FEBS Letters 571 (2004) 124–128 FEBS 28619

Oxygen-dependent H_2O_2 production by Rubisco ☆

Kangmin Kim[a], Archie R. Portis Jr.[a,b,*]

[a] United States Department of Agriculture, Agricultural Research Service, Photosynthesis Research Unit, USA
[b] Department of Crop Sciences, University of Illinois, 190 ERML, 1201 W Gregory Ave., Urbana, IL 61801, USA

Received 12 May 2004; accepted 16 June 2004

Available online 6 July 2004

Edited by Richard Cogdell

Abstract Oxygen and ribulose-1,5-bisphosphate dependent, H_2O_2 production was observed with several wild type Rubisco enzymes using a sensitive assay. H_2O_2 and D-*glycero*-2,3-pentodiulose-1,5-bisphosphate, a known and potent inhibitor of Rubisco activity, are predicted products arising from elimination of H_2O_2 from a peroxyketone intermediate, specific to oxygenase activity. Parallel assays using varying CO_2 and O_2 concentrations revealed that the partitioning to H_2O_2 during O_2 consumption by spinach Rubisco was constant at 1/260–1/270. High temperature (38 °C), which reduces Rubisco specificity for CO_2 versus O_2, increased the rates of H_2O_2 production and O_2 consumption, resulting in a small increase in partitioning to H_2O_2 (1/210). Two Rubiscos with lower specificity than spinach exhibited greater partitioning to H_2O_2 during catalysis: *Chlamydomonas reinhardtii* (1/200); and *Rhodospirillum rubrum* (1/150).

Keywords: Oxidation; Carbon metabolism; Photosynthesis

1. Introduction

Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) catalyzes both photosynthetic carbon fixation and photorespiratory oxygen incorporation in plants. In addition to these primary reactions, Rubisco can also catalyze other side reactions by misprotonation, rearrangement, or elimination of various enzyme-bound intermediates [1,2]. The catalytic activity of many Rubiscos also exhibits a slow decline in activity in vitro, often called 'fallover' [3]. Several products arising from the side reactions, like D-xylulose-1,5-bisphosphate (XuBP), 3′-ketoarabinitol-1,5-bisphosphate (KABP), D-*glycero*-2,3-pentodiulose-1,5-bisphosphate (PDBP), and 2′-carboxytetritol-1,5-bisphosphate are obvious causative compounds in the slow decline in activity because they may be slow to dissociate from the active sites after their formation. Indeed Rubisco activase, which facilitates the release of a wide variety of sugar phosphates from Rubisco, can eliminate 'fallover' during Rubisco assays [4,5].

XuBP, formed by epimeric reprotonation of the enediol formed from D-ribulose-1,5-bisphosphate (RuBP), has been observed to be produced once every 400–600 carboxylation turnovers [6,7] and a strong inhibitory effect of purified XuBP on Rubisco also has been observed [7,8]. However, the contribution of XuBP to the slow inactivation of Rubisco is still uncertain because XuBP can be slowly carboxylated and shows more favorable binding to uncarbamylated Rubisco form [7,9–11]. Also the use of aldolase to rapidly remove any XuBP resulted in no alteration in the kinetics of 'fallover' [3] and when XuBP formation was suppressed by increasing CO_2 and O_2, a substantial loss of Rubisco activity still occurred [11].

KABP was also reported to accumulate during Rubisco catalysis, remaining tightly bound to Rubisco active sites and exhibiting oxygen-dependent formation [6,11]. However, it is likely that the compound identified in these reports was actually PDBP, based on the method and conditions used for identification [12].

