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CO_2 concentrating mechanisms and environmental change

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ABSTRACT

The diversity of CCMs among aquatic oxygenic photolithotrophs causes difficulties in generalising about their responses to environmental change. A frequent response of organisms with CCMs to increasing CO_2 is increased organic carbon content, even if (as is often the case) the growth rate is not increased. In the few studies performed, the internal carbon dioxide concentration relative to the growth carbon dioxide concentration is decreased for organisms grown at elevated CO_2, but not sufficiently so to alter the degree of Rubisco saturation with CO_2. The more commonly measured affinity for external CO_2 generally also decreases with increased CO_2 concentration for growth. The present global distribution of cyanobacteria and algae with CCMs over a wide temperature range contrasts with terrestrial C_4 plants which mainly grow in warmer habitats. This suggests that algae and cyanobacteria will benefit less from increasing global temperatures in terms of geographical range than will terrestrial C_4 plants. The general correlate of increased temperature and a shallowing (shoaling) of the thermocline means that phytoplankton will experience a higher mean PAR and UV-B flux, and a decreased availability of combined nitrogen, phosphorus and iron. The general conclusion is that this combination of environmental changes will in part offset the decreased CCM activity in response to increased CO_2. The few data available on responses to environmental change of the minority of aquatic oxygenic photolithotrophs lacking CCMs, suggest some similarities of responses of these organisms and those with CCMs. Organic carbon burial ("blue carbon") by undisturbed seagrass beds will probably increase under environmental change.

1. Introduction

CCMs such as C_4 photosynthesis and Crassulacean Acid Metabolism (CAM) in terrestrial vascular land plants were discovered at a time (in the mid-1960s; see the review by Hatch, 2002 for an historical perspective) when the CO_2 affinity of C_3 vascular plants was thought to involve diffusive CO_2 entry followed by its assimilation by what was then known as ribulose diphosphate carboxylase, and now as ribulose bisphosphate carboxylase-oxygenase (Rubisco) (Raven, 1970; Smith, 1985; Portis and Parry, 2007). However, at that time the highest CO_2 affinity of the carboxylase extracted from C_3 vascular plants was too low to account for in vivo photosynthetic CO_2 affinity, and it was not then known that the oxygen inhibition of C_3 photosynthesis could be accounted for by the oxygenase activity of the carboxylase. While the former problem was not resolved until the work of Bahr and Jensen (1974) and of Lilley and Walker (1975), the latter issue was elucidated by Bowes et al. (1971) and by Ogren and Bowes (1971). These two findings, later expanded to the kinetics of a range of forms of Rubisco (Whitney and Andrews 1998; Tcherkez et al., 2006; Portis and Parry, 2007), underpin the requirement of some photosynthetic organisms for CCMs, accounting for the kinetics of in vivo photosynthesis with respect to CO_2 and O_2 concentrations, and others to function as C_3 plants with diffusive CO_2 flux to Rubisco. Although there were earlier suggestions of CCMs in aquatic organisms (e.g. Raven, 1970; Raven and Glidewell, 1978, reviewed by Smith, 1985), the clinching evidence came from work of Badger et al. (1980) on *Chlamydomonas reinhardtii* and Kaplan et al. (1980) on *Anabaena cylindrica*, who demonstrated that in these organisms active transport of inorganic carbon from the bulk medium into the cell caused an increase in CO_2 concentration within the cell (and by implication at the active site of Rubisco). This accumulation of CO_2 above what would be expected from diffusional uptake driven by concentration and pH gradients is the lynchpin of CCMs. The various mechanisms by which this concentration of CO_2 is achieved are described below.

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The work of Bowes and colleagues has been very important in establishing the role of CCMs in aquatic macrophytes, especially in relation to the role of C_4 metabolism and seasonal variability in expression of CCMs. Bowes et al. (1978) and Salvucci and Bowes (1981) emphasised the diversity of inorganic carbon acquisition kinetics in freshwater aquatic macrophytes, in contrast to the clear distinction between C_3 and C_4 vascular plants on land. This was amplified by Maberly and Spence (1983) for freshwater macrophytes and microphytes and for marine macrophytes by Maberly (1990), as well as many others, with information for marine macrophytes reviewed by Koch et al. (2013).

Returning to the contribution of Bowes and colleagues, the early work on freshwater submersed flowering plants demonstrated inducible C_4 photosynthesis in *Hydrilla verticillata* but not in *Myriophyllum spicatum* as components of their CCMs when grown under conditions producing a low CO_2 compensation concentration. This was followed by a very detailed analysis of the single-cell C_4 metabolism in *H. verticillata* including studies of the biochemical pathways, enzymes and molecular genetics and its relationship to the acid-base polarity of leaf surfaces (Reiskind et al., 1997; Magnin et al., 1997; van Ginkel et al., 2001; Rao et al., 2002, 2006a, 2006b, 2008; Estavillo et al., 2007). Reiskind et al. (1988) and Reiskind and Bowes (1991) demonstrated C_4 biochemistry in photosynthesis of the one ulvophycean green macroalga, *Udotea flabellum*, but not in another, *Codium decorticatum.* The relation of these aquatic macrophyte single-celled C_4 mechanisms to other aquatic CCMs and to terrestrial C_4 mechanisms has been reviewed by Bowes et al. (2002), Maberly and Madsen (2002), Raghavendra and Sage (2011) and Koch et al. (2013). The work of Bowes and colleagues helps interpret the observed continuum of CCM kinetics, and consequently in the extent to which inorganic C can be extracted from a water body (Maberly and Spence, 1983; Maberly, 1990), with substantial variation even found within a genus (Kevekordes et al., 2006).

