A model for inorganic carbon fluxes and photosynthesis in cyanobacterial carboxysomes

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Received August 5, 1990

REINHOLD, L., KOSLOFF, R., and KAPLAN, A. 1991. A model for inorganic carbon fluxes and photosynthesis in cyanobacterial carboxysomes. Can. J. Bot. **69**: 984–988.

A barrier to CO_2 diffusion within the cyanobacterial cell has been regarded as essential for the inorganic carbon concentrating mechanism. We present here an extension of our earlier quantitative model demonstrating that it may be unnecessary to postulate any barrier other than the ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) molecules themselves. It is proposed that carbonic anhydrase is located in the interior of the carboxysome and that the CO_2 generated is largely fixed as it diffuses outwards past Rubisco sites located along the diffusion path. Equations have been developed, by combining a mass balance equation with Fick's Law and the Michaelis-Menten equation (representing CO_2 fixation), estimate the value that must be assigned to the diffusion coefficient for CO_2 within the carboxysome if the CO_2 concentration is to be reduced to near zero at the carboxysome outer surface. A solution has been obtained for two limiting cases, that where CO_2 concentration is nearly saturating and that where it is at the $K_m(CO_2)$ value or below. These two estimates predict that the permeability constant for the Rubisco zone in the carboxysome would have to be 10^{-2}–10^{-3} cm·s^{-1}, a value that we suggest is reasonable for three-dimensional diffusion through a densely packed protein layer. The concentration gradient in the inward direction, for substrates penetrating the carboxysomes from the cytoplasm, is shown to be relatively flat, owing to the concentrating effect experienced by solutes passing from the periphery to the center of a sphere.

Key words: cyanobacteria, carboxysomes, inorganic carbon fluxes, photosynthesis, model.

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On a considéré comme essentielle l'existence d'une barrière à la diffusion du CO_2 à l'intérieur de la celle cyanobactérienne, pour le mécanisme de concentration du carbone inorganique. Les auteurs présentent une extension de leur modèle quantitatif démontrant qu'il pourrait être inutile de postuler l'existence d'une quelconque barrière autres que les molécules de Rubisco elles-mêmes. On propose que l'anhydrase carbonique est localisée à l'intérieur du carboxysome et que le CO_2 généré est principalement fixé lorsqu'il diffuse vers l'extérieur, au delà des sites Rubisco localisés le long du sentier de diffusion. Des équations ont été développées, en combinant une équation de balance de masses avec la loi de Frick et l'équation de Michaelis-Menten (représentant la fixation du CO_2) afin d'estimer la valeur qui doit être assignée au coefficient de diffusion du CO_2 à l'intérieur du carboxysome, si la concentration en CO_2 doit être réduite près de zéro à la surface externe du carboxysome. On a pu obtenir une solution pour deux cas de limitation, soit celui où la concentration en CO_2 est presque saturante et celle où est atteint une valeur égale ou inférieure au K_m (CO_2). Ces deux estimations permettent de prédire que la constante de perméabilité pour la zone de Rubisco dans le carboxysome devrait être de 10^{-2} à 10^{-3} cm s^{-1}, soit une valeur qui ne nous semble pas déraisonnable pour une diffusion en trois dimensions à travers une couche de protéines densément ramassées. Le gradient de concentration dans la direction interne, pour des substrats pénétrant le carboxysome à partir de cytoplasme, apparaît relativement plat, dû à l'effet concentrateur rencontré par les solutés allant de la périphérie au centre d'une sphère.

Mots clés : cyanobactérie, carboxysome, flux de carbone inorganique, photosynthèse, modèle.

