Upper ocean oxygenation, evolution of RuBisCO and the Phanerozoic succession of phytoplankton

Rosalind E.M. Rickaby*, M.R. Eason Hubbard

Department of Earth Sciences, University of Oxford, South Parks Road, Oxford, OX1 3AN, UK

ABSTRACT

Evidence is compiled to demonstrate a redox scale within Earth’s photosynthesisers that correlates the specificity of their RuBisCO with organismal metabolic tolerance to anoxia, and ecological selection by dissolved O2/CO2 and nutrients. The Form 1B RuBisCO found in the chlorophyte green algae, has a poor selectivity between the two dissolved substrates, O2 and CO2, at the active site. This enzyme appears adapted to lower O2/CO2 ratios, or more “anoxic” conditions and therefore requires additional energetic or nutrient investment in a carbon concentrating mechanism (CCM) to boost the intracellular CO2/O2 ratio and maintain competitive carboxylation rates under increasingly high O2/CO2 conditions in the environment. By contrast the coccolithophores and diatoms evolved containing the more selective Rhodophyte Form 1D RuBisCO, better adapted to a higher O2/CO2 ratio, or more oxic conditions. This Form 1D RuBisCO requires lesser energetic or nutrient investment in a CCM to attain high carboxylation rates under environmentally high O2/CO2 ratios. Such a physiological relationship may underpin the speciation of the ocean from domination by the green algae to that of the red algae lineage at the Mesozoic. We aim to highlight the contrasting evolutionary trajectories of the green algal lineage with those of the red algal lineage.

1. Aim

This hypothesis paper aims to integrate recent measurements of RuBisCO kinetic parameters across the phytoplankton and terrestrial plants together with data on the physiology, ecology and evolution of oxygenic photosynthesisers. New evidence suggests increased oxygenation of the ocean could have been a selective force in the transformation of the ocean from domination by the green algae to that of the red algal lineage at the Mesozoic. We aim to highlight the contrasting evolutionary trajectories of the green algal lineage with those of the red algal lineage from different ends of the redox spectrum and how selection for different biochemical parameters of the RuBisCO enzyme has worked through time and space. We explore the geological factors that may have triggered a perturbation in the dissolved O2/CO2 of the ocean leading to an environmental selection towards the mineralising red algal lineage.

2. Phanerozoic phytoplankton and upper ocean oxygenation

Three independent lines of evidence demonstrate that the Phanerozoic ocean was dominated by a succession of phytoplankton: microfossils, molecular biomarkers, and molecular clocks for individual clades. The low C28/C29 ratios of the sterane profiles of Paleozoic rocks are most likely driven by early diverging prasinophyte green algae, and chlorophyte green algae that produce high abundances of C29 relative to C27 and C28 sterols as found from a large, phylogenetically based survey of sterol profiles from the kingdom Plantae [1]. The Devonian saw an expansion of more derived prasinophyte algae (Chlorophyta) at the expense of the incumbent phytoplankton as evidenced by an extremely high sterane/hopane ratio in sedimentary lipids [2], and elevated C28:C29 sterane ratios [1,3]. The later and more derived groups of green algae produce a greater abundance of C28 relative to C27 and C28 sterols [1]. Later, the Mesozoic Ocean was taken over by the chlorophyll a+c containing phytoplankton of the haptophytes (e.g. coccolithophores) and heterokont (e.g. diatom) lineages, whose plastids are derived from red algae (Rhodophyta) via secondary endosymbiosis [4–6]. In each case the larger cell sizes of the phytoplankton, and in the latter, the addition of mineralising skeletons, added power to the biological pump of carbon and nutrients from the surface ocean to the deep, propagating oxygenation and with ramifications throughout the ecosystem.

Recent evidence based on I/Ca in carbonates, a redox proxy...
sensitive to suboxia, identified an excursion in recorded I during the Devonian and a step change at 200 Ma, coincident with each of these micro-faunal revolutions [7]. Elevated I/Ca indicates increased ocean oxygenation, and is interpreted as a deepening of the oceanic oxygen minimum zone at each of these times achieving more persistently oxygenated modern day conditions at the Paleozoic-Mesozoic transition. This new record of I/Ca correlates with the abundance of the biomarker C28/C29 steranes, markers of the radiation in more derived green algae (Chlorophyta and Streptophyta) and the succession of the modern phytoplankton groups from a compilation of rock and oil samples (Fig. 1 [2,5,6]). Such a similarity between these two very different datasets found on contrasting samples and geochemical analysis is strongly suggestive that there could be a common driver to both, such as an increase in surface water oxygenation.

3. Chlorophyll a + c algae have a higher RuBisCO specificity than chlorophyll b algae

The form and specificity of Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO, EC 4.1.1.39), the enzyme that catalyzes CO2 fixation during oxygenic photosynthesis, is also transformed across the open ocean upon the transition from a chlorophyll b to a chlorophyll a + c algal lineage dominated assemblage. It is thought that all Forms of RuBisCO arose from a Form III RuBisCO within an Archaean methanogen [9–11]. This ancestral form was found in prokaryotes such as Prochlorococcus spp., while Form IB is found in higher plants, green algae and β-cyanobacteria. Form IC is found in some photosynthetic bacteria e.g. Rhodobacter sphaeroides and Form ID is found in all non-green eukaryotic algae (i.e. red and chromist algae, except Form II-containing dinoflagellates) [14].

