Subcellular localization of glutathione and cysteine in cyanobacteria

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Glutathione is an important antioxidant in plants and animals. It plays key roles in the activation of defense genes, redox buffering, sensing and detoxifying reactive oxygen species (ROS) and by participating in ROS-signaling pathways. Glutathione can also be found in many bacteria where it represents the predominant intracellular thiol compound [1]. The protection of glutathione against ROS is especially important for cyanobacteria that rely on photosynthesis in order to produce sufficient carbon for growth and cell metabolism. As high levels of ROS are commonly formed during photosynthesis and have the potential to destroy cellular components, glutathione and related derivatives are important for cell survival. Even though the roles of glutathione in bacteria are well investigated its subcellular distribution is still unclear as methods for its detection within cyanobacteria are not available.

In the present study we present a method that allows the detection and quantification of subcellular glutathione and cysteine levels with computer-supported transmission electron microscopy in *Synechocystis* sp. This method has been previously applied on plant samples and has given valuable information about compartment specific glutathione metabolism [2]. In order to verify the accuracy of the immunogold labelling method we have quantified subcellular glutathione and cysteine contents in *Synechocystis* sp. grown in a medium with sulphur and also in cells that were transferred into sulphur depleted medium for 48 hours.

Ultrastructural studies revealed similar ultrastructural preservation of cells grown in medium with or without sulphur (Fig. 1). Cells from both media showed a dense cytosol with well preserved thylakoid membranes, globules and small vacuoles. Immunogold labelling of glutathione and cysteine revealed that both components are more or less equally distributed within the cells and that no glutathione and cysteine is located in intrathylakoidal spaces. Nevertheless, glutathione and cysteine could commonly be observed in interthylakoidal spaces (Fig.1). No, or only very little glutathione and cysteine labelling was found inside the cell walls and vacuoles. The accuracy of the labelling results was verified by the observation that glutathione labelling in cells grown in sulphur depleted medium was reduced to background levels and that cysteine labelling in the same cells was completely missing (Figs 1 and 2). These results also demonstrate that cyanobacteria rely on a sufficient supply of sulphate in order to maintain cysteine and subsequently glutathione levels within the cytosol.

In conclusion, the present study represents the first report of a method that allows the quantification of subcellular glutathione and cysteine contents in cyanobacteria and can now be used to study the importance of glutathione metabolism in these unicellular organisms (e.g. during different environmental stress situations), thus allowing a deeper insight into the importance of subcellular glutathione metabolism in cyanobacteria.