[Traduit par la rédaction]

Introduction

We have recently proposed a quantitative model for inorganic carbon fluxes and photosynthesis in cyanobacteria (10, 11). A central feature of this model was the transfer of the barrier to CO_2 diffusion, regarded as essential to the concentration mechanism and hitherto assigned to the plasmalemma, to the carboxysome surface. This transfer had two major advantages: we could now attribute to the plasmalemma a permeability coefficient for CO_2 (P_{CO_2}) compatible with the known properties of polar lipid bilayers; second, the model allowed photosynthetically evolved O_2 to escape from the cell unhindered by the barrier that retained CO_2, thus obviating problems owing to build up of high internal O_2 concentrations. An important aspect of the model was that no matter which inorganic carbon (C_i) species was supplied, HCO_3^- was the species that arrived at the cytoplasmic surface of the plasmalemma. This proposal was based on experimental findings that led us to suggest that the membrane transport protein involved might serve as a vectorial carbonic anhydrase (13, 16). We further predicted that carbonic anhydrase (CA) was absent from the cytoplasm and that HCO_3^- and CO_2 would not reach equilibrium in this compartment. The accumulated HCO_3^- ions would penetrate the carboxysomes where the presence of CA at low concentration (our model showed that only a very low level of catalysis was required) would lead to CO_2 generation and subsequent fixation by ribulose 1,5-bisphosphate carboxy-

lase/oxygenase (Rubisco). The rates of CO_2 fixation predicted by our model accorded well with experimentally observed rates. One aspect of the model has recently been elegantly tested by Price and Badger (8) who expresed human CA in *Synechococcus* cells and observed that they had thereby caused them to lose the ability to accumulate internal C_i. They concluded that the latter ability depended on the absence of CA from the cytosol and its specific localization in the carboxysomes, as our model predicted.

We now wish to present a model that indicates that the requirement for a substantial barrier to CO_2 diffusion in the cell can probably be dispensed with altogether.

The model

This model was already foreshadowed in our earlier publications, when we suggested that there might be a steep gradient in CO_2 concentration within the carboxysome. Such a gradient, we pointed out, might arise if the CA were placed centrally in the interior of the carboxysome, and much of the CO_2 generated was fixed as it diffused outwards past Rubisco sites located along the diffusion path (see (10), Fig. 4). This proposal has now been quantitatively examined.

Consider a source of CO_2 of radius r_o immersed in the volume of the carboxysome (Fig. 1). Generating reactions keep the CO_2 concentration at this source constant at c_o. The goal is to be able to deduce the concentration (c) at various distances (r) from the source, assigning various values for D, the diffusion coefficient for CO_2 within the carboxysome, or alternatively, to deduce what value must be assigned to D to reduce the concentration at a known distance from the source to some predetermined level. The first step is to make a mass balance, which is most conveniently accomplished in spherical coordinates originating from the centre of the sphere. A mass balance on a spherical shell like the rubber shell of a balloon, located at distance r from the sphere, of surface area $4\pi r^2$, and thickness Δ takes the form

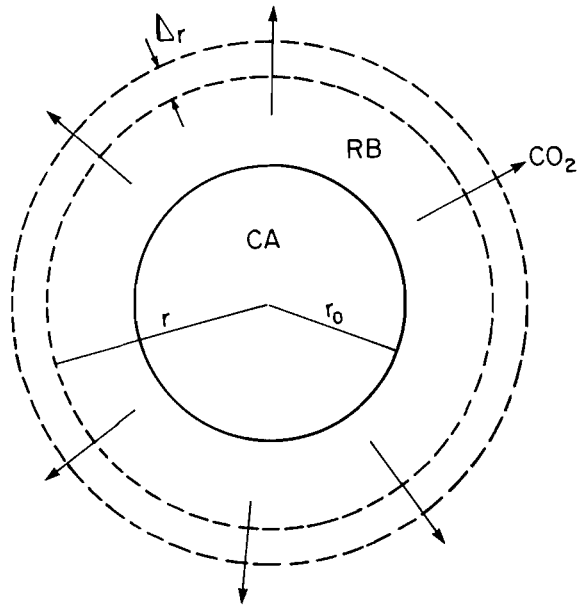
$$[1] \qquad \frac{d}{dt}(4\pi r^2 \Delta r c) = (4\pi r^2 j_i)_r - (4\pi r^2 j_i)r + \Delta r$$

(solute accumulation within the shell is equal to diffusion into the shell minus diffusion out of the shell)

where j_i is the CO_2 flux.

