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Inter- and intracellular distribution of modulated glutathione in plant tissues

Maria MÜLLER, Bernd ZECHMANN, Andreja URBANEK & Günther ZELLNIG

Institute of Plant Physiology, University of Graz, Schubertstraße 51, 8010 Graz, Austria
e-mail: maria.mueller@uni-graz.at

Abstract. In the present study we want to summarize data dealing with the inter- and intracellular distribution of glutathione in different plant tissues under various environmental conditions. Monochlorobimane fluorescence of reduced glutathione is described for light microscopical investigations and allows distinguishing between glutathione in cytoplasm and in the nucleus. Further, a specific antibody, which recognizes both the reduced and oxidized form of glutathione, was used in order to demonstrate glutathione in different cell compartments. The labelling showed different glutathione concentrations in the cell compartments with a high staining intensity in mitochondria.

Keywords: Glutathione, immunolabelling, monochlorobimane, plant tissues, ultrastructure

Introduction

Glutathione (GSH) is considered to have a broad spectrum of functions in plants. It is a cellular protectant, signal substance and a major reservoir of non-protein reduced sulphur in plants. Glutathione is present in all organs, with different concentrations in the organs or within the same organ at different developmental stages (Foyer & Rennenberg 2000), but thus far little is known about the GSH distribution in cellular compartments. Intercompartmental variations in glutathione concentration are supposed to be crucial in signalling processes. Noctor & al. (2002) discuss varying concentrations of glutathione in chloroplasts, which are reported between 8 % and 50 % of the leaf glutathione depending on the isolation media and the methods.

Our study presents an overview of a histochemical and an immunocytochemical method for the determination of inter- and intracellular glutathione contents in plant tissues.

Material and methods

Plant material

Epidermal cells of *Allium cepa* L. as well as leaves of *Cucurbita pepo* L. were used as described elsewhere (Müller & al. 1999 a, 2002 b).

Monochlorobimane (BmCl) labelling of thiols (modified according to MÜLLER & al. 1999 a, 2002 a).

A stock solution of BmCl (Molecular Probes) was diluted in phosphate buffer, pH 7.2, immediately prior to use to obtain the required concentration of 50 mM BmCl. All plant material was exposed to BmCl from 5 to 20 minutes (depending on the used material). After removing the staining solution, the samples were washed with buffer to remove excess staining solution. Single cells and single cell layers were investigated immediately under the fluorescence microscope.

Immunogold-analysis (modified according to MÜLLER & al. 2002 b)

Small sections of the plant material were fixed according to a standard fixation protocol (cf. ZELLNIG & al. 2000) or in 0.5 % glutaraldehyde/2.5 % paraformaldehyde, dehydrated in a graded series of ethanol and embedded in LR-White resin. Ultrathin sections were incubated with an anti-glutathione rabbit polyclonal IgG (Signature Immunologics, Inc.) followed by a 10 nm gold-labelled secondary antibody (British Biocell Int.). The antiserum was shown to react selectively with glutathione, although it does not differentiate between the reduced and oxidized forms of glutathione. To confirm the selectivity of the immunolabelling in the present set of experiments, the tissue sections were either incubated with the primary antibody, which was exposed to 5 mM of GSH prior to the application, followed by the secondary antibody or treated with the secondary antibody alone. Both treatments resulted in a negative immunolabelling.

