Possible CO_2 Concentrating Mechanism in Chloroplasts of C_3 Plants. Role of Carbonic Anhydrase

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Abstract. The possibility of a specific CO_2 concentrating mechanism present in chloroplasts of C_3 plants is analyzed. Proton gradient between thylakoids and the stroma is assumed to be the driving force for this process. The possible CO_2 concentrating mechanisms are: 1. HCO_3^- permeation into thylakoids, its dehydration there and diffusion of CO_2 formed into the stroma; 2. Dehydration of HCO_3^- present in the stroma at the thylakoid surface in a reaction with H^+ leaving the thylakoids through: a) channels of membrane-bound carbonic anhydrase; b) channels of the ATPase complex. A system of equations describing CO_3^- and CO_2 diffusion as well as CO_2 assimilation and formation was used. The increase in photosynthesis rate, upon CO_2 diffusion being facilitated in the presence of carbonic anhydrase, and due to the action of CO_2 concentrating mechanisms, was numerically estimated. The CO_2 concentrating mechanism was shown to function effectively only with the entire chloroplast being the CO_2 concentrating zone. This is the case when the bulk of stromal carbonic anhydrase is localized near the inner chloroplast envelope. The existence of CO_2 concentrating mechanisms around a single granum or around thylakoids is hardly possible. Approaches enabling the detection of similar concentrating mechanisms are discussed.

Key words: Photosynthesis — Chloroplast — Carbonic anhydrase — CO_2 concentrating mechanism

Introduction

Photosynthetic capacity of C_3 plants is supposed to increase upon increased CO_2 concentration in chloroplasts. Introduction of C_4 plants key enzymes into C_3 cells, as well as active transport of bicarbonate (HCO_3^-) into chloroplast have

been suggested as possible CO_2 concentrating mechanisms (Nasyrov 1978; Bassham and Buchanan 1982).

CO_2 concentrating may also be due to processes operative in chloroplasts. A mechanism based on the existence of an appreciable pH gradient between the stroma and thylakoids may be assumed. The reaction of reversible hydration of CO_2 within physiological pH is known: $CO_2 + H_2O \rightleftarrows HCO_3^- + H^+$ (Porker and Bjorkquist 1977; Reed and Graham 1981). The stroma of illuminated chloroplasts has a pH of about 8, while the thylakoid inside has a value of about 5 (Heldt et al. 1973; Witt 1979). In the alkaline milieu of the stroma CO_2 is hydrated to form HCO_3^-, and in the acidic milieu of thylakoids HCO_3^- becomes dehydrated to CO_2.

CO_2 concentration at the sites of its assimilation may be increased when HCO_3^- from the stroma becomes dehydrated in the acidic milieu of thylakoids (Pronina et al. 1981; Pronina and Semenenko 1984).

Spontaneous reversible hydration of CO_2 is extremely slow, but carbonic anhydrase found in chloroplasts may markedly accelerate the reaction (Porker and Bjorkquist 1977; Reed and Graham 1981).

Several CO_2 concentrating mechanisms are possible:

1. According to Pronina et al. (1981) and Pronina and Semenenko (1984), significant amounts of HCO_3^- may pass into thylakoids of illuminated chloroplasts, where HCO_3^- becomes dehydrated. CO_2 formed in the thylakoids diffuses from them forming a zone of increased CO_2 concentration in the stroma.

2. Membrane-bound carbonic anhydrase seems to be present in thylakoids (Pronina et al. 1981; Komarova et al. 1982; Pronina and Semenenko 1984). According to Wistrand (1984), the membrane-bound carbonic anhydrase of mammalian cells may form membrane channels. Assuming that the membrane-bound thylakoid carbonic anhydrase also forms such channels, it may be supposed that H^+ crosses the thylakoid membrane via the channels and reacts with HCO_3^- on the thylakoid surface in the presence of carbonic anhydrase.

3. It can be speculated that carbonic anhydrase is localized near the channels through which H^+ is transferred from the thylakoids, e.g., to bind to the ATPase complex. These H^+ ions may be employed to dehydrate HCO_3^-, and CO_2 may appear at the thylakoid surface as under 2.

The present work was aimed at analyzing the possibility of the presence of a CO_2 concentrating mechanism.

Theoretical consideration

The actual chloroplast structure is too complicated to enable calculations of diffusion fluxes of CO_2 and HCO_3^-. We considered two simplified cases.

a. Homogeneous spherical chloroplast (Fig. 1*A*). The volume of thylakoids and the surface of thylakoid membranes per unit of chloroplast volume correspond to the specific thylakoid volume and specific thylakoid surface in chloroplasts.

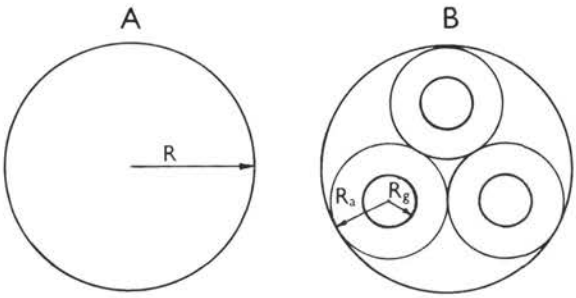

Fig. 1. *A*. Schematic representation of a chloroplast with uniformly distributed thylakoids. *R* is the radius of the chloroplast. *B*. A single granum inside a chloroplast. R_g is the radius of the granum, R_a is the radius of the border line region of the stroma surrounding the granum.

b. Spherical granum consisting of several thylakoids localized in a homogeneous stroma (Fig. 1*B*). The grana were supposed to be spheres with radii equal to that of a thylakoid and with a surface determined by the outer thylakoid surface of the real grana. The intragranal space was considered as homogeneous and having all the characteristics of the real intrathylakoid milieu. The radius of the grana surrounding stroma was taken as half of the average distance between grana in a real chloroplast.

According to Heldt et al. (1973) and Walz et al. (1974), both the intrathylakoid space and the stroma contain significant amounts of protonophores, which more than 100-fold exceed the HCO_3^- concentration in chloroplasts equilibrated with intercellular air. Due to such an amount of protonophores, the H^+ concentration profile is thought to be uniform throughout the stroma or in single thylakoid, for that reason HCO_3^- flux would be limited by its diffusion rate only (Gutknecht et al. 1977).

Let us consider steady-state diffusion of CO_2 and HCO_3^- in spherical chloroplasts or around a spherical granum. Keeping in mind the spherical symmetry of the system the first Fick's law for total fluxes may be written as

$$J_{cs} = -D_c S \frac{dC_s}{dr} \tag{1}$$

$$J_{bs} = -D_b S \frac{dB_s}{dr} \tag{2}$$

where C_s and B_s are concentrations of CO_2 and HCO_3^- respectively; J_{cs} and J_{bs} are fluxes of CO_2 and HCO_3^- across the surface (S) of a sphere with a radius r ($S = 4\pi r^2$); D_c and D_b are effective coefficients of diffusion of CO_2 and HCO_3^- respectively.

