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Quantitative evaluation of the role of a putative CO_2-scavenging entity in the cyanobacterial CO_2-concentrating mechanism

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Abstract

This paper assesses the contribution of a postulated CO_2-scavenging system to the efficient operation of the CO_2-concentrating mechanism (CCM) in cyanobacteria. A quantitative model for the CCM is presented which incorporates an energy-dependent carbonic anhydrase-like entity located at or near the inner surface of the plasma membrane. This entity, which converts CO_2 to HCO_3^- against the thermodynamic potential, scavenges CO_2 leaking outward from the carboxysomes, and, further, converts CO_2 entering from the medium to HCO_3^-, thus maintaining an inward diffusion gradient along which CO_2 enters passively. The model resembles our earlier models in postulating that CO_2 and HCO_3^- are not at equilibrium throughout the greater part of the cell, and that CO_2 is generated in high concentration at carbonic anhydrase sites within the carboxysomes. The model further takes into account the concentric thylakoid membranes which surround the carboxysomes, and events in the periplasmic space and the unstirred layer surrounding the cell. Implications of the predicted steady state fluxes of CO_2 and HCO_3^-, and of their steady state concentrations in various cellular compartments, are discussed. The plasma membrane carbonic anhydrase-like activity lowers the photosynthetic K_m for external Ci, as well as decreasing the inorganic C 'leak', but it may not save on energy expenditure.

Keywords: Mathematical model; Plasmalemma carbonic anhydrase; Inorganic carbon fluxes

1. Introduction

It is currently widely accepted that a CO_2-concentrating mechanism (CCM) operates in cyanobacteria, compensating for the large difference between the $K_m(CO_2)$ of their Rubisco and the concentration of CO_2 at equilibrium with air

Abbreviations: Ci, inorganic carbon; CA, carbonic anhydrase; CCM, CO_2 concentrating mechanism; PS, periplasmic space; Rubisco, ribulose 1,5-bisphosphate carboxylase/oxygenase. The subscripts m, p, e, c and x designate medium, periplasmic space, outer cytoplasm, inner cytoplasm and carboxysome, respectively.

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(Lucas and Berry, 1985). Quantitative models which have been put forward for the CCM (Badger et al., 1985; Reinhold et al., 1987, 1989) predict that during its operation there would be a substantial leak of accumulated CO_2 from the cell. The notion of a 'CO_2-scavenging' system to reduce the CO_2 leak has been put forward by Espie et al. (1991), who proposed on the basis of their experimental results that their postulated plasmalemma CO_2 transporter would act as such a scavenger. In the present communication, we evaluate the contribution of an added 'CO_2-scavenging' entity to the efficiency of operation of the CCM. To enable quantitative assessment of the role of such an entity, we present here a model for inorganic carbon (Ci) fluxes and photosynthesis in these organisms based on a system of simultaneous differential equations. As in the case of our previous models (Reinhold et al., 1989; Reinhold et al., 1991), the central premise is that the dominant Ci species in the cytoplasm is HCO_3^-, and that the generation of CO_2 from accumulated HCO_3^- occurs at carbonic anhydrase (CA) sites within the carboxysomes, in close proximity to carboxylation sites on Rubisco molecules where a substantial proportion is immediately fixed. The present model differs from previous models, however, in the following important respect: whereas we earlier suggested (Volokita et al., 1984) that the transport proteins mediating CO_2 uptake through the plasmalemma acted as a vectorial carbonic anhydrase releasing HCO_3^- into the interior, we now postulate that CO_2 enters the cell by diffusion, along a diffusion gradient maintained by the operation of an energy-dependent CA-like activity immediately within the plasmalemma (possibly attached to its inner surface). This CA-like activity not only converts entering CO_2 to HCO_3^- but scavenges CO_2 diffusing outwards from the carboxysomes (as well as that produced in the cytoplasm by uncatalyzed dehydration of HCO_3^-). In analogy with active transport systems, where it is commonly envisaged that energy input is linked to the maintenance of unequal (operative) affinities for the substrate at the two faces of the membrane, we suggest that binding of ATP — or some other form of energy input — might bring about a conformational change in the CA such as to lower its affinity for HCO_3^- and/or raise its affinity for CO_2. The hydration reaction would thus be promoted and the dehydration reaction depressed. Plasmalemma-located CA activity has recently been reported by Bedu et al. (1992). The model presented here also takes account the zone of concentric thylakoid membranes which surround the carboxysomes, and includes events in the periplasmic space and unstirred layer surrounding the cells.

