Effects of Arsenite, Sulfite, and Sulfate on Photosynthetic Carbon Metabolism in Isolated Pea (Pisum sativum L., cv Little Marvel) Chloroplasts

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ABSTRACT

Photosynthetic CO2-fixation in isolated pea (Pisum sativum L., cv Little Marvel) chloroplasts during induction is markedly inhibited by 0.4 millimolar sulfite. Sulfate at the same concentration has almost no effect. The 14CO2-fixation pattern indicates that the primary effect of sulfite is inhibition of the reaction catalyzed by ribulose bisphosphate carboxylase and a stimulation of export of intermediates out of the chloroplasts. Inhibition of light modulation of stromal enzyme activity does not appear to account for the toxicity of SO2 in this Pisum variety. Arsenite at 0.2 millimolar concentrations inhibits light activation and inhibits photosynthetic CO2 fixation. The 14CO2-fixation pattern indicates that the primary effect of arsenite is inhibition of light activation of reductive pentose phosphate pathway enzyme activity.

Several sites of action have been proposed to explain the effect of SO2, one of the major air pollutants, on plants. In chloroplasts sulfite (at pH 8.2: 9.2% HSO3- and 90.8% SO32-) and effectors induced by SO2 fumigation can attack reactions of photosynthetic electron transport (7, 26), photophosphorylation (5, 27), and the reductive pentose phosphate pathway (10, 14, 17, 24, 29, 31–33). Light modulation of stromal enzyme activity in the chloroplast is known to be sulfite sensitive (3, 20, 21).

In previous experiments involving 14CO2 fixation in intact chloroplasts the RPP pathway intermediates were only partially resolved (16). To verify the reported SO2 effects and to determine their significance in situ, we have examined the effect of sulfite on the 14CO2 fixation pattern and the distribution of 14C-labeled metabolites between chloroplasts and the incubation medium during photosynthetic induction.

SO2-fumigated plants can accumulate sulfate (9, 30), which has been reported to be both relatively harmless (8, 27) and to quite effectively inhibit photosynthesis in isolated chloroplasts (4, 27). We treated isolated pea chloroplasts with sulfate in order to compare effects with those of sulfite.

The major causes of the sulfite-induced reduction in the photosynthetic rate in these experiments are obviously the inhibition of the RuBP carboxylation reaction and an increased export of intermediates (first of all TP) out of the chloroplasts. A direct or indirect inhibitory action of sulfite on electron transport, photophosphorylation, or light modulation of enzyme activity cannot be excluded, but these are at least in the model system used here, most likely of secondary importance. Sulfite had little or no effect on photosynthetic CO2 fixation or the pattern of 14CO2 fixation.

Arsenite is known to inhibit photosynthetic CO2 fixation if it is added prior to illumination of the chloroplast suspension (11). Since the only known effect of arsenite on photosynthetic carbon metabolism is inhibition of light modulation, it has been suggested that inhibition of light modulation affects the buildup of photosynthetic intermediates during the induction phase of photosynthesis (1). Our results show that, in contrast to sulfite, arsenite effectively inhibits light activation in intact chloroplasts. This inhibition is evident both in experiments where enzyme activity is monitored and where the 14CO2 fixation pattern is followed.

MATERIALS AND METHODS

Cultivation of pea plants (Pisum sativum L., cv Little Marvel), isolation of chloroplasts and determination of Chl concentration was as described previously (18). The chloroplast incubation mixture was kept at 25°C, had a total volume of 2 ml, and contained 330 mM sorbitol, 50 mM Hepes-KOH (pH 8.2), 5 mM [14C]NaHCO3, 0.2 mM K2HPO4, 5 mM Na2S03, 0.2 mM ATP, 200 units catalase ml⁻¹, and an amount of chloroplasts equivalent to 130 μg Chl ml⁻¹. After 4 min incubation in the dark in the presence of 5 mM [14C]NaHCO3 (5 Ci, 185 GBq/mol) the chloroplasts were illuminated with a light intensity of 6 × 10⁶ ergs cm⁻² s⁻¹ (60 mW cm⁻²). The light from a General Electric cool beam lamp (300 W, 120 V) was focused through a round bottom flask filled with 1% (w/v) CuSO4. Where indicated, Na2S03, Na2HPO4, or Na2SO3 was added to the chloroplasts to a concentration of 0.4 mM, or NaAsO2 was added to a concentration of 0.2 or 0.3 mM, as indicated, 2 min before illumination.

Separation of the chloroplasts from the incubation medium, preparation of chloroplast extracts, and the separation by HPLC and detection of the 14C-labeled metabolites were performed according to the procedures described in Marques et al. (19).