The equation for steady-state diffusion is

$$\frac{D}{r^2}\frac{d}{dr}\left(r^2\frac{dY_s}{dr}\right) - Q_s = 0 \tag{3}$$

where Y_s is CO_2 or HCO_3^- concentration, Q_s is evolution or absorption rate of CO_2 or HCO_3^- in a volume unit, D is the effective diffusion coefficient of substrate.

From Eqs. 1 and 2

$$\frac{dC_s}{dr} = -\frac{J_{cs}}{4\pi r^2 D_c} \tag{4}$$

$$\frac{dB_s}{dr} = -\frac{J_{bs}}{4\pi r^2 D_b} \tag{5}$$

Let us suppose that only CO_2 assimilation, reversible CO_2 hydration, and CO_2 evolution from stromal HCO_3^- occur. Then, substituting dC_s/dr and dB_s/dr from Eqs. 4 and 5 into Eq. 3 gives

$$\frac{dJ_{cs}}{dr} = 4\pi r^2(-F_s - H_s + G_s) \tag{6}$$

$$\frac{dJ_{bs}}{dr} = 4\pi r^2(H_s - G_s) \tag{7}$$

where F_s is CO_2 assimilation rate in the stroma, H_s is reversible CO_2 hydration rate in the stroma, G_s is HCO_3^- absorption and CO_2 evolution rates in thylakoids which are equally distributed in chloroplasts. In case with an isolated spherical granum $G_s = 0$, for thylakoids are assumed to be absent in stroma surrounding the granum.

The CO_2 assimilation rate in a stroma with saturated illumination and limited CO_2 concentration is approximated (cf. Farquhar et al. 1980; Raven and Glidewell 1981) by:

$$F_s = \frac{V_c C_s}{C_s + K_c(1 + O/K_o)} \tag{8}$$

where O is O_2 concentration, V_c is the maximal rate of CO_2 assimilation in

chloroplast, K_c is the Michaelis constant for CO_2, and K_o is the inhibition constant for O_2.

The value of the Michaelis constant for reversible hydration of CO_2 by carbonic anhydrase considerably exceeds CO_2 concentration in water equilibrated with air (cf. Tables 1 and 2); owing to this, a linear equation for C_s and B_s can be written:

$$H_s = \left(k'_h + \frac{k_h Z}{K_h}\right)\left(C_s - \frac{B_s}{K}\right) \tag{9}$$

Here, Z is the carbonic anhydrase concentration, k_h is the turnover number of carbonic anhydrase, K_h is the Michaelis constant for reversible hydration of CO_2, K is the $[HCO_3^-]/[CO_2]$ ratio at equilibrium, k'_h is the CO_2 hydration constant in water. Constants k_h, K_h, k'_h and K are pH-dependent.

The equation for G_s has different forms depending on the CO_2 concentrating mechanism considered. Let us consider HCO_3^- penetration into thylakoids (the first mechanism).

The steady-state flux of HCO_3^- into thylakoid equals the CO_2 flux leaving the thylakoid, since CO_2 cannot be assimilated in thylakoids. Then, the rate of HCO_3^- dehydration per volume unit of chloroplast is

$$G_s = P_c S_t (C_t - C_s) = P_b S_t (B_s - B_t) \tag{10}$$

where C_t and B_t are the respective concentrations of CO_2 and HCO_3^- in thylakoids; C_s and B_s are concentrations of CO_2 and HCO_3^- in the stroma near the thylakoids respectively; P_c and P_b are the respective permeability coefficients of thylakoid membranes for CO_2 and HCO_3^-; S_t is the specific membrane surface accessible for CO_2 and HCO_3^-.

Using Eqs. 9 and 10, and assuming that CO_2 and HCO_3^- concentrations are uniform inside a thylakoid (as it is small enough) we obtain:

$$C_t = \frac{P_c C_s (P_b + A/K_t) + P_b B_s A/K_t}{P_c (P_b + A/K_t) + A P_b} \tag{11}$$

$$B_t = \frac{(P_c + A) P_b B_s + A P_c C_s}{P_c (P_b + A/K_t) + A P_b} \tag{12}$$

where

$$A = \left(k'_{ht} + \frac{k_{ht} Z_t}{K_{ht}}\right)\frac{V_t}{S_t} \tag{13}$$

Z_t is the carbonic anhydrase concentration in thylakoids, V_t is thylakoid volume per volume unit of chloroplast. The subscript (t) shows that the coefficients are to be estimated for intrathylakoidal conditions.

The value of G_s can be obtained from Eq. 10 by substituting of C_t or B_t.

The Michaelis constant for HCO_3^- dehydration by soluble carbonic anhydrase is at least 30 mmol.l^{-1} (Reed and Graham 1981). This value is much greater than HCO_3^- concentration in a stroma equilibrated with air. If we assume the same kinetic characteristics of carbonic anhydrase for concentrating mechanisms 2 and 3 (without HCO_3^- entering the thylakoids), then the HCO_3^- dehydration rate at the thylakoid surface will be linearly dependent on HCO_3^- concentration:

$$G_s = E_s B_s \tag{14}$$

where E_s is the proportion coefficient dependent on the rate of H^+ outward flux and on some other factors.

The amount of HCO_3^- entering thylakoids (or CO_2 formed in thylakoids) in an entire chloroplast (G_H) can be calculated using the integral

$$G_H = 4\pi \int_R^0 G_s r^2 \, dr \tag{15}$$

Now, using Eq. 10, we can write for steady state HCO_3^- absorption or CO_2 formation for a single granum (case b)

$$J_{cg} = P_c S_g (C_{tg} - C_{sg}) = P_b S_g (B_{sg} - B_{tg}) \tag{16}$$

where J_{cg} is CO_2 outward flux (equal to HCO_3^- inward flux); C_{sg} and C_{tg} are CO_2 concentrations around the granum and inside it respectively; B_{sg} and B_{tg} are HCO_3^- concentrations next to the granum and inside it respectively; S_g is the granum surface accessible to HCO_3^-.

The values of C_{tg} and B_{tg} can be obtained from Eqs. 11—13 by substituting C_{sg}, B_{sg}, S_g and V_g for C_s, B_s, S_t and V_t respectively. Here, V_g is the thylakoid volume in the granum.

If we assume that HCO_3^- cannot enter the granum thylakoids (case 2 and 3), the amount of CO_2 appearing on thylakoid surfaces may be written as in Eq. 14

$$J_{cg} = E_{sg} B_{sg} \tag{17}$$

where E_{sg} is the proportion coefficient.