All Form I enzymes are structurally similar with 422 symmetry (tetragonal-trapezoidal crystal structure) and a core consisting of four LSU dimers (L2) arranged around a four-fold axis, capped at each end with four SSUs [13]. Forms IA – ID can be differentiated according to their amino acid sequence. Forms IA and IB are about 80% similar, as are the forms IC and ID. Between Forms IA/B and IC/D there is only about 60% sequence similarity [15,16]. Despite the different forms of I Rubisco, they all have the same functional active site [17].

During oxygenic photosynthesis, RuBisCO catalyzes two competitive reactions; fixation of CO2 for photosynthesis (carboxylation) and energy wasting photorespiration using O2 (oxygenation). The ability of a particular RuBisCO to discriminate between the non-polar, structurally similar substrates CO2 and O2 is determined by the kinetic properties of the enzyme, denoted as the specificity factor (Ω):

$$\Omega = \frac{V_c K_r}{V_o K_c}$$

where $V_c$ and $V_o$ are maximal velocities of the carboxylase and oxygenase reactions and $K_r$ and $K_c$ are the Michaelis constants for the substrates CO2 and O2. The carboxylation:oxygenation efficiency of the net reaction must also account for the CO2 and O2 concentrations at the catalytic site of the enzyme:

$$\text{carboxylation/oxygenation} = \frac{(V_c K_r/V_o K_c)^*(\text{[CO}_2]/\text{[O}_2])}{1 + \frac{K_r}{K_c} + \frac{K_o}{K_c} + \frac{K_r}{K_o}}$$

RuBisCO kinetic characterization from a diversity of organisms shows specificity that range from about 4 to 240 [18–21]. As seen in Fig. 2, a replacement of Form IB-containing green algae and β-cyanobacteria across the ocean, by Form ID-containing haptophytes and heterokonts represents an approximate doubling in RuBisCO specificity [18–20, Supplementary Table 1] in the open ocean.

4. Organisms with higher specificity RuBisCOs are selected by higher environmental O₂/CO₂

All else being equal, according to equation (2), an increase in the O₂/CO₂ ratio in the environment will decrease carboxylation relative to oxygenation for a given RuBisCO and net photosynthetic efficiency. Phytoplankton may adapt to such an environmental pressure by development of a carbon concentrating mechanism (CCM) to internally elevate CO2 relative to O2 around RuBisCO and restore net carbon fixation rates [21–23], by expression of a higher specificity RuBisCO should two different enzymes be available in the genome, or by improvement of their RuBisCO selectivity.

Many extant aquatic photosynthesisers possess a CCM [21,25,26]. Key constituents of the CCM include: (i) plasma- and chloroplast-membrane inorganic carbon transporters; (ii) a suite of carbonic anhydrase enzymes in strategic locations; and usually (iii) a micro-compartiment in the chloroplast in which most Rubisco aggregates (the pyrenoid) [20,27]. Generally, RuBisCO enzymes from algae have evolved a lower affinity for CO2 when the algae have adopted a strategy that employs a CCM to help optimise for CO2 fixation [20]. The phylogenetic progression in green RuBisCO kinetic properties suggest that RuBisCO substrate affinity for CO2 demonstrates a systematic relaxation.
in response to the origins and effectiveness of a CCM [27]. In land plants, it has been established that positive selection in rbcL emerges coincident with the development of a C4 CCM which elevates CO2 to almost saturation at the site of RuBisCO [28,29]. This relaxes pressure for RuBisCO to have a high affinity for CO2-so KC of C4 plants is generally higher than the KC of C3 plants. The development of a CCM masks any selective pressure exerted by rising external O2/CO2 on RuBisCO because it shields the enzyme within a high CO2 microenvironment. The extent of relaxation of selective pressure due to the presence of a CCM depends on its efficiency as there is great diversity in the structure and function of CCMs across the aquatic photosynthesisers.

Despite the prevalence of a CCM in aquatic photosynthesisers, there is considerable evidence that environmental O2/CO2 has frequently exerted a selective pressure on Rubisco specificity, or the use of a higher specificity Rubisco where two forms exist. A higher O2/CO2 ratio can elevate the intracellular CO2/O2 and over time the relative substrate affinities for O2 than CO2 and thus appear to compensate for the 16-fold excess of dissolved O2 relative to CO2 (Fig. 2). It is not clear why there should be such an apparent tuning of the relative affinities of RuBisCO to compensate for the modern environmental ratio of O2/CO2.

One plausible mechanism may be the action of a CCM which acts to elevate the intracellular CO2/O2 ratio and over time the relative substrate affinities of RuBisCO evolve in response to this compensating in-tracellular ratio.

