Plant Physiol. (1984) 76, 723–729
0032-0889/84/76/0723/07/$01.00/0

Assimilatory Power (Postillumination CO_2 Uptake) in Leaves

MEASUREMENT, ENVIRONMENTAL DEPENDENCIES, AND KINETIC PROPERTIES

Received for publication March 8, 1984 and in revised form July 18, 1984

AGU LAISK,* OLAVI KIIRATS, AND VELLO OJA
Institute of Astrophysics and Atmospheric Physics, Estonian Academy of Sciences, 202444 Tôravere, Tartu, Estonia, U.S.S.R

ABSTRACT

Assimilatory power was measured in ten C_3 species by means of a rapid-response gas exchange device as the total amount of CO_2 fixed in N_2-CO_2 atmosphere after switching the light off. Different steady-state levels of the assimilatory power were obtained by varying light intensity and O_2 and CO_2 concentrations during the preexposition periods in the leaf chamber.

Within the limits of the linear part of the CO_2 curve of photosynthesis in N_2, the assimilatory power is constant, being sufficient for the assimilation of about 20 nanomoles CO_2 per square centimeter leaf. The pool starts to decrease with the onset of the CO_2 saturation of photosynthesis. Increase in O_2 concentration from 0 to 100% at 350 microliters CO_2 per liter produces a considerable decrease in the assimilatory power.

The mesophyll conductance (M) was found to be proportional to the assimilatory power (A): M = mA. The most frequently occurring values of the proportionality constant (m) (called the specific efficiency of carboxylation) were concentrated between 0.03 and 0.04 centimeter per second per nanomole A per square centimeter but the measured extreme values were 0.01 and 0.06 centimeter per second per nanomole A per square centimeter. The specific rate of carboxylation (the rate per unit A) showed a hyperbolic dependence on CO_2 conentration with the most frequent values of K_m (CO_2) ranging from 25 to 35 micromolar in the liquid phase of mesophyll cells (extremes 23 and 100 micromolar).

It is concluded that the CO_2- and light-saturated rate of photosynthesis is limited by the reactions of the formation of the assimilatory power and not by ribulose-1,5-bisphosphate carboxylase. O_2 is a competitive consumer of the assimilatory power, and the inhibitory effect of O_2 on photosynthesis is caused mainly by a decrease in the pool of the assimilatory power at high O_2 concentrations. In intact leaves, the kinetic properties of ribulose-1,5-bisphosphate carboxylase seem to be variable.

McAlister (10) observed a postillumination CO_2 uptake in wheat leaves that was related to previous photosynthesis. Using ^{14}C as a tracer, Calvin and Benson (4) and Benson and coworkers (3) found out that in green algae a 30-s preillumination time was enough for the formation of about 80 to 100% of the full CO_2-fixing capacity. In a series of extensive studies carried out on *Chlorella*, Tamiya, Miyachi, and their associates showed that the ability for light-enhanced dark CO_2 fixation was closely parallel to the photosynthetic rates of cells during their life cycle (15). The substrate for the postillumination CO_2 uptake was formed photochemically concomitant with O_2 evolution. The stationary level of the substrate pool was achieved after 40 to 50 min illumination of dark-adapted cells in an atmosphere of N_2. The stationary level of the substrate pool was diminished by raising the O_2 concentration. O_2 also accelerated the self-decay of the pool in darkness in the absence of CO_2 (14). When it appeared possible to evoke the postillumination reduction of DPIP[1] in the Hill reaction in *Chlorella* cells in amounts comparable with the light-enhanced dark CO_2 uptake, a conclusion was drawn that the light-generated substance should be a reducing agent. But no correlation was found between the changes of the pools of reduced pyridine nucleotides and the postillumination CO_2 uptake in light-dark transients (16), nor were the reduced sugars found in significant portion among the products of the light-enhanced dark CO_2 fixation (7). As it has been shown that RuBP in *Chlorella* cells can be accumulated in significant amounts, it seemed reasonable to postulate that the dominant substrate for the postillumination CO_2 uptake in C_3 plants is RuBP (12).

The phenomenon of the postillumination CO_2 uptake has also been observed in isolated chloroplasts and in higher plants (13). Assuming that in C_3 plants its substrate is mostly RuBP, studies of the kinetics of CO_2 fixation with respect to the pool of the acceptor and CO_2 concentration would give us information about the kinetic properties of the carboxylation reaction *in vivo*.

In this study the postillumination CO_2 uptake of leaves from various C_3 plants is studied by gas exchange technique with the aim of elucidating the limiting role of the substrate compound in photosynthesis and kinetics of the caboxylation reaction *in vivo*. Possibly, the postillumination CO_2 uptake does not proceed at the expense of a pool of one concrete substrate compound (*e.g.* RuBP), but as a result of rapid interconversions of intermediates of the Calvin cycle, a number of chemical compounds may be to some extent involved in the process (*e.g.* ATP, NADPH, the pool of H^+ ions in thylakoids). On this basis there arises a question of terminology. McAlister (10) called the compound capable of postillumination binding of CO_2 the 'CO_2 combining intermediate,' while Calvin and Benson (4) named it a 'reducing power.' Miyachi (12) prefers to use the experimental terminology 'light-enhanced dark CO_2 fixation' (LED). Here we adopt the term 'assimilatory power' from Arnon and coworkers (1), widening its scope from the original meaning of NADPH + ATP to the sum of all energetically rich compounds causing the postillumination gulp of CO_2 in a photosynthetic organism. However, it is most likely that the major part of the postillumination CO_2 uptake in C_3 plants is related to the pool of RuBP.

MATERIALS AND METHODS

Plants of sunflower (*Helianthus annuus* L.) and bean (*Phaseolus vulgaris* L.) were grown in a growth chamber in 5-L pots filled with soil. Combined nutrients were added (10 g/pot). Growth parameters: irradiation density 16 klux, 18/6-h day/night cycle, air temperature 27 ± 2°C/17 ± 2°C day/night, RH

[1] Abbreviations: DPIP, 2,6-dichlorophenolindophenol; RuBP, ribulose-1,5-bisphosphate; PEP, phosphoenolpyruvate.

