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Interaction of Hydrogen Peroxide with Ribulose-1,5-bisphosphate Carboxylase/Oxygenase from Rice

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Abstract—The properties of rice-derived ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco) in different concentrations of hydrogen peroxide (H_2O_2) solutions have been studied. The results indicate that at low H_2O_2 concentrations (0.2-10 mM), the properties of rubisco (e.g., carboxylase activities, structure, and susceptibility to heat denaturation) change slightly. However, at higher H_2O_2 concentrations (10-200 mM), rubisco undergoes an unfolding process, including the loss of secondary and tertiary structure, forming extended hydrophobic interface, and leading to cross-links between large subunits. High concentrations of H_2O_2 can also result in an increase in susceptibility of rubisco to heat denaturation. Further pre-treatments with or without reductive reagents to rubisco show that the disulfide bonds in rubisco help to protect the enzyme from damage by H_2O_2 as well as other reactive oxygen species.

Key words: rubisco, hydrogen peroxide, unfolding, inactivation

Ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco, EC 4.1.1.39) plays an essential role in CO_2 fixation during photosynthesis and photooxidation of photorespiration in plants [1, 2]. It catalyzes the initial reaction in the Calvin cycle, the carboxylation of ribulose-1,5-bisphosphate (RuBP) to form two molecules of 3-phosphoglycerate. The physiological role of rubisco and its regulatory factors has been well discussed and documented [3-5]. Rubisco has a molecular weight (MW) close to 550,000 and is composed of eight identical large subunits (L, MW ≈ 56,000) and eight identical small subunits (S, MW ≈ 12,500), and therefore it has the general formula of L_8S_8 [6]. The folding and unfolding of rubisco under different conditions have been studied extensively [7-14]. Many investigations only concerned its conformational changes when the enzyme binds to its substrate RuBP or is activated by its reactive enzyme–carbamate–magnesium (ECM) complex using the approaches of chemical modification [12], fluorescence [13], or difference spectrum [14]. In our previous work, we have studied SDS-induced conformational changes and inactivation of rice rubisco. It showed that even at low SDS concentrations rubisco completely lost its carboxylase activity and most of the thiol groups were exposed, while at higher SDS concentrations dissociation of small subunits and significant conformational changes occurred [15].

Hydrogen peroxide (H_2O_2) is one of the most stable reactive oxygen species in plants. Modifications of proteins by reactive oxygen species usually include amino acid alterations, increased hydrophobic interactions, partial denaturation (unfolding), and covalent cross-linking [16]. Protein oxidation is also related to increased proteolytic susceptibility as has now been reported by several laboratories [17, 18]. Although the oxidative effect of H_2O_2 is quite weak, it is considered to be an important oxidant in plant cells. H_2O_2 is mainly produced in chloroplasts by the electron transfer to O_2 from photosystem I (P700). The excess H_2O_2 can be eliminated by the scavenger system in higher plants [19]. During leaf senescence, H_2O_2 accumulates rapidly in chloroplasts to a high

Abbreviations: ANS) 1-anilino-8-naphthalenesulfonate; DTT) dithiothreitol; RuBP) ribulose-1,5-bisphosphate; DTNB) 5,5′-dithiobis-(2-nitrobenzoic acid).

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content as the photosynthetic functions of plants decline sharply [20]. Moreover, it was reported that H_2O_2 acts as signal of cell apoptosis, which also suggests H_2O_2 playing an important role in decay of plant photosynthetic functions [21]. Because H_2O_2 is kinetically stable and electrically neutral, it is able to pass through cell membranes and reach cell locations remote from its formation site [22]. Therefore, the effects of H_2O_2 on rubisco are of physiological significance. Here we characterize the inactivation, unfolding, and changes in susceptibility to heat denaturation of rubisco which was treated with different concentrations of H_2O_2. The results show that at low concentrations (0.2-10 mM), H_2O_2 had little impact on rubisco. However, at higher concentrations, H_2O_2 greatly influenced the carboxylase activity, the structure, and the susceptibility to heat denaturation of rubisco.

MATERIALS AND METHODS

Chemicals. 3-Phosphoglyceric phosphokinase, glyceraldehyde-3-phosphate dehydrogenase, ribulose-1,5-bisphosphate (RuBP), catalase, and 1-anilino-8-naphthalenesulfonate (ANS) were purchased from Sigma (USA); dithiothreitol (DTT) was a product of Promega (USA); 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) was from Serva (USA). Other reagents were local products of analytical grade.