2. The model

For the purpose of modelling the CCM, the cyanobacterial cell is considered as approximating

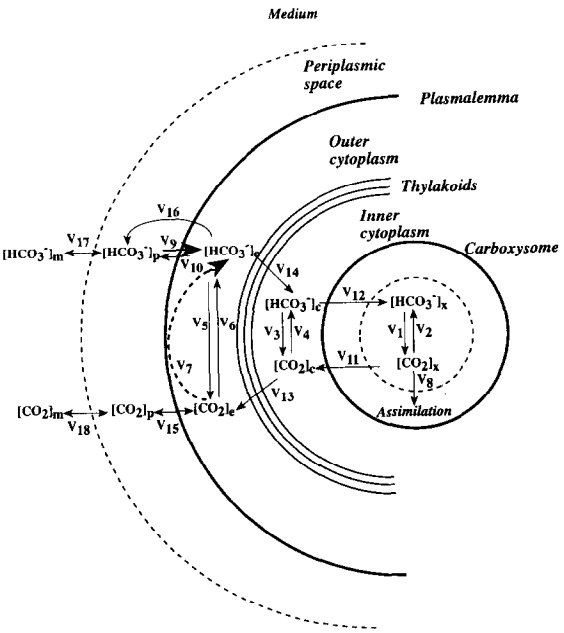

Fig. 1. Schematic diagram (not drawn to scale) of a cyanobacterial cell, indicating the various CO_2 and HCO_3^- fluxes which take part in the CO_2 concentrating mechanism. Only one carboxysome is shown. The indicated periplasmic layer is the inner region of the unstirred layer. The outer region of the unstirred layer is considered in this model only as creating a diffusion barrier (indicated by the dotted line) between periplasmic space and medium. For explanation of the numbered fluxes see text. The subscripts m, p, e, c, and x designate concentrations in the medium, periplasmic space, outer cytoplasmic region, inner cytoplasmic region and carboxysome, respectively. Energized fluxes are indicated by enlarged arrow heads.

to a sphere with six carboxysomes (see Reinhold et al., 1991) near its centre. Three concentric thylakoid layers, each consisting of two lipid membranes (Allen, 1968) divide the cytoplasm into an inner and an outer region (see Fig. 1). The carboxysomes are regarded as comprising an inner 'CA zone' where CO_2 concentration is very high; and an outer rubisco zone where part of the CO_2 is fixed (Reinhold et al., 1991). For ease of calculation, the unstirred layer surrounding the cell (the thickness of which may equal the radius of the cell — Raven, 1991) has been divided into two sections. The inner layer, adjacent to the plasmalemma and 0.1 mm thick, will be termed periplasmic space. The outer layer is considered only as creating a diffusion barrier between the bulk medium and the periplasmic space. The distribution of CO_2 and HCO_3^- has been taken as uniform within each of the five compartments (inner carboxysomal space, inner and outer cytoplasm, periplasmic space and medium). Diffusion resistances of defined magnitude separate the compartments.

The model envisages the following component processes:

(1) Active transport of HCO_3^- across the plasmalemma by a reversible membrane transport system.
(2) Diffusion flux of HCO_3^- between inner and outer cytoplasmic regions through the thylakoid membranes and through water channels in the thylakoid system; and between the inner cytoplasmic region and the carboxysomes. Also, passive diffusion of HCO_3^- through the plasmalemma.
(3) Diffusion flux of CO_2 across the plasmalemma; between the inner and outer cytoplasmic regions; and between the inner cytoplasmic regions and the carboxysomes.
(4) Conversion of CO_2 to HCO_3^- in the cytoplasm against the thermodynamic equilibrium by an energy-dependent CA-like entity located on or near the inner surface of the plasmalemma.
(5) As in our previous models, it is envisaged that interconversion between Ci species in the cytoplasm is uncatalyzed; within the carboxysomes interconversion is catalyzed at CA sites in the close vicinity of Rubisco.
(6) Fixation by Rubisco of part of the CO_2 generated at the CA sites.
(7) In the medium, diffusion of HCO_3^- and of CO_2 between the external medium and the periplasmic space through the unstirred layer. Since the volume of the periplasmic space is small, the uncatalyzed interconversion between CO_2 and HCO_3^- here has been neglected.

2.1. Formulation of the model

Fig. 1 gives a schematic diagram of the model. It is assumed that the kinetics of the plasmalemma HCO_3^- transport system approximates to the simple Michaelis-Menten formulation and that, as in many active transport mechanisms, V_{max} is the same at both outer and inner membrane interfaces but that the K_m differs. Therefore

$$v_9 = \frac{V_t[HCO_3^-]_p}{[HCO_3^-]_p + K_t} \quad (1)$$

$$v_{10} = \frac{V_t[HCO_3^-]_e}{[HCO_3^-]_p + K_r} \quad (2)$$

where V_t is the maximum rate of HCO_3^- transport per cell (1.6×10^{-11} μmol s^{-1}, twice the maximum rate of CO_2 fixation); K_t and K_r are the Michaelis constants for transport in the inward and outward directions respectively (80 μM (cf. Volokita et al., 1984) and 200 000 μM respectively), allowing a maximum accumulation ratio of 25 000 (see Reinhold and Kaplan 1984) and the subscripts p and e denote HCO_3^- concentration in the periplasmic space and the external cytoplasmic compartment respectively.