This equation must now be combined with Fick's Law and, further, a term must be inserted representing the CO_2 fixation process. This involves what M. H. Jacobs, author of a celebrated monograph on diffusion (6), has termed "very formidable mathematical difficulties." For the present purposes, we have solved an equation for two limiting cases. First, the case where the rate of the fixation process is almost independent of CO_2 concentration, that is, where the CO_2 concentration is saturating or nearly so and $V = V_{max}$. Second, for the case where the carboxysome CO_2 concentration is at about the K_m (CO_2) for Rubisco, or below, so that it is permissible to regard fixation rate as V_{max}/K_m times concentration.

Clearly, as CO_2 diffuses outwards and its concentration falls from a saturating concentration at the source to a very low one at the periphery, we shall be passing from the first case to the second case. The precise values for the parameters we are investigating will lie somewhere between the prediction for these two cases.



accumulation within the shell = diffusion into the shell − diffusion out of the shell

$$\frac{\partial}{\partial t}(4\pi r^2 \Delta r c)_r = (4\pi r^2 j)_r - (4\pi r^2 j)_{r+\Delta r}$$

FIG. 1. Diagram indicating outward diffusion of CO_2 from a spherical source. For an explanation see text. CA, carbonic anhydrase; RB, Rubisco.

The formulation of the first case, developed from [1] by considering the situation at the steady state (see Appendix), can be stated (after certain simplifying steps) as

$$[2] \qquad \frac{d^2c}{dr^2} + \frac{2dc}{rdr} = \frac{\alpha}{D}$$

where α is the constant rate of CO_2 fixation (V_{max}) of a unit volume of carboxysome. After various integration steps, and taking as boundary conditions $r = r_o$, $c = c_o$, and $r = r_f$, $dc/dr = 0$, the following solution is obtained:

$$[3] \qquad c_r = c_o + \frac{\alpha}{6D}(r^2 - r_o^2) + 2r_f^3\left(\frac{1}{r} - \frac{1}{r_o}\right)$$

Next, we have to arrive at an estimate of r_o, the radius of the source (the CA space) as compared with that of the Rubisco space (r). Holthuijzen *et al.* (4) and Shively *et al.* (14) in their electron microscope (EM) studies of *Thiobacillus* carboxysomes deduced that the radius of a Rubisco molecule was 5 nm. The electron micrograph of the *Anabaena* cell used for our previous model (9, 10) indicated a radius of about 200 nm for the carboxysome. Making appropriate allowance for the fact that small spheres closely packed into a larger sphere only occupy about 60% of the volume, we estimate that the carboxysome could contain $200^3 \times 0.6)/5^3 = 38\,400$ molecules Rubisco.

Joseph Berry has pointed out (personal communication) that if we want about half of the CO_2 produced at a CA site to be

fixed before it escapes we need about 17 000 Rubisco sites (2125 molecules) per CA molecule. This follows from the fact that the catalytic constant of CA is 10^5 s^{-1}, while that of Rubisco is 3–5 s^{-1}. This consideration suggests that we only need about 20 or 30 molecules of CA per *Anabaena* carboxysome. This number would be higher if the CA were substrate-limited; $K_m(HCO_3^-)$ is larger for CA than is $K_m(CO_2)$ and might be as high as 100 mM (9). If this were so, the HCO_3^- concentration predicted for the carboxysome compartment at just saturating external C_i concentration for photosynthesis (10, 11) would be suboptimal for CA.

We shall be on the conservative side and assign larger dimensions to the CA space than necessary on the basis of these considerations; besides, it may contain something apart from CA. For the moment we regard it as a sphere at the centre of the carboxysome of radius 1/20 that of the Rubisco space.