50 to 60%, soil moisture 80% of the field capacity. At 25 d, the sunflower plants had developed 17 leaves. Excised upper leaves were used in experiments. Except with beans, for ecological measurements leaves were taken from plants growing around the Tôravere Observatory near Tartu, South Estonia, in August 1982. During the experiments, the petioles of the leaves were kept in distilled H_2O.

The gas exchange apparatus has been described elsewhere (17). Two identical independent open systems for CO_2 exchange measurements (further called 'channels') containing two gas analyzers may be tuned to different background CO_2 concentrations (0–2000 μl/l) and O_2 concentrations (0–100%). A leaf chamber (44 × 44 × 3 mm^3) may be rapidly switched into the chain of either the first or the second channel. The flow rate of gas through the chamber is 20 cm^3/s. By reducing the dimensions of all the elements of the system and by using thermostated condenser-tubes for presetting a constant low humidity before the entrance of gas into the analyzers, the response time (99.5% of full deflection) of the system was reduced to 2.3 s (with the analyzer 'Infralyt IV', 100- × 20-mm cuvettes). The leaf transpiration rate was measured by means of psychrometers (one in each channel). The CO_2 exchange rate and the intracellular CO_2 concentration (considering also CO_2 solubility in the liquid phase of mesophyll cells) were calculated as described in Laisk (8).

The methods were elaborated for the maximum precision of measuring the total amount of CO_2 assimilated by the leaf after the interruption of illumination. Though the transient process of reducing the rate of CO_2 uptake after switching the light off could be directly recorded, the hypothetical response-curve of the apparatus for the momentary stopping of the CO_2 uptake had to be theoretically redesigned for each measurement (Fig. 1a). To avoid this task, the leaf chamber was usually switched into the other channel simultaneously with darkening (Fig. 1b).

Measurements were carried out according to the following program. The gas exchange rate of a leaf was brought into a steady state under desired experimental conditions in the first channel. In the second channel, the CO_2 concentration was preset equal to the CO_2 concentration in the first channel (when not otherwise stated) using N_2 as carrier gas ($[O_2] < 0.01\%$). This avoided competitive consumption of the assimilatory power by O_2 during the measurement. Simultaneously with darkening, the leaf chamber was switched from the first channel into the second

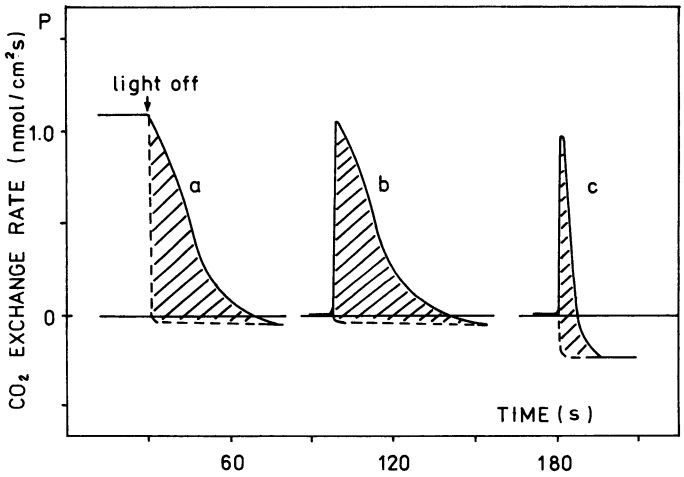


FIG. 1. Measurement of the photosynthetic assimilatory power. ——, Recorded curve of the postillumination CO_2 uptake without (a) and with switching the leaf chamber into the second channel simultaneously with darkening (b, c). – – –, hypothetical response curve of the gas analyzer to respiratory CO_2 evolution without O_2 (a, b) and in the presence of O_2 (c). Hatched area, photosynthetic assimilatory power.

one. An example of the recorded output signal of the gas analyzer in the second channel is shown in Figure 1b. The hatched area represents the integral of the postillumination CO_2 uptake. When using this method, no experimental error caused by the inertia of the system was introduced into the results.

The time of exposure of the leaf in darkness during each measurement of the assimilatory power did not exceed 2 min at low CO_2 concentrations (at higher CO_2 concentrations, the assimilatory power was consumed faster). During this time, the preexposition conditions in the first channel were changed, the leaf chamber was switched back to the first channel, and the light was turned on. The stabilization of the photosynthetic rate at new preexposition conditions occurred within 5 min. During the preexposition of leaves at low CO_2 concentrations in N_2, a slow tendency to photoinhibition could be observed (18), but this effect did not exceed a few percent from the steady-state rate of photosynthesis.

To obtain the full amount of carboxylation, the bottom line of the hatched area in Figure 1 should follow the course of respiration. In the absence of O_2 during the preconditioning periods, only CO_2 evolution from mitochondrial (dark) respiration has to be considered. By extrapolating the photosynthesis *versus* intracellular CO_2 concentration curves (PC_w curves) to the zero CO_2 concentration in the mesophyll cells, it was found that in full-grown sunflower leaves the rate of mitochondrial respiration in the light and in the absence of O_2 was reduced to a very low value. On this basis, the rate of mitochondrial respiration was not determined in any particular case, but assumed to be close to zero at the moment of switching the light off. For the absence of any information about the course of respiration during the postillumination CO_2 uptake, a straight line was drawn from zero to the respiration rate after the full cessation of CO_2 uptake. In case the leaf was preconditioned in an atmosphere containing O_2, photorespiratory CO_2 evolution was determined by the extrapolation of the PC_w curve in any particular case (Fig. 1c). Due to the rapid change of CO_2 concentration in the chamber (0.5 s) and rapid reaction of the apparatus, the rate of CO_2 evolution from the leaf right after switching the chamber to the zero CO_2 concentration was considered to be close to the photorespiration rate under the steady-state (non-zero CO_2) conditions of photosynthesis. The following transient rise and fall of the rate of CO_2 evolution under zero CO_2 concentration could be easily recorded. The error introduced by incorrect accounting of respiration is negligible in an anaerobic atmosphere where respiration is low, but may be significant at high concentrations of O_2.

