Photosynthesis Research 16: 141–154 (1988)

Minireview

Uptake and utilization of inorganic carbon by cyanobacteria

JOHN PIERCE[1] & TATSUO OMATA[2]

[1] *Central Research and Development Department, E.I. Du Pont de Nemours and Company, Experimental Station, Building 402, Room 2230, Wilmington, Delaware 19898, USA;* [2] *Solar Energy Research Group, The Institute of Physical and Chemical Research (RIKEN), Wako-shi, Saitama, 351-01, Japan*

Received 6 October 1987; accepted 9 December 1987

Key words: carbonic anhydrase, carboxysomes, CO_2 transport, cyanobacteria, inorganic carbon utilization, ribulose bisphosphate carboxylase

Abstract. In the cyanobacteria, mechanisms exist that allow photosynthetic CO_2 reduction to proceed efficiently even at very low levels of inorganic carbon. These inducible, active transport mechanisms enable the cyanobacteria to accumulate large internal concentrations of inorganic carbon that may be up to 1000-fold higher than the external concentration. As a result, the external concentration of inorganic carbon required to saturate cyanobacterial photosynthesis in vivo is orders of magnitude lower than that required to saturate the principal enzyme (ribulose bisphosphate carboxylase) involved in the fixation reactions. Since CO_2 is the substrate for carbon fixation, the cyanobacteria somehow perform the neat trick of concentrating this small, membrane permeable molecule at the site of CO_2 fixation. In this review, we will describe the biochemical and physiological experiments that have outlined the phenomenon of inorganic carbon accumulation, relate more recent genetic and molecular biological observations that attempt to define the constituents involved in this process, and discuss a speculative theory that suggests a unified view of inorganic carbon utilization by the cyanobacteria.

Abbreviations: C_i – Inorganic carbon, H-cells – Cells grown under high CO_2, L-cells – Cells grown under low CO_2, RuBP – Ribulose-1,5-bisphosphate, WT – Wild type

Introduction

Cyanobacteria generally utilize CO_2 as their primary carbon source. The supply of CO_2 often limits their growth, and this limitation can be broadly seen to derive from both physical and chemical factors. The low partial pressure of CO_2 in the atmosphere ($\sim 0.035\%$) and the slow diffusion of CO_2 through water conspire to lower intrinsic rates of delivery of CO_2 to the cyanobacterium. In addition, the primary agent of CO_2 fixation, ribulose bisphosphate (RuBP) carboxylase, suffers from a low intrinsic affinity for CO_2 and is also inhibited by the presence of O_2, an alternate substrate that

is produced in large quantity by the primary light reactions of photosynthesis.

The detailed manner by which the cyanobacteria utilize inorganic carbon (C_i) is not known at present, but a variety of biochemical and physiological experiments have sketched the broad outlines of the problem. The techniques of molecular biology and genetic analysis are just now being used to address these questions. In this review, we will briefly outline the biochemical and physiological experiments on C_i transport, accumulation, and fixation that form the basis of our present knowledge of C_i utilization by the cyanobacteria. In addition, we will mention the more recent genetic experiments that have have provided interesting insights into the process. Finally, we will discuss some more speculative aspects of C_i utilization that may be of use in providing a logical framework for future research in the area. The interested reader is referred to a recent symposium proceedings for further information (Lucas and Berry 1985)

RuBP carboxylase and CO_2 fixation by cyanobacteria

The cyanobacteria fix CO_2 by the Calvin cycle of reductive photosynthesis, and are therefore susceptible to the usual inefficiencies associated with this type of metabolism. (Although there is evidence for appreciable levels of phospho-enol pyruvate carboxylase in the cyanobacteria (Codd and Stewart 1973, Colman et al. 1976), detailed, quantitative studies of the flux of carbon in cyanobacterial photosynthesis indicate that this C-4 type of carbon fixation does not predominate (Creach et al. 1981).) The inefficiency is due to the molecular properties of RuBP carboxylase, which catalyzes the condensation of RuBP with CO_2 in the commencing reaction of the Calvin cycle as well as the oxygenation of RuBP with O_2 in the commencing reaction of photorespiration.

Cyanobacterial RuBP carboxylases are large (530 kD), hexadecameric enzymes (L_8S_8) comprising 8 large catalytic subunits (51 kD) and 8 small subunits (15 kD) of unknown function (Takabe et al. 1976; Andrews et al. 1981). These two polypeptides are encoded by adjacent structural genes which are co-transcribed onto a single mRNA (Shinozaki and Sugiura 1985). It is presumably by this method of co-transcription that the 1:1 stoichiometry of large and small subunits is maintained by the organism. This simple arrangement has also made it possible to express both subunits of cyanobacterial RuBP carboxylases in *E. coli*, whereby large quantities of the enzyme have been made available for study (Gatenby et al. 1985, Tabita and Small 1985). The cyanobacterial RuBP carboxylases possess extraor-

dinarily low affinities for CO_2 (200–300 μM) and extraordinarily high maximum turnover numbers ($12\,s^{-1}$) compared to RuBP carboxylases from other sources (Badger 1980, Andrews and Abel 1981, TJ Andrews, personal communication). In addition, the relative specificity of these enzymes for CO_2 versus O_2 is low (~ 50) compared with that of higher plant enzymes (Jordan and Ogren 1981, 1983). As a result, the oxygenase activity of these enzymes is appreciable in solutions equilibrated in air.