As CO_2 diffuses outwards from this space there will be a very considerable fall in its concentration quite apart from that due to the fixation reaction that consumes it. The mere fact of three-dimensional diffusion in a spherical situation would reduce the CO_2 concentration at the carboxysome surface to a very small fraction of that at the source. This dilution has, of course, no impact on the calculated rate of loss of CO_2 by diffusion out of the carboxysome and eventually out of the cell; as the radius increases, the drop in rate of loss per unit surface area of a spherical shell owing to attenuation of CO_2 is compensated for by the increase in total surface area of the shell. Because of this compensation effect we are going to be on the safe side in the discussion that follows. We are not merely going to seek a value for D that would reduce total outward flux at the carboxysome surface to about half total outward flux at the source (i.e., a situation where half of the CO_2 generated would be fixed before it escaped). We shall ask, what is the value of D necessary to bring the CO_2 concentration at the carboxysome surface down to zero, i.e., 100% fixation of generated CO_2?

Let us set source CO_2 concentration (C_o in eq. 2) at 1 mM and α (i.e., V_{max}) at 40 $\mu mol \cdot cm^{-3}$ carboxysome$\cdot s^{-1}$ (derived from our earlier model (10, 11) if one assumes six carboxysomes per cell. We think this is probably a better estimate of the number of carboxysomes per cell than our earlier guess of 60). This estimate of V_{max} compares with the value 60 $\mu mol \cdot cm^{-3}$ carboxysome$\cdot s^{-1}$, which can be calculated from Price and Badger's data (8). The radius of the carboxysome r is again 2×10^{-5} cm, as in our previous model, r_f is taken as 4×10^{-5} cm, and r_o is 10^{-6} cm. If c, the concentration at the carboxysome surface, is to be zero, we find that we must assign a value of about 10^{-6} $cm^2 \cdot s^{-1}$ to D.

Turning now to the second limiting case, where fixation rate is considered as a function of concentration, i.e., $(V_{max}/K_m)c$, again combining [1] with Fick's Law, and inserting a term for fixation rate, gives

$$[4] \qquad \frac{D}{r^2}\frac{d}{dr}r^2\frac{dc}{dr} - kc = 0$$

The following solution has been reached:

$$[5] \qquad c(r) = \frac{A\,e^{-\left(\frac{K}{D}\right)^{1/2} \cdot r}}{r}$$

where $A = c_o r_o e^{\left(\frac{K}{D}\right)^{1/2} \cdot r_o}$

Using the same dimensions for r_o and r as before, the same V_{max}, $K_m = 0.25$ $\mu mol \cdot mL^{-1}$, and $c_o = 0.25$ $\mu mol \cdot mL^{-1}$, we find that when $D = 10^{-8}$ $cm^2 \cdot s^{-1}$, $c = 10^{-4}$ $\mu mol \cdot mL^{-1}$. The total flux out of the carboxysome, i.e., the CO_2 escaping, will only be one-third of that generated at the source.

Discussion

Taking these two estimated values of a suitable D together, the permeability constant for the Rubisco zone in the carboxysome would work out at about 10^{-2} or 10^{-3}. Gutknecht *et al.* (2) deduced that the permeability of aqueous unstirred layers to CO_2 was 1.8×10^{-3} $cm^{-1} \cdot s^{-1}$, corresponding to $D = 4 \times 10^{-5}$ $cm^2 \cdot s^{-1}$. We shall come presently to the question as to whether it is reasonable to accept the above values for P and D for the carboxysome, but first we wish to consider the implications for the gradients in concentration in the other direction of essential substrates, HCO_3^- and ribulose 1.5-bisphosphate (RuBP), that must diffuse in to the carboxysome from the cytoplasm. It transpires that there is no problem here. Solutes diffusing from the periphery to the centre of a sphere are strongly concentrated as they go, the inverse of the dilution necessarily accompanying outwards diffusion. Equations have already been worked out for quantitative evaluation of diffusion inwards from the surface of a sphere under conditions where the diffusion substance is consumed as it passes (e.g., the diffusion of oxygen into respiring cells), though again, because of the complexity of the mathematics it has been assumed that respiration is almost independent of O_2 tension (3). Diffusion into a sphere is given by

