Light and Stomatal Metabolism

II. EFFECTS OF SULFITE AND ARSENITE ON STOMATAL OPENING AND LIGHT MODULATION OF ENZYMES IN EPIDERMIS

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ABSTRACT

The effect of sulfite and arsenite on stomatal opening and light modulation of enzymes was examined in isolated epidermal strips of Pisum sativum L. var Little Marvel leaves. Sulfite or arsenite at 10 micromolar rapidly inhibited the stomatal opening process in light. Light activation of phosphoenolpyruvate carboxylase and NADP-malate dehydrogenase was completely diminished when the epidermal strips were incubated for 2 hours in light with either sulfite or arsenite at 10 micromolar. The data obtained suggest that the inhibition of stomatal opening by sulfite or arsenite in light might result from the inhibition of light modulation of key enzymes in guard cells.

In recent years, considerable interest has been shown in testing the stomatal responses to air pollutants since stomata are the principal portals for entry of air pollutants (5, 6, 8, 9). SO2 absorbed by plant leaves through stomata is transformed into sulfite and/or bisulfite ions on the wet surface of guard cells and in cytoplasmic fluid, resulting in proton generation (17). With SO2 exposure, stomata of tomato, perilla, peanut, spinach, and grape closed (7, 15), whereas stomata of ginkgo and American elm showed opening (10). Stomata of pea plants closed consistently with injurious levels of SO2 and also showed a closing response with noninjurious levels (11).

Light modulation of enzyme activity in higher plants involves generation of membrane-bound vicinal dithiol groups within the chloroplasts, apparently by the reduction of disulfide bonds (2, 3). Sulfitolation of these disulfide bonds is particularly harmful to the plant since the regulation of carbon metabolism will be disrupted. The capacity for light modulation of enzyme activity in chloroplasts was reduced or eliminated when the chloroplast membrane fraction was exposed to sulfite in light or darkness (3). Anderson and Duggan (4) investigated the effect of sulfite on the activities of light-modulated enzymes in leaf chloroplast systems and the effect of SO2 on enzyme activity in whole plants. Light modulation of enzyme activity was diminished by brief exposure to sodium sulfite or atmospheric SO2.

The effects of SO2 on chloroplast enzyme systems have been studied using the hydration products of bisulfite and sulfite (18). The toxic effects of SO2 on components of the photosynthetic apparatus are various. Exposure to SO2 has been reported to inhibit O2 evolution (16), photophosphorylation (16, 18), and CO2 fixation (18). Sulfite concentrations as low as 125 μm favored the formation of low-mol-wt forms of NAD- and NADP-malate dehydrogenases (18).

Anderson and Avron (3) showed that arsenite reacts with vicinal dithiols and reduces or eliminates the light modulation of enzyme activity in pea leaf chloroplasts. CO2 fixation in spinach chloroplasts was inhibited by arsenite under conditions where electron transport and photophosphorylation are not affected (3).

Recently, we showed that light modulates the activity of a number of key enzymes involved in stomatal movement (13). The experiments reported here indicate that the sulfite or arsenite effect on stomatal opening in the light is a consequence of the inhibition of light modulation of enzyme activity in pea leaf epidermis.

MATERIALS AND METHODS

Plant Material. The fully expanded first pair of leaves from 10- to 12-d-old pea (Pisum sativum L. var Little Marvel) plants grown in vermiculite in a greenhouse, during winter, were used for the studies. Preparation of epidermal strips was carried out as described by Rao and Anderson (13).

Incubation of Epidermal Strips. The epidermal strips (5 × 5 mm) were transferred to small Petri dishes (5 cm) containing 10 ml of treatment solution. The solution contained 10 mM Pipes-KOH buffer (pH 6.8), 50 mM KCl, 0.1 mM CaCl2, and with or without 10 μM sodium sulfite or sodium arsenite. The strips were incubated at 25°C in light or darkness in a waterbath. The light intensity was 140 w·m−2 (Yellow Springs Instruments Kettering model 65 Radiometer), provided by two General Electric 120-v, 150-w reflector flood lamps. Stomatal aperture was measured at regular time intervals under a microscope (×1000) using a precalibrated ocular micrometer. At least 10 strips were chosen from each treatment, and the widths of five stomata selected at random from each strip were recorded.

Preparation of Epidermal Tissue Extracts. Epidermal tissue incubated as described above was then homogenized in a precooled homogenizer with 7 ml of the extraction medium containing 50 mM Tris (pH 8.3), 1 mM EDTA, and 10 mM MgCl2. After incubation in sulfite or arsenite, the epidermal tissue was washed twice with the extraction medium to eliminate surface contamination of the tissue with sulfite or arsenite. The homogenate was centrifuged at 1000g for 10 min, and the supernatant was retained for enzyme and protein assay.

Enzyme Assays. PEP carboxylase (EC 4.1.1.31) and NADP-malate dehydrogenase (EC 1.1.1.37) were assayed as described in Rao and Anderson (13). NAD-malate dehydrogenase (EC 1.1.1.37) was assayed as was the NADP-linked enzyme, but with
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The influence of much higher concentrations of sulfite or arsenite on PEP carboxylase and NAD-malate dehydrogenase is shown in Figure 3. PEP carboxylase was slightly inhibited by sulfite and markedly activated by arsenite (at lower concentrations). The activity of NAD-malate dehydrogenase was markedly decreased by the increase in the concentration of sulfite in the reaction mixture, while arsenite at very high concentrations (500 μM) gave only slight inhibition of the enzyme activity. At the low concentrations used in the experiments dealing with stomatal opening, no effects on enzyme activity were seen. Clearly, the effect of these compounds on stomatal opening cannot be attributed to a direct effect on these two enzymes.