The E48Q mutant of *Rhodospirillum rubrum* Rubisco [12] produces PDBP as a major product under aerobic conditions with a rapid decline in its activity, whereas the wild-type enzyme does not. Using this mutant, it was shown that H_2O_2 was produced in nearly equal amounts, demonstrating that PDBP arises from the elimination of H_2O_2 from a peroxyketone intermediate, specific to reaction with O_2. Nevertheless, a significant role for PDBP in the decline of Rubisco activity with wild-type plant Rubiscos during catalysis, which is suggested by a reevaluation of the reports detecting KABP formation, has remained questionable due to several observations. PDBP can accumulate in stored RuBP preparations as a result of non-enzymatic oxidation and thereby result in considerable variation in Rubisco 'fallover' assays [13]. This being the case, a parallel non-enzymatic oxidation of RuBP is difficult to exclude during the long term assays required to kinetically analyze the 'fallover' process. Partially purified PDBP obtained by chemical oxidation of RuBP with Cu^{2+}, was a very potent inhibitor of Rubisco, but it also is unstable under many conditions [7,13]. This makes its isolation and quantitative recovery from Rubisco reactions an uncertain task. Based on these reports, the previous determinations of PDBP (as KABP) may not represent a actual production of this compound during catalysis in vitro and the degree to which wild-type

☆ Mention of a trademark, proprietary product or vendor does not constitute a guarantee or warranty of the product by the US Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

* Corresponding author. Fax: +1-217-244-4419.
E-mail address: arportis@uiuc.edu (A.R. Portis Jr.).

Abbreviations: KABP, 3′-ketoarabinitol-1,5-bisphosphate; PDBP, D-*glycero*-2,3-pentodiulose-1,5-bisphosphate; Rubisco, ribulose-1,5-bisphosphate carboxylase/oxygenase; RuBP, D-ribulose-1,5-bisphosphate; XuBP, D-xylulose-1,5-bisphosphate

0014-5793/$22.00 © 2004 Published by Elsevier B.V. on behalf of the Federation of European Biochemical Societies.
doi:10.1016/j.febslet.2004.06.064

Rubisco enzymes might produce PDBP and the other product, H_2O_2, has remained unknown.

In this report, using a sensitive assay we demonstrate the production of H_2O_2, and by inference PDBP, during catalysis in real time by various Rubiscos and thereby determine the partitioning ratio between the production of H_2O_2 and normal oxygenation. The implications of this side reaction for understanding the decline in Rubisco activity during assay are discussed.

2. Materials and methods

2.1. Materials

Scopoletin, horse radish peroxidase type VI-A (EC 1.11.1.7.) and carbonic anhydrase (EC 4.2.1.1.) were purchased from Sigma (St. Louis, MO, USA). *N*-Acetyl-3,7-dihydroxyphenoxazine (Amplex Red) was obtained from Molecular Probes (Eugene, OR, USA). RuBP was prepared with minor modifications of the method described in [13]. Rubisco was purified from spinach (*Spinacia oleracea* L.) and *Chlamydomonas reinhardtii*, as reported previously [14], and *R. rubrum* rubisco was a kind gift from M. Chatfield and W.L. Ogren.

2.2. Oxygenase activity assay

Oxygen depletion by the oxygenase activity of Rubisco was measured with a Hansatech D.W. oxygen electrode unit (Norfolk, England). To attain the desired O_2 concentrations, the reaction mixtures were sparged with various gas mixtures (i.e., N_2, 50% and 100% O_2) except for the ambient oxygen level (21%). O_2 consumption and H_2O_2 production were monitored in parallel 0.5 mL reaction mixtures containing 100 mM Tricine–NaOH (pH 8), 20 mM $MgCl_2$, 40 mM KCl, 25 U carbonic anhydrase, 50 μM scopoletin, 10 U peroxidase. Dithiothreitol was excluded from reaction mixture due to its ability to decrease scopoletin fluorescence. After O_2 in reaction mixture reached the desired concentration, half of the initial 1 mL volume was moved to the fluorescence cuvette (for H_2O_2 assay). Then, the reaction chamber was sealed and various concentrations of $NaHCO_3$ were added using a syringe. Catalysis was initiated by addition of Rubisco (250 μg/mL) and RuBP (5 mM) and O_2 depletion was monitored for about 10 min. Data were acquired with a PowerLab interface to a computer (ADInstruments, Grand Junction, CO, USA).

