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Uptake and Fixation of CO_2 in Lichen Photobionts

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Abstract

Lichens are the symbiotic phenotype of nutritionally specialised fungi that live in symbiosis with photosynthesising algal and/or cyanobacterial photobionts. Although the initiation and maintenance of the metabolic activity of lichens require that water is taken up and stored, excess water may potentially limit the photosynthetic activity of the lichen if this causes swelling of the fungal hyphae, which may impede the diffusion of CO_2 to the photobiont. In free-living algae and cyanobacteria this potential limitation of photosynthesis has partly been compensated for by the evolution of a CO_2 concentrating mechanism (CCM). This mechanism operates under conditions of low CO_2 availability in their environment, such as when the diffusion of CO_2 is slow or when HCO_3^- is the dominating inorganic carbon source. This paper gives a brief presentation of the function of the CCM in free-living cyanobacteria and microalgae and summarises recently obtained evidence for the presence of this mechanism in lichens with cyanobacterial *Nostoc* and green algal *Trebouxia* photobionts. However, the CCM is absent in some photobiont genera; evidence for this is also presented and possible reasons for this absence are discussed.

Keywords: CO_2 concentrating mechanism, lichen, photobiont, photosynthesis, Rubisco

Abbreviations: CA = Carbonic anhydrase (E.C. 4.2.1.1); CCM = CO_2 concentrating mechanism, DIC = dissolved inorganic carbon; EZA = ethoxyzolamide (6-ethoxy-2-benzothiazole-2-sulfonamide); K_m = Michaelis-Menten constant for an enzyme reaction; Rubisco = Ribulose-1,5-bisphosphate carboxylase-oxygenase (E.C. 4.1.1.39); ($S_{C/O}$) = relative specificity for CO_2 as opposed to O_2 of Rubisco.

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1. Introduction

CO_2 is an obligatory intermediate in the fixation and reduction of dissolved inorganic carbon (DIC) by photosynthetic organisms as this DIC species is the substrate for the key enzyme, Rubisco, in this process. Terrestrial plants obtain CO_2 directly from the atmosphere, while aquatic photosynthetic organisms must obtain their CO_2 from the DIC pool in the surrounding water. The acquisition of CO_2 from an aquatic environment presents problems, largely as a result of the physical chemistry of the DIC species in solution. The diffusion rate of CO_2 is some 10^4 times lower in aqueous media compared to air and this also applies to the other major forms of DIC, bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) (Badger, 1987). The chemical equilibria between the DIC species are also influenced by the pH, and above pH 6.5, there is an increasing predominance of the HCO_3^- ion. In an aqueous medium in equilibrium with the gas phase the CO_2(aq) concentration increases slightly with decreasing temperature but is independent of the pH. However, the $HCO_3^- + CO_3^{2-}$ concentration will increase about 10-fold with each unit of pH increase above 7. Water in equilibrium with ambient air has a CO_2(aq) concentration of about 10–12 μM at 25° C and of about 20 μM at 10° C, while the HCO_3^- concentration may thus be up to several orders of magnitude higher and hence represent a potentially major pathway for photosynthetic CO_2 supply (Stumm and Morgan, 1981). However, the relatively slow conversion of HCO_3^- to CO_2 means that this may limit the CO_2 supply, especially if photosynthesis is rapid (Lucas, 1975).

The major strategy that has evolved at all systematic levels of aquatic organisms (cyanobacteria, microalgae, macroalgae and aquatic angiosperms) to overcome the potential problem with CO_2 limitation of photosynthesis, is a mechanism for the active transport and accumulation of DIC within the cell (Lucas, 1975; Badger, 1987; Kaplan et al., 1991; Raven et al., 1990; Coleman, 1991; Badger and Price, 1992). The requirement of a CO_2 concentrating mechanism (CCM) in aquatic photosynthesising organisms is also closely related to the kinetic properties of Rubisco. This enzyme is bifunctional and can both carboxylate and oxygenate ribulose-1,5-bisphosphate which results in the competitive inhibition of CO_2 fixation by O_2, so called photorespiration. As the CCM functions to elevate CO_2 around the active site of Rubisco, photorespiration will be depressed and the affinity for CO_2 of intact cells will increase (Badger, 1987). However, as the CCM is driven by photosynthetic electron transport (Spalding and Ogren, 1982) this increased CO_2 use efficiency is attained at the cost of a decreased light use efficiency of photosynthesis (Falk and Palmqvist, 1992; Palmqvist et al., 1994a).

Two major reasons suggest that one might also expect the CCM to be operating in lichens. First, because it is such a widespread mechanism among free-living algae and cyanobacteria and second because it may confer a particular advantage to lichens, as CO_2 diffusion may be slow in these (Cowan et al., 1992). Indeed, the presence of a CCM in lichens and their photobionts has now been demonstrated by several investigators using different types of measurements (Raven et al., 1990; Badger et al., 1993; Palmqvist, 1993; Máguas et al., 1993; Palmqvist et al., 1994b). However, the CCM is not present in all lichenised algae, which will be discussed later. This paper sets out to present some of these recent advances in our understanding of the different strategies of DIC acquisition that have evolved in lichens and their photobionts, especially in relation to what is known about these processes in free-living cyanobacteria and green microalgae.