Results and discussion

The technique of histochemical glutathione tracing by monochlorobimane (BmCl) in fluorescence microscopy was developed for single cell layers (*Allium* epidermis – MÜLLER & al. 1999 b) and for suspension cell cultures (root protoplasts of *Allium* – MÜLLER & al. 1999 a). The method relies on conjugation of BmCl to GSH within intact tissues resulting in a fluorescent GS-bimane conjugate (Fig. 1). The GS-bimane fluorescence levels are well known as an indicator of GSH in living mammalian tissues and are also described for plants (FRICKER & al. 2000, MEYER & FRICKER 2000, MEYER & al. 2001, SÁNCHEZ-FERNÁNDEZ & al. 1997). A photoactivation of fluorescence from non-conjugated BmCl after UV excitation, as previously mentioned by FRICKER & al. (2000) was not observed. Further data dealed with the manipulation of the intensity of the fluorescence that is observed after modification of the glutathione status of the cells, either by application of oxidants (MÜLLER & al. 1999 c) or by inhibiting glutathione synthesis by buthionine sulphoximine (MÜLLER & al. 1999 a). For the tissue- and subcellular imaging of glutathione concentrations in sections of intact root tissues (*Brassica oleracea*), where the glutathione contents were manipulated by H_2S fumigation, the staining procedure had to be adjusted (MÜLLER & al. 2002 b). In the roots of *Brassica,* a sulphur demanding plant, the H_2S treatment resulted in an increased fluorescence in the meristem cells, implying an enhanced glutathione content in the cytoplasm as well as in the nucleoplasm. These results showed the sensitivity of this method, making it possible to distinguish in the glutathione contents not only between different tissues but also in different cell compartments at the light microscopic level.

With this background, it was of interest to collect more precise data on GSH localization and GSH concentrations in the cells by using transmission electron microscopy. The subcellular localization of glutathione was investigated with an indirect immunogold-labelling method. On all investigated cell sections gold particles were present after the immunoreaction, but the labelling intensity varied over a wide range between the compartments. Very low intensities of gold labelling were found in peroxisomes and the ER; high intensities could be detected in the mitochondria (Fig. 2). The density of gold particles in chloroplasts and especially in the nuclei was lower (Fig. 2) than expected from either biochemical data (NOCTOR & al. 2002) or from staining with bimane derivates (MÜLLER & al. 2002 b).

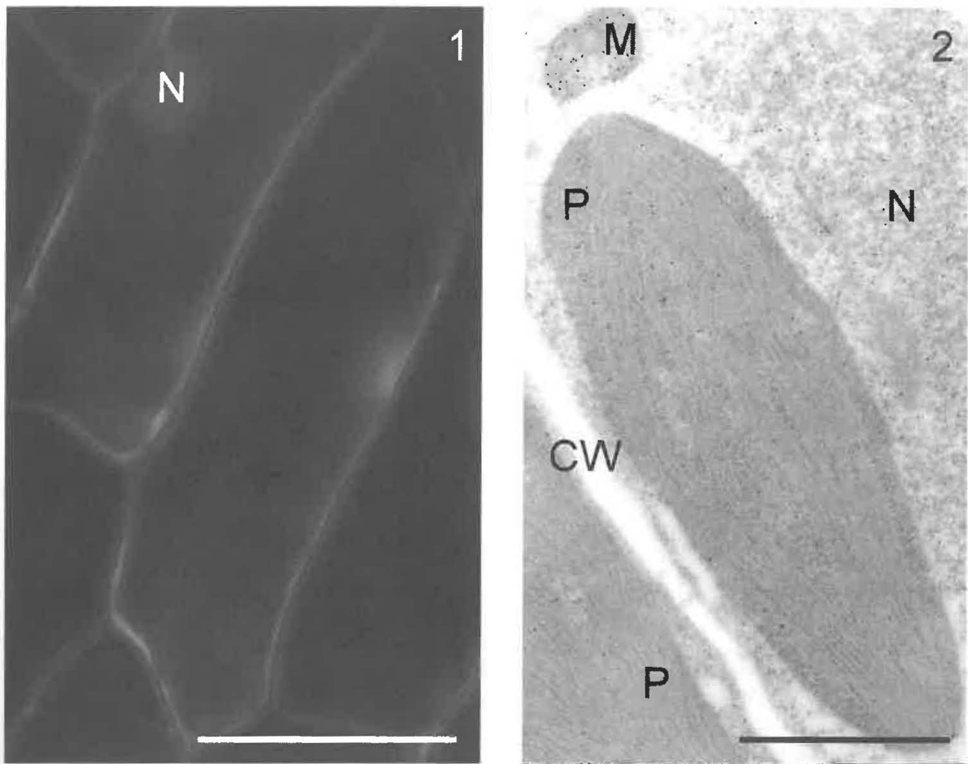

Figure 2: Part of a mesophyll cell of *Cucurbita pepo* L. Electron micrograph showing the distribution of GSH in various compartments after immunolabelling; gold particles are present in the chloroplast (P), the nucleus (N) and in an increased number in the mitochondria (M). Bar = 1 mm.

