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Degradation of Glutathione (GSH) in Heterotrophic Tobacco Cells

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Summary

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In addition to cysteine (Cys), glutathione (GSH) and γ-glutamyl-cysteine (γ-EC), the dipeptide cysteinyl-glycine (CG) was detected for the first time as an intermediate of GSH metabolism in heterotrophic tobacco cells. Cellular concentrations of the dipeptide were low (0.07 ± 0.02 µmol g dry weight^{-1}), but showed strong dependency on the sulfur source offered to the cells. Inhibition of GSH synthesis by buthioninesulfoximine (BSO) did not cause measurable changes in γ-EC concentrations, as would be expected if γ-EC is degradation product of GSH. On the other hand, we observed an inhibition of GSH degradation in the presence of the γ-glutamyl-transpeptidase (γ-GT) inhibitor 6-diazo-5-oxo-L-norleucine (DON) indicating a role of this enzyme in GSH degradation via CG.

Introduction

GSH, the predominant long distance transport and storage form of reduced sulfur in many plant species (RENNENBERG & LAMOUREUX 1990) is taken up by tobacco cells (RENNENBERG 1981, STEINKAMP & RENNENBERG 1984, SCHNEIDER & al. 1992). As tobacco cells grow with GSH as the sole source of sulfur its degradation must proceed inside the plant cell to make the reduced sulfur in form of Cys available for numerous biosynthetic processes. Previous investigations showed clear evidence for γ-EC being the first degradation product of GSH in photoheterotrophic tobacco cells (STEINKAMP & RENNENBERG 1985). In animal cells, however, GSH degradation proceeds extracellularly with CG as the first degradation product

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(HAHN & al. 1978, GRIFFITH & MEISTER 1979). The present work provides evidence for an intracellular degradation of GSH via CG in heterotrophic tobacco cells.

Materials and Methods

Tobacco suspension cultures (*Nicotiana tabacum* L. var. "Samsun") were grown for 6 days in a sulfate (1.73 mM) containing, modified Murashige and Skoog-medium (M+S-medium) (LOGEMANN & BERGMANN 1974) on a rotary shaker (100 rpm) in the dark. Previous to preculture (2 days) the cells were harvested on a stainless steel sieve (pore size 65 µm), washed with sulfur deficient M+S-medium and inoculated into either sulfate (1 mM) or GSH (1 mM) containing M+S-medium. For degradation experiments cells were incubated for 24 h in sulfur deficient M+S-medium. In radioactive labelling experiments tobacco cells were exposed to 1 mM ^{35}S-GSH as sulfur source during incubation (24 h). Thiol extraction and analysis were carried out by a modification of the method described by SCHUPP & RENNENBERG 1988. The remaining pellets were used for the extraction of the radioactive labelled cellular protein fraction. Radioactivity was determined by liquid scintillation counting (SCHNEIDER & al. 1992).

Results and Discussion

In heterotrophic tobacco cells four low molecular thiols were detected. In addition to Cys, GSH and γ-EC the cells contained the dipeptide CG. The average intracellular concentrations (µmol g dry weight^{-1}) of these compounds were: Cys 0.37 ± 0.22, GSH 11.46 ± 3.16, γ-EC 0.06 ± 0.03 and CG 0.07 ± 0.02, but showed strong dependency on the sulfur source offered to the cells (data not shown). Thiol pools of the cells increased considerably after preculture of the cells in GSH containing M+S-medium (Fig. 1 A, B; 0 h). A rapid decline in GSH and Cys concentration was observed during the following 24 h period of sulfur starvation (Fig. 1 A), indicating degradation of intracellular GSH and the use of its Cys moiety for biosynthetic processes. Simultaneously, a decrease of the dipeptide concentrations occurred under these conditions (Fig. 1 B). Inhibition of GSH synthesis by BSO slightly increased the decline of GSH as compared to the controls (Fig. 1 A, C). The Cys pool increased during the first 3 h of exposure to BSO and then also started to decline (Fig. 1 C). This observation indicated a short term accumulation of Cys as a result of inhibition of γ-EC-synthetase by BSO, normally depleting the Cys pool by *de novo* γ–EC synthesis. The γ-EC pool itself remained almost constant under these conditions (Fig. 1 D), whereas the CG concentration decreased more strongly than observed in controls (Fig. 1 B, D).

γ-GT is abundant in many plant species (KASAI & LARSON 1980) and was also characterized in the tobacco cells used in the present study (STEINKAMP & RENNENBERG 1984). In animal cells γ-EC is responsible for the first step in GSH degradation (HAHN & al. 1978, GRIFFITH & MEISTER 1979) resulting in CG and γ-glutamyl-amino acid as degradation products. The competitive inhibitor of tobacco γ-GT *in vitro*, DON (STEINKAMP & RENNENBERG 1984), caused a severe inhibition

of GSH degradation in heterotrophic tobacco cells (Fig. 1 E). Simultaneously, the CG concentration (Fig. 1 F) decreased more strongly than observed without inhibitor (Fig. 1 B). These data provided evidence for a participation of γ-GT in GSH degradation in heterotrophic tobacco cells.