2.3. H_2O_2 assay

Hydrogen peroxide formation during Rubisco catalysis was detected by the decrease in scopoletin fluorescence due to peroxidase catalyzed oxidation by H_2O_2 [15]. The response to the addition of small amounts of H_2O_2 showed that there was sufficient peroxidase activity to permit a real time assay of H_2O_2 production. Fluorescence was monitored using a LS-5 fluorescence spectrophotometer (Perkin–Elmer, Boston, MA) and data acquired with a PowerLab (ADInstruments, Grand Junction, CO) interface to a computer. Excitation and emission wavelengths were 366 and 460 nm, respectively. The decrease in scopoletin fluorescence was converted to H_2O_2 based on standard curves obtained using the reaction mixture. After the transfer from the O_2 electrode chamber (described above), the reaction mixtures were flushed with same gas mixture for an additional 5 min. Rubisco catalysis was initiated in the same way as described above.

3. Results

3.1. Detection of H_2O_2 production and mechanism for the formation of H_2O_2 and PDBP during Rubisco catalysis

Due to its high specificity and sensitivity, scopoletin has been used to assess H_2O_2 production by various systems [15]. To assess the reliability of detection, we also compared the scopoletin assay with a similar Amplex Red H_2O_2 detection method [16]. Standard curves for H_2O_2 from 0 to 20 μM using each method were linear and similar to each other (data not shown). No fluorescence change in the absence of Rubisco, peroxidase, or RuBP in the reaction mixture indicated that the assay system is specific for H_2O_2 production by Rubisco catalytic activity. A time-dependent decrease in scopoletin fluorescence (or increase with Amplex Red, not shown) was easily observed when all three were present. The calculated amounts of H_2O_2 produced under varied CO_2 concentrations were similar with the two detection methods (data not shown).

The enediol of RuBP forms a peroxyketone intermediate when O_2 reacts instead of CO_2 during Rubisco catalysis (Fig. 1). This intermediate is then normally hydrated and cleaved to 3-phosphoglycerate and phosphoglycolate. However, as clearly demonstrated with the E48Q *R. rubrum* mutant [12], the peroxyketone can also eliminate H_2O_2 and be converted to PDBP. In some cases with mutant enzymes, PDBP has been observed to undergo subsequent rearrangement to carboxytetritol bisphosphate [17]. Based on current knowledge, an accurate measurement of H_2O_2 production during Rubisco catalysis would directly reflect the de novo formation of PDBP.

3.2. Low CO_2 and high O_2 increased both the oxygenase activity and the production of H_2O_2 by Rubisco

To determine the oxygen and carbon dioxide dependence of H_2O_2 production, real-time H_2O_2 production was measured using the scopoletin assay with various O_2 and CO_2 concentrations. Simultaneously, O_2 consumption was measured in parallel by using an oxygen electrode. Upon increasing the O_2 levels from anaerobic conditions to 100%, both H_2O_2 production (Fig. 2A) and O_2 consumption (Fig. 2B) were increased as a function of time. In presence of 10 mM $NaHCO_3$, H_2O_2 production after 5 min with 50% O_2 (1860 pmol/mg) and 100% O_2 (2590 pmol/mg) increased 2.0- and 2.9-fold compared to that at the ambient O_2 level (890 pmol/mg), respectively.

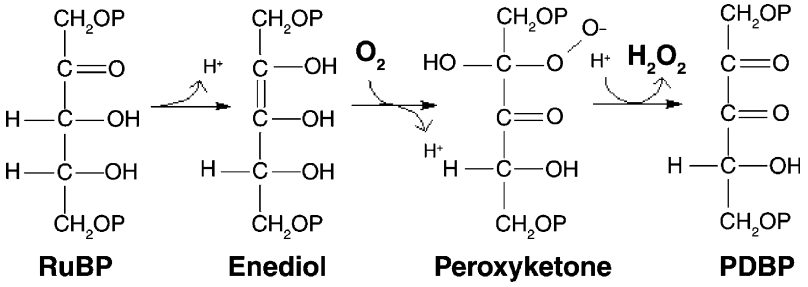

Fig. 1. Pathway for the formation of H_2O_2 and pentodiulose-1,5-bisphosphate during Rubisco catalysis with oxygen (adapted from [12]).