FBPase activity was determined as in Marques and Anderson (18) with FBP 0.5 mM and MgCl2, 10 mM. Na2AsO2 was added to the chloroplasts 2 min before illumination to a concentration of 0.3 mM.

RESULTS

In the experiment shown in Figure 1 the maximal photosynthetic CO2 fixation rate in the control chloroplasts was 0.92 μmol
Effects of Sulfite and Arsenite on Photosynthesis

Fig. 1. Effect of sulfite (△) and sulfate (▽) on incorporation of $^{14}$CO$_2$ into total soluble $^{14}$C (A), soluble $^{14}$C within the chloroplasts (B), and the percentage of the total fixed soluble $^{14}$C exported (C) in isolated pea chloroplasts during photosynthetic induction. Control, (Ο). The pH of the incubation medium was 8.2 and the temperature was 25°C. Sulfite and sulfate were added 2 min before illumination. For detailed conditions see "Materials and Methods." After 10 min of illumination the $^{14}$C-activity in the insoluble fraction was 2.2% of the total fixed $^{14}$C in the control (sulfite, 0.9%; sulfate, 1.6%).

mg$^{-2}$ Chl min$^{-1}$. Addition of sulfite (final concentration: 0.4 mM) to the chloroplasts 2 min before illumination resulted in a 54% inhibition in the photosynthetic rate after 6 to 8 min of illumination. Since the photosynthetic rate in the sulfite-treated chloroplasts still increased after 8 min whereas that of the controls remained constant (reflected in the total soluble fraction shown in Fig. 1), after 8 to 10 min the rate of photosynthesis was reduced by only 28%. Sulfate (0.4 mM) inhibited the maximal photosynthetic rate by about 16%.

The difference in the $^{14}$C fixed into the soluble fraction within the chloroplasts between control and sulfite-treated chloroplasts was more distinct (Fig. 1) than in the total chloroplast suspension (chloroplasts and incubation medium) indicating an increased export of intermediates after addition of sulfite. TP, PGA, and HMP were the major components contributing to the sulfite-induced change of the distribution of total soluble $^{14}$C between chloroplasts and medium (Fig. 3). Sulfite caused just a slight increase in the export rate of metabolites (Fig. 1), in a similar way as phosphate did when added to the chloroplasts at the same concentration (Fig. 2).

Sulfite also caused a change in the $^{14}$CO$_2$ fixation pattern of RPP cycle intermediates within the chloroplasts (Fig. 4). Most conspicuous is the percent increase of radioactive RuBP after sulfite treatment. Sulfate, likewise, increased the percent $^{14}$C-activity in RuBP compared to the controls but to a much lesser extent than sulfite (Fig. 4).

The percentage of total soluble radioactivity within chloroplasts in PGA, FBP, and HMP (Fig. 4), and in the insoluble fraction (after 10 min illumination: control, 2.2% of total fixed $^{14}$C; sulfite, 0.9%; sulfate, 1.6%) was lower after addition of sulfite, compared to sulfite-treated and control chloroplasts. Ratios of incorporation into substrate/product pairs for several enzymes are shown in Figure 5. Obviously, several steps in the RPP cycle are influenced by sulfite. Sulfite treatment resulted in

Fig. 2. Distribution of fixed $^{14}$C between chloroplasts and medium (Ο) after addition of Na$_2$SO$_3$ (0.4 mM; △) or Na$_2$HPO$_4$ (0.4 mM; ▽) to the assay medium (0.2 mM Pi + 5 mM PPI) 2 min before illumination. Total soluble $^{14}$C outside is plotted versus total soluble $^{14}$C inside the chloroplasts after 4, 6, 8, and 10 min of illumination. The maximal CO$_2$ fixation rate in the control of this experiment was 0.82 μmol mg$^{-2}$ Chl min$^{-1}$. 

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a significantly higher ratio of RuBP to PGA and a lower PGA to TP ratio. The TP/FBP ratio was also increased after addition of sulfite but there was no apparent difference in the TP/SBP ratio. Whereas the FBP/HMP ratio was lower, the ratio of SBP to HMP was the same or higher than in the control in sulfite-treated chloroplasts after 4 min of illumination. In repeated experiments the ratio of SBP to HMP after 4 min was never lower after sulfite-treatment.

Addition of arsenite (0.2 mM) to the chloroplasts before illumination resulted in a clear extension of the lag in CO2 fixation (Fig. 6A; see also discussion of experiment in Fig. 8 immediately below). In arsenite treated chloroplasts the percentage of radioactivity was high in PGA, SBP, and FBP, but low in HMP and RuBP compared to control chloroplasts (Figs. 6, 7).

