

## CARBOXYLATION OF RIBULOSE-1,5-BISPHOSPHATE AND THE CONCENTRATION OF CO<sub>2</sub>: WHERE IS SATURATION?

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**Abstract.** The rate of carboxylation in wheat *Triticum aestivum* leaves was measured at different CO<sub>2</sub> concentrations in the steady state of photosynthesis or after the transfer to a higher CO<sub>2</sub> concentration. In the latter case, CO<sub>2</sub> uptake rates may be observed by an order of magnitude higher than the CO<sub>2</sub>-saturated steady-state rate of photosynthesis of these leaves. It is suggested that the immediate reaction of the binding of CO<sub>2</sub> representing a partial step of carboxylation should be saturated only at very high CO<sub>2</sub> concentrations if at all.

**Key words:** carboxylation, ribulose-1,5-bisphosphate, CO<sub>2</sub> concentration.

Photosynthesis of leaves of C<sub>3</sub> plants is saturated at concentrations of CO<sub>2</sub> of about 600 to 1500 ppm. Usually, at CO<sub>2</sub> saturation, carboxylation rate is limited by the concentration of ribulose-1,5-bisphosphate (RuBP) because an increase in the ambient CO<sub>2</sub> concentration causes a decrease in the level of RuBP [1–4]. Therefore, it is difficult to study *in vivo* the CO<sub>2</sub> dependence of the carboxylation reaction saturated with RuBP, especially under steady-state conditions of photosynthesis. Nevertheless, this can be done in experiments with leaves photosynthesizing at a very low [CO<sub>2</sub>], or illuminated for a few seconds in the absence of CO<sub>2</sub>. Such a treatment allows to accumulate concentrations of RuBP significantly in excess to those of the carboxylation centres of ribulose-1,5-bisphosphate carboxylase (Rubisco). The subsequent transfer of these leaves to different concentrations of CO<sub>2</sub> allows to study the dependence of the carboxylation rate with the RuBP-saturated enzyme.

$K_M(\text{CO}_2)$  of Rubisco has been estimated as being 10 to 25  $\mu\text{M}$  [5–7]. This corresponds to about 250 to 625 ppm CO<sub>2</sub> in the gas phase in equilibrium, and suggests that the CO<sub>2</sub>-saturation arrives at about 750 to 1900 ppm CO<sub>2</sub>. In transient state experiments where a leaf photosynthesizing at a steady-state rate, is transferred to a very high [CO<sub>2</sub>], uptake rates significantly higher than the CO<sub>2</sub>-saturated steady-state photosynthesis may be observed. In bean leaves preilluminated in the normal atmosphere and in the saturating light, the CO<sub>2</sub>-binding rate as high as 3000 nmol·dm<sup>-2</sup>·s<sup>-1</sup> has been observed immediately after the transfer of the leaf to 15,000 ppm CO<sub>2</sub> [3]. This exceeded 20 fold the CO<sub>2</sub>-saturated steady-state photosynthesis of these leaves. The uptake represented genuine chemical binding, as it could be seen from the product, phosphoglyceric acid. This observation has provoked the question about the nature and the real level of the CO<sub>2</sub>-saturation of the carboxylation of RuBP.

We have studied the dependence of the initial carboxylation rate on  $[\text{CO}_2]$  at sharp transfers of leaves photosynthesizing at a steady-state rate or illuminated for a few seconds in the absence of  $\text{CO}_2$  to different  $\text{CO}_2$  concentrations. Data discussed in this paper show that the RuBP-saturated carboxylation reaction increases in its rate with  $[\text{CO}_2]$  up to its very high levels and there is no sign of saturation up to 5000 to 8000 ppm.