Net passive diffusion of CO_2 between periplasmic space and cytoplasm is a function of the diffusion gradient, and of the permeability coefficient and surface area of the boundary membrane.

$$v_{15} = P_b A([CO_2]_p - [CO_2]_e) \quad (3)$$

where P_b is the permeability coefficient of the plasmalemma for CO_2 (taken as that for a lipid bilayer membrane, see Table 1); A is the area of the

Table 1
Values adopted for geometric parameters and for permeability and diffusion coefficients

Symbol	Description	Value	Reference
N	Number of carboxysomes per cell	6	Reinhold et al., 1991
r_1	Radius of CO_2 source zone ('CA space') within carboxysomes	0.01 μm	see Reinhold et al., 1991
r_2	Radius of carboxysome	0.2 μm	Reinhold et al., 1989
r_3	Radius of inner cytoplasmic region (up to thylakoid zone)	1.73 μm	Allen 1968
r_4	Radius of inner + outer cytoplasmic regions (up to plasmalemma)	1.77 μm	Derived from cell volume as given in Reinhold et al., 1989
r_5	Radius of cytoplasm + periplasmic space (PS)	1.87 μm	PS taken as arbitrary fraction of unstirred layer, see text.
r_6	Radius of cell + unstirred layer	3.54 μm	2 × r_4 (see text)
P_b	Permeability coefficient of lipid bilayer membrane to CO_2	3.5 × 10^3 μm s^{-1}	Gutknecht et al., 1977
D_C	Coefficient for diffusion of CO_2 in water	1.88 × 10^3 μm^2 s^{-1}	Mazarei and Sandall, 1980
D_H	Coefficient for diffusion of HCO_3^- in water	1.15 × 10^3 μm^2 s^{-1}	Walker et al., 1980

plasmalemma (calculated from the radius r_4, Table 1).

Conversion of CO_2 into HCO_3^- at (or near) the inner surface of the plasmalemma by energy-dependent CA-like activity was also presumed to approximate to Michaelis Menten kinetics.

$$v_7 = \frac{V_c[CO_2]_e}{[CO_2]_e + K_{mc}} \quad (4)$$

V_c (maximum rate of CO_2 conversion per cell) was taken as 3.5 times the maximum rate of CO_2 fixation and K_{mc}, the apparent K_m for CO_2 conversion, as 0.4 μM, corresponding to the parameters observed for CO_2 uptake (Espie et al., 1991).

Diffusion of HCO_3^- across the plasmalemma is given by:

$$v_{16} = P_{pH}A([HCO_3^-]_e - [HCO_3^-]_p) \quad (5)$$

where $A = 4\pi r_4^2$ (for r_4 see Table 1), and P_{pH} is the permeability coefficient of the plasmalemma for HCO_3^-. Eq. 5 somewhat underestimates v_{16} as it omits the electric field across the plasmalemma (see Stein 1986, p. 45). However, the inaccuracy involved in this approximation will have been small in comparison with the uncertainty necessarily involved in estimating P_{pH}. The value of the latter has been taken as 0.3 × 10^{-2} μm s^{-1}.

Net inward diffusion of accumulated HCO_3^- from the outer to the inner cytoplasmic regions, through the concentric thylakoid membranes, is given by

$$v_{14} = P_{tH}A([HCO_3^-]_e - [HCO_3^-]_c) \quad (6)$$

where $A = 4\pi r_3^2$ (see Table 1), the subscript c denotes the inner cytoplasmic region, and P_{tH} is the general permeability coefficient through the six lipid membranes and the water channels in the thylakoid system. The latter value may be calculated according to the general equation

$$P = (1/p_1 + 1/p_2 + ...+ 1/p_n)^{-1} \quad (6a)$$

provided the thickness of the membranes is negligible and they are uniformly distributed. It is assumed as a first approach that the permeability coefficient for bicarbonate in a thylakoid membrane layer is 100 times less than that for CO_2 given in Table 1.

Carboxysomes can be regarded as spheres densely packed with enzyme molecules which may constitute a substantial barrier to diffusion (Reinhold et al., 1991).

