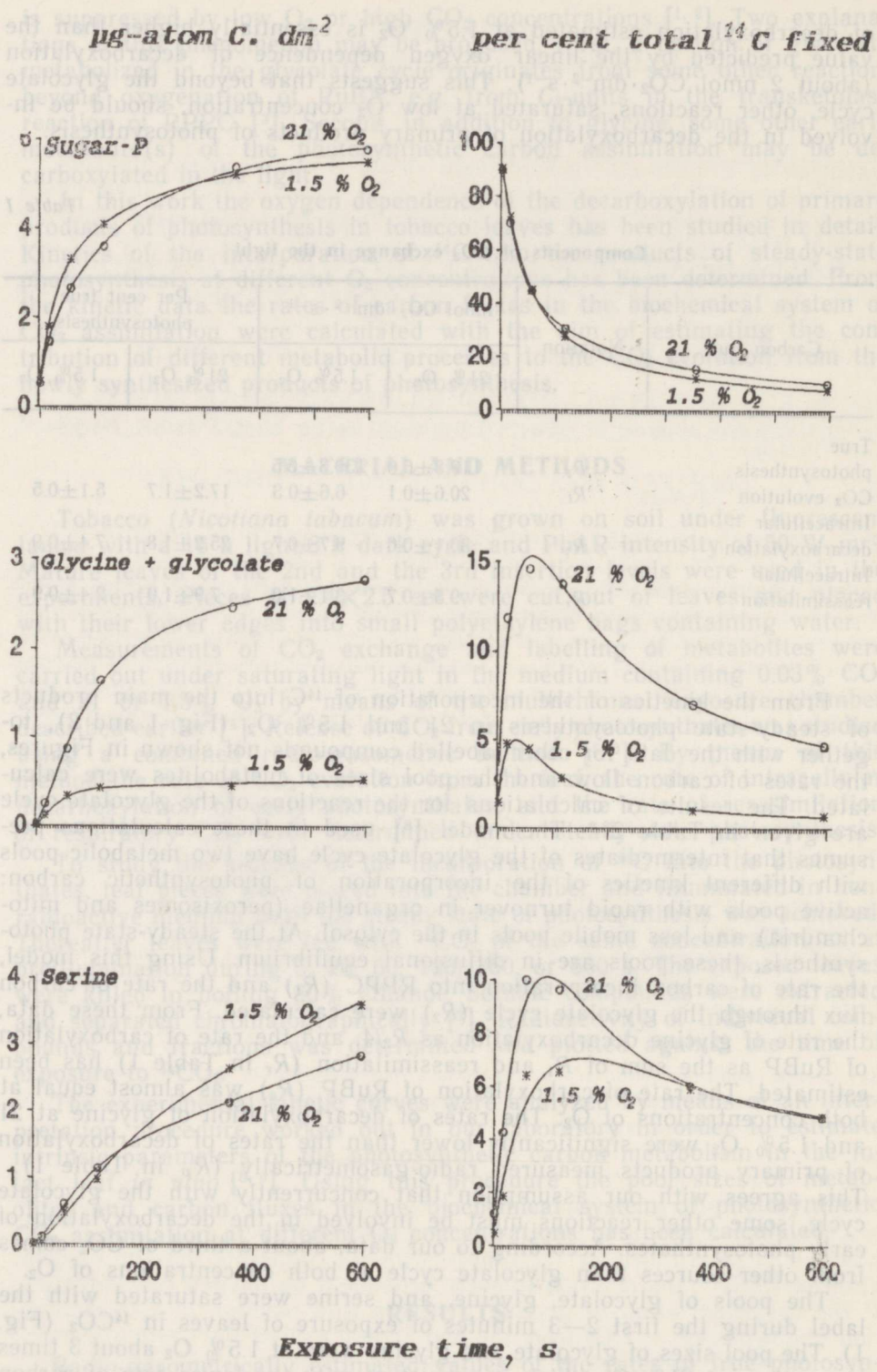

Fig. 1. The kinetics of ^{14}C incorporation into the intermediates of the glycolate cycle. Points represent the experimental values, solid lines are theoretical curves calculated from the two-component model [8] by non-linear regression procedure.

