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Pressure effect on rate of production of glucose-equivalent in plant cells

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Abstract. The rate of glucose equivalent production in C_4 green plants is investigated as a function of the intercellular partial pressure of CO_2, so as to find the precise physical chemistry of photosynthesis. Expressions are first formulated for the dependence of photochemical efficiency and of rubisco activation on pressure. Then a pressure-dependent rate law is derived. The latter is successfully tested for two specific C_4 plants, namely, *Panicum antidotale* and *Panicum coloratum*.

Keywords. C_4 Green plants; rate of equivalent production; pressure effect.

1. Introduction

Research on green plant photosynthesis has gained a tremendous momentum for several decades.[1–4] The group of C_4 plants constitutes a major part of plant population, and several experimental studies have been performed on them.[2] An extensive amount of research has been carried out on the effect of intensity of light, the duration of exposure to sun rays, temperature, CO_2 pressure, and stomatal conductance.[1,3] Ghannoum *et al* investigated the photosynthetic mechanism of young C_4 leaves and showed its differences with C_3-photosynthetic mechanism.[2b] Crafts–Brandner and Salvucci analysed the impact of high temperature and CO_2 internal pressure on the net rate of photosynthesis.[3i] Research has also been conducted on the activity of the enzymes involved in the photosynthetic process, especially on the activation and competitive inhibition processes of the enzyme 1,5-ribulose biphosphate carboxylase/oxygenase (rubisco).[1d,3] Rubisco is the most influential enzyme of the whole process as it catalyses the conversion of CO_2 to 3PGA. Farquhar carried out a modelling investigation to determine the kinetic properties of Rubisco and the dependence of electron transport on photon flux.[4] Harley *et al* modified this model so that the parameters used could be established from whole-leaf gas-exchange measurements.[5] The latter authors also combined this model with the equations developed by Tenhunen *et al*[6] to investigate the interactive effects of the incident irradiance, leaf temperature, and partial pressures of CO_2 and O_2. The behaviour of photosynthetic enzyme under elevated CO_2 pressure was experimentally investigated by Vu *et al.*[7] Portis *et al* examined the activation of rubisco at physiological CO_2 concentration by another enzyme, called rubisco activase.[8a] Cen *et al* demonstrated the regulation of the rubisco activity in response to variation in ambient CO_2 pressure and leaf temperature.[8b] In fact, the CO_2 assimilation rate as function of the intercellular CO_2 pressure has been measured.[2] Despite these advances, a quantitative expression for the dependence of the rate of glucose-equivalent production on the intercellular CO_2 partial pressure is lacking in literature.

Recently, we have formulated an integrated rate equation for the production of glucose-equivalents in C_4 green plants under the condition of a high and constant partial pressure of CO_2 in chloroplast.[9a] We use the term glucose-equivalent for photosynthates like sucrose, starch, etc., which produce glucose in the plant cell by self-degradation. The rate equation was established by considering the so-called photochemical reactions (light reactions) and biosynthetic reactions (dark reactions) of photosynthesis in green plants. In a subsequent paper,[9b] the validity of the rate expression was examined for five specific C_4 plants.

In the present work, we have theoretically investigated the dependence of the rate of glucose-

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equivalent production on the intercellular CO_2 partial pressure (p_{CO_2}) in C_4 plants. To the best of our knowledge, this is the first effort in this direction. We specifically show that the reaction sequence due to King and Altman[10] and Farquhar,[1j] along with the steps of competitive inhibition of the inactive form of the enzyme and denaturation of the active form, can successfully explain the pressure-dependence of glucose-equivalent production. We have organized the present paper in the following way. Modifications of the rate equation have been described in section 2, and the photochemical efficiency is discussed in section 3. Section 4 deals with the effect of pressure on enzyme activity. In section 5, results of our formulation have been discussed for two specific plants, and a summary of our conclusions has been given in the last section.

