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| Phyton (Horn, Austria) Special issue: "Sulfur-Metabolism" | Vol. 32 | Fasc. 3 | (37)-(40) | 18. 12. 1992 |
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Trans-Tonoplast Transport of the Sulfur Containing Compounds Sulfate, Methionine, Cysteine and Glutathione

By

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Key words: Amino acid transport, barley, mesophyll, protoplasts, vacuole.

S u m m a r y

DIETZ K.-J., BRUNE A. & PFANZ H. 1992. Trans-tonoplast transport of the sulfur containing compounds sulfate, methionine, cysteine and glutathione. - *Phyton* (Horn, Austria) 32 (3): (37)-(40).

The role of the vacuole for cellular compartmentation of sulfur-containing compounds was investigated using vacuoles isolated from barley mesophyll protoplasts. In addition to transport functions for sulfate and methionine, a MgATP-activated translocator allows for uptake of oxidized - but not of reduced - glutathione from the surrounding medium. Cysteine is only taken up at a low basal rate which can be attributed to diffusion through the lipid membrane.

M a t e r i a l s a n d M e t h o d s

Barley was grown in soil. Primary leaves of 10 days old plants were used for isolation of mesophyll protoplasts and vacuoles (MARTINOIA & al. 1985). Compartmentation and transport studies were performed as described by KAISER & al. 1989). The total sulfur content was determined with an induced coupled plasma atomic emission spectrometer (ICP), sulfate with an HPLC-based anion chromatograph (cf. KAISER & al. 1988) and glutathione with an enzymatic procedure. For the latter suspensions of isolated mesophyll protoplasts and vacuoles were treated with 3.3 % sulfosalicylic acid (total GSH + GSSG) or additionally with 6 µl triethanolamine and 2 µl vinylpyridine in 150 µl final volume; the enzymatic assay contained phosphate/EDTA-buffer, NADPH, DTNB, sample and 1 Unit glutathione reductase in a final volume of 1 ml. (cf. GRIFFITH 1985). The data for the cytoplasm were calculated by subtraction of the vacuolar from the protoplast values. Transport studies were performed with radioactive substrates (³⁵S) using the silicone oil centrifugation technique as previously described by MARTINOIA & al. 1985 and for amino acids by DIETZ & al. 1990a.

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Results and Discussion

Vacuoles of barley mesophyll cells may contain a considerable portion of the cellular sulfur, mainly in the form of sulfate (cf. RENNENBERG 1984). They also contain some glutathione and methionine (Table 1). Vacuolar cysteine-levels were usually below or close to the detection limit of analysis. In order, to understand the pattern of compartmentation of sulfur-containing compounds, the transport across the tonoplast of isolated vacuoles was studied.

Uptake of ^{35}S -sulfate was measured as described by MARTINOIA & al. 1985. Conditions were chosen according to DIETZ & al. 1990b since ATP and Mg^{2+} at concentrations of 5 and 10 mM had proven to be optimal for anion translocation into the vacuole. Under these conditions, we observed saturation kinetics which were different from the more or less linear concentration dependence described by KAISER & al. 1989. The K_m for uptake was 3.8 mM. Sulfate transport was unidirectional. Endogenous sulfate present in the vacuoles was not mobilized from the vacuoles under any condition tested so far (data not shown).

Table 1. Distribution of total sulfur and sulfur-containing compounds in mesophyll protoplasts isolated from primary leaves of 10 days old barley grown in soil culture. Sulfur compound contents are either expressed as $\mu\text{mol } 10^7 \text{ protoplasts}^{-1} \text{ h}^{-1}$ (total sulfur, sulfate, methionine) or as $\text{nmol } 10^7 \text{ protoplasts}^{-1} \text{ h}^{-1}$ (GSH, GSSG and total glutathione) and represent the mean of 3 to 6 measurements. 10^7 protoplasts originated from 1 g of leaf fresh weight and correspond to 1 mg of chlorophyll; in suspension they occupied a volume of 200 μl .

| Compound | Mesophyll protoplasts | Vacuole | Cytoplasm |
|--------------|-----------------------|-----------------|-----------|
| total sulfur | 6.2 | 2.5 | 3.7 |
| sulfate | 1.5 | 1.3 | 0.2 |
| methionine | 0.27 | 0.16 | 0.11 |
| glutathione | 117 ± 32 | 2.4 ± 0.2 | |
| GSH | 105 ± 40 | 0.93 ± 0.61 | |
| GSSG | 6.2 ± 0.2 | 0.74 ± 0.30 | |
| GSH:GSSG | 17 | 1.3 | |

Table 2. Transport of cysteine and methionine across the tonoplast as compared to arginine, aspartic acid and phenylalanine. Transport activities were related to 10^7 vacuoles and extrapolated from 20 min to 1 h incubation time. The hydropathy index (H) of the amino acids is given according to KYTE & DOOLITTLE 1982. Positive H-values indicate hydrophobic and negative values hydrophilic properties of the corresponding amino acid.