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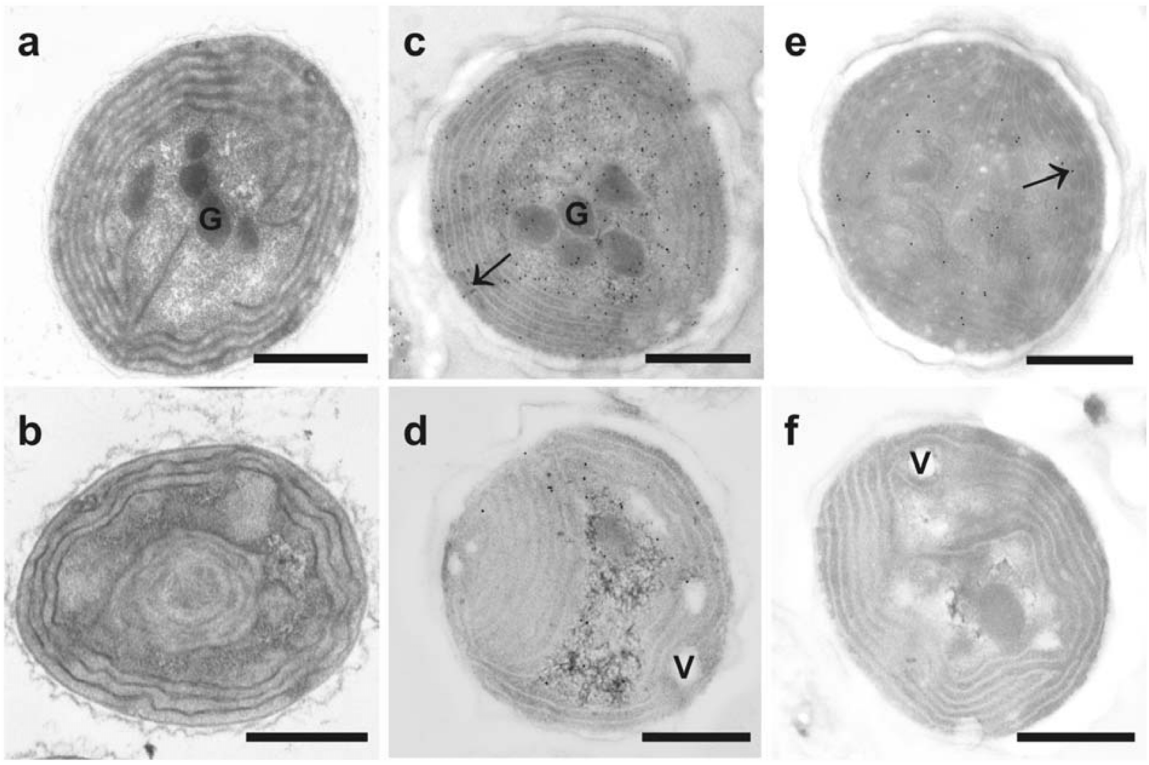

Figure 1. Transmission electron micrographs showing the ultrastructure (a,b) and gold particles bound to glutathione (c,d) and cysteine (e,f) within *Synechocystis* sp. cells grown in media with (a, c, e) and without (b, d, f) the addition of sulphur. Note that gold particles bound to glutathione and cysteine can be found in interthylakoidal (arrows) but not in intrathylakoidal spaces. Bars=0.5µm. G=globules, V=small vacuoles.

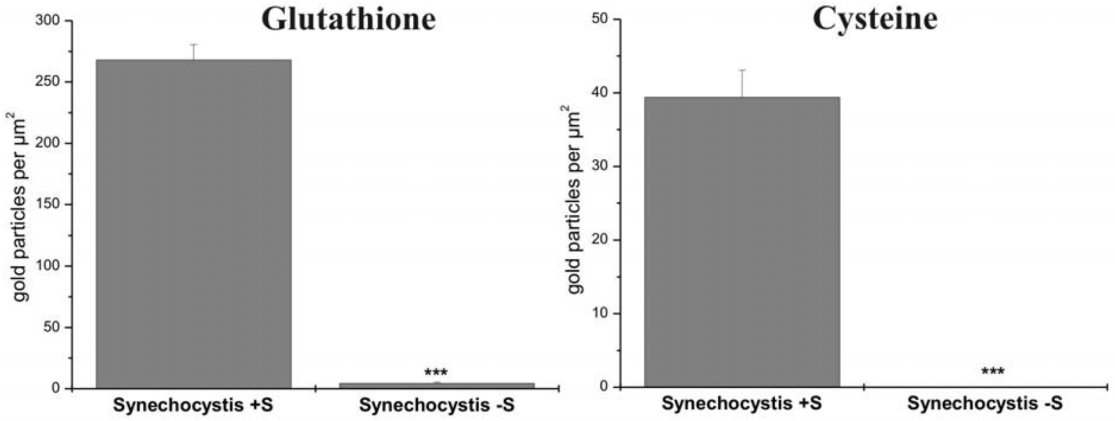

Figure 2. Graphs show means with standard errors and document the amount of gold particles bound to glutathione and cysteine per µm^2 in *Synechocystis* cells grown in media with (+S) and without sulfur (-S). Note that samples grown in sulfur depleted medium contain significant less glutathione and cysteine than samples grown in media with sulfur. Significant differences between samples were calculated using the Mann-Whitney U test and are indicated with (***) which means that $P<0.001$. $n>50$.

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