## MATERIAL AND METHODS

Experiments were carried out with the first leaf of 9 day-old plants of wheat (*Triticum aestivum*, var. 'Saratovskaya 29') grown under fluorescent lamps in containers with the soil. The light intensity on the level of leaf tips was  $9 \text{ mW}\cdot\text{cm}^{-2}$ . Excised leaves were put with their lower ends into a plastic bag containing water and then placed into the exposure chamber. The construction and characteristics of the chamber have been described earlier [8]. The leaves were preilluminated in the chamber in the light of saturating intensity,  $40 \text{ mW}\cdot\text{cm}^{-2}$ , and flushed with  $\text{N}_2$  containing 300, 1000, 1700, or 3000 ppm  $^{12}\text{CO}_2$  and 1.5%  $\text{O}_2$ , until they reached the steady-state of photosynthesis (monitored by the infrared gas analyzer). The state of stomata during preillumination was monitored by the transpiration rate by means of a psychrometer. When a constant rate of photosynthesis had been achieved,  $^{12}\text{CO}_2$  was replaced with  $^{14}\text{CO}_2$  (300, 1000, 1700, or 3000 ppm) and the leaves were fed with the tracer for 0.2 s. In another set of experiments, leaves in the steady state of photosynthesis were, before labelling, kept in the light in the  $\text{CO}_2$ -free atmosphere for 6 s to allow them to accumulate maximum amounts of RuBP.

The chamber temperature was  $30^\circ\text{C}$ . After the labelling, the leaves were dropped into boiling 80% ethanol. From the radioactivity of the material, the amount of the  $\text{CO}_2$  taken up was calculated. Constants of the chamber applied at calculations have been published earlier [8]. Due to alterations made in the gas flow system, the time of equilibration of the gas composition in the chamber was 0.3 s in the experiments referred to in this paper. This lag has been taken into account at the calculations of data.  $^{14}\text{CO}_2$  concentrations given in Tables represent the real concentration in the chamber during the 0.2 s exposure. The RuBP content of the leaves was estimated by the postillumination  $\text{CO}_2$  uptake as described earlier [3]. Each experimental point in figures and in tables represents the average of 6 to 18 leaves.

## RESULTS

Efficiency of the illumination in the  $\text{CO}_2$ -free atmosphere, for accumulation of RuBP, was checked with leaves preilluminated at different  $[\text{CO}_2]$ . Table 1 compares the concentration of RuBP at three different  $\text{CO}_2$  concentrations applied during preillumination (the steady-state photosynthesis) and after a 6 s illumination in the  $\text{CO}_2$ -free atmosphere. The steady-state level of RuBP was inversely related to the  $\text{CO}_2$  concentration.  $[\text{RuBP}]$  was markedly increased by illumination in the  $\text{CO}_2$ -free gas phase.

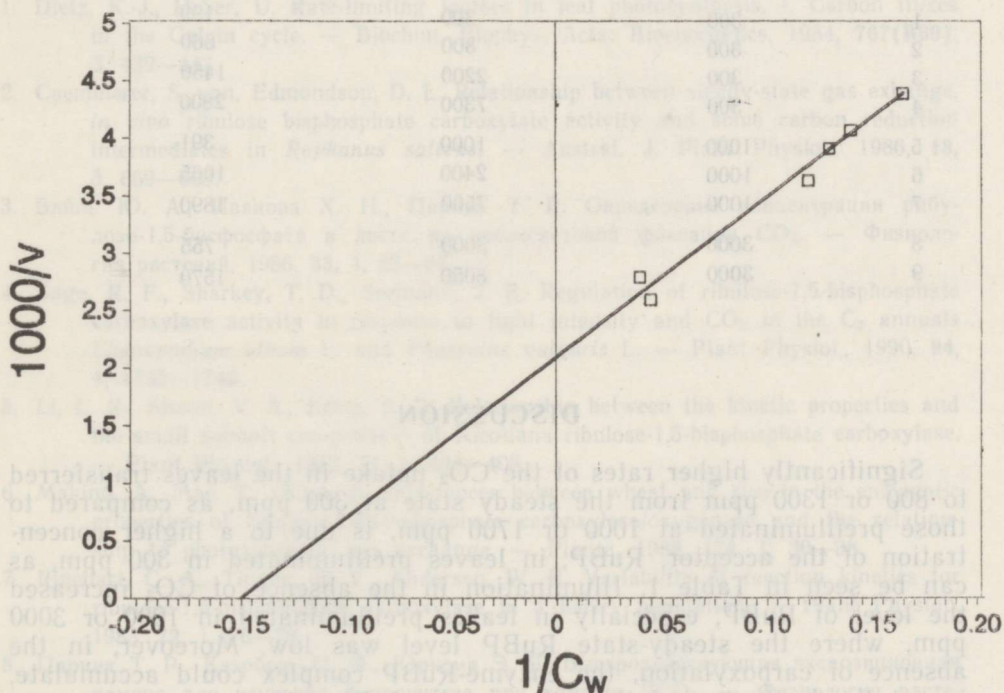
Figure depicts the double reciprocal plot of the  $\text{CO}_2$  uptake rate ( $v$ ) vs. the intracellular concentration of  $\text{CO}_2$  ( $C_w$ ) at the steady-state photosynthesis. The curve intersects the  $1/C_w$  axis and the  $1/v$  axis at points  $-0.151$  and  $2.1\cdot 10^{-3}$ , respectively, corresponding to  $K_M(\text{CO}_2) = 6.6 \mu\text{M}$  and  $V_{\max} = 481 \text{ nmol}\cdot\text{dm}^{-2}\cdot\text{s}^{-1}$ . Such a  $K_M$  predicts the saturation of

