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Effects of Light and Atmospheric H_2S on Amino Acid Composition in Spinach Leaves

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S u m m a r y

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The effect of fumigation with 0.25 $\mu l\ l^{-1}$ H_2S on changes in the composition of the free amino acid pool showed a consistent decrease in serine, precursor for cysteine synthesis, in the light and to a lesser extent in the dark. Comparison of the serine data with data on thiol level, from experiments performed under the same experimental conditions, shows that the decrease in serine is of the same order of magnitude as the increase in thiols over the same period. This suggests that the decline in serine level when plants are fumigated with H_2S is the result of a drain of serine for thiol synthesis.

Introduction

Exposure of plants to atmospheric H_2S may cause reduction in growth at concentrations of 0.03 $\mu l\ l^{-1}$, and higher (DE KOK & al. 1998). H_2S is readily absorbed and metabolised by plants and leads to accumulation of free thiol compounds, including glutathione (GSH: γ-Glu-Cys-Gly), γ-glutamyl-cysteine and cysteine (BUWALDA & al. 1988, 1994). In spinach, accumulation of γ-glutamyl-cysteine was only found in the dark; after a dark-light transition it was incorporated into glutathione (BUWALDA & al. 1994). Addition of external glycine prevented the accumulation of γ-glutamyl-cysteine in the dark, and resulted in accumulation of GSH (BUWALDA & al. 1990, 1993). Since synthesis of GSH requires specific amino acids, its accumulation in H_2S exposed plants forms a sink for free amino

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acids. VAN DIJK & al. 1986 found that fumigation of spinach plants with 0.25 H_2S $\mu l\ l^{-1}$ resulted in changes in the composition of the free amino acid pool during the light period. Fumigation with a ten times higher concentration of H_2S, which caused leaf necrosis, also resulted changes in composition of the free amino acid pool (STEUBING & JÄGER 1978).

The aim of the present investigation was 1) to study the effect of H_2S in the light and in the dark on changes in free amino acid levels, and 2) to relate these changes to the utilisation of amino acids for synthesis of GSH.

Materials and Methods

Spinach plants were grown as described previously (BUWALDA & al. 1990). The first leaf pair of three- to four-week old plants was used for the experiments. Plants were exposed to H_2S in fumigation cabinets as described by MAAS & al. 1985. Experiments were performed with intact plants, or detached leaves, prepared and treated as described previously (BUWALDA & al. 1990). The plant material was harvested and stored after BUWALDA & al. 1990. Levels of free amino acids in freeze-dried material were assayed by HPLC as described in BUWALDA & al. 1990.

Results and Discussion

Both light and the exposure to atmospheric H_2S (0.25 $\mu l\ l^{-1}$) affected the composition of the free amino acid pool in the first leaf pair of intact spinach plants after 12 h in the light (PAR 180 $\mu mol\ m^{-2}\ s^{-1}$) or in the dark. In control plants (0 $\mu l\ l^{-1}$ H_2S) the levels of aspartate, serine, glycine and glutamine were higher in the light than in the dark, while the levels of alanine, glutamate, isoleucine, leucine, tyrosine and asparagine were lower in the light than in the dark (Table 1). These effects were found consistently in two other experiments involving light-dark comparisons (results not shown). The effect of light on the other amino acids in Table 1 was small, and not consistent between the experiments. A light dependent increase in serine and glycine levels was found for pea leaves (BAUER & al. 1977). However, several other light-dependent changes in amino acid composition did not match the effects reported here for spinach leaves, indicating that a generalisation of these observations is not possible.

After fumigation with 0.25 μl^{-1} H_2S for 12 h, serine content level was decreased significantly, especially in the light, and to a lesser extent in the dark. This effect was found consistently in all three experiments. No consistent and/or significant changes in glycine level were found. In the light the increase in methionine level in the H_2S plants was small but significant, which was not found by VAN DIJK & al. 1986. In the light glutamate also increased, but this effect was not consistent. The effects of H_2S on the other amino acids in Table 1 were generally small, and not consistent between the experiments. The decrease in serine level after fumigation with H_2S in the light was very fast. Another experiment, in which plants were fumigated for 48 h in either continuous light or darkness, showed that serine level of the plants fumigated in the light decreased significantly within 1.5 h. After 3 h a level close to those of the other treatments was reached (Fig. 1). The effect of H_2S

on serine level in the light is in agreement with the results of VAN DIJK & al. 1986 for spinach upon fumigation with 0.25 μl l^{-1} H_2S for 48 h with a 12 h day and 12 h night cycle. They found a significant increase in glycine, no change in glutamate, and changes in other amino acids, which were not found in the present study. The discrepancy between the present results and those of VAN DIJK & al. 1986 may be due to the fact that different leaf pairs were used. DE KOK 1989 reported differences in thiol accumulation between leaf pairs.

Table 1. Effects of fumigation with 0 (control) or 0.25 μl l^{-1} H_2S for 12 h on the composition of the free amino acid (F.A.A.) pool of the first leaf pair of spinach plants. Values (μmol g^{-1} DW ± SD) are the means of four independent measurements, with two plants in each. For each amino acid, mean values followed by the same character are not significantly different ($P>0.05$).