2. Rate equation

The reaction sequence of photosynthesis including both the light reactions and the dark reactions is extraordinarily complicated. Many of these steps are co-regulatory, and it is always better to use a systems approach including all known steps of reasonable kinetic behavior.[11] One should also consider the regulation of the light reactions via the so-called non-photochemical quenching as well as through the lumen pH-driven regulation at the level of cytochrome-b6f complex. In fact, Laisk and Edwards constructed a numerical model of C_4 photosynthesis considering all enzymatic reactions and most of the regulatory functions.[12] Their model provides good simulations for various rates of intermediate steps under varying light intensity and concentrations of CO_2 and O_2. It also relates production rate of NADPH with that of ATP. However, this strategy makes the whole formulation very complicated, to be tracked only numerically, and a physical understanding of the integrated process is invariably lost.

The plant cell being a highly compartmentalized system, the application of any arbitrary treatment like chemical kinetics in a solution is not appropriate. *A conceptual understanding is obtained when we rely on experimental methodologies like apparent equilibrium treatments and steady state approximations, and group the entire photosynthetic process into only a few classes of reactions*. Therefore, in our previous work, we simplified the whole reaction sequence into seven groups of reactions.[9] This is also the basis of the present work.

In our previous derivation of the integrated rate expression for C_4 plants,[9a,b] it was assumed that these plants are not under any environmental stress and the intercellular partial pressure of CO_2 is high. Thus the effect of any change of CO_2 partial pressure on the glucose equivalent production rate was not considered. Recent studies on C_4 photosynthesis show that the rate of CO_2 assimilation (A_{CO_2}) as well as photochemical efficiency (ϕ) increase with intracellular CO_2 concentration that is dependent on the intercellular CO_2 partial pressure.[3,7] Following the treatment in references by Mehta *et al*,[9a,b] the rate of production of glucose-equivalent is written as

$$R_{\text{glucose}} = (8/3)\, R_1' v_L \phi(T, p)\, g(T, p) \\ ([\text{G3P}]/[\text{P}_i]^2)_{\text{leaf}}\, Q_{\text{G3P} \rightarrow \text{Glc-6-P}} \times \text{PPFD}, \quad (1)$$

where R_1' is the theoretically calculated rate of NADPH production in mol (L of lumen)$^{-1}$ s^{-1} given by $R_1' = 3{\cdot}235 \times 10^{-7}\ [672{\cdot}5 - 197{\cdot}3e^{-(1{\cdot}795+0{\cdot}000462T)}]$,[9c] v_L is the lumen volume in L per m^2 of leaf area, $\phi(T, p)$ is the photochemical efficiency, $g(T, p)$ is the enzyme activation quotient, [G3P] and [P_i] are the global concentrations of glyceraldehydes-3-phosphate and inorganic phosphates inside the leaf, Q is the concentration reaction quotient for the overall conversion of G3P into Glc-6-P^{2-}, and PPFD is the photosynthetic photon flux density.

The modifications required here involve treating ϕ and g in (1) as functions of not only temperature but also pressure.

3. Photochemical efficiency

The CO_2 leaf concentration can be expressed as

$$[\text{CO}_2(\text{BS})] = [\text{CO}_2(\text{BS})]_{\text{sat}}\, \tilde{P}_{\text{sat}}(T, p_{\text{CO}_2}), \quad (2)$$

where $[CO_2(BS)]_{sat}$ is the limiting value of the concentration of CO_2 in the bundle sheath, and $\tilde{P}_{\text{sat}}(T, p_{\text{CO}_2})$ is the saturation probability at a particular temperature T and a specific CO_2 partial pressure p_{CO_2}. Because T and p_{CO_2} are two independent quantities, to the lowest order $\tilde{P}_{\text{sat}}$ can be expressed as a multiplicative function

$$\tilde{P}_{\text{sat}}(T, p) = P_{\text{sat}}(T)\, f(p). \quad (3)$$

It is known that the photochemical efficiency for the C_4 plants rises very sharply with pressure and ulti-

mately reaches a constant value at the high pressure limit.[13] In the high pressure limit, the probability $f(p)$ should be unity, and

$$\tilde{P}_{\text{sat}}(T, p_{\text{CO}_2}^{\text{high}}) = P_{\text{sat}}(T), \tag{4}$$

which was the condition used in our previous work.[9a,b] It was also shown that with time the NADPH concentration gradually builds up from an initial zero value and ultimately reaches a saturation value. The variation of NADPH concentration with time is given by[9a,b]

$$[\text{NADPH}](t) = \sqrt{\frac{R_1}{b}}\ \frac{e^{2\sqrt{R_1 b}t} - 1}{e^{2\sqrt{R_1 b}t} + 1}, \tag{5}$$

where R_1 is the net rate of production of NADPH in chloroplast,[9a] a product of the maximum possible NADPH production rate (R_1') and the survival probability of the membrane. Besides, b is given by

$$b = 2k_3^{\text{eff}}[\text{CO}_2(\text{BS})] + 3k_3^{\text{eff}}[\text{CO}_2(\text{BS})]_{\text{sat}}. \tag{6}$$

