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RIBULOSE BISPHOSPHATE CARBOXYLASE AND GLYCOLLATE OXIDASE FROM JACK PINE: EFFECTS OF SULPHUR DIOXIDE FUMIGATION

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Key Word Index—*Pinus banksiana*; Pinaceae; jack pine; ribulose bisphosphate carboxylase; glycollate oxidase; sulphur dioxide; SO_3^{2-}; SO_4^{2-}.

Abstract—Ribulose bisphosphate (RuBP) carboxylase and glycollate oxidase were partially purified from jack pine (*Pinus banksiana* Lamb.) needles. Preincubation of RuBP carboxylase with HCO_3^- and Mg^{2+} markedly stimulated its activity. RuBP carboxylase showed hyperbolic reaction kinetics with respect to HCO_3^-, Mg^{2+}, and RuBP. Both SO_3^{2-} and SO_4^{2-} inhibited RuBP carboxylase, but SO_3^{2-} was more inhibitory than SO_4^{2-}. The SO_3^{2-} inhibition was competitive with respect to HCO_3^- (whether SO_3^{2-} was present during activation or was added to the activated enzyme), while the SO_4^{2-} inhibition was non-competitive with respect to HCO_3^-. Glycollate oxidase was inhibited more severely by low concentrations of SO_3^{2-} than by SO_4^{2-}. Fumigation of jack pine seedlings with 0.34 ppm sulphur dioxide for 24 and 48 hr produced a considerable decline in the activities of these enzymes, but 1 hr of fumigation produced no effect. During the longer exposures the sulphur content of the needles increased considerably, although the needles showed no visible injury. It is suggested that the accumulation of SO_3^{2-} and SO_4^{2-} in the needles following sulphur dioxide exposure influenced the enzyme activities.

INTRODUCTION

In leaves, photosynthetic carbon dioxide fixation is primarily carried out by ribulose bisphosphate (RuBP) carboxylase, an enzyme which is widely distributed in plant leaves. In C_3 plants, RuBP carboxylase can account for up to 60% of the soluble proteins in the leaves [1]. In *Pinus banksiana* Lamb. needles, photosynthetic carbon dioxide fixation occurs exclusively by the C_3-type mechanism; as in other C_3 species, RuBP carboxylase represents a major component of the total soluble proteins [Khan, A. A. and Malhotra, S. S., unpublished results]. In C_3 plants, carboxylation by RuBP carboxylase may be a rate-limiting reaction since changes in carbon dioxide concentration or enzyme activity affect photosynthesis [2–4]. This enzyme also acts as an oxygenase [5–7], catalysing cleavage of RuBP to phosphoglycollic and phosphoglyceric acids. Glycollic acid, derived from phosphoglycollic acid, is further metabolized by glycollate oxidase in the photorespiratory pathway. The activities of RuBP carboxylase and glycollate oxidase are, therefore, important for the photosynthetic process. In spite of the key roles of these enzymes in photosynthesis, their activities in conifers have received little attention. In this study we characterized and partially purified RuBP carboxylase and glycollate oxidase from *P. banksiana*.

Sulphur dioxide, one of the major airborne pollutants, can penetrate plant foliage through stomata and can cause metabolic or physical injury. Inhibition of net photosynthesis in crop species [8–12] and forest vegetation [13] has often been observed after sulphur dioxide fumigation.

A reduction in photorespiration has been suggested as the reason for decreased synthesis of glycine and serine following exposure to sulphur dioxide [14]. Since these amino acids can also be synthesized by alternate pathways, a decrease in their biosynthesis cannot be linked exclusively to inhibition of photorespiration. A direct sulphur dioxide effect on reactions linked to photorespiration therefore remains to be demonstrated [15]. In view of the essential roles of RuBP carboxylase and glycollate oxidase in photosynthesis and photorespiration, respectively, we examined the effects of gaseous sulphur dioxide, SO_3^{2-} and SO_4^{2-} on their activities.