Boundary conditions for chloroplasts are as follows: CO_2 concentration on the inside surface of the chloroplast envelope (C_h) is determined by CO_2 concentration in the cytoplasm. The HCO_3^- flux across a chloroplast envelope is zero, because of the unpermeability of the latter for HCO_3^- (Heber and Heldt 1981). Owing to the assumed spherical symmetry there are no CO_2 and HCO_3^- fluxes in the center of a chloroplast. Hence,

$$C_s = C_h \quad \text{and} \quad J_{bs} = 0 \quad \text{if} \quad r = R$$
$$J_{cs} = 0 \quad \text{and} \quad J_{bs} = 0 \quad \text{if} \quad r = 0$$

where R is the chloroplast radius.

The boundary conditions for a single granum are: CO_2 concentration at the border of the stromal region surrounding the granum was taken as the CO_2 concentration near the internal envelope of the chloroplast. Since grana are supposed to be uniformly distributed in chloroplasts, HCO_3^- fluxes between them would be zero. The HCO_3^- flux directed towards the granum surface and the CO_2 flux from the granum surface are equal for any of the CO_2 concentrating mechanisms under study:

$$C_s = C_h \quad \text{and} \quad J_{bs} = 0 \quad \text{if} \quad r = R_a$$
$$J_{cs} = -J_{bs} \quad \text{if} \quad r = R_g$$

where R_a is the radius of the region in which CO_2 becomes concentrated.

The values of concentration and rate constants adopted are shown in Tables 1 and 2. The concentrations of dissolved CO_2 and O_2 as well as the rate constants are shown for 25 °C.

Table 1. Concentrations and dimensions

$C = 7.7\ \mu mol.l^{-1}$,	CO_2 concentration in water equilibrated with intercellular air
$C_h = 7\ \mu mol.l^{-1}$,	CO_2 concentration in the stroma at the chloroplast envelope
$O = 250\ \mu mol.l^{-1}$,	O_2 concentration in the stroma
$Z = 2\ mmol.l^{-1}$,	average concentration of carbonic anhydrase in chloroplasts
$R = 2.21\ \mu m$,	average radius of a chloroplast
$Chl = 83\ mg.cm^{-3}$,	average concentration of chlorophyll in a chloroplast
$V_t = 0.33$,	relative volume of thylakoids in a chloroplast
$S_t = 16.7\ \mu m^2.\mu m^{-3}$,	thylakoid surface in a chloroplast
$R_g = 0.2\ \mu m$,	average granum radius
$N = 10$,	average number of thylakoids in a granum
$S_g = 1.38\ \mu m^2$,	average surface of thylakoids in a granum, accessible for to HCO_3^-
$V_g = 0.282$,	the share of granal thylakoids volume in the chloroplast volume
$R_a = 0.277\ \mu m$,	the radius of the CO_2 concentrating sphere around a granum

The CO_2 and O_2 concentration shown in Table 1 is taken for water solution in equilibrium with intercellular air (cf. Osmond et al. 1982). The CO_2 concentration at the internal envelope of chloroplasts is assumed to be by about 10% less due to diffusion resistance for CO_2 flux between intercellular space and chloroplasts (cf. Nobel 1974; Raven and Glidewell 1981).

The concentration of carbonic anhydrase in chloroplasts apparently depends on the plant species and growth conditions (Reed and Graham 1981). The value for spinach shown in Table 1 is taken from report by Jacobson et al. (1975).

Table 2. Constants

$P_c = 0.35\ cm\ .\ s^{-1}$,	CO_2 permeability coefficient of the thylakoid membrane
$D_{cw} = 1.88 \times 10^{-5}\ cm^2\ .\ s^{-1}$,	CO_2 diffusion coefficient in water
$D_{bw} = 1.15 \times 10^{-5}\ cm^2\ .\ s^{-1}$,	HCO_3^- diffusion coefficient in water
$D_c = 0.5D_{cw}$,	effective CO_2 diffusion coefficient in chloroplasts
$D_b = 0.5D_{bw}$,	effective CO_2 diffusion coefficient in chloroplasts
$V_c = 18.1\ mmol\ .\ l^{-1}\ .\ s^{-1}$,	maximal rate of CO_2 fixation per unit volume of chloroplasts
$K_c = 15.4\ \mu mol\ .\ l^{-1}$,	Michaelis constant for CO_2 assimilation
$K_o = 393\ \mu mol\ .\ l^{-1}$,	O_2 inhibition constant of CO_2 assimilation
$k'_h = 3.5 \times 10^{-2}\ s^{-1}$,	pH 8 rate constant for non-catalyzed
$k'_{ht} = 2.9 \times 10^{-2}\ s^{-1}$,	pH 5 CO_2hydration
$K = 62.2$,	pH 8 equilibrium ratio of HCO_3^- to CO_2
$K_t = 0.066$,	pH 5 concentration
$k_h = 3.8 \times 10^5\ s^{-1}$,	pH 8 turnover number of carbonic
$k_{ht} = 0.15 \times 10^5\ s^{-1}$,	pH 5 anhydrase
$K_h = 20\ mmol\ .\ l^{-1}$,	pH 8 Michaelis constant of reversible
$K_{ht} = 20\ mmol\ .\ l^{-1}$,	pH 5 CO_2 hydration

Chloroplast size as well as its pigment concentration may vary significantly (Nobel 1974; Mokronosov 1981). We used the values for a potato leaf at the age of 20 days (cf. Mokronosov and Nekrasova 1977). Table 1 shows the calculated radius of a spherical chloroplast having a volume of 45 μm^3. The mean chlorophyll concentration in the chloroplast was calculated assuming the number of chlorophyll molecules in it to be 2.5×10^9 (cf. Mokronosov and Nekrasova 1977). Both chloroplast volume and its chlorophyll concentration are close to mean values for several plant species (Mokronosov 1981).

The share of thylakoids on chloroplast volume may vary greatly, but in most papers known to us (Wrischer 1978; Silaeva and Silaev 1979; Macovec and Volfova 1981) it has been reported to be approximately 1/3.

The thylakoid surface accessible for ions is difficult to estimate. However, evidence has been presented for the existence of gaps between granal thylakoids (Nir and Pease 1973); owing to this, some portions of the surface between thylakoids can be assumed to be accessible to ions. We shall thus assume that ions are able to contact a half of the granal thylakoid surfaces only, and the entire surface of intergranal thylakoids.

The average diameter of a granal thylakoid and its surface area were derived according to values reported by Nobel (1974) and Barber (1972). It was assumed that a granum contains 10 thylakoids (cf. Nobel 1974).

The radius of stromal CO_2 concentrating sphere around a granum was estimated assuming the volume of granal thylakoids to be about 28% of the sphere volume (cf. Silaeva and Silaev 1979). Only half of the granal thylakoid surface was assumed to be accessible for ions.

The permeability coefficient of the thylakoid membrane for CO_2 is unknown and was taken as that for a lipid bilayer (cf. Gutknecht et al. 1977). A significant amount of protein present in lipid thylakoid membrane can change the value of the coefficient. Diffusion coefficient for CO_2 in water was taken according to Mazarei and Sandall (1980) and that for HCO_3^- in water according to Walker et al. (1980).