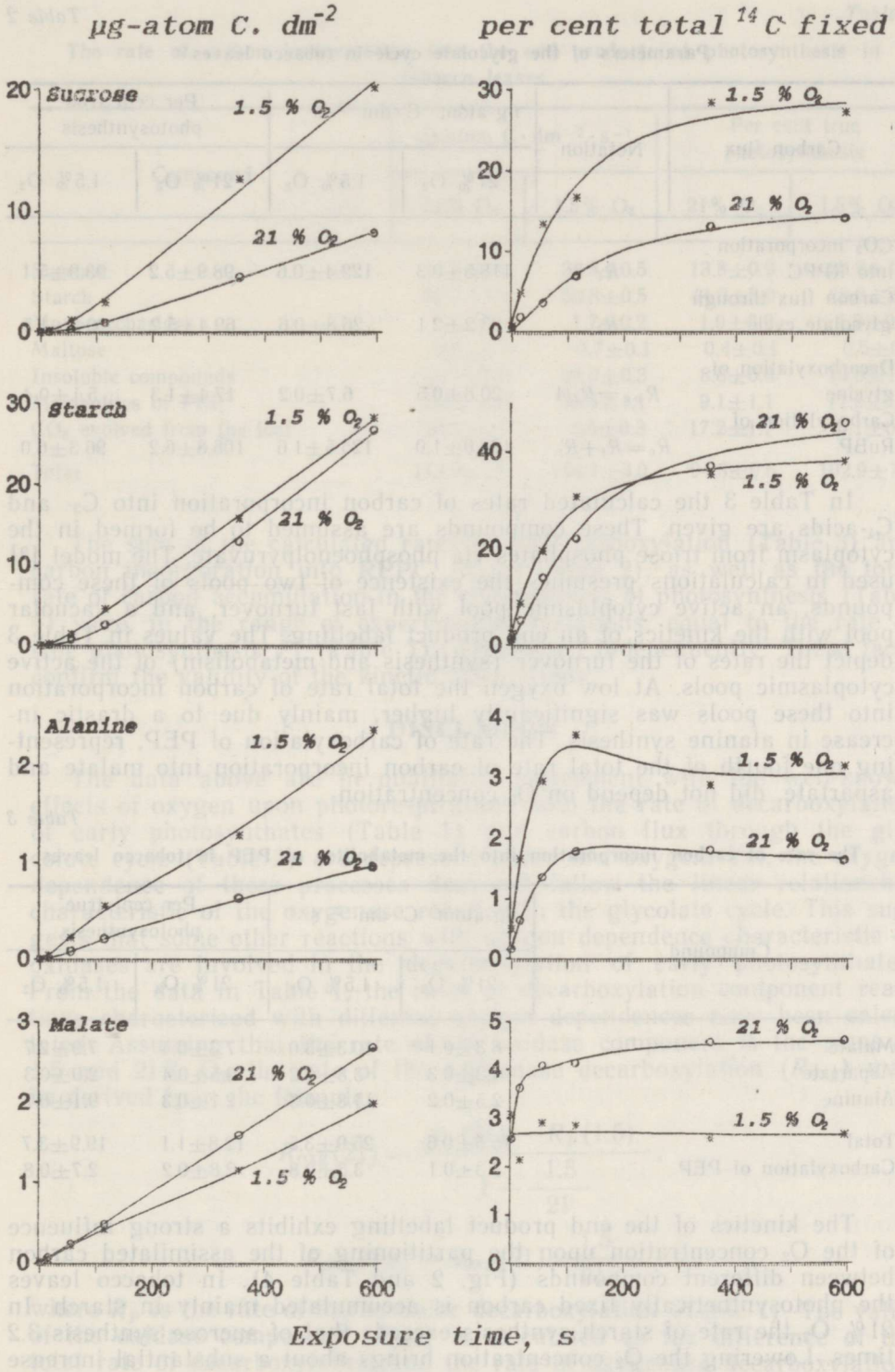

Fig. 2. The kinetics of ^{14}C incorporation into the end products of photosynthesis. See explanations to Fig. 1.

Table 2

Parameters of the glycolate cycle in tobacco leaves

Carbon flux	Notation	ng-atom C · dm^{-2} · s^{-1}		Per cent true photosynthesis	
		21% O_2	1.5% O_2	21% O_2	1.5% O_2
CO_2 incorporation into RPPC	R_1	118.5±0.3	122.4±0.6	98.9±5.2	93.9±5.1
Carbon flux through glycolate cycle	R_2	83.2±2.1	26.8±0.6	69.4±5.2	20.6±1.5
Decarboxylation of glycine	$R_{pg}=R_2/4$	20.8±0.5	6.7±0.2	17.4±1.3	5.1±0.4
Carboxylation of RuBP	$R_c=R_1+R_r$	128.0±1.0	125.5±1.6	106.8±6.2	96.3±6.0