To reduce the experimental error of measurements, the following corrections were taken into account. The bottom line of respiration was bent at the beginning according to the response curve of the apparatus (Fig. 1). Though background CO_2 concentrations in both the channels were equal, the actual CO_2 concentration in the leaf chamber was lower in the preconditioning channel due to leaf CO_2 uptake. Together with the assimilatory power in the leaf, a certain amount of gas equal to the volume of the leaf chamber and of the connecting tubes (about 8 cm^3) with the lower CO_2 concentration was also transmitted from the first into the second channel, that brought about additional deflection not produced by the assimilatory power. This correction was proportional to the difference between the CO_2 concentrations in the channels and it was taken into account in calculations. Its value varied from 0.3 to 0.5 $nmol/cm^2$ when the background concentrations of CO_2 in both the channels were equal, but could be larger if measurements were carried out at CO_2 concentrations different from those during the preconditioning of the leaf.

At low CO_2 concentrations, the time required for the full exhaustion of the assimilatory power lengthened to several tens of seconds. During this time a considerable self-decay of the

assimilatory power occurred. The rate coefficient of the self-decay of the assimilatory power was determined and the process was taken into account in calculations of the actual initial values of the assimilatory power. This correction was about 10% at low CO_2 concentrations.

Of all the sources of error, the most essential is the unknown course of photorespiration during the measurements of the assimilatory power. A rough estimate for the total error of the method yields a value of 0.5 nmol CO_2/cm^2.

Below, some experimental data will be given in MA axes where M is the mesophyll conductance (the initial slope of the CO_2 curve of photosynthesis with respect to the intracellular CO_2 concentration [PC_w curve]) and A is the pool of the assimilatory power measured as postillumination CO_2 uptake. It will be shown that the carboxylation rate follows Michaelis-Menten type kinetics with respect to CO_2 at a constant pool of the assimilatory power. From measurements of the net rate of CO_2 uptake (P) at different intracellular CO_2 concentrations (C_w), the initial slope of the PC_w curve (M) was calculated and plotted against the value of A.

RESULTS

Self-Decay of the Assimilatory Power. This was measured in darkness in a CO_2- and O_2-free atmosphere. It was assumed that under these conditions the reactions consuming the assimilatory power are depressed and self-decay is the only cause of the decrease of the pool in the dark.

The leaf gas exchange rate was stabilized in N_2, at the background CO_2 concentration C_o = 232 μl/l, irradiation density I = 42.8 mw/cm^2 (absorbed PAR), leaf temperature t_l = 23.6°C. Then the leaf chamber was switched into the second channel in which the zero CO_2 concentration in N_2 had been preset, and it was simultaneously darkened. After the time of exposure to darkness, the leaf chamber was switched back into the first channel to C_o = 232 μl CO_2/l without opening the light source. The resulting deflection in the output signal of the gas analyzer in the first channel, similar to that in Figure 1b, was due to the assimilatory power left in the leaf after the time of exposure in the dark. The experiment was repeated with different times of exposure in the second channel (Fig. 2). The curve in Figure 2 is exponential with the time constant of 128 s. Evidently, the self-decay of the assimilatory power proceeds according to the first-order kinetics with the rate constant $k_s = 7.8 \cdot 10^{-3}$ s^{-1} at 22.5°C (leaf temperature in the dark). This value of k_s was used for calculations of correction for the self-decay of the assimilatory power in other experiments.

Dependence of the Assimilatory Power on CO_2 Concentration. The CO_2 exchange rate of the leaf was brought into a steady state at different CO_2 concentrations from 90 to 1960 μl/l at a saturating irradiation density 43.2 mw/cm^2, t_l = 23.7°C. At every CO_2 concentration, the assimilatory power was measured (Fig. 3). The pool of the assimilatory power remains high over the CO_2-limited range of photosynthesis. In this experiment, the maximum assimilatory power in a sunflower leaf was 21 nmol/cm^2, but in some cases values as high as 27 nmol/cm^2 could be recorded. It means that the pool of the assimilatory power is capable of maintaining an average rate of CO_2 uptake 2 nmol/cm^2·s (typical rate at the atmospheric CO_2 concentrations in N_2 in sunflower leaves) during 10 to 14 s after the interruption of illumination.

When the CO_2 concentration was raised to the plateau of the CO_2 curve of photosynthesis, the pool of the assimilatory power decreased. For example, at 1960 μl/l (CO_2 concentration in the liquid phase of cells C_w = 56 nmol/cm^3) the pool of only 5 nmol/cm^2 was measured.

Effect of O_2 on the Pool of the Assimilatory Power. This was measured at a constant background CO_2 concentration C_o = 350 μl/l after reaching the steady-state conditions of photosynthesis at different O_2 concentrations. The presence of O_2 brings about significant decrease in the assimilatory power and in the net rate of CO_2 uptake (Fig. 4). In 100% O_2, the pool is only 2.7 nmol/cm^2.

Dependence of the Assimilatory Power on Irradiation Density. Starting from the steady-state photosynthesis at C_o = 184 μl/l in N_2 at an irradiation density I = 52.9 mw/cm^2, t_l = 23.9°C, the light intensity was reduced by means of neutral filters. After 60 s, the rate of CO_2 uptake reached minimum. This moment was

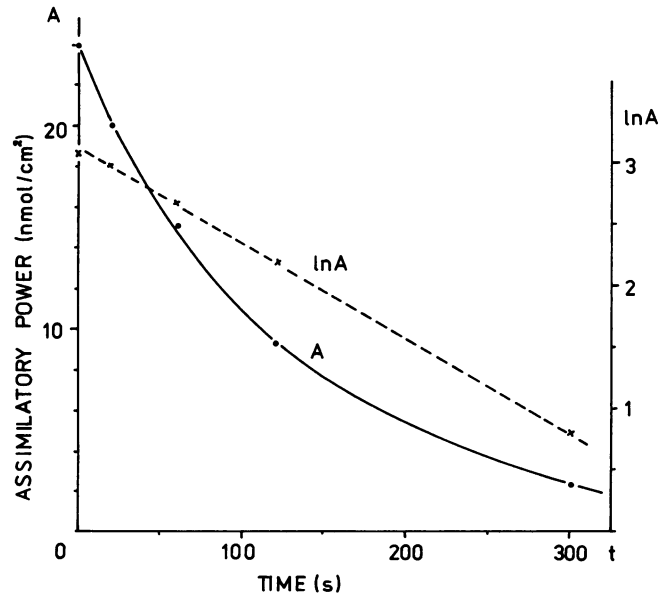


FIG. 2. Self-decay of the assimilatory power. Sunflower leaf preexposed to 232 μl/l CO_2 in N_2, irradiation density 42.8 mw/cm^2 absorbed PAR, leaf temperature 23.6°C, then switched to 0 μl/l CO_2 and darkened, after the time interval (t) switched back to 232 μl/l CO_2, the following total dark CO_2 assimilation measured and plotted against t.