RESULTS AND DISCUSSION

Extraction, partial purification and characterization of RuBP carboxylase

RuBP carboxylase activity in the jack pine needle tissue was 50% higher in the mature, dark green needles than in the pale green, growing needles; therefore, mature needles were used for experimental purposes. Extraction of the enzyme was best achieved with a phosphate buffer (pH 7.5, 0.1 M) containing PVP-10 and magnesium chloride. The use of other buffers (Tris, Tes and borate) resulted in either no improvement or a partial loss of the enzyme activity. Similarly, use of PVP-40 or insoluble PVP

resulted in preparations with a lower enzyme activity than PVP-10. Addition of dithioerythritol (DTE) and ascorbic acid did not improve the enzyme activity but prevented browning of the enzyme extract due to oxidation of tissue phenolics.

The partial purification of RuBP carboxylase from pine needles indicated that *ca* 95% of the total activity present in the initial homogenate was present in the 120000 *g* supernatant (Table 1). A major portion of this activity precipitated upon 60% saturation with ammonium sulphate. In order to assay enzyme activity at this stage, residual ammonium sulphate was removed by filtration on Biogel P-2. The enzyme extract was then further purified on a column of Sepharose 6B. The activity was eluted immediately after the void volume in a colourless fraction. Other protein-containing fractions were primarily brown and were devoid of enzyme activity. The overall recovery of the enzyme activity in the colourless fraction was 60–70%.

The enzyme was stable (85–90% retention of activity) at room temperature for at least 12 hr, but cold storage (0° or in frozen condition) resulted in a considerable decrease in its activity and the appearance of fine protein fibres in the solution. RuBP carboxylase from tobacco leaves has also been shown to undergo cold inactivation [16], and recently it was demonstrated that the enzyme from puma rye undergoes reversible cold inactivation, probably as a result of conformational alterations [17].

Pine RuBP carboxylase showed optimum activity at pH 7.5 in Tris buffer. The activity was stimulated considerably by preincubation with HCO_3^-, $MgCl_2$ or RuBP, but the stimulation was greatest when the enzyme was preincubated simultaneously with HCO_3^- and $MgCl_2$. Similar results have been reported with RuBP carboxylase from other plants [18–21]. A preincubation time of 2–5 min produced optimum activity.

The reaction kinetics of RuBP carboxylase with respect to RuBP, magnesium chloride and HCO_3^- were found to be hyperbolic in nature (Michaelis–Menten type). The apparent K_m values for RuBP and Mg^{2+} were 0.3 and 1.5 mM, respectively. Since the binding of HCO_3^-/CO_2 with the enzyme is known to influence the enzyme's conformation and affinity for HCO_3^-/CO_2 in preparations from other plants [22–24], the substrate kinetics for HCO_3^- with pine needle enzyme were studied under two sets of conditions. In one set, the enzyme was preincubated with varying amounts of HCO_3^-, and the reaction was started with RuBP. The apparent K_m in this system was 11.8 mM. In the other set, the enzyme was activated first by preincubating it with 10 mM HCO_3^- plus 20 mM magnesium chloride. The assay was then started using the enzyme and varying amounts of HCO_3^- (allowance was made for the residual amount of HCO_3^- in the enzyme extract). In this case, the apparent K_m for HCO_3^- was 3.6 mM. This indicates that initial binding of the enzyme with HCO_3^- markedly increased the enzyme's affinity for HCO_3^- during RuBP carboxylation and further shows that RuBP carboxylase from pine needles behaves similarly to that from spinach [22]. While this work was in progress, Gezelius and Hallgren [25] reported similar K_m values for HCO_3^- with unpurified extracts of *Pinus silvestris*.

Effects of SO_3^{2-} and SO_4^{2-} on RuBP carboxylase

The addition of SO_3^{2-} or SO_4^{2-} markedly inhibited RuBP carboxylase activity. SO_3^{2-} was more inhibitory than SO_4^{2-}: 5 mM SO_3^{2-} produced a 50% reduction in activity, while 10 mM or higher concentrations of SO_4^{2-} were required for a similar effect. This is in contrast to the unpurified enzyme from *P. silvestris* [25], for which both SO_3^{2-} and SO_4^{2-} were equally inhibitory.