This putative selective pressure exerted by environmental O2/CO2 on the RuBisCO Ko/Kc ratio may, paradoxically, also explain why some photosynthesisers have been found to have a Ko/Kc so extreme as to be apparently tuned to conditions outside the geologically recent range in atmospheric O2/CO2. During the ice ages of the Pleistocene, atmospheric CO2 concentrations have varied in parallel with the temperature fluctuations by ∼100 ppmV [34] but atmospheric O2 has stayed near constant [35]. The dissolved O2/CO2 has fluctuated from ∼13 to 19 in interglacial to ∼39 to 50 during glacial periods (Fig. 2 [33]). Compared to this range, the Ko/Kc of the 1D RuBisCO of two red algae ∼13 to 19 in 39 to 50 during glacial periods (Fig. 2 [33]). Compared to this range, the Ko/Kc of the 1D RuBisCO of two red algae ∼13 to 19 in 39 to 50 during glacial periods (Fig. 2 [33]). Compared to this range, the Ko/Kc of the 1D RuBisCO of two red algae ∼13 to 19 in 39 to 50 during glacial periods (Fig. 2 [33]). Compared to this range, the Ko/Kc of the 1D RuBisCO of two red algae 

Fig. 2. a) The sensitivity of the equilibrium dissolved ratio of O2/CO2 (CO2 [22]) (O2 [23]) concentrations to temperature and salinity (S; 0, open circles, and 35 ppt, closed circles) for the modern (with an atmosphere of 400 ppmV) compared to that at the LGM with invariant O2 but a CO2 atmosphere of 180 ppmV. This environmental O2/CO2 provides a calibration for the redox gradient to RuBisCO of different algal groups and their ecology showing the relative substrate affinities Ko/Kc of RuBisCO as a first order determinant of RuBisCO specificity. Species abbreviations label: Rhodophyta: Gsu Galdiera sulfuraria, Gmo Gm衮 giarinus, moni Musselithium cruentum, Haptothyes: Pfl Pavlovale lutheri, Pfl Porphyridium furcatus, Haptophyte: Plu Pavlovale lutheri, Pfl Plu Pavlovale lutheri, Pfl Pavlovale lutheri. Thermochromis: Cyl Phaeodactylum tricornutum, Cyl Lyngbya sps, Cta Cyanobacterium caldarium. Tps Thalassiosira pseudonana, Sco: Skeletonema costatum, Green algae: Cce Chloraminus rehundtii, Sob Scenedesmus obliquus, Egi Euglena gracilis, Cyanobacteria: Syn synchococcus, Pro Prochlorococcus Plants: C3: Trt Trichomonas CA Zea Mays, Anaerobes: Tde Thiobacillus dentitans, Rep Rhodobacter sphaeroides, Rru Rhodospirillum rubrum, Mhe Methanococccoides burtonii, Thermoanaerobacter kodakarenensis. Also labeled are mean ecologies of different groups of algae ranging from obligate anaerobes, through facultative anaerobes to obligate aerobes and hyperoxic tolerant. b) The number of anaerobic metabolic pathways in the genomes (PFL, PFL-AE, PFO/PNO, HYDA, HYDE, HYD, ADH, ACK, PTA, ASCT, ADP-ACS) of the labeled organisms where RuBisCO specificity has also been determined taken from Atteia et al., [24]. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.).
their CCM [19] and their ecology. Some diatoms (Cylindrotheca fusiformis, and Phaeodactylum tricornutum) have higher specificities (∼110–120) than others. These species have a distinct ecology compared to diatoms reported with lower specificities (∼80), being found in coastal regions, estuaries, mud flats and rock pools compared to the lower specificity open ocean marine diatoms. These highly specific diatom RubisCOS have a very high Kc relative to their Ks which may be a result of living in these more restricted hypoxic coastal/intertidal zones, or even subaerially exposed, compared to open ocean conditions. Similarly two diatom strains of Skeletonema costatum, and Chaetoceros calcitrans have outstandingly low specificities (specificity of 30.3 and 56.7 respectively). These species are known to have resting stages that persist in oxygen-deficient sediments [37] for decades.

At the other extreme of the environmental scale, the form II RuBisCO of Methanococcales bursontii, Thermococcus kodakarenis, Hydrogenovibrio marinus, T. denitrificans, Rhodobacter sphaeroides and Rhodospirillum rubrum is expressed only under anaerobic conditions i.e. with extremely low O2/CO2 ratios. Within this context, the lower Kc/Ks (4–8) of the cyanobacteria RubisCO compared to the RuBisCO of green algae, haptophytes and heterokonts is consistent with the ability of the β-cyanobacteria to flourish under eutrophication. Such an observation may be supported by the correlation of abundance of microbial carbonates in the geological record with inferred periods of a more poorly oxygenated ocean [38].

This analysis reveals that organisms containing the 1D Form RuBisCO within the chlorophyll a+c eukaryotes are better adapted to an open ocean environment with an O2/CO2 ratio (∼16 to 35) that is approximately double that to which the RuBisCO of the cyanobacteria appears to be adapted (O2/CO2 ratio of 4–8). This further supports the hypothesis that a step change in upper ocean oxygenation contributed to the changing success of these different algal groups across the Paleozoic/Mesozoic boundary. Should the cyanobacteria Kc/Ks be tuned to the paleozoic dissolved O2/CO2 ratio in the oceans, before the haptophytes and heterokonts took over then atmospheric compositions at that time, could have fluctuated around 10% O2 and 400 to 800 ppm CO2 when cyanobacteria were dominant. Even if this link between the Kc/Ks and the O2/CO2 of the environment is only qualitative, the Kc/Ks does provide some indication of ecological O2/CO2 ratios and is a first order determinant of RuBisCO specificity.