The net diffusion flux of HCO_3^- into and out of the carboxysomes can be calculated by the following form of the equation developed for diffusion in spheres (Jacobs, 1935, p. 125)

$$v_{12} = \frac{N4\pi D_{xH} r_1 r_2([HCO_3^-]_c - [HCO_3^-]_x)}{r_2 - r_1} \quad (7)$$

where N = number of carboxysomes and D_{xH} = diffusion coefficient for HCO_3^- in the carboxysome. This diffusion coefficient is likely to be considerably lower than the corresponding coefficient for CO_2, since HCO_3^- has to pass through water channels in the densely packed proteins in the carboxysome (see under V_{11}below). D_{xH} cannot yet be accurately predicted, and it was taken as 0.09 D_{xC}, the corresponding diffusion coefficient for CO_2 (see below). The uncatalyzed interconversion between HCO_3^- and CO_2 in the outer cytoplasmic region is given by the following equations:

$$v_5 = K_3 V_e [HCO_3^-]_e \tag{8}$$

$$v_6 = K_4 V_e [CO_2]_e \tag{9}$$

and in the inner cytoplasmic region by

$$v_3 = K_3 V_c [HCO_3^-]_c \tag{10}$$

$$v_4 = K_4 V_c [CO_2]_c \tag{11}$$

where v_3 and v_5 indicate the dehydration of HCO_3^- and v_4 and v_6 the hydration of CO_2; K_3 the overall rate constant for dehydration of HCO_3^- (2.63×10^{-3} s^{-1}) and K_4 the overall rate constant for hydration of CO_2 (37.2×10^{-3} s^{-1}) (recalculated from Spalding and Portis, 1985, for pH 7.6); V_e and V_c are the volumes of the outer and inner cytoplasmic regions respectively.

The interconversion between HCO_3^- and CO_2 within the carboxysomes, catalyzed by CA, is given by the equations (see Spalding and Portis, 1985)

$$v_1 = \frac{V_{ba} K_{ca} [HCO_3^-]_x}{K_{ba} K_{ca} + K_{ca} [HCO_3^-]_x + K_{ba} [CO_2]_x} \tag{12}$$

$$v_2 = \frac{V_{ca} K_{ba} [CO_2]_x}{K_{ba} K_{ca} + K_{ca} [HCO_3^-]_x + K_{ba} [CO_2]_x} \tag{13}$$

where v_1 and v_2 are the rates of dehydration and hydration respectively; V_{ca} is the maximum rate of CO_2 hydration per cell (taken as 8×10^{-10} μmol s^{-1} at pH 7.6, 100 times the maximum rate of CO_2 assimilation); V_{ba} is the maximum rate of HCO_3^- dehydration (recalculated from Spalding and Portis (1985) as 1.18 V_{ca}); K_{ca} is the K_m of CA for HCO_3^- (30 133 μM) and K_{ba} that for CO_2 (1800 μM) as given in Spalding and Portis, 1985)

Fixation of the CO_2 generated within the carboxysomes is given by:

$$v_8 = \frac{V_p [CO_2]_x}{[CO_2]_x + K_c} \tag{14}$$

where V_p is the maximum rate of CO_2 fixation per cell (8×10^{-12} μmol s^{-1}, Reinhold et al., 1991); K_c is the apparent $K_{m(CO_2)}$ for Rubisco (250 μM, Badger et al., 1985).

Net outward diffusion from the carboxysomes of such CO_2 as is not fixed there is given by:

$$v_{11} = \frac{N 4\pi D_{xC} r_1 r_2 ([CO_2]_x - [CO_2]_c)}{r_2 - r_1} \tag{15}$$

(see Jacobs (1935) as for Eq. 7). D_{xC}, diffusion coefficient for CO_2 through the densely packed proteins in the carboxysome, may be substantially smaller than that in water (see Reinhold et al., 1991). Values for D in proteins from 10 to 100 times lower than that in water have been suggested (Verkhivker et al., 1992). (Fixation of CO_2 at carboxylation sites along the diffusion path within the carboxysomes (see Reinhold et al., 1991) would have the apparent effect of further reducing D.) As a first approximation D_{xC} has been taken as 1/80 that in water (see Table 1).

Net outward diffusion of CO_2 from the inner to the outer cytoplasmic compartment, through the concentric thylakoid membranes is given by:

$$v_{13} = P_{cc} A ([CO_2]_c - [CO_2]_e) \tag{16}$$

where A is again $4\pi r_3^2$ (see Table 1) and P_{cc} is the general permeability coefficient through the six lipid membranes, calculated according to Eq. 6a above.

