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RUBISCO: STRUCTURAL AND FUNCTIONAL ASPECTS WITH SPECIAL REFERENCE TO CATALYTIC IMPERFECTION

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Carbon fixation is one of the most fundamental aspects of plant physiology. It is carried out by RuBisCO (**r**ibulose **bis**phosphate **c**arb**o**xylase), an enzyme that also catalyzes oxygenation reaction yielding 'toxic' intermediates. At optimal conditions, RuBisCO preferentially incorporates inorganic carbon into organic carbon skeletons (RuBP, ribulose-1,5-bis phosphate), generating two molecules of 3-phosphoglycerate. This simple compound is subsequently used to build other organic molecules of life and contributes significantly to plant growth and increased biomass. Thus, RuBisCO is a potential target for genetic manipulation to improve plant yield by strengthening source-sink relationship.

Key words: RuBisCO, catalytic imperfection, carbamylation, oxygenation, photosynthesis, trade off and photorespiration

RuBisCOis used as an acronym for ribulose-1,5-bisphosphate carboxylase/oxygenase. It is a multimeric protein complex that catalyzes the first step (rate-limiting) in photosynthetic carbon fixation and photo respiratory carbon oxidation process *(Hudson et al*. 1992) (Figure 1). RuBisCO is found in almost all autotrophic organisms including photosynthetic bacteria, cyanobacteria, algae, archaea and higher plants *(Hudson et al*. 1992, Mueller and Badger, 2007). It has been estimated that RuBisCO constitutes about 50% of soluble protein in chloroplasts (Spreitzer and Salvucci 2002). It is not only the most abundant protein in chloroplasts but also the most abundant enzyme in the global carbon cycle that literally feeds life on earth and without it no plant would grow and there will be no food to eat (Ellis, 1979). This large amount of RuBisCO is attributed to its slow activity (Zhou and Whitney 2019), regulated by relative concentration of CO_2 and O_2, Mg^{2+} and pH of stroma region.

Structural details of RuBisCO

RuBisCO is a hexadecameric protein consisting of 16 subunits which are grouped into two categories based on their size and nature: Eight identical large **(L_8)** subunits-extremely conserved (Andersson and Taylor, 2003; Andersson and Backlund 2008) and eight identical small **(S_8)** subunit (Figure 2). Further, RuBisCO is a dimer of octamers**(L_8S_8)**. Large subunits (50-55kDa) are encoded by chloroplast ***rbc*L** (ribulose-1,5-bisphosphate carboxylase/oxygenase L) gene. Each such subunit consists of a catalytic site (Andrews 1988, Morell *et al.* 1997) and a

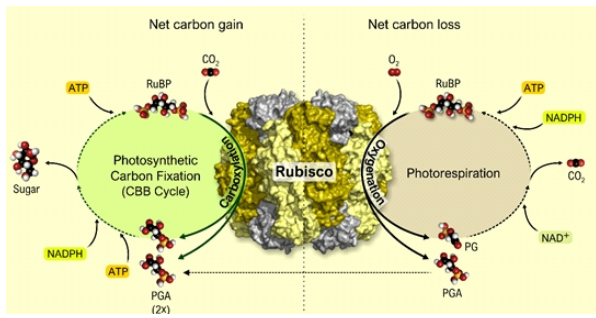

Figure 1: RuBisCO catalyzing both carboxylation and oxygenation reaction (reproduced with the permission of authors and Agrisera). Under optimal conditions, RuBisCo catalyses carboxylation of ribulose-1,5-bisphosphate (RuBP) to form two molecules of 3-Phosphoglyceric acid (PGA). Under high temperature and Low CO_2 concentration, RuBisCO catalyses oxygenation reaction (photorespiration) yielding one molecule of 2-Phosphoglycolate (2-PG) and one molecule of 3-Phosphoglyceric acid. Phosphoglyceric acid is subsequently used to build other organic molecules of life.

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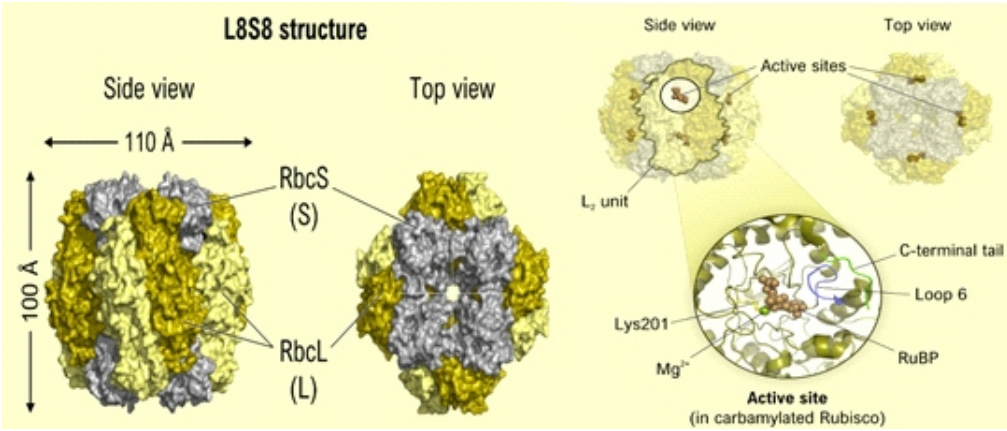