It is known that the magnitude of the self-diffusion coefficient of water in chloroplasts is approximately half that in water (Karimova et al. 1975). We assume, therefore, that CO_2 and HCO_3^- diffusion coefficients in chloroplast medium are also half those in water.

The rate constants in Eq. 8 were taken according to Farquhar et al. (1980). Maximal CO_2 assimilation rate was calculated using data for maximal rate per chlorophyll unit (cf. Farquhar et al. 1980) and chlorophyll concentration (Table 1).

The coefficients for non-catalyzed CO_2 hydration were calculated according to Eq. 5 in Pocker and Bjorkquist (1977).

The $[HCO_3^-]/[CO_2]$ ratios for pH 8 and pH 5 were obtained using data of Magid and Tusbeck (1968).

The turnover number of carbonic anhydrase at pH 8 for spinach leaf enzyme was taken from Fig. 6 in Pocker and Ng (1973), and interpolated for pH 5 from the same figure.

The value of the Michaelis constant for CO_2 hydration varies with different plant species, ranging between 1.5 and 42.4 mmol $.l^{-1}$ (Reed and Graham 1981). We employed an approximate mean value. The dependence of results on the value of the constant is discussed below.

A system of differential equations (4—7) was solved by the fourth order Runge-Kutta method. The stationary solutions under the above boundary conditions were obtained using a special computer program. For homogeneous chloroplast, the program changed the values of CO_2 flux (J_{ch}) and HCO_3^- concentration near the internal envelope of the chloroplast so as to observe the boundary conditions in the centre of the chloroplast.

For the case of a single granum (case b), the CO_2 flux and HCO_3^- concentration at the border of a CO_2 concentrating sphere were selected by the procedure described above. In this case, the HCO_3^- flux towards the region adjacent to the granum is equal to CO_2 outward flux.

Calculations for the CO_2 concentrating mechanism 1 have shown that HCO_3^- concentration in the thylakoids might not exceed 1% of its concentration in the stroma. Thus, Eqs. 10 and 16 can be reduced to a linear dependence on HCO_3^- concentration in the stroma. Solutions obtained for mechanisms 2 and 3 are thus similar to that of the first mechanism assuming $E_s = P_b S_t$ and $E_{sg} = P_b S_g$, respectively.

The steady-state CO_2 assimilation rate calculated for a chloroplast was equal to CO_2 flux across the chloroplast envelope. For a granum, this assimilation rate corresponds to CO_2 flux across the concentrating sphere border.

Results

Facilitation of CO_2 diffusion

Fig. 2 shows the numerical solution to the system of equations (4—7) assuming that carbonic anhydrase is equally distributed in a chloroplast, and that any of the concentrating mechanisms are not active. It was shown that at pH 8, CO_2 hydration occurs in the stroma at the chloroplast envelope. HCO_3^- concentration increases and diffusion of HCO_3^- to the centre of the chloroplast is accelerated. In the central part of the chloroplast CO_2 is consumed, and the reaction is shifted to the HCO_3^- dehydration side. The concentration of CO_2 becomes almost uniform throughout the chloroplast volume. CO_2 assimilation rate increases by about 9% as compared to that in absence of carbonic anhydrase.

When carbonic anhydrase is absent, the HCO_3^- flux induced by reversible

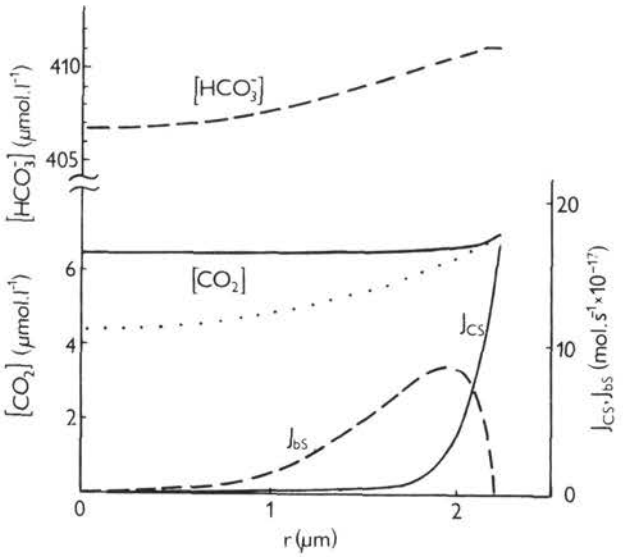

Fig. 2. CO_2 diffusion facilitation in a chloroplast having carbonic anhydrase, r is the distance from the chloroplast centre; calculated CO_2 concentration profile ($[CO_2]$) and CO_2 flux (J_{cs}) across the spheric surface with a radius r; HCO_3^- concentration profile ($[HCO_3^-]$) and HCO_3^- flux (J_{bs}) across the spheric surface with a radius r; CO_2 concentration profile in absence of carbonic anhydrase (dotted line)

CO_2 hydration is negligible and cannot significantly change CO_2 distribution.

Also, increase in CO_2 assimilation rates due to facilitation of CO_2 diffusion around the single granum is less than 0.01%.

CO_2 concentrating

A region of a raised CO_2 concentration may exist around individual grana if any of the CO_2 concentrating mechanisms discussed is active. Fig. 3 shows results of the calculation. Due to the fact that CO_2 becomes consumed in the stroma only, the maximal CO_2 consumption rate per unit volume around a granum was repeatedly calculated.

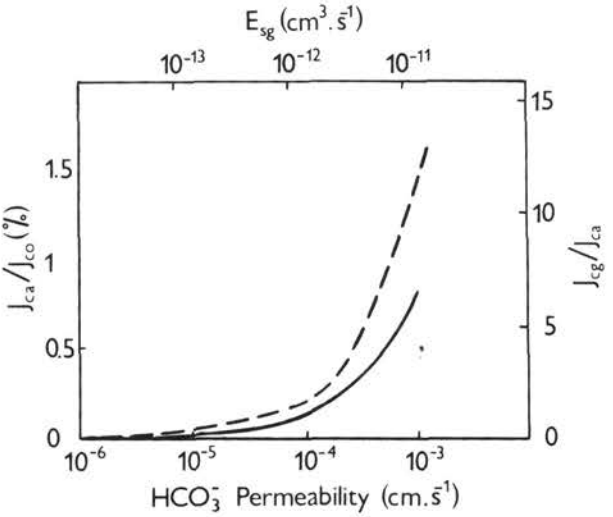

Fig. 3. Relative increase in CO_2 assimilation rate, CO_2 being concentrated around a single granum, depending on HCO_3^- permeability coefficient of the thylakoid membrane (for mechanism 1), or on coefficient E_{sg} (for mechanisms 2 and 3). J_{co} and J_{ca} are CO_2 fluxes into the stromal CO_2 concentrating region in absence and presence of carbonic anhydrase respectively, with any of the CO_2 concentrating mechanisms active; J_{cg} is HCO_3^- flux to (or CO_2 formation in) a granum (for mechanism 1), or in the region near the granum (for mechanisms 2 and 3); increase in CO_2 assimilation rate with an active CO_2 concentrating mechanism expressed as J_{ca}/J_{co} ratio (solid line); numbers of HCO_3^- molecules dehydrated in the region around the granum per one CO_2 molecule assimilated (J_{cg}/J_{ca}) (interrupted line). $V_c = 29.0$ mmol.l^{-1}.s^{-1}.