In Table 3 the calculated rates of carbon incorporation into C_3- and C_4-acids are given. These compounds are assumed to be formed in the cytoplasm from triose phosphates via phosphoenolpyruvate. The model [9] used in calculations presumes the existence of two pools of these compounds, an active cytoplasmic pool with fast turnover, and a vacuolar pool with the kinetics of an end product labelling. The values in Table 3 depict the rates of the turnover (synthesis and metabolism) of the active cytoplasmic pools. At low oxygen the total rate of carbon incorporation into these pools was significantly higher, mainly due to a drastic increase in alanine synthesis. The rate of carboxylation of PEP, representing one fourth of the total rate of carbon incorporation into malate and aspartate, did not depend on O_2 concentration.

Table 3

The rate of carbon incorporation into the metabolites of PEP in tobacco leaves

Compound	ng-atom C · dm^{-2} · s^{-1}		Per cent true photosynthesis	
	21% O_2	1.5% O_2	21% O_2	1.5% O_2
Malate	8.8±0.1	10.3±3.0	7.3±0.4	7.9±2.7
Aspartate	4.2±0.3	3.8±0.2	3.5±0.4	2.9±0.3
Alanine	2.5±0.2	11.8±0.3	2.1±0.3	9.1±0.7
Total	15.5±0.6	25.9±3.5	12.8±1.1	19.9±3.7
Carboxylation of PEP	3.3±0.1	3.5±0.8	2.8±0.2	2.7±0.8

The kinetics of the end product labelling exhibits a strong influence of the O_2 concentration upon the partitioning of the assimilated carbon between different compounds (Fig. 2 and Table 4). In tobacco leaves the photosynthetically fixed carbon is accumulated mainly in starch. In 21% O_2 the rate of starch synthesis exceeds that of sucrose synthesis 3.2 times. Lowering the O_2 concentration brings about a substantial increase in the rates of accumulation of sucrose, C_3- and C_4-acids, and unidentified insoluble compounds. Consequently, at low oxygen the overall carbon balance is rearranged in such a manner that at the unchanged rate of carboxylation of RuBP (R_c in Table 2) the limitation of photorespiratory CO_2 efflux is compensated by the higher rates of carbon accumulation in sucrose and metabolites of PEP.

Table 4

The rate of carbon incorporation into the end products of photosynthesis in tobacco leaves

Compound	ng-atom C · $dm^{-2} \cdot s^{-1}$		Per cent true photosynthesis	
	21% O_2	1.5% O_2	21% O_2	1.5% O_2
Sucrose	16.5±0.2	38.8±0.5	13.8±0.9	29.8±1.9
Starch	52.7±3.3	50.8±0.5	44.0±5.0	39.0±2.3
Monosaccharides	1.2±0.2	1.7±0.2	1.0±0.2	1.3±0.2
Maltose	0.5±0.1	0.7±0.1	0.4±0.1	0.5±0.1
Insoluble compounds	10.5±0.2	20.0±0.3	8.8±0.6	15.3±1.0
Metabolites of PEP	10.9±0.8	15.5±1.1	9.1±1.1	11.9±1.4
CO_2 evolved from the leaf	20.6±0.1	6.6±0.3	17.2±1.7	5.1±0.5
Total	112.9±4.9	134.1±3.0	94.3±9.6	102.9±7.4

The sum of the calculated rates of PEP carboxylation (Table 3) and carbon incorporation into RPPC (R_1 in Table 2), as well as the total rate of carbon accumulation in the end products of photosynthesis (Table 4) were, in the range of experimental deviations, equal to the rate of true photosynthesis P_t (Table 1), determined independently. These facts confirm the validity of the kinetic calculations.