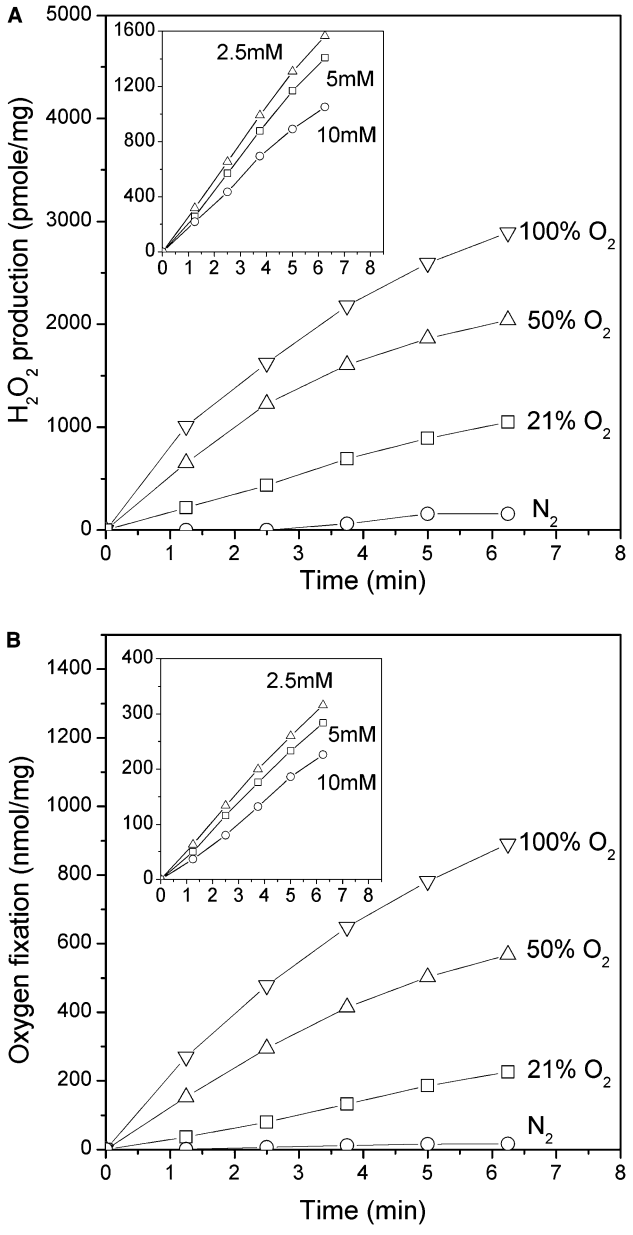

Fig. 2. Production of H_2O_2 and oxygen consumption during spinach Rubisco catalysis. (A) Production of hydrogen peroxide as detected by scopoletin oxidation during the Rubisco catalysis. (B) Oxygen consumption measured by an oxygen electrode during Rubisco catalysis. Fully activated Rubisco was used for the assays. H_2O_2 production was calculated from fluorescence changes using standard curves. 10 mM of $NaHCO_3$ was added after reaching the desired O_2 level, followed by initiation of the reaction by adding Rubisco and RuBP. Insets show the results with lower $NaHCO_3$ concentrations and 21% O_2.

