

Review Paper

Rubisco: Limitations and Re-engineering for a Better Enzyme

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Ribulose-1, 5-bisphosphate carboxylase-oxygenase (Rubisco) is the principal catalyst for photosynthesis and is the basic means by which living creatures acquire the carbon necessary for life. Yet, with a turnover rate of 3 sec⁻¹, Rubisco is one of the slowest enzymes known for various reasons. Re-engineering of the enzyme leads to the improvement of the functional property to optimize its activity. An ideal engineering strategy combines Bioinformatics and computational modelling with our knowledge of the natural variation in Rubisco sequence, structure and activity to help fine tune the design process. Improved Rubisco holds promise to bring renaissance in global agriculture.

Keywords: Rubisco; enzyme; engineering; carboxylation; Photosynthesis

INTRODUCTION

Ribulose-1,5-bisphosphate carboxylase/oxygenase, most commonly known by the shorter name Rubisco, is a bifunctional enzyme (EC 4.1.1.39) that is used in the Calvin cycle to catalyze the first major step of carbon fixation, a process by which the atoms of atmospheric carbon dioxide (CO₂) are made available to organisms in the form of energy-rich molecules. Besides its carboxylase activity, Rubisco also acts as an oxygenase in a reaction involving competition between O₂ and CO₂ for reaction with RuBP (ribulose-1, 5- bisphosphate) (Nishitani et al., 2010, Wostrikoff & Stern, 2007). Thus, while photosynthesis is initiated by the carboxylase activity, the oxygenase activity produces a by-product which needs to be recycled in the photorespiratory pathway, a process which wastes carbon and energy and greatly reduces the overall efficiency of photosynthesis (Andersson & Backlund, 2008). Rubisco is thought to be

the most abundant protein in the world and comprises up to 50% of all soluble proteins in plants (Spreitzer & Salvucci, 2002). It provides the only link between pools of inorganic and organic carbon in the biosphere. Thus its importance would be hard to overestimate.

Structure and different forms of Rubisco

Rubisco is a very large and complex protein. In plants, algae, cyanobacteria, and phototropic and chemoautotrophic proteobacteria the enzyme usually consists of two types of protein subunit, called the large subunit (LSU, about 55,000 Da) and the small subunit (SSU, about 13,000 Da) (Dhingra et al., 2004). The LSU gene is part of the chloroplast DNA molecule in plants. There are typically several related SSU genes in the nucleus of plant cells and the SSU are imported to the stromal compartment of chloroplasts from the cytosol by crossing the outer chloroplast membrane. The enzymatically active substrate (ribulose 1,5-bisphosphate) binding sites are located in the large chains that form dimers in which amino acids from each large chain contribute to the binding sites. A total of eight large chain dimers and eight small chains assemble into a larger complex of about 540,000 Da (Jeremy M. Berg,

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Abbreviations

RuBP, D-ribulose 1,5-bisphosphate; Rubisco,
RuBP carboxylase/ oxygenase;
LSU, Large Sub Unit; SSU, Small Sub Unit

2002). It has been found that (in some proteobacteria and dinoflagellates) Rubisco contain only large subunits (crystallography).

Magnesium ions (Mg^{2+}) are essential for its enzymatic activity. Proper positioning of Mg^{2+} in the active site of the enzyme involves addition of an "activating" carbon dioxide molecule (CO_2) to a lysine in the active site (forming a carbamate)[5]. Alkaline pH helps in the formation of the carbamate. The pH and the concentration of magnesium ions in the fluid compartment (in plants, the stroma of the chloroplast) increases in the light (Harvey Lodish, 2000).

There are four forms of ribulose 1, 5-bisphosphate carboxylase/oxygenase (Rubisco) found in nature. Forms I, II, and III catalyse the carboxylation and oxygenation of ribulose 1,5-bisphosphate, while form IV, also called the Rubisco-like protein (RLP), does not catalyse either of these reactions (Tabita et al., 2008).

Rubisco's limitations

Most enzymes typically can carry out thousands of chemical reactions each second. In contrast, RuBisCO is notoriously inefficient as a catalyst for the carboxylation of RuBP, being able to "fix" only about 3 CO_2 molecules each second. Nevertheless, because of its extremely large concentration, under most conditions and when light is not limiting photosynthesis, the reaction of Rubisco responds positively to increasing CO_2 concentration. Thus, it is usually the concentration of CO_2 which is limiting (Nishitani et al., 2010).