Purification of rubisco from rice and determination of its carboxylase activity. The purification of rubisco was as described [15]. The purified rubisco was pooled and judged to be homogeneous on the basis of SDS-PAGE and native PAGE. The purified rubisco molecules were treated with Tris-HCl buffer containing different H_2O_2 concentrations for 6 h. Catalase was added into the reaction solution to the final concentration of 5 μg/ml to remove the remaining H_2O_2. The carboxylase activity was determined spectrophotometrically at 25°C in an assay medium containing 100 mM Tris-HCl (pH 8.0), 1 mM EDTA, 10 mM $MgCl_2$, 5 mM DTT, 70 mM $NaHCO_3$, 10 mM ATP, 0.4 mM NADH, 0.4 mM RuBP, 3-phosphoglyceric phosphokinase (8 units/ml), and glyceraldehyde-3-phosphate dehydrogenase (5 units/ml). The total volume of the assay mixture was 600 μl. Rubisco was preincubated with assay medium without RuBP before activity assay. The reaction was initiated by the addition of RuBP, and the activity was calculated from the decrease of A_{340} [23]. The enzyme concentration was determined using Coomassie Brilliant Blue G-250 dye with BSA as the standard protein [24].

Determination of the number of reactive thiol groups. The number of DTNB-reactive thiol groups was determined spectrophotometrically at 25°C by measuring the absorbance at 412 nm with the molecular extinction coefficient $\varepsilon = 13{,}600\ M^{-1}\cdot cm^{-1}$ after incubation with DTNB for at least 1 h [25].

Gel electrophoresis of rubisco pretreated with H_2O_2. Rubisco samples were incubated with different H_2O_2 concentrations for 6 h and then catalase was added to remove the remaining H_2O_2. Samples were then boiled with the sample application buffer with or without β-mercaptoethanol and fractionated by SDS-PAGE. The electrophoresis was carried out in gels consisting of 3.75% polyacrylamide for the stacking gel and 12% polyacrylamide for the separating gel for 1 h at a constant voltage of 160 V using a Bio-Rad Mini-PROTEAN cell (USA). The same samples were used for non-denaturing PAGE consisting of 7.5% polyacrylamide for the separating gel. All gels were stained with Coomassie Brilliant Blue R-250.

Spectrometry for monitoring structural changes in rubisco. Fluorescence emission spectra were measured using a Hitachi 850 (Japan) spectrofluorimeter. The excitation wavelength was 280 nm. The ANS fluorescence emission spectra were excited by light with wavelength of 380 nm. The molecular ratio of ANS to rubisco was 125 : 1. Circular dichroism (CD) spectra were recorded on a Jasco 715 circular dichroism spectropolarimeter. All measurements were carried out in 50 mM Tris-HCl buffer (pH 8.0) at 25°C.

RESULTS

Structural changes of rubisco molecules in H_2O_2 solutions. The intrinsic fluorescence emission spectra of rubisco in different H_2O_2 concentrations are shown in Fig. 1. The fluorescence emission spectrum of the native enzyme has a peak at 337 nm. With increasing H_2O_2 concentrations, the fluorescence emission intensity decreased and the emission peak red-shifted. The emission peak red shifted to 345 nm and the fluorescence intensity decreased to a minimum value in 400 mM H_2O_2.

The fluorescence emission of ANS is known to increase when the dye binds to the hydrophobic regions of a protein [26]. Figure 2 shows the fluorescence emission spectra of ANS bound to rubisco in the absence of H_2O_2 and in the presence of different H_2O_2 concentrations. The fluorescence emission peak of ANS bound by native rubisco blue-shifted from 540 to 485 nm. Increasing of H_2O_2 concentrations caused the fluorescence emission intensity of rubisco-bound ANS to increase with a blue-shift of the emission peak. The fluorescence emission intensity of rubisco-bound ANS decreased slightly but was still much stronger than that of ANS bound by native rubisco as the H_2O_2 concentration was increased up to 200 mM. Control experiment (Fig. 2) showed that H_2O_2 had little effect on the fluorescence properties of ANS within the concentration range of H_2O_2 from 0 to 200 mM as used in the present investigation.

