Review

Plant Biotechnology, 16(1), 33–37 (1999)

Super-RuBisCO: The Key for Improvement of Plant Photosynthesis

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Plants receive various kinds of stresses from the environment and are obliged to reduce their productivity. These stresses are often lethal. Changing the present plants to ones resistant to these stresses is important for guaranteeing the next generation the food and the environment. One of the most severe factors that influence the productivity is the shortage of water for growth. Under water limitation, plants close the stomata and the excess light energy captured on the thylakoid membranes is directed to formation of active oxygens. Our previous efforts successfully rendered a model plant, tobacco, to be active oxygen-resistant. However, the active oxygen-resistant tobacco could not grow at all under water deficit because of closure of the stomata. The plant to be created must fix CO_2 for growth even if the stomata is almost closed and the rate of the flow of CO_2 from the atmosphere into the leaf is strongly reduced. Our knowledge in plant physiology tells us plants can fix CO_2 even under water deficit if the active site of the CO_2-fixing enzyme RuBisCO strongly discreminates against dioxygen. The present plant RuBisCO fixes one molecule of dioxygen for every 3 CO_2 fixations. We have found that RuBisCO from *Galdieria partita*, an acidic, thermophilic red alga, discreminates against dioxygen 3 times more strongly than the plant enzyme. The affinity of the *Galdieria* enzyme for CO_2 was 2 times higher than the plant enzyme. Physiological calculations tell that the *Galdieria* enzyme can render tobacco to fix CO_2 at the range of the CO_2 concentration where the natural plants cannot if the genes are successfully introduced into tobacco.

1. Intruduction

The present urgent, world-wide issue is the global warming caused by the increase in the atmospheric concentration of CO_2. The concentration is deduced to increase 2-fold in the next one century and the global atmospheric temperature would increase 2 to 3 degrees higher than the presnt value. Under the estimated circumstances, the next generation of people will loose the land for cultivation and the ecosystem that supports their lives will be changed. It should be essential for the present scientists to devise technology for relieve our ecosystem from these crises.

Photosynthetic organisms have contributed to adsorption of the atmospheric CO_2 for over the last 4 billion years. The enourmous economic and social acitivities of human beings, however, are releasing CO_2 at a much higher rate than that of CO_2 fixation by photosyntheis of plants. This is causing our ecosystem to be polluted and damaged. The deforestration in the tropical regions promotes the disruction of the ecosystem. One of the most plausible approach to halt the ecosytem distruction would be increasing the land area for plantation and greening these areas and regions for sequestration of the atmospheric CO_2 into plants. This may be done by changing our crop plants to have habitats to live under severe conditions.

Plants that can grow on poor, arid lands may be created by improving their physiology in growth perofor-mances. Photosynthesis converts high energy photons to chemical energies in chloroplasts. The photosynthetic carbon reduction (PCR) cycle utilizes the energies for reduction of CO_2 incorporated by RuBisCO from the atmonsphere (**Fig. 1**). This review mentions the importance of the maintenance of the energy balance between photon energy trapping and its utilization and discuss how we are able to fortify the catapcity of plants to balance them in arid lands. The target is RuBisCO.

2. Photon utilization and photodamages in photosynthesis

Balacing the rate of the conversion of photon energy into chemical energies such as NADPH and ATP and those of the inflow of CO_2 from the atmosphere and of the reduction of the CO_2 fixed is the critical point for land plants. If the rate of the energy utilization is lowered by the decreased inflow of CO_2 through the stomata, chloroplants are obliged to direct the energies to photorespiration and reduction of oxygen molecule to superoxide radicals (O_2^-) [1]. O_2^- is dismutated to hydrogen peroxide by superoxide dismutase. Hydrogen peroxide is a potent oxidant of the some PCR enzymes, which lose their acitivies by the oxidation of the fuctional vicinal sulfhydryl groups.