DISCUSSION

The data above are in qualitative agreement with the well-known effects of oxygen upon photorespiration: both the rate of decarboxylation of early photosynthates (Table 1) and carbon flux through the glycolate cycle (Table 2) are depressed under low oxygen. Yet, the oxygen dependence of these processes does not follow the linear relationship characteristic of the oxygenase reaction in the glycolate cycle. This suggests that some other reactions with oxygen dependence characteristic of oxidases are involved in the decarboxylation of early photosynthates. From the data in Table 1, the rates of decarboxylation component reactions characterized with different oxygen dependences have been calculated. Assuming that the rate of the oxidase component is the same at 1.5 and 21% O_2, the rate of the oxygenase decarboxylation (R_{oxg}) may be derived from the formulae

$$R_{oxg}(21) = \frac{R_p(21) - R_p(1.5)}{1 - \frac{1.5}{21}},$$

$$R_{oxg}(1.5) = R_{oxg}(21) \cdot \frac{1.5}{21},$$

where R_p is the rate of intracellular decarboxylation (Table 1). The value of the oxidase component (R_{oxd}) is calculated as the difference of the total rate of decarboxylation and the rate of oxygenase decarboxylation. As it follows from Table 5, more than 1/4 of the total amount of CO_2 evolved at 21% O_2 is derived from the oxidase decarboxylation. At 21% O_2 the rate of oxygenase decarboxylation is approximately equal to the rate of glycine decarboxylation (R_{pg} in Table 2). This is not the case at 1.5% O_2 where the rate of glycine decarboxylation is several times higher than the calculated rate of oxygenase decarboxylation.

Table 5

Components of the decarboxylation of early photosynthates in tobacco leaves

Carbon flux	Notation	nmol $CO_2 \cdot dm^{-2} \cdot s^{-1}$		Per cent true photosynthesis	
		21% O_2	1.5% O_2	21% O_2	1.5% O_2
Oxygenase decarboxylation	R_{oxg}	22.0±1.4	1.6±0.1	18.4±2.1	1.2±0.1
Oxidase decarboxylation	R_{oxd}	8.1±0.8	8.1±0.8	6.8±1.0	6.2±0.9
Total	R_p	30.1±0.6	9.7±0.7	25.2±1.8	7.4±0.9

The most probable substrates of oxidase decarboxylation are C_3- and C_4-acids formed from primary triose phosphates and oxidized in the terminal steps of glycolysis and in the Krebs cycle—the processes saturated at low oxygen concentrations [10]. In this work the rate of decarboxylation of these compounds has been estimated by the analysis of labelling kinetics. The results of the analysis of ^{14}C incorporation into malate are shown in Fig. 3 and Table 6. According to the two-pool model [9] of malate turnover, the kinetics of its labelling is divided into three components describing: (I) the incorporation of ^{14}C into the C-4 atom of malate molecule in the reactions catalyzed by PEP carboxylase and malate dehydrogenase, (II) the incorporation of ^{14}C from triose phosphates into C-1, C-2 and C-3 atoms of malate, and (III) accumulation of malate as an end product of photosynthesis (dashed lines, Fig. 3). Using the nonlinear regression procedure the model was fitted to the experimental data and the components of malate turnover were calculated. The results in Table 6 demonstrate that at both O_2 concentrations the rate of synthesis of malate exceeds that of its accumulation. The difference of these rates may be regarded as the rate of malate decarboxylation. Its value covers about a half of the rate of oxidase decarboxylation calculated in Table 5. The remaining proportion of oxidase decarboxylation may be derived from other reactions of glycolysis and the Krebs cycle, e.g. decarboxylation of pyruvate. The 14-fold decrease in O_2 concentration brought about only a 3-fold decrease in the calculated rate of carbon flux through the glycolate cycle (Table 2). This result points to the possibility that a portion of glycolate, metabolized via the glycolate cycle, had been synthesized in reactions other than oxygenation of RuBP, probably in the transketolase reaction of RPPC. This reaction may be regarded as an extra output of RPPC which is switched on under the conditions where the rate of RuBP carboxylation exceeds the potential capacity of the consumption of fixed carbon for the end product synthesis, including CO_2 evolution from the glycolate cycle. This may be the case at low oxygen concentration where the interruption of carbon efflux from RPPC via the RuBP oxygenation may have not been fully compensated by the acceleration of other pathways of the end product synthesis. The formation of glycolate by a non-oxygenase mechanism has been demonstrated in experiments where the sucrose synthesis was suppressed blocking the assimilate export from the leaf [11].