Conclusions

We can conclude that with the use of the above-described methods it is possible to obtain data about glutathione concentrations in different compartments by light and electron microscopy. But there is still a need of quantification analyses of the total pool of glutathione and till now no systematic investigations of the subcellular expression of glutathione, and the distribution of γ-glutamylcysteine, the immediate precursor of glutathione, were done with plant tissues. Therefore the investigations have to be continued and intensified in this field.

Literature

FRICKER M. D., M. MAY, A. J. MEYER, N. SHEARD, & N. S. WHITE 2000: Measurement of glutathione levels in intact roots of *Arabidopsis*. J. Microsc. **198**: 162–173.

FOYER C. H. & H. RENNENBERG 2000: Regulation of glutathione synthesis and its role in abiotic and biotic stress defence. In: Brunold C., H. Rennenberg, L.J. De Kok, I. Stulen, J. C. Davidian (eds.): Sulfur Nutrition and Sulfur Assimilation in Higher Plants, Paul Haupt Publishers, Berne, pp. 127–153.

MEYER A. J. & M. D. FRICKER 2000: Direct measurement of glutathione in epidermal cells of intact *Arabidopsis* roots by two photon laser scanning microscopy. J. Microsc. **198**: 174–181.

MEYER A. J., M. J. MAY & M. FRICKER 2001: Quantitative in vivo measurement of glutathione in *Arabidopsis* cells. Plant J. **27**: 67–78.

MÜLLER M., M. TAUSZ & D. GRILL 1999A: Histochemical tracing of glutathione by fluorescence microscopy and image analysis system in living plant cells. In: DENKE A., K. DORNISCH, F. FLEISCHMANN, J. GRAßMANN, I. HEISER, S. HIPPELI, W. OßWALD & H. SCHEMPF *(eds.):* Different Pathways through Life – Biochemical Aspects of Plant Biology and Medicine, pp. 189–197.

MÜLLER M., M. TAUSZ, H. GUTTENBERGER & D. GRILL 1999B: Histochemical localization of glutathione in plant tissues: A comparison of two fluorescence staining methods. Phyton (Horn, Austria) **39**: 69–74.

MÜLLER M., M. TAUSZ, A. WONISCH & D. GRILL 1999C: Effects of an oxidizing agent (hydrogen peroxide) on the glutathione system in epidermal cells of *Allium cepa* L. investigated by histochemical staining. Free Rad. Res. **31**: 121–127.

MÜLLER M., L.J. DE KOK, W. WEIDNER & M. TAUSZ 2002A: Differential effects of H_2S on cytoplasmic and nuclear thiol concentrations in different tissues of *Brassica* roots. Plant Physiol. Biochem. **40**: 585–589.

MÜLLER M., B. ZECHMANN, M. TAUSZ & G. ZELLNIG 2002B: Subcellular distribution of glutathione – a high resolution immunogold analysis in leaves of pumpkin (*Cucurbita pepo* L.). *In:* Davidian J. C., D. Grill, L.J. De Kok, I. Stulen, M. J. Hawkesford, E. Schnug, & H. Rennenberg *(eds.): Sulfur transport and assimilation in plants – Regulation, interaction and signalling*, Backhuys Publishers, Leiden, pp.295–297.

NOCTOR G., L. GOMEZ, H. VANACKER & C. H. FOYER 2002: Interactions between biosynthesis, compartmentation and transport in the control of glutathione homeostasis and signalling. J. Exp. Bot. **53**: 1283–1304.

SÁNCHEZ-FERÁNDEZ R., M. D. FRICKER, L. B. CORBEN, N. S. WHITE, N. SHEARD, C. J. LEAVER 1997: Cell proliferation and hair tip growth in the Arabidopsis root are under mechanistically different forms of redox control. Proc. Natl. Acad. Sci. USA **94**: 2745–2750.

ZELLNIG G., M. TAUSZ, B. PEŠEC, D. GRILL & M. MÜLLER 2000: Structural and ultrastructural changes in root cells of spruce trees (*Picea abies* (L.) Karst.) caused by exogenously applied glutathione. Protoplasma **212**: 227–235.