The calculations showed that the efficiency CO_2 concentrating at a single thylakoid is much less than that for an individual granum. Therefore, CO_2 concentrating around a single thylakoid can be ignored.

The weak CO_2 concentrating power around an individual thylakoid or a

granum is related to rapid hydration of CO_2 formed in the surrounding stroma. To increase the concentrating efficiency the rapid hydration of CO_2 near the granum must be prevented. This can occur when all of the carbonic anhydrase is localized at the chloroplast envelope. Fig. 4 shows the corresponding solution.

It should be emphasized that thylakoids usually have no contacts with the chloroplast envelope. Thus, it was assumed that no thylakoidal HCO_3^- dehydration occurs in the region occupied by carbonic anhydrase. We had, therefore, to recalculate the values of Z, V_t and S_t.

The CO_2 concentrating mechanism may be explained by the hydration of CO_2 diffusing from the cytoplasm and from the central part of the chloroplast, by diffusion of HCO_3^- formed near the chloroplast envelope to the chloroplast centre, and by the formation of CO_2 due to HCO_3^- dehydration in the central part of the chloroplast (Fig. 4).

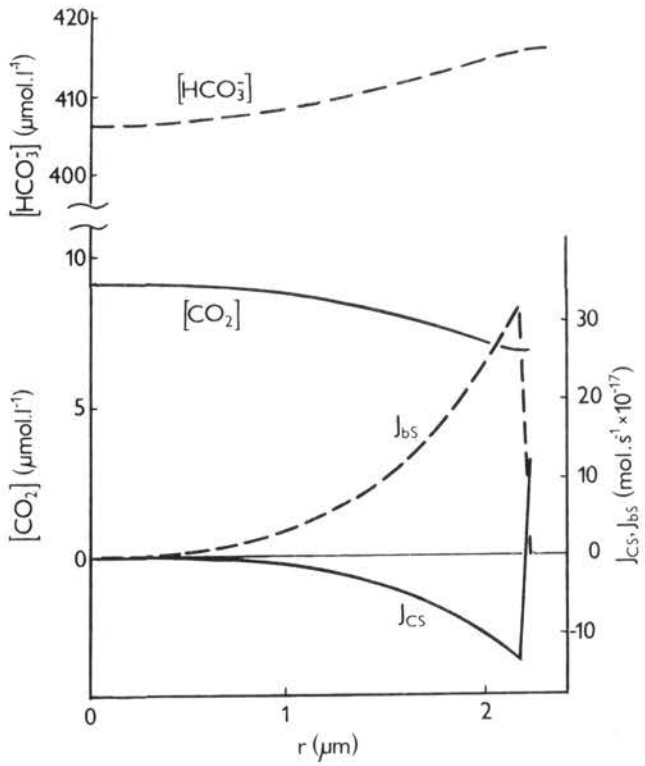

Fig. 4. CO_2 concentrating inside the chloroplast. For symbols see Fig. 2. HCO_3^- permeability coefficient of the thylakoid membrane is 10^{-4} cm . s^{-1} ($E_s = 16.2$ s^{-1}). The carbonic anhydrase concentration in the thylakoids assumed to be equal to the mean values found in chloroplasts (for mechanism 1). The stromal carbonic anhydrase is supported to be localised in a 0.05 μm layer below the chloroplast envelope. $Z = 20.1$ mmol . l^{-1}, $V_t = 0.357$ and $S_t = 17.9$ μm^{-1}.

Fig. 5 shows the relative increases in CO_2 assimilation rates, the number of HCO_3^- molecules entering thylakoids (or numbers of dehydrated molecules for cases 2 and 3) upon one CO_2 molecule being consumed.

A substantial decrease of CO_2 concentrating efficiency can be observed only when the carbonic anhydrase concentration in the thylakoids decreases more than 100-fold, with its concentration in the stroma remaining constant.

When value of permeability coefficient of thylakoid membrane for CO_2 was varied by several fold (concentrating mechanism 1), the results of the numerical experiment were changed insignificantly because the CO_2 penetration across the membrane remained to be considerably higher than penetration of HCO_3^-.

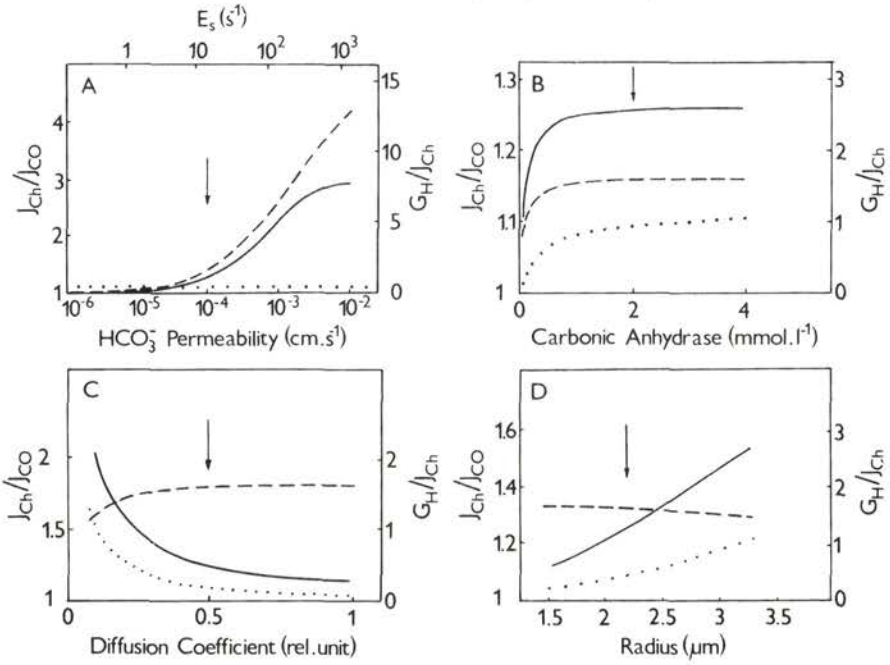

Fig. 5. Increase in CO_2 assimilation rate and energy expenditure for CO_2 diffusion facilitation and CO_2 concentrating in chloroplasts: relative increase in CO_2 assimilation rate as a result of CO_2 diffusion facilitation (J_{ch}/J_{co}) (dotted lines); the same with an active CO_2 concentrating mechanism (J_{ch}/J_{co}) (solid lines); HCO_3^- molecules dehydrated per one CO_2 molecule assimilated (G_H/J_{ch}) with an active CO_2 concentrating mechanism (interrupted lines). J_{co} is CO_2 flux into a chloroplast in absence of carbonic anhydrase, J_{ch} is CO_2 flux into a chloroplast upon CO_2 diffusion facilitation or with an active CO_2 concentrating mechanism. Arrow: solution for coefficients as taken from Fig. 4. *A*. dependence on HCO_3^- permeability coefficient of the thylakoid membrane (or on E_s for mechanisms 2 and 3); for other coefficients see Fig. 4. *B*. dependence on mean carboanhydrase concentration in chloroplasts. For local distribution of the enzyme see Fig. 4. *C*. dependence on relative diffusion coefficients of CO_2 (D_c/D_{cw}) and HCO_3^- (D_b/D_{bw}). This ratio for water solution is taken as a unity. *D*. dependence on chloroplast radius. All calculations were made assuming all the stromal carbonic anhydrase as being localized in a 0.05 μm layer below the chloroplast envelope.