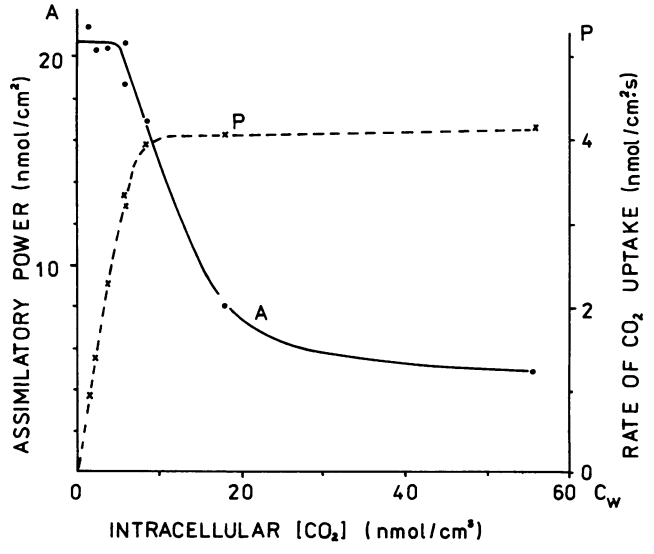


FIG. 3. CO_2 curves of the rate of CO_2 uptake (P) and the assimilatory power (A) of a sunflower leaf. The leaf was preexposed to different CO_2 concentrations in the atmosphere of N_2 at the saturating irradiation density 43.2 mw/cm^2 absorbed PAR, leaf temperature 23.7°C. Under steady-state conditions of photosynthesis, light was interrupted and the assimilatory power measured as shown in Figure 1b.

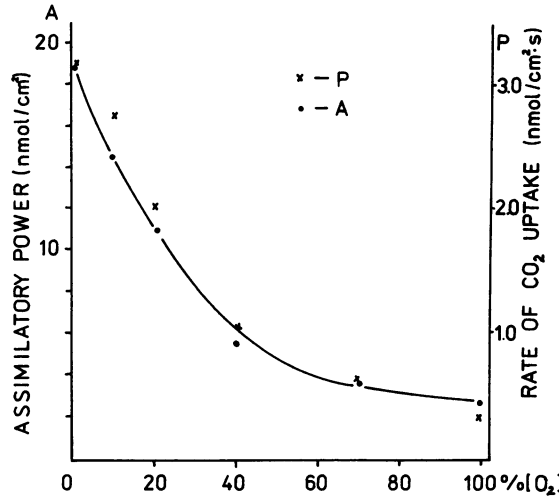


FIG. 4. Effect of O_2 on the pool of the assimilatory power (A) and the net rate of CO_2 uptake (P). Sunflower leaves were preexposed to 350 μl/l CO_2 and different O_2 concentrations, irradiation density 43.2 mw/cm², leaf temperature 23.7°C, then light was interrupted and the postillumination CO_2 uptake was measured in N_2 at 350 μl/l CO_2 as shown in Figure 1c.

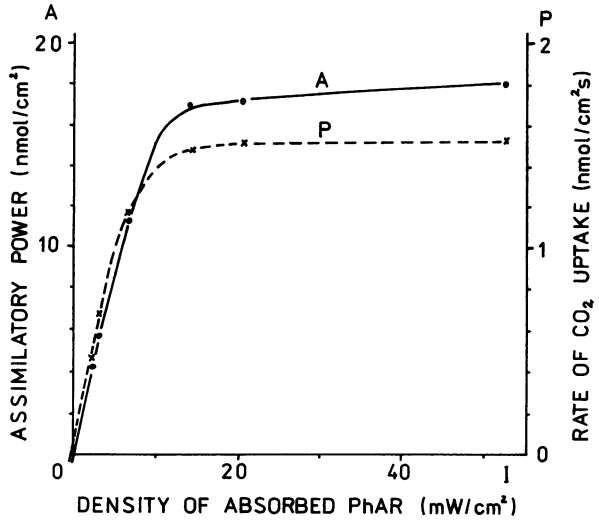
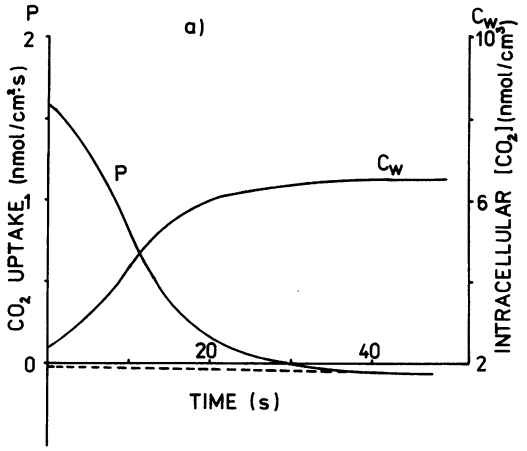


FIG. 5. Light curves of the rate of CO_2 uptake (P) and of the assimilatory power (A). Sunflower leaf was preexposed to 184 μl/l CO_2 in N_2 at irradiation density 52.9 mw/cm², leaf temperature 23.9°C. Then irradiation density was reduced, after 60 s illumination was interrupted, and the postillumination CO_2 uptake was measured. Befure measurements at each reduced irradiation density, the photosynthetic rate was stabilized at 52.9 mw/cm².

chosen for measuring the assimilatory power proceeding from the considerations that at this moment its pool had already obtained a new equilibrium value while other light-dependent readjustments in the photosynthetic apparatus were only beginning. Measurements at different light intensities were started from the stabilization of the photosynthetic rate at the full light intensity. From Figure 5, it is evident that the light curve of the assimilatory power matches the course of the light curve of photosynthesis.

