

Enhancing Photosynthetic Efficiency and Carbon Capture in Algae Through Targeted Rubisco Mutations: A Theoretical Approach

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Abstract:

The accelerating pace of climate change, driven by rising levels of atmospheric CO₂, necessitates innovative strategies for carbon capture and sequestration. Photosynthetic organisms play a pivotal role in the global carbon cycle, converting CO₂ into organic matter through the process of photosynthesis. Enhancing the efficiency of this process in microalgae, such as *Chlamydomonas reinhardtii*, presents a promising avenue for both reducing atmospheric CO₂ and producing sustainable biofuels.

Rubisco (Ribulose-1,5-bisphosphate carboxylase/oxygenase) is a critical enzyme in the Calvin cycle, catalyzing the first step of carbon fixation. However, Rubisco is inherently inefficient because it can also react with oxygen, leading to photorespiration—a process that reduces the overall photosynthetic capacity and efficiency of CO₂ assimilation. This inefficiency makes Rubisco a key target for genetic improvement. Theoretical approaches to improve Rubisco's efficiency in *C. reinhardtii* involve introducing specific mutations known to enhance the enzyme's catalytic properties. This paper proposes three such mutations: Lys334→Ser, Ala340→Gly, and Asp473→Glu, aimed at increasing CO₂ specificity, catalytic turnover, and thermal stability, respectively. These modifications are expected to not only boost the photosynthetic efficiency of *C. reinhardtii* but also significantly enhance its potential for carbon capture and biomass production.

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Model Organism for Carbon Capture:

Chlamydomonas reinhardtii is a widely studied model organism in the field of photosynthesis and bioenergy due to its ease of genetic manipulation and rapid growth rates. It can grow autotrophically, directly fixing atmospheric CO₂ into organic compounds, thus serving as a natural carbon sink. The alga's ability to thrive under various environmental conditions, including variable light intensities and temperatures, makes it a robust platform for experimental modifications aimed at enhancing photosynthetic efficiency. Furthermore, the scalability of *C. reinhardtii* cultivation in bioreactors allows for large-scale production, which is crucial for applications in biofuel production and carbon sequestration technologies.

Proposed Mutations and Theoretical Justification:

Lys334→Ser: Increasing CO₂/O₂ Specificity

The substitution of lysine with serine at position 334 is thought to increase the CO₂/O₂ specificity factor of Rubisco. Lysine at this position plays a significant role in stabilizing the transition state of the carboxylation reaction. By replacing lysine with serine, a smaller amino acid, the enzyme's active site undergoes subtle changes that could reduce its affinity for O₂, thereby minimizing the oxygenase reaction that leads to photorespiration. Studies on tobacco (*Nicotiana tabacum*) have demonstrated that similar mutations have been successful and increase Rubisco's CO₂ specificity, resulting in a significant reduction in photorespiration and an increased net rate of photosynthesis (Parry et al., 2002). This increased specificity for CO₂ would enhance the overall efficiency of carbon fixation, thereby maximizing the amount of CO₂ assimilated into organic matter. In *C. reinhardtii*, this mutation would decrease the energy and carbon losses associated with photorespiration, leading to more efficient carbon capture.

Ala340→Gly: Enhancing Catalytic Turnover (k_{cat})

The Ala340→Gly mutation is hypothesized to increase the catalytic turnover rate of Rubisco. Alanine at position 340 contributes to the rigidity of the enzyme's active site. By substituting glycine, which has a

smaller and more flexible side chain, the enzyme's active site might gain greater conformational flexibility during the catalytic cycle. This increased flexibility could facilitate the transition state, potentially lowering the activation energy required for the carboxylation reaction and thus increasing the rate of CO₂ fixation. Although direct studies on this specific mutation in Rubisco are limited, similar alterations in cyanobacteria have led to increased catalytic efficiency, enabling the enzyme to stabilize the transition state more effectively and reducing the energy barrier for the carboxylation reaction (Andersson & Backlund, 2008). If applied to *C. reinhardtii*, this mutation could result in more efficient CO₂ conversion into organic compounds, thereby enhancing biomass production and carbon capture.

Asp473→Glu: Improving Thermal Stability

The replacement of aspartic acid with glutamic acid at position 473 is proposed to enhance the thermal stability of Rubisco. Aspartic acid contributes to the formation of salt bridges that help maintain the structural integrity of the enzyme under varying thermal conditions. Glutamic acid, with its longer side chain, could strengthen these interactions, potentially providing additional stability. Research on similar modifications in thermophilic bacteria suggests that such changes could enhance Rubisco's thermal stability, allowing it to maintain activity at higher temperatures by reinforcing the enzyme's structural integrity and preventing denaturation (Cummins et al., 2018). In *C. reinhardtii*, this mutation would ensure that the enzyme remains active and efficient even as global temperatures rise, thereby preserving the alga's ability to fix CO₂ effectively. The improved thermal stability is especially relevant for large-scale cultivation in outdoor environments, where temperature fluctuations are common.

Hypothetical Mechanisms and Benefits:

Combined Effects of Mutations: The synergistic effect of the Lys334→Ser, Ala340→Gly, and Asp473→Glu mutations could significantly enhance the photosynthetic efficiency of *C. reinhardtii*. By increasing CO₂ specificity, the Lys334→Ser mutation reduces the loss of fixed carbon to photorespiration, conserving energy and enhancing net carbon capture. The Ala340→Gly mutation boosts the catalytic efficiency of the enzyme, allowing for a higher rate of CO₂ assimilation and thus more rapid biomass accumulation. The Asp473→Glu mutation ensures that these benefits are retained across a broader temperature range, protecting the enzyme from thermal inactivation. Collectively, these modifications are poised to maximize the carbon capture potential of *C. reinhardtii*, making it a more effective organism for biotechnological applications aimed at reducing atmospheric CO₂ levels.

Potential for Increased Biomass Production and Carbon Sequestration:

The enhanced efficiency in CO₂ fixation and biomass production positions *C. reinhardtii* as a valuable tool for carbon sequestration. The increased biomass yield can be harnessed for biofuel production, providing a renewable energy source that further contributes to carbon neutrality. The ability to capture and convert substantial amounts of atmospheric CO₂ into biomass makes *C. reinhardtii* an integral component of integrated carbon management strategies. By optimizing the Rubisco enzyme, this theoretical framework lays the groundwork for developing robust algal strains that can be deployed at scale for environmental and industrial applications.

Conclusion:

The proposed Rubisco mutations—Lys334→Ser, Ala340→Gly, and Asp473→Glu—in *Chlamydomonas reinhardtii* offer a promising theoretical approach to enhancing photosynthetic efficiency and carbon capture capability. By targeting the enzyme's CO₂ specificity, catalytic turnover, and thermal stability, these mutations address key limitations in the photosynthetic process. The resulting increase in carbon fixation efficiency and biomass production positions *C. reinhardtii* as a powerful organism for biofuel production and carbon sequestration. This research highlights the potential of genetic engineering to create more efficient photosynthetic organisms, contributing to sustainable solutions for climate change mitigation.

References:

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