Similarly, O_2 consumption after 5 min with 50% O_2 (503 nmol/mg) and 100% O_2 (782 nmol/mg) were 2.7 and 4.2 times more than with 21% O_2 (186 nmol/mg) and 10 mM $NaHCO_3$. H_2O_2 production and O_2 consumption were also increased by decreasing the bicarbonate (CO_2) concentration, but to a lesser extent (Fig. 2, inset). Under anaerobic conditions (N_2-flushing), the production of H_2O_2 and O_2 consumption was very low. To facilitate accurate measurements of O_2 consumption, a high Rubisco concentration was used with substantial depletion of the oxygen substrate. Thus O_2 consumption and H_2O_2 production in these experiments probably decreased with time at a rate faster than that expected from 'fallover'. We did not attempt to kinetically analyze 'fallover' using the real-time capacity of the H_2O_2 assay with lower Rubisco concentrations, but this may be possible with a more sensitive spectrofluorometer.

Although the initial rates of H_2O_2 production and oxygen consumption varied widely in these experiments, there was a linear correlation between them (Fig. 3). The slope in this figure indicates that partitioning of the peroxyketone intermediate in the oxygenase reaction pathway is constant at a value of 1 H_2O_2 (and PDBP) being produced for every 270 reactions of RuBP with O_2 at 25 °C. The lack of an effect of CO_2 on the partitioning ratio is consistent with H_2O_2 being produced only after the initial reaction of RuBP with O_2 and in competition with the normal production of phosphoglycerate and phosphoglycolate as end products.

3.3. Effect of higher temperature on oxygen consumption and H_2O_2 production

Temperature increases the catalytic activity of Rubisco with both CO_2 and O_2 as substrates, but it does so unequally and the specificity value (relative reaction rate) decreases from about 88 at 25 °C to 64 at 35 °C with spinach Rubisco [18]. It was therefore of interest to investigate the effect of temperature on H_2O_2 production by the oxygenase activity of spinach Rubisco. At 38 °C, both oxygen consumption and H_2O_2 production increased (not shown) and the relative rate of H_2O_2 production to oxygenase activity slightly increased. Using both CO_2 and O_2 concentration to vary the rates, the partitioning

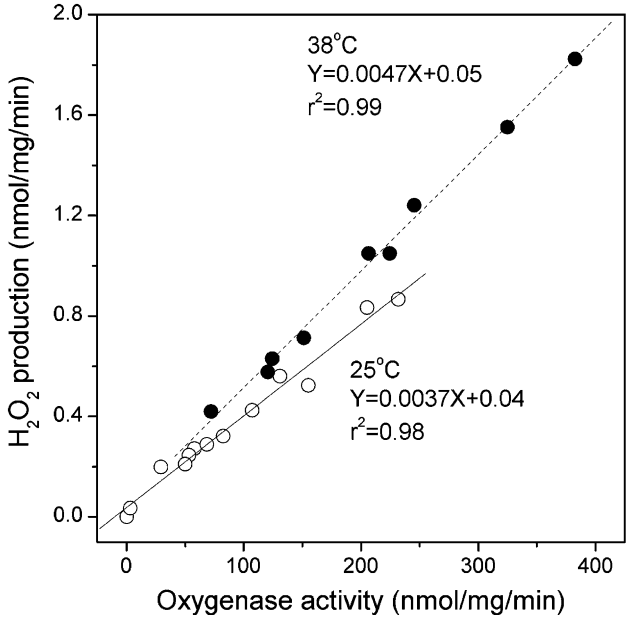

Fig. 3. Correlation between H_2O_2 production and oxygenase activity of spinach Rubisco measured at 25 and 38 °C. H_2O_2 production and oxygen consumption after the first 1 min with various O_2 (0–50%) and CO_2 (2.5–10 mM of $NaHCO_3$) concentrations are plotted. Temperature in the oxygen electrode and spectrophotometer cell was controlled by a water bath and monitored simultaneously. Linear regressions are shown and the difference was significant at 99% confidence level ($t = 7.25$, $P < 0.001$) by Student's t-test.

ratio at this temperature was one H_2O_2 per every 210 reactions with O_2 (Fig. 3).

The declines in the rate of H_2O_2 production and the oxygenase activity at this temperature also occurred faster than at 25 °C (data not shown). As mentioned earlier, due to the use of a high amount of Rubisco and depletion of oxygen in the assays, measurement of the 'fallover' rate was not attempted.