To assess the specific nature of SO_3^{2-} and SO_4^{2-} inhibition on the enzyme, we used the enzyme preparations purified on Sepharose 6B. This was necessary in order to remove interference from SO_3^{2-} oxidizing enzyme systems. Furthermore, the assays were run under a nitrogen atmosphere. In experiments with SO_3^{2-}, the enzyme activity was measured under two sets of experimental conditions. In set 1, SO_3^{2-} was present during the activation step (preincubation), while in set 2 it was added to the activated enzyme. In both, the SO_3^{2-} effect was studied with respect to HCO_3^-. The results in Figs. 1 and 2 show that under both conditions SO_3^{2-} caused an inhibition that was competitive with respect to HCO_3^-. The values of apparent K_i for SO_3^{2-} were 2.2 mM for set 1 and 0.9 mM for set 2. A similar type of competitive inhibition has been reported for spinach chloroplast enzyme when SO_3^{2-} was added during the activation step [26]. The K_i for SO_3^{2-} for spinach enzyme was 3 mM, which is close to the value reported here (2.2 mM). Recently, however, Gezelius and Hallgren [25] reported that SO_3^{2-} inhibition was non-com-

Table 1. RuBP carboxylase partial purification from *P. banksiana* needles

Purification	Total activity (units)	Total protein (mg)	Specific activity (units/mg protein)	Overall recovery (%)
Initial homogenate (crude extract)	57	223	0.26	100.0
120000 *g* supernatant	54	87	0.62	94.6
$(NH_4)_2SO_4$ (0–60% fraction)	47	71	0.66	82.2
Sepharose 6B fraction	38	29	1.30	66.5

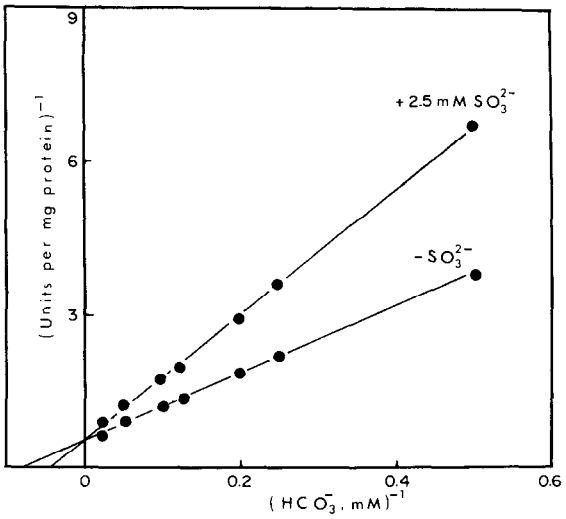

Fig. 1. Effect of SO_3^{2-} on RuBP carboxylase. A double reciprocal plot.

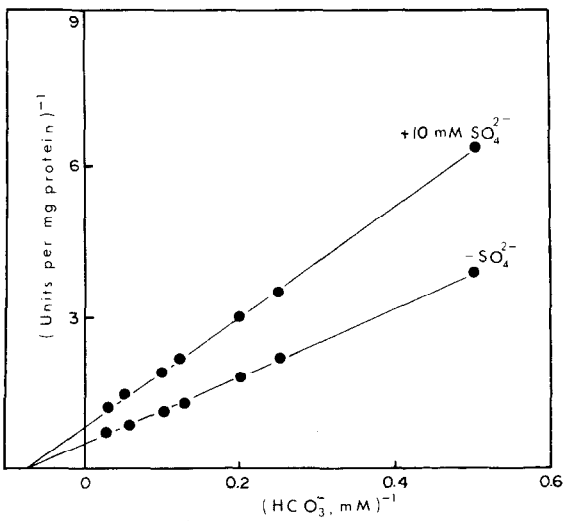

Fig. 3. Effect of SO_4^{2-} on RuBP carboxylase. A double reciprocal plot.

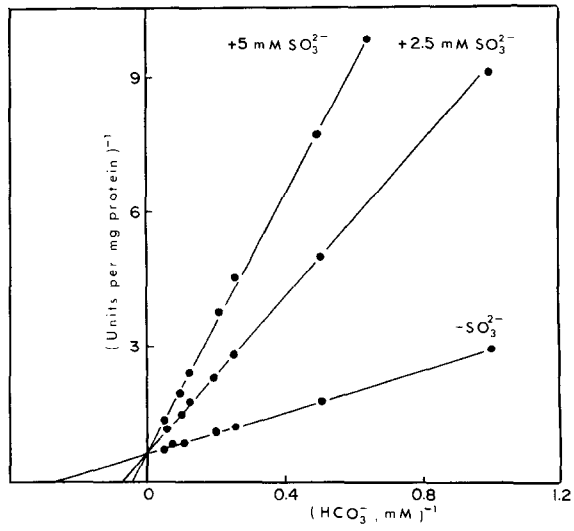

Fig. 2. Effect of SO_3^{2-} on activated RuBP carboxylase. A double reciprocal plot.

petitive with respect to HCO_3^- in both *P. silvestris* and spinach enzymes.