Figure 2: Structural organization of RuBisCO (reproduced with the permission of authors and Agrisera). RuBisCO is composed of 8 large (L8) and 8 small (S8) subunits. Large subunits are encoded by plastid located ribulose-1,5-bisphosphate carboxylase/oxygenase L gene (rbcL) where as small subunits are encoded by ribulose-1,5-bisphosphate carboxylase/oxygenase S gene (rbcS) located in the nucleus.

regulatory site. A catalytic site can fix 3 molecules of CO_2 per second. Therefore, a total of 24 CO_2 molecules are fixed by eight catalytic centers (Andersson *et al.* 1989,Knight, Andersson and *Branden* 1990; Lorimer and Miziorko 1980) of RuBisCO. However, the small subunits (12-14 kDa) are encoded by a family of closely related nuclear (***rbc*S**) genes (Spreitzer 2003). They are not critical for carboxylation (Lee, Read and Tabita 1991). However, they are thought to stabilize and enhance the catalytic activity of the L subunits.

RuBisCO protein subunits are organized in a specific manner. The eight large subunits form a central core and are arranged in two superimposed tiers of four each around a 4-fold axis. This central octameric core is capped on either side by 4 small subunits (Knight *et al.* 1990) (Figure 3).

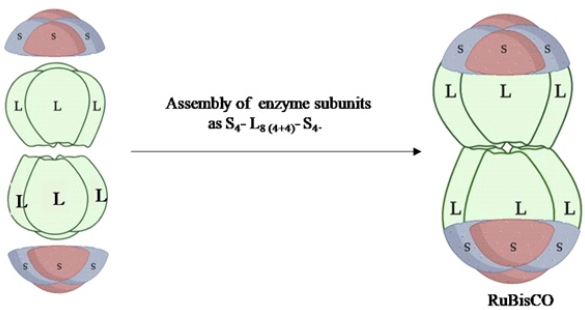

Figure 3: *Assembly of 16 enzyme subunits (L_8S_8).The eight large subunits are arranged in two superimposed tiers of four each ($L_4 + L_4$) forming a central core capped on either side by 4 small subunit (S_4).*

Catalytic mechanism and role of Mg^{2+} ions: The catalytic mechanism of RuBisCO has been extensively investigated through various structural studies, chemical modifications and direct mutagenesis (Hartman and Harpel 1994, Cleland *et al.* 1998, Erb, Tobias and Zarzycki 2017). It has been observed that RuBisCO cannot directly fix atmospheric CO_2 into organic molecules. To catalyze this carboxylation reaction, it requires prior activation. RuBisCO activation is achieved by carbamylation of ε-amino group of the lysine 201 residue in the active site (Lorimer and Miziorko 1980) by a non-substrate CO_2 molecule. In order to stabilize negative charge, Mg^{2+} ion joins carbamate (carbonic acid amide) to form carbamate-Mg^{2+} complex (active). Mg^{2+} operates by driving deprotonation of the Lys-210 residue, causing it to rotate by 120 ° (Schreuder *et al.* 1993) to the trans