In the above k_3^{eff} is the effective rate constant for the ultimate conversion of CO_2(BS) to Glyceraldehyde-3-phosphate (G3P).

Using (2) in (6) we get

$$b = k_3^{\text{eff}}[\text{CO}_2(\text{BS})]\left[2 + \frac{3}{\tilde{P}_{\text{sat}}(T, p)}\right]. \tag{7}$$

The importance of this relationship is discussed in the following. The photochemical efficiency (ϕ) is defined as the ratio of the number of CO_2(BS) used up and the number of photons consumed. Considering the membrane survival probability $P_{\text{mem}}(T)$, we get[9]

$$\phi = \frac{1}{8}\left(\frac{\text{Rate of production of G3P}}{\text{Rate of production of NADPH}}\right) \times P_{\text{mem}}(T), \tag{8}$$

where $P_{\text{mem}}(T)$ varies in the range $0 \le P_{\text{mem}}(T) \le 1$ from the high temperature to a moderate temperature. The rate of production of G3P ($2R_3$) is given by

$$2R_3 = \frac{2\tilde{P}_{\text{sat}}(T, p)}{2\tilde{P}_{\text{sat}}(T, p) + 3} R_1. \tag{9}$$

Therefore, ϕ can be written as

$$\phi = \frac{1}{4}\left(\frac{\tilde{P}_{\text{sat}}(T, p)}{2\tilde{P}_{\text{sat}}(T, p) + 3}\right). \tag{10}$$

The factor 1/4 comes from the consideration that 4 photons are required to produce 1 molecule of NADPH.[13]

From the experimental data, it is evident that as $p_{\text{CO}_2} \to 0$, $\phi \to 0$, that is, $f(p) = \tilde{P}_{\text{sat}}(T, p)/\tilde{P}_{\text{sat}}(T, \infty) \to 0$. The variation of $f(p)$ with p can be predicted as

$$\text{d}f(p)/\text{d}p = 2\gamma(1 - f)p, \tag{11}$$

that is first order with respect to $(1 - f)$ and first order with respect to p_{CO_2}. This yields the solution $f(p) = 1 - \exp(-\gamma p^2)$. Therefore, we have chosen a function of the form $f_n = 1 - \exp(-\gamma p^n)$ and compared with the experimental efficiency curve by a least square fitting. The theoretically determined curve along with the experimental curve of ϕ versus p_{CO_2} is shown in figure 1. The experimental points have been taken for an average C_4 plant.[13] The least square analysis yields $n = 1{\cdot}98$ and $\gamma = 0{\cdot}169$ as the best parameters. Obviously, n is more or less equal to 2.

Although a formulation is given here for the photochemical efficiency, the parametrized expression for ϕ is not used in our treatment while determining the glucose-equivalent production rate. Instead, we have relied on the experimental photochemical efficiency data. The merit of this discussion is that it lends credence to the use of $\tilde{P}_{\text{sat}}(T, p)$ as a multiplicative function, as in (3).

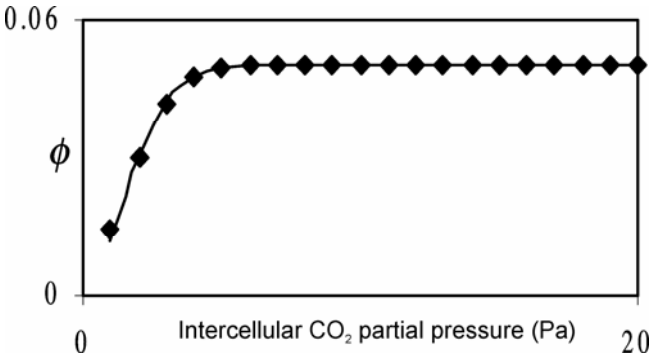

Figure 1. Average photosynthetic efficiency (ϕ) versus intercellular CO_2 partial pressure curve. Solid line shows the theoretically determined plot with $n = 2$ and $\gamma = 0{\cdot}169$ and dots represent the experimental data points. Here we take $T = 303{\cdot}15$ K and $P_{\text{sat}}(303{\cdot}15) = 1$.