	Light		Dark	
F.A.A.	Control	H_2S	Control	H_2S
Ser	12.9 ± 2.2^{c}	7.5 ± 1.7^{b}	6.3 ± 0.4^{b}	5.1 ± 0.6^{a}
Gly	1.5 ± 0.1^{b}	2.2 ± 1.1^{b}	0.8 ± 0.2^{a}	0.6 ± 0.2^{a}
Glu	20.7 ± 2.4^{a}	26.0 ± 0.9^{b}	33.4 ± 1.8^{c}	43.8 ± 1.2^{c}
Met	0.1 ± 0.0^{a}	0.4 ± 0.1^{b}	0.1 ± 0.0^{a}	0.2 ± 0.1^{a}
Ala	3.0 ± 0.4^{a}	2.9 ± 0.2^{a}	9.1 ± 1.5^{b}	9.3 ± 0.9^{b}
Arg	0.3 ± 0.0^{b}	0.2 ± 0.0^{a}	1.2 ± 0.2^{c}	1.8 ± 0.3^{d}
Asp	24.1 ± 2.9^{b}	23.0 ± 2.9^{b}	10.8 ± 1.4^{a}	10.6 ± 2.3^{a}
His	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	0.5 ± 0.2^{b}	0.6 ± 0.3^{b}
Ileu	0.9 ± 0.2^{a}	1.1 ± 0.3^{a}	2.2 ± 0.4^{b}	2.9 ± 0.4^{c}
Leu	0.5 ± 0.0^{a}	0.6 ± 0.1^{a}	2.9 ± 0.6^{b}	3.9 ± 0.3^{b}
Lys	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	1.6 ± 0.5^{b}	1.6 ± 0.6^{b}
Phe	0.8 ± 0.1^{a}	1.2 ± 0.3^{ab}	1.3 ± 0.3^{bc}	1.7 ± 0.3^{c}
Pro	1.0 ± 0.2^{a}	1.9 ± 1.1^{a}	1.0 ± 0.3^{a}	1.0 ± 0.2^{a}
Thr	2.2 ± 0.4^{b}	1.2 ± 0.0^{a}	2.1 ± 0.5^{b}	2.5 ± 0.7^{b}
Tyr	0.8 ± 0.2^{a}	0.9 ± 0.2^{a}	1.1 ± 0.2^{ab}	1.5 ± 0.4^{b}
Val	2.0 ± 0.2^{a}	2.0 ± 0.4^{a}	3.3 ± 0.6^{b}	4.1 ± 0.4^{c}
Asn	2.5 ± 0.3^{b}	1.8 ± 0.2^{a}	4.9 ± 0.4^{c}	5.6 ± 0.4^{d}
Gln	23.4 ± 3.7^{b}	24.6 ± 4.2^{b}	13.2 ± 2.9^{a}	14.5 ± 2.8^{a}

The effect of H_2S on the composition of the free amino acid pool might be related to the extra sink for of the amino acids involved in GSH synthesis, viz. serine, glycine and glutamate. The only consistent effect found was a marked decrease in serine, the precursor for cysteine synthesis, in the light and to a lesser extent in the dark. Comparison of the present data with data on thiol level, from experiments performed under the same experimental conditions, shows that the decrease in serine level in the light (5.4 μmol g^{-1} DW, Table 1) is of the same order of magnitude

as the increase in thiols over the same period (7.5 μmol g^{-1} DW; BUWALDA & al. 1993). For dark-fumigated plants the data were 1.2 and 5.0 μmol g^{-1} DW for the decrease in serine, and the increase in GSH, respectively. The decrease in serine level, therefore, might be related to the drain of serine for the synthesis of GSH. Other experiments with spinach showed that the availability of O-acetylserine, the substrate for cysteine synthase, the enzyme most likely involved in the fixation of H_2S by the plant (DE KOK & al. 1998) apparently limited the rate of uptake of H_2S at a concentration of 0.75 μl l^{-1}, but not at 0.25 μl l^{-1} (BUWALDA & al. 1992).

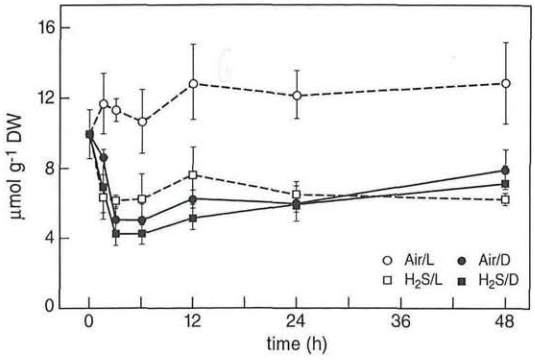

Fig. 1. Effect of fumigation with 0 (control; ○) or 0.25 μl l^{-1} H_2S (□) on serine level in continuous light (open symbols) or in the dark (closed symbols). Fumigation was started after the plants had been in the light for 3 h. Data represent the means of four independent measurements with pooled material from 3 plants in each sample (± SD).

The present experiments showed that the glycine pool was not affected by H_2S and much smaller than the serine pool. In the dark pool size was lower than in the light, and probably limiting for GSH synthesis, since addition of glycine prevented the accumulation of γ-glutamylcysteine (BUWALDA & al. 1988). Nevertheless, there is still a considerable accumulation of thiols in the dark (BUWALDA & al. 1993), which shows that a serine source other than through the photorespiratory pathway may be involved in GSH synthesis. However, it should be kept in mind that 1) a level is a reflection of a dynamic pool, which for serine as well as for glycine is also affected by light, and 2) levels in subcellular compartments are not known. Therefore, more research on fluxes through pathways, and subcellular concentrations of the various compounds is needed before conclusions on the relation between serine and glycine levels on the one hand, and GSH levels on the other hand, can be drawn.

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