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The Flux of Atmospheric H_2S to Spinach Leaves can be affected by the Supply of O-Acetylserine

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Summary

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H_2S can be actively taken up and metabolized by plant leaves and at high atmospheric concentrations, its flux to shoots was limited by the internal resistance of the leaves to H_2S. Feeding of O-acetylserine, the substrate for O-acetylserine sulfurylase, to detached leaves resulted in a decreased internal resistance of the leaves, and hence an increased H_2S flux to the leaves. This demonstrated that the flux of H_2S to leaves is determined by its metabolism.

Introduction

Atmospheric H_2S is rapidly taken up and metabolized by plants; fumigation generally results in increased levels of water-soluble, non-protein sulfhydryl compounds (DE KOK 1990). H_2S flux to leaves was strongly dependent on metabolic activity of the tissue and on the atmospheric H_2S concentration (DE KOK & al. 1989, 1991). At concentrations of H_2S lower than 0.3 μl l^{-1}, its flux was limited by stomatal conductance, demonstrating a low internal resistance of the leaves to H_2S. At higher H_2S concentrations, the flux was limited by an increase in internal resistance (DE KOK & al. 1989, 1991). It was proposed, that the internal resistance to H_2S is determined by its rate of incorporation into cysteine (DE KOK & al. 1989, 1991). H_2S may be incorporated into cysteine by O-acetylserine sulfurylase, using O-acetylserine (ANDERSON 1980). There is circumstantial evidence that cysteine desulfhydrase, operating in reversed mode, might also be

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involved (SCHÜTZ & al. 1991).

In order to determine the significance of O-acetylserine sulfurylase and the availability of its substrate O-acetylserine as the rate limiting step for H_2S flux to leaves at high atmospheric concentrations, we tested the effect of the addition of O-acetylserine on H_2S flux and sulfhydryl accumulation in detached spinach leaves exposed to 0.25 or 0.75 µl l^{-1} H_2S in the light.

Materials and Methods

Spinach plants were grown as described previously (BUWALDA & al. 1990). In the experiments, the first leaf pair of seedlings was used 21 to 23 days after germination. One hour before transferring of the leaves to the fumigation cuvette, the leaves were detached as described previously (BUWALDA & al. 1990) and placed with their petioles in tap water or in 10 mM O-acetylserine in tap water (pH adjusted to 7.0 with NaOH) in 20 ml glass vials. H_2S flux and water vapour efflux were measured at 20°C, a photon flux density of 160 µmol m^{-2} s^{-1} (within the 400 - 700 nm range) and a relative humidity of 35 %, as described by DE KOK & al. 1991. Within 1 min. after opening the cuvette, the fresh weight of the leaves was determined, and the leaves were frozen in liquid N_2. Water-soluble, non-protein sulfhydryl compounds in freeze-dried material were assayed by HPLC as described previously (BUWALDA & al. 1988, 1990).

Results and Discussion

Our experiments demonstrated that both the supply of O-acetylserine and H_2S did not affect the transpiration rate ($J(H_2O)$) of detached spinach leaves (Table 1). The values of diffusive conductance of detached leaves to aqueous vapour efflux ($g(H_2O)$) were close to those reported for intact spinach plants (DE KOK & al. 1989). The H_2S flux ($J(H_2S)$) to detached leaves at an atmospheric concentration of 0.75 µl l^{-1} H_2S was only slightly higher than that at 0.25 µl l^{-1} (Table 1). The ratio of H_2S deposition velocity ($g(H_2S)$, defined as the H_2S flux divided by the atmospheric H_2S concentration (DE KOK & al. 1991)) to $g(H_2O)$ at an atmospheric concentration of 0.25 µl l^{-1} H_2S was 0.7 or higher, indicating a low internal resistance of the leaves to H_2S. However, at 0.75 µl l^{-1} this ratio was lower than 0.27, which indicated a high internal resistance. These observations on detached leaves are in close agreement with results for shoots of intact spinach plants (DE KOK & al. 1991).

