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
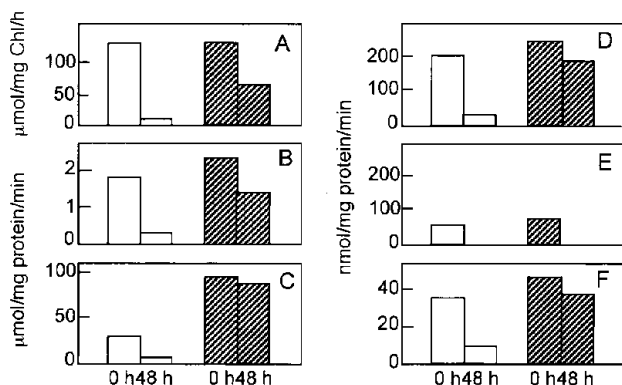

Fig. 3 Effects of drought stress on photosynthetic activities in wild tobacco (open bars) and the transformant (shaded bars). Experimental conditions are detailed in the text. A, photosynthetic CO_2 gas exchange, B, ribulose 5-phosphate kinase; C, catalase; D, cytosolic APX; E, plastid APX; F, glutathione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

crop productiviy.

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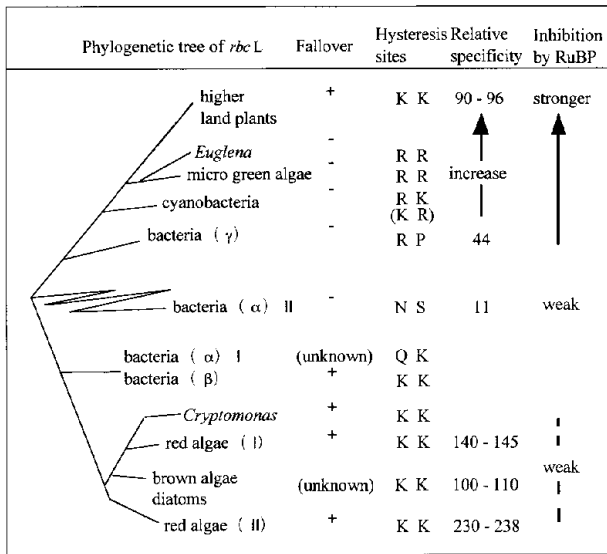
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Fig. 4 Evolutionary grouping of structures and functions of RuBisCO among photosynthetic organisms. Amino acid residues of the hysteresis sites are abbreviated to one character names.

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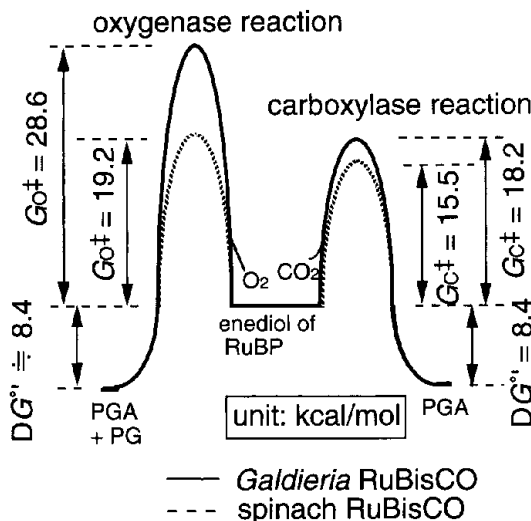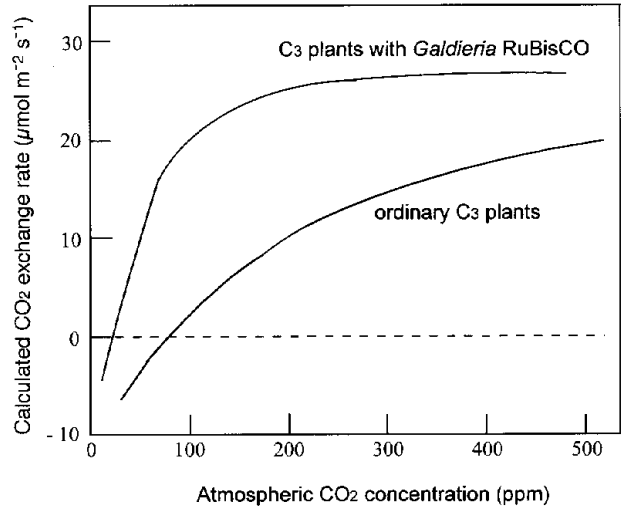
plant RuBisCO, the realizable transgenic plants will have the CO_2 compensation point at 16 ppm CO_2. The phase transition will occurr around 70 ppm CO_2. This predicts that the introduced *Galdieria* enzyme will utilyze the photosynthetic chemical energies efficiently even in the presence of low concentrations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

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