A recent study showed that *Synechocystis* 6803 contained an amount of RuBP carboxylase that was ~ 1.6–2 fold higher than that required to maintain maximum rates of light-driven CO_2 fixation (Pierce et al. 1988). This amount of extractable carboxylase activity (measured at saturating concentrations of substrates) was equivalent to the maximum rate of photosynthetic electron transport (measured in the presence of methylviologen). Since we are not certain of the activation level of this enzyme, nor of the exact concentrations of intracellular CO_2 and O_2 maintained during steady state metabolism, it is not clear whether RuBP carboxylase activity is limiting to overall photosynthesis. However, an enzyme with an intrinsic $K_m(CO_2) = 300\,\mu M$ operating in air-saturated solutions ($[O_2] \sim 255\,\mu M$) would, due to O_2 inhibition, possess an effective $K_m(CO_2) \sim 600\,\mu M$. Under these conditions, the organism would be required to maintain a steady state internal $[CO_2]$ of 600–1000 μM (~ 30–50 mM total internal C_i at an internal pH of 7.8) just to support the maximum observed rates of photosynthesis.

The measurement of extractable enzyme activities and quantitative extrapolation of these to in vivo activities is, of course, subject to numerous difficulties. However, regardless of whether the activity of RuBP carboxylase is cleanly rate limiting to photosynthesis, it has become clear that there is no overabundance of this activity. In studies with *Synechocystis* 6803, the natural RuBP carboxylase gene was replaced with that of the RuBP carboxylase from *Rhodospirillum rubrum* (Pierce et al. 1988). As a result of this mutagenesis, the mutant organism grew photosynthetically by virtue of the action of the foreign carboxylase. Maximum rates of photosynthetic electron transport were similar to WT levels, but the maximum rate of light-driven CO_2 fixation was ~ 2-fold lower and the amount of extractable RuBP carboxylase activity was ~ 3-fold lower in this mutant than in WT cells. In addition, the maximum growth rate of the mutant was ~ 2-fold lower than that of the WT cells. It seems clear that photosynthesis in the mutant is limited by RuBP carboxylase. Further, since a three-fold diminution in carboxylase activity was associated with a two fold diminution in photosynthesis, it appears that levels of RuBP carboxylase activity in WT cells are not in large excess, but are rather fairly well matched to photosynthetic electron transport rates.

The low affinity of cyanobacterial RuBP carboxylases for CO_2 is exacerbated by the high levels of O_2 produced during photosynthetic electron transport. Even at air levels of CO_2 and O_2, these enzymes spend ~30–35% of their time functioning as RuBP oxygenases, and the phosphoglycolate so produced must be metabolized to avoid further metabolic complications (phosphoglycolate is a fairly potent inhibitor of triose phosphate isomerase). For instance, phosphoglycolate was shown to accumulate to high levels when *Anabaena variabilis*, grown under high CO_2, was placed in CO_2 free air for a short time (Marcus et al. 1983). However, in cells which had adapted to growth in air, only very low levels of phosphoglycolate were observed. Clearly, the cyanobacteria must be able to metabolize this compound, although the detailed manner in which they do so is unclear. Possible mechanisms include dephosphorylation to glycolate with subsequent excretion or respiratory metabolism (Codd and Sallal 1978, Bergman et al. 1984, 1985). Although the details of glycolate metabolism in the cyanobacteria are complex (indeed, multiple pathways may be operative (Codd and Stewart 1973), it is clear that the cell loses both energy and carbon as a result of having to dispatch the phosphoglycolate produced by RuBP oxygenase activity.

Uptake of C_i by cyanobacteria

To enhance their capacity to fix CO_2, the cyanobacteria have elaborated an active mechanism for accumulating intracellular C_i (Kaplan et al. 1980, Miller and Colman 1980, Badger and Andrews 1982). An internal C_i concentration of over 100 mM and an accumulation ratio ($[C_i]_{in}/[C_i]_{out}$ as high as 1000 have been reported (Badger and Andrews 1982, Miller and Colman 1980, Badger et al. 1985). With such elevated internal concentrations, the rate of RuBP carboxylation is increased, the rate of RuBP oxygenation is decreased, and the losses associated with phosphoglycolate production are minimized. The accumulation of intracellular C_i is made possible by an active transport system for C_i. C_i transport activity is higher in cells grown under air levels (0.035%) of CO_2 (L-cells) than in cells grown under elevated levels (1 to 5% in air) of CO_2 (H-cells) (Kaplan et al. 1980). Transfer of H-cells to low CO_2 conditions increases their C_i-transporting activity and photosynthetic affinity for C_i, and de novo protein synthesis is required for this adaptation (Marcus et al. 1982, Omata and Ogawa 1986).