3.4. R. rubrum *and* C. reinhardtii *Rubisco produce more* H_2O_2 *than spinach Rubisco*

Rubisco enzymes isolated from cyanobacteria and green algae have higher specific activities, discriminate less between reaction with CO_2 versus O_2 and usually exhibit much less 'fallover' compared to higher plant species [19–22]. To investigate the relationship between these differences and the ability to produce H_2O_2 during the oxygenase reaction, we determined the relative partitioning to H_2O_2 by Rubiscos isolated from *R. rubrum*, *C. reinhardtii*, and spinach at 25 °C (Fig. 4). Initial oxygenase activity of both *R. rubrum* (187 nmol/mg/min) and *C. reinhardtii* (55 nmol/mg/min) Rubisco measured with 10 mM $NaHCO_3$ were higher than those of spinach Rubisco (43 nmol/mg/min). Similarly, *R. rubrum* (1.3 nmol/mg/min) and *C. reinhardtii* (0.36 nmol/mg/min) Rubiscos generated more H_2O_2 than spinach Rubisco. Using various O_2 and CO_2 concentrations, the partitioning between H_2O_2 production and oxygenase activity were significantly different among three Rubiscos. The stoichiometry between H_2O_2 production and oxygen consumption for *R. rubrum*, *C. reinhardtii*, and spinach Rubiscos were 1/150, 1/200, and 1/260, respectively. Our data clearly demonstrate that *R. rubrum* and *C. reinhardtii* Rubisco produce more H_2O_2 (and thus PDBP) than spinach, even though H_2O_2 formation was not detectable with *R. rubrum* wild-type Rubisco using a different assay in a previous report [17].

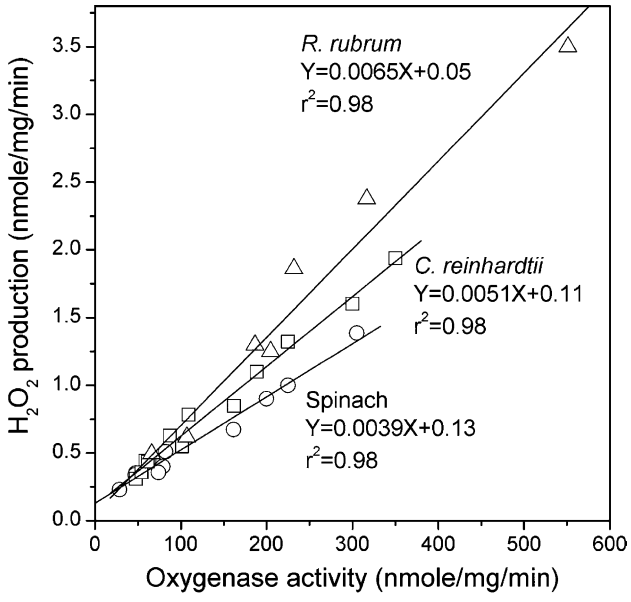

Fig. 4. Correlation between H_2O_2 production and oxygenase activity by spinach, *R. rubrum*, and *C. reinhardtii* Rubisco enzymes at 25 °C. Each Rubisco was fully activated by addition of 10 mM Mg^{2+} and 10 mM $NaHCO_3$ (66 mM for *R. rubrum*). H_2O_2 production and oxygen consumption after the first 1 min under various O_2 (0–50%) and CO_2 (2.5–10 mM of $NaHCO_3$) concentrations are plotted. Linear regressions are shown and the slopes for *R. rubrum* ($t = 4.84$, $P < 0.001$) and *C. reinhardtii* ($t = 4.01$, $P < 0.001$) enzymes were significantly different from the spinach enzyme at 99% confidence level by Student's *t*-test.