Diffusion of HCO_3^- between the bulk medium and the PS through the unstirred layer is given by

$$v_{17} = \frac{4\pi D_H r_5 r_6 ([HCO_3^-]_m - [HCO_3^-]_p)}{r_6 - r_5} \tag{17}$$

(see Jacobs (1935) as for Eqs. 7 and 15).

Similarly, diffusion of CO_2 between these two zones is given by

$$v_{18} = \frac{4\pi D_C r_5 r_6([CO_2]_m - [CO_2]_p)}{r_6 - r_5} \quad (18)$$

The subscript m denotes concentrations in the medium. Table 1 gives values for D_C, D_H and the radii.

The system of differential equations for the rate of change in the concentrations of the Ci species in the various compartments may be written as follows:

$$d[CO_2]_x/dt = \{v_1 - v_2 - v_8 - v_{11}\}/V_x$$

$$d[HCO_3^-]_x/dt = \{v_2 + v_{12} - v_1\}/V_x$$

$$d[CO_2]_c/dt = \{v_3 + v_{11} - v_4 - v_{13}\}/V_c$$

$$d[HCO_3^-]_c/dt = \{v_4 + v_{14} - v_3 - v_{12}\}/V_c$$

$$d[CO_2]_e/dt = \{v_5 + v_{13} + v_{15} - v_6 - v_7\}/V_e$$

$$d[HCO_3^-]_e/dt = \{v_6 + v_7 + v_9 - v_5 - v_{10} - v_{16}\}/V_e$$

$$d[CO_2]_p/dt = \{v_{18} - v_{15}\}/V_p$$

$$d[HCO_3^-]_p/dt = \{v_{10} + v_{16} + v_{17} - v_9\}/V_p$$

where V is volume and the subscripts x, c, e and p designate carboxysomes, inner cytoplasm, outer cytoplasm, and the PS respectively. The fourth order Runge-Kutta method was applied to solve this system of differential equations. The calculations were performed up to the time that corresponds to a steady-state. External pH was taken as 8.0; both cytoplasmic and carboxysomal pH as 7.6.

3. Results and discussion

Figs. 2 and 3 present the steady state concentrations of the two Ci species in the various compartments, and their steady state fluxes, for external concentrations of 10 μM (K_m for photosynthesis) and 500 μM respectively. They indicate that catalysed dehydration of the accumulated HCO_3^- which reaches the carboxysomes will produce steady state CO_2 concentrations in these bodies adequate to support photosynthesis at expected rates; the relatively low permeability of the carboxysomes to CO_2 restricts CO_2 leak sufficiently to achieve this. The concentric thylakoid system surrounding the carboxysomes would also add somewhat to the diffusion resistance. These findings strengthen the validity of our previously published model (Reinhold et al., 1989; Reinhold et al., 1991) which postulates that the need to assign a high resistance to CO_2 diffusion to the plasmalemma can be dispensed with if CA-catalyzed CO_2 generation is confined to the carboxysomes. The ratio of CO_2 leaked/CO_2 fixed may in fact be expected to be rather lower than indicated in Fig. 2, since the strong diminution in CO_2 concentration as it diffuses outwards through the carboxysome past Rubisco sites where it is consumed (see Reinhold et al., 1991) has not

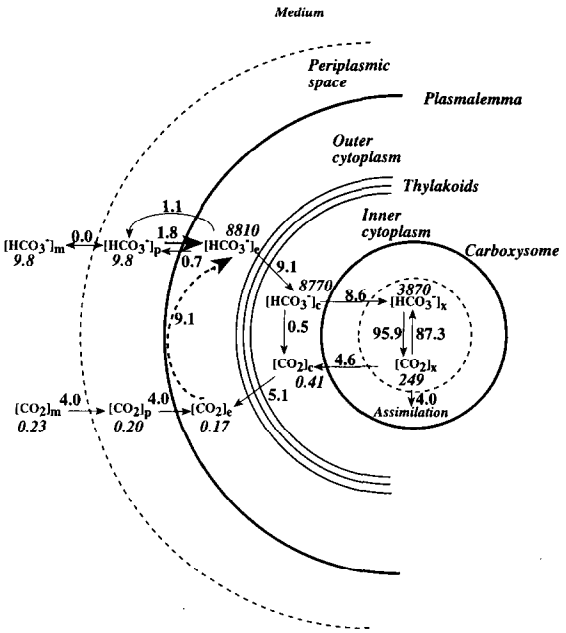

Fig. 2. Predicted steady state concentrations of Ci species in the various compartments of a photosynthesizing cyanobacterial cell and in the surrounding medium, together with the predicted steady state values for the Ci fluxes which constitute the CO_2 concentrating mechanism, when the external Ci concentration is the approximate $K_{1/2}$ for photosynthesis (10 μM). The pH of the medium is taken as 8, and of the cytoplasm as 7.6. Steady state concentrations (italic figures) are given as μM. The units for the fluxes (bold figures beside arrows) are 10^{-12} μmol s^{-1} $cell^{-1}$. Fluxes below 0.05×10^{-12} μmol s^{-1} $cell^{-1}$ are not shown. Other details as for Fig. 1.