Because Ziegler [26] and Gezelius and Hallgren [25] studied the nature of SO_3^{2-} inhibition with unpurified preparations of the enzyme, it is difficult to assess the interference due to oxidation of SO_3^{2-} to SO_4^{2-}. Although the latter workers have suggested that in their assays flushing of the reaction tubes with nitrogen gas would prevent oxidation of SO_3^{2-}, it is, however, possible that their crude enzyme preparations may have contained an active SO_3^{2-} oxidizing system that could rapidly oxidize added SO_3^{2-} before complete anaerobic conditions were established. We have found that crude extracts and chloroplasts of *P. banksiana* needles contain very active SO_3^{2-} oxidizing systems [Khan, A. A. and Malhotra, S. S., unpublished results]. If crude extracts of the enzymes used by Gezelius and Hallgren [25] also contained active SO_3^{2-} oxidizing systems, it can be assumed that an appreciable amount of the added SO_3^{2-} was converted to SO_4^{2-} during flushing with nitrogen gas. In any case, with partially purified RuBP carboxylase of *P. banksiana* (which contained no SO_3^{2-} oxidizing activity) we were unable to obtain non-competitive inhibition by added SO_3^{2-}. Furthermore, we observed that our purified enzyme was inhibited by SO_4^{2-} and that the inhibition was non-competitive with respect to HCO_3^- (Fig. 3). Similar results have been reported for spinach enzyme [27]. The K_i for SO_4^{2-} was considerably higher (17.5 mM) than the K_i for SO_3^{2-} (2.2 mM). The similarity between the non-competitive inhibition of SO_4^{2-} that we found using a partially purified enzyme preparation and that of SO_3^{2-} in a crude extract of *P. silvestris* [25] suggests that in *P. silvestris* the SO_3^{2-} was oxidized to SO_4^{2-}.

Effects of thiol binding reagents on RuBP carboxylase

Thiol-group binding reagents [*p*-hydroxymercuribenzoate (*p*HMB) and *N*-ethylmaleimide (NEM)] strongly inhibited RuBP carboxylase activity (Table 2). The addition of excess dithioerythritol (DTE) protected the enzyme from the inhibitory effect of NEM, indicating that sulphydryl groups of the enzyme were essential for enzyme activity. The obligatory involvement of sulphydryl groups for structural conformation of this enzyme protein has also been shown for spinach and *Chromatium* enzymes [28, 29].

Table 2. Inhibition of RuBP carboxylase by thiol binding compounds and its reversal by DTE

Treatment	Enzyme activity (units/mg protein)
Control	1.66
+ *p*HMB (4 mM)	0.01
+ NEM (2 mM)	0.57
+ NEM (4 mM)	0.12
+ NEM (2 mM) + DTE (10 mM)	1.69

Characterization of glycollate oxidase from P. banksiana

As with RuBP carboxylase, the activity of glycollate oxidase also increased (70–80%) during greening and maturation of the needles. Mature needles were therefore used in all experiments. The enzyme showed optimum activity at pH 8 (Tris buffer), and the rate of reaction was linear up to 15 min. The extraction of enzyme was best achieved in a phosphate buffer—PVP-10 medium similar to that described for RuBP carboxylase. The enzyme from the 120000 *g* supernatant was partially purified by fractionation with ammonium sulphate. Most of the activity (80%) was precipitated at 25–50% ammonium sulphate saturation, resulting in a 50–60% increase in specific activity over that of the supernatant. The enzyme was stable for several days when stored in a frozen condition. The enzyme showed hyperbolic reaction kinetics with respect to its substrate, glycollic acid (K_m was 36 mM).