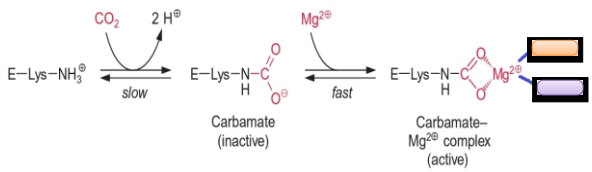

Figure 4: Carbamylation of Lys-201 residue

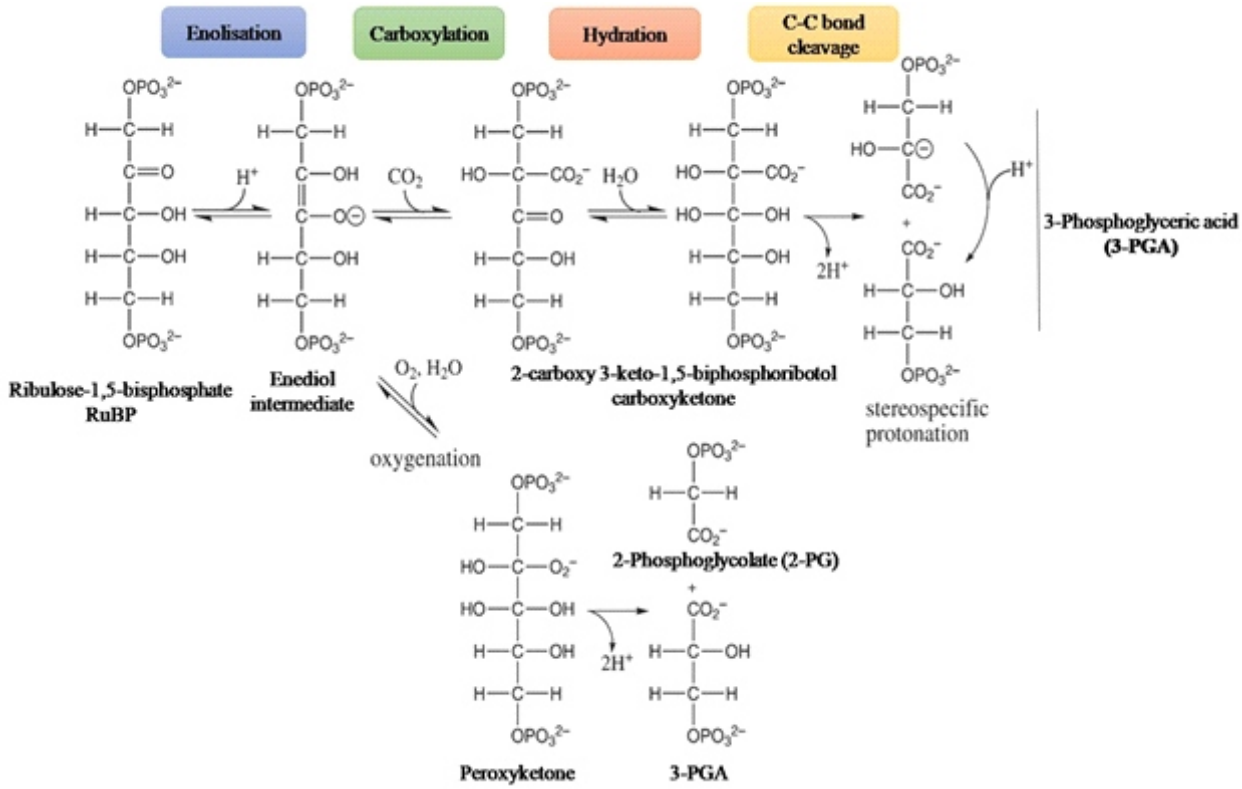

Figure 5: *Carboxylation/oxygenation reactions catalyzed by RuBisCO. Ribulose-1,5-bisphosphate (RuBP) is isomerized to an enediol intermediate before carboxylation or oxygenation. The enediol intermediate reacts with CO_2 or O_2. During carboxylation, RuBP (enediol) irreversibly reacts with CO_2 to form 2-carboxy 3-keto-1,5-biphosphoribotol (carboxyketone). Through hydration and cleavage, this unstable six-carbon intermediate is broken down to produces two molecules of 3-phosphoglycerate (3-PGA). If oxygenation occurs, besides 3-PGA, one molecule of 2-Phosphoglycolate (2-PG) is also generated, which is subsequently salvaged in photorespiration.*

conformation. This conformational change significantly decreases the distance between the nitrogen of Lys and the carbon of CO_2 and brings them in close proximation. The close proximity allows for the formation of a covalent bond between the two and creates a negatively charged carbamyl group that facilitates metal-ion binding, resulting in the formation of carbamate-Mg^{2+} complex (Figure 4). The bound Mg^{2+} ion is in turn coordinated with Asp-203 and Glu-204 (Andersson *et al.* 1989). These amino acid residues together with $Mg^{2+}$and His-335 (Stec and Boguslaw 2012) bring about final conformational change in the RuBisCO protein, that results in exposure of the active site on each large subunit, thereby rendering the enzyme active for CO_2 fixation.

The activation of RuBisCO is dependent on another enzyme called RuBisCO activase. This enzyme is ATP dependent (Spreitzer and Salvucci 2002, Spreitzer 2003) and remains inactive until ε-amino group of Lys 201 is carbamylated. Non-carbamylated, inactive form of RuBisCO binds RuBP very tightly, resulting in the inhibition of the enzyme. During the hydrolysis of ATP, RuBisCO activase induces structural changes in RuBisCO that allows the release of tightly bound ribulose-1,5-bisphosphate (RuBP) and thus enables the carbamylation of the free enzyme. This ATP dependence of RuBisCO activase coordinates RuBisCO activity with the light reactions that provides assimilatory powers such as ATP and NADPH (Shevela, Bjorn and Govindjee 2019, Andrews and

Lorimer 1987). The carboxylation is a complex multistep process involving 4 discrete steps (Figure 5); **i)** Enolization of ribulose-1,5-bisphosphate (RuBP) **ii)** Carboxylation of 2,3-enediol **iii)** Hydration of resulting ketone **iv)** Carbon splitting and stereospecific protonation.