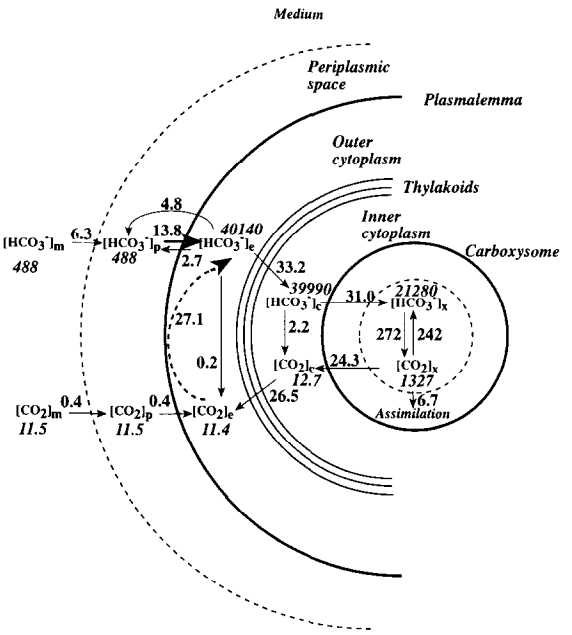

Fig. 3. Predicted steady state Ci concentrations and fluxes as in Fig. 2 when the external Ci concentration is 500 μM.

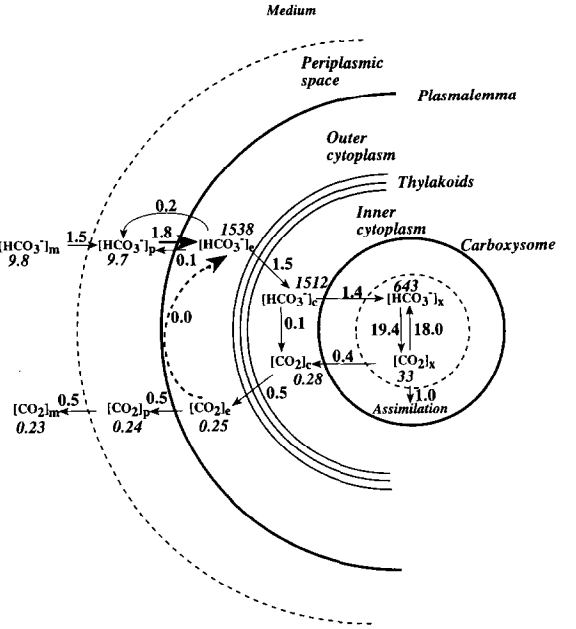

Fig. 4. Predicted steady state Ci concentrations and fluxes as in Fig. 2, when the plasmalemma CA-like activity is set at zero.

been taken into account here; in order to simplify calculation, the concentrations in each compartment have been regarded as uniform.

An important modification which is introduced into the model in the present investigation is the placement of energy-dependent CA-like activity at or near the inner surface of the plasmalemma, where it can act as an effective scavenger of CO_2 leaking into the cytoplasm from the carboxysomes. The notion of a scavenger system was earlier proposed by Espie et al. (1991) who pointed out that their postulated plasmalemma CO_2 transporter would be expected to retrieve part of the CO_2 leaked into the medium during photosynthesis. A scavenger operating within the cytoplasm, as suggested here, before escaping CO_2 has been dispersed by three-dimensional diffusion through the plasmalemma, would be a more efficient arrangement.

The contribution of the scavenger system can be assessed by comparing Fig. 2 with Figs. 4 and 5, in which the plasmalemma CA-like activity has been set at zero. When this comparison is made at