Changes in the secondary structures of rubisco during denaturation were also studied using far-UV CD

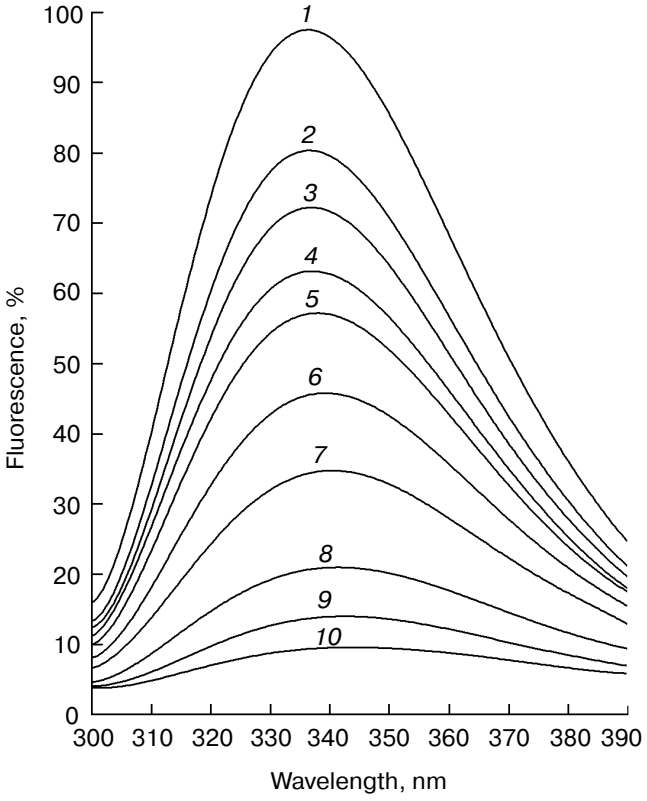

Fig. 1. Fluorescence emission spectra of rice rubisco in H_2O_2 solutions. Rubisco was dissolved in 50 mM Tris-HCl, pH 7.8, containing H_2O_2 at different concentrations. The solution was incubated for 6 h at 25°C in the dark before fluorescence measurements with excitation wavelength of 280 nm. The final rubisco concentration was 0.5 μM. The H_2O_2 concentrations for the curves *1-10* were 0, 10, 20, 30, 40, 60, 100, 200, 300, and 400 mM, respectively.

spectra. Figure 3 shows the CD spectra of the enzyme at different H_2O_2 concentrations. The CD spectra for the denatured enzyme show that as the H_2O_2 concentration increased, the average molecular ellipticity decreased in magnitude. These results indicate that the presence of H_2O_2 greatly affected the secondary structures of rubisco.

DTNB is thought to react only with exposed protein thiol groups (-SH) and is used to detect the number of thiol groups on a protein surface. The results of Ellman's reaction show that the thiol groups of rubisco were oxidized by H_2O_2. The number of the exposed thiol groups decreased from approximately 30 to 21 at H_2O_2 concentration of 5 mM and remained at this value as the H_2O_2 concentration increased from 5 to 20 mM. In the higher H_2O_2 concentration range (≥20 mM), increasing of H_2O_2 concentration caused the number of the exposed thiol groups to be markedly decreased to the minimum value (all exposed thiol groups oxidized by H_2O_2) at 100 mM H_2O_2 (see Fig. 7).

The SDS-PAGE results presented in Figs. 4a and 4b show the influences of H_2O_2 on the structure of rubisco. The data in Fig. 4a show that H_2O_2 did not cause detectable degradation of the large and small subunits of rubisco. The elimination of β-mercaptoethanol from a parallel set of samples was expected to maintain the thiol groups in an oxidized configuration and thus enable cross-linked proteins to migrate more slowly under non-reducing but denaturing conditions (Fig. 4b). The data in Fig. 4b show a protein band on the top of the separating gel in H_2O_2-treated rubisco samples run under non-reducing conditions, the appearance of which occurred concomitant with the disappearance of some large subunits at ~56 kD. These results indicate that large subunits in the enzyme are cross-linked via disulfide bonds when H_2O_2 is present at high concentrations. However, the concentration of the small subunits of rubisco appeared not to change under the same conditions. The non-denaturing PAGE gel presented in Fig. 4c shows that rubisco aggregated when treated with H_2O_2 at high concentrations (40-200 mM). The aggregates were too large to enter the stacking gel (3.75% polyacrylamide).