4. Discussion

An accelerated rate of decline in the activity of Rubisco caused by oxygen, which is associated with the formation of a tight-binding inhibitor identified as KABP, was reported by Zhu et al. [11]. Their proposed mechanism for the oxygen dependence of KABP formation involves a re-release of O_2 after formation of a superoxide radical anion in a radical pair, a process that would seem unlikely. The observations [12] that PDBP can be misidentified as KABP and that PDBP production with the E48Q *R. rubrum* mutant is O_2 dependent, provides an alternative explanation. However, PDBP can be found in RuBP preparations at levels similar to those expected to be produced in 'fallover' assays [13]. Thus demonstrating that it is produced catalytically in an oxygen-dependent manner by a wild-type plant enzyme has been problematic. We took advantage of recently developed sensitive assays for H_2O_2 and the observation that H_2O_2 is produced along with PDBP in an oxygen-dependent reaction by the mutant *R. rubrum* enzyme to demonstrate that H_2O_2 is also produced by several wild-type enzymes in an oxygen-dependent manner during catalysis (Figs. 2–4). While H_2O_2 production almost certainly serves as a sensitive assay for PDBP production, the subsequent fate of this compound in the active sites of Rubisco or in solution remains to be determined.

Assays conducted with varying CO_2 and O_2 concentrations demonstrated that H_2O_2 production (and by inference PDBP) is directly correlated with O_2 consumption at a ratios varying from 1/150 (*R. rubrum*) to 1/270 (spinach). The spinach value is comparable to the rate of formation of XuBP by the spinach enzyme (1/400–600 carboxylations). However, the formation of XuBP, which proceeds from the enediol intermediate, is expected to be in competition with reaction with CO_2 or O_2. XuBP formation is suppressed by high CO_2 and O_2 [11], but a detailed analysis of the CO_2 and O_2 dependence of XuBP production has not been reported. Nevertheless, with the current estimates for partitioning to the two side reactions and a ratio of carboxylation to oxygenation of about 3.5/1 at atmospheric levels of CO_2 and O_2 the relative rates of XuBP and PDBP production would be about 2/1. Therefore, both compounds have the potential to play a major role in the 'fallover' process, but their potential to cause a decline in Rubisco activity also depends on how fast they are released or undergo further processing. Considerable progress in further understanding the interactions of XuBP with Rubisco has recently been reported [7] and similar studies are needed for PDBP. It should be possible to assay both XuBP and H_2O_2 production in parallel, which will facilitate such studies and provide a better understanding of the relative roles of these compounds in the 'fallover' process.

Partitioning to H_2O_2 (PDBP) production by spinach Rubisco was increased to 1/210 at a higher temperature (38 °C), which also decreases the partitioning (specificity value) of the preceding enediol between CO_2 and O_2 from 88 at 25 °C to <64 at 38 °C [18]. Increased partitioning to H_2O_2 with two other enzymes, *R. rubrum* and *C. reinhardtii*, exhibiting less

specificity than spinach, was also observed. These similar trends suggest that structural changes in the active site that decrease specificity for reaction with CO_2 also result in a decreased ability to partition the peroxyketone intermediate towards the normal oxygenase products.

The decline in Rubisco activity during catalysis is also increased by temperature [23]. It is likely that the increased oxygenase activity and partitioning to PDBP formation contribute significantly to the increased rates of 'fallover' observed at higher temperatures. However, detailed kinetic studies of both the 'fallover' process and the rates of XuBP and PDBP formation at high temperature will be required to completely understand the effects of temperature on the 'fallover' process. Such knowledge will contribute to a better understanding of the effects of temperature on photosynthesis in planta.

Although they exhibit much less 'fallover' in their catalytic activities, *R. rubrum* and *C. reinhardtii* Rubisco produced more H_2O_2 (and thus PDBP) than spinach (Fig. 4). Therefore, Rubisco 'fallover' may not only be due to PDBP production itself during catalysis, but also the tightness of the binding of PDBP in the active sites of Rubisco and/or the ability of Rubisco to process PDBP further (i.e., cleavage or rearrangement). Previously, it was shown that, *R. rubrum* Rubisco converts PDBP to carboxytetritol bisphosphate [17,24] and the E48Q mutant could process PDBP to phosphoglycolate and phosphoglycerate in the absence of borate, a stabilizer of PDBP [12]. These products did not appear to remain tightly bound to the active sites. Kane et al. [13] suggested that inability of higher-plant Rubiscos to catalyze PBDP to less tight binding products may be an explanation for susceptibility to 'fallover'. Our data indirectly support this hypothesis. Investigation of the inhibition kinetics of Rubiscos isolated from spinach, *R. rubrum*, and *C. reinhardtii* with purified PDBP will give more insights into the slow inactivation of Rubisco during catalysis.