Dynamic Relationships between Photosynthesis and the Assimilatory Power. The simplest way to obtain different pairs of values of the rate of CO_2 uptake and the pool of the assimilatory power is monitoring the postillumination decay-curve of photosynthesis. In the course of the curve, the assimilatory power and



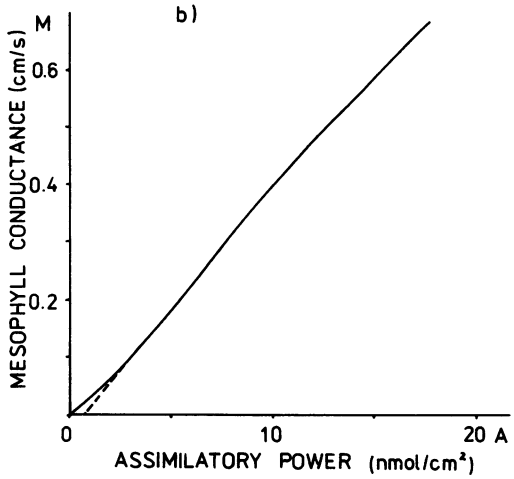


FIG. 6. Postillumination decay of the rate of net CO_2 uptake (P) in a sunflower leaf and the corresponding course of the CO_2 concentration in mesophyll cells (C_w) (a) and relationship between the mesophyll conductance (M) and the pool of the assimilatory power (A) calculated from data in 'a' (b). The leaf was exposed to 206 μl/l CO_2 in N_2, irradiation density 38 mw/cm², leaf temperature 23.7°C in a steady state, and light was disrupted at the time 0.

the rate of carboxylation decline from their initial values to zero. At low CO_2 concentrations, the process is sufficiently slow to be traced by the rapid-response recording system. In Figure 6a, the postillumination decay curve of the CO_2 uptake rate (P) and the correspnding change in the CO_2 concentration in the mesophyll cells (C_w) are shown for a sunflower leaf. Within 30 s, the rate of CO_2 uptake declines from 1.6 nmol/cm²·s to zero. At the same time the CO_2 concentration in the mesophyll cells rises from 2.3 to 6.5 nmol/cm³. Within the first 1.2 s, the decay curve of photosynthesis may be distorted by the inertia of the recording system. The results show that the rate of CO_2 uptake starts to decline immediately after switching the illumination off. There is no horizontal part at the beginning of the decay curve of photosynthesis which would indicate the saturation of the carboxylation reaction with the acceptor of CO_2.

Kinetics of Carboxylation with Respect to the Assimilatory Power and CO_2 in Leaves. The pool of the assimilatory power (A) at different moments of the decay-curve of photosynthesis (Fig. 6) was found by integrating the rate of carboxylation from the particular moments to the end of the curve. This procedure revealed the capacity of the leaf for further fixing CO_2 at any moment of the postillumination decay curve of carboxylation.

At the same points of the curve, the mesophyll conductance (M) was calculated. The curve relating the mesophyll conductance to the pool of the assimilatory power (the MA curve) is shown in Figure 6b. The curve is slightly sigmoidal. This sigmoidity may be caused by the beginning inactivation of RuBP carboxylase to the end of the decay curve of photosynthesis in the dark; or it may be an apparent result of the presence of chloroplasts having different proportionality constants between M and A in the leaf; it may also reflect the presence of β-carboxylation of PEP. Leaving aside the initial concave part of the curve, a reasonable approximation of the relationship between the assimilatory power (A) and the mesophyll conductance (M) is proportional dependence M = mA. In this experiment, the value of m = 0.040 cm/s·nmol·cm^2. Within the proportionality, constant m will be referred to as the 'specific efficiency of carboxylation.'

The values of the mesophyll conductance were also calculated for the steady-state experimental data presented in Figures 5 and 6 and plotted against the assimilatory power. The points lay almost on a straight line when K_m (CO_2) = 30 μM was used in the calculations (Fig. 7). The specific efficiency of carboxylation obtained from these experiments was m = 0.035 or 0.038 cm^3/s·nmol, respectively.

The kinetics of carboxylation with respect to CO_2 can be obtained from CO_2 curves of photosynthesis and the assimilatory power (Fig. 3). To reduce the measured rates of CO_2 uptake to a constant pool of the assimilatory power, we shall introduce the term of the 'specific rate of carboxylation' (E) which denotes the rate of carboxylation at a given CO_2 concentration per unit assimilatory power: E = F/A. In Figure 8, the specific rate of carboxylation is plotted against the CO_2 concentration in the mesophyll cells. The curve resembles a typical Michaelis-Menten hyperbola for which the K_m (CO_2) value of about 55 μM may be obtained from the double-reciprocal plot.

Ecophysiological Dispersion of the Kinetic Properties of the Carboxylation Reaction. To establish the kinetics of carboxylation with respect to CO_2 and the assimilatory power being similar or not in different species, leaves of various plants growing in the vicinity were taken to the laboratory. Applying different CO_2 concentrations and light intensities, pairs of steady-state values of the CO_2 uptake rate and the pool of the assimilatory power were obtained. Characteristic parameters of kinetics of CO_2 uptake in these species obtained from MA and EC_w plots and the postillumination curves of photosynthesis, as shown above, are summarized in Table I. Cabbage and potato excluded, all the other experiments revealed the values of m between 0.03 and 0.04 cm^3/s·nmol A. In many cases, the value of K_m (CO_2) ranged between 25 and 35 μM, but values as high as 83 and 100 μM also occurred. However, the accuracy of determination of the K_m (CO_2) is rather low and the high values may well be artifacts.

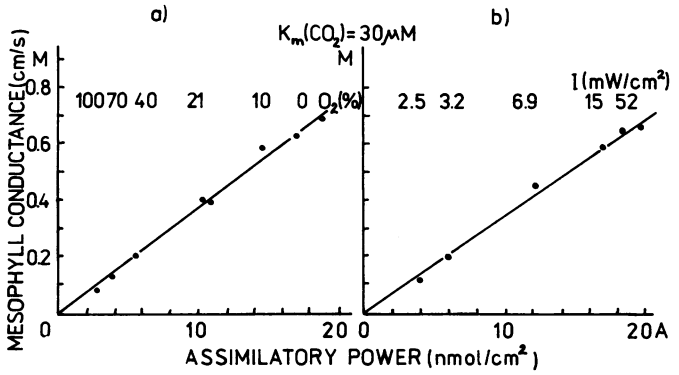


FIG. 7. Relationships between the mesophyll conductance (M) and the pool of the assimilatory power (A) at different O_2 concentrations (a) and light intensities (b) calculated from the steady-state experiments in Figures 4 and 5.