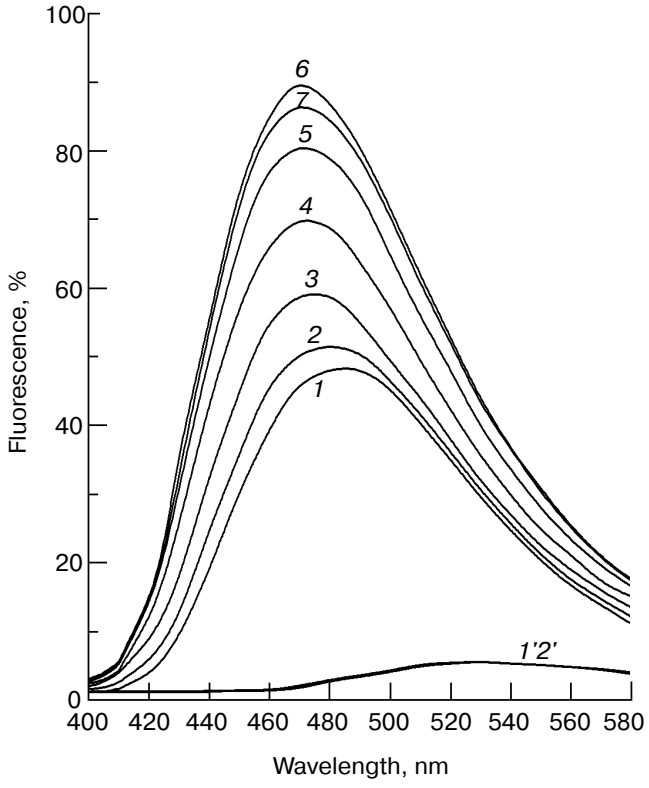

Fig. 2. ANS binding characteristics of rice rubisco in H_2O_2 solutions. Rubisco samples were incubated with H_2O_2 for 6 h at 25°C in the dark before ANS fluorescence measurements with excitation wavelength of 380 nm. The final concentrations of rubisco and ANS were 0.75 and 30 μM, respectively. The H_2O_2 concentrations for curves *1′* and *2′* (in the absence of rubisco) were 0 and 200 mM; the H_2O_2 concentrations for curves *1-7* (in the presence of rubisco) were 0, 10, 20, 40, 60, 100, and 200 mM, respectively.

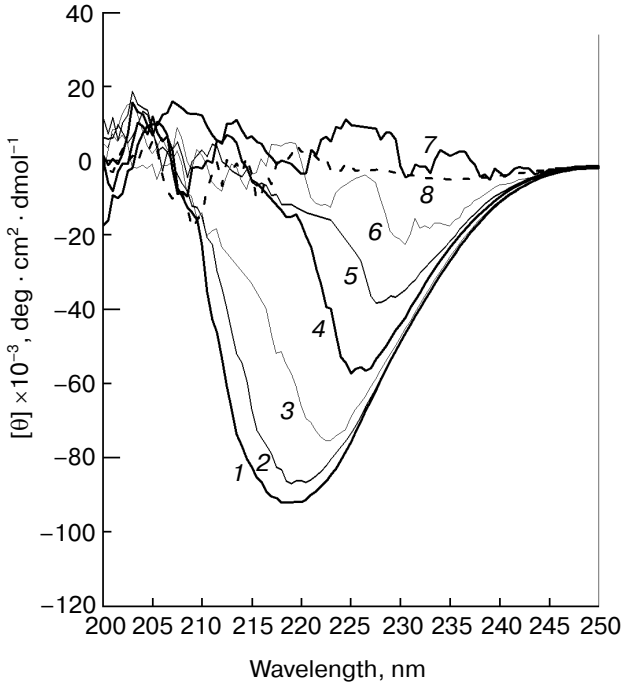

Fig. 3. Far-ultraviolet CD spectra of rice rubisco in H_2O_2 solutions of different concentrations. Rubisco samples were incubated with H_2O_2 for 6 h at 25°C in the dark before CD spectra measurements. The final rubisco concentration was 1.0 μM. The H_2O_2 concentrations for curves *1-7* were 0, 10, 20, 40, 60, 100, and 200 mM, respectively. Curve *8* represents the CD spectrum of rubisco denatured in 6 M guanidine hydrochloride for 6 h.