4. Pressure effect

The CO_2 concentration plays a vital role on the activation of enzyme rubisco. Mate *et al* have showed that the carboxylation of Lys-residue within rubisco's catalytic sites takes place before carboxylation of RuBP.[7c] This actually activates rubisco by allowing the binding of Mg^{2+}. *In vivo*, this process of activation and maintenance of rubisco in active form is facilitated by a second enzyme called rubisco-activase. The activity of rubisco-activase is pressure dependent. Therefore, the enzyme activity quotient must be expressed as a function $g(T, p)$ that depends on both temperature and CO_2 pressure.

4.1 *Activation quotient*

To determine the functional form of $g(T, p)$, the detailed mechanism of the rubisco activation process has been considered. The reaction sequence can be represented by following the methods of King and Altman[10] and Farquhar.[1f] This is written as

$$E^{\text{inact}} + CO_2 \rightleftharpoons ECO_2 \quad (K_1) \tag{12}$$

$$ECO_2 + Mg^{2+} \rightleftharpoons E^{\text{act}} \quad (K_2), \tag{13}$$

where E^{inact} is the inactive form of the enzyme, ECO_2 is an intermediate and E^{act} is the activated form of the enzyme, and K_1 and K_2 are the two equilibrium constants. This mechanism is generally valid for all higher plants.

It is well-known that the enzymatic reaction is inhibited by oxygen or other molecules. The competitive inhibition can be described as

$$E^{\text{inact}} + O \rightleftharpoons EO \quad (K_3). \tag{14}$$

To represent the denaturation of the activated enzyme, we write another additional step,

$$E^{\text{act}} + mCO_2 \rightleftharpoons E^{\text{den}} \quad (K_4), \tag{15}$$

where K_4 is the corresponding equilibrium constant. The number m can vary as 0, 1, 2, … The third and fourth steps may or may not be necessary. Later it is shown that only with these additional steps and the specific value of $m = 1$ the observed data can be best explained.

It is well established that carbon assimilation in the bundle sheath cells of C_4 plants are solely controlled by the flux of CO_2.[14] However, in presence of carbonic anhydrase the assimilated CO_2 sets up a rapid equilibrium with the existing bicarbonate pool. It is also known that HCO_3^- is easily permeable to BS cells, but it can not penetrate chloroplast at significant rates.[15] Even if it enters into the chloroplast it does not take part in carbon assimilation. This shows that we have an equilibrium like

$$CO_2(\text{meso}) + H^+ + H_2O \xrightleftharpoons{\text{Carbonic anhydrase}} HCO_3^-(\text{meso})$$

$$HCO_3^-(\text{meso}) \xrightleftharpoons{\text{Series of reaction}} HCO_3^-(\text{BS})$$

$$\xrightleftharpoons{\text{Carbonic anhydrase}} CO_2(\text{BS}) + H^+ + H_2O\,. \tag{16}$$

The lack of experimental procedure to determine the level of CO_2 in each compartment of leaf invokes one to carry out modelling work to determine the inorganic carbon pools in the leaves.[14] However, the models reported so far determine fluxes of carbon empirically and are unable to take detailed account of intracellular CO_2 distribution. In addition, the *p*H and the permeability of bicarbonate also affect the size and composition of such inorganic carbon pools. The situation becomes more complicated due to the uneven distribution of carbonic anhydrase in different locations and different compartments. Therefore, one is left only with the consideration that in the steady state, the intracellular solvated CO_2 is in a constant proportion with the intercellular CO_2 (g). Hence one can write the intracellular CO_2 concentration as