The CO_2-concentrating mechanism of free-living cyanobacteria and green microalgae

Our understanding of the CCM has made considerable progress during the last couple of years, largely as a result of molecular genetic approaches. These findings are presented and discussed in detail in a number of recent reviews (Badger, 1987; Coleman, 1991; Kaplan et al., 1991; Badger and Price, 1992; Sültemeyer et al., 1993). Cyanobacteria are probably entirely dependent on their CCM, as the cyanobacterial Rubisco has a much lower affinity for CO_2 as compared to that of higher plants and green algae (Table 1) (Jordan and Ogren, 1981; Badger and Andrews, 1987; Kane et al., 1994). Green algae, however, appear to have evolved a number of different mechanisms for the acquisition of CO_2 (Hogetsu and Miyachi, 1977; Badger 1987; Munoz and Merrett, 1989; Coleman, 1991), but as the fresh-water alga *Chlamydomonas reinhardtii* has been studied in most detail among the species possessing a CCM, this alga will serve as the model species in this presentation.

A simplified model of the cyanobacterial CCM is presented in Fig. 1A. The figure is based on experiments with the marine cyanobacterium *Synechococcus sp.* which have been reviewed and/or presented elsewhere (Badger, 1987; Kaplan et al., 1991; Price et al., 1992). A system which is capable of active DIC transport is of central importance for the functioning of the CCM. So far, however, it has not been possible to isolate any specific proteins associated with such a putative pump although several pieces of evidence support the existence of a single transporter, located in the plasma membrane, which is able to use either CO_2 or HCO_3^- as substrate. Na^+ is also involved in the transporting process, most likely through its role in pH regulation through the operation of the Na^+/H^+ antiport system (Fig. 1B) (Badger and Price, 1992).

Table 1. Characteristics of Rubisco isolated and purified from different species. Values for $K_m(CO_2)$ and the ratio of carboxylation to oxygenation, the CO_2/O_2 specificity factor ($S_{C/O}$) of Rubisco were obtained as described in the cited articles. The cyanobacteria and the green algae, except *Coccomyxa*, are known to possess a CCM (Badger, 1987).

Species	$K_m(CO_2)$, μM	$S_{C/O}$
C_3 plants		
Spinacea oleracea	14 [a]	82.1 ± 0.8 [b]
Nicotiana tabacum	11 [a]	81.9 ± 1 [b]
Green algae		
Coccomyxa PA	11.9 ± 3.0 [d]	82.9 ± 2.2 [d]
Chlamydomonas reinhardtii	29 ± 1 [c]	62 ± 1 [c]
Euglena gracilis	25 [a]	54 ± 2 [a]
Scenedesmus obliquus	38 [a]	63 ± 2 [a]
Cyanobacteria		
Cocochloris peniocystis	121 [a]	47 ± 2 [a]
Synechococcus	185 [e]	42.5 ± 1 [b]

[a] Jordan and Ogren, 1981; [b] Kane et al., 1994; [c] Chen et al., 1988; [d] Palmqvist et al., 1995; [e] Badger and Andrews, 1987.

Carboxysomes are small polyhedral-shaped protein bodies, which are present in the cytosol of several species of cyanobacteria. The possibility that the carboxysome may be the actual site of CO_2 elevation and thus be an important part of the CCM emerged gradually and was formalised in a model by Reinhold et al. (1989). This model has been experimentally tested and confirmed and it is now quite clear that the accumulated HCO_3^- is indeed dehydrated to CO_2 only within the carboxysomes, where the majority of the cell's Rubisco is located, and that this dehydration is facilitated by a low level of carbonic anhydrase (CA) (Price et al., 1992). The model also postulated that CA, which catalyses the reversible hydration of CO_2 to HCO_3^-, should be absent from the cytosol, so that the slow uncatalysed conversion between HCO_3^- and CO_2 would minimise wasteful leakage of CO_2 out of the cell. This prediction has also been tested and experimentally confirmed (Badger and Price, 1992).

Our understanding of the microalgal CCM is much less clear, even though especially one freshwater microalga, i.e. *Chlamydomonas reinhardtii*, has been quite extensively studied (Spalding and Ogren, 1982; Moroney et al., 1985; Badger, 1987; Coleman, 1991; Badger and Price, 1992; Falk and Palmqvist, 1992;