It has been shown that both CO_2 and HCO_3^- are actively removed from the medium by cyanobacterial cells and used for photosynthesis (Badger and Andrews 1982, Volokita et al. 1984, Miller and Canvin 1985). It is not

known whether CO_2 and HCO_3^- uptake are due to common or different transporters. Recent observations have shown that H-cells utilize mainly CO_2, while L-cells utilize both CO_2 and HCO_3^- (Abe et al. 1987, Miller and Canvin 1987). Although CO_2 is taken up faster than HCO_3^- at lower concentrations of C_i in the medium (Badger and Andrews 1982, Volokita et al. 1984), HCO_3^- appears to be the C_i species which arrives at the inner side of the cytoplasmic (plasma) membrane, regardless of the C_i species supplied (Volokita et al., 1984, Badger et al. 1985, Ogawa and Kaplan 1987). That is, CO_2 transport appears to occur with a concomitant hydration. This important result suggests that a carbonic anhydrase-like moiety may be present in the vicinity of the transporter and may participate in CO_2 uptake.

Na^+ plays an important role in C_i transport, though the Na^+ requirement for C_i uptake appears to vary depending on the growth conditions experienced by the cells. In L-cells of *Anabaena variabilis* (Kaplan et al. 1984, Reinhold et al. 1984) and *Anacystis nidulans* (Miller and Canvin 1985), the affinity for HCO_3^- transport increases markedly as Na^+ concentrations are increased through the millimolar range. In *A. nidulans*, however, Na^+-independent HCO_3^- uptake has also been demonstrated in cells from standing cultures (Espie and Canvin 1987). CO_2 uptake by *A. nidulans* is independent of Na^+ in L-cells (Miller and Canvin 1985), but variations in $[Na^+]$ though the μM range do affect CO_2 uptake in H-cells (Miller and Canvin 1987). In contrast to the case in *A. nidulans*, CO_2 uptake by L-cells of *A. variabilis* is stimulated by low levels of Na^+ (Abe et al. 1987). It has yet to be determined whether these different modes of C_i uptake are due to different C_i transporters, or perhaps to modification of common transporter(s).

Mechanism of C_i transport

C_i uptake is light-dependent, though there have been contradictory observations as to the requirement for PS I and PS II reactions. It is known that C_i uptake is inhibited by DCMU (Miller and Colman 1980, Badger and Andrews 1982, Ogawa et al. 1985). However, in the presence of iodoacetamide, which inhibits CO_2 fixation, C_i uptake is insensitive to DCMU and shows action spectra typical of PS I reactions (Ogawa and Ogren 1985, Ogawa et al. 1985). Recently, it was shown that light is required not only for energization but also for a time-dependent activation of the C_i-transporting system. This activation, which occurs over a 5–10 min period when dark adapted *A. nidulans* cells are exposed to light, requires only a very low activity of PS II and is similar to the induction of photosynthesis that is observed during dark–light transitions (Kaplan et al. 1987). DCMU inhibits

the activation, whereas addition of DTT suppresses the inhibitory effect of DCMU. It was concluded that operation of PS I is sufficient for energization of the C_i-transporting system, while PS II provides reducing equivalents needed for activation of the system. Energization of the system by PS I suggests that the transport is driven by ATP produced by cyclic photophosphorylation.

Hyperpolarization of the cytoplasmic membrane upon addition of HCO_3^- indicates that the C_i transporting mechanism involves a primary electrogenic pump (Kaplan et al. 1982), though mechanistic details are lacking. It is not yet known, for instance, whether C_i uptake is due to a primary, active HCO_3^- pump or to a secondary active transporter powered by a primary pump for another ion. Attempts to detect C_i stimulated ATPase activity in the cytoplasmic membrane, which might account for the observed activity of C_i transport, have so far been unsuccessful (see Kaplan, 1985). It is worth noting, however, that the mechanistic possibilities of HCO_3^--H^+ symport (or HCO_3^--OH^- antiport) secondary to a H^+-extrusion pump have been discounted (Zenvirth et al. 1984). The paucity of data concerning Na^+ involvement in C_i uptake accommodates various mechanisms (Kaplan 1985, Reinhold et al. 1984): Na^+-HCO_3^- symport secondary to an active extrusion of Na^+ (though evidence for Na^+ flux during C_i uptake is lacking (Miller and Canvin 1985); a direct effect of Na^+ on the affinity of the transporter for HCO_3^-; regulation of intracellular pH during CO_2 fixation via a presumed Na^+-H^+ antiport system. It is clear from these various possibilities that the mechanism of C_i uptake is completely obscure, and alternate approaches will be required before gaining a useful understanding of this process.