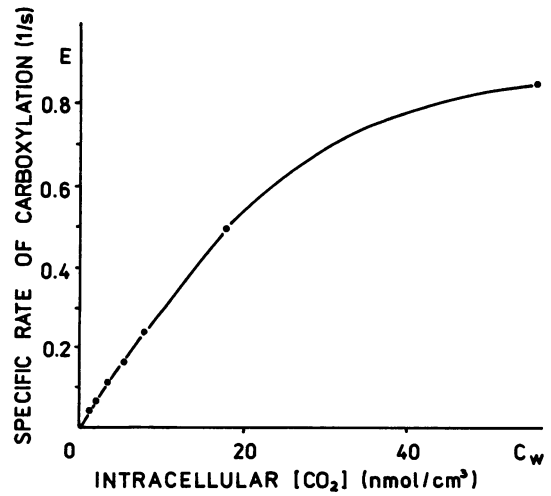


FIG. 8. Relationship between the specific rate of carboxylation E = F/A and the intracellular CO_2 concentration C_w calculated from the experiment in Figure 3.

DISCUSSION

At limiting CO_2 concentrations, the pool of the assimilatory power in sunflower leaves is capable of binding about 20 nmol CO_2/cm^2 of the leaf. As the dry weight of the leaves used in experiments was on an average 5 mg/cm^2, the pool is about 4 nmol/mg. In *Chlorella ellipsoidea*, the pool of R varied from 5 to 6 nmol/mg (23) which is in approximate agreement with our results. The rate constant of self-decay of the assimilatory power from our experiments ($k_s = 7.8 \cdot 10^{-3}$ s^{-1}) is in good agreement with the values of the same constant for *Chlorella* (7.6–10.5·10^{-3} s^{-1} (11).

Assuming that the assimilatory power is in a one-to-one stoichiometric ratio with fixed CO_2, and there is 30 to 45 μl chloroplast stroma per mg Chl (2), the approximate concentration of the assimilatory power in chloroplasts would be about 11 to 17 mM. In relation to the leaf Chl content, the concentration would be about 400 nmol/mg Chl or 0.36 mol/mol Chl. This is close to the ratio found by McAlister for wheat (10).

An interesting question is the chemical nature of the assimilatory power. The simplest version would be to identify the assimilatory power with the acceptor of CO_2 RuBP. This assumption is supported by the kinetic properties of the carboxylation reaction with respect to the assimilatory power and CO_2 found above. The pools of RuBP measured in cells and chloroplasts appear to be close to the pool of the assimilatory power. In *Chlamydomonas reinhardtii*, the concentration of RuBP at low partial pressures of CO_2 and O_2 was found to be 325 nmol/mg Chl (5). In spinach chloroplasts, the RuBP levels reached 240 nmol/mg Chl in the absence of O_2 and CO_2, and dropped when adding CO_2 (22). In wheat seedlings at 0.1% O_2 and 100 μl/l CO_2, over 400 nmol RuBP/mg Chl accumulated, while at the air level of O_2 the maximum 300 nmol/mg Chl was observed at 350 μl CO_2/l (19). In soybean leaflets, RuBP was in maximum at low levels of CO_2 and O_2, and dropped when the concentration of either CO_2 or O_2 was raised, but the absolute levels of RuBP did not exceed 70 nmol/mg Chl (6). The dependencies of RuBP pools on intracellular CO_2 pressure in bean leaves quite similar to that presented in Figure 3 (for the assimilatory power) were obtained in Badger *et al.* (2). The maximum RuBP pool (about 30 nmol/mg protein or 34 nmol/cm^2) was observed at 20 mbar O_2 and below 200 μbar CO_2. The RuBP level started to decrease

Table I. *Kinetic Characteristics of the Photosynthetic CO_2 Uptake of Leaves of Different Species Revealed from Gas Exchange Measurements (22°C)*

Exp. No.	Species	m_d[a]	m_s[a]	K_m (CO_2)[b]	V_{max}[c]	A_m[d]	ζ[e]	A_m/ζ	M_m[f]	F_m[g]
1.	Alder, *Alnus incana* (L.) Moench	0.028	0.032			18.7	5.93	3.15	0.49	4.04
2.	Lilac, *Syringa vulgaris* L.	0.033	0.030	67	2.0	21.1	10.23	2.07	0.53	3.32
3.	Willow, *Salix caprea* L.	0.037	0.034	83	2.9	16.3	5.72	2.85	0.52	4.96
4.	Foalfoot, *Tussilago farfara* L.	0.034	0.033	100	3.3	13.0	5.54	2.34	0.40	3.09
5.	Burdock, *Arctium tomentosum* Mill.	0.037	0.032	33.3	1.05	9.6	2.95	3.25	0.34	2.15
6.	Cabbage, *Brassica oleracea* L.	0.010	0.0087	30.3	0.26	53.0	9.07	5.84	0.43	
7.	Wheat, *Triticum aestivum* L.	0.036	0.027			19.0			0.53	4.0
8.	Potato, *Solanum tuberosum* L.	0.096	0.062	23.2	1.43	7.2	4.49	1.60	0.44	2.43
9.	Potato, *Solanum tuberosum* L.	0.053	0.033			18.0	4.43	4.06	0.61	4.19
10.	Potato, *Solanum tuberosum* L.	0.027				26.0			0.62	
11.	Sunflower,[h] *Helianthus annus* L.	0.035	0.036	31.2	1.11	16.5	3.38	4.88	0.58	6.58
12.	Sunflower, *H. annuus* L.		0.042	26.3	1.11	17.5			0.61	4.55
13.	Sunflower, *H. annuus* L.		0.035	31.3	1.11	26.2	3.45	7.55	0.90	5.63
14.	Sunflower, *H. annuus* L.		0.035	26.3	0.91	16.4	4.70	3.49	0.56	5.08
15.	Sunflower, *H. annuus* L.		0.030	55.0	1.67	21.4	3.54	6.04	0.65	4.16
16.	Sunflower, *H. annuus* L.	0.062				15.2			0.88	
17.	Bean, *Phaseolus vulgaris* L.	0.057	0.048	20.0	0.91	9.8			0.48	2.15