5. Higher specificity RubisCOS are selected by organisms with aerobic physiology

In addition to environmental O2/CO2 ratios exerting a selective influence on organisms harbouring different specificity RubisCOS, there is also a correlation between the physiological adaptation of the photosynthetic organism to aerobic/anaerobic conditions and RuBisCO specificity (Fig. 2). Obligate anaerobes, or facultative anaerobes which express a form II RuBisCO under anaerobic conditions, all have the lowest RuBisCO specificities (between 1 and 16). There is then a distinction between a group containing the higher specificity 1B land plants and 1D containing haptophytes and heterokonts (obligate aerobes) with a specificity between 80 and 120, compared to the group containing the 1B containing green algae and cyanobacteria (facultative anaerobes) with a specificity between 30 and 60. When investigating the differences between these photosynthesizing organisms, a distinctive physiology for the green algae and cyanobacteria emerges compared to other oxygenic autotrophs. They all have the ability to undergo indirect water photolysis to generate H2 if grown under anaerobic or low sulfate conditions. Anaerobic metabolic pathways allow unicellular organisms to tolerate or colonize anoxic environments. Green algae, such as Chlamydomonas reinhardtii, and Scenedesmus obliquus, all have the ability to ferment their plastidic starch to a variety of end products including acetate, ethanol, formate, glycerol, lactate, H2 and CO2. Cyanobacteria, depending on the species, utilize both nitrogenases and hydrogenases in the pathway of H2 production [39], whereas the green algae rely solely on hydrogenases [40]. Nitrogenases have the advantage that they act unidirectionally, whereas hydrogenases are bidirectional [41]. The high O2 sensitivity of both enzymes requires the separation of H2 evolution and CO2 fixation, temporally or spatially.

An overview of the presence of anaerobic metabolic pathways from whole genome analysis confirms such a gradient to the O2 tolerance of these physiologies ([24]; Fig. 2b). The red algae may be considered an “oxic” endmember to the algae. They are nearly devoid of any of the pathways involved in anaerobic metabolism. They also appear to have undergone a significant genome reduction in their evolutionary history, which could be responsible for the loss of the ancestral anaerobic pathways from the primary endosymbiosis [42]. Similarly the haptophytes and heterokonts are also lacking many of the anaerobic metabolic genes found in the green algae. In a parallel to the large diversity of diatom RuBisCO specificity, the greatest diversity in anaerobic gene presence is also found among the diatoms. By contrast, many green algae have an abundance of anaerobic pathways. Some were lost via gene reductions (such as in Ostreococcus tauri), but C. reinhardtii is adapted to both aerobic and anaerobic conditions. In a survey on different algal groups, redox-regulation of some parts of the Calvin Benson Cycle was also found to be variable with the greatest degree of regulation in green algae, but there was little or no redox-regulation in a red alga or in most lineages with red-algal derived plastids (including the diatoms) [43].

It was from within the Chlorophyte green algae, through the sister group of the Chlorophyte green algae, that the land plants emerged with their facultative anaerobic metabolism capable of living on land and becoming truly complex multicellular organisms (defined by three-dimensional body plans and multiple cell types). Meanwhile the red algal lineages have been limited in stature, multicellularity and ability to make roots [44] and were restricted to marine environments. The first steps onto land would have required the ability to differentiate cells to make roots to obtain nutrients from sediments or soils in periodically inundated and anoxic soils (e.g.Refs. [45,46]). Indeed it may have been the cellular differentiation into roots/shoots versus leaves harbouring RubisCO that allowed the physical segregation between the anaerobic metabolic pathways in the roots and the chloroplasts containing RubisCO in the leaves that allowed the plant RubisCOS to make the step change towards a higher RubisCO specificity, indicative of an aerobic environment, in the leaves.

6. Direction of evolution of RuBisCO specificity and nutrient requirement for a CCM

Eukaryotes with a green plastid possess Form IB Rubiscos thought to have arisen through endosymbiosis of a Form IB containing cyanobacteria. Within this lineage therefore, the Form IB RubisCO improved its specificity in response to increasing O2/CO2 ratios over time, as seen in the land plants compared to the green algae and cyanobacteria. The emergence of a CCM was required to generate high intracellular CO2/O2 to maintain photosynthetic efficiency with the poorer specificity RubisCO found in the cyanobacteria. These CCMs arose relatively late in geological time, ∼420 Ma, after CO2 concentrations in the atmosphere and ocean declined from their initially high levels and dissolved O2 levels rose [33,47].

It is hard to decipher the direction of evolution of the kinetics of RubisCO within the form 1D RubisCO containing lineages. Eukaryotes with a red plastid have Form ID that was originally derived from a γ-proteobacteria [9]. The red algae putatively diverged from the eukaryote tree of life ∼1.1 billion years ago and provided the plastids in the secondary endosymbiotic event that gave rise to the heterokonts and haptophytes. Did the haptophytes and heterokonts inherit a relatively low-specificity RubisCO from the ancestral rhodophyte via secondary endosymbiosis, which has been retained in most extant haptophyte/heterokont species? Was a lower specificity RubisCO of the
haptophytes and heterokonts then shielded from the evolving environment by emergence of CCM pathways in response to rising O2/CO2? Did this lower specificity Rubisco then become more specific in other species, such as Pavlova lutheri, that lack a CCM and inhabit higher O2/CO2 environments? An increase in Rubisco specificity in the Pavlovales may have occurred concurrently with the selection of a higher Rubisco specificity in red algae (as Fig. 2 shows P. lutheri has closest K/K to the red algal species). Alternatively, did the haptophytes and stramenopiles inherit an already highly specific Rubisco from the rhodophytes, which then relaxed under the persistent induction of a CCM elevating internal CO2/O2 in the haptophytes and heterokonts (as proposed by Young et al. [48])?