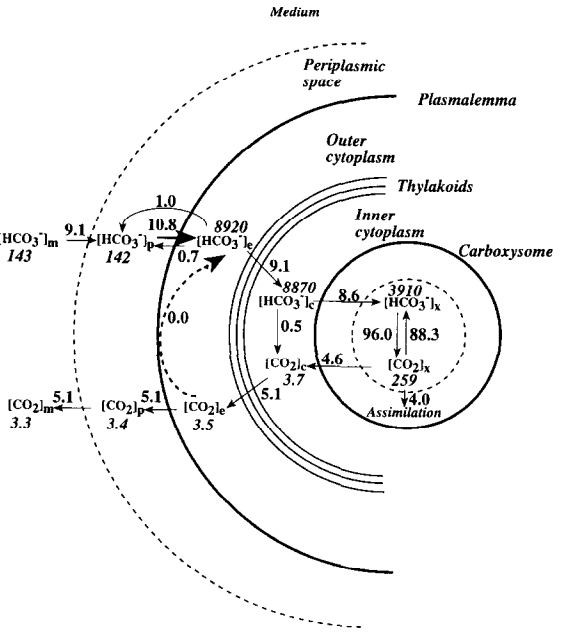

Fig. 5. Predicted steady state Ci concentrations and fluxes as in Fig. 2, when the plasmalemma CA-like activity is set at zero, but the external Ci concentration is raised so as to achieve the same rate of assimilation as in Fig. 2.

equal external Ci concentration (compare Figs. 2 and 4) it is seen that assimilation rate is reduced from four to one if this CA-like activity is abolished. In order to achieve the same rate of assimilation as in the presence of plasmalemma CA-like activity, it is necessary to raise the external Ci concentration to about 15 times that supplied in Fig. 2 (compare Figs. 2 and 5). A biological advantage of the plasmalemma CA is thus that it lowers the photosynthetic K_m for external Ci. Further, the Ci leaked from the cell by net passive diffusion ($CO_2 + HCO_3^-$) is 6.1×10^{-12} μmol s^{-1} $cell^{-1}$ in Fig. 5 and only 1.1×10^{-12} in Fig. 2. As a consequence, the ratio CO_2 fixed/Ci leaked is 0.66 in the absence of the CA-like activity as compared with 4.0 in its presence. However, in spite of the large difference in size of leak of accumulated Ci, the energy cost of the system per CO_2 fixed appears to be the same in the two cases. The total 'energy-requiring' flux is 10.9×10^{-12} μmol s^{-1} $cell^{-1}$ in Fig. 2 (active uptake, 1.8; plasmalemma CA activity, 9.1); in Fig. 5 it is 10.8×10^{-12} (active HCO_3^- uptake). Therefore, if it is assumed that the ATP requirement for CO_2 hydration on the plasmalemma is equal to that for HCO_3^- transport (see section on energy consumption) the energy expenditure is equal. The CO_2 which effluxes from the carboxysomes leaks from the cell in Fig. 5; in Fig. 2 it is 'recycled'. Energy saving would thus ensue if re-cycling demands less energy than membrane transport of HCO_3^-.

Apart from re-cycling effluxing CO_2, the energy dependent CA also converts newly-entering CO_2 to HCO_3^-, in which form there can be net diffusion into the carboxysomes. As a result of these two actions the CA-like activity maintains an inward concentration gradient for CO_2 across the plasmalemma, allowing CO_2 to enter the cell by diffusion (see section on mechanism of CO_2 uptake). The model thus predicts that Ci efflux from the cell is chiefly in the form of HCO_3^-.

The proportion of Ci taken up which is fixed decreases with increasing external Ci concentration (cf. Figs. 2 and 3). This is the inevitable outcome of the fact that the K_m and V_{max} for the HCO_3^- transport process are higher than those for CO_2 assimilation (see also Kaplan et al., 1980), and the predicted curve for uptake therefore continues to rise after the curve for assimilation has flattened (cf. Figs. 6 and 7. Also compare the curves for cytoplasmic HCO_3^- content with that for assimilation in Fig. 6). It is possible that in nature a control system exists which switches off or slows down transport when internal HCO_3^- concentration has reached a level high enough to saturate photosynthesis. The fact that, when the plasmalemma CA operates, Ci efflux from the cell is in the form of HCO_3^- (Figs. 2 and 3) rather than preponderately CO_2 (Fig. 5) could then be of further advantage. The slow down of carrier transport due to the control mechanism is likely to affect the outward as well as the inward operation of the system, and leak of accumulated Ci would

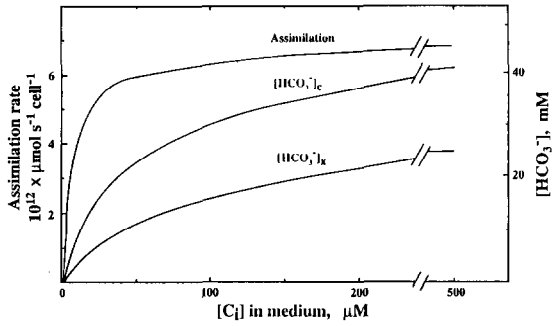

Fig. 6. Predicted dependence of assimilation rate, and of HCO_3^- concentration in the cytoplasm and the carboxysomes, on external Ci concentration. The subscripts c and x denote concentrations in the inner cytoplasmic region and the carboxysomes, respectively.