Reasons for inefficiency

This slow and inefficient activity of Rubisco can be attributed to several factors. First, the competitive inhibition by O_2 . Second, the dead-end inhibition by RuBP. Third, inactivation by loss of carbamylation. Fourth, Rubisco's multi-step mechanism (five separate reactions catalysed in the same active site) may constitute a barrier to evolution of its carboxylase efficiency (Portis, 2003). Fifth, catalytic improvements may be limited by intrinsic chemical (energetic) constraints. Sixth, Rubisco has a very conserved active site that may be resistant to the normal evolutionary adaptation by single-step mutations. That is, it may need suitable multiple mutations to improve and because these are rare the enzyme is very slow to adapt. Seventh, during Rubisco's evolution from the first photosynthetic organism, the concentrations of CO_2 and O_2 in the atmosphere have changed very greatly from one with high CO_2 and low O_2 to one of low CO_2 and high O_2 . This makes the evolutionary adaptation of the active site to keep up with changes to minimize the oxygenase reaction and maximize the carboxylase reaction a difficult

one (Jin et al., 2004). These inadequacies make Rubisco rate limiting for photosynthesis and an obvious tempting target for investigation in order to increase agricultural productivity. The most interesting avenue to explore are the reasons which deal with possible chemical and evolutionary blocks (Tabita et al., 2007, Satagopan et al., 2009).

Reengineering of Rubisco

Application of multi-method approach has pioneered the process of reengineering Rubisco. In an innovative computational approach, scientists reconsidered Rubisco's catalysis and discovered a novel mechanism. New roles to several active-site residues were found, which in turn were supported by existing experimental data (King et al., 1998).

These new mechanistic findings have been coupled with Rubisco sequence, x-ray structural and other experimental data using computational biology tools. These helped researchers in making rational predictions of multiple mutations expected to increase Rubisco's efficiency. The re-design strategy is based entirely on the LSU (Large Sub Unit) as it is the prime location of determining its catalytic efficiency. Predictions have been validated in experimental conditions.

Understanding evolution of Rubisco can help a great deal in re-designing of a better enzyme. Land plants have evolved under natural conditions where the availability of water and/or nitrogen often limits photosynthesis. As shown by the many studies involving CO_2 -enrichment, increasing the rate of carboxylation by Rubisco will increase plant yield, provided that sufficient nitrogen is available for increased protein synthesis. Improving the catalytic efficiency of Rubisco will have the same effect but require less nitrogen to implement. More detail insight into Rubisco evolution holds promise for better understanding its complex and multistep reaction mechanism and for understanding how to modify the properties of the active site (responsible for catalysis) by introducing mutations to give rise to better enzymatic activity. As we arrive in an era of technological advancement, the prospects for improving Rubisco are excellent (Mauser et al., 2001).

An ideal approach:

An ideal approach should include doing computational simulations of the two reaction (oxygenation, carboxylation) catalysed by Rubisco in the complete environment of several representative species (green plant, red algae, cyanobacteria etc, some other species may be included to make the study a more representative one and statistically significant) to understand how evolution has adapted the reaction in distant branches of

photosynthetic organisms. One particularly interesting aspect is to design or predict multiple Rubisco mutants with improved activity. Biochemical and structural analysis of mutants will also be part of the proposed study. Exhaustive analysis of its active site at sequence level and by homology modelling and molecular dynamics is some other methods that has to be used. Bibliometric and Bioinformatic analysis will come in handy in designing predicted mutants in silico. Kinetic measurements of the mutant enzyme or other natural Rubiscos of interest in the evolutionary study will help to validate the results. The evolutionary analysis with the integration of bioinformatics tools and experimental validation will bring out the best results in the Rubisco research.

DISCUSSION AND CONCLUSIONS

It is hard to overestimate the economic and social benefits that would ensue from improving the ability of plants (primarily crops) to use their water, light and nitrogen resources more efficiently and increase their capacity to sequester carbon dioxide (CO₂). Scientists should aim to effect such changes by replacing the CO₂-fixing enzyme, Rubisco, with more efficient forms. By interacting with atmospheric carbon dioxide, Rubisco - creates the carbohydrates, proteins, and fats that sustain plants and other living beings. But ironically, Rubisco, the world's most abundant protein is perhaps the most incompetent enzyme. Hence, the quest for a more efficient Rubisco to increase agricultural productivity is considered as "a holy grail in plant biology."

Fuelled by evolutionary engineering strategy we can integrate computational biology tools and experimental approaches with our existing knowledge of Rubisco sequence, structure and activity across species to fine tune the process. Photosynthesis as a whole is not particularly efficient; a crop plant that stores as much as 1% of the total received solar energy is exceptional. Since Rubisco is by far the biggest drag in the process of photosynthesis, in a world where the population of the poorest nations still increases exponentially, speeding up the slowest step in the photo assimilation of carbon for increasing crop yield might be a worthy aim. The tools that more than doubled the world's grain harvests since

1960 have lost their edge. There are enormous signs indicating familiar tools of the Green Revolution are providing diminishing returns. Thus, Rubisco has become an increasingly tempting target.

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