Regarding the direction of evolution of Rubisco specificity in the different lineages, three lines of evidence suggest that the latter hypothesis may be the more likely scenario i.e. that the haptophytes and stramenopiles inherited an already highly specific Rubisco which then relaxed in specificity over time. Firstly, the ancient fossil record of the red algae, Bangiophyta, places them as continuous inhabitants of the hyperoxygenic peri-to-supra-tidal environment [49]. The peri-tidal zone is distinct for harboring hyperoxic conditions of elevated O2 and much diminished CO2 concentrations during daily light-driven photosynthesis [36], and the supratidal zone sees highly elevated atmospheric O2/CO2 ratio with million fold faster diffusion rates. Another representative of the red algae, the Porphyra and its ancestors, have competed successfully in this dynamic and severe intertidal environment for over a billion years [44]. Similarly, the relatively morphologically simple Pavlovales have always been restricted to near-shore, brackish, or freshwater environments often with semibenthic modes of life, and this may mirror the ancestral ecological strategy of the Paleozoic haptophytes [5]. Even under a poorly oxygenated atmosphere, it is likely that these restricted coastal zones where photosynthesis was rife were consistently hyperoxic. Over this billion year timeframe, these hyperoxic conditions exerted a selection pressure beyond that of typical ocean conditions and selected for Rubiscos that were better adapted to these rather more extremely oxygenated conditions, a trait inherited by the secondary endosymbiotic lineages. To obtain a competitive edge in this environment, algae could have additionally induced a CCM. It is through the persistent induction of a CCM to elevate internal CO2/O2 ratios that eventually the Rubisco specificity relaxed during the speciation events that founded the lineages including the haptophytes and heterokonts.

The strongest signal of positive selection in haptophyte Rubiscos is at the divergence between the Pavlovophyceae and Prymnesiophyceae [48] with a step change in Rubisco specificity (from 125 to 90) between respective representatives P. lutheri and Isochrysis galbana (Fig. 3). Distinct differences in Rubisco specificities within the haptophyte lineage Rubisco correlate with the formation of a pyrenoid and/or presence of a CCM [20]. Pavlova lutheri has a low cellular affinity for carbon, negligible change in this affinity when adapted to high or low external carbon conditions (Rae et al., unpubl data) and lacks a pyrenoid [50,51], suggesting that it has no CCM [20]. By contrast both Isochrysis galbana and Pleurochrysis carterae contain a pyrenoid [50,64,67] and are known to possess CCMs (including carbonic anhydrases) and lower specificity Rubiscos compared to P. lutheri.

The winners of the competition for success in the open ocean then derives from the nutrient efficiency with which algal lineages can maintain high rates of carboxylation relative to oxygenation. Across a rise in ocean O2/CO2 at the Mesozoic, as a result of their poorer Rubisco specificity, β-cyanobacteria need to invest greater nutrient resource in fixing carbon via a more efficient CCM than the haptophytes and heterokonts with a more highly specific Rubisco and lesser need of investment in proteins for a CCM to succeed within this niche. A hint of this higher nutrient requirement for C fixation in species with a lower Rubisco specificity is afforded by a comparison of the Redfield ratio of a range of species measured under identical laboratory conditions in the same study (Fig. 4a). There is such plasticity to the Redfield ratio that direct comparison across a broad range of species from the exact same conditions is the only way to obtain a direct comparison. If a higher C:N reflects a higher efficiency C fixation process per protein expressed, then species with higher Rubisco specificities indeed obtain greater C fixation rates per nitrogen fixed as a result of requiring less proteins for the CCMs. In addition, green seaweeds from the upper intertidal zone but same location have been found to have lower C:N ratios (~10) than both red (~13) and brown seaweeds (~16) for the lower intertidal zone [76] suggestive that these different Redfield ratios may be more broadly characteristic of these algal lineages. Upon oxygenation and increasing oligotrophy of the open ocean, haptophytes and heterokonts could outcompete the green algae and β-cyanobacteria in terms of net carbon fixation relative to oxygenation per nutrient required.

Such nutrient selection and open ocean or coastal selection of CCM efficiency is evident amongst the different groups of cyanobacteria, compensating for a lesser Rubisco efficiency. The open ocean α-cyanobacteria with form IA Rubiscos have a restricted suite of HCO3− accumulation processes and little capacity to acclimatize to decreased inorganic C availability [47,77,78]. The oceanic α-cyanobacteria may have developed a physiology where they may not have the ability to acquire or induce high-affinity carbon transport systems, and in some species no active CO2 uptake system may be present which makes them nutrient efficient and well adapted to the open ocean. On the other hand, many β-cyanobacteria have the ability to induce various CO2 and HCO3− transport systems as their environmental conditions change [47,77,78] and so flourish in coastal nutrient rich zones. Indeed the sensing of oxygen may play a key role in the induction of a CCM in the cyanobacteria [79].