Effects of SO_3^{2-} and SO_4^{2-} on glycollate oxidase

Glycollate oxidase was much more sensitive to SO_3^{2-} than was RuBP carboxylase. More than 50% inhibition was observed at 10 μM SO_3^{2-} (Table 3), and the inhibition became more severe at higher concentrations. SO_3^{2-} inhibited the enzyme competitively with respect to glycollic acid (K_i for SO_3^{2-} was 5.7 μM). Glycollate oxidase from spinach has also been shown to be very sensitive to SO_3^{2-} [30, 31] due to the formation of glyoxylate bisulphite, a potent competitive inhibitor of the enzyme [31]. The addition of up to 2.5 mM SO_4^{2-} did not produce any appreciable effect on the enzyme activity (Table 3).

Effects of sulphur dioxide fumigation on RuBP carboxylase and glycollate oxidase

Accumulation of SO_3^{2-} and SO_4^{2-} in plant leaves is known to occur upon sulphur dioxide fumigation [32]. Above a certain concentration the absorbed sulphur dioxide (or its accumulation products) is expected to affect sensitive metabolic processes. This was tested by fumigating pine seedlings with 0.34 ppm gaseous sulphur dioxide and measuring its effect on the activities of RuBP carboxylase and glycollate oxidase. Fumigation for 1 hr did not produce any change in the activities of these enzymes (Table 4). This lack of response could be due to: (a) rapid air movement (as a result of a high airflow rate) that caused little or no change in the carbon dioxide level in the cuvette air; (b) the presence of active SO_3^{2-} oxidizing systems in the needles [Khan, A. A. and Malhotra, S. S., unpublished results]; and (c) the reduction of initially absorbed sulphur dioxide (or its oxidation products) in the chloroplasts either to the normal metabolic products of the reductive sulphur cycle or to hydrogen sulphide and its release from the needle tissues [32].

Table 3. Effects of SO_3^{2-} and SO_4^{2-} on glycollate oxidase

SO_3^{2-} (μmol)	Enzyme activity (units/mg protein)	SO_4^{2-} (mmol)	Enzyme activity (units/mg protein)
0	0.188	0	0.172
2.5	0.168	1.0	0.172
5.0	0.120	2.5	0.167
10.0	0.086		

Table 4. Effects of sulphur dioxide fumigation (0.34 ppm) on RuBP carboxylase and glycollate oxidase activities and sulphur content of fumigated pine needles

Duration of SO_2 fumigation* (hr)	Enzyme activity: RuBP carboxylase (% control)	Enzyme activity: Glycollate oxidase (% control)	Sulphur† (% control)
1	100.0	100.0	100.5
24	88.9	91.3	165.0
48	70.8	76.6	200.0

*As described in the text, plants were fumigated with an 18 hr photoperiod/day.

†The sulphur content in the unfumigated needles was 1.00 ± 0.2 mg/g dry wt of needles.

Rapid airflow in the cuvette is expected to keep the carbon dioxide level in plant tissues high enough to maintain RuBP carboxylase in its activated form. Furthermore, the amount of initially absorbed sulphur dioxide in the tissues would be considerably lower than the amount of carbon dioxide (due to a large difference in carbon dioxide and sulphur dioxide levels in the cuvette air). This would, therefore, limit the availability of carbon dioxide-binding sites for sulphur dioxide action. Stomatal closure that generally occurs at high carbon dioxide levels [32] may also be another factor that restricts initial sulphur dioxide absorption.

The presence of SO_3^{2-} oxidizing systems in the needles would rapidly convert small amounts of toxic species of dissolved sulphur dioxide (SO_3^{2-} and HSO_3^-) to a relatively less toxic form, SO_4^{2-}. It has been reported that SO_4^{2-} is relatively harmless to photosynthesis [33, 34]. Both RuBP carboxylase and glycollate oxidase in *P. banksiana* were found to be less sensitive to SO_4^{2-} than to SO_3^{2-}; therefore, it would appear that SO_3^{2-} toxicity depends on the efficiency of the SO_3^{2-} oxidizing system of the tissues. Furthermore, the changes in the tissue balance of sulphydryl and disulphide groups in the fumigated plant tissues [32] can affect the activities of enzymes requiring these groups.