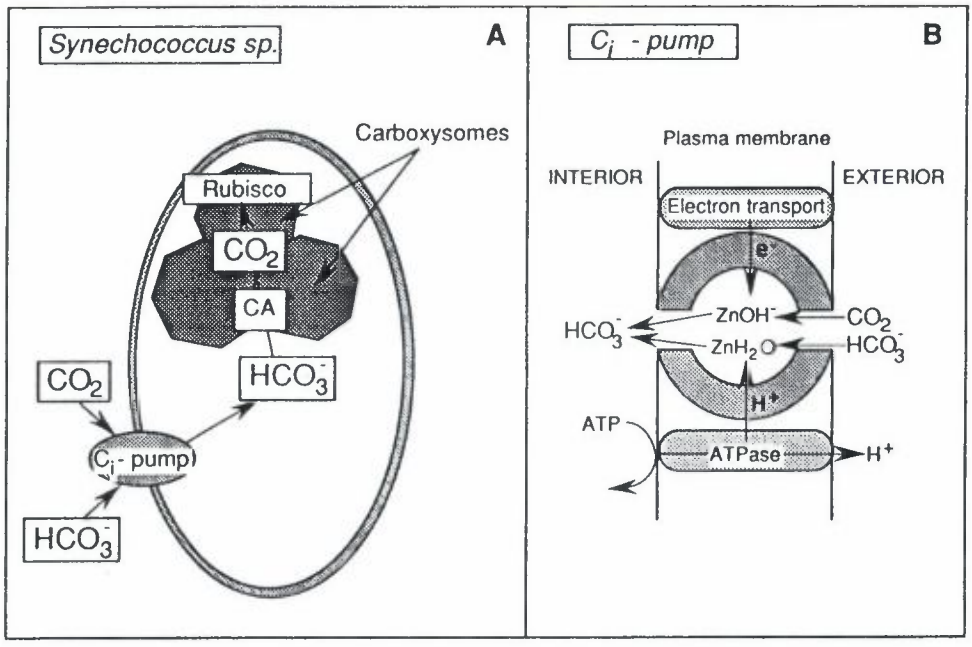

Figure 1. **A**. Simplified model of the cyanobacterial CO_2 concentrating mechanism, based on experimental data obtained with cultures of the marine cyanobacterium *Synechococcus sp,*. DIC is actively transported across the cell membrane, most probably by a single transporter, which can use either CO_2 or HCO_3^- as a substrate, even though HCO_3^- appears to be the DIC species delivered to the interior of the cell. The accumulated HCO_3^- is most probably dehydrated to CO_2 and fixed by Rubisco only within a subcellular compartment, the carboxysomes. These are small polyhedral-shaped protein bodies containing both Rubisco and the enzyme carbonic anhydrase (CA).
B. A speculative model of the DIC transporter in cyanobacteria as proposed by Badger and Price (1992). Their model is based on a transporter with a "CA-like" active site, containing a Zn metal ion. The mechanism for this reaction is drawn by analogy to H^+ transfer reactions catalysed by Zn bound to the active site of CA as discussed by Silverman (1991).

Sültemeyer et al., 1993; Palmqvist et al., 1994c). The model presented in Fig. 2 is therefore largely hypothetical and summarises the different possibilities that are currently discussed. There is evidence for active DIC-transport both across the plasma membrane and the chloroplast envelope. These uptake processes both appear to involve a close coupling with membrane bound H^+-ATPase activity, which would indeed be necessary for internal pH regulation when HCO_3^- is taken up. There is also a role for several isozymes of CA in the

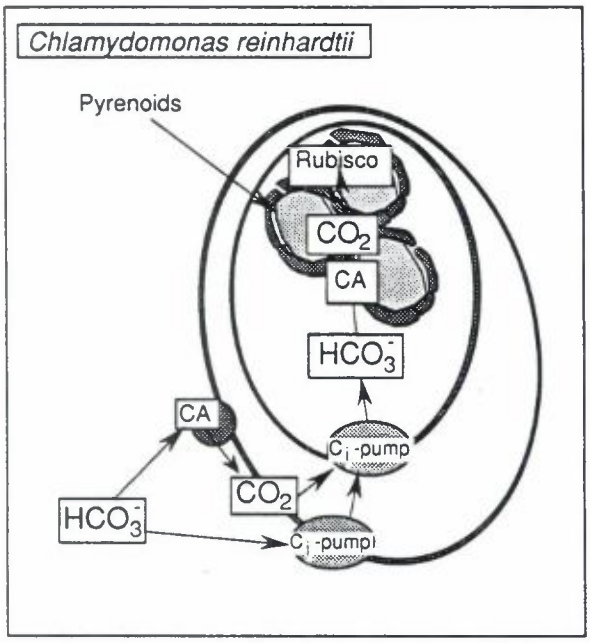

Figure 2. Simplified and hypothetical model of the CO_2 concentrating mechanism in the green microalga *Chlamydomonas reinhardtii*. There is evidence that both CO_2 and HCO_3^- are actively transported and that both an extracellular as well as one or several internally located CAs are important for the function of the CCM in this alga. It is, however, unclear whether the DIC-transporter is located at the plasma membrane, the chloroplast envelope or at both membranes. It was recently suggested that the pyrenoid, a starch-coated proteinaceous structure present in the chloroplast stroma of many eucaryotic algae, may play a similar role as the carboxysome in cyanobacteria (Kuchitsu et al., 1991), even though there is no firm evidence to support this hypothesis.

