Sulphur study in *Arabidopsis thaliana* using X-ray Absorption Spectroscopy

M.T. Klepka[1], A. Wolska[1], A. Drzewiecka-Antonik[1], A. Maximenko[2], K. Ławniczak-Jabłońska[1], J. Piotrowska[3], M. Olszak[3], A. Wawrzynska[3], A. Sirko[3]

[1]Institute of Physics Polish Academy of Sciences, Warsaw, Poland
[2] SOLARIS National Synchrotron Radiation Centre, Krakow, Poland
[3]Institute of Biochemistry and Biophysics Polish Academy of Sciences, Warsaw, Poland

Motivation

Sulphur is an essential macronutrient for the proper growth and development of plants. It is taken up from the soil as inorganic sulphate, reduced to sulphite and sulphide and then incorporated into variety of organic compounds. Sulphur is essential for the biosynthesis of amino acids such as cysteine and methionine incorporated into proteins, glutathione, coenzymes and vitamins. In addition, sulphur-containing metabolites are involved in the response of plants to biotic and abiotic stresses. Transcription of LSU (RESPONSE TO LOW SULFUR) genes is strongly activated by sulphur deficiency, however the biological function of these plant-specific proteins is unknown.

X-ray absorption near-edge structure (XANES), due to its high sensitivity (~ppm) can be used to study compounds with very low concentration. Therefore, it could be a perfect tool to investigate chemical state of elements in plant tissues.

Purpose of the project

The aim of this project was to prove suitability of XANES for detection of forms of Sulphur chemical state in plant material. In further perspective we would like to verify the hypothesis that proteins are involved in modulation of the sulphate assimilation pathway in plants and establish the appropriate profile of sulphur-containing metabolites in plant tissues. Additionally, we wanted to identify the form of Phosphorus present in plant samples.

Experimental

The preliminary XANES experiment was performed at ASTRA beamline at SOLARIS. The Sulphur and Phosphorus K edges were measured in a transmission mode.

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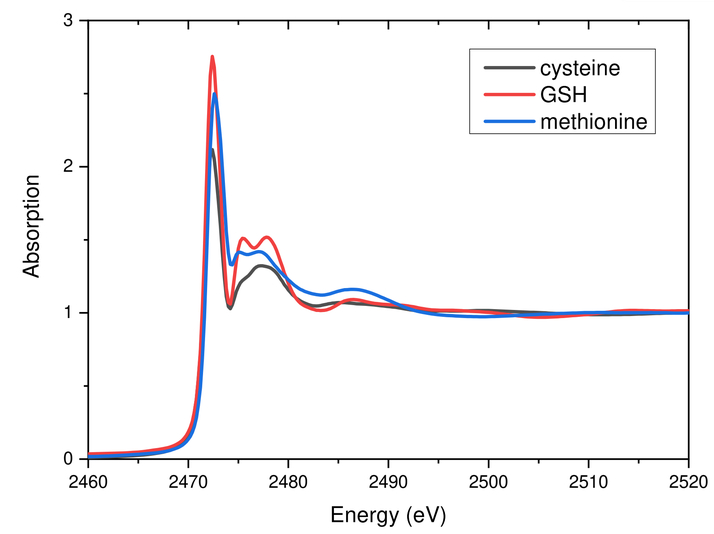
Samples

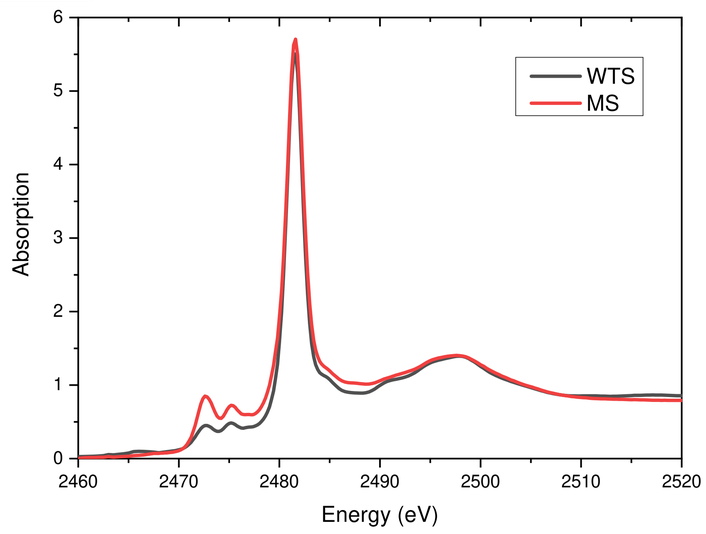
4 samples of the leaf tissues were investigated:
* from the wild-type plants grown in nutrient solution with sufficient S (WTS)
* from the wild-type plants grown in nutrient solution without S (WT)
* from the lsu-KO mutant (lacking LSU proteins) grown in nutrient solution with sufficient S (MS)
* from the lsu-KO mutant (lacking LSU proteins) grown in nutrient solution without S (M)