Fumigation of intact plants of *Glycine max* with $^{35}SO_2$ has been shown to result in the accumulation of mainly $^{35}SO_4^{2-}$ [35]. In our experiment (1 hr fumigation), the pine needles probably did not accumulate sufficient quantities of SO_3^{2-} to be toxic. This is supported by the fact that the sulphur content of the needles did not increase after the exposure. Both enzymes, however, were markedly inhibited after exposure of pine seedlings to sulphur dioxide for 24 and 48 hr (Table 4). These exposures did not produce any visible symptoms of injury. The inhibitory effect was accompanied by an increase in the sulphur content of the needles, perhaps indicating an accumulation of toxic levels of SO_3^{2-} and SO_4^{2-} in the tissues. The decrease in RuBP carboxylase and glycollate oxidase therefore appeared to be related to

increased sulphur dioxide uptake and the sensitivity of the enzymes to accumulated SO_3^{2-} and SO_4^{2-}. Glyoxylate bisulphite, a competitive inhibitor of glycollate oxidase, has been shown to accumulate in rice plants after large doses of $^{35}SO_2$ [36]. If small amounts of glyoxylate bisulphite are continuously formed during long sulphur dioxide exposures, it would affect glycollate oxidase and its dependent processes. For example, glycollic acid is known to affect stomatal opening in light [37], which in turn affects photosynthesis.

EXPERIMENTAL

Plant growth. Jack pine (*Pinus banksiana* Lamb.) seedlings were grown in peat moss in styroblock trays [38] placed in a greenhouse at 20–24° under natural daylight supplemented by 12 klx fluorescent light (16 hr). Mature needles from 4–5-month-old plants were used.

Fumigation of plants with SO_2. Plants were transferred from the styroblock trays to fumigation cuvettes as described previously [39]. The construction and operation of the cuvettes were essentially the same as described before [39] except that the cuvettes used in the present study were modified for a high airflow rate (*ca* 100 l/min).

For each expt, sets of *ca* 12 seedlings of uniform age and growth were transferred to two cuvettes. One cuvette received clean air (control), while the other cuvette received air containing 0.34 ppm SO_2. Prior to fumigation, the cuvettes containing the plants were placed in a controlled environment chamber for 48 hr and were maintained at 20 klx light intensity (18 hr photoperiod). The temp. in light and dark periods was 22 and 18°, respectively. Relative humidity inside the cuvettes was maintained at 60–65% by using prehumidified air. The rate of airflow in the cuvettes was 100 l/min. During fumigation the concn of SO_2 was maintained at 0.34 ppm by a feedback controller and was continuously monitored by a Phillips PW 9700 SO_2 analyser. A fan at the inlet port of each cuvette provided uniform air mixing. Plants were harvested after fumigation, and the mature needles were used for experimental purposes.

Extraction and purification of enzymes. Needles (1 cm sections) were homogenized for 1 min with a Brinkman Polytron Homogenizer (Model PT-10) at full power in 10 vol. of a chilled soln of 0.1 M KPi buffer (pH 7.5) containing 1% PVP-10, 1 mM DTE, and 1 mM $MgCl_2$. The homogenate was filtered through Miracloth and centrifuged at 120000 *g* for 30 min. The supernatant was then used for the purification of RuBP carboxylase and glycollate oxidase.

RuBP carboxylase was purified from the 120000 *g* supernatant by an initial fractionation with $(NH_4)_2SO_4$. The enzyme was pptated by adding solid $(NH_4)_2SO_4$ to the supernatant to a 60% satn point. The pptated proteins were removed by centrifugation, dissolved in 0.1 M Tris buffer (pH 7.5), and desalted by passing through a small column (2 × 15 cm) of Biogel P-2. The enzyme was then further purified by gel filtration on Sepharose 6B columns (2 × 45 cm). Equilibration and elution of the columns was done with 0.1 M Tris buffer (pH 7.5); 3.5 ml enzyme fractions were collected and analysed for enzyme activity and protein content.

Glycollate oxidase was partially purified by precipitating the enzyme with $(NH_4)_2SO_4$. The protein fraction obtained at 25–50% satn was dissolved in 0.1 M Tris buffer (pH 7.5) and used in the enzyme reaction.

For both RuBP carboxylase and glycollate oxidase purifications, all of the above operations were carried out at 0–4°. In the fumigation expts, the enzyme activity was assayed in the 120000 *g* supernatant because the change in enzyme activities was similar whether they were assayed after partial purification or in the 120000 *g* supernatant.