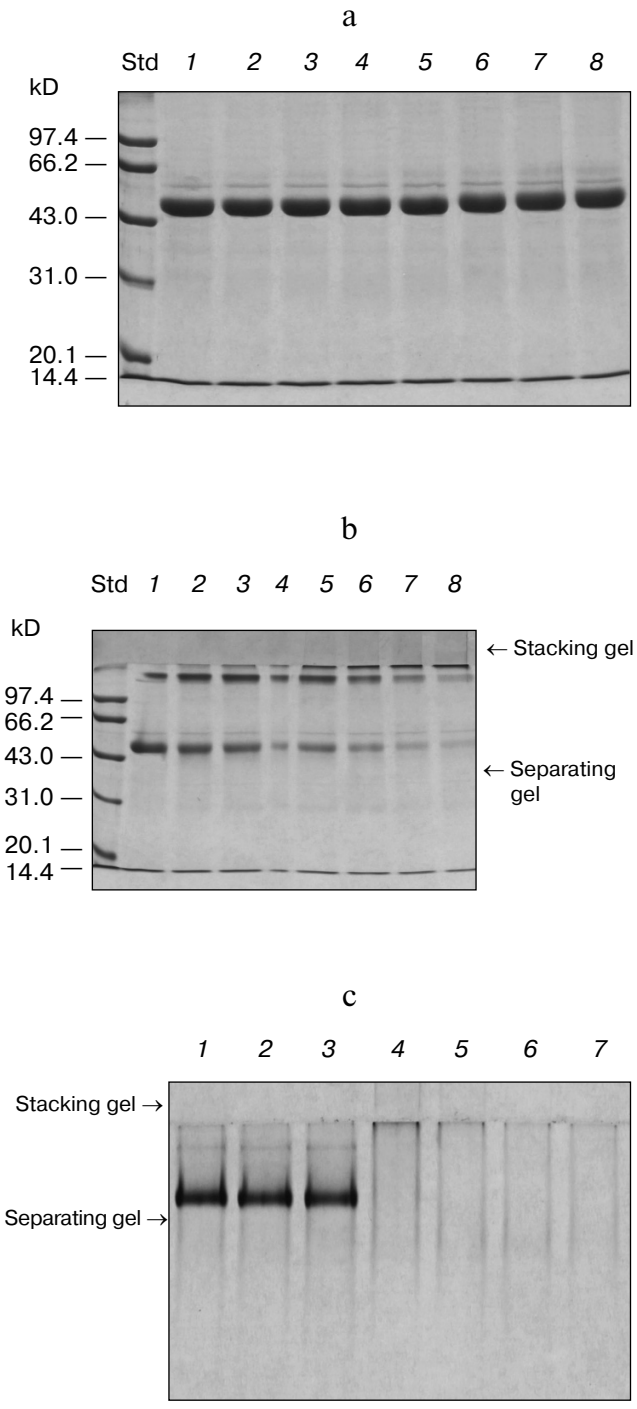

Fig. 4. Electrophoresis patterns for rice rubisco denatured in various H_2O_2 concentrations. a) Reductive SDS-PAGE pattern for rice rubisco denatured in H_2O_2. The H_2O_2 concentrations used for denaturation were 0, 10, 20, 30, 40, 60, 80, and 100 mM for lanes *1-8*. b) Non-reducing SDS-PAGE pattern for rice rubisco denatured in H_2O_2. The H_2O_2 concentrations used for denaturation were 0, 10, 20, 30, 40, 60, 80, and 100 mM for lanes *1-8*. c) Non-denaturing PAGE pattern for rice rubisco denatured in H_2O_2. The H_2O_2 concentrations used for denaturation were 0, 10, 20, 40, 60, 100, and 200 mM for lanes *1-7*.

Changes in susceptibility to heat denaturation of rubisco in H_2O_2 solutions. The changes of susceptibility to heat denaturation of rubisco molecules when treated with different H_2O_2 concentrations were investigated by measuring the content of stable rubisco at 60°C (Fig. 5). The results show that low H_2O_2 concentrations (0-20 mM) did not apparently change the susceptibility of rubisco to heat denaturation. However, higher H_2O_2 concentrations (20-200 mM) increased the heat susceptibility of rubisco so that it was more easily denatured and aggregated by heat treatment.

Inactivation of carboxylase activity of rubisco in H_2O_2 solutions. The extent of inactivation of rubisco carboxylase activity in solutions with different H_2O_2 concentrations is shown in Fig. 6. The data show that at low H_2O_2 concentrations the carboxylase activity changed slightly when increasing the H_2O_2 concentration up to 10 mM. Further increases in the H_2O_2 concentration caused the carboxylase activity of rubisco to decrease sharply. The rubisco carboxylase activity was completely lost when the H_2O_2 concentration was at 120 mM.

The extent of inactivation of carboxylase activity in different H_2O_2 concentrations was compared between non-pretreated rubisco samples and samples pretreated