Sulfite and arsenite effects on light modulation of enzyme activities in epidermis are detailed in Table I. Enzyme activities are expressed on a buffer soluble protein basis. Incubation of epidermis in light for 2 h increased the activity of PEP carboxylase (2-fold) and NADP-malate dehydrogenase (3-fold). The light activation of PEP carboxylase and NADP-malate dehydrogenase was completely inhibited by sulfite or arsenite treatment. The total activity of the above two enzymes was also markedly decreased by sulfite (67% and 79%) and arsenite (50% and 63%).

DISCUSSION

Stomatal movements are caused by changes in guard cell turgor arising from the movement of K+ and H+ with electroneutrality being maintained by movement of Cl− or internal production of malate (12, 14). In the previous paper, we reported the possible involvement of light modulation of enzymes in stomatal movement in pea leaf epidermis (13). The present study indicates that the inhibition of light modulation of enzymes is one of the primary sensitive sites of action for the sulfite- or arsenite-induced stomatal closure in light. The inhibition of stomatal opening by sulfite in light in pea leaf epidermal strips is consistent with the observations of Olszyk and Tibbits (11) on intact pea leaves with SO2 fumigation. The stimulation of stomatal opening by arsenite in darkness indicates that the site of action for sulfite and arsenite in darkness might be different. The reasons for the observed increase in the activity of PEP carboxylase at lower concentrations of arsenite added to the reaction mixture are not known. But the decrease in the activity of NAD-malate dehydrogenase with increasing concentrations of sulfite is consistent with the observations of Ziegler (18).

Several mechanisms for the regulation of stomatal aperture changes during exposure were proposed on the basis of the present knowledge of factors controlling stomatal aperture, and the physiological and biochemical effects of SO2 within plants (6). SO2 exposure decreased the pH of the cell cytoplasm (17). Acidification of the cytoplasm could result in changes in the structure of the diphosphopyridinenucleotide as substrate.

Protein Assay. Protein was estimated as described in the preceding paper (13).

Reagents and Seed Material. All biochemicals were obtained from Sigma. Other chemicals were analytical reagent grade. Pea seeds were obtained from Northrup and King, Chicago.

RESULTS

The effect of sulfite and arsenite on the time course of stomatal opening in light or darkness in the epidermal strips of pea leaf is shown in Figure 1. The stimulation of stomatal opening was greater in the light than in darkness. There was more stimulation of stomatal opening at 3 h after incubation than at 2 h. Sulfite or arsenite at 10 μM inhibited the stomatal opening appreciably in light. In darkness, arsenite stimulated the stomatal opening while sulfite showed a slight inhibition.

The effect of different levels of sulfite or arsenite on stomatal opening in light or darkness in isolated epidermal strips is shown in Figure 2. Sulfite or arsenite at 10 μM concentrations elicited a marked inhibition of stomatal opening in light. The higher concentrations (50 and 100 μM) of sulfite or arsenite also inhibited the stomatal opening in light. In the epidermal strips incubated in darkness, sulfite did not show definite effect on stomatal movement, whereas arsenite stimulated the stomatal opening process with the increase in concentration.
membranes, thus inhibiting the influx of $K^+$ necessary to maintain the turgidity and result in closure (14). Higher levels of malic, citric, and isocitric acids were observed in open stomata than in closed stomata (12). The marked inhibition of the light modulation of the enzymes involved in malate formation and metabolism, PEP carboxylase and NADP-malate dehydrogenase, by sulfite or arsenite could result in the decreased production of malate and would lead to the decrease in stomatal opening.

Light modulates the activity of several enzymes in photosynthetic species ranging from cyanobacteria to higher plants (2). Anderson and Avron (3) envisaged that there might be thylakoid-bound LEM located at the reducing side of PSI to mediate the transfer of electrons from the photosynthetic electron transport system. They found that if the thylakoids were poisoned by being exposed to arsenite or sulfite, light modulation of enzyme activity was inhibited in the reconstituted system. Sulfite was effective whether the thylakoids were exposed to the poison in light or darkness. But in darkness, arsenite had no effect and could be washed out of the thylakoids. These experiments suggested that a disulfide bond was involved in light modulation. Alscher-Herman (1) has found that light activation of fructose 1,6-bisphosphatase in an $SO_2$-sensitive soybean cultivar is sulfite sensitive, while light activation in a $SO_2$-insensitive cultivar is not affected by sulfite. These experiments indicate that $SO_2$ susceptibility is related to the LEM system.

The presence of both photosystems in guard cells has been demonstrated (12). It was suggested that one of the important physiological changes associated with light inhibition of photosynthetic electron transport might be modulation of enzyme activity in guard cells. In the previous paper, we provided experimental evidence for the possible involvement of light modulation of enzymes in the mechanism of stomatal movement (13). The present study indicates that sulfite and arsenite-induced inhibition of stomatal opening in light may be due to the inhibition of light

![Graph](https://www.plantphysiol.org)
modulation of key enzymes in guard cells.

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LITERATURE CITED