| Amino acid | Transport rate ($\text{nmol } 10^7 \text{ vacuoles}^{-1} \text{ h}^{-1}$) | | | |
|---------------|---|--------------|-----------------------|------|
| | control | ATP/EDTA | ATP/ Mg^{2+} | H |
| arginine | 19 | 279 | 30 | -4.5 |
| aspartic acid | 4 | 16 | 7 | -3.5 |
| glutamine | 25 ± 9 | 58 ± 26 | 31 ± 14 | -3.5 |
| cysteine | 34 ± 5 | 33 ± 4 | 34 ± 2 | 2.5 |
| methionine | 44 ± 25 | 132 ± 24 | not measured | 1.9 |
| phenylalanine | 45 ± 13 | 73 ± 16 | 60 ± 12 | 2.8 |

Uptake of cysteine and methionine was measured by using the same uptake system. There was a significant capacity to transport methionine across the tonoplast (Table 2, cf. DIETZ & BUSCH 1990). Similar to the translocation of other amino acids, ATP stimulated the translocation even in the absence of Mg^{2+} . Transport in the absence of ATP is likely to reflect permeation of the amino acids by diffusion through the membrane. The ATP-activated component is carrier-mediated. It is interesting to note that there was a good correlation between the basal amino acid uptake rate and the hydropathy index (H) of the corresponding amino acid. The basal transport rate of arginine was higher due to a second high affinity/low V_{max} -transport system for arginine and lysine (see MARTINOIA & al. 1991),

In contrast to methionine, basal ^{35}S -cysteine transport into the vacuoles could not be stimulated by ATP. These data indicate that there is no system at the tonoplast that catalyzes the uptake of cysteine.

In another set of experiments, vacuoles were incubated with ^{35}S -glutathione either in the reduced (GSH) or in the oxidized form (GSSG). Figure 1 shows that the uptake of GSH by vacuoles was negligible. However, in the presence of MgATP, linear uptake of GSSG occurred. At a concentration of 1 mM GSSG, the uptake rate was 173 ± 63 nmol 10^7 vacuoles $^{-1}$ h $^{-1}$. Paper chromatography revealed that the radioactive label taken up by the vacuoles migrated with GSSG and neither with GSH nor with a lower molecular weight degradation product of higher mobility (result not shown). No transport was observed in the presence of ATP/EDTA. Thus stimulation of transport of GSSG differs from the activation profile described for the tonoplast amino acid carrier (DIETZ & al. 1990).

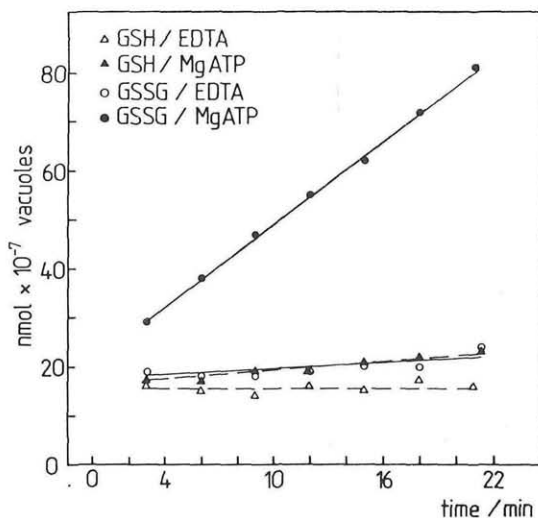


Fig. 1. Uptake of ^{35}S -glutathione by intact vacuoles. Incubation conditions were as denoted in the figure and as described in DIETZ & al. 1990a. Concentrations of GSSG and GSH were 1 mM each. GSSG was taken up only when Mg^{2+} and ATP (10 mM and 5 mM, respectively) were present in the incubation medium.

In contrast to the tonoplast membrane, oxidized glutathione was not transported across the plasma membrane. However, GSH was taken up by mesophyll protoplasts. Uptake rates were similar in the light and in the dark and roughly linear with time. In the presence of 1 mM substrate concentrations, GSH was taken up at a rate of 0.11 and 0.15 $\mu\text{mol } 10^7 \text{ protoplasts}^{-1} \text{ h}^{-1}$ in the light and in the dark, respectively. The corresponding rates for GSSG were between 0.01 and 0.02.

These results illustrate the important function of the vacuole for compartmentation of sulfur containing compounds. They suggest that vacuoles may also be of importance for the redox-balance of the cytoplasm, allowing deposition of GSSG under oxidizing conditions. This hypothesis is supported by the low ratio of GSH to GSSG in the vacuole of 1.3 as compared to 17 in whole protoplast extracts. It will be interesting to investigate the function of the vacuole in glutathione compartmentation under conditions which lead to an accumulation of cellular GSSG (for example after exposure of plants to excess sulfur (BUWALDA & al. 1988) or after inhibition of the catalase (SMITH 1985).

Acknowledgements

This work was supported within the "Sonderforschungsbereiche" 176 and 251 of the "Deutsche Forschungsgemeinschaft".

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Jahr/Year: 1992

Band/Volume: [32_3](#)

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Artikel/Article: [Trans-Tonoplast Transport of the Sulfur Containing Compounds Sulfate, Methionine, Cysteine and Glutathione. 37-40](#)