Discussion

Increase in CO_2 assimilation due to the facilitation of CO_2 diffusion

The saturation of CO_2 assimilation upon increasing carbonic anhydrase concentration (Fig. 5*B*) may be explained by the fact that the increase in the assimilation rate reaches the limit when CO_2 becomes uniformly distributed inside the chloroplast.

The diffusion coefficients were assumed to be half of those in water (Table 2). If the real values were smaller, the CO_2 assimilation rate would increase significantly (Fig. 5*C*).

The larger the chloroplast, the higher the increase in CO_2 assimilation rate due to the facilitation of CO_2 diffusion (Fig. 5*D*).

These effects may be explained by a marked decrease of CO_2 concentration in the centre of the chloroplast upon a decrease of diffusion coefficients of CO_2 or upon an increase in the chloroplast radius. Thus, if the CO_2 concentration profile becomes more uniform due to the facilitation of diffusion, the CO_2 assimilation rate increases more significantly.

The results obtained for facilitated diffusion of CO_2 to the spheric chloroplast are similar to those reported by Yokum and Lommen (1975), who considered chloroplasts as homogenous flat layers.

Permeability of thylakoid membranes for HCO_3^-

The coefficient of HCO_3^- permeability of thylakoid membranes is crucial for the establishment of the possible existence of the first CO_2 concentrating mechanism (Fig. 5*A*). Recent publications have not dealt with HCO_3^- permeability coefficients for thylakoid membranes.

It is known, however, that the passive transport of HCO_3^- into erythrocytes is characterized by permeability coefficients ranging between 1.2×10^{-4} and 4.75×10^{-4} cm . s^{-1} (Silverman 1974; Chow et al. 1976; Chow and Chen 1982). If these values also hold for the thylakoid membranes, the increase in CO_2 assimilation rate for a single granum may be 0.13—0.5% only (Fig. 3), but for chloroplasts it may even be 1.3—1.8-fold (Fig. 5*A*).

There are some indirect data for chloroplasts indicating that HCO_3^- significantly enters the thylakoids. The increased rate of efflux of H^+ from thylakoids illuminated for several minutes in the presence of HCO_3^- (Jagendorf 1972; Cohen and Jagendorf 1974) may be accounted for by the penetration of HCO_3^- into thylakoids followed by dehydration and CO_2 diffusion into the stroma, where CO_2 becomes hydrated to HCO_3^- and H^+. It is obvious that such a

mechanism may represent an indirect proton carrier in a way proposed for the explanation of weak acids effect on thylakoids (cf. Strotmann and Thiel 1973).

Unusual high rates of HCO_3^- penetration into thylakoids have been observed by Molotkovsky and Jakovleva (1980) who studied the swelling rate of thylakoids in media containing various anions.

Also, it is known that in illuminated chloroplasts an electric potential gradient is maintained, which accelerates the passive flux of anions from the stroma into thylakoids (Vredenberg 1976).

Recently, a specific protein which induces anion transport has been observed in thylakoids (Vambutus et al. 1984).

Little is known on possible H^+ penetration rates through channels of membrane-bound carbonic anhydrase (mechanism 2) and on the amount of H^+, which is free for HCO_3^- dehydration (mechanism 3).

Energy consumption of CO_2 concentrating mechanisms

Mechanisms 1 and 2 require energy only to pump H^+ into thylakoids. To dehydrate one HCO_3^- molecule one proton is required which needs one quantum of light to be pumped into a thylakoid (Vredenberg 1976; Witt 1979). Thus, the number of light quanta additionally needed for the assimilation of one CO_2 molecule with an active concentrating mechanism, is determined by the ratio of HCO_3^- ions transferred into thylakoids to CO_2 molecules assimilated in the stroma.

The energy requirements are predominantly determined by the permeability of thylakoid membranes to HCO_3^- (for mechanism 1) and by the value of E_s (for mechanism 2) (Figs. 3 and 5*A*).

Even a negligible concentrating of CO_2 around a single granum requires a great number of quanta (Fig. 3). In a chloroplast as a whole, the energy requirements are substantially lower. For example, a twofold increase in CO_2 assimilation rate can be reached with only 5 extra quanta (Fig. 5*A*), while no less than 12 quanta are necessary for the assimilation of a single CO_2 molecule (Osmond et al. 1982).

Mechanism 3 may use the energy of protons leaving thylakoids. If, for instance, each H^+ transferred by an ATPase complex reacts with HCO_3^- to give one CO_2 molecule, then the amounts of CO_2 molecules formed in a volume unit of a chloroplast should be at least 12 times as many as those consumed. For a single granum at $J_{cg}/J_{ca} = 12$ the rate of CO_2 assimilation may increase by less than 1% (Fig. 3), while in a chloroplast at $G_H/J_{ch} = 12$ the CO_2 assimilation rate may increase severalfold (Fig. 5*A*). Such a great increase must occur without any additional light quanta consumption.

We can conclude that the CO_2 concentrating mechanism may be advantageous for a chloroplast as whole, and that such a mechanism is unlikely to exist around a single granum.

The CO_2 concentrating efficiency depends on carbonic anhydrase; when the activity of the latter is decreased, the photosynthetic efficiency as well as the number of light quanta required additionally to concentrate CO_2 diminish (Fig. 5*B*). This may be attributed to the fact that carbonic anhydrase is necessary to operate the CO_2 concentrating mechanism.

The CO_2 concentrating efficiency may be higher and extra energy may be less when the diffusion coefficients of CO_2 and HCO_3^- in the chloroplast stroma are assumed to be lower than those shown in Table 2 (Fig. 5*C*) or when the chloroplast radius is larger (Fig. 5*D*). The increase in efficiency may be accounted for by the accumulation of CO_2 in the central part of the chloroplast as a result of a retardation of CO_2 diffusion to the envelope.