Biochemical studies of the C_i transport mechanism

Identification of the protein(s) involved in C_i transport is important for elucidating the molecular mechanism of the transport. However, difficulties attend this identification: C_i transport is measurable only with intact cells or spheroplasts; there is no known biochemical activity that is specific to the transport system and measurable in a cell-free system; no specific inhibitor of the transport is known. Under these circumstances, analysis of the adaptation process of H-cells to low CO_2 conditions has been used as an alternative approach to ascertain what proteins might be involved in C_i transport. Since de novo protein synthesis is required for the adaptation (Marcus et al. 1982, Omata and Ogawa 1986), it was expected that analysis of protein synthesis during adaptation would reveal proteins (not necessarily transporter(s)) involved in the transport activity.

A large amount of a 42-kD protein is present in the cytoplasmic membrane of L-cells of *A. nidulans* (Omata and Ogawa 1985). This protein was shown to be the only major protein that is actively synthesized during adaptation from high CO_2 to low CO_2 conditions (Omata and Ogawa 1986). Studies with high CO_2-requiring mutants of *A. nidulans* R2 also indicated that the synthesis of the protein is one of the characterizing features of the adaptation (Ogawa et al. 1987, Omata et al. 1987). These observations suggested that the 42-kD protein might be an essential component for the adaptation. Recently, the structural gene for this protein was cloned and used to construct a defined mutant of *A. nidulans* R2 which is totally deficient in the protein (Omata et al. in preparation). Unexpectedly, the resulting mutant was still capable of adapting to low CO_2 conditions with an increase in C_i-transporting activity. The results indicate that neither adaptation nor C_i-transport require this protein, and the physiological role of the 42-kD protein remains obscure. Proteins that *are* essential for adaptation and transport may prove to be minor components which went un-undetected in the previous studies.

High CO_2-requiring mutants of cyanobacteria

Another approach for studying C_i transport and accumulation processes is to obtain mutants which require high CO_2 for growth. Among the high CO_2-requiring mutants isolated from *A. nidulans* R2 and *Synechocystis* 6803 (Carlson and Pierce, unpublished observations) after chemical mutagenesis, the mutants from *A. nidulans* R2 have been biochemically and physiologically characterized (Marcus et al. 1986, Ogawa et al. 1987, Omata et al. 1987). The mutant E1 (Marcus et al. 1986) has C_i-transporting activity, wild type RuBP carboxylase activity, and is capable of adaptive transformation to low CO_2 conditions (Omata et al. 1987). Yet, it is incapable of photosynthesis under low CO_2. Although it can produce and maintain elevated levels of intracellular C_i, the mutant appears to be incapable of utilizing this pool. It has been suggested that the mutant lacks intracellular carbonic anhydrase activity which converts the intracellular pool of C_i (mostly HCO_3^-) into CO_2. However, this hypothesis is difficult to prove since even wild type levels of carbonic anhydrase are so low that accurate measurements of its activity are quite difficult. Though its genotype is unknown, the E1 mutant has proven to be useful for studies of the C_i-transporting mechanism, since C_i uptake can be measured in the effective absence of CO_2 fixation (Ogawa and Kaplan 1987).

Another mutant, RK1, was also shown to be defective in its ability to

utilize the intracellular C_i pool (Ogawa et al. 1987). In contrast to the E1 mutant, RK1 showed no sign of adaptation nor increased amounts of the 42-kD protein upon exposure to low CO_2 conditions. The mutant may be deficient in a component which transmits the low CO_2 signal for inducing the functional and compositional changes that are observed in the wild type strain during adaptation to low CO_2. Interestingly, revertants of RK1 capable of growth in air regained the ability to adapt to low CO_2 conditions and also synthesized increased amounts of the 42-kD protein under inducing conditions (Omata and Pierce, unpublished observations). This result suggests that, although the 42-kD protein is clearly not required for growth in air (see above), the 42-kD gene may be somehow linked with the gene responsible for the RK1 phenotype.

At present, no mutants with specific defects in C_i transport have been obtained. Cloning of these transport-specific genes and the genes responsible for the mutations in E1 and RK1 would provide valuable information on the mechanisms of C_i utilization by cyanobacteria. Since *Anacystis nidulans* R2 and related strains are transformable by foreign DNA, it should be quite straightforward to clone the genes responsible for a given phenotype by complementation with plasmid libraries containing wild type DNA. We may reasonably expect reports along these lines in the near future.