Plants have a machinery to decompose hydrogen peroxide in chloroplasts [1]. The enzyme functioning for the decomposition is ascorbate peroxidase (APX). In this reaction, ascorbate is oxidized to monodehydro-

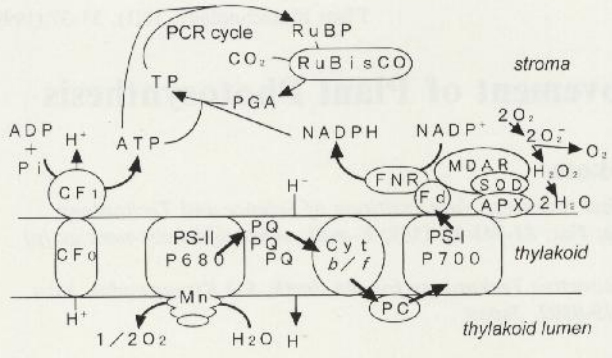

Fig. 1 Overall reactions in photosynthesis. PS, photosystem; PQ, plastoquinon; Cyt, cytochrome; Fd, ferredoxin; FNR, ferredoxin: $NADP^+$ reductase; MDAR, monodehydroascorbate reductase; SOD, superoxide dismutase; APX, ascorbate peroxidase; RuBP, ribulose 1,5-bisphosphate; PGA, 3-phosphoglycerate; TP, triose phosphate; CF, coupling factor.

ascorbate, which is then re-reduced by ferredoxin or monodehydroascorbate reductase. In other words, plant photosynthesis depends on the acitve oxygen-scavenging system to prevent if from oxidative damages. However, APX cannot always decompose hydrohgen peroxide effectively. The stability of the enzyme is severely dependent on the presence of the reductant ascorbate. When obliged to react with hydrogen peroxide in the absence of or in the presence of low concentrations of ascorbate, APX is easily decomposed through the adduct between APX and hydrogen peroxide. This is a phenomenon that the enzyme encounters when chloroplasts cannot use the chemical energies efficiently. This happens when water use by plants is limited in the presence of full sunlight.

To improve this situation in plant photosynthesis, we introduced a bacterial catalase into tobacco chloroplasts with aid of *Agrobacterium*. The transformation vector was designed for the catalase to target chloroplasts. Several transformed tobacco lines were recovered with high catalase acitivities. Although plant cells have an endogenous high activity of catalase in peroxisomes, the activities in some tarnsformants exceeded 2 to 3-fold that of the wild type.

The growth of the transformants in the green house were quite similar to that of the wild plants. Both wild and transformed tobacco were grown for 5 weeks with enough watering in the green house and then transferred to a high light condition (1,500 μmol photons m^{-2} s^{-1}) (**Fig. 2**). The plants did not receive no water after the transfer. Wild tobacco revealed chlorosis 48 hours after the start of the treatment, and chlorosis was spreaded over the leaves after 72 hours. In contrast, there was seen no visible phenomenon in the transformant T43-1 during the treatment. Figure 3 shows physiological and biochemical changes of both types of tobacco after 48 hours of the treatment.

