

Determination of ferrocene-derivatized phytochelatins by LC/ESI-MS and LC/ICP-MS

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Plants evolved numerous strategies to deal with heavy metals that represent both essential nutrients (e.g. Zn, Cu, Fe) and toxic agents (e.g. Cd, Pb, Hg). Plants react to Cd stress by synthesis of metal coupling thiol rich peptides, so called phytochelatins (PCs).

PCs are glutathione-derived peptides with the general primary structure $(\gamma\text{-Glu-Cys})_n\text{-Gly}$. The amount of $\gamma\text{-Glu-Cys}$ units depends on the organism and metal exposure time. The peptides are able to bind metals such as Cd via coupling by thiol groups, according to the HSAB concept. Thus, toxicity as well as the detoxification of the metal is connected to its affinity to sulfhydryl groups.

Former studies have shown a broad variety of PC and iso PCs synthesized in *C. reinhardtii* after Cd exposition (Bräutigam et al. Analytical and Bioanalytical Chemistry, submitted). Up to now, a suitable method for the precise quantification of phytochelatins does not exist. Therefore, a new method for the quantitative determination of phytochelatins in the green alga *Chlamydomonas reinhardtii* is presented. This alga appears in fresh water and soil. The polar peptides were derivatized using a ferrocene-based derivatizing agent. Hereby, the cysteine residues react quantitatively with a maleimide group to form the corresponding stable thioethers. Through this derivatizing process unpolar reaction products are formed which are suitable for the separation on reversed phase columns.

The labeled biomolecules are characterized by liquid chromatography (LC) coupled with electrospray ionization (ESI) and inductively coupled plasma (ICP) mass spectrometric (MS) detection. Several PCs and PC Isoforms extracted from *C. reinhardtii* could be derivatized, separated and identified using RP-HPLC with coupled ESI-MS. As complementary data, ICP-MS measurements show a high potential for absolute quantification.