Light activation of FBPase in intact chloroplasts was, as expected (19), inhibited after addition of arsenite (0.3 mM), whereas addition of sulfite (0.4 mM) to the chloroplasts (Fig. 8) as well as to the enzyme assay directly (data not shown) slightly increased the activity of FBPase. Maximal CO2-dependent O2-evolution (0.79 μmol mg−1 Chlorophyll min−1) in the experiment shown in Figure
EFFECTS OF SULFITE AND ARSENITE ON PHOTOSYNTHESIS

8 was inhibited 45% by 0.4 mM sulfite and 51% by 0.3 mM arsenite. Arsenite extended the lag phase from 3.8 min (control chloroplasts) to 5.2 min. The lag phase after addition of sulfite was 3.6 min.

DISCUSSION

Sulfite has been reported both to stimulate and to inhibit photosynthetic CO₂ fixation (16, 23, 28). We observed an even higher inhibition (54% with 0.4 mM sulfite, see “Results”) than has been reported previously (50% with 5 and 1.6 mM sulfite, respectively, depending on the PPI concentration, [27]). This may be because we treated the chloroplasts with sulfite in the dark prior to illumination, since in the light sulfite is assimilated and oxidized to sulfate (9, 30). Damage of pea and tomato plants after SO₂ exposure in the dark is reported to be greater than in the light (22).

Sulfate was much less toxic than was sulfite (Fig. 1). The slight decrease in the photosynthetic rate with sulfate is probably, in part, due to an increased export of triosephosphates from the chloroplasts (Fig. 3). Sulfite caused a much more significant increase in the export rate of intermediates (especially TP, see Fig. 3) than phosphate (Fig. 2) or sulfate (Figs. 1, 3). Besides a possible stimulatory effect of sulfite on the Pi-translocator (12), the higher permeability of metabolites in the presence of sulfite might simply be due to nonspecific disruption of membranes (17, 25). The rate of uptake of sulfite is reported to be lower than the rate of phosphate uptake in spinach chloroplasts (12).

The marked increase in the percentage of radioactivity in RuBP and the decrease in PGA in the presence of sulfite (Figs. 4, 5) clearly demonstrates that one of the most sulfite-sensitive steps of the RPP pathway is the RuBP carboxylation reaction. Sulfite is competitive with CO₂ (14, 31, 32). Reported Ki (sulfite) values for RuBP carboxylase range from 0.9 to 18.5 mM (10, 14, 31, 32). Since the photosynthetic rate of the pea chloroplasts was already reduced more than 50% when sulfite was added to 0.4 mM (a concentration 2-fold lower than the lowest reported Ki for RuBP carboxylase), if the sensitivity of the carboxylase is the cause of the inhibition, then either (a) sulfite accumulates within the chloroplasts, (b) the CO₂ concentration in the vicinity of the carboxylase is relatively low allowing sulfite to compete effectively with CO₂, or (c) *Pisum* carboxylase is more sensitive than other carboxylases to sulfite. Sulfate has little or no effect on RuBP carboxylation (Fig. 4). This is consistent with the high Ki value for sulfate (17.5 mM) for the *Pinus banksiana* Lamb. carboxylase (14) and with experiments with sulfate and sulfite on bean (24) and *P. banksiana* carboxylase (17). In *Pinus silvestris*, however, RuBP carboxylase is inhibited to the same extent by 10 mM sulfite or sulfate (10).

The ratios of ¹⁴C-labeled intermediates shown in Figure 5

![Figure 5](https://academic.oup.com/plphys/article-fig/82/2/488/6083908) of ¹⁴C-label in an intermediate to that in its immediate product in the RPP pathway (O). Effects after addition of 0.4 mM Na₂SO₃ (Δ) and 0.4 mM Na₂SO₄ (△) 2 min before illumination. Same experiment as Figure 1.
suggest that besides RuBP carboxylase, there may be an additional sulfite-sensitive enzyme in the RPP pathway, namely aldolase. Aldolase activity, assayed as FBP formation or cleavage, was not affected when sulfite was added to the chloroplast suspension (up to 1.6 mM) or directly to the assay cuvette (up to 3 mM) (data not shown). Possibly part of the glyceraldehyde-3-P was converted to the sulfonate which might be produced at higher sulfite levels (13). Or the aldolase activity may be reduced because the TP levels are lower, shifting the TP/FBP ratio toward TP (Fig. 5). Inhibition of aldolase would not explain the lower

FIG. 6. A, Total soluble 14C fixed in the total chloroplast suspension (∆, ▲) and within chloroplasts (○, ●) in the presence (∆, ▲) and absence (∆, ○) of 0.2 mM arsenite. Arsenite was added to the chloroplast suspension (120 μg Chl ml−1) 2 min before illumination. B, 14C incorporation into glyceraldehyde-3-phosphate (PGA; □), triose-P (TP; ▲), sedoheptulose-1,7-bisphosphate (SBP; ●), fructose-1,6-bisphosphate (FBP; ○), hexose- and heptose-P (HMP; ●), and ribulose-1,5-bisphosphate (RuBP; ▲) expressed as percent of total soluble 14C within the chloroplasts. C, Conditions the same as in B, except addition of 0.2 mM arsenite 2 min before illumination.