Carbonic anhydrase and the CO_2 diffusion problem

As previously discussed, it appears that the major species of C_i reaching the cytoplasm of cyanobacteria is in the form of HCO_3^-, and it is this form that accumulates to high internal concentrations. Since CO_2 is the species utilized by RuBP carboxylase, the HCO_3^- must be dehydrated prior to use, and a possible role for carbonic anhydrase in CO_2 utilization is easily envisioned. However, (and despite clear evidence for the involvement of carbonic anhydrase in CO_2 utilization by micro-algae such as *Chlamydomonas reinhardtii* (for a review, see Aizawa and Miyachi 1986), experimental evidence for carbonic anhydrase in cyanobacteria remained elusive until fairly recently. In a careful study using sophisticated techniques, Badger et al. (1985) showed that there was very low, but detectable carbonic anhydrase activity in a *Synechococcus* species. Importantly, these workers also provided theoretical arguments indicating that, even though carbonic anhydrase activity was low, it was nevertheless required by this organism to maintain known rates of CO_2 fixation. Rather higher levels of carbonic anhydrase activity have been found in *Anabaena variabilis*, although the distribution of the enzyme (soluble or insoluble activity) varied among different strains of

A. variabilis (Yagawa et al. 1984). Recently a particulate carbonic anhydrase activity has also been recovered from *Chlorogloeopsis fritschii* cells (Lanaras et al. 1985).

The requirement of RuBP carboxylase for CO_2 and the presence of carbonic anhydrase activity in cyanobacteria pose interesting problems in understanding CO_2 utilization in these organisms. If C_i accumulation is to be effective in supplying useful concentrations of CO_2, then the internal CO_2 concentration must be large compared to external concentrations. To avoid a large penalty due to diffusion of this CO_2 out of the cell, a permeability barrier must exist. In fact, by assuming that a cyanobacterial cell contains a single impermeability barrier (the plasmalemma), a very low CO_2 permeability (10^{-5} cm s^{-1}) can be measured (Zenvirth and Kaplan 1984, Badger et al. 1985). This value is extraordinarily low for a polar lipid bilayer, and prompts a further concern emphasized by Badger et al. If one makes the reasonable assumption that the permeability of O_2 is of the same order as that for CO_2, then the O_2 released by photosynthesis would build up to concentrations of a few mM ($\sim$2–6 atmospheres) when photosynthesis is saturated by C_i. The dilemma, of course, is that such high levels of O_2 would be likely to cause oxidative damage and would be expected to severely inhibit CO_2 fixation by RuBP carboxylase, the enhancement of which appears to be a major purpose for C_i accumulation in the first place.

A unified view of C_i utilization by cyanobacteria

Recently, an interesting hypothesis has been advanced that obviates the apparent dilemma of a toxic buildup of oxygen without requiring a lipid bilayer that is selectively impermeable to CO_2. This hypothesis (Reinhold et al. 1987) ascribes a major, functional role to carboxysomes, the subcellular inclusion bodies in which cyanobacterial RuBP carboxylase is normally sequestered. These polyhedral bodies, which are found in a wide range of autotrophic organisms (Codd and Marsden 1984), contain a very limited distribution of polypeptides, of which up to 50% appears to be RuBP carboxylase. The hypothesis of Reinhold et al. suggests that both RuBP carboxylase and carbonic anhydrase are localized in cyanobacterial carboxysomes, and that this localization can serve to allow profitable use of C_i that is transported into the cytoplasm. Briefly, the HCO_3^- that builds up to high levels in the cytoplasm may diffuse into the carboxysome, where it is dehydrated to CO_2 by carbonic anhydrase and subsequently utilized by RuBP carboxylase. In this manner, CO_2 is produced and utilized in the same subcellular location away from the site of photosynthetic O_2 generation. The

small amount of CO_2 produced by non-enzymatic dehydration in the cytoplasm and the large amount of photosynthetically produced O_2 would be free to diffuse across a permeable plasmalemma. It is this slow production of cytoplasmic CO_2 which would give rise to the apparently low rate of CO_2 diffusion out of the cell. In this manner, the problems of high internal O_2 tensions and low permeability of the plasmalemma to CO_2 outlined above would be avoided.

To be sure, there are difficulties associated with this model for CO_2 utilization by cyanobacteria: 1) the model requires a carboxysome membrane that is relatively impermeable to CO_2, or an internal carboxysome structure which maintains a higher CO_2 concentration in the vicinity of RuBP carboxylase than at the carboxysome membrane (Reinhold et al. 1987); 2) the model requires a carboxysomal location for carbonic anhydrase, though as mentioned above, carbonic anhydrase has been found in both soluble and particulate fractions.[1] However, regardless of the detailed mechanism of CO_2 utilization, other evidence and considerations tend to support a functional role for carboxysomes. To date, all cyanobacteria which have been shown to actively accumulate C_i also contain carboxysomes. Furthermore, since a large fraction of the total cyanobacterial RuBP carboxylase resides in the carboxysome (Coleman et al. 1982), and since there is no overabundance of RuBP carboxylase activity (see above), it would appear that carboxysomal CO_2 fixation is required. Carboxysomes isolated by differential centrifugation are indeed capable of RuBP dependent CO_2 fixation (Codd and Stewart 1976), and therefore appear to be permeable to RuBP. This is an important finding (consistent with evidence for a cytoplasmic location for ribulose 5-phosphate kinase (Hawthornthwaite et al. 1985) and would indicate that most of the reactions of the Calvin cycle could take place cytoplasmically. Permeability to such a highly charged molecule as RuBP may indicate either a specific transporter or a very unusual carboxysome membrane. In addition, a mutant of *Synechocystis* 6803 has been obtained which requires high CO_2 levels for growth, is