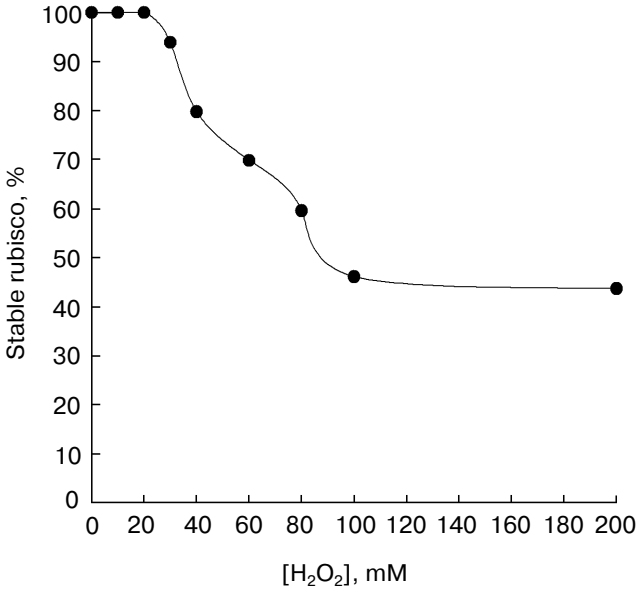

Fig. 5. Increased susceptibility to heat denaturation of H_2O_2-treated rubisco. Rubisco samples were treated with H_2O_2 for 6 h at 25°C in the dark. After removal of the remaining H_2O_2, one aliquot of rubisco (at each H_2O_2 concentration) was incubated at 60°C while another was incubated at 25°C. After 2-h incubation, all samples were centrifuged at 3000 rpm for 15 min. The concentration of soluble rubisco remaining in the supernatant was determined with the Coomassie Brilliant Blue G-250 reagent. Stable rubisco percentage was determined by rubisco solubility as follows: stable rubisco percentage = $C_2/C_1 \cdot 100$ (C_1 represents the concentration of soluble rubisco after incubation at 25°C, C_2 represents the concentration of soluble rubisco after incubation at 60°C).

with reductive reagents (10 mM DTT or 20 mM β-mercaptoethanol) (Fig. 6). The results show that rubisco samples pretreated with reducing reagents lost their carboxylase activity more easily than non-pretreated samples in H_2O_2 solutions.

DISCUSSION

The interactions of H_2O_2 with rubisco have been studied previously, but all the studies only concentrated on the inhibitory kinetics of the carboxylase activity and the oxygenase activity, in which H_2O_2 was only used as a kind of inhibitor [27, 28]. Comprehensive studies of the influence of H_2O_2 on the structure and function of rubisco have not been reported.

The present investigation has explored the structural changes of rubisco treated with different H_2O_2 concentrations by means of fluorescence spectroscopy, circular dichroism spectroscopy, Ellman's reaction, and electrophoresis. The results show that low H_2O_2 concentrations (0.2-10 mM) had little effect on the structure of rubisco. H_2O_2 mainly changed the surface characteristics of rubisco including oxidation of some reactive thiol groups and formation of more hydrophobic surface.

Higher H_2O_2 concentrations (10-200 mM) significantly changed the structure of rubisco. The fluorescence results show that the tertiary structure of rubisco was impaired and more aromatic amino acid residues were exposed to the polar solvent (Fig. 1). The ANS fluorescence results indicate the exposure of hydrophobic residues such as tryptophan (Fig. 2). The CD results show that the secondary structures of rubisco were destroyed in the presence of H_2O_2 (Fig. 3). High H_2O_2 concentrations can also induce the aggregation of rubisco molecules. The non-reducing SDS-PAGE results show that the cross-linking via disulfide bonds between the large subunits of rubisco molecules could be one of the reasons for its aggregation. However, since the treatment with H_2O_2 usually leads to the increasing of the hydrophobicity of rubisco, which was indicated by the ANS fluorescence experiment (Fig. 2), another possible reason might be that H_2O_2 promoted the formation of unspecific hydrophobic interactions between rubisco molecules.

At high H_2O_2 concentrations (20-200 mM), the resistance of rubisco to heat denaturation decreased. This can be explained by the partial unfolding and increased exposure of hydrophobic moieties. The increased non-electrostatic interactions between exposed nonpolar amino acid side chains and the aqueous environment