[a] Specific efficiency of carboxylation, $cm^3/s \cdot nmol$; d, from transient; s, from steady-state measurements.
[b] Michaelis-Menten constant of carboxylase *in vivo*, μM.
[c] Maximum turnover rate of the carboxylation per unit assimilatory power, 1/s.
[d] Maximum pool of the assimilatory power, $nmol/cm^2$.
[e] Dry weight of the leaf blade, mg/cm^2.
[f] Mesophyll conductance at A_m cm/s (directly measured).
[g] Maximum CO_2 uptake rate measured at CO_2 and light saturation, $nmol/cm^2 \cdot s$.
[h] Sunflower grown in the field.

just after the onset of CO_2 saturation of photosynthesis when CO_2 concentration was gradually raised. At 21% O_2, the maximum RuBP pool did not exceed 15 nmol/mg protein at 200 μl CO_2/l.

These results indicate that the maximum absolute value and the kinetic behavior of the RuBP pool under various CO_2 and O_2 concentrations are similar to the absolute value and to the CO_2 and O_2 dependencies of the assimilatory power.

Assuming that the assimilatory power is identical to the pool of RuBP, there remains one contradiction to be solved. The sunflower leaves used in experiments contained 34% protein by dry weight. Using the correlation between the concentration of RuBP carboxylase and the total protein content found for spinach and soybean leaves in Seeman and Berry (21), a value for the carboxylase concentration would be 0.25 mg/cm^2 = 3.6 nmol active sites/cm^2 = 80 μmol active sites/g Chl. If the amount of RuBP exceeds this value, the free RuBP would be saturating for the carboxylase reaction since the K_m (RuBP) of carboxylase from sunflower is only about 120 μM (20). From this, one can deduce that the time course of the postillumination decrement of the rate of CO_2 uptake should be a two-phase curve: at first, the rate of CO_2 uptake has to remain constant until 50 to 60% of the assimilation power will be consumed and only after that an exponential drop will be observed. In actually recorded curves (Fig. 6), the saturation phenomenon is absent which means that the actual number of RuBP molecules in chloroplasts does not considerably exceed the number of active sites of the enzyme. Badger *et al.* (2) have faced the same controversy and propose the following possible explanations: (a) the number of RuBP-carboxylase active sites per molecule is higher than assumed; (b) protein measurements are erroneous; (c) activation of carboxylase may vary in parallel with the RuBP pool size; (d) K_m (RuBP) *in vivo* is higher than measured *in vitro*. We believe that 3- to 4-fold underestimation of the RuBP carboxylase content in the leaf is rather unlikely. The time constant for the postillumination decay of the CO_2 uptake rate is firmly dependent on the CO_2 concentration at whatever the assimilatory power is consumed, being the longer the lower is the CO_2 concentration (data not shown). Therefore, it is also rather unlikely that the postillumination drop of the CO_2 uptake rate is primarily caused by inactivation of the carboxylase. The latter is generally a slower process than the postillumination decay of photosynthesis and it should be even more rapid at lower CO_2 concentrations. We have no evidence against the explanations concerning the number of active sites of the carboxylase or its K_m (RuBP) *in vivo*.

Dependencies of the assimilatory power on CO_2 and O_2 concentrations and light intensity enable one to make conclusions about its place in the sequence of the limiting reactions of photosynthesis. From Figure 2, it can be seen that if the carboxylation rate is limited by the low concentration of CO_2, the internal mechanism of synthesis of the assimilatory power will maintain a high and constant pool of it. But at higher CO_2 concentrations, the reactions of synthesis of the assimilatory power can no more meet the growing requirements of the carboxylation process and limitation is transferred to the chain of synthesis of the assimilatory power. The raising of the CO_2 concentration in the limits of the plateau of the CO_2 curve of photosynthesis leads to a decrease in the pool of RuBP. Therefore, in accordance with Laisk and Oja (9) the CO_2- and light-saturated (potential) rate of photosynthesis is determined by the capacity of reactions of formation of the assimilatory power and not by the maximum turnover rate of RuBP carboxylase.

Oxygen is a competitive consumer of the assimilatory power (23). At an ambient CO_2 concentration of 350 $\mu l/l$, the presence of 21% O_2 causes a 42% decrease in the pool of the assimilatory power and 36% decrease in the net rate of CO_2 uptake compared to the anaerobic atmosphere. The effect of oxygen on the net rate of CO_2 uptake is compensated, compared to its effect on the mesophyll conductance, by an increase in the intracellular CO_2 concentration at lower rates of CO_2 uptake. From these experiments, it follows that the inhibitory effect of O_2 on gross photosynthesis is mainly the result of a decrease in the pool of the

assimilatory power caused by its competitive consumption by atmospheric oxygen.

The dependence of the mesophyll conductance on the pool of the assimilatory power (the MA curve) turned out to be almost linear. Sometimes there was observed a slight concavity of the dynamic MA curve at the low levels of the assimilatory power. The existence of a small activity of β-carboxylation of PEP in sunflower leaves that decreases more slowly in the dark than the carboxylation of RuBP would explain this result (7).

The kinetic properties of the carboxylation reaction with respect to CO_2 resemble the kinetics of carboxylase to this substrate. K_m (CO_2) of the carboxylation reaction at a constant pool of the assimilatory power ranges frequently between 25 and 35 μM (Table I). In later studies, the most frequently occurring values of the K_m (CO_2) of carboxylase vary between 10 and 40 μM (24, 25). The lowest *in vivo* values of the K_m (CO_2) seem to be somewhat higher than the lowest values obtained *in vitro*. Variations in K_m (CO_2) in Table I are mostly produced by variations in the V_{max}, that suggests that the turnover rate of one active site of carboxylase may be a variable parameter in intact leaves. But, in any case, variations in the V_{max} of the enzyme are not reflected in the maximum value of the CO_2-saturated photosynthetic rate (F_m) (Table I) since the latter is limited by the rate of resynthesis of the acceptor of CO_2 and not by the V_{max} of carboxylase. For instance, in experiment 13 with sunflower the measured F_m was 5.63 nmol/cm^2·s, but if the maximum pool of the assimilatory power (A_m) had been maintained at saturating CO_2 concentrations, the rate would have been $26.2 \times 1.1 = 29.1$ nmol/cm^2·s. Such a high turnover rate of carboxylase *in vivo* is not only theoretically estimated, but values as high as 20 nmol/cm^2·s are also measured in short pulses of CO_2 (9).