microalgal CCM (Moroney et al., 1985; Badger and Price, 1992; Sültemeyer et al., 1993; Palmqvist et al., 1994c). One of these is an extracellular CA, which is induced in large amounts when *Chlamydomonas* is grown under limiting CO_2 supply. It has generally been assumed that the main function of this CA is to facilitate the utilisation of external HCO_3^-, but since it is now apparent that *Chlamydomonas* has the ability to actively transport HCO_3^- across the plasma membrane the role of periplasmic CA probably has to be reassessed (Palmqvist et al., 1994c). If DIC is accumulated as HCO_3^- within the cell there is also an obvious role for an internal CA in the vicinity of Rubisco, analogous to the carboxysomal CA of cyanobacteria. The presence of one or several internal CA(s) has been demonstrated by several experimental approaches and it has

also been possible to establish that internal CA is required for the functioning of the CCM, even though it has not been possible to find the precise location of this (these) (Moroney et al., 1985; Badger and Price, 1992; Sültemeyer et al., 1993; Palmqvist et al., 1994c). Whether DIC is accumulated within the whole cell or only within the chloroplast is also an open question. However, interesting analogies occur between the microalgal chloroplast and cyanobacteria in that much of the Rubisco is localised within discrete structures, so called pyrenoids, which are separate from the soluble stroma. Recently, these have been suggested to play a similar role in algae as the carboxysomes in cyanobacteria (Kuchitsu et al., 1991). However, this hypothesis remains to be experimentally tested.

Evidence for a CCM in lichens with Nostoc and Trebouxia photobionts

With the aim of screening for the possible presence of the CCM in lichens and their photobionts the photosynthetic properties of a range of lichens has recently been examined (Raven et al., 1990; Badger et al., 1993; Palmqvist, 1993; Máguas et al., 1993; Palmqvist et al., 1994b). Both cyanobacterial (*Calothrix* and particularly *Nostoc spp*) as well as green algal (*Trebouxia spp*) associations have been included in these investigations. In addition, a few species of tripartite lichens have also been studied in some detail. The tripartite lichens had a green algal photosynthesising photobiont of either the genus *Coccomyxa* or *Dictyochloropsis* and a cyanobacterial, nitrogen-fixing, secondary photobiont of the genus *Nostoc*. The *Nostoc* cells in these associations are usually confined to specific structures, cephalodia, and may therefore be separated from the green algal parts of the lichen thallus.

All lichens with *Nostoc* as primary photobiont were found to have a high photosynthetic affinity for CO_2, i.e. the rate of increase in photosynthesis at low and limiting CO_2 concentrations, referred to as the carboxylation efficiency (CE) was high and the CO_2 compensation concentration was low (Palmqvist, 1993). Also consistent with the presence of a CCM there were no signs of photorespiration in these lichens and photosynthesis was clearly inhibited by ethoxyzolamide (EZA), a potent inhibitor of both carboxysomal CA and the DIC-transporter in free-living cyanobacteria (Badger and Price, 1992). Direct evidence for the presence of a CCM in lichenised *Nostoc* was obtained in another recent investigation (Badger et al., 1993), where fast CO_2 gas exchange transients where measured. This technique made it possible to detect an internal inorganic carbon pool in lichenised as well as free-living *Nostoc*, which was accumulated in the light and was released in the dark. It was also shown that EZA decreased the size of this pool and that the pool accumulated in the presence of the carbon-reduction-cycle inhibitor, glycolaldehyde. A

typical dark-light-dark transient experiment with a *Nostoc*-lichen is shown in Fig. 3. Direct and indirect evidence for a CCM in cyanobacterial lichens have until now been obtained for the following species; *Lichina pygmea* with *Calothrix*-photobionts (Raven et al., 1990), *Leptogium azureum, Lobaria scrobiculata, Nephroma bellum, Peltigera canina, Peltigera malacea, Peltigera neopolydactyla, Peltigera rufescens, Pseudocyphellaria knightii* and *Sticta fuliginosa* with *Nostoc* -photobionts (Badger et al., 1993; Máguas et al., 1993; Palmqvist, 1993; Palmqvist, unpublished).

In terms of photosynthetic CO_2 use efficiency, lichens with green algal primary photobionts could be divided into at least two groups (Palmqvist, 1993; Palmqvist et al., 1994b), the *Trebouxia*-lichens on the one hand and the tripartite lichens on the other. The tripartite lichens had a lower CO_2-use