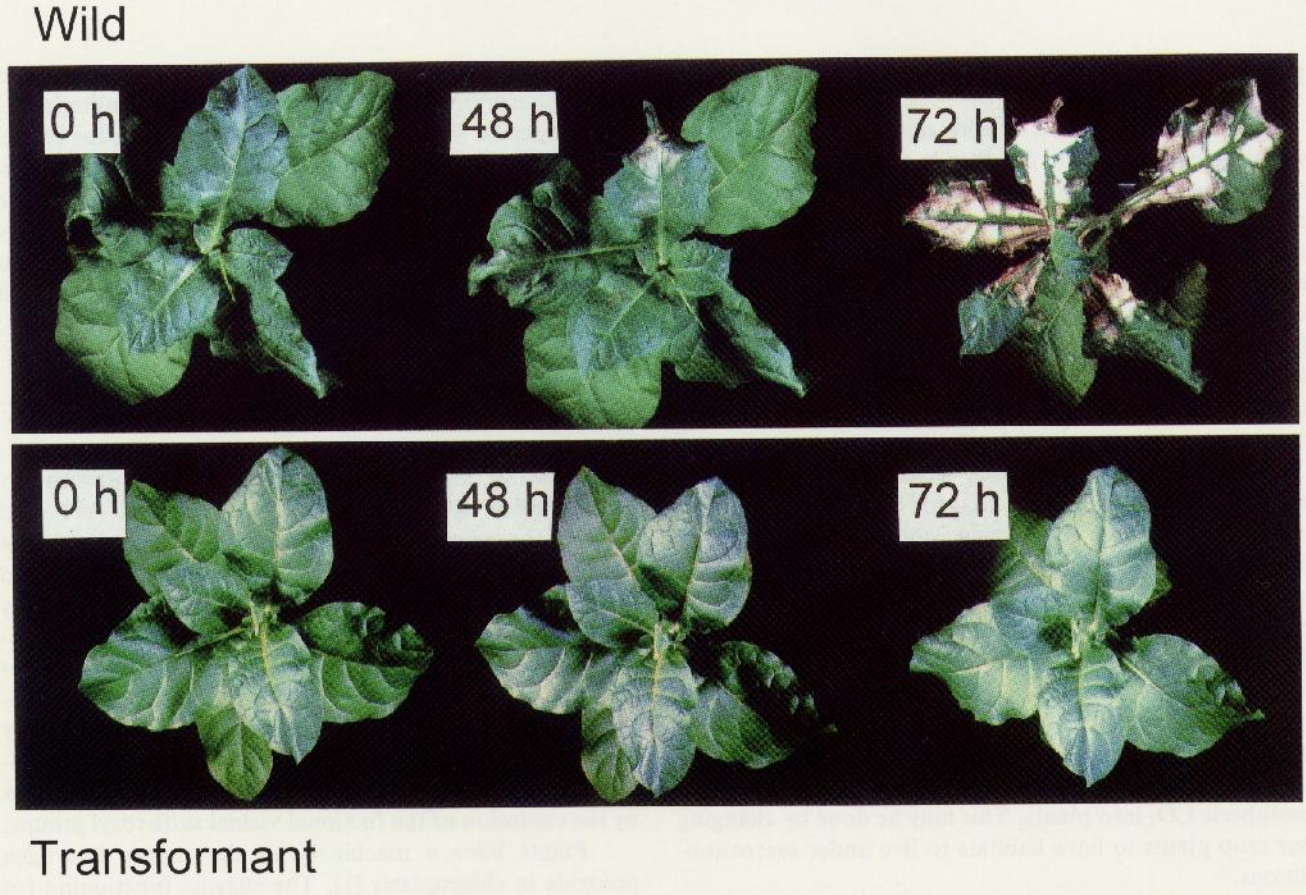

Fig. 2 Drought-stress responses of wild tobacco and the transformant having bacterial catalase in chloroplasts. See the text for details.

Photosynthetic CO_2 fixation was decreased to 10% of the control received no drought treatment (**Fig. 3**). Similar large decreases were also encountered for the activities of phosphoribulokinase and other photosynthesic enzymes. On the contrary, the CO_2 fixation and photosynthetic enzymes were still active after 48-hour treatment in the transformant with bacterial catalse in chloroplasts. The only exception was the activity of chloroplast APX; no activity was detected even in the transformant. The observed strong difference in the sensititvity to drought between APX and phosphoribulokinase implies that APX is much more sensitive to hydrogen peroxide than the PCR key enzyme *in vivo*.

In conclusion, the introduced catalase can be substituted for endogenous APX in tobacco chloroplasts. Since catalase itself is not inactivated by hydrogen peroxide, the enzyme may have functioned to decompose the active oxygen effectively under the severe drought conditions used here.

3. Photosynthetic CO_2 fixation and RuBisCO

The above study clearly shows that it is possible to improve the endogenous active oxygen-scavenging system by introducing bacterial catalase into plant chloroplasts. However, one should not ignore the fact that the transformants can be alive for longer periods without any growth. This kind of approach in creating arid-philic plants would not be the goal for changing the present plants into ones that can sequestrate the atmospheric CO_2 by growing on unused, deforestrated and arid lands. The plants we should seek for will be ones that are still productive in photosynthesis under these growth conditions. A plausible target for this purpose is RuBisCO [4].

The CO_2-fixation step catalyzed by RuBisCO in photosynthesis is the important rate-limiting step. The control coefficient of the enzyme in photosynthesis is over 0.5 in the presence of full sunlight. This fact tells us that improving the enzymatic efficiency is a meaningful direction for improvements of plant water-use efficiency and crop productiviy.

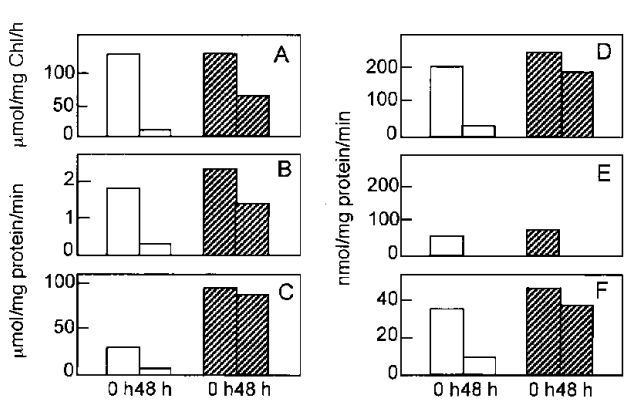

Fig. 3 Effects of drought stress on photosynthetic activities in wild tobacco (open bars) and the transformant (shaded bars). Experimental conditions are detailed in the text. A, photosynthetic CO_2 gas exchange, B, ribulose 5-phosphate kinase; C, catalase; D, cytosolic APX; E, plastid APX; F, glutathione reductase.