Acknowledgements: The present work was supported in part by a Grant (97ER20268) from the US Department of Energy.

References

[1] Roy, H. and Andrews, T.J. (2000) in: Photosynthesis – Physiology and Metabolism (Leegood, R.C., Sharkey, T.D. and von Caemmerer, S., Eds.), pp. 53–83, Kluwer Academic Publishers, The Netherlands.
[2] Hartman, F.C. and Harpel, M.R. (1994) Annu. Rev. Biochem. 63, 197–234.
[3] Edmondson, D.L., Badger, M.R. and Andrews, T.J. (1990) Plant Physiol. 93, 1376–1382.
[4] Robinson, S.P. and Portis Jr., A.R. (1989) Plant Physiol. 90, 968–971.
[5] Portis Jr., A.R. (2003) Photosynth. Res. 75, 11–27.
[6] Edmondson, D.L., Kane, H.J. and Andrews, T.J. (1990) FEBS Lett. 260, 62–66.
[7] Pearce, F.G. and Andrews, T.J. (2003) J. Biol. Chem. 278, 32526–32536.
[8] McCurry, S.D. and Tolbert, N.E. (1977) J. Biol. Chem. 252, 8344–8346.
[9] Yokota, A. (1991) Plant Cell Physiol. 32, 755–762.
[10] Zhu, G. and Jensen, R.G. (1991) Plant Physiol. 97, 1354–1358.
[11] Zhu, G., Bohnert, H.J., Jensen, R.G. and Wildner, G.F. (1998) Photosynth. Res. 55, 67–74.
[12] Chen, Y.R. and Hartman, F.C. (1995) J. Biol. Chem. 270, 11741–11744.
[13] Kane, H.J., Wilkin, J.M., Portis Jr., A.R. and Andrews, T.J. (1998) Plant Physiol. 117, 1059–1069.
[14] Larson, E.M., O'Brien, C.M., Zhu, G., Spreitzer, R.J. and Portis Jr., A.R. (1997) J. Biol. Chem. 272, 17033–17037.
[15] Staniek, K. and Nohl, H. (1999) Biochim. Biophys. Acta 1413, 70–80.
[16] Zhou, M., Diwu, Z., Panchuk-Voloshina, N. and Haugland, R.P. (1997) Anal. Biochem. 253, 162–168.
[17] Harpel, M.R., Serpersu, E.H., Lamerdin, J.A., Heng, Z.H., Gage, D.A. and Hartman, F.C. (1995) Biochemistry 34, 11296–11306.
[18] Jordan, D.B. and Ogren, W.L. (1984) Planta 161, 308–313.
[19] Andrews, T.J. and Ballment, B. (1984) Proc. Natl. Acad. Sci. USA 81, 3660–3664.
[20] Yokota, A. and Kitaoka, S. (1989) Plant Cell Physiol. 30, 183–192.
[21] Uemura, K. et al. (1998) Plant Cell Physiol. 39, 212–219.
[22] Jordan, D.B. and Ogren, W.L. (1981) Nature 291, 513–515.
[23] Crafts-Brandner, S.J. and Salvucci, M.E. (2000) Proc. Natl. Acad. Sci. USA 97, 13430–13435.
[24] Harpel, M.R., Chen, Y. and Hartman, F.C. (1995) in: Photosynthesis – From Light to the Biosphere (Mathis, P., Ed.), pp. 159–162, Kluwer Academic Publishers, Dordrecht, The Netherlands.