Table 1. The effect of O-acetylserine (OAS) feeding on the flux of H_2S to detached spinach leaves. Data from 2 experiments on $J(H_2O)$, transpiration rate; $J(H_2S)$, H_2S flux to leaves; $g(H_2S)$, H_2S deposition velocity; $g(H_2O)$, diffusive conductance of leaves to aqueous vapour efflux, are presented.

OAS (mM)	H_2S ($\mu l\ l^{-1}$)	$J(H_2O)$ (mmol g FW^{-1} h^{-1})		$J(H_2S)$ (μmol g FW^{-1} h^{-1})		$g(H_2S)/g(H_2O)$	
0	0.25	10.1	8.1	0.10	0.09	0.69	0.84
0	0.75	11.4	9.3	0.14	0.12	0.20	0.27
10	0.25	9.6	7.7	0.10	0.10	0.74	1.04
10	0.75	10.8	8.5	0.34	0.28	0.70	0.77

Feeding O-acetylserine to detached spinach leaves had no substantial effect on $J(H_2S)$ and $g(H_2S)/g(H_2O)$ at an atmospheric H_2S concentration of 0.25 $\mu l\ l^{-1}$. However, at 0.75 $\mu l\ l^{-1}$ O-acetylserine feeding strongly increased both $J(H_2S)$ and the ratio of $g(H_2S)/g(H_2O)$. The latter finding demonstrated, that at a high atmospheric H_2S concentration, its metabolism is restricted by the availability of substrate for O-acetylserine sulfurylase. Again, this strongly supports the suggestion, that H_2S flux directly depends on its metabolism in the plants. Besides, our results indicated that the internal resistance of leaves to H_2S may not only be determined by the affinity of the enzyme(s) involved in cysteine synthesis but also, at least in the case of O-acetylserine sulfurylase, by the availability of its substrate. Further research is needed to establish the importance of cysteine desulfhydrase in the fixation of H_2S (SCHÜTZ & al. 1991).

It was calculated that 60 to 90 % of the absorbed H_2S could be revealed in the sulfhydryl fraction (data from tables 1 and 2), again demonstrating the significance of direct metabolism of H_2S. After a one hour H_2S-fumigation, cysteine and glutathione accumulated to the same extent (Table 2). Detached leaves also accumulated detectable amounts of γ-glutamyl-cysteine in the light, in contrast to leaves of intact plants (BUWALDA & al. 1988). The increased flux of H_2S to leaves in response to O-acetylserine at 0.75 $\mu l\ l^{-1}$ H_2S resulted in a relatively large increase in the cysteine fraction.

Table 2. The effect of a one hour O-acetylserine (OAS) feeding and H_2S fumigation on the sulfhydryl content of detached spinach leaves. Thiol content is expressed as μmol g FW^{-1} and represents the mean of 2 experiments with 3 independent measurements on 3 leaves each. In each column, values followed by the same character are not significantly different ($p > 0.05$). Control values were not significantly different from the initial values.

OAS (mM)	H_2S ($\mu l\ l^{-1}$)	Cysteine	γ-Glutamyl-cysteine	Glutathione	Total SH
0	0.00	0.01 ± 0.00a	0.00 ± 0.00a	0.14 ± 0.03ab	0.15 ± 0.03a
0	0.25	0.04 ± 0.01b	0.01 ± 0.00b	0.16 ± 0.01bc	0.21 ± 0.02b
0	0.75	0.04 ± 0.01b	0.02 ± 0.01cd	0.16 ± 0.01bc	0.22 ± 0.02b
10	0.00	0.01 ± 0.00a	0.00 ± 0.00a	0.12 ± 0.01a	0.13 ± 0.01a
10	0.25	0.04 ± 0.01b	0.02 ± 0.01bc	0.17 ± 0.02c	0.23 ± 0.01b
10	0.75	0.16 ± 0.03c	0.03 ± 0.01d	0.17 ± 0.03c	0.35 ± 0.06c

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