[1] These findings should not be taken as strictly disallowing the model. Although the model's absolute requirement for a carboxysomal location of carbonic anhydrase should provide a rigorous test of its veracity, the experimental difficulties in performing the test are great. As mentioned above, only a very low (and easily overlooked) level of carbonic anhydrase activity is required to support cyanobacterial photosynthesis. Difficulties in experimental interpretation would be compounded if the carbonic anhydrase-like activity responsible for the concomitant production of HCO_3^- during CO_2 transport (see above) were, under certain circumstances, to masquerade as a particulate carbonic anhydrase. Carboxysomes are easily broken during cell disruption, and carboxysomal carbonic anhydrase would appear as a soluble activity. Finally, it is conceivable that carbonic anhydrase exists in more than one location.

extremely sensitive to changes in the CO_2/O_2 ratio supplied during growth, and is unable to grow at all in air. This mutant also lacked microscopically observable carboxysomes (Pierce et al. 1988). All of these phenotypes were due to a single genetic lesion, the replacement of the WT carboxylase gene with the corresponding gene from *Rhodospirillum rubrum*. Altogether, the mutant's extreme sensitivity to O_2 was much higher than could be expected from the differences in gaseous substrate specificity between the *R. rubrum* and WT cyanobacterial enzymes, and it is interesting to speculate (in the manner of Reinhold et al.) that this sensitivity is due to the absence of functional carboxysomes. For instance, it may be that the *R. rubrum* enzyme, as a consequence of its dimeric, quaternary structure (c.f., the hexadecameric cyanobacterial enzyme), was not sequestered in the carboxysome, thereby rendering these subcellular structures un-observable. If carboxysomes do indeed play a role in effective CO_2 utilization, their functional absence in the mutant might explain its anomalous O_2 sensitivity.

The model of Reinhold et al. is important in providing a unified view of C_i utilization in cyanobacteria and in suggesting useful experiments concerning the role of carboxysomes in C_i utilization. In particular, it states that effective accumulation of C_i requires not only an active transport mechanism, but also a diffusional barrier provided by the carboxysome. Experiments designed to validate the model should be most informative, even if they result in the model's dismissal.

Concluding remarks

In this review, we have sketched the broad outlines of C_i utilization by the cyanobacteria. It is our opinion that, due essentially to their procaryotic nature, the mechanisms for C_i utilization in the cyanobacteria will prove to be quite different from that in higher plants and eucaryotic algae. It will also be evident from our liberal use of speculation, that many aspects of C_i utilization by the cyanobacteria remain obscure. It would appear however, that the irreducible requirements for effective assimilation of CO_2 include an energy dependent transport process, a barrier to outward diffusion of internal CO_2, and the ability to catalyze the dehydration of HCO_3^-. These aspects of C_i utilization are beginning to be addressed by genetic and molecular biological studies. These approaches complement the earlier physiological studies and should provide for a molecular understanding of the temporal and spatial nature of C_i utilization by the cyanobacteria.

References

Abe T, Tsuzuki M and Miyachi S (1987) Transport and fixation of inorganic carbon during photosynthesis in cells of *Anabaena* grown under ordinary air III. Some characteristics of the HCO_3^- transport system in cells grown under ordinary air. Plant Cell Physiol 28: 867–874

Aizawa K and Miyachi S (1986) Carbonic anhydrase and CO_2 concentrating mechanisms in microalgae and cyanobacteria. FEMS Microbiol Rev 39: 215–233

Andrews TJ and Abel KM (1981) Kinetics and subunit interactions of ribulose bisphosphate carboxylase-oxygenase from the cyanobacterium, *Synechococcus* sp. J Biol Chem 256: 8445–8451

Andrews TJ, Abel KM, Menzel E and Badger MR (1981) Molecular weight and quaternary structure of ribulose bisphosphate carboxylase from the cyanobacterium, *Synehococcus* sp. Arch Microbiol 130: 344–348

Badger MR (1980) Kinetic properties of RuBP carboxylase from *Anabaena variabilis*. Arch Biochem Biophys 201: 247–254

Badger MR and Andrews TJ (1982) Photosynthesis and inorganic carbon usage by the marine cyanobacterium, *Synechococcus* sp. Plant Physiol 70: 517–523

Badger MR, Bassett M and Comins HN (1985) A model for HCO_3^- accumulation and photosynthesis in the cyanobacterium *Synechoccus* sp. Plant Physiol 77: 465–471