At low oxygen concentration, the decrease in the rate of the carbon flux through the glycolate cycle was accompanied by a nearly proportional decrease in the pools of glycolate and glycine, whereas the pool size of serine remained almost unchanged (Fig. 1). An increase of the ratio of serine/glycine pools has also been observed when the oxy-

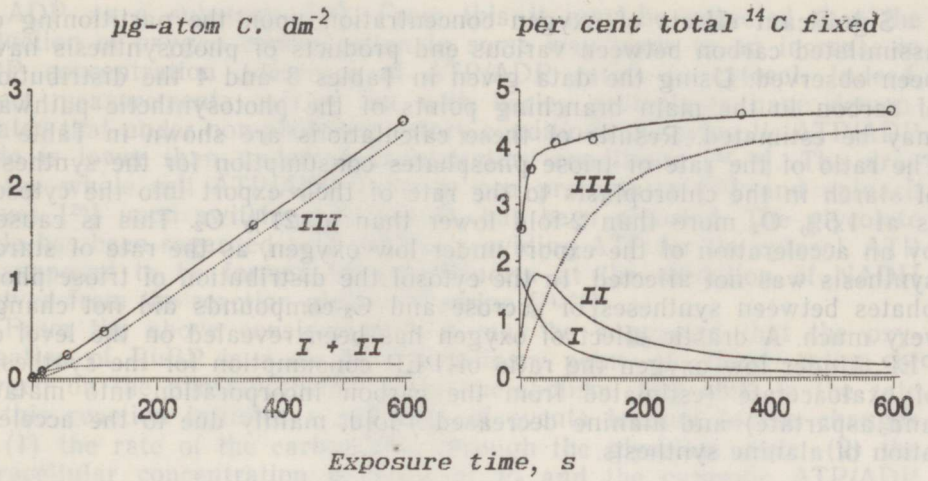

Fig. 3. Analysis of the kinetics of ^{14}C incorporation into malate at 21% O_2. Explanations in the text.

Table 6

The rate of synthesis and accumulation of malate in tobacco leaves

	ng-atom C · dm^{-2} · s^{-1}		Per cent true photosynthesis	
	21% O_2	1.5% O_2	21% O_2	1.5% O_2
Synthesis	8.8±0.1	10.3±3.0	7.3±0.4	7.9±2.7
Accumulation	4.6±0.1	3.7±0.3	3.8±0.3	2.8±0.4
Difference	4.2±0.2	6.6±3.3	3.5±0.7	5.1±3.1

genation of RuBP was partially blocked by high CO_2 concentrations, low light intensity, or restricted assimilate export from the leaf [9, 12]. Relatively high levels of accumulation of carbon in serine under the conditions which depressed the glycolate cycle has been observed in several laboratories [13–15]. This phenomenon may be explained on the basis of the stoichiometry of the reactions of the glycolate cycle. Assuming that under suppressed rate of the RuBP oxygenation the rate constants of other enzymes of the glycolate cycle remain unchanged, the pool sizes of the metabolites of one-substrate reactions must decrease proportionally to the rate of carbon flux. It is valid for glycolate and glycine with the rate of turnover described by the formula:

$$v = k_1 \cdot [\text{glycolate}] = k_2 \cdot [\text{glycine}].$$

Serine is metabolized by the serine glyoxylate aminotransferase, i.e. in a two-substrate reaction:

$$v = k_3 \cdot [\text{glyoxylate}] \cdot [\text{serine}].$$

If the pool size of glyoxylate decreases proportionally to the rate of carbon flux, then the pool size of serine must remain unchanged to retain the steady-state rate of its turnover. Consequently, any decrease in the level of the RuBP oxygenation inevitably leads to an increase in the relative content of serine among the metabolites of the glycolate cycle.

Significant effects of oxygen concentration upon the partitioning of assimilated carbon between various end products of photosynthesis have been observed. Using the data given in Tables 3 and 4 the distribution of carbon in the main branching points of the photosynthetic pathway may be estimated. Results of these calculations are shown in Table 7. The ratio of the rate of triose phosphates consumption for the synthesis of starch in the chloroplasts to the rate of their export into the cytosol is at 1.5% O_2 more than 2-fold lower than at 21% O_2. This is caused by an acceleration of the export under low oxygen, as the rate of starch synthesis was not affected. In the cytosol the distribution of triose phosphates between syntheses of sucrose and C_3-compounds did not change very much. A drastic effect of oxygen has been revealed on the level of PEP. Under low oxygen the ratio of PEP consumption for the synthesis of oxaloacetate (estimated from the carbon incorporation into malate and aspartate) and alanine decreased 4-fold, mainly due to the acceleration of alanine synthesis.