Enzyme assays. RuBP carboxylase. A regular assay in a final vol. of 0.2 ml involved preincubation of the enzyme for 3 min at 30° in a reaction medium containing 10 μmol Tris buffer (pH 7.5), 2 μmol $MgCl_2$ and 4 μmol [^{14}C]$NaHCO_3$ (9.8×10^5 cpm). Where indicated, specified test compounds were included in the preincubation period. The enzyme reaction was started by adding 150 nmol of RuBP. After 1–3 min at 30° the reaction was terminated by adding 0.1 ml HCO_2H (88%). In some kinetic expts (Fig. 2), the activity of the purified enzyme was assayed according to the procedure outlined in ref. [22] with some modifications. This procedure involved activation of the enzyme by preincubating it for 3 min in 10 mM [^{14}C]$NaHCO_3$ and 20 mM $MgCl_2$ at 30°. The activated form of the enzyme was then used to start the reaction under N_2 gas. The assay mixture in a final vol. of 0.2 ml contained 10 μmol Tris buffer (pH 7.5), 2 μmol $MgCl_2$, varying concns of [^{14}C]$NaHCO_3$, 150 nmol RuBP, activated enzyme, and H_2O or a specified amount of SO_3^{2-} (in assays involving SO_3^{2-} effect). The amount of HCO_3^- present in the enzyme aliquot was taken into account in calculating its total concn in each assay. The reaction was run for 3 min at 30° and stopped by adding 0.1 ml HCO_2H (88%). An aliquot from either procedure was then transferred to a 7-ml scintillation vial, and the contents of the vial were flushed with N_2 gas to almost dryness on a hot-water bath. Incorporation of ^{14}C into acid-stable products was then determined by dissolving the contents of the vial in 0.1 ml H_2O and mixing them with 5 ml of a scintillation fluid [0.4% Omnifluor (New England Nuclear) dissolved in 30% EtOH in toluene]; counting was done with a liquid scintillation spectrometer. A reaction control in which RuBP was not added during the assay was always run and the counts, if any, were subtracted from those of the treatment assays. A unit of enzyme activity was defined as 1 μmol HCO_3^- incorporated into acid-stable products/min under assay conditions. *Glycollate oxidase.* Enzyme activity was assayed according to ref. [40] with some modifications. The assay system in a final vol. of 2 ml contained 100 μmol Tris buffer (pH 8.0), 15 μmol glycollic acid (pH adjusted to 7.0), 5 μmol phenylhydrazine HCl (pH adjusted to 7.0), enzyme soln and H_2O. The reaction was run for 1–10 min. The increase in A_{324nm} was followed, using a molar extinction coefficient of 17×10^3/cm/mol [41] for calculating the activity. A unit of enzyme activity was defined as 1 μmol glyoxylate formed/min under assay conditions. *Other estimations.* Protein was estimated according to ref. [42] after pptation with TCA and washing the ppt with 80% Me_2CO. Bovine serum albumin was used as a standard. Needle dry wt was measured by oven-drying the fresh needles at 80° for 24 hr. For S analysis the dried samples were powdered and a portion was used for O_2-flask combustion [43] and S was measured according to ref. [44].

All expts were repeated several times and the trends were found to be highly reproducible. The data in the tables are taken from representative expts.

