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An Appraisal of Existing and Additional Strategies for Enhancing Photosynthetic CO_2 Fixation and Elimination of Oxygenase Activity in Rubisco

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Abstract

Researchers need to understand the function of Rubisco as a storage protein, in addition to its enzymatic function, and the same has been emphasized. New speculative strategies suggested aims to complement/accelerate the existing strategies. Accordingly, a study of Rubisco and other enzyme sequences viz; oxygenases, oxidases, other carboxylases and oxygen/CO_2 carrying hemoglobins have been emphasized. Further, the approach of cloning of relevant genes in chloroplasts is suggested which obviates the need for creating Kranz anatomy. Though achieving the ultimate goal is of a long term nature speculative/ theoretical strategies suggested will in the short term advance our current understanding of plant metabolism particularly in relation to photosynthesis and photorespiratory pathways and also of manipulating other pathways/enzymes in desired ways.

Keywords:* *Rubisco; Photosynthesis; Photorespiration; Oxygenases, Oxidases; Carboxylases

Introduction

In olden days scientists went to the extent of saying that "the seeds of the second Green Revolution lies in eliminating photorespiration". Though this wishful vision is yet to be realized, since then until now, the scenario has changed a lot. Particularly in recent times-strategies for enhancing photosynthetic fixation, manipulating Rubisco, reducing photorespiration and understanding of the significance of photorespiration, etc has changed considerably. So, there exists much information both on highly speculative strategies yet to have experimented and which have been experimented to varying degrees and still continuing. Even if they come to fruition, it is in the very long term [1-9]. Though speculative strategies have been suggested they have been reticent with respect to the following points:

A). Rubisco is not just a CO_2 fixing enzyme but acts as a storage protein. This point seems to be overlooked/relegated. During senescence, Rubisco is degraded and the resulting amino acids either as such and/or after metabolic reworking are translocated through the phloem to serve as a source of Nitrogen, Carbon, and Sulfur to the developing grains/seeds [10].

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Therefore certain strategies already speculated need a rethinking.

B). Although, Rubisco exhibits slow catalysis and the inability to discriminate CO_2 and O_2, yet at the same time, a point to be noted is that it is the most abundant enzyme available. The reason for its abundance is mentioned above that it acts as a storage protein to be utilized during grain development. Thus, for an enzyme that is so abundant, probably there is no need for high catalytic efficiency. In other words, if an enzyme is catalytically very efficient, there is no need for it to be present in such large quantities. In case Rubisco's abundance is reduced, then its role as a storage protein would be affected and may result in decreased crop yields [10]. In case abundance remains the same and catalytic efficiency also is very high then it is difficult to imagine the outcome-the rate of growth of the plant, nutrients and water utilization, biological and economic yields, etc.

C). The only point in olden days in favor of the photorespiration process was that it protects the plant from photo-inhibition under conditions of high light intensities, high temperature, water stress, etc. But now paramount importance is being attributed to photorespiration, and the reaction responsible for this attribution is the conversion of glycine into serine in mitochondria during which THF (Tetra Hydro Folate) is converted to methylene-THF [11,12]. THF one-carbon metabolism is intimately connected to so many indispensable primary metabolic networks and hence the photorespiratory process is inevitable at least to an extent in all plants [13-15]. A fact to be reflected is that in the conversion of glycine to serine, both CO_2 and NH_3 are released. However, while ammonia is apparently totally re-fixed considerable CO_2 is lost. Theoretically, it is possible that carbon dioxide and ammonia released in mitochondria during photorespiration can be combined in a carbamoyl phosphate synthase type of reaction and thereby conserve both carbon dioxide and ammonia. But no such event happened during evolution. No one seems to have tried cloning carbamoyl phosphate synthase (I and II) genes to result in mitochondria and see the outcome.

After a critical review of the speculative and partly experimented strategies mentioned above, the following additional and continuation of existing strategies are suggested to realize the above-mentioned goals.:

I. Rubisco large subunit has two CO_2 binding sites-activation sites where only CO_2 binds and substrate binding sites where O_2 competes with CO_2 and the large sub-unit cannot discriminate between the two. Hence the amino acid sequence at and around the activation site need to be studied in more depth in more plants and organisms and compare and contrast the substrate-binding sites in order to make decisions for suitable amino acid substitutions at the substrate-binding site to make it exclusively specific for CO_2.

Protein engineering of Rubisco-particularly large sub-unit-needs to be done on a large scale for deciphering the specificities. For example, in Subtilisin enzyme protein, more than 50% of the amino acids in the protein have been mutated, and several aspects of its nature and function studied [16]. Accordingly, mutations on a large scale need to be studied in Rubisco.

II. Further, it is known that the activation site is not the same in all plants but can differ. Hence, studying the activator CO_2 binding site in different plants can throw considerable light on the exclusive specificity for CO_2. Not only a large sub-unit, but even a small subunit of Rubisco can also bind to CO_2 alone [17,18] . Hence these binding sites also need to be studied to proceed as above. It is also known that a large sub-unit by itself without the need for a small subunit can catalyze the fixation of CO_2. Therefore the sequence and role of small sub-unit need to be studied critically.