$$[6] \qquad c_x = c_o = \frac{\alpha}{6D}(R^2 - r^2)$$

where R is the radius of the sphere and α is again the rate of the consumption reaction. Taking $r = 0$ (i.e., assessing concentration at the very centre of the sphere), c_o for RuBP as 0.5 $\mu mol \cdot mL^{-1}$, and α as V_{max} for photosynthesis, 40 $\mu mol \cdot mL^{-1}$ carboxysome $\cdot s^{-1}$, then c_x (the concentration at the centre) is over 90% of that at the surface even though RuBP is being consumed in photosynthesis. If one considers HCO_3^-, the estimated cytoplasmic concentration is about 17 mM (10, 11) at external c just saturating for photosynthesis, and this would drop by under 1% if D were 10^{-7} $cm^2 \cdot s^{-1}$. (If D were 10^{-8} $cm^2 \cdot s^{-1}$ the RuBP and HCO_3^- concentrations at the centre would be 0.25 and 16.7 $\mu mol \cdot mL^{-1}$, respectively.)

Quantitative treatment of the photorespiration profile along the row of Rubisco molecules will depend on the estimation of CO_2 and O_2 concentrations in the various layers; however, the degree of activation by CO_2 of the successive Rubisco molecules will also have considerable importance, and of this we as yet have no knowledge.

It is reasonable to accept the proposition that D for CO_2 diffusion through the Rubisco layer of the cytoplasm is so much smaller than that for diffusion in an aqueous unstirred layer? Diffusion in proteins is in fact known to be very slow in comparison with that through water. The welded three-dimensional structure of the protein usually leaves open only a few well-defined diffusion paths, whereas in water CO_2 will of course

diffuse in all directions. Moreover, diffusion along these paths is an activated "gated" reaction; that is, there is an energy barrier, usually associated with a conformation change that opens a "gate" through which the diffusing solute must squeeze (15). We should like to suggest that at least part of the significance of the dense packing of Rubisco in the carboxysomes is the substantial barrier to diffusion that such an arrangement affords. Whether a diffusion coefficient of the order predicted here is in fact appropriate will depend on the structure of the protein as well as on the packing. The tortuosity of the diffusion path between and through the protein molecules, effectively increasing r, may also play a significant part; a substantial increase in r would allow a compensatory increase in D.

However, there is of course another problem arising out of this model. If the CO_2 generated in the carboxysome is to be largely consumed before it reaches the surface, then by definition the peripherally sited Rubisco molecules are going to be operating at a very low carboxylating velocity unless source concentrations are extremely high, in which case leak rate would also be very high. (In the interests of energy conservation one would expect a trans-inhibition mechanism to slow down C_i uptake before such wasteful leak rates were reached.) Is there any evidence that there is surplus Rubisco in the carboxysomes and might Rubisco concentration not be the limiting factor in CO_2 fixation? Price and Badger (8) report a carboxysome volume of 4.2×10^{-14} $cm^3 \cdot cell^{-1}$. If the carboxysomes are fully packed with Rubisco molecules, allowing for the packing factor, this would give 5×10^4 molecules/cell, which is 5×10^{-14} μmol (4×10^{-13} sites). Using the catalytic constant 4 s^{-1}, this suggests a maximum fixation rate of 1.6×10^{-12} $\mu mol \cdot s^{-1} \cdot cell^{-1}$, somewhat lower than the authors' quoted experimentally observed V_{max} Rubisco of 2.6×10^{-12} $\mu mol \cdot cell^{-1}$.

A similar calculation for *Anabaena*, taking the number of carboxysomes per cell as 6, estimates maximum fixation rate as 6×10^{-12} $\mu mol \cdot s^{-1} \cdot cell^{-1}$, as compared with the observed V_{max} for photosynthesis of 8×10^{-12} $\mu mol \cdot s^{-1} \cdot cell^{-1}$. But both these calculations may overestimate the number of molecules by underestimating their volume; the molecules may approximate to cyclinders rather than spheres (12) and the electron micrographs may show their bases.