The results obtained rise a question concerning a possible increase in concentrating efficiency at the expense of the cytoplasm surrounding the chloroplast. If HCO_3^- can penetrate the chloroplast envelope and carbonic anhydrase is localized not only in thylakoids but in the cytoplasm as well, the concentrating region may be formed in the cytoplasm around the chloroplast. In this case CO_2 concentration in the chloroplast may be increased due to increasing diffusion resistance for CO_2 leaving the region of its assimilation.

The magnitude of the Michaelis constant of carbonic anhydrase for reversible CO_2 hydration reported in the literature may vary considerably. It can be shown that the CO_2 concentrating efficiency is little changed upon varying the constant.

Localization of carbonic anhydrase

In higher plants carbonic anhydrase is mostly localized in chloroplasts (Jacobson et al. 1975; Reed and Graham 1981). Membrane-bound as well as soluble forms of the enzyme have been found. At least a portion of carbonic anhydrase may thus be assumed to be localized in thylakoids (Pronina et al. 1981; Pronina and Semenenko 1984). Using the histochemical techniques the bulk of carbonic anhydrase in chlorella chloroplasts has been shown to be localized in thylakoids (Aiken and Romanovicz 1980). In higher plants, the enzyme may be bound to chloroplast envelope (Rathnam and Das 1975). Hence, the assumption concerning the localization of carbonic anhydrase both in thylakoids and at the chloroplast envelope, does not contradict experimental data; nevertheless, further studies are required to be able to draw decisive conclusions.

Indirect evidence of CO_2 concentrating mechanism presence in plants

The occurrence of a CO_2 concentrating mechanism in C_4 plants has been related to adaptation of the plants to water deficiency under intensive illumination and at elevated temperatures (Nasyrov 1978; Osmond et al. 1982). The concentrating mechanisms discussed above may be operative in C_3 plants adapted to similar conditions. Conclusions concerning the presence of a similar mechanism may be drawn based on indirect evidence.

If HCO_3^- can enter thylakoids and bind H^+ there, it becomes an uncoupler of photophosphorylation. Photophosphorylation uncoupling by HCO_3^- should be expected when H^+ penetrates membranes through channels of membrane-bound carbonic anhydrase (mechanism 2). Both the rate of HCO_3^- penetration into thylakoids (mechanism 1) and the rate of its dehydration on the surface of thylakoids (mechanism 2) must increase when HCO_3^- concentration in the stroma increases (Eqs. 10, 14). Hence, both the first and the second CO_2 concentrating mechanisms have to result in a decrease of photophosphorylation rates upon an increase in CO_2 concentration.

However, increase of photophosphorylation rate upon increasing CO_2 concentrations can also be observed. This may be accounted for by conformation changes in thylakoids (Cohen and Jagendorf 1974; Cohen and Mac Peek 1980). The direction of changes in photophosphorylation rate upon increasing CO_2 concentrations may depend on the plant species. Reutskii and Kozlova (1980) have reported a decrease of photophosphorylation rates upon increasing the CO_2 concentration in broken chloroplasts of xeromorphous and related species.

An unusual photosynthesis response to CO_2 may be also considered as indirect evidence for the possible existence of an energy-consuming CO_2 concentrating mechanism in some C_3 plants. For example, a decrease of CO_2 assimilation rate in cotton and sunflower, i.e. plants usually grown in intense light with water deficiency, can be observed when CO_2 concentrations in the intercellular space are slightly increased as compared to those in the air (Canvin 1979; Woo and Wong 1983). These phenomena may be a result of a decrease of photophosphorylation rate upon increasing CO_2 concentration. It should be emphasized, however, that any energy-consuming CO_2 concentrating mechanism must show similar results.

The driving force of CO_2 concentrating is a proton gradient formed between the thylakoid and the stroma. This would be of certain advantage to the above CO_2 concentrating mechanisms. However, the existence of such mechanisms in higher plants or algae needs further experimental verification.

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References

Aiken D. G., Romanovicz D. K. (1980): Ultrastructural demonstration of carbonic anhydrase in the chloroplast of *Chlorella vulgaris*. J. Cell Biol. **87**, 187 a

Barber J. (1972): Stimulation of millisecond delayed light emission by KCl and NaCl gradients as a means of investigating the ionic permeability properties of the thylakoid membranes. Biochim. Biophys. Acta **275**, 105—116

Bassham J. A., Buchanan B. B. (1982): Carbon dioxide fixation pathways in plants and bacteria. In: Development, Carbon Metabolism and Plant Productivity. Vol. 2 (Ed. Govindjee), pp. 141—189, Acad. Press, New York

Canvin D. T. (1979): Photorespiration: comparison between C_3 and C_4 plants. In: Encyclopedia of Plant Physiology, New Ser., vol. 6: Photosynthesis II. Photosynthetic Carbon Metabolism and Related Processes (Eds. M. Gibbs and E. Latzko), pp. 368—396, Springer, Berlin—Heidelberg—New York

Chow E. I., Chen D. (1982): Kinetic characteristics of bicarbonate-chloride exchange across the neonatal human red cell membrane. Biochim. Biophys. Acta **685**, 196—202

Chow E. I., Grandall E. D., Forster R. E. (1976): Kinetics of bicarbonate-chloride exchange across the human red blood cell membrane. J. Gen. Physiol. **68**, 633—652

Cohen W. S., Jagendorf A. T. (1974): Further studies on the bicarbonate stimulation of photophosphorylation in isolated chloroplasts. Plant Physiol. **53**, 220—223

Cohen W. S., Mac Peek W. A. (1980): A proposed mechanism for the stimulatory effect of bicarbonate ions on ATP synthesis in isolated chloroplasts. Plant Physiol. **66**, 242—245

Farquhar G. D., Caemmerer S. von, Berry J. A. (1980): A biochemical model of photosynthetic CO_2 assimilation in leaves of C_3 species. Planta **149**, 73—90

Gutknecht J., Bisson M. A., Tosteron D. C. (1977): Diffusion of carbon dioxide through lipid bilayer membranes. Effects of carbonic anhydrase, bicarbonate and unstirred layers. J. Gen. Physiol. **69**, 779—794

Heber U., Heldt H. W. (1981): The chloroplast envelope: structure, function and role in leaf metabolism. Annu. Rev. Plant Physiol. **32**, 139—168

Heldt H. W., Werdan K., Milovancev M., Geller G. (1973): Alkalinization of the chloroplast stroma caused by light-dependent proton flux into the thylakoid space. Biochim. Biophys. Acta **314**, 224—241

Jacobson B. S., Fong F., Heath R. L. (1975): Carbonic anhydrase of spinach. Studies on its location, inhibition and physiological function. Plant Physiol. **55**, 468—474

Jagendorf A. T. (1972): Proton flux and ATP formation in chloroplast. In: Proc. IInd Internat. Congress Photosynthesis Res. Vol. 2 (Eds. G. Forti, M. Avron, A. Melandri), pp. 1057—1064, Junk, The Hague

