Phyton (Horn, Austria) Special issue: "Sulfur-Metabolism"	Vol. 32	Fasc. 3	(51)-(54)	18. 12. 1992

Specificity of Glutathione (GSH) Mediated Inhibition of Sulfate Uptake into Excised Tobacco Roots

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K e y w o r d s : *Nicotiana tabacum*, amino acids, ophthalmic acid, γ-glutamyl-cysteine, oxidized glutathione.

S u m m a r y

GUNZ G., HERSCHBACH C. & RENNENBERG H. 1992. Specificity of glutathione (GSH) mediated inhibition of sulfate uptake into excised tobacco roots. - Phyton (Horn, Austria) 32 (3): (51)-(54).

The specificity of GSH mediated inhibition of sulfate uptake has been investigated by measuring sulfate transport into excised tobacco roots. In the presence of the GSH derivatives γ-glutamyl-cysteine (γ-Glu-Cys), ophthalmic acid (OPA) and oxidized glutathione (GSSG) sulfate uptake decreased significantly. These results indicated that GSH may not be a highly specific inhibitor of sulfate transport. Also the sulfur-free amino acids L-alanine (Ala), L-aspartate (Asp) and L-glutamine (Gln) inhibited sulfate uptake into tobacco roots significantly. This observation provided evidence for an interpathway control of nitrogen and sulfur metabolism at the level of sulfate uptake.

Introduction

Sulfur is available to plants in the soil predominantly in the form of sulfate. It is taken up by the roots (RENNENBERG 1984) and is transported with the transpiration stream to the leaves (RENNENBERG & al. 1979), where it is reduced and incorporated into organic sulfur compounds (ANDERSON 1980, 1990). Reduced sulfur is translocated from the shoot to the root in the phloem mainly in the form of GSH to cover the demand of root tissues for reduced sulfur (RENNENBERG & LAM-

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OUREUX 1990). From investigations with tissue cultures it has been suggested that GSH is involved in the interorgan regulation of sulfate uptake (RENNENBERG & al. 1988). In heterotrophic but not in green tobacco cells sulfate influx is inhibited by GSH (RENNENBERG & al. 1988); GSH also inhibited sulfate uptake and xylem loading of sulfate in excised tobacco roots (HERSCHBACH & RENNENBERG 1991). The present investigations were performed to test whether sulfate uptake of excised tobacco roots is specifically inhibited by GSH.

Materials and Methods

Tobacco roots (*Nicotiana tabacum* L. var. "Samsun") were grown from seeds in a 1/5 modified MURASHIGE & SKOOG 1962 medium for a total of 27 to 29 days. Cultivation was conducted in an environmental growth chamber under long day conditions as described by HERSCHBACH & RENNENBERG 1991. Uptake, xylem loading and exudation of ^{35}S-sulfate in excised roots was investigated using an incubation chamber originally described by PITMAN 1971. Sulfate transport into roots was initiated by addition of ^{35}S-sulfate to the medium in compartment A of the incubation chamber (HERSCHBACH & RENNENBERG 1991). To determine sulfate exudation out of the cut ends of the roots an aliquot of the solution in compartment C of the incubation chamber was removed every hour and replaced by the same amount of non radioactive transport medium. Incubation was terminated after 6 h. Roots were cut into 3 segments corresponding to the compartments A, B and C. Radioactivity of the root segments and of the solution of compartment C was determined by liquid scintillation counting.

Results and Discussion

Exposure of excised tobacco roots to 0.1 mM GSH reduced sulfate uptake and sulfate exudation to less than 20 % of the controls within 6 h (HERSCHBACH & RENNENBERG 1991). In the present study the influence of various GSH derivatives on the sulfate transport of excised tobacco roots was examined. As shown in Fig. 1, γ-Glu-Cys (0.1 mM), a metabolic product of GSH, inhibited sulfate uptake to 16 % and sulfate exudation out of the cut ends of the roots to 10 % of controls within 6 h. Also the relative amount of the sulfate taken up that was loaded into the xylem, was reduced from 48 % to 34 %. As γ-Glu-Cys is an intermediate product of GSH the effect of γ-Glu-Cys might be brought about by GSH. On the other hand, the inhibition of sulfate uptake by GSH might be caused by γ-Glu-Cys. GSSG, the oxidized form of GSH, also showed an inhibitory effect on sulfate transport. Sulfate uptake was decreased to 39 % and sulfate exudation to 36 % by incubating the roots in 0.1 mM GSSG for 6 h. There was also a decline of the relative amount of the sulfate taken up that was loaded into the xylem (Fig. 1). These findings supported the idea that the thiol moiety of GSH is not essential for its inhibitory effect on sulfate transport. To examine this assumption, roots were incubated with 0.1 mM OPA, an analog of GSH without thiol moiety. During the 1 h incubation, sulfate uptake decreased to 24 % and sulfate exudation to 16 % of controls. Also the relative amount of the sulfate taken up that was loaded into the xylem was reduced. The findings that the GSH derivatives inhibited uptake and xylem loading

of sulfate into tobacco roots suggested that GSH may not be a highly specific inhibitor of sulfate uptake into tobacco roots.