FIG. 7. Ratios of 14C label in FBP (○, △) and SBP (●, ▲) to those in HMP within control (○, ●) and arsenite treated (△, ▲) pea chloroplasts. Data were used from the experiment shown in Figure 6.

FIG. 8. Light activation of fructose-1,6-bisphosphatase (○) from isolated pea chloroplasts during photosynthetic induction. The enzyme assay (pH 8.0, total volume: 1 ml) was with FBP 0.5 mM, MgCl2 10 mM and 25 μl chloroplast suspension. Effects after addition of 0.4 mM Na2SO3 (△) and 0.3 mM NaAsO2 (□) to the chloroplasts 2 min before illumination.
ratio of FBP to HMP after sulfite-treatment. The ratio of HMP to the insoluble fraction was not increased in the presence of sulfite (data not shown).

The light activation of FBPase may have been slightly stimulated by sulfite, which could explain the lower ratio of FBP to HMP in the presence of sulfite (Fig. 5). The slightly higher light-activated FBPase activity after addition of sulfite to the chloroplasts (Fig. 8) supports this suggestion. Alternatively, the activity of transketolase may be reduced by lower levels of glyceraldehyde-3-P and causing an accumulation of radioactivity in the HMP-fraction.

Since the ratio of PGA to TP is lower in sulfite-treated chloroplasts (Fig. 5), the availability of ATP after addition of sulfite does not seem to be critical under our experimental conditions (cf., 5, 27). Another possible consequence of exposing illuminated chloroplasts to sulfite is the accumulation of H$_2$O$_2$ (29). Since we added catalase to the chloroplast incubation medium, effects of H$_2$O$_2$ probably were eliminated. Furthermore, effects observed after H$_2$O$_2$ treatment (6, 15, 29) are very different from those of sulfite.

Arsenite treatment, which is known to inhibit light activation of RPP pathway enzymes (2), resulted in a completely different $^{14}$CO$_2$-fixation pattern than sulfite treatment. In the experiment shown in Figures 6 and 7 the percent of activity in hexose- and pentose-phosphate-P$_i$ was higher in arsenite-treated than in control chloroplasts, which indicates that light activation of FBPase and SBPase was inhibited in the poisoned chloroplasts. One would also expect to see evidence for inhibition of light activation of G3P dehydrogenase and Ru5P kinase. Arsenate caused an increase in the percentage of $^{14}$C-label in PGA, but no corresponding decrease of labeled TP (Fig. 6, B and C). The expected decrease is probably eliminated as a result of the accumulation of FBP and SBP (Fig. 7). The lower level of $^{14}$C-label in RuBP in arsenite treated chloroplasts probably reflects the lowered F6P and 7P poolsizes as well as inhibition of light activation of Ru5P kinase.

Addition of sulfite to the chloroplasts did not, in contrast to addition of arsenite, result in inhibition of light activation of FBPase (Fig. 8).

We are able to show the relative effects of sulfite on ribulose bisphosphate carboxylase activity, light activation, and metabolite export in a single experiment. The inhibition of light modulation when thylakoids are treated with sulfite (3, 21) does not appear to be sufficient to account for the inhibition of CO$_2$ fixation in the chloroplasts used here. Instead, in these experiments inhibition of RuBP carboxylase and the increase in export of intermediates appear to be responsible for the effect of sulfite on photosynthetic CO$_2$ fixation. Long-term exposure of the light modulation system to $SO_3^-$ (= sulfite) might, however, affect light activation of the enzymes of photosynthetic CO$_2$ fixation. In experiments in this laboratory with two Pismus varieties chosen for differences in $SO_3^-$ tolerance, differences in photosynthetic sensitivity paralleled differences in the sensitivity of the light modulation system (21). It seems likely that the relative importance of the various factors involved in $SO_3^-$-sensitivity differs between species and between cultivars.

Arsenite clearly affects light modulation and photosynthetic induction. The extension of the lag period by arsenite must then be due to inhibition of light activation of RPP cycle enzymes.

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LITERATURE CITED