$$[CO_2] = \xi p_{CO_2}, \tag{17}$$

where ξ is a proportionality constant. Solving (12)–(15) and (17), we get

$$g(T, p) = \frac{[E^{\text{act}}]}{[E]} = \frac{p}{a + bp + cp^{m+1}}, \tag{18}$$

where

$$a = (1 + K_3[O])/K_{\text{eff}}\,\xi,$$

$$K_{\text{eff}} = K_1K_2[Mg^{2+}], \tag{19}$$

$$b = 1 + 1/K_2[Mg^{2+}],$$

$$c = K_4\xi^m.$$

At high p_{CO_2}, beyond an optimum temperature (T_{opt}) the activation quotient $g(T, p)$ abruptly decreases indicating that reaction (15) is highly endothermic, with K_4 increasing very fast with temperature. Reactions (12) and (13) are also endothermic, and they contribute to the initial rise in activation (for $T < T_{opt}$) through the terms a and b in (18).

The equilibrium constants K_1 and K_2 are known from experiment, and $[Mg^{2+}]$ can be obtained from measured data on chloroplast.

4.2 *Production of glucose equivalent*

Equation (1) is valid for all temperature and a large range of pressure. The most remarkable aspect of this rate law is that all quantities except $K_3[O]$, K_4, m and ξ that occur in $g(T, p)$ are generally known from experiment. So, a direct comparison with the experimental data becomes possible. The molecularity m can be determined from the shape of $R_{glucose}$ versus p_{CO_2} plot. For $K_3[O]$ and ξ relative values can be obtained from coefficients a and c in (18).

In literature, the rate of the glucose-equivalent production is normally expressed in terms of the rate of CO_2 assimilation (A_{CO_2}) that is six times the former rate, that is, $A_{CO_2} = 6R_{glucose}$. From here onwards we will use A_{CO_2} in place of $R_{glucose}$.

5. Results and discussion

5.1 *Choice of variables*

The CO_2 assimilation rates at different pressures have been considered for *two specific C_4 species*, *P. coloratum* and *P. antidotale* (figure 2).[2] As the photochemical efficiency for these two plants are not available, we have used the experimental average of photochemical efficiency values for C_4 plants from.[13] The volume of thylakoid lumen for a general higher plant leaf is estimated to be $0{\cdot}0012\ Lm^{-2}$.[13] The ratio $[G3P]/[P_i]^2$ and the concentration reaction quotient Q are the other experimental data which we have used in this work. All the data selected are for C_4 plants or for a general leaf.

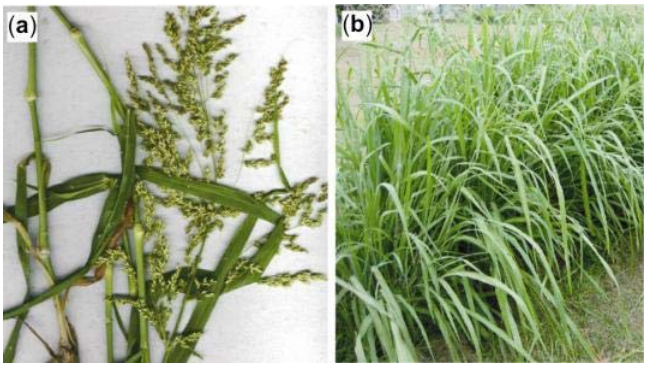

Figure 2. Plants under investigation: (**a**) *P. antidotale*, a typical Indian plant, (http://en.wikipedia.org/wiki/Panacium_antidotale) and (**b**) *P. coloratum*, a plant commonly found in Australia (with permission from Bruce-Cook © DPI&F, http://www.tropicalforges.info).

The plant cell is extensively compartmentalized. Compartmentation controls metabolism as well as transport. For example, the allocation of triose phosphates is largely controlled by the respective concentrations of G3P and P_i in the stroma and in the cytosol. The conversion of G3P to sucrose and starch depends heavily on the quoted P_i concentrations, which are constantly changing, let alone the vacuolar P_i concentration. Even the total P_i concentration changes from one species to another, and depends strongly on the physiological conditions such as soil, tissue, development stage, etc. Because of these reasons, we have adopted the average global (leaf) concentration of P_i^{2-} (100 mM) as well as the average global leaf concentration of G3P (0·032 mM).[13] This gives the ratio $([G3P]/[P_i^{2-}]^2)_{leaf}$ as approximately equal to 0·0032 in molar unit. The concentration values are correct only through two digits. This leaves a large margin of error.