It is worth noting that this consideration above yields contrasting nutrient gradients for CCMs to succeed in different O2/CO2 environments between the chlorophyte and chromalveolate lineages. Amongst the green algae with a poorly specific Rubisco, a great energetic or nutrient investment in a CCM is necessary to inhabit conditions of elevated O2/CO2. By contrast, amongst the haptophytes and heterokonts, the greater nutrient requirement for a CCM, which over time relaxes Rubisco specificity, allows them to extend their ecology into lower O2/CO2 environments. A second argument to support the red algae having consistently expressed high specificity Rubiscos rather than evolving towards a higher specificity Rubisco under rising O2/CO2 comes from C isotopic fractionation. A more highly specific Rubisco binds the CO2 substrate more tightly and thus results in a larger kinetic fractionation associated with C fixation compared to a lower specificity Rubisco (Fig. 4b). The larger C isotopic swings of oceanic δ13C in the Neoproterozoic which dampen towards the modern day [80] could either reflect variations in the burial rate of isotopically light carbon (e.g. Ref. [81]) or burial of different sources of organic carbon with more extreme C isotopic signatures than those of the modern day. If burial of organic matter oscillated between the much lighter isotopic carbon produced by the highly specific Rubisco in the coastal Pavlovales and Porphyra, and the much isotopically heavier organic matter of the cyanobacteria [82], this could contribute to the higher amplitude oscillations of the early part of the δ13C record (see Figure 2c of [81]). The oceanic δ13C oscillations became damped as CCMs were induced and the more intermediate specificity Rubiscos of the terrestrial plants, haptophytes and heterokonts became increasingly prevalent throughout the Phanerozoic contributing to burial of organic matter with less extreme carbon isotopic compositions due to the smaller isotopic fractionations factors of these more intermediate specificity Rubiscos. There are hints that some seaweed δ13C and P. lutheri δ13C may be extremely isotopically light [83,84] which could be consistent with a large carbon isotope discrimination factor of a highly specific Rubisco.

Thirdly, there is little difference in Rubisco specificity between the cyanobacteria (the primary endosymbiont) and the chlorophyte green algae nor between the red alga, P. cruentum (a putative secondary endosymbiont) and the haptophyte P. lutheri suggestive that the ancestor
of the endosymbiont and secondary lineages bearing that endosymbiont are similar in their RuBisCO specificity. Furthermore, the positive selection and change in RuBisCO specificity are coincident at the speciation events [48]. This might suggest that the process of endosymbiosis itself did not change the specificity, and that it was the later environmental change around the RuBisCO that effected a change in the RuBisCO of the land plants and that of the haptophytes and heterokonts.

7. Tempo of evolution of RuBisCO kinetics

7.1. Slow to generate specificity

Despite the general correlation of RuBisCO specificity with relative substrate affinity across the continuum of change in Kc/Ko, there are discrete groups of RuBisCO specificities characterized by a range of Kc/Ko values (Fig. 3). This is suggestive that Kc/Ko may evolve very quickly, and in response to environmental or physiological conditions, but a change in specificity takes much longer. If net carboxylation is the single rate-limiting step for the growth and replication of a single-celled photosynthesizing microorganism, then strong positive selection will be exerted upon the enzyme that catalyzes this step i.e. RuBisCO. A strong selection pressure from an increasing O2/CO2 at the active site can impart drastic improvements in a short period of time, yielding an evolved enzyme that is no longer the weak link in the metabolic network of the cell, hence step changes in specificity [85]. Once that link is no longer the weakest, selection pressure shifts to another point in that physiological pathway such as the proteins of the CCM.

For a particular RuBisCO specificity, a CCM may be responsible for some of the variance in the Kc/Ko due to its ability to boost the internal CO2/O2 ratio (C:N measured in cells under exponential growth, light green bars [73]). These are data measured on a wide variety of species under the exact relationship between higher RuBisCO specificity and C:N is higher when carbon fixation is more nutrient efficient and requires less proteins of a CCM. B) The propensity for plasticity in the Redfield ratio (C:N measured in cells from Boller et al., [75]). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
over those substrates faster so C₄ plants require less nitrogen to achieve a given CO₂ fixation capacity. But due to these correlative biochemical constraints, a CCM appears to yield no change in specificity over relatively short timescales.

In comparison, when considering the largely CCM-lacking Pavlo concession of the CCM-bearing Prymnesiophyceae of the haptophyte lineage, it is evident that the evolution of a CCM in the latter has both altered the substrate affinities and relaxed the specificity of the RuBisCO enzyme (Fig. 3). A possible explanation for the difference between the evolution of specificity in RuBisCOs of the haptophyte lineage and the C₄/C₃ plants is that it takes a long time to accumulate sufficient mutations to effect a change in RuBisCO specificity, on the order of 10⁰s of millions of years. The signal of positive selection in haptophyte RuBisCOs at the branch between the Pavlovophyceae and Prymnesiophyceae [48] is dated to between 300 and 400 Ma. The step change in 1B RuBisCO specificity accompanies the divergence between the filamentous green algae and the land plants around 410 Ma, so about 200 Myrs after the rise to dominance of the chlorophytes ~650 Ma [3]. By contrast, C₄ plants have only been present for the last ~30 Myrs [86]. It seems to take >100 Myrs to accumulate sufficient mutations to evolve an improved functional RuBisCO specificity.

These step changes in the RuBisCO specificity align with analyses of gene sequence for the RbcL gene across the algal phylogenies that find evidence for positive selection in the gene sequence clustered only at the base of the divergence of the modern algal lineages [48]. So RuBisCO undergoes major changes at the establishment of the haptophyte, and heterokont groups relative to the red algae [48]. This is consistent with the former use of RbcL genes for phylogenetic reconstruction i.e. as a species specific marker.