Table 7

Distribution of carbon in the main branching points of the photosynthetic pathway in tobacco leaves

Branching point	Ratio of rates	
	21% O_2	1.5% O_2
TP in chloroplasts		
Starch : export	1:0.5	1:1.2
TP in the cytosol		
Sucrose : PEP	1:0.7	1:0.6
PEP in the cytosol		
Oxaloacetate : Alanine	1:0.3	1:1.1

Export of triose phosphates from chloroplasts into cytosol is mediated by the phosphate translocator and depends on the concentration gradient of orthophosphate (P_i). Enhancement of the export under reduced oxygen suggests that the P_i gradient may have increased. This might be caused by a decrease in the rate of the P_i release from the phosphoglycolate phosphatase reaction in chloroplasts at low O_2 concentration [16]. As a result, the concentration of P_i in chloroplasts decreases, intracellular concentration gradient increases, and more triose phosphates are exported into cytosol.

The published data about the influence of oxygen concentration on starch synthesis are contradictory. In experiments with bean [7, 16] and soybean [17], the rate of starch synthesis increased in response to the reduction of O_2 concentration. The opposite effect was found in studies carried out with beet, spinach [18], and potato [19]. In our experiments with tobacco no significant effect of oxygen on starch synthesis was detected. Apparently, the direction of the oxygen effect depends on the ratio of the capacities of different pathways of end product synthesis. This ratio is determined genetically and may vary over a wide range among different species. It has been suggested that one of the key enzymes determining the rate and ratio of end product synthesis is sucrose phosphate synthase [20, 21].

The drastic increase in the alanine synthesis under low oxygen points to the acceleration of PEP consumption in the reaction catalyzed by pyruvate kinase. The rate of this reaction depends on the concentration

of ADP as a substrate [22]. From this it may be expected that the reduction of oxygen concentration in some way leads to an increase in ADP concentration (decrease of ATP/ADP ratio) in cytosol. Indeed, direct measurements carried out with barley protoplasts have demonstrated that under non-photorespiratory conditions the cytosolic ATP/ADP ratio is lower than under photorespiratory conditions [23, 24]. The drop of the whole cell ATP/ADP ratio in pea protoplasts [25] and spinach leaves [26] upon switching to low O_2 has been reported. The glycolate cycle has been regarded as a process providing ATP for the cytosol. ATP is supposed to be formed in mitochondria at the oxidation of NADH derived from the reaction glycine → serine [24, 25].

From the above considerations it may be suggested that the oxygenation of RuBP is a single and primary point of direct action of oxygen on the photosynthetic carbon metabolism. Any shift in the rate of this reaction initiates a sequence of events leading to the changes in (1) the rate of the carbon flux through the glycolate cycle, (2) the intracellular concentration gradient of P_i and the cytosolic ATP/ADP ratio, and (3) the rate of triose phosphate export from the chloroplasts into the cytosol and the partitioning of assimilated carbon between different end products of photosynthesis.

ACKNOWLEDGEMENTS

The authors wish to express their sincere appreciation to Dr. Juta Viil for her valuable discussions.

REFERENCES

1. Bravdo, B.-A., Canvin, D. Effect of carbon dioxide on photorespiration. — Plant Physiol., 1979, 63, 2, 399—401.
2. Viil, J., Pärnik, T. Parameters of the reductive pentose phosphate cycle and of the glycolate pathway under different concentrations of oxygen. — Z. Pflanzenphysiol., 1979, **95**, 3, 213—225.
3. Eickenbusch, J. D., Scheibe, R., Beck, E. Activated glycol aldehyde and ribulose diphosphate as carbon source for oxidative glycolate formation in chloroplasts. — Z. Pflanzenphysiol., 1975, **75**, 4, 375—380.
4. Пярник Т. Р., Кээрберг О. Ф., Юрисма Э. Я. Быстродействующая экспозиционная камера для изучения фотосинтеза при помощи $^{14}CO_2$. — Физиология растений, 1987, **34**, 4, 837—845.
5. Pärnik, T., Keerberg, O., Viil, J. $^{14}CO_2$ evolution from bean leaves at the expense of fast labelled intermediates of photosynthesis. — Photosynthetica, 1972, **6**, 1, 66—74.
6. Пярник Т., Кээрберг О., Вийль Ю. Радиоизотопный метод определения фотодыхания растений. Препринт Э-1 АН ЭССР, Таллинн, 1977.
7. Voskresenskaya, N. P., Viil, Y. A., Grishina, G. S., Pärnik, T. R. Effects of oxygen concentration and light intensity on the distribution of labelled carbon in photosynthesis products in bean plants. — Photosynthetica, 1970, **4**, 1, 1—8.
8. Кээрберг О. Ф., Вийль Ю. А. Количественная характеристика путей превращения углерода при фотосинтезе. — In: Ничипорович А. А. (ed.). Фотосинтез и продукционный процесс. Наука, Москва, 1988, 40—53.
9. Кээрберг О. Ф. Количественная характеристика путей превращения углерода при фотосинтезе. Doctoral dissertation, Москва, 1989.
10. Пярник Т. Р., Кээрберг О. Ф., Кээрберг Х. И. Выделение CO_2 из листьев фасоли за счет декарбоксилирования C_3- и C_4-кислот, образующихся при фотосинтезе. — In: Вознесенский В. Л. (ed.). Вопросы взаимосвязи фотосинтеза и дыхания. Томск, 1988, 77—83.
11. Чиков В. И. Фотосинтез и транспорт ассимилятов, Наука, Москва, 1987.