We have used the relation $\Delta G_E^S = \Delta G^0 - \Delta G^S = -RT \ln Q_{G3P \rightarrow Glc\text{-}6\text{-}P^{2-}}$. The quantity ΔG_E^S is estimated to be about $-2{\cdot}5$ kcal mol^{-1} by considering the relevant steps as discussed in table 4 of ref. 16 and the activity of water in vivo.[9b,9d,16] See table 1. Previously, we found that the value of $-2{\cdot}55$ kcal mol^{-1} accounts for about 80% conversion of G3P into glucose-equivalents in average C_4 plants.[9a] Therefore, we have retained the same value.

The equilibrium constants K_1 and K_2 were determined by Laing *et al*,[15] $K_1 = 1{\cdot}0989 \times 10^4\ M^{-1}$ and $K_2 = 684{\cdot}21\ M^{-1}$. *P. antidotale* and *P. coloratum* have Mg^{2+} ions to the extent of 0·49% and 0·67% of dry mass.[17] The dry mass for a general leaf is about $0{\cdot}04\ Kg\ m^{-2}$ and the average leaf volume is $0{\cdot}3\ L\ m^{-2}$,[13] and the same data can be adopted for C_4 plants. The average number of chlorophyll is $5{\cdot}6 \times 10^{-4}\ M\ m^{-2}$, accounting for about 0·00187 M as the concentration of 'bound' Mg^{2+}. Therefore, the concentration of extra-chlorophyll Mg^{2+} ion is 0·0253 M in *P. antidotale* and 0·0354 M in *P. coloratum*.

The rubisco activation quotient $g(T, p)$ varies in different species. It is known to vary even for the

Table 1. Free energy changes in kcal mol^{-1} of the relevant steps for the enzymatic conversion of G3P to Glc-6-P, from ref 16. The quantity ΔG^S is the free energy change at experimental condition and ΔG^0 is the corresponding standard free energy change.

Reactions	ΔG^0	ΔG^S	$\Delta G^0 - \Delta G^S$
Glyceraldehyde-3-P^{2-} → dihydroxyacetone-P^{2-}	–1·8	–0·2	–1·6
Glyceraldehyde-3-P^{2-} + dihydroxyacetone-P^{2-} → Fru-1,6-P_2^{4-}	–5·2	–0·4	–4·8
Fru-1,6-P_2^{4-} + H_2O → Fru-6-P^{2-} + P_i^{2-}	–3·4	–6·5	3·1
Fru-6-P^{2-} → Glc-6-P^{2-}	–0·5	–0·3	–0·2
ΔG_E^S			–2·5[1]

[1]The activity of water is considered to be diminished *in vivo*, thereby increasing ΔG_E^S by about 1·0 kcal mol^{-1}.[16]

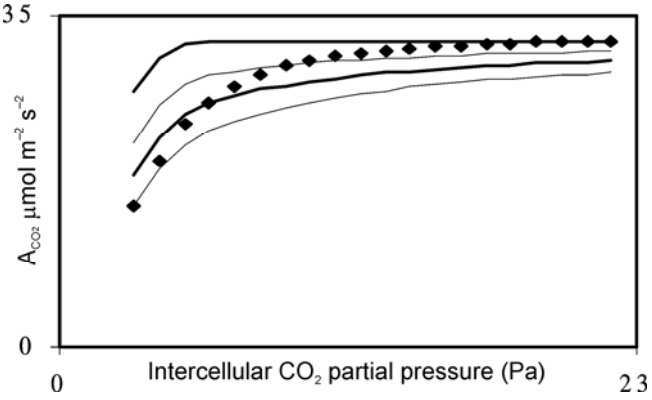

Figure 3. Plot of A_{CO_2} (= 6 $R_{glucose}$) calculated with m = 0 for *P. antidotale*. PPFD used is 940 μmol quanta $m^{-2}s^{-1}$. The plot shows that a $g(T, p)$ of the form $p/(a + bp)$ can never explain the experimental data (represented by ♦). A similar plot is obtained for *P. coloratum.*

same plant in different seasons throughout the year.[18] A detailed study on the temperature activation of rubisco for various plants by Crafts–Brandner and Salvucci reveals that in average the activation quotient varies in the range from 71% to 83% from 298 K to 308 K.[19]