RuBisCO, even of higher land plants, has several disadvantages as an enzyme [4]. The reaction turnover rate is up to 3/sec/reaction site; 1/100 to 1/1,000 of enzymes found in nature. The affinity of the enzyme for CO_2 is 10 to 15 μM; just a quater of the enzyme in chloroplasts can participate in photosynthesis. Much worse is occurrence of the unavoidable oxygenase reaction. Plant RuBisCO well adapted to the present oxygenic atmosphere still fixes O_2 once for every 2 to 3 CO_2 fixations in chloroplasts. A part of the reaction product is oxidized to CO_2 in the subsequent glycolate pathway. Totally, the oxygenase reaction reduces the productivity of crop plants up to 60%.

We have been trying to improve the enzyme based on the molecular and biochemical mechanisms of the evolution and adaptation of the enzyme to the present atmosphere after the appearance of the enzyme in the nature. Information on the structure-function relationship of the RuBisCO evolution will be highly expected to give us various approaches useful for the improvement of the enzyme. Particularly, removing the oxygenase reaction will render plants to be resistant to drought and the plant with this RuBisCO will show the significant net CO_2 fixation in photosynthesis under drought conditions [4].

The biphasic reaction course, fallover, of carboxylation catalysed by RuBisCO has been known as a characteristic of the enzyme from higher land plants [5]. Fallover consists of hysteresis in the reaction seen during the initial several minutes and a subsequent, very slow suicide inhibition by inhibitors formed from the substrate ribulose-1,5-bisphosphate (RuBP) [6]. This study examined the relationship between occurrence of fallover, the putative hysteresis-inducible sites (Lys-21 and Lys-305 of the large subunit in spinach RuBisCO), and the relative specificity in the carboxylase and oxygenase reactions amongst RuBisCOs of a wide variety of photosynthetic organisms. Figure 4 shows the relationship between occurrence of fallover, amino acid residues at the hysteresis-inducible sites and the relative specificity of RuBisCOs [7].

The phylogenetic tree for the evolution of the gene for the large subunits of RuBisCO, *rbc*L, has been well accepted [8, 9]. Occurrence of fallover and the hysteresis-inducible sites well followed the sequence of the adaptation of photosynthetic organisms to the terrestrial habitat or the increase in the relative specificity of RuBisCO.

From this line of our studies, we expected that introduction of the hysteresis-inducible sites into the photosynthetic bacterial (γ) enzyme would give rise to an increase in the relative specificity of the bacterial enzyme. However, this was not the case. The mutant *Chromatium vinosum* RuBisCO having lysine residues at R21 and P305 showed fallover, but its relative specificity was very similar to that of the wild enzyme [10].

Another interesting point is occurence of the hysteresis-inducible sites in β-purple bacteria and non-green algae (**Fig. 4**). The relative specificity of the non-green algae was much higher than that of higher C3-plants. Interestingly, red algae are dividied into two groups in the phylogenetic tree of *rbc*L. The group including *Porphiridium* and *Porphyra* live at moderate temperatures in

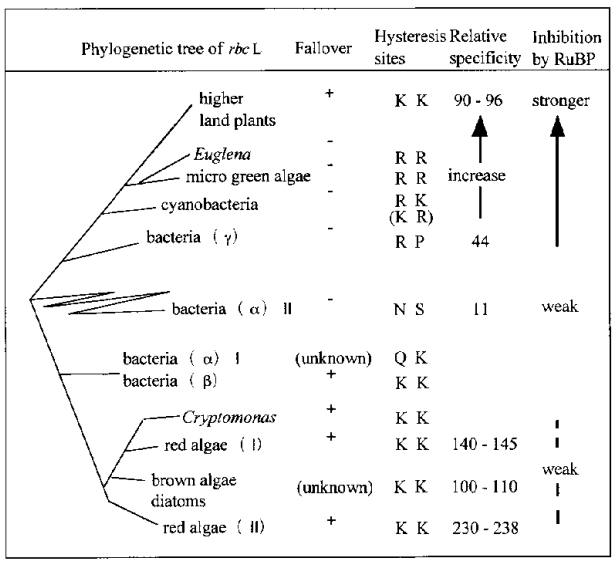

Fig. 4 Evolutionary grouping of structures and functions of RuBisCO among photosynthetic organisms. Amino acid residues of the hysteresis sites are abbreviated to one character names.