12. Расулов Б., Х., Пярник Т. Р., Иванова Х. Н., Кээрберг О. Ф. Компоненты CO_2-газообмена и фотосинтетический метаболизм углерода листьев хлопчатника в условиях заторможенного экспорта ассимилятов. — Физиология растений, 1990, **37**, 1, 12—21.
13. Lawyer, R. L., Cornwell, K. L., Gee, S. L., Bassham, J. A. Effects of glycine hydroxymate, carbon dioxide, and oxygen on photorespiratory carbon and nitrogen metabolism in spinach mesophyll cells. — Plant Physiol., 1982, **69**, 5, 1136—1139.
14. Coudret, A., Ferron, F., Laffray, D. High CO_2 partial pressure effects on dark and light CO_2 fixation and metabolism in *Vicia faba* leaves. — Photosynthesis Research, 1985, 7, 2, 115—126.
15. Kleczkowski, L. A., Givan, C. V. Serine formation in leaves by mechanisms other than the glycolate pathway. — J. Plant Physiol., 1988, **132**, 641—652.
16. Viil, J., Ivanova, H., Pärnik, T. Effects of O_2 and irradiance on CO_2 assimilation in *Phaseolus vulgaris*. — Photosynthetica, 1985, **19**, 1, 88—97.
17. Servaites, J. C., Ogren, W. L. Oxygen inhibition of photosynthesis and stimulation of photorespiration in soybean leaf cells. — Plant Physiol., 1978, **61**, 1, 62—67.
18. Dietz, K.-J. A possible rate-limiting function of chloroplast hexose monophosphate isomerase in starch synthesis of leaves. — Biochim. Biophys. Acta, 1985, **839**, 3, 240—248.
19. Sharkey, T. D., Vassey, T. L. Low oxygen inhibition of photosynthesis is caused by inhibition of starch synthesis. — Plant Physiol., 1989, **90**, 2, 385—387.
20. Champigny, M. L., Foyer, C. Nitrate activation of cytosolic protein kinases diverts photosynthetic carbon from sucrose to amino acid biosynthesis — basis of a new concept. — Plant Physiol., 1992, **100**, 1, 7—12.
21. Galtier, N., Foyer, C. H., Huber, J., Voelker, T. A., Hüber, S. C. Effects of elevated sucrose-phosphate synthase activity on photosynthesis, assimilate partitioning, and growth in tomato (*Lycopersicon esculentum* var. UC82B). — Plant Physiol., 1993, **101**, 2, 535—543.
22. Podesta, F. E., Plaxton, W. C. Plant cytosolic pyruvate kinase: a kinetic study. — Biochim. Biophys. Acta, 1992, **1160**, 2, 213—220.
23. Gardeström, P. Adenylate ratios in the cytosol, chloroplasts and mitochondria of barley leaf protoplasts during photosynthesis at different carbon dioxide concentrations. — FEBS Letters, 1987, **212**, 1, 114—118.
24. Gardeström, P., Wigge, B. Influence of photorespiration on ATP/ADP ratios in the chloroplasts, mitochondria, and cytosol, studied by rapid fractionation of barley (*Hordeum vulgare*) protoplasts. — Plant Physiol., 1988, **88**, 1, 69—76.
25. Кузьмин А. Н., Маслов А. И. Участие фотодыхания в поддержании концентрации АТФ *in vivo*. — Докл. АН СССР, 1980, **251**, 2, 510—512.
26. Sharkey, T. D., Stitt, M., Heineke, D., Gerhardt, R., Raschke, K., Heldt, H. W. Limitation of photosynthesis by carbon metabolism. II. O_2-insensitive CO_2 uptake results from limitation of triose phosphate utilization. — Plant Physiol., 1986, **81**, 4, 1123—1129.