These figures therefore do not encourage one to suppose that there is surplus Rubisco in the carboxysomes. Admittedly, however, a surplus of, say two times would be easily missed in such rough estimations. Mayo *et al.* (7) in a careful examination of the factors limiting photosynthesis in *Synechococcus* reported that *in vitro* Rubisco activity was always greater than the inorganic carbon-saturated photosynthetic rate. Moreover, taking their figure for site density, and catalytic constant 4 s^{-1}, one can calculate that the potential V_{max} for fixation was five times the observed V_{max}. It may be recalled that Beudecker *et al.* (1) concluded that in *Thiobacillus* Rubisco was not rate limiting for fixation.

The problem of rapidly decreasing CO_2 concentration along a radial row of Rubisco molecules would, of course, vanish if there were only one Rubisco molecule in the row, i.e., if the CA zone were surrounded by a single circle of Rubisco molecules. That is one of the reasons that makes Holthuijzen *et al.*'s (4) suggestion that there may be only one layer of Rubisco attached to the outer envelope through the small subunit so interesting. This suggestion was based largely on the finding that after solubilization procedures only the large subunit was released into the medium; the small subunit was always recovered together with the envelope. Incidentally, this group also obtained immunological evidence (5) that the large subunits are not exposed to the outer surface of the carboxysome. This evidence is in keeping with our central hypothesis that there is an area in the carboxysome interior where CO_2 is generated and that the fixation sites border this area.

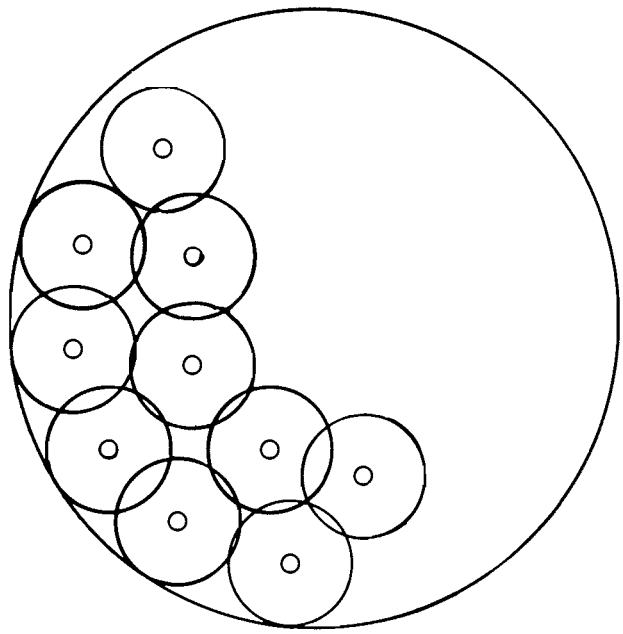


FIG. 2. Possible organization of carboxysome. Carbonic anhydrase sites (small circles) surrounded by zones containing Rubisco molecules.

What sort of permeability coefficient would one have to assign to this layer if one aimed at allowing only one in two CO_2 molecules to escape, at an internal CO_2 concentration just saturating photosynthesis? We can obtain a rough answer to this by treating this layer as a membrane (it would be 17 nm thick according to electron micrographs, a 7-nm (4) envelope plus a layer of Rubisco 10 nm in diameter). The surface area would be about 4×10^{-9} cm^{-2} (for our *Anabaena* carboxysome). Taking CO_2 concentration in the inner space as 1 mM, in the cytoplasm as negligible (cf. our earlier model), then outwards diffusive flux equals $4 \times 10^{-9} \cdot P_{CO_2}$. We are assuming this flux equals the rate of CO_2 fixation, about 10^{-12} $\mu mole \cdot s^{-1}$, and P_{CO_2} would thus be 2.5×10^{-4} $cm^{-1} \cdot s^{-1}$.