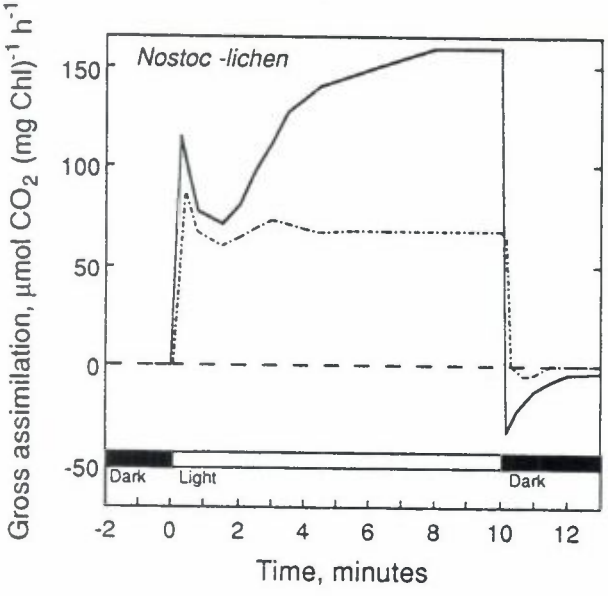

Figure 3. Kinetics of light-dark courses of CO_2 gas exchange for the cyanobacterial (*Nostoc*) lichen *Peltigera canina*. Small pieces of lichen thalli (4–5 cm^2) were incubated in 20 mM EPPS, pH 8.0 (control) (solid line) or in the same buffer containing 500 µM EZA for 1 h (dotted line) prior to the measurements. The samples were dark adapted for 10 min prior to illumination. The initial peak in CO_2 uptake is due to the filling of an internal DIC pool, which can only be detected when the enzymes of the CO_2 fixation process have been given sufficient time to be inactivated, i.e. at least 10–15 min of darkness. The rapid evolution of CO_2 when the light is switched off represents the post-illumination release of the internal DIC pool. These patterns of exchange are discussed in detail in Badger et al. (1993). The measurements were performed at ambient CO_2 (35 Pa) and at 20° C in an open CO_2 gas-exchange system as described in Palmqvist et al. (1994b). The data presented in this figure has not been published previously.

efficiency of photosynthesis as compared to the *Trebouxia*-lichens, including a higher CO_2 compensation concentration, a lower CE and significant photorespiration. In addition, tripartite lichens also have a much higher discrimination against the carbon isotope ^{13}C compared to *Trebouxia* -lichens, thus being more similar to higher plants with C_3 photosynthesis (Máguas et al., 1993). Taken together, these results led to the conclusion that the CCM is most probably absent in the primary photobiont of the investigated tripartite lichens, which includes the following species; *Lobaria ampilissima, Lobaria pulmonaria, Nephroma arcticum, Peltigera aphthosa, Solorina crocea, Sticta aurata* (Máguas et al., 1993; Palmqvist, 1993; Palmqvist et al., 1994b).

From the photosynthesis measurements it was not possible, however, to establish whether the CCM was present in *Trebouxia* or not. But as for the cyanobacterial lichens, the presence of a CCM in *Trebouxia* could be demonstrated by measuring fast gas-exchange transients (Badger et al., 1993; Palmqvist et al., 1994b) (Fig. 4). It was found though, that *Trebouxia* accumulates a much smaller pool of DIC compared to *Nostoc* and that EZA causes an increase in the size of the pool in many of the species, although photosynthesis is inhibited by the same CA inhibitor (Palmqvist et al., 1994b). These differences most probably reflect the inherent differences in the functioning of the CCM between cyanobacteria and microalgae, such as the location(s) and precise function(s) of CA isozymes. Direct and indirect evidence for the presence of a CCM has now been obtained for a range of *Trebouxia* lichens, such as *Cetraria spp., Evernia prunastri, Hypogymnia physodes, Lasilla pustulata, Parmelia spp., Platismatia glauca, Ramalina spp., Usnea spp.* (Badger et al., 1993; Máguas et al., 1993; Palmqvist, 1993; Palmqvist et al., 1994b; Palmqvist, unpublished). It may thus be concluded that the CCM appears to be a general characteristic of this photobiont genera.

CO_2 acquisition in Coccomyxa

The photosynthesis measurements thus suggested that the primary photobionts (*Coccomyxa* and *Dictyochloropsis*) of the tripartite lichens do not have a CCM. This conclusion was further supported by their patterns of fast CO_2 gas exchange upon dark-light-dark transitions, showing no, or very weak, signs of a DIC pool (Fig. 5) (Palmqvist et al., 1994b). Interestingly, the apparent absence of a CCM in *Coccomyxa* and *Dictyochloropsis* is also correlated with the absence of pyrenoids in the chloroplast of these species (Tschermak-Woess, 1988). This correlation is thus in agreement with the hypothesis of an important role of these structures in the microalgal CCM (Kuchitsu et al., 1991). The discovery of the absence of a CCM in these photobionts was interesting and several studies have already been done with