III. On the extreme, there is Rubisco Like Proteins (RLP's) that do not catalyze either carboxylase or oxygenase reactions [19]. Hence their protein sequence may yield useful information regarding the binding of CO_2 and O_2, which can be utilized for the manipulation of Rubisco.

IV. Greater effort and out of the box thinking is required in understanding natural diversity in the catalytic properties of the Rubisco so as to accordingly think of modifying the amino acids in the enzyme sequence. That natural diversity is existing is a

clear indication that evolutionary changes have been taking place [19,20]. Hence Rubisco sequences have to be compared from a wide variety of organisms and from a wide variety of environments, and there is a need for more Rubisco's to be sequenced from the above cases.

Sequencing studies of various proteins reveal that not only sequences with low similarity but even sequences with as high as 90% similarity can show vast differences in catalytic activity. Therefore one needs to scan the sequences accordingly to understand the necessary amino acid modifications to be made.

In addition to, the attempts made to modify the catalytic site to avoid oxygenase activity, a novel approach of creating a fusion/hybrid protein of Rubisco i.e., Rubisco large sub-unit partly substituted by another suitabsle oligo/polypeptide sequence which can result in exclusively binding CO_2 and avoid oxygen may be attempted. Such suitable polypeptide sequences may be searched from the sequence of existing exclusive carboxylases. Making several such fusion/hybrid combinations and screening them should be attempted. Fusion proteins did evolve in nature, for example, Propionyl Co-A synthase [21]. One should be optimistic that there should be some polypeptide sequence that can discriminate between CO_2 and O_2 that can be used to make a fusion/hybrid Rubisco protein. In reality, there are enzymes that are absolutely specific to their substrate (s), and enzymes with broad specificity to substrates. Hence, theoretically, it is justified to speculate the possibility of understanding the nature of the above specificities and accordingly thinks of manipulating an enzyme towards altered/ desired specificity.

Attempts have been made to understand the mechanism of carboxylases other than Rubisco [8,22,23]. However, a fundamental difference with these carboxylases is that they are Biotin Co-factor dependent. It’s the part of the co-factor which carries the CO_2 apart from the influence of the amino acids nearby. Apart from using this information, there is a need to study a number of protein sequences of Biotin independent carboxylases to make decisions. It appears useful information of only one Biotin independent carboxylase is available.

Plant genomes possess a large and diverse array of both mono- and di-oxygenases that exclusively bind oxygen. These enzymes are also present in other organisms [24] sequence information of some oxygenases is available, and there is a further need to sequence as many mono-and di-oxygenases to arrive at a correct understanding of the oxygen-binding sites. Based on the above information strategies for due mutagenesis of the oxygen-binding/catalytic site of Rubisco to eliminate oxygenase activity can be designed. As in the case of carboxylases, there are both Co-factor dependent and independent oxygenases-Co-factor being heme-iron. However, as mentioned above, there are specific and variety of oxygenases. Similarly, there is a diverse array of oxidases which use oxygen as a substrate [25-27]. Hemoglobin and Myoglobin, both are heme iron proteins and can bind both oxygen and CO_2. Hence understanding of the above proteins with respect to oxygen and/ or CO_2 binding can throw light on the specificity of oxygen and CO_2 binding sites. Several hundred sequences of the above-mentioned enzymes put together are available in the protein sequence databases. These sequences may be analyzed for further understanding and still, the number of the above-mentioned enzymes may be sequenced from different organisms to gain insights about manipulating Rubisco.

Now the technology for chloroplast genetic engineering is developed [12]. Hence the possibility of cloning minimum required C4 genes into C3 plants may be attempted to reduce photorespiration without the need for creating a Kranz anatomy and keeping the original Rubisco cycle intact.

Feasibility of execution to fruition of the suggested strategies –as in the case of earlier speculated strategies, only time has to say, but efforts need to be made as made in the past with other speculations in view of the problem being an intellectually highly challenging one with potential for great advancements in Biochemistry and Biotechnology and for human welfare in the long run.

Concluding Remarks

With depleting natural resources and projections of the continued increase in the global population and the fears of negative impacts of climate change on agriculture, the pressure to increase food production is severe in the coming decades. Hence, both short and long-term strategies are required and recommended. No doubt, the complexity of suggested strategies is daunting but there is a dire need to enhance global food production. Hence, till the final goal is achieved all strategies need to be explored. Present understanding and future advances in molecular biology and genetic engineering and protein sequence databases coupled with the already existing information provide a reasonable basis to work on the suggested long term strategies with optimism. While pursuing the suggested strategies it is essential to understand the function of Rubisco as a storage protein; the presumed essential role of photorespiration; nature of binding sites exclusive to CO_2 and exclusive to O_2 in some enzymes/proteins and influence of neighboring regions on the proteins on the substrate binding affinities. While preliminary and short term experiments have to be/can be at any level, for realizing the needed ultimate goal, it is essential to take forward the experiments to field level and record data on plant growth parameters and yield and quality parameters to assess the outcome of the strategies pursued.

Compliance with Ethical Standards

This is a theoretical paper and has no funding. Thus no experiments and any approval by Ethics Committee.

Competing Interests

Adhikarla S Rao is the sole author, and the manuscript content has no conflicts of interest.

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