2. Distribution of CCMs among aquatic plants

Aquatic oxygenic photosynthetic organisms do not all have the capacity to express CCMs. Organisms lacking CCMs include all algae from the Chrysophyceae and Synurophyceae (Raven et al., 2005a; Maberly et al., 2009) and freshwater members of the Rhodophyta (Raven et al., 2005a) as well as a significant minority of marine florideophycean red algae (Maberly, 1990; Maberly et al., 1992; Raven et al., 2002a,b; Moulin et al., 2011; Raven and Hurd, 2012; Koch et al., 2013) and some ulvophycean marine green algae (Raven et al., 2002a,b; Kevekordes et al., 2006; Raven and Hurd, 2012). Among embryophytes it seems that all aquatic bryophytes lack CCMs (Raven, 1970; Maberly, 1985a,b; but see Salvucci and Bowes, 1981; Peñuelas, 1985 for evidence of HCO_3^- use in submerged mosses), as do a number of freshwater aquatic flowering plants (Maberly and Madsen, 2002; Pedersen et al., 2013). Many seagrasses are not carbon dioxide saturated for growth (Koch et al., 2013), though this may be associated with enhanced storage in rhizomes at higher CO_2.

All remaining aquatic photosynthetic organisms, including all cyanobacteria, have CCMs, driven either through active transport of protons or some inorganic carbon species across one or more membranes between the medium and Rubisco, or through the use of C_4, C_3–C_4 intermediate or Crassulacean Acid Metabolism (CAM), operating singly or in various combinations (Badger et al., 1980; Kaplan et al., 1980; Maberly, 1990; Maberly et al., 1992; Keeley, 1998; Maberly and Madsen, 2002; Giordano et al., 2005; Kevekordes et al., 2006; Roberts et al., 2007; Klavsen et al., 2011; Moulin et al., 2011; Reinfelder, 2011; Koch et al., 2013).

From the nature of CCMs it might be expected that organisms with CCMs (or the capacity to express CCMs) would be CO_2-saturated and show little or no stimulation of photosynthesis and growth by increasing CO_2 levels beyond those in equilibrium with present day atmospheres. In practice, there are circumstances in which primary production by aquatic photoautotrophs may be C-limited (e.g. Jansson et al., 2012) either because the organisms involved lack a CCM capacity (such as occurs with the Chrysophyceae and Synurophyceae and freshwater members of the Rhodophyta, see above) or because CCM activity is down-regulated because of environmental conditions. Given the large changes in global environment, including ocean acidification, that the planet is currently undergoing, it is important to consider how environmental conditions will modulate CCM activity.

3. CCMs today: categories and functioning under environmental change

3.1. Active influx of bicarbonate

The great majority of aquatic organisms with CCMs are able to take up bicarbonate from the environment, as indicated by carbon isotope disequilibrium and by membrane-inlet mass spectrometry on organisms with no extracellular carbonic anhydrase, either because this activity is not expressed, or because an inhibitor of extracellular carbonic anhydrase is present (Espie and Colman, 1986; Badger et al., 1994; Rost et al., 2007). While these techniques do not give 'false positives' in indication of bicarbonate entry, they can over-emphasize the quantitative importance of bicarbonate uptake relative to the carbonic anhydrase-catalysed extracellular conversion of bicarbonate to carbon dioxide, with the subsequent entry of carbon dioxide. As pointed out by Raven (1970; see Smith, 1967 and Raven, 1968), active (against the electrochemical gradient) transport is needed if bicarbonate transport across the plasmalemma is the only means of increasing intracellular carbon dioxide to the steady-state concentration needed in a CCM. While claims of active bicarbonate transport should be measured against the arguments of Walker et al. (1980; see also Ferrier, 1980) discussed below, there are now well-established plasmalemma-located bicarbonate transporters in alpha and beta cyanobacteria, *Chlamydomonas*, *Emiliania*, *Ulva* and diatoms (Giordano et al., 2005; Koch et al., 2013; Nakajima et al., 2013). There is also the possibility of active bicarbonate influx in the mechanism involving localised surface acidification. There is also evidence of active bicarbonate uptake by chloroplasts of *C. reinhardtii* (Moroney et al., 1987) and, probably, diatoms (Hopkinson et al., 2011). A final point is that an anion channel, permitting energetically downhill transport of bicarbonate, is required for the CCM of *Chlamydomonas* involving the thylakoid lumen-located carbonic anhydrase *Cah3* (Raven, 1997). It is probable that bicarbonate active influx is the only mechanism of bicarbonate use in very small organisms (10 μm effective spherical diameter or less) because of problems of maintaining large pH differences along the cell surface in organism with such a small surface area. However, no data are, to our knowledge, available for bicarbonate transporters in embryophytes.

Where investigated, the fraction of the inorganic carbon flux related to CCMs involving active bicarbonate influx at the plasmalemma is decreased by carbon dioxide levels above the present concentration (Burkhardt et al., 2001; Rost et al., 2003, 2006b).

3.2. CO_2 entry as part of CCMs

As discussed above for bicarbonate, the great majority of aquatic organisms with CCMs are able to take up carbon dioxide from