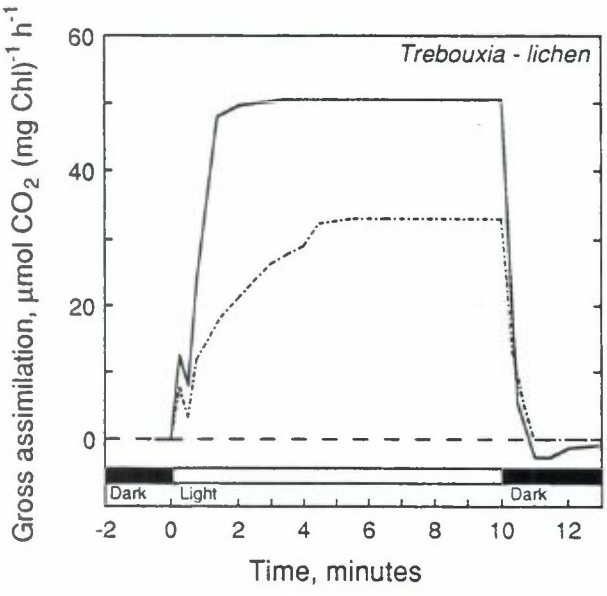

Figure 4. Kinetics of light-dark courses of CO_2 gas exchange for the green algal (*Trebouxia*) lichen *Lasilla pustulata* The measurements were performed at 15°C, but otherwise as described in the legend to Fig. 3. The solid line represents the control and the dotted line a sample pre-treated in EZA. In contrast to the *Nostoc* -lichens, there is generally no significant release of the DIC pool when the light is switched off in *Trebouxia* -lichens. This is probably due to continuing of CO_2 fixation in the dark (Badger et al., 1993). The data presented in this figure has not been published previously.

Coccomyxa cells, isolated from the tripartite lichen *Peltigera aphthosa* (Palmqvist, 1993; Ögren, 1993; Hiltonen et al., 1995; Palmqvist et al., 1994a; Palmqvist et al., 1995). This is because, in view of the detailed information that already exists on the photosynthetic properties of algae that possess this mechanism, it has been regarded to be of interest to examine the photosynthetic performance of a species which lack the CCM. So far, the *Coccomyxa* studies have shown that this alga is more similar to higher C_3 plants than to *Chlamydomonas*, both in terms of light- and CO_2 utilisation efficiency of photosynthesis (Palmqvist et al., 1994a), and with respect to amount, activity and possible location of internal CA (Hiltonen et al., 1995).

The relative requirement of a CCM is probably also related to the properties of Rubisco, as it has been found that organisms possessing a CCM generally have a lower affinity for CO_2 and a relatively higher oxygenation activity of their Rubisco as compared to organisms with passive CO_2 acquisition. It was

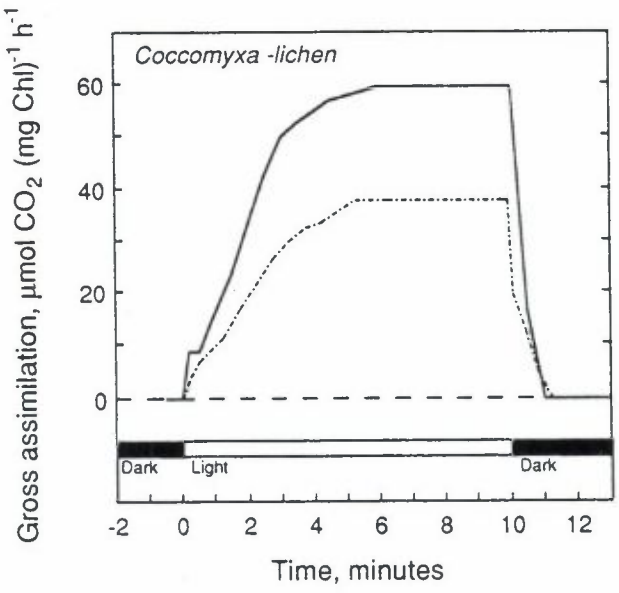

Figure 5. Kinetics of light-dark courses of CO_2 gas exchange for the tripartite lichen *Nephroma arcticum*, where the green alga *Coccomyxa* constitutes the primary photobiont. Measurements were performed with cephalodial free (i.e. *Nostoc*-free) parts of the thalli, with the aim of separating the responses of *Coccomyxa* from those of *Nostoc*. The measurements were performed at 15°C, but otherwise as described in the legend to Fig. 3. The solid line represents the control and the dotted line a sample pre-treated in EZA. This figure has been re drawn from the data presented in Palmqvist et al. (1994b).

therefore hypothesised that algae without the CCM would possibly have a more efficient Rubisco than algae with this mechanism (Palmqvist et al., 1994a). When Rubisco was purified from *Coccomyxa* it was indeed found that this was also the case, as both its affinity and specificity for CO_2 was much more similar to the higher plant Rubisco than to that of *Chlamydomonas* and the other green algae (Table 1) (Palmqvist et al., 1995). These results thus suggest that the requirement of a CCM may rather be related to the inherent characteristics of Rubisco than to CO_2 limitation imposed by the environment. However, the occurrence of CCMs appear to be taxonomically rather randomly distributed and may have evolved polyphyletically (Raven, 1991). At this stage, it is therefore difficult to speculate about the relations between Rubisco evolution and the requirements of possessing a CCM. At present, it is not even clear how *Coccomyxa* is related to the other green microalgae as this genera has not been included in any recent cladistic analysis (Mishler et al., 1994;

Friedl, 1994). Finally, it has also been suggested that one of the functions of the CCM may be to increase the nitrogen-use efficiency, as less nitrogen has to be diverted to the synthesis of Rubisco, and possibly, photorespiratory enzymes (Beardall et al., 1982; Raven et al., 1985). In this context, it is particularly intriguing that the CCM appears to be absent only in the tripartite lichens, which should generally be less limited by nitrogen compared to the *Trebouxia*-lichens, due to the nitrogen fixation activity of cephalodial *Nostoc* (Rai, 1988).