Bergman B, Codd GA and Hällbom L (1984) Glycollate excretion by N_2-fixing cyanobacteria treated with photorespiratory inhibitors. Z Pflanzenphysiol 113: 451–460

Bergman B, Codd GA, Hällbom L and Codd GA (1985) Effects of amino-oxyacetate and aminoacetonitrile on glycolate and ammonia release by the cyanobacterium *Anabaena cylindrica*. Plant Physiol 77: 536–539

Codd GA and Marsden WJN (1984) The carboxysomes (polyhedral bodies) of autotrophic prokaryotes. Biol Rev 59: 389–422

Codd GA and Sallal A-KJ (1978) Glycolate oxidation by thylakoids of the cyanobacteria *Anabaena cylindrica*, *Nostoc muscorum*, and *Chlorogloea fritschii*. Planta 139: 177–181

Codd GA and Stewart WDP (1973) Pathways of glycollate metabolism in the blue—green alga *Anabaena cylindrica*. Arch Microbiol 124: 149–154

Codd GA and Stewart WDP (1976) Polyhedral bodies and ribulose 1,5-diphosphate carboxylase of the blue-green alga *Anabaena cylindrica*. Planta 130: 323–326

Coleman JR, Seeman JR and Berry JA (1982) RuBP carboxylase in carboxysomes of blue-green algae. Carnegie Inst of Wash Ybk 81: 83–87

Colman B, Cheng K-H and Ingle RK (1976) The relative activities of PEP carboxylase and RuDP carboxylase in the blue-green algae. Plant Science Letters 6: 123–127

Creach E, Codd GA and Stewart WDP (1981) Primary products of photosynthesis and studies of carboxylating enzymes in the filamentous cyanobacterium *Anabaena cylindrica*. In: G. Akoyunoglou (ed.) Photosynthesis IV. Regulation of Carbon Metabolism, pp 49–56. Balaban International Science Services, Philadelphia, USA

Espie GS and Canvin DT (1987) Evidence for Na^+-independent HCO_3^- uptake by the cyanobacteriuim *Synechococcus leopoliensis*. Plant Physiol 84: 125–130

Gatenby AA, van der Vies S and Bradley D (1985) Assembly in *E. coli* of a functional multisubunit ribulose bisphosphate carboxylase from a blue green alga. Nature 314: 617–620

Hawthornthwaite AM, Lanaras T and Codd GA (1985) Imuno-electronmicroscopic localization of Calvin cycle enzymes in *Chlorogloeopsis fritschii*. J Gen Microbiol 131: 2497–2500

Jordan DB and Ogren WL (1981) Species variation in the specificity of ribulose bisphosphate carboxylase/oxygenase. Nature 291: 513–515

Jordan DB and Ogren WL (1983) Species variation in the kinetic properties of ribulose 1,5-bisphosphate carboxylase/oxygenase. Arch Biochem Biophys 227: 425–433

Kaplan A (1985) Adaptation to low CO_2 levels: Induction and the mechanism for inorganic carbon uptake. In: Lucas WJ and Berry JA (eds) Inorganic Carbon Uptake by Aquatic Photosynthetic Organisms, pp 325–328. Waverly Press/American Society of Plant Physiologists

Kaplan A, Badger MR and Berry JA (1980) Photosynthesis and the intracellular inorganic carbon pool in the bluegreen alga *Anabaena variabilis*: Response to external CO_2 concentration. Planta 149: 219–226

Kaplan A, Volokita M, Zenvirth D and Reinhold L (1984) An essential role for sodium in the bicarbonate transporting system of the cyanobacterium *Anabaena variabilis*. FEBS Lett 176: 166–168

Kaplan A, Zenvirth D, Marcus Y, Omata T and Ogawa T (1987) Energization and activation of inorganic carbon uptake by light in cyanobacteria. Plant Physiol 84: 210–213

Kaplan A, Zenvirth D, Reinhold L and Berry JA (1982) Involvement of a primary electrogenic pump in the mechanism for HCO_3^- uptake by the cyanobacterium *Anabaena variabilis*. Plant Physiol 69: 978–982

Lanaras T, Hawthornthwaite AM and Codd GA (1985) Localization of carbonic anhydrase in the cyanobacterium *Chlorogloeopsis fritschii*. FEMS Microb Lett 26: 285–288

Lucas WJ and Berry JA (1985) Inorganic carbon uptake by aquatic photosynthetic organisms. Waverly Press, Baltimore, pp 1–480

Marcus Y, Harel E and Kaplan A (1983) Adaptation of the cyanobacterium *Anabaena variabilis* to low CO_2 concentration in their environment. Plant Physiol 71: 208–210

Marcus Y, Schwarz R, Friedberg D and Kaplan A (1986) High CO_2 requiring mutant of *Anacystis nidulans* R2. Plant Physiol 82: 610–612