7.2. Fast evolution of Ko/Kc

By contrast, manipulations of the environment or intracellular O₂/CO₂ appear to yield changes to the RuBisCO substrate affinities at rates as fast as the timescale of decades. Assuming that the relative substrate affinity for RuBisCOs compensate for environmental O₂/CO₂ as proposed in Ref. [33], this timescale for adaptation derives from the degree at which these affinities appear to have kept pace with the documented changes in the environment. The Kₚ/Kₑ of most C₄ and C₃ plants cluster at a level that could compensate for a Pleistocene glacial period when there was an excess of O₂/CO₂ between 39 and 50 fold (Fig. 2), or somewhere in between the extrema of the glacial-interglacial variance. It is reasonable to compare plant RuBisCO kinetics to dissolved gas ratios since RuBisCO experiences those substrates in a dissolved state. The Kₚ/Kₑ of algal RuBisCOs appear better tuned to current conditions (O₂/CO₂ ratio of 13–19), a dissolved ratio that is lower than that experienced during the last 1 million years of the Pleistocene glacial cycles because of the anthropogenic rise in pCO₂ over the last two centuries. Consequently the fine-tuning of RuBisCO’s relative affinity for substrates appears to evolve in response to environmental change over timescales of tens of kyrs in the plants, and hundreds of years in the algae. This observation supports the emerging view that RuBisCO may be optimized to its environment [74]. Its kinetic performance can even be classified as moderately efficient when compared to a global overview of enzyme kinetic rates [87–89].

Adaptation processes may work differently in relatively small, subdivided populations of terrestrial organisms and astronomically large populations of marine phytoplankton inhabiting a fairly homogenous environment. Population size is one of the most important parameters that determines the amount of new genetic variation introduced into a population via mutation (the more individuals, the more copies of a gene in a population to mutate every generation), as well as the dynamics of spread and loss of the mutations by chance or selection [90]. It has further been shown that molecular evolution is proportional to generation time in plant lineages and microbial lineages [91,92]. Perennials, with longer generation times, have been shown to accumulate substitutions more slowly than rapidly maturing annual plants. It is likely therefore that the substrate affinity of algal RuBisCOs can be fine tuned to environmental change more quickly and potentially keep pace with anthropogenically diminishing O₂/CO₂ ratios, compared to the slower evolving plant RuBisCO which appear to be stuck in glacial times. The rate of evolution of algal RuBisCOs is potentially orders of magnitude faster than that of plants due to their small generation time compared to that of plants. There is a hint that some diatom species (two strains of P. tricornutum and C. fusiformis) are also better adapted to glacial O₂/CO₂, potentially due to longer generation times as a result of resting spore formation, akin to the slower evolution rates in spore-forming bacteria.

8. Implications for optimizing photosynthesis

Photosynthesisers have evolved two strategies for achieving similar rates of net carboxylation at a given environmental O₂/CO₂. The evolution of a CCM maintains a more ancient O₂/CO₂ ratio, shielding the RuBisCO against the environment and keeping a lower specificity RuBisCO competitive for carboxylation (e.g. the cyanobacteria whom likely increase internal CO₂ 10-fold above the environment) but at an additional nutrient cost. By contrast, the CCM lacking species succumb to the selection pressure of the environmental O₂/CO₂ resulting in a more specific RuBisCO (e.g. the coastal P. lutheri).

Given sufficient time (10⁰s of millions of years), the persistent expression of a CCM reduces the specificity of RuBisCO so a natural limit emerges as to how far net carboxylation, and RuBisCO can improve, within the bounds of evolution. The brown algae, which diversified between 150 and 200 Ma, live ecologically at greater elevation relative to the tide than the red algae. They are distinct for employing iodinated peroxidases [93] which suggests that their more recent divergence has allowed them to take advantage of the rise in ocean iodate documented by the carbonates [7] as part of their antioxidant strategy, and they are well adapted to high O₂/CO₂. The brown algae could harbor the Rolls Royce of RuBisCOs, currently limited to this supratidal zone of hyperoxic conditions and are a worthy candidate of characterization of RuBisCO kinetics in the quest to find the most efficient RuBisCO.

At the other end of the spectrum, the cyanobacteria and green algae appear to have a RuBisCO which, as a bare enzyme, is poorly optimized for the modern oxygenated environment. Within the β-cyanobacteria, an exceptional CCM has evolved including the carboxysome (e.g. Ref. [94]) that compensates for the low specificity RuBisCO. But given that better RuBisCOs exist, has the improvement of RuBisCO within the cyanobacteria been limited by some other factor? It has been speculated that a first Calvin cycle might have evolved from ancient nucleotide metabolism and initially served in redox cofactor balancing and/or mixotrophy, before developing autotrophic function [11]. The 1B form of RuBisCO has also been invoked to be involved in anaerobic methionine sulphur salvage metabolism with the suggestion that the active site of RuBisCO has evolved to insure that this enzyme maintains both key functions [95]. Each of these RuBisCO functions may be lost with an evolution to a higher specificity RuBisCO. As a result the green algal and cyanobacteria RuBisCO may be limited to lower intracellular redox conditions that allows the maintenance of some anaerobic pathways to enable success in environments of fluctuating oxygenation.