photosynthesis of these leaves at the ambient concentration of CO<sub>2</sub> of about 600 ppm. Still, as it can be seen from Table 2, significantly higher carboxylation rates than the CO<sub>2</sub>-saturated steady-state rate (5 and 6) may be obtained if the leaves were transferred from 300 ppm to 800 or 1300 ppm. Even a larger increase was observed if the leaves had been illuminated for 6 s in the CO<sub>2</sub>-free atmosphere. In this case, a rate 10 fold in excess to the CO<sub>2</sub>-saturated steady-state photosynthesis was obtained when the leaves were exposed to 11,000 ppm CO<sub>2</sub>. In another experiment, leaves photosynthesizing in the steady state in 300, 1000, or 3000 ppm CO<sub>2</sub> were transferred to a CO<sub>2</sub>-free medium, and illuminated there for 6 s. Thereafter the leaves were fed with <sup>14</sup>CO<sub>2</sub> of the steady-state concentration or higher. From Table 3 it may be seen that in all cases (except 300 ppm <sup>14</sup>CO<sub>2</sub>) significantly higher rates than at the saturated steady-state photosynthesis were observed (compare with Set 5 and 6, Table 2).

Table 1

Concentration of RuBP in wheat leaves at the steady-state photosynthesis and after 6 s illumination in the absence of CO<sub>2</sub>

[CO <sub>2</sub> ] at steady state, ppm	RuBP, nmol·dm <sup>-2</sup>	
	Steady-state level	After 6 s without CO <sub>2</sub>
300	881	926
1000	577	732
3000	171	587



Double reciprocal plot of the steady-state photosynthesis ( $v$ ) vs. intracellular CO<sub>2</sub> concentration ( $C_w$ ) in wheat leaves in the saturating light.  $v$ , nmol·dm<sup>-2</sup>·s<sup>-1</sup>;  $C_w$ , μM.

Table 2

Dependence of the RuBP carboxylation rate on the concentration of CO<sub>2</sub> during preillumination and the exposure

Exp. No	CO <sub>2</sub> concentration, ppm		Carboxylation, nmol·dm <sup>-2</sup> ·s <sup>-1</sup>	
	Preillumination	Exposure	Immediately after steady state	After 6 s illumination without CO <sub>2</sub>
1	300	300	179	187
2	300	800	500	664
3	300	1300	565	885
4	300	11000	—	2410
5	1000	1000	222	—
6	1000	3000	400	—
7	1000	5000	591	—
8	3000	3000	234	—

Table 3

The initial carboxylation rate after a 6 s illumination in the absence of CO<sub>2</sub>

Exp. No	CO <sub>2</sub> concentration, ppm		Carboxylation, nmol·dm <sup>-2</sup> ·s <sup>-1</sup>
	Preillumination	Exposure	
1	300	300	199
2	300	800	660
3	300	2200	1450
4	300	7300	2800
5	1000	1000	391
6	1000	2400	1065
7	1000	7500	1890
8	3000	3000	755
9	3000	8050	1570