When cells were fed ^{35}S-GSH (Tab. 1) Cys was identified as degradation product of GSH. Approximately 12 % of the radioactivity taken up by the cells was incorporated into cellular protein. Radioactive labelling of the dipeptide pools was not observed. Inhibition of GSH synthesis by BSO caused a decrease in the GSH content after 24 h (data not shown) accompanied by a two-fold higher incorporation of radiolable into cellular protein (Tab. 1). The total amount of ^{35}S-GSH taken up by the cells was not altered under these conditions, confirming the earlier findings that GSH is taken up into tobacco cells as intact molecule (RENNENBERG 1981, STEINKAMP & RENNENBERG 1984, SCHNEIDER & al. 1992). The γ-GT inhibitor DON, however, caused a strong inhibition of GSH uptake as indicated by the low specific activities in the Cys and GSH pools. Whether there was also an inhibition of GSH degradation by DON could not be concluded from the experiments performed. The high rate of radioactivity incorporated into protein under these conditions remained obscure.

The present experiments provide evidence for the degradation of GSH via CG catalyzed by γ-GT in heterotrophic tobacco cells, whereas degradation of GSH via γ-EC was observed in photoheterotrophic cells (STEINKAMP & RENNENBERG 1984). More information is necessary to elucidate the role of CG in GSH metabolism in plant cells. Pulse labelling experiments will provide a useful tool for future research on this topic.

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Table 1: Distribution of radioacivity in cellular thiol and protein fractions. Heterotrophic tobacco cells precultured for 2 days in sulfate containing M+S-medium were incubated for 24 h with ^{35}S-GSH in the presence or absence of inhibitor. Subsequently, the cells were harvested and low molecular weight thiols were extracted and analysed by HPLC. The protein fraction was obtained by extracting the remaining pellets with 1.5 % (v/v) NaOH. Radioactivity was determined by liquid scintillation counting. Data shown are means ± s.d. of at least 2 independent experiments with 3 replicates each.

	Incubation conditions		
	GSH	GSH + BSO	GSH + DON
Distribution of radioactivity (μCi g dry weight^{-1})			
Cys	0.421 ± 0.038	0.399 ± 0.035	0.062 ± 0.004
GSH	5.319 ± 0.085	4.394 ± 0.769	0.814 ± 0.083
Protein	0.793 ± 0.153	1.919 ± 0.491	0.985 ± 0.148
Total	6.533 ± 0.525	6.712 ± 1.138	1.861 ± 0.485
Specific activity (μCi μmol^{-1})			
Cys	0.248 ± 0.027	0.321 ± 0.044	0.065 ± 0.006
GSH	0.303 ± 0.017	0.507 ± 0.068	0.071 ± 0.008

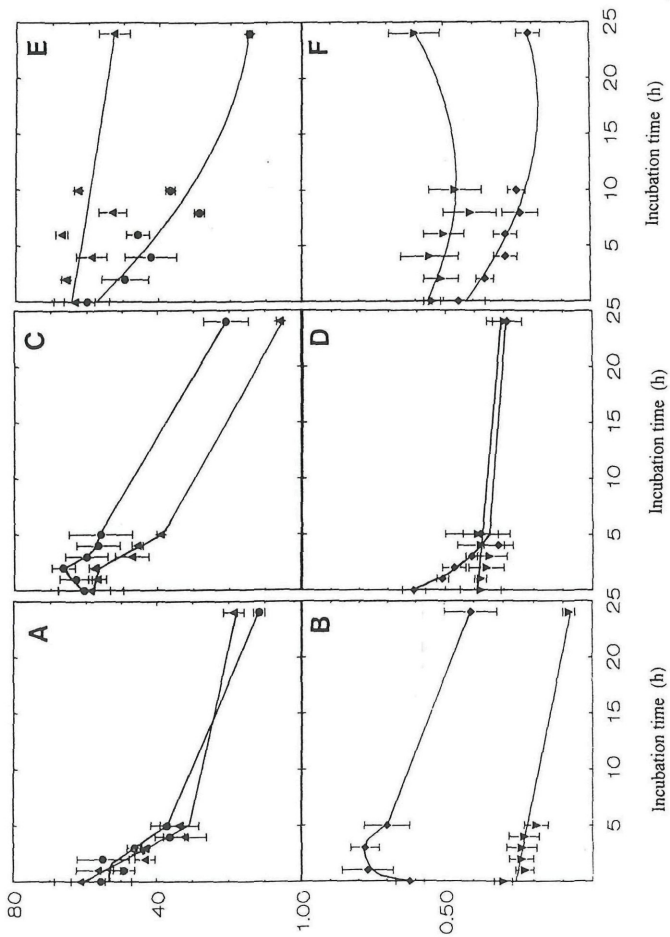

Fig. 1. Time dependent changes in the thiol contents during sulfur starvation. Heterotrophic tobacco cells were cultured for 6 days in sulfate containing M+S-medium and were subsequently precultured 2 days in GSH (1 mM) containing M+S-medium. Incubation was carried out under sulfur starvation for 24 h with or without inhibitors. Extraction and analysis of low molecular weight thiols was performed by HPLC. A, C, E: (•) Cys, (Δ) GSH; B, D, F: (▽) γ-EC, (◆) CG. A, B: incubation without inhibitor (control); C, D: incubation in the presence of BSO; E, F: incubation in the presence of DON. Data shown are means ± s.d. of at least 3 independent experiments with 3 replicates each.