Table I shows that the specific efficiency of carboxylation (m) is also a variable parameter. In most cases, the values of m are concentrated in the range between 0.03 and 0.04 cm^3/s·nmol A, but values as low as 0.01 cm^3/s·nmol A and as high as 0.06 cm^3/s·nmol A also occurred.

As the specific efficiency of carboxylation (m) was measured with greater precision than K_m (CO_2), this confirms the conclusion that the kinetic parameters of th RuBP-carboxylating system *in vivo* may be subject to internal regulation. The high values of m (up to 0.06 cm^3/s·nmol) measured in potato and sunflower leaves are interesting. It may mean that in intact leaves the RuBP carboxylase is usually partially activated and the potential capability of the enzyme is higher than can be realized under *in vivo* conditions in leaves.

LITERATURE CITED

1. ARNON DI, FR WHATLEY, MB ALLEN 1958 Assimilatory power in photosynthesis. Science 127: 1026–1034
2. BADGER MR, TD SHARKEY, S VON CAEMMERER 1984 The relationship between steady-state gas exchange of bean leaves and the levels of carbon-reduction-cycle intermediates. Planta 160: 305–313
3. BENSON AA, M CALVIN, VA HAAS, S ARONOFF, AG HALL, JA BASSHAM, JW WEIGL 1949 C^{14} in photosynthesis. *In* J Franck, WE Loomis, eds, Photosynthesis in Plants. Iowa State College Press, Ames, p 381
4. CALVIN M, AA BENSON 1948 The path of carbon in photosynthesis. Science 107: 476–478
5. COLLATZ GJ 1978 The interaction between photosynthesis and ribulose P_2 concentration—effects of light, CO_2 and O_2. Carnegie Inst Year Book 77: 248–251
6. HITZ WD, CR STEWART 1980 Oxygen and carbon dioxide effects on the pool size of some photosynthetic and photorespiratory intermediates in soybean (*Glycine max* L. Merr.). Plant Physiol 65: 442–446
7. HOGETSU D, S MIYACHI 1970 Effect of oxygen on the light-enhanced dark carbon dioxide fixation in *Chlorella* cells. Plant Physiol 45: 178–181
8. LAISK A 1977 Kinetics of Photosynthesis and Photorespiration of C_3-Plants. Nauka, Moscow (in Russian)
9. LAISK A, V OJA 1974 Leaf photosynthesis under short pulses of carbon dioxide: carboxylation reaction in vivo. Fiziol Rastenii (Sov Plant Physiol) 21: 1123–1131 (in Russian)
10. MCALISTER ED 1939 The chlorophyll-carbon dioxide ratio during photosynthesis. J Gen Physiol 22: 613–636
11. MIYACHI S 1959 Effect of poisons upon the mechanism of photosynthesis as studied by the preillumination experiments using carbon-14 as a tracer. Plant Cell Physiol 1: 1–15
12. MIYACHI S 1979 Light-enhanced dark CO_2 fixation. *In* M Gibbs, E Latzko, eds, Encyclopedia of Plant Physiology, New Series, Vol 6, Photosynthesis II. Springer-Verlag, Heidelberg, pp 68–71
13. MIYACHI S, D HOGETSU 1970 Light-enhanced carbon dioxide fixation in isolated chloroplasts. Plant Cell Physiol 11: 927–936
14. MIYACHI S, S IZAWA, H TAMIYA 1955 Effect of oxygen on the capacity of carbon dioxide fixation by green algae. J Biochem 42: 221–244
15. NIHEI T, T SASA, S MIYACHI, K SUZUKI, H TAMIYA 1954 Change of photosynthetic activity of *Chlorella* cells during the course of their normal life cycle. Arch Microbiol 21: 156–166
16. OH-HAMA T, S MIYACHI 1959 Effects of illumination and oxygen supply upon the levels of pyridine nucleotides in *Chlorella* cells. Biochim Biophys Acta 34: 202–210
17. OJA V 1983 A quick-response gas measuring device for studying the kinetics of leaf photosynthesis. Fixiol Rastenii (Sov Plant Physiol) 30: 1045–1052 (in Russian)
18. OSMOND CB 1981 Photorespiration and photoinhibition. Some implications for the energetics of photosynthesis. Biochim Biophys Acta 639: 77–98
19. PERCHOROWICZ JT, RG JENSEN 1983 Photosynthesis and activation of ribulose bisphosphate carboxylase in wheat seedlings. Regulation by CO_2 and O_2. Plant Physiol 71: 955–960
20. RANTY B, G CAVALIER1982 Purification and properties of ribulose 1,5-bisphosphate carboxylase from sunflower leaves. Planta 155: 388–391
21. SEEMAN JR, JA BERRY 1982 Interspecific differences in the kinetic properties of RuBP carboxylase protein. Carnegie Inst Year Book 81: 78–83
22. SICHER RC, AL HATCH, DK STUMPF, RG JENSEN 1981 Ribulose 1,5-bisphosphate and activation of the carboxylase in the chloroplast. Plant Physiol 68: 252–255
23. TAMIYA H, S MIYACHI, T HIROKAWA 1957 Some new preillmination experiments with carbon-14. *In* H Gaffron, AH Brown, CS French, R Livingstone, EI Rabinovich, BL Strehler, NE Tolbert, eds, Research in Photosynthesis. Interscience Publishers, New York, pp 213–223
24. YEOH HH, MR BADGER, L WATSON 1980 Variations in K_m (CO_2) of ribulose-1,5-bisphosphate carboxylase among grasses. Plant Physiol 66: 1110–1112
25. YEOH HH, MR BADGER, L WATSON 1981 Variations in kinetic properties of ribulose-1,5-bisphosphate carboxylases among plants. Plant Physiol 67: 1151–1155