To conclude, it is now apparent that the photobionts of lichens have evolved a range of different CO_2 acquisition strategies. *Nostoc* and *Calothrix* appear to possess a CCM, similar to that of free-living species of cyanobacteria. *Trebouxia* also appear to possess a CCM, although more work is needed before the precise mechanisms involved in the uptake and fixation of CO_2 in this photobiont genus can be fully described. *Coccomyxa* represents a photobiont genus without a CCM and has a photosynthetic CO_2 acquisition strategy which is more similar to that of higher plants with C_3 photosynthesis than to other algae with a CCM. It is possible that these different strategies may also affect the photosynthetic performance of the whole lichen, as lichens with a CCM may both be less inhibited by high water contents, if this results in slow diffusion of CO_2 and also have a higher nitrogen-use efficiency. However, until these questions are more clearly addressed in studies of lichen physiology and ecophysiology, the putative relations between the CO_2 acquisition strategy of the photobiont and the ecological and perhaps evolutionary adaptations of the whole lichen symbiosis may not be fully understood.

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REFERENCES

Badger, M.R. 1987. The CO_2 concentrating mechanism in aquatic phototrophs. In: *The Biochemistry of Plants: A Comprehensive Treatise*. M.D. Hatch and N.K. Boardman, eds., Academic Press, Inc., San Diego, USA, Vol. 10, pp. 219–274.

Badger, M.R. and Andrews, T.J. 1987. Co-evolution of Rubisco and CO_2 concentrating mechanisms. In: *Progress in Photosynthesis Research*. J. Biggins, ed., Martinus Nijhoff, Dordrecht, The Netherlands, Vol. III, pp. 601–609.

Badger, M.R., Pfanz, H., Büdel B., Heber, U., and Lange, O.L. 1993. Evidence for the functioning of photosynthetic CO_2 concentrating mechanisms in lichens containing green algal and cyanobacterial photobionts. *Planta* **191**: 57–70.

Badger, M.R. and Price, G.D. 1992. The CO_2-concentrating mechanism in cyanobacteria and microalgae. *Physiologia Plantarum* **84**: 606–615.

Beardall, J., Griffiths, H., and Raven, J.A. 1982. Carbon isotope discrimination and the CO_2 accumulating mechanism in *Chlorella emersonii*. *Journal of Experimental Botany* **33**: 729–737.

Chen, Z., Chastian, C.J., Al-Abed, S.R., Chollet, R., and Spreitzer, R.J. 1988. Reduced CO_2/O_2 specificity of ribulose-bisphosphate carboxylase/oxygenase in a temperature sensitive chloroplast mutant of *Chlamydomonas*. *Proceedings of the National Academy of Sciences, USA* **85**: 4696–4699.

Coleman, J.R. 1991. The molecular and biochemical analyses of CO_2-concentrating mechanisms in cyanobacteria and microalgae. *Plant, Cell and Environment* **14**: 861–867.

Cowan, I.R. Lange, O.L., and Green, T.G.A. 1992. Carbon-dioxide exchange in lichens: determination of transport and carboxylation characteristics. *Planta* **187**: 282–294.

Falk, S. and Palmqvist, K. 1992. Photosynthetic light utilisation efficiency, photosystem II heterogeneity and fluorescence quenching in *Chlamydomonas reinhardtii* during the induction of the CO_2 concentrating mechanism. *Plant Physiology* **100**: 685–691.

Friedl, T. 1994. Assessing the relationships of some coccoid green lichen algae and the microthamniales (Chlorophyta) with 18S ribosomal RNA gene sequence comparisons. *Journal of Phycology* **30**: 500–506.

Hiltonen, T., Karlsson, J., Palmqvist, K., Clarke, A.K., and Samuelsson, G. 1995. Purification and characterisation of an intracellular carbonic anhydrase from the unicellular green alga *Coccomyxa*. *Planta* **195**: 345–351.

Hogetsu, D. and Miyachi, S. 1977. Effects of CO_2 concentration during growth on subsequent photosynthetic CO_2 fixation in *Chlorella*. *Plant and Cell Physiology* **18**: 347–352.

Jordan, B.J. and Ogren, W.L. 1981. Species variation in the specificity of ribulose bisphosphate carboxylase/oxygenase. *Nature* **291**: 513–515.

Kane, H.J., Viil, J., Entsch, B., Paul, K., Morell, M.K., and Andrews, T.J. 1994. An improved method for measuring the CO_2/O_2 specificity of ribulosephosphate carboxylase-oxygenase. *Australian Journal of Plant Physiology* **21**: 449–461.

Kaplan, A., Schwarz, R., Lieman-Hurwitz, J., and Reinhold, L. 1991. Physiological and molecular aspects of the inorganic carbon-concentrating mechanism in cyanobacteria. *Plant Physiology* **97**: 851–855.

Kuchitsu, K., Tsuzuki, R.K., and Miyachi, S. 1991. Polypeptide composition and enzyme activities of the pyrenoid and its regulation by CO_2 concentration in unicellular green algae. *Canadian Journal of Botany* **69**: 1062–1069.