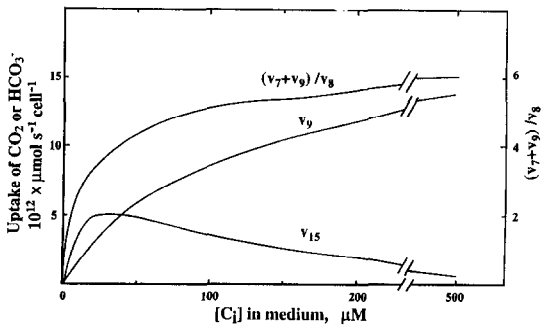

Fig. 7. Predicted dependence of mediated HCO_3^- uptake (v_9), net CO_2 uptake (v_{15}), and the ratio rate of energy-dependent steps/assimilation rate $[(v_7 + v_9)/v_8]$ on external Ci concentration.

thus be substantially slowed. The interesting curve for net rate of CO_2 uptake (Fig. 7) which first rises with increasing external Ci concentration, then falls, is due to the fact that after photosynthesis and the plasmalemma CA have been saturated, CO_2 concentration in the cytoplasm increases and approaches that in the medium, lessening the inward diffusion gradient for CO_2 (cf. Figs. 2 and 3).

3.1. The energy consumption of the CCM

According to the model, metabolic energy input (presumably directly or indirectly in the form of ATP) is required for active transport of HCO_3^- through the plasmalemma and for hydration and release of cytoplasmic CO_2 by the plasmalemma CA. The energy cost can be evaluated by comparing the sum of the rates of these two processes with the rate of CO_2 fixation. Fig. 7 indicates that the ratio $[v_7 + v_9]/v_8$ (ie; the rates of transfer + hydration divided by rate of photosynthesis) rises from about two at low external Ci concentration to about six at saturating external Ci. Thus assimilation of one CO_2 molecule requires transport or hydration of two to six Ci molecules. According to Grassl (1991) three molecules of HCO_3^- can be transported through the membrane for every molecule of ATP expended. If the same number of CO_2 molecules can be hydrated at the plasmalemma per ATP molecule, the operation of the CCM would require expenditure of one to two additional ATP molecules for each CO_2 molecule assimilated.

On the assumption that 3 H^+ are required for the generation of one ATP molecule and that 3 H^+ are accumulated for two quanta absorbed (Rich 1988), it can be calculated that two to four additional quanta are needed to produce these additional one to two ATP molecules. This conclusion is in good agreement with data indicating that the operation of the CCM in microalgae requires additional expenditure of about three to four quanta per each assimilated CO_2 molecule (Burger et al., 1988)

3.2. Effect of uncoupling the energy supply

De-energization of the HCO_3^- carrier system (e.g. by transition to darkness) would be expected to convert it from an active transport mode to a facilitated diffusion mode, in which form it would tend to equilibrate HCO_3^- across the plasmalemma. De-energization of the energy-dependent CA-like entity, it may be suggested, might convert it to a conventional CA, in which case substantial amounts of CO_2 would immediately be generated. (CA activity associated with the plasmalemma has in fact been observed by Bedu et al., 1992.) The result of both these processes would be dramatic loss of Ci from the cell, in the latter case in the form of CO_2, in the former as HCO_3^-.

3.3. Mechanism of CO_2 uptake

It has been inferred from the results of a number of investigations that CO_2 uptake is mediated (Volokita et al., 1984) and even active (Aizawa and Miyachi 1986; Miller et al., 1988; Espie et al., 1991; Badger and Price 1992). However, mediated transport might be considered improbable in the case of a bilipid membrane with high CO_2 permeability. The model developed here explains CO_2 influx on the basis of passive diffusion through the plasmalemma followed by conversion of CO_2 into HCO_3^- (see Figs. 2 and 3). Saturation kinetics would be observed, since maintenance of the inward diffusion gradient would depend on the conversion reactions. The latter would be the rate-limiting step controlling CO_2 entry. The observed kinetics would be 'replacement kinetics' (CO_2 molecules would diffuse inwards to replace those converted to HCO_3^-) and uptake would thus reflect the kinetics of the energy-dependent CA. Derivation of the kinetic parameters would, however, be complicated by the fact that the CO_2 of exogenous origin would be competing for the enzyme with CO_2 diffusing into the cytoplasm from the carboxysomes, as well as with that formed in the cytoplasm by uncatalysed dehydration of HCO_3^-. The evidence cited for active CO_2 transport (e.g. saturation kinetics, effects of various inhibitors) can be explained on the basis of this model.

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