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REFERENCES

1. Ku, M. B. S., Schmitt, M. R. and Edwards, G. E. (1979) *J. Exp. Botany* **30**, 89.
2. Wareing, P. F., Khalifa, M. M. and Treharne, K. J. (1968) *Nature (London)* **220**, 453.
3. Bjorkman, O. (1968) *Physiol. Plant.* **21**, 1.
4. Anderson, W. R., Wildner, G. F. and Criddle, R. S. (1970) *Arch. Biochem. Biophys.* **137**, 84.
5. Bowes, G., Orgen, W. L. and Hageman, R. H. (1971) *Biochem. Biophys. Res. Commun.* **45**, 716.
6. Andrews, T. G., Lorimer, G. H. and Tolbert, N. E. (1973) *Biochemistry* **12**, 11.
7. Lorimer, G. H., Andrews, T. G. and Tolbert, N. E. (1973) *Biochemistry* **12**, 18.
8. Thomas, M. D. and Hill, G. R. (1937) *Plant Physiol.* **12**, 309.
9. Oshima, Y., Ushijima, T. and Tazaki, T. (1973) *Environ. Control Biol.* **11**, 103.
10. Bull, J. N. and Mansfield, T. A. (1974) *Nature (London)* **250**, 443.
11. Black, V. G. and Unsworth, M. H. (1979) *J. Exp. Botany* **30**, 473.
12. Furukawa, A., Natori, T. and Tatsuka, T. (1980) *Natl. Inst. Environ. Stud. Jpn. Res. Rep.* **11**, 1.
13. Malhotra, S. S., Addison, P. A. and Khan, A. A. (1980) *Research Report, LS* 3.1. Alberta Oil Sands Environmental Research Program, Edmonton.
14. Koziol, M. J. and Cowling, D. W. (1978) *J. Exp. Botany* **29**, 1431.
15. Hallgren, J. E. (1978) in *Sulfur in the Environment, Part II: Ecological Impacts* (Nriagu, J. O., ed.) p. 163. John Wiley, New York.
16. Chollet, R. and Anderson, L. L. (1977) *Biochim. Biophys. Acta* **482**, 228.
17. Hurner, N. P. A. and Macdowall, F. D. H. (1978) *Can. J. Biochem.* **56**, 1154.
18. Pon, N. G., Rabin, B. R. and Calvin, M. (1963) *Biochem. Z.* **338**, 7.
19. Tabita, F. R. and McFadden, B. A. (1974) *J. Biol. Chem.* **249**, 3453.
20. Lorimer, G. H., Badger, M. R. and Heldt, H. W. (1978) in *Photosynthetic Carbon Assimilation* (Siegelman, H. W. and Hind, G., eds.) p. 283. Plenum Press, New York.
21. Paech, C., McCurry, S. D., Pierce, J. and Tolbert, N. E. (1978) in *Photosynthetic Carbon Assimilation* (Siegelman, H. W. and Hind G., eds.) p. 227. Plenum Press, New York.
22. Andrews, T. J., Badger, M. R. and Lorimer, G. H. (1975) *Arch. Biochem. Biophys.* **171**, 93.
23. Badger, M. R. and Lorimer, G. H. (1976) *Arch. Biochem. Biophys.* **175**, 723.
24. Laing, W. A. and Christeller, J. T. (1976) *Biochem. J.* **159**, 563.
25. Gezelius, K. and Hallgren J. E. (1980) *Physiol. Plant.* **49**, 354.
26. Ziegler, I. (1972) *Planta* **103**, 155.
27. Trown, P. W. (1965) *Biochemistry* **4**, 908.
28. Sugiyama, T., Nakayama, N., Ogawa, M., Akawaza, T. and Oda, T. (1968) *Arch. Biochem. Biophys.* **125**, 98.
29. Takabe, T. and Akazawa, T. (1975) *Arch. Biochem. Biophys.* **169**, 686.
30. Zelitch, I. (1957) *J. Biol. Chem.* **224**, 251.
31. Zelitch, I. (1958) *J. Biol. Chem.* **233**, 1299.
32. Malhotra, S. S. and Khan, A. A. (1982) in *Air Pollution and Plant Life* (Treshow, M., ed.). John Wiley, London (in press).
33. Hill, D. J. (1974) *New Phytol.* **73**, 1193.
34. Ferguson, P. and Lee, J. A. (1979) *New Phytol.* **82**, 703.
35. Garsed, S. G. and Read, D. J. (1977) *New Phytol.* **99**, 583.
36. Tanaka, H., Takanashi, T. and Yatazawa, M. (1972) *Water Air Soil Pollut.* **1**, 205.
37. Zelitch, I. and Walker, D. A. (1964) *Plant Physiol.* **39**, 856.
38. Malhotra, S. S. (1976) *New Phytol.* **76**, 239.
39. Malhotra, S. S. and Khan, A. A. (1978) *Phytochemistry* **17**, 241.
40. Hess, J. L. and Tolbert, N. E. (1967) *Plant Physiol.* **42**, 371.
41. Dixon, G. H. and Kornberg, H. L. (1959) *Biochem. J.* **72**, 3.
42. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265.
43. Chan, C. C. Y. (1975) *Analyt. Letters* **8**, 655.
44. Carson, J. A., Crepin, J. M. and Nemunis Siugzdines, P. (1972) *Can. J. Soil Sci.* **52**, 278.