Karimova F. G., Rybkina G. V., Sedych N. V., Ratushnjak Ju. M., Belcevich T. M., Chalidullina N. G., Biglova S. G., Velicanova G. A. (1975): Influence of drought on water regime of chloroplasts. In: Water Exchange of Plants in Unfavourable Environment (Ed. N. S. Petinov), pp. 89—92, Shtinica, Kishinev (in Russian)

Komarova Yu. M., Doman N. G., Shaposhnikov G. L. (1982): Two forms of carboanhydrase from bean chloroplasts. Biokhimiya (USSR) **47**, 1027—1034 (in Russian)

Macovec P., Volfova A. (1981): Influence of senescence and nitrogen fertilization on the ultrastructural characteristics of barley chloroplasts. Photosynthetica **15**, 145—147

Magid E., Tusbeck B. O. (1968): The rates of the spontaneous hydration of CO_2 and the reciprocal reaction in neutral aqueous solution between 0° and 38 °C. Biochim. Biophys. Acta **165**, 515—524

Mazarei A. F., Sandall D. C. (1980): Diffusion coefficients for helium, hydrogen and carbon dioxide in water at 25 °C. AIChE J. **26**, 154—157

Mokronosov A. T. (1981): Ontogenetic Aspect of Photosynthesis. Nauka, Moscow (in Russian)

Mokronosov A. T., Nekrasova G. F. (1977): Ontogenetic aspect of photosynthesis studied with potato leaf. Fiziologiya Rasteniy **24**, 458—465 (in Russian)

Molotkovsky Yu. G., Yakovleva G. A. (1980): The permeability to anions of thylakoid membranes. Fiziologiya Rasteniy **27**, 453—469 (in Russian)

Nasyrov Y. S. (1978): Genetic control of photosynthesis and improving of crop productivity. Annu. Rev. Plant Physiol. **29**, 216—237

Nir I., Pease D. C. (1973): Chloroplast organization and the ultrastructural localization of photosystems I and II. J. Ultrastruct. Res. **42**, 534—550

Nobel P. S. (1974): Introduction to Biophysical Plant Physiology. W. H. Freeman Co., San Francisco

Osmond C. B., Winter K., Ziegler H. (1982): Functional significance of different pathways of CO_2 fixation in photosynthesis. In: Encyclopedia of Plant Physiology, New Ser., Vol. 12B: Physiological Plant Ecology II. Water Relation and Carbon Assimilation (Eds. O. L. Lange, P. S. Nobel, C. B. Osmond, H. Ziegler), pp. 479—547, Springer, Berlin—Heidelberg—New York

Pocker Y., Bjorkquist D. M. (1977): Stopped — flow studies of carbon dioxide hydration and bicarbonate dehydration in H_2O and D_2O acid-base and metal ion catalysis. J. Amer. Chem. Soc. **99**, 6537—6543

Pocker Y., Ng J. S. Y. (1973): Plant carbonic anhydrase. Properties and carbon dioxide hydration kinetics. Biochemistry **12**, 5127—5134

Pronina N. A., Semenenko V. E. (1984): Localization of membrane-bound and soluble forms of carboanhydrase in Chlorella cells. Fiziologiya Rasteniy **31**, 241—251 (in Russian)

Pronina N. A., Avramova S., Georgiev D., Semenenko V. E. (1981): A pattern of carbonic anhydrase activity in *Chlorella and Scenedesmus* on cell adaptation to high light intensity and low CO_2 concentration. Fiziologiya Rasteniy **28**, 43—52 (in Russian)

Rathnam C. K. M., Das V. S. R. (1975): Inter- and intracellular distribution of carbonic anhydrase, PEP carboxylase and RuDP carboxylase in leaves of *Eleusine coracana*, a C-4 plant. Z. Pflanzenphysiol. **75**, 360—364

Raven J. A., Glidewell S. M. (1981): Processes limiting photosynthetic conductance. In: Physiological Processes Limiting Plant Productivity (Ed. C. B. Johnson), pp. 109—136, Butterworths, London

Reed M. L., Graham D. (1981): Carbonic anhydrase in plants: distribution, properties and possible physiological roles. In: Progress in Phytochemistry, Vol. 7, pp. 47—94. Pergamon Press, Oxford New York

Reutskii V. G., Kozlova Zh. I. (1980): The CO_2 effect on light reactions of chloroplasts in C-3 plants of various ecological groups. Dokl. Akad. Nauk BSSR **24**, 944—947 (in Russian)

Silaeva A. M., Silaev A. V. (1979): Methods for quantitative analysis of electron-microscopic images of chloroplats. Fiziologiya i Biochimiya Kulturnych Rasteniy. **11**, 547—562 (in Russian)

Silverman D. N. (1974): A new approach to measuring the rate of rapid bicarbonate exchange across membranes. Mol. Pharmacol. **10**, 820—836

Strotmann H., Thiel A. (1973): Zum Mechanismus der Entkopplung der Photophosphorylierung durch Anionen schwacher Säuren. Ber. Deutsch. Bot. Ges. **89**, 209—212

Vambutus V., Beattie D. S., Bittman R. (1984): Isolation of protein(s) containing chloride ion transport activity from thylakoid membranes. Arch. Biochem. Biophys. **232**, 538—548

Vredenberg W. J. (1976): Electrical interaction and gradients between chloroplast compartments and cytoplasm. In: The Intact Chloroplast (Ed. J. Barber), pp. 53—88, Elsevier, Amsterdam —New York—Oxford

Walker N. A., Smith F. A., Cathers I. R. (1980): Bicarbonate assimilation by fresh-water charophytes and higher plants: 1. Membrane transport of bicarbonate ions is not proven. J. Membrane Biol. **57**, 51—58

Walz D., Goldstein L., Avron M. (1974): Determination and analysis of the buffer capacity of isolated chloroplasts in the light and in the dark. Eur. J. Biochem. **47**, 403—407

Wistrand P. J. (1984): Properties of membrane-bound carbonic anhydrase. In: Biology and Chemistry of the Carbonic Anhydrases. Annu. New York Acad. Sci., Vol. 429 (Eds. R. E. Tashian and D. Hewett-Emmett), pp. 195—206, New York Acad. Sci., New York

Witt H. T. (1979): Energy conversion in the functional membrane of photosynthesis analysis by light pulse and electric pulse methods. The central role of the electric field. Biochim. Biophys. Acta **505**, 355—427

Woo R. C., Wong S.C.(1983): Inhibition of CO_2 assimilation by supraoptimal CO_2: effect of light and temperature. Aust. J. Plant Physiol. **10**, 75—85

Wrischer M. (1978): Ultrastructural changes in plastids of detached spinach leaves. Z. Pflanzenphysiol. **86**, 95—106

Yokum S., Lommen P. W. (1975): Mesophyll resistances. In: Perspectives of Biophysical Ecology. Ecol. Stud., Vol. 12 (Eds. Gates D. M. and Schmerl R. D.), pp. 45—54, Springer, New York

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