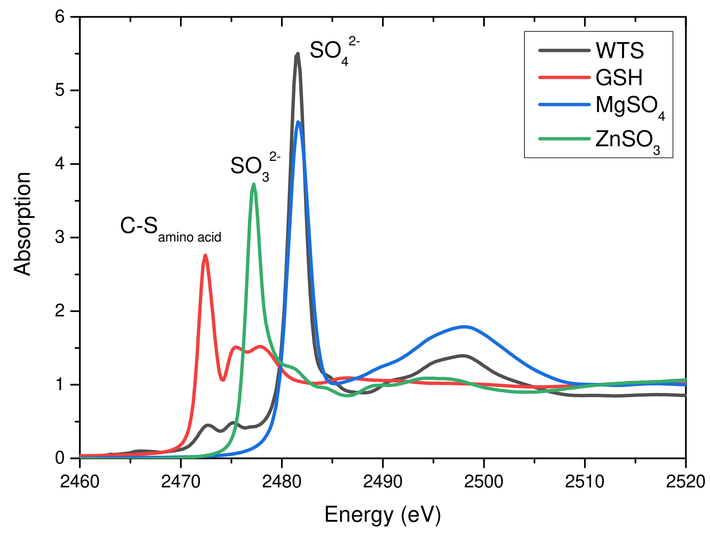
Reference samples

Organic - cysteine, GSH, methionine
Inorganic- $MgSO_4$, $ZnSO_3$, KH_2PO_4

S K-edge

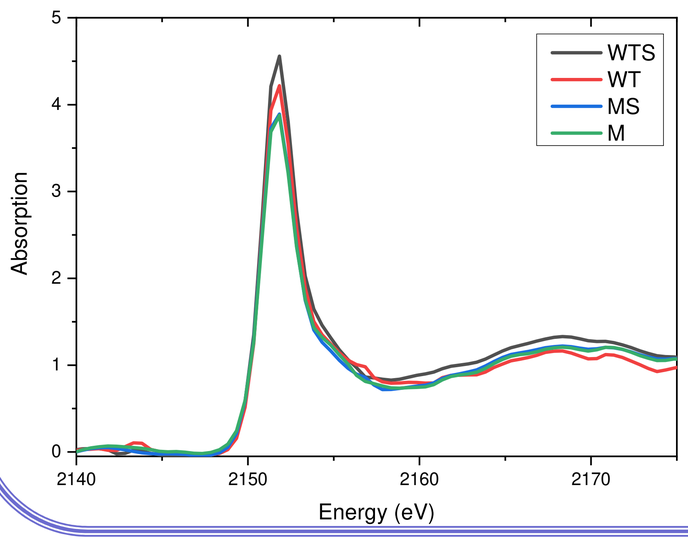


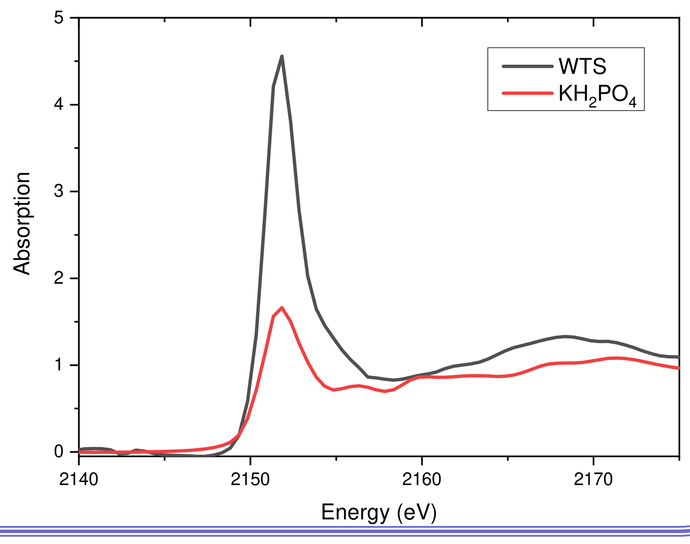




No Sulphur was detected where the nutrient solution without Sulphur was used (samples WT and M).

P K-edge





XANES results

* Despite low concentration of both Sulphur and Phosphorus it was possible to get good quality data already in a transmission mode.

* Inorganic SO_4^{2-} anion and C-$S_{amino\ acid}$ organic bond were identified.

* Phosphorus was found to be at 5+ oxidation state in all investigated compounds.