Lucas, W.J. 1975. Photosynthetic assimilation of exogenous HCO_3^- by aquatic plants. *Journal of Experimental Botany* **26**: 331–346.

Máguas, C., Griffiths, H., Ehleringer, J., and Serôdio, J. 1993. Characterisation of photobiont associations in lichens using carbon isotope discrimination techniques. In: *Perspectives of Plant Water Relations from Stable Isotopes*. J. Ehleringer, A.E. Hall, and G.D. Farquhar, eds., Academic Press, Inc., New York, USA, pp. 201–212.

Mishler, B.D., Lewis, L.A., Buchheim, M.A., Renzaglia, K.S., Garbary, D.J., Delwiche, C.F., Zechman, F.W., Kantz, T.S., and Chapman, R.L. 1994. Phylogenetic relationships of the green algae and bryophytes. *Annals of the Missouri Botanical Garden* **81**: 451–483.

Moroney, J.V., Husic, H.D., and Tolbert, N.E. 1985. Effect of carbonic anhydrase inhibitors on inorganic carbon accumulation by *Chlamydomonas reinhardtii*. *Plant Physiology* **79**: 177–183.

Munoz, J. and Merrett, M.J. 1989. Inorganic carbon transport in some marine eukaryotic microalgae. *Planta* **178**: 450–455.

Ögren, E. 1993. Convexity of the photosynthetic light-response curve in relation to intensity and direction of light during growth. *Plant Physiology* **101**: 1013–1019.

Palmqvist, K. 1993. Photosynthetic CO_2-use efficiency in lichens and their isolated photobionts: the possible role of a CO_2-concentrating mechanism. *Planta* 191: 48–56.

Palmqvist, K., Ögren, E., and Lernmark, U. 1994a. The CO_2 concentrating mechanism is absent in the green alga *Coccomyxa*. A comparative study of photosynthetic CO_2 and light responses of *Coccomyxa, Chlamydomonas reinhardtii* and barley protoplasts. *Plant, Cell and Environment* **17**: 65–72.

Palmqvist, K., Samuelsson, G., and Badger, M.R. 1994b. Photobiont related differences in carbon acquisition among green-algal lichens. *Planta*. **195**: 70–79.

Palmqvist, K., Yu, J-W. and Badger, M.R. 1994c. Carbonic anhydrase activity and inorganic carbon fluxes in low- and high-C_i cells of *Chlamydomonas reinhardtii* and *Scenedesmus obliquus*. *Physiologia Plantarum* **90**: 537–547.

Palmqvist, K., Sültemeyer, D., Baldet, P., Andrews, T.J., and Badger, M.R. 1995. Characterisation of inorganic carbon fluxes, carbonic anhydrase(s) and Rubisco in the unicellular green alga *Coccomyxa*. Comparisons with low-CO_2 cells of *Chlamydomonas reinhardtii*. *Planta*. In press.

Price, G.D., Coleman, J.R. and Badger, M.R. 1992. Association of carbonic anhydrase activity with carboxysomes isolated from the cyanobacterium *Synechococcus* PCC7942. *Plant Physiology* **100**: 784–793.

Raven, J.A. 1991. Implications of inorganic C utilisation: Ecology, evolution and geochemistry. *Canadian Journal of Botany* **69**: 908–924.

Raven, J.A., Johnston, A.M., Handley, L.L., and McInroy, S. 1990. Transport and assimilation of inorganic carbon by *Lichina pygmea* under emersed and submersed conditions. *The New Phytologist* **114**: 407–417.

Raven, J.A., Osborne, B.A., and Johnston, A.M. 1985. Uptake of CO_2 by aquatic vegetation. *Plant, Cell and Environment* **8**: 417–425.

Rai, A.N. 1988. Nitrogen metabolism. In: *Handbook of Lichenology*. M. Galun, ed., CRC Press, Boca Raton, FL, Vol. I, pp. 201–237.

Reinhold, L., Zviman, M., and Kaplan, A. 1989. A quantitative model for inorganic carbon fluxes and photosynthesis in cyanobacteria. *Plant Physiology and Biochemistry* **27**: 945–954.

Silverman, D.N. 1991. The catalytic mechanism of carbonic anhydrase. *Canadian Journal of Botany* **69**: 1070–1078.

Spalding, M.H. and Ogren, W.L. 1982. Photosynthesis is required for induction of the CO_2-concentrating mechanism in *Chlamydomonas reinhardtii*. *FEBS Letters* **145**: 41–44.

Stumm, W. and Morgan, J.J. 1981. *Aquatic Chemistry. An Introduction Emphasizing Chemical Equilibria in Natural Waters*. 2nd edition. John Wiley & Sons, Inc., New York, Chichester, Brisbane, Toronto, Singapore.

Sültemeyer, D., Schmidt, C., and Fock, H.P. 1993. Carbonic anhydrases in higher plants and aquatic micro-organisms. *Physiologia Plantarum* **88**: 179–190.

Tschermak-Woess, E. 1988. The algal partner In: Handbook of Lichenology. M. Galun, ed., CRC Press, Boca Raton, FL, Vol. I, pp. 39–92.