This model thus requires a substantial diffusion barrier, constituted either by the Rubisco molecules or the envelope or both together. The main obstacle to accepting this model, however, is the fact that the number of Rubisco molecules that could fit onto the external skin of a carboxysome is not large enough to account for observed fixation rates. Price and Badger (8) calculated the surface are of their *Synechococcus* carboxysomes to be about 1.3×10^{-9} cm^2, which is large enough for about 1300 Rubisco molecules of diameter 10 nm to be attached to it. In passing, we will point out that this is not very far from Joseph Berry's "minimum carboxysome" of about 2000 Rubisco molecules. However, as we saw earlier even 5000 molecules (the figure if the carboxysome is completely packed with Rubisco) is barely large enough to account for observed fixation rates.

Let us close by considering very briefly an alternative pattern for the carboxysome, not one CA zone but many, possibly as many as there are CA molecules within it (see Fig. 2). Even doubling or trebling, for the reasons I listed earlier, Berry's estimate of one CA molecule for 2000 Rubisco molecules, this still means that many hundreds of Rubisco molecules would have to be arranged round each CA molecule, necessarily in multiple rows. We are thus back to the first model on a smaller scale: in each of the smaller "solar systems," if consumption of generated CO_2 is efficient, the outer rows of Rubisco are severely limited by low CO_2 concentrations.

In summing up, then, I think one can conclude that the proposal that a carboxysome consists of a single row of Rubisco molecules attached to the envelope by their small subunits will only be acceptable if our notions of the relative geometry and dimensions of carboxysomes and Rubisco molecules undergo drastic revision. While this interesting role proposed for the small subunit does not at present seem tenable, the latter's role may nevertheless be concerned with the correct steric organization of the carboxysome. I also believe that we have reasonable grounds for concluding that, given a carboxysome filled with closely packed Rubisco molecules, it may become unnecessary to postulate any barrier to CO_2 diffusion in the cell other than the Rubisco molecules themselves.

Acknowledgment

This research was supported by a grant from the United States – Israel Binational Science Foundation.