A discussion on PPFD is due here. It is known that excess light causes extensive photodamage.[20] When the photon flux density is high, the excess energy is dissipated by non-photochemical quenching (NPQ). Ghannoum *et al* carried out a detailed investigation on the photochemical behaviour of both the species, *P. antidotale* and *P. coloratum*.[2] We have taken the experimental data from their paper at PPFD = 940 μmol quanta $m^{-2}s^{-1}$. Whatever may be the effect of NPQ is inherent in their data.

The constant ξ for the equilibrium between atmospheric CO_2 and the CO_2 dissolved in water is estimated to be $9{\cdot}77 \times 10^{-3}$ M Pa^{-1} at STP. The estimation is based on the well-known solubility of CO_2, 1·45 g L^{-1}. This is several orders higher than the steady state ξ for the cells of the two plant species under investigation. A possible reason for this is the presence of the bundle-sheath membrane. The steady-state ratio of the intracellular CO_2 and the intercellular CO_2 is controlled by diffusion through membrane which lowers the ξ value by several orders.

Figure 3 shows that the observed CO_2 assimilation rate can never be explained by an activation quotient of the form $p/(a + bp)$. This initiates the necessity of including the fourth step (denaturation of the activated enzyme) with $m \neq 0$. In fact, Crafts–Brandner *et al* have experimentally shown that activation of Rubisco decreases sharply after reaching the optimum temperature at around 35°C.[18,19] With the rise of temperature, the enzyme is denatured by heat. For $m = 1$ one obtains a good fit with the experimental rates, both for *P. antidotale* and *P. coloratum*, when b is fixed as $(1 + 1/K_2[Mg^{2+}])$. We have used K_2 = 684·21 M^{-1} and $[Mg^{+2}]$ = 0·0253 M and 0·0354 M for *P. antidotale* and *P. coloratum* respectively. Also any change of b from $(1 + 1/K_2[Mg^{2+}])$ leads to a very strong digression from the experimental curves. The latter observation firmly establishes the existence of the two initial steps. These steps were originally proposed by King and Altman,[10] Laing and Christeller,[15] and Farquhar[1j] from *in vitro* analyses. For $m > 1$, the fitting worsens. We emphasize that the fitting with the observed data is extremely sensitive, and a good fit is obtained only with $m = 1$ and $b = 1 + 1/K_2[Mg^{2+}]$.

For *P. antidotale* and *P. coloratum*, b equals 1·058 and 1·041 respectively. The least square fitting with $m = 1$ yields the optimized parameters a = 3·215, $c = 0{\cdot}2672 \times 10^{-2}$ ($\sigma^2 = 0{\cdot}4133$), and $a = 2{\cdot}666$, $c = 1{\cdot}042 \times 10^{-2}$ ($\sigma^2 = 0{\cdot}1074$), for *P. antidotale* and *P. coloratum* respectively. The corresponding plots

are given in figures 4 and 5. If we neglect inhibition (Step 3), we get $K_4 = 1{\cdot}645 \times 10^3\ M^{-1}$ and $\xi_{p.anti} = 1{\cdot}624 \times 10^{-6}\ MPa^{-1}$ for *P. antidotale* and $K_4 = 7{\cdot}395 \times 10^3\ M^{-1}$ and $\xi_{p.col} = 1{\cdot}409 \times 10^{-6}\ MPa^{-1}$ for *P. coloratum*. Thus K_4 is of the order of $10^3\ M^{-1}$, and ξ is of the order of $10^{-6}\ MPa^{-1}$, that is, the order of the Bunsen absorption coefficient of CO_2 in water at 30°C which is about $0{\cdot}7 \times 10^{-6}\ MPa^{-1}$.[21] The quantity $K_{eff}\xi$ is a constant of the order of unity in Pa^{-1} unit.

