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Sulfite oxidation in, and sulfate uptake from the cell wall of leaves. *In muro* studies.

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K e y w o r d s : Air pollution, apoplast, detoxification, sulfur dioxide, proton concentration.

S u m m a r y

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In muro studies on sulfite oxidation and sulfate uptake were performed using primary leaves of barley. Experiments carried out with leaves infiltrated with solutions containing sulfite and/or sulfate revealed that oxidation of sulfite as well as the uptake of sulfate were strictly dependent on the apoplastic pH; the rates of sulfite oxidation were higher at alkaline than at acidic pH values.

I n t r o d u c t i o n

Plants are able to cope with air pollutants under various circumstances. Avoidance, detoxification, and repair mechanisms may be involved in their survival in a moderately polluted atmosphere (HÄLLGREN al. 1982, RENNENBERG al. 1990). In addition to oxidation and reduction of sulfite in cytoplasmic organelles (ROTHERMEL & ALSCHER 1985, DITTRICH & al. 1992), oxidation of sulfite also occurs in the cell wall and may prevent toxic effects of SO_2. The importance of the plant cell wall is obvious, as this "compartment" represents the first phase, gaseous pollutants have to cross on their diffusional path to the leaf cells. *In vitro* studies of sulfite oxidizing activities with isolated cell wall enzymes have shown that sulfite oxidation rates can be high under optimum conditions (PFANZ & al. 1990, PFANZ & OPPMANN 1991). The present paper was aimed to study sulfite oxidation in intact leaves.

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Materials and Methods

Barley (*Hordeum vulgare* L. var. Alraune) was grown in soil in a growth cabinet (12 h light at 21° C; 15° C in the dark). 10 days old primary leaves were used for the experiments. After cutting, the leaves were infiltrated with the test solutions. For sulfite oxidation the test solutions contained 100 mM sorbitol, 1 mM $CaCO_3$, 4 mM KCl, 4 mM $KHCO_3$, and 50 mM buffer. The buffer used at the pH 4-6 range was Mes, and at pH 7 and 8 Hepes. If not stated otherwise, the sulfite concentration was 2 mM. For sulfate uptake studies, solutions were identical, but Na_2SO_4 was used instead of Na_2SO_3. After infiltration, the leaves were incubated in humid chambers at 22 °C in the light (800 µmol m^{-2} s^{-1}). At different time intervals, leaves were carefully blotted dry and centrifuged, and an intercellular washing fluid was obtained as described in PFANZ & OPPMANN 1991. Aliquots of this liquid were used to determine sulfate concentrations using anion chromatography.

Results and Discussion

The ability to oxidize sulfite to sulfate was largely dependent on the pH of the aqueous phase of the cell wall (Fig. 1). Experimental adjustment of the proton concentration in the cell wall was obtained by the infiltration of buffers at high concentrations (50 mM; cf. PFANZ & al. 1988). Initial sulfite oxidation was very rapid at neutral to alkaline pH values (pH 7-8) and slower at slightly acidic pH values (pH 4-6). After 3 to 4 min the oxidation ceased. No further accumulation of sulfate was observed, but a decrease in sulfate in the washing fluids was found instead (for explanation see Fig. 3). Although 2 mM sulfite were used for infiltration in the experiment of Fig. 1, the maximum concentration of sulfate measured in the re-extracted solution was only 1.5 mM. This is, on the one hand, due to the uptake of sulfate (and sulfite, cf. HAMPP & ZIEGLER 1977, SPEDDING & al. 1980, PFANZ & al. 1987), but may also be due to the fact that the infiltrated solution is diluted inside the leaf with the cell wall water (for barley: approx. 274 µl intercellular air space/80 µl aqueous cell wall volume). As the normal apoplastic sulfate concentration is approximately 0.5 mM in barley leaves, calculation gives a concentration of maximum 1.7 mM extractable sulfate after complete oxidation of 2 mM sulfite. Measurements of sulfite oxidation in the cell wall at different sulfite concentrations demonstrated that the initial oxidation rates were high (Fig. 2) and increased linearly with the sulfite concentration applied (not shown). Saturation was not reached within the concentration range applied (5 mM sulfite). This finding is in accordance with data published earlier on sulfite oxidation *in vitro* (PFANZ & OPPMANN 1991). Also here, a decrease in the amount of sulfate formed was obtained after continuing incubation. This leads to the conclusion that part of the sulfate formed is taken up by the leaf cells via the plasmalemma.