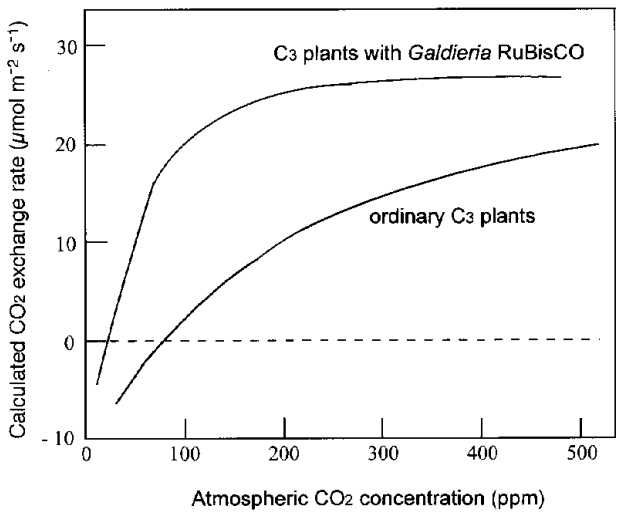

Fig. 6 Calculation of the photosynthetic CO_2 gas exchange rates of ordinary C3-plants and of realizable C3-plants in which *Galdieria* RuBisCO is functioning in place of the original enzyme. Calculations were done using the kinetic parameters for spinach and *Galdieria* RuBisCOs [11] and equations for photosynthetic gas exchange [12].

the presence of salts. The other group contains *Cyanidium* and *Galdieria*, which grow at higher temperatures. The relative specificity of RuBisCOs from the latter group were the extremes of RuBisCOs examined so far [11]. The higher specificity for CO_2 fixation in these RuBisCOs was partly due to their higher affinities for CO_2 (6.6 μM) and partly to an higher activation energy in the oxygenation reaction (28.6 kcal mol^{-1}) **(Fig. 5)**.

Figure 6 shows the A/Ci curve for the ordinary C3-plants, calculated by the equations of Farquhar and von Caemmerer [12] with the reported kinetic values for plant RuBisCO. The CO_2 compensation point is 50 ppm intercellular CO_2 and the CO_2 fixation shifts from the RuBisCO-limiting phase to the RuBP-regeneration-limiting phase at 170 ppm CO_2. If RuBisCO is substituted for

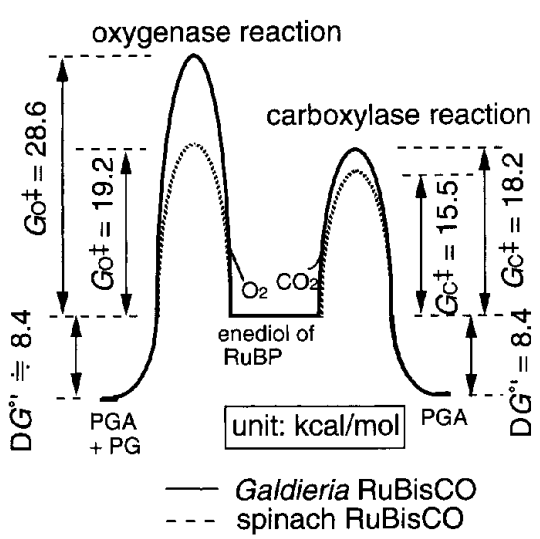

Fig. 5 Activation energies of carboxylase and oxygenase reactions of spinach and *Galdieria* RuBisCO.

plant RuBisCO, the realizable transgenic plants will have the CO_2 compensation point at 16 ppm CO_2. The phase transition will occurr around 70 ppm CO_2. This predicts that the introduced *Galdieria* enzyme will utilyze the photosynthetic chemical energies efficiently even in the presence of low concentrations of CO_2.

These considerations teaches us that changing the enzymatic properties of RuBisCO of C3-plants is the meaningful direction for improvement of plant productivity [4]. Particularly, increasing the relative specificity and the affinity for CO_2 of RuBisCO is the meaningful direction in plant biotechnology.

Acknowledgements

This work was partly supported by PEC/MITI of Japan.

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