However, K_4 is an equilibrium constant that should remain same for both the species, and it is impossible to account for different *ac* products without involving the third step (inhibition). It is well established that Rubisco is subject to competitive inhibition by O_2.[22] When all the four steps are taken into consideration, the *c* values show $\xi_{p.anti}/\xi_{p.col} = 0{\cdot}26$. In *P. antidotale* the steady state concentration of CO_2 is about 70% less than that of *P. coloratum*. It can be easily shown that from the *a* values one gets that ratio $(1 + K_3[O]_{p.anti})/(1 + K_3[O]_{p.col}) = 0{\cdot}31$. Thus the inhibitor in the *P. antidotale* is also about 70% less. This makes the net CO_2 assimilation rate in *P. coloratum* comparable with that of *P. antidotale* in spite of the lower steady state concentration.

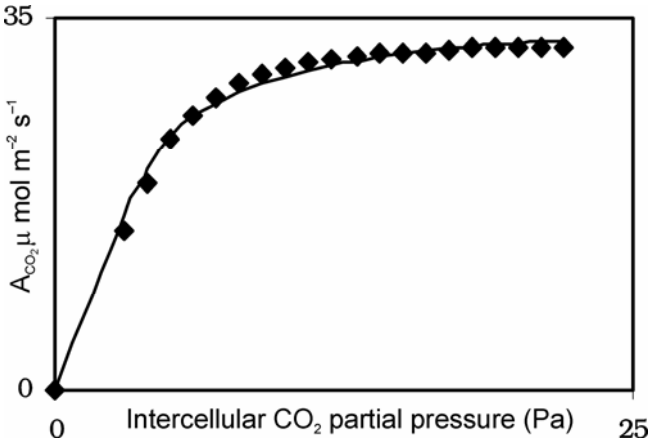

Figure 4. A_{CO_2} ($= 6R_{glucose}$) versus intercellular CO_2 pressure for *P. antidotale* at PPFD = 940 μ mol quanta $m^{-2}\ s^{-1}$ and $T = 303{\cdot}15$ K. The solid line is the theoritically calculated plot (this work), and the dots are the experimental data points from (Ghannoum *et al* 1998).

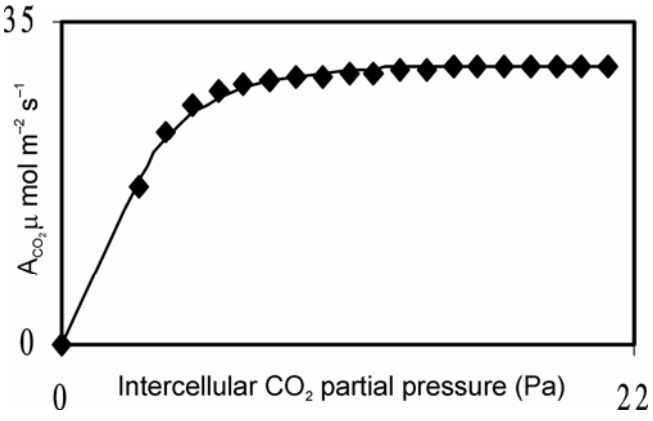

Figure 5. A_{CO_2} ($= 6R_{glucose}$) versus intercellular CO_2 pressure for *P. coloratum* at PPFD = 940 μ mol quanta $m^{-2}s^{-1}$ and T = 303·15 K. The solid line is the theoritically calculated plot (this work), and the dots are the experimental data points from (Ghannoum *et al* 1998).

6. Conclusion

In this work, we have derived expressions for (i) the photochemical efficiency, (ii) the activation quotient of enzyme, and (iii) the rate of glucose-equivalent production in C_4 plants. These expressions contain the explicit pressure dependence of the respective quantities. The activation process has been considered along with inhibition of the inactive enzyme and denaturation of the activated form, and the best activation quotient is realized in the form of (18) with $m = 1$. Finally, we have obtained a pressure and temperature-dependent expression for the rate of glucose-equivalent production. To the best of our knowledge, this is the first time an analytical expression is derived for the pressure activation quotient of rubisco. The final rate equation is, of course, based on the theory[23] and numerical simulation[9c] of exciton dynamics on thylakoid membrane to find the rate of NADPH production. It also relies on the seven groups of biosynthetic reactions which we considered in our previous work.[9a,b]

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