To gain more insight into the disappearance of sulfate in our experimental system, leaves were infiltrated with solutions containing sulfate (2 mM). As in the sulfite oxidation experiments, uptake was studied with illuminated leaves at room temperature (cf. JESCHKE & SIMONIS 1965). Fig. 3 clearly demonstrates that there was an uptake of sulfate within the same time scale as in the experiments described above (Figs. 1 and 2). The disappearance of sulfate was very rapid during the first

minutes of the experiment and decreased thereafter. Furthermore, uptake was dependent on the pH of the solution applied (see also Fig. 1). Uptake rates were higher at higher proton concentrations. This is in agreement with sulfate uptake data in root systems, isolated cells and isolated cell organelles (NISSEN 1971, VANGE & al. 1974, SMITH 1975, SPEDDING & al. 1980, Cram 1990). Also for intact *Lemna* plants evidence for a proton/sulfate co-transport is provided (LASS & ULLRICH-EBERIUS 1984).

In conclusion, *in situ* sulfite oxidation in the cell wall of leaf cells can indeed be measured. In contrast to the situation with isolated peroxidases, the *in muro* reaction does not need additional cofactors (like hydrogen peroxide or lignin precursors; PFANZ & OPPMANN 1991). The sulfate formed in this reaction is taken up by the leaf cells; therefore sulfate is not thought to accumulate in the leaf apoplast to a large extent (cf. KAISER & al. 1989).

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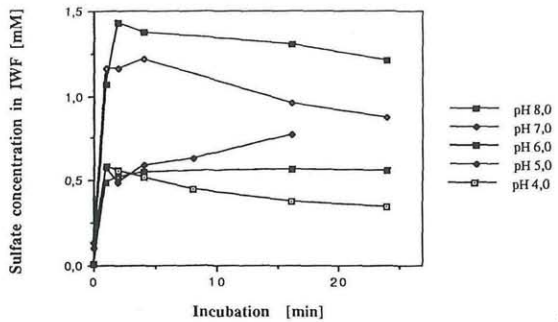

Fig. 1. Effect of pH on *in muro* sulfite oxidizing acitivities in the cell wall of illuminated barley leaves. The sulfate concentration of the intercellular washing fluid after infiltration, incubation and re-extraction is given. The initial sulfite concentration before infiltration was 2 mM.

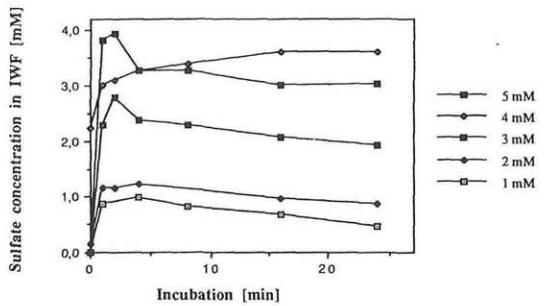

Fig. 2. Time course of sulfite oxidation in the cell wall of illuminated barley leaves at different sulfite concentrations. The sulfate concentration of the intercellular washing fluid after infiltration, incubation and re-extraction is given. Experiments were performed at pH 7.

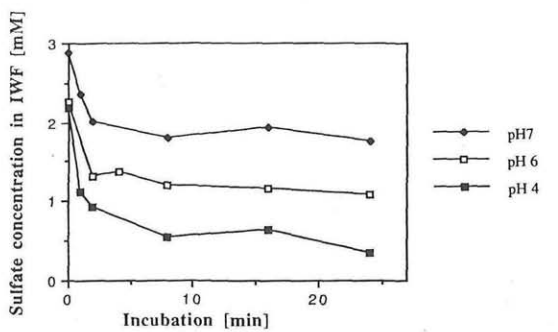

Fig. 3. Uptake of sulfate from the apoplast into the cells of 10 day old primary leaves of barley. Uptake was recorded as disappearance of sulfate from solutions infiltrated into and re-extracted from illuminated leaves (22° C). The sulfate concentration in the infiltration medium was 2 mM.