1. Beudeker, R. F., Cannon, G. C., Kuenen, J. G., and Shively, J. M. 1980. Relations between D-ribulose-1-5-bisphosphate carboxylase, carboxysomes, and CO_2 fixing capacity in the obligate chemolithotroph *Thiobacillus neapolitanus* grown under different limitations in the chemostat. Arch. Microbiol. **124**: 185–189.
2. Gutknecht, J., Bisson, M. A., and Tosteson, F. C. 1977. Diffusion of carbon dioxide through lipid bilayer membranes. J. Gen. Physiol. **69**: 779–794.
3. Harvey, E. N. 1928. The oxygen consumption of luminous bacteria. J. Gen. Physiol. **11**: 499–575.
4. Holthuijzen, Y. A., van Breemen, J. F. L. Konings, W. N., and van Bruggen, E. F. J. 1986. Electron microscopic studies of carboxysomes of *Thiobacillus neapolitanus*. Arch. Microbiol. **144**: 256–262.
5. Holthuijzen, Y. A., Kuenen, J. G., and Konings, W. N. 1987. Activity of ribulose bisphosphate carboxylase in intact and disrupted carboxysomes of *Thiobacillus neapolitanus*. FEMS Microbiol. Lett. **42**: 121–124.
6. Jacobs, M. H. 1935. Diffusion processes. Ergeb. Biol. **XII**: 1–159.
7. Mayo, W. P., Elrifi, I. R., and Turpin, D. H. 1989. The relationship between ribulose bisphosphate concentration, dissolved inorganic carbon (DIC) transport, and DIC-limited photosynthesis in the cyanobacterium *Synechococcus leopeliensis* grown at different concentrations of inorganic carbon. Plant Physiol. **90**: 720–727.
8. Price, G. D., and Badger, M. R. 1989. Expression of human carbonic anhydrase in the cyanobacterium *Synechococcus* PCC7942 creates a high CO_2-requiring phenotype. Plant Physiol. **91**: 505–513.
9. Reed, M. L., and Graham, D. 1981. Carbonic anhydrase in plants: distribution, properties and possible physiological functions. *In* Progress in phytochemistry. *Edited by* L. Reinhold, J. B. Harborne, and T. Swain. Pergamon Press, Oxford. pp. 47–94.
10. Reinhold, L., Zviman, M., and Kaplan, A. 1987. Inorganic carbon fluxes and photosynthesis in cyanobacteria — a quantitative model. *In* Progress in photosynthesis research Vol. 4. *Edited by* J. Biggins Martinus Nijhoff, Dordrecht. pp. 289–296.
11. Reinhold, L., Zviman, X., and Kaplan, A. 1989. A quantitative model for carbon fluxes and photosynthesis in cyanobacteria. Plant Physiol. Biochem. **27**: 945–954.
12. Schneider, G., Lindquist, Y., Branden, C. I., and Lorimer, G. 1987. Plant molecular biology. *Edited by* D. von Wettstein and N-H. Chua. Plenum Press, New York. pp. 1–8.
13. Schwarz, R., Friedberg, D., and Kaplan, A. 1988. Is there a role for the 42 kilodalton polypeptide in inorganic carbon uptake by cyanobacteria? Plant Physiol. **88**: 284–288.
14. Shively, J. M., Ball, F. L., and Kline, B. W. 1973. Electron microscopy of the carboxysomes (polyhedral bodies) of *Thiobacillus neapolitanus*. J. Bacteriol. **116**: 1105–1111.
15. Szabo, A., and Shoup, D. 1982. Stochastically gated diffusion influenced reactions. J. Chem. Phys. **77**: 4484–4493.
16. Volokita, M., Zendvirth, D., Kaplan, A., and Reinhold, L. 1984. Nature of the inorganic carbon species actively taken up by the cyanobacterium *Anabaena variabilis*. Plant Physiol. **76**: 599–602.

Appendix

In this appendix the basic reaction diffusion equations are presented. The mass balance equation (eq. 1 in text) combined with the loss term in the differential form gives

$$[A1] \qquad \frac{dc}{dt} = \nabla J - \frac{V_{max} \cdot c}{k_m + c}$$

where c is the local concentration and J the local flux of the solute. The loss term is assumed to be of the Michaelis-Menten type. The flux is related to the gradient of the concentration by Fick's law

$$[A2] \qquad J = D \cdot \nabla \cdot c$$

where D is the diffusion tensor. Combining [A1] with [A2] one obtains

$$[A3] \qquad \frac{dc}{dt} = \nabla \cdot D \cdot \nabla \cdot c - \frac{V_{max} \cdot c}{k_m + c}$$

In general the diffusion tensor is dependent on position and on concentration. Assuming a constant diffusion tensor and assuming spherical symmetry, [A3] can be written as

$$[A4] \qquad \frac{dc}{dt} = D \frac{1}{r^2} \frac{\partial}{\partial r} r^2 \frac{\partial}{\partial r} c - \frac{\alpha \cdot c}{k_m + c}$$

where $\alpha = V_{max}$. A steady-state solution is sought for this equation. A boundary condition is imposed of a constant concentration c_0 at a radius r_0. The general solution of [A4] requires a numerical approach. A closed form solution can be obtained in two limiting cases. If the concentration c is considerably larger than k_m then the righthand term in [A4] can be replaced by α. A simple integration leads to [3]. The other limit is obtained when c is much smaller than k_m. This limit is obtained for relatively large radii where the concentration c becomes small. In this case the steady-state solution of [A4] can be transformed by substituting $c = ru$ to obtain

$$[A5] \qquad D \frac{\partial 2 \cdot u}{\partial \cdot r^2} - k \cdot u = 0$$

that by simple integration leads to [5].