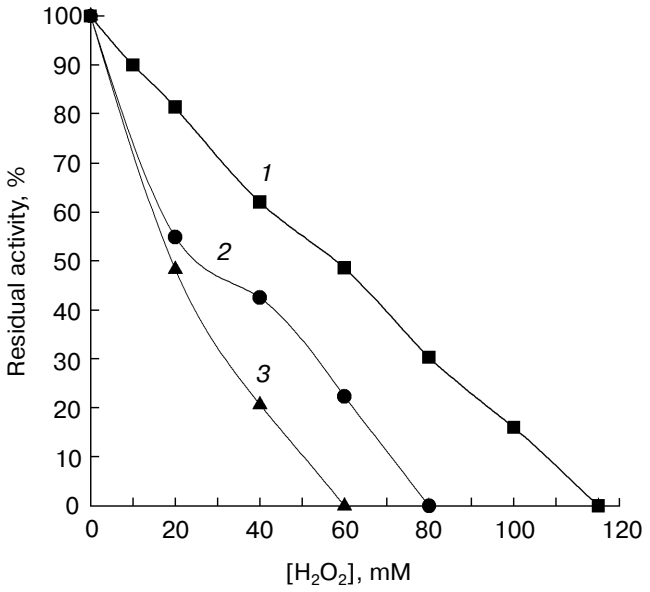

Fig. 6. Inactivation of rice rubisco in H_2O_2 solutions of different concentrations. Rubisco samples without pretreatment (*1*), pre-treated by 10 mM DTT (*2*), and pre-treated with 20 mM β-mercaptoethanol (*3*) were incubated with H_2O_2 for 6 h at 25°C in the dark. Catalase was added to remove the remaining H_2O_2 before carboxylase activity was measured. The final rubisco concentration was 0.5 μM.

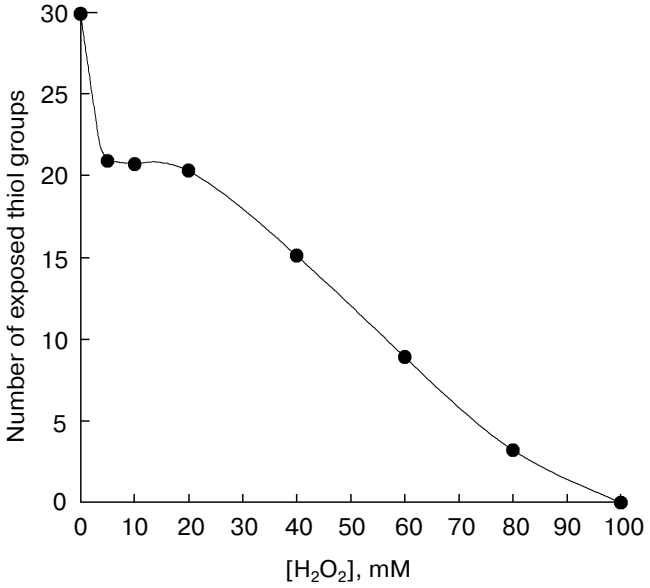

Fig. 7. Changes in number of exposed thiol groups of rice rubisco in H_2O_2 solutions of different concentrations. Rubisco samples were incubated with H_2O_2 for 6 h at 25°C in the dark. Samples were then incubated with DTNB for 1 h and the number of DTNB-reactive thiol groups was determined spectrophotometrically by measuring the absorbance at 412 nm. The concentration of rubisco was 0.5 μM. The molecular ratio of DTNB to rubisco was 6250 : 1.

would promote the instability and precipitation of rubisco at high temperatures.

Low H_2O_2 concentrations (0.2-10 mM) had little effects on the carboxylase activity of rubisco, which however decreased dramatically at higher H_2O_2 concentrations (20-200 mM). It is excluded that H_2O_2 could act as an inhibitor to the carboxylase because catalase was used to remove the residual H_2O_2 before carboxylase activity was measured. Another interpretation is that inactivation of the enzyme was partially caused by its structural changes in H_2O_2 solutions. This could be one but not the main reason because no significant structural changes have been observed in the same H_2O_2 concentration range (10-120 mM). H_2O_2 and other reactive oxygen species have usually been found to be able to modify the chemically reactive groups (such as thiol groups) of amino acid residues. The redox state of Cys247 in large subunit was thought to determine the sensitivity of rubisco in plants (*Spirodela oligorrhiza* and *Triticum aestivum* L.) to inactivation and cross-linking caused by oxidative stress [29]. The highly conserved residue Cys172, which is adjacent to the active site, was found by Marcus et al. to play a role in redox regulation of rubisco activity [30]. We think the main reason for inactivation of rubisco is oxidative modification of the reactive thiol groups that exist near the active site of rubisco and are essential for its carboxylase activity.

We also found that rubisco molecules lost their carboxylase activity more easily when pretreated with reductive reagents. It was already known that disulfide bonds do exist between large subunits in rubisco of higher plants [31]. The existence of one protein band at ~110 kD in the non-reducing SDS-PAGE (Fig. 4b) indicates that there exist disulfide bonds between large subunits in the rice-derived rubisco we studied. It has been reported that reduction of disulfide bonds with DTT caused increase in thermal inactivation for rubisco from the green alga *Chlamydomonas reinhardtii* [32]. We think reducing reagents can break the disulfide bonds between large subunits and this leads to the increasing susceptibility of rubisco to the inactivation by H_2O_2. Therefore, the existence of disulfide bonds in the rubisco oligomer can help to protect the enzyme from damage by H_2O_2 or other reactive oxygen species.