The inhibitory effect of OPA on sulfate transport indicated that products of nitrogen metabolism may influence sulfate uptake into tobacco roots. To test this hypothesis tobacco roots were exposed to various sulfur-free amino acids. After 6 h incubation with 0.1 µM amino acids, the rate of sulfate uptake was significantly inhibited by Ala (42 %), Asp (35 %) and Gln (27 %; fig. 2). Also sulfate exudation out of the cut ends of the roots was reduced. The relative amount of the sulfate taken up that was loaded into the xylem was not affected by these amino acids. These results may be interpreted as a first indication of an interpathway control between sulfur and nitrogen metabolism, not only at the level of reduction as reported by BRUNOLD 1990, but also at the level of uptake.

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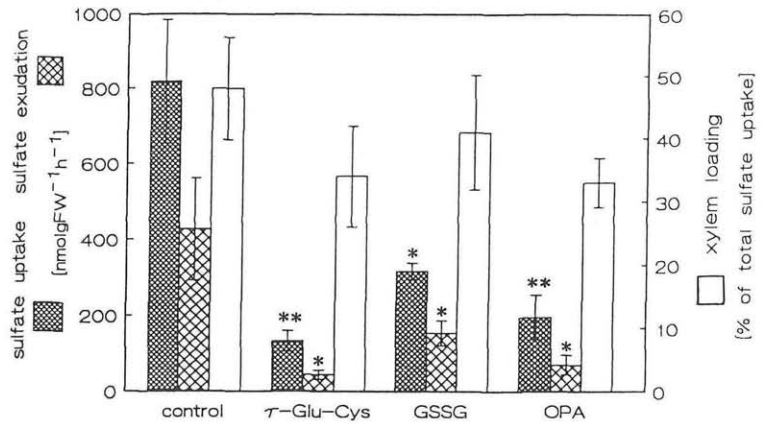

Fig. 1. Effect of GSH derivatives (0.1 mM) on the uptake and xylem loading of sulfate. After 2 h equilibration with sulfur-free transport medium excised tobacco roots were exposed to the GSH derivatives shown. Transport was initiated by addition of ^{35}S-sulfate to the medium. Radioactivity in the root segments and in the medium of compartment C was determined after 6 h incubation by liquid scintillation counting. Data given are means of four independent experiments (± s.d.) with ten roots each. Indices show significant differences to the controls (* = $p \leq 0.05$; ** = $p \leq 0.01$).

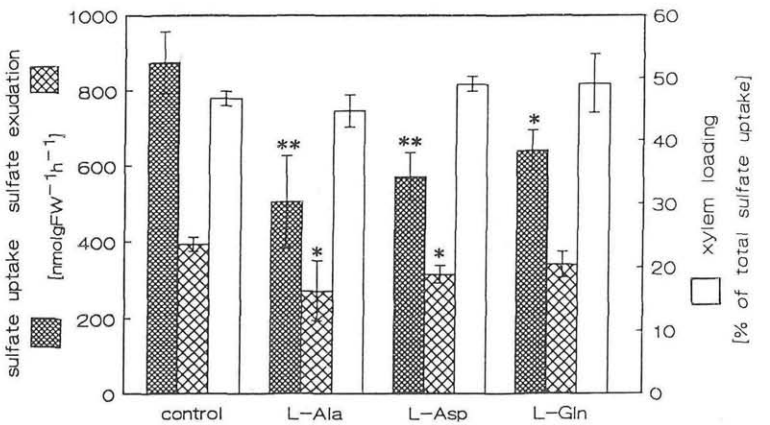

Fig. 2. Effect of amino acids (0.1 µM) on the uptake and xylem loading of sulfate. After 2 h equilibration with sulfur-free transport medium excised tobacco roots were exposed to the amino acids shown. Transport was initiated by addition of ^{35}S-sulfate to the medium. Radioactivity in the root segments and in the solution of compartment C was determined after 6 h incubation by liquid scintillation counting. Data given are means of four independent experiments (± s.d.) with ten roots each. Indices show significant differences to the controls (* = $p \leq 0.05$; ** = $p \leq 0.01$).