9. Geological implications

A prolonged increase in dissolved ocean or intracellular O₂/CO₂ can precipitate rapid change in the RuBisCO enzyme. Although it is tempting to speculate that improvements in RuBisCO might increase carbon fixation rates and oxygen production rates and set the atmospheric composition [96], the majority of change in RuBisCO is an adaptation to an environment which is less favourable to net carboxylation. Any adaptation in terms of specificity and/or induction of a CCM helps sustain carboxylation rates as the environment O₂/CO₂...
becomes less favourable.

In terms of the ocean atmosphere budget of CO₂ and O₂, it is likely that the ratio underwent distinct step changes through geological history. The two atmospheric gases are inversely linked via the burial of carbon in its reduced organic carbon form, the dominant geological driver, that acts to decrease CO₂ at the same time as driving O₂ increase. An interrogation of a recent compilation of Phanerozoic δ¹³C of the ocean points towards a first order monotonic rise in ocean δ¹³C through the Paleozoic indicative of an increasing proportional burial of organic carbon relative to carbonate peaking with the heaviest through the Palaeozoic indicative of an increasing proportional burial of organic carbon in its reduced organic carbon form, the dominant geological surface ocean [99] due to the deepening of the oxidative remineralisation of organic matter. So this persistent oxygenation of the upper ocean and the amalgamation of plates uplifted significant shelf organic carbon onto the continents and out of the geological carbon cycle. Alternatively, submarine fans that accumulate during mountain erosion are efficient at burying large quantities of organic carbon from a vegetated land surface [97]. Pangaea was the first time in geological history that the continents amalgamated, contained significant mountains after plate collision and were covered by terrestrial biota. This maximal sink of organic carbon could have shifted the redox balance of the atmosphere/ocean towards a final rise in atmospheric O₂ and lowered CO₂. It may have been this peak organic carbon burial that tipped the environmental balance in the surface ocean towards the chlorophyll c containing lineages and propagated the positive feedbacks towards deepening OMZs, increasing pH and enhanced oligotrophy. There is a hint that the first sedimentary evidence for the coccolithophores (∼220 Ma), and the biomarker change may have predated the deepening of the OMZ by ∼20 million years (Fig. 1 [98]).

9.1. The deepening of the OMZs

A small trigger such as those described above can easily propagate to a large selective force by a positive feedback and co-evolution between the environment and phytoplankton physiology at the start of the Mesozoic. An increase in the redox or Eh of the upper ocean, or a deepening of an OMZ goes hand in hand with an increase in pH of the surface ocean [99] due to the deepening of the oxidative remineralisation of organic matter. So this persistent oxygenation of the upper waters also accompanied a persistent alkalinisation of the surface ocean helping the advent of mineralised skeletons and carbonate buffering of the deep ocean [100]. There is a positive feedback between the deepening of the oxygen minimum zone due to the enhanced ballast [7,101], increased alkalinisation of the surface ocean, aiding calcification and contributing to the persistent oxygenation. Such ballasting also deepens nutrient remineralisation leaving the surface ocean increasingly deploited - a condition less tolerated by the more nutrient hungry cyanobacteria and green algae that flourish under eutrophication than the nutrient-lean red algae. Concurrently the macro fauna themselves may not just be recipients of additional energy, but by their change in lifestyle as a result of the increasing transfer of nutrients from the lower echelons of the ecosystem they may also be implicit in driving the deepening of the OMZs and oxygenation of the upper ocean by their daily vertical migration [102]. There is a feedback loop between deepening OMZs, persistent oxygenation, alkalinisation, and oligotrophy which once set in motion creates an aggravating selective force towards the mineralising coccolithophores and diatom success over the incumbent green algae setting the scene for the advent of the modern ocean and its biota.

10. Conclusions

The different photosynthetic lineages, and their expressed RuBisCO specificities appear to evolve from contrasting redox endmembers towards similar “redox poise” under modern oxygenated conditions. The view of RuBisCO kinetic data, here, and the fossil record suggests that the red algae and other lineages with red algal-derived plastids (e.g. haptophytes, heterokonts) with a superior RuBisCO specificity were restricted to the oxic intertidal oasis through the Paleozoic. A step change in upper ocean oxygenation, enhanced oligotrophy, and elevated surface ocean pH at the start of the Mesozoic allowed them to inundate the open ocean. By contrast, the β-cyanobacteria and green algae with a greater nutrient requirement to support the CCM supply of carbon for their lesser specificity RuBisCO were restricted to the nutrient rich coastal ocean. This photosynthetic strategy is better adapted to fluctuating anoxic conditions which may have been a key to their successful invasion of the land through periodically inundated and anoxic soils. We explore the tempo of adaptation within RuBisCO kinetistics to changing environmental O₂/CO₂ ratios and show that evolution of a CCM, at a nutrient cost, acts to relax RuBisCO efficiency and so imposes a natural limit to the improvement of RuBisCO over Earth’s history.

Acknowledgments

REMR is grateful to the ERC Starting Grant (SP2-GA-2008-200915), ERC Consolidator Grant (681746) and a Wolfson Research Merit Award from the Royal Society, UK for financial support of this work on RuBisCO and for the incredible work and discussions of the GRACE team including Ana Heureux, Jodi Young, Ben Rae, Renee Lee, Harry McClelland and collaborator Spencer Whitney and Rob Sharwood at ANU. The manuscript has benefited from discussions with Nick Butterfield, Steven Kelly, Emily Flashman, Simon Conway Morris, Erdem Idiz, Rachel Wood and Sinead Collins.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.freeradiobiomed.2019.05.006.

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