## DISCUSSION

Significantly higher rates of the CO<sub>2</sub> uptake in the leaves transferred to 800 or 1300 ppm from the steady state at 300 ppm, as compared to those preilluminated at 1000 or 1700 ppm, is due to a higher concentration of the acceptor, RuBP, in leaves preilluminated in 300 ppm, as can be seen in Table 1. Illumination in the absence of CO<sub>2</sub> increased the level of RuBP, especially in leaves preilluminated in 1000 or 3000 ppm, where the steady-state RuBP level was low. Moreover, in the absence of carboxylation, the enzyme-RuBP complex could accumulate, i.e. at the beginning of the subsequent labelling its concentration was at maximum. In such a case, the initial CO<sub>2</sub> uptake rate reflected the primary binding of CO<sub>2</sub> to the complex and it did not involve the time required for the binding of RuBP and the formation of the complex.

It occurs that the binding of  $\text{CO}_2$  must be a markedly faster process than the binding of RuBP and the release of the product. An intriguing fact is that no  $\text{CO}_2$  saturation was revealed at its concentrations 10 to 15 fold in excess to those saturating the steady-state photosynthesis, even if applied immediately to leaves in the steady state.

It must be taken into account that during a 0.2 s exposure to different  $\text{CO}_2$  concentrations, the mean level of the enzyme-RuBP complex is different. The initial level of RuBP was equal for all sets preilluminated at the same  $[\text{CO}_2]$ , but the amount of  $\text{CO}_2$  bound for 0.2 s and, logically, that of consumed RuBP was several times higher if exposed to high  $\text{CO}_2$  concentrations, the average level of RuBP being lower. Therefore at higher  $\text{CO}_2$  concentrations the uptake rate is somewhat underestimated.

The highest concentration of  $^{14}\text{CO}_2$  applied in this work — 11,000 ppm — corresponds to about 0.5 mM dissolved carbon dioxide at the equilibrium with the ambient atmosphere. This is nearly 20 times higher than is required for the saturation of photosynthesis, but still, by an order of magnitude, it is lower than the concentration of carboxylation centres in the chloroplast stroma. If  $\text{CO}_2$  is bound to special centres of the enzyme molecule, prior to being bound to the acceptor, RuBP, such a concentration seems to be far below the one capable to saturate these centres. Nevertheless, the partial reaction of carboxylation representing the primary binding of  $\text{CO}_2$  is too fast to be a pacemaker at the  $\text{CO}_2$ -saturated photosynthesis.

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## RIBULOOS-1,5-BISFOSFAADI KARBOKSÜÜLIMINE JA CO<sub>2</sub> KONTSENTRATSIOON: KUS SAABUB KÜLLASTUS?

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Mõõdeti karboksüülimise kiirus nisulehtedes CO<sub>2</sub> erineva kontsentratsioonil puhul statsionaarsel fotosünteesil ja üleminekul kõrgemale kontsentratsioonile. Viimasel juhul täheldati CO<sub>2</sub> sidumise kiirust, mis ületas CO<sub>2</sub>-ga küllastatud fotosünteesikiiruse terve suurusjärgu võrra. Järeldatakse, et vahetu CO<sub>2</sub> sidumise reaktsioon, mis kujutab endast karboksüülimise osareaktsiooni, küllastub kas alles väga kõrgel CO<sub>2</sub> kontsentratsioonil või ei küllastu üldse.

## КАРБОКСИЛИРОВАНИЕ РИБУЛОЗО-1,5-БИСФОСФАТА И КОНЦЕНТРАЦИЯ СО<sub>2</sub>: ГДЕ ДОСТИГАЕТСЯ НАСЫЩЕНИЕ?

Юта ВИЙЛЬ, Тийт ПЯРНИК, Хийе ИВАНОВА

Определена скорость карбоксилирования в листьях пшеницы при разных концентрациях СО<sub>2</sub> в стационарном состоянии фотосинтеза или после перехода к более высокой концентрации СО<sub>2</sub>. В последнем случае скорость связывания СО<sub>2</sub> может превышать скорость стационарного СО<sub>2</sub>-насыщенного фотосинтеза на целый порядок. Предполагается, что непосредственная реакция связывания СО<sub>2</sub>, представляющая собой частный этап карбоксилирования, насыщается только при очень высоких концентрациях, если вообще насыщается.

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