HAPNIKU KONTSENTRATSIOONI TOIME SÜSINIKUVOOGUDE KIIRUSELE CO_2 ASSIMILATSIOONI BIOKEEMILISES SÜSTEEMIS

Hiie IVANOVA, Olav KEERBERG, Tiit PÄRNIK

Uuriti fotosünteesi esmasproduktide dekarboksüülimist ja ^{14}C statsionaarse fotosünteesi produktidesse lülitumise kineetikat tubaka lehtedes O_2 erineva kontsentratsiooni puhul (21 ja 1,5%). Kineetilistest andmetest arvutati CO_2 assimilatsiooni biokeemilise süsteemi metaboliitide fondide suurused ja süsinikuvoogude kiirused. 1,5% O_2 puhul oli esmasproduktide dekarboksüülimise kiirus oluliselt suurem väärtusest, mis tulenenuks RuBP oksügeenimisele ja glükolaatsele tsüklile iseloomulikust lineaarsest hapnikusõltuvusest. Sellest järeldub, et lisaks glükolaatse

tsükli metaboliitidele võivad dekarboksüülimisreaktsioonide substraatideks olla ka fotosünteesi teised esmasproduktid, eeldatavasti C_3- ja C_4-happed, mis oksüdeeritakse hapniku madalal kontsentratsioonil küllastuvates reaktsioonides. O_2 kontsentratsiooni 14-kordne vähendamine kutsus esile ainult 3-kordse languse glükolaatset tsüklit läbiva süsinikuvoo kiiruses. See fakt viitab võimalusele, et vähese hapnikusisaldusega keskkonnas sünteesitakse osa glükolaadist mitteoksügenaasse mehhanismi vahendusel, tõenäoselt taandusliku pentoosfosfaatide tsükli transketolaasses reaktsioonis. O_2 kontsentratsiooni vähendamisel kiirenes oluliselt sahharoosi ja C_3-hapete, eriti alaniini süntees tsütosoolis. Seejuures ei muutunud tärklise sünteesi kiirus kloroplastides. Nende tulemuste põhjal oletatakse, et hapnik avaldab kaudset toimet ATP/ADP suhtele tsütosoolis ning ortofosfaadi rakusisesele kontsentratsioonigradiendile, mille muutused kutsuvadki esile jälgitud nihked assimilaatide transpordis ja jaotumises.

ДЕЙСТВИЕ КОНЦЕНТРАЦИИ КИСЛОРОДА НА СКОРОСТИ ПОТОКОВ УГЛЕРОДА В БИОХИМИЧЕСКОЙ СИСТЕМЕ АССИМИЛЯЦИИ CO_2

Хийе ИВАНОВА, Олав КЭЭРБЕРГ, Тийт ПЯРНИК

Исследованы декарбоксилирование ранних продуктов фотосинтеза и кинетика включения ^{14}C в продукты стационарного фотосинтеза в листьях табака при разных концентрациях O_2 (21 и 1,5%). Из кинетических данных рассчитаны скорости потоков углерода и величины фондов метаболитов в биохимической системе ассимиляции CO_2. При 1,5% O_2 скорость декарбоксилирования ранних продуктов была существенно выше значения, рассчитанного на основе линейной кислородной зависимости, характерной для оксигенирования РуБФ в гликолатном цикле. Из этого вытекает, что кроме метаболитов гликолатного цикла субстратами декарбоксилирования могут быть и другие ранние продукты фотосинтеза, наиболее вероятно C_3- и C_4-кислоты, которые окисляются в реакциях, насыщающихся при низких концентрациях кислорода. 14-кратное снижение концентрации O_2 привело лишь к 3-кратному уменьшению скорости потока углерода через гликолатный цикл. Этот факт указывает на возможность того, что при низком содержании кислорода часть гликолата синтезируется по неоксигеназному механизму, вероятно, в транскетолазной реакции восстановительного пентозофосфатного цикла. Уменьшение концентрации O_2 вызывало существенное ускорение синтеза сахарозы и C_3-кислот, особенно аланина, в цитозоле. При этом скорость синтеза крахмала в хлоропластах оставалась неизменной. На основе этих данных выдвинуто предположение о косвенном действии кислорода на внутриклеточный градиент ортофосфата и на соотношение АТФ/АДФ в цитозоле, что в конечном итоге приводит к рассмотренным сдвигам в транспорте и распределении ассимилятов в фотосинтезирующей клетке.