REFERENCES

1. Jensen, R. G., and Bahr, J. T. (1977) *Ann. Rev. Plant Physiol.*, **28**, 379-400.
2. Hartman, F. C., and Harpel, M. R. (1994) *Ann. Rev. Biochem.*, **63**, 197-234.
3. Miziorko, H. M., and Lorimer, G. H. (1983) *Ann. Rev. Biochem.*, **52**, 507-535.
4. Portis, A. R. (1992) *Ann. Rev. Plant Physiol.*, **43**, 415-437.
5. Jordan, D. B., and Chollet, R. (1983) *J. Biol. Chem.*, **258**, 13751-13758.
6. Libouga, D. G., Aguie-Beghin, V., and Douillard, R. (1996) *Int. J. Biol. Macromol.*, **19**, 271-277.
7. Viitanen, P. V., Lubben, T. H., Reed, J., Goloubinoff, P., O'Keefe, D. P., and Lorimer, G. H. (1990) *Biochemistry*, **29**, 5665-5671.
8. Gatenby, A. A., and Ellis, R. J. (1990) *Annu. Rev. Cell Biol.*, **6**, 125-149.
9. Van der Vies, S. M., Viitanen, P. V., Gatenby, A. A., Lorimer, G. H., and Jaenicke, R. (1992) *Biochemistry*, **31**, 3635-3644.
10. Eckardt, N. A., and Pell, E. J. (1995) *Plant Phys. Biochem.*, **33**, 273-282.
11. Jiang, R. F., Wang, Z. X., and Xu, G. J. (1997) *Biochim. Biophys. Acta*, **1343**, 95-101.
12. Schloss, J. V., and Hartman, F. C. (1977) *Biochem. Biophys. Res. Commun.*, **77**, 230-238.
13. Chollet, R., and Anderson, L. L. (1977) *Biochim. Biophys. Acta*, **482**, 228-240.
14. Kwok, S. Y., and Wildman, S. G. (1974) *Arch. Biochem. Biophys.*, **161**, 354-359.
15. Lu, W., Li, S., Li, G. F., Gong, Y. D., Zhao, N. M., Zhang, R. X., and Zhou, H. M. (2002) *Biochemistry* (Moscow), **67**, 940-944.
16. Giulivi, C., Pacifici, R. E., and Davies, K. J. A. (1994) *Arch. Biochem. Biophys.*, **311**, 329-341.
17. Pacifici, R. E., Kono, Y., and Davies, K. J. A. (1993) *J. Biol. Chem.*, **268**, 15405-15411.
18. Cervera, J., and Levine, R. L. (1988) *FASEB J.*, **2**, 2591-2595.

19. Brennan, T., Rychter, A., and Frenkel, C. (1979) *Bot. Gaz.*, **140**, 384-388.
20. Ferguson, I. B., Watkins, C., and Harman, J. (1983) *Plant Physiol.*, **71**, 182-186.
21. O'Brien, I. E. W., Baguley, B. C., Murray, B. G., Morris, B. A. M., and Ferguson, I. B. (1998) *Plant J.*, **13**, 803-814.
22. Woitaszek, P. (1997) *Biochem. J.*, **332**, 681-692.
23. Lorimer, G. H., Andrews, T. J., and Tolbert, N. E. (1975) *Meth. Enzymol.*, **42**, 484-487.
24. Bradford, M. M. (1976) *Analyt. Biochem.*, **72**, 248-262.
25. Ellman, G. L. (1959) *Arch. Biochem. Biophys.*, **82**, 70-77.
26. Stryer, L. (1965) *J. Mol. Biol.*, **13**, 482-495.
27. Badger, M. R., Andrews, T. J., Canvin, D. T., and Lorimer, G. H. (1980) *J. Biol. Chem.*, **255**, 7870-7875.
28. Edmondson, D. L., Badger, M. R., and Andrews, T. J. (1990) *Plant Physiol.*, **93**, 1376-1382.
29. Mehta, R. A., Fawcett, T. W., Porath, D., and Mattoo, A. K. (1992) *J. Biol. Chem.*, **267**, 2810-2816.
30. Marcus, Y., Altman-Gueta, H., Finkler, A., and Gurevitz, M. (2003) *J. Bacteriol.*, **185**, 1509-1517.
31. Knight, S., Andersson, I., and Branden, C. I. (1990) *J. Mol. Biol.*, **215**, 113-160.
32. Moreno, J., and Spreitzer, R. J. (1999) *J. Biol. Chem.*, **274**, 26789-26793.