Catalytic imperfection

RuBisCO makes mistakes because of inbuilt catalytic imperfection. Besides carboxylation reaction, RuBisCO also catalyzes a non-productive side reaction during photorespiration (Knight, Andersson and Branden 1990, Erb, Tobias and Zarzycki, 2017). The side reaction takes place when an enodiolate intermediate of RuBP is attacked by O_2 and produces onemolecule of 3-phosphoglycerate and one molecule of 2-phosphoglycolate. 2-phosphoglycolateis a toxic unwanted metabolic intermediate that is subsequently salvaged in an energy demanding process (photorespiration). This side reaction with molecular oxygen not only reduces functional activity of RuBisCO but also drains a pool of RuBP and decreases the overall efficiency of carbon fixation by up to 50% (Andrews and Lorimer 1987, Sage 2002).

Under optimal conditions, the ratio of oxygenation to carboxylation during photosynthesis is 1:4; this implies that every fifth ribulose-1,5-bisphosphate molecule is consumed in the oxygenation reaction (Buchanan, Gruissem & Jones 2015). This catalytic imperfection has evolutionary reason. RuBisCO evolved at a time when the earth was reducing with no trace of molecular oxygen (O_2). In CO_2 rich environment, there was no selection pressure on RuBisCO. Therefore, it was notoriously inefficient in discriminating CO_2 and O_2 (Tabita *et al.* 2008, Young *et al.* 2012, Jordan and Ogren 1981). This ineffiecient discrimination is also attributed to similar electrostatic potential of CO2 and O2 (Gready 2008). Due to oxygenic photosynthesis in cyanobacteria, algae and land plants, huge amount of CO_2 was fixed into biomass. At the same time, equimolar amount of O_2 was released, making O_2 the second most abundant gas in today's atmosphere. With the rise of atmospheric O_2 concentration, RuBisCO had to learn to discriminate between CO_2 and O_2. Increased O_2 concentration induced selection pressure that brought some changes in RuBisCO's structural design, the significant one was tradeoff between specificity and turnover rate (Spreitzer and Salvucci 2002). The turnover rate of RuBisCO is positively correlated with its CO_2 affinity and negatively correlated with the CO_2/O_2 specificity ratio of the enzyme (Bainbridge *et al.* 1995, Tcherkez, Farquhar and Andrews 2006). The efficiency of RuBisCO is thus dependent on the relative concentration of CO_2 and O_2 in the atmosphere and the ability of RuBisCO to discriminate between the two. The specificity ratio *S* is defined as: *S* =VcKo/VoKc, where Vc and Vo are maximal velocity for carboxylation and oxygenation, and Kc and Ko are the relative Michaelis constants for CO_2 and O_2, respectively. The specificity ratio has implications for the crop yield, nitrogen and water utilization in plants, and the global carbon cycle (Lorimer and Andrews 1973). RuBisCO specificity is also attributed to the temperature. When the temperature rises, the CO_2/O_2 specificity of the RuBisCO decreases (Studer *et al.* 2014, Christin *et al.* 2008). This is due to the decreased solubility of CO_2 in cytoplasm and stroma region of the chloroplast. However, the solubility of O_2 remains unaffected (Shevela *et al.* 2020, Parry *et al.* 2012, Ogren 1984, Salvucci *et al.* 1985).Increased temperature also ensures forced closure of stomata to minimize evapotranspiration loss at the cost of CO_2acquisition.

CONCLUSION

It is an established fact that enzyme activity and specificity are reciprocally linked with each other and so is the case with RuBisCO. Because discrimination usually comes at the

cost of reduced catalytic rate, a more specific enzyme almost inevitably becomes a slower catalyst. As a consequence, the RuBisCO enzyme apparently became trapped in a tradeoff between enzyme catalysis and specificity. In order to fix this catalytic imperfection, some plants adapted to live in hot climates have evolved local carbon dioxide concentrating mechanisms to maximize the carboxylase activity of RuBisCO. The high concentration of CO_2 at the site of RuBisCO allows a lower specificity ratio of CO_2/O_2 and therefore an increase in turnover rate and thus efficiency. However, in C_3 plants with no carbon concentrating mechanism in place, a better RuBisCO can be engineered by improving specificity for CO_2 over O2 and by enhancing carboxylation efficiency.

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