Marcus Y, Zenvirth D, Harel E and Kaplan A (1982) Induction of HCO_3^- transporting capability and high photosynthetic affinity to inorganic carbon by low concentration of CO_2 in *Anabaena variabilis*. Plant Physiol 69: 1008–1012

Miller AG and Canvin DT (1985) Distinction between HCO_3^- and CO_2-dependent photosynthesis in the cyanobacterium *Synechococcus leopoliensis* based on the selective response of HCO_3^- transport to Na^+. FEBS Lett 187: 29–32

Miller AG and Canvin DT (1987) Na^+-stimulation of photosynthesis in the cyanobacterium *Synechococcus* UTEX 625 grown on high levels of inorganic carbon. Plant Physiol 84: 118–124

Miller AG and Colman B (1980) Active transport and accumulation of bicarbonate by a unicellular cyanobacterium. J Bacteriol 143: 1253–1259

Ogawa T and Kaplan A (1987) The stoichiometry between CO_2 and H^+ fluxes involved in the transport of inorganic carbon in cyanobacteria. Plant Physiol 83: 888–891

Ogawa T and Ogren WL (1985) Action spectra for accumulation of inorganic carbon in the cyanobacterium *Anabaena variabilis*. Photochem Photobiol 41: 583–587

Ogawa T, Kaneda T and Omata T (1987) A mutant of *Synechococcus* PCC 7942 incapable of adapting to low CO_2 concentration. Plant Physiol 84: 711–715

Ogawa T, Miyano A and Inoue Y (1985) Photosystem-I-driven inorganic carbon transport in the cyanobacterium, *Anacystis nidulans*. Biochem Biophys Acta 808: 77–84

Omata T and Ogawa T (1985) Changes in the polypeptide composition of the cytoplasmic membrane in the cyanobacterium *Anacystis nidulans* during adaptation to low CO_2 conditions. Plant Cell Physiol 26: 1075–1081

Omata T and Ogawa T (1986) Biosynthesis of a 42-kD polypeptide in the cytoplasmic membrane of the cyanobacterium *Anacystis nidulans* strain R2 during adaptation to low CO_2 concentration. Plant Physiol 80: 525–530

Omata T, Ogawa T, Marcus Y, Friedberg D and Kaplan A (1987) Adaptation to low CO_2 levels in a mutant of *Anacystis nidulans* R2 which requires high CO_2 for growth. Plant Physiol 83: 892–894

Pierce J, Carlson TJ and Williams JGK (1988) Anomalous oxygen sensitivity in a cyanobacterial mutant requiring the expression of ribulose bisphosphate carboxylase from a photosynthetic anaerobe. Submitted to Proc Nat Acad Sci

Reinhold L, Volokita M, Zenvirth D and Kaplan A (1984) Is HCO_3^- transport in *Anabaena* a Na^+ symport? Plant Physiol 76: 1090–1092

Reinhold L, Zviman M and Kaplan A (1987) Inorganic carbon fluxes and photosynthesis in cyanobacteria — a quantitative model. In: Biggins J (ed.) Progess in Photosynthesis Research, Vol 4, pp 289–296. Dordrecht: Martinus Nijhoff

Shinozaki K and Sugiura M (1985) Genes for the large and small subunit of ribulose bisphosphate carboxylase/oxygenase constitute a single operon in a cyanobacterium *Anacystis nidulans*. 6301. Mol Gen Genet 200: 27–32

Spalding MH, Spreitzer RJ and Ogren WL (1983) Reduced inorganic carbon transport in a CO_2-requiring mutant of *Chlamydomonas reinhardtii*. Planta 159: 261–266

Tabita FR and Small CL (1985) Expression and assembly of active cyanobacterial ribulose-1,5-bisphosphate carboxylase/oxygenase in *Escherichia coli* containing stoichiometric amounts of large and small subunits. Proc Nat Acad Sci USA 82: 6100–6103

Takabe T, Nishimura M and Akazawa T (1976) Presence of two subunit types in ribulose 1,5-bisphosphate carboxylase from blue-green algae. Bioch Biophys Res Commun 68: 537–544

Volokita M, Zenvirth D, Kaplan A and Reinhold L (1984) Nature of inorganic carbon species actively taken up by the cyanobacterium *Anabaena variabilis*. Plant Physiol 76: 599–602

Yagawa Y, Shiraiwa Y and Miyachi S (1984) Carbonic anhydrase from the blue-green alga (cyanobacterium) *Anabaena variabilis*. Plant and Cell Physiol 25: 775–783

Zenvirth D and Kaplan A (1981) Uptake and efflux of inorganic carbon in *Dunaliella salina*. Planta 152: 8–12

Zenvirth D, Volokita M and Kaplan A (1984) Evidence against H^+-HCO_3^- symport as the mechanism for HCO_3^- transport in the cyanobacterium *Anabaena variabilis*. J Membrane Biol 79: 271–274