



Looking for Glutathione Transferase substrates and ligands

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Thematic action concerned: WP1 Transversal

Context —

Glutathione transferases (GSTs) constitute a multigenic family of enzymes widely distributed in living organisms. In plants, they are notably involved in the detoxification of exogenous toxic compounds such as herbicides, but also in the synthesis or transport of specialized metabolites. Transcriptomic studies have also shown that the expression of genes coding for these enzymes is strongly regulated during abiotic and biotic stress. From a biochemical point of view, the functions carried out by these enzymes are either based on the catalytic transformation of substrates, mainly glutathione conjugation and deglutathionylation reactions; or on their so-called "ligandine" property dedicated to the transport or storage of various (non-catalyzed) ligands. However, the substrates/ligands as well as the associated physiological functions of these enzymes remain mostly unknown.

Objectives —

The main objective of this project is to identify ligands and substrates of some poplar GSTs using *in vivo* and *in vitro* trapping approaches of these molecules and to characterize the identified molecular interactions. The approaches that will be developed will have the potential to determine precisely the roles and functions of some poplar GSTs and notably those encoded by genes whose expression is strongly regulated during the host-pathogen interaction between poplar and the obligate biotrophic fungus *Melampsora larici-populina* (*Mlp*), to understand how some specialized metabolites are synthesized and transported or stored, and to identify new classes of valuable natural products.

Approaches —

The project is divided into three main axes:

(1) Purification of targeted GSTs as recombinant proteins and preparation of biological material

This axis is devoted to the expression and purification of targeted GSTs after overproduction in the bacterium *E. coli* which will be used as baits for the search for their substrates and ligands. It also includes the preparation of poplar extracts enriched in specialized metabolites and their metabolic profiling in collaboration with the "Mass Spectrometry" team of the Institute of Chemistry of Natural Substances (ICSN, <https://icsn.cnrs.fr/recherche/cbsa/ms>).

(2) Identification of molecules interacting with targeted poplar GSTs

This axis aims at identifying and characterizing molecules (ligands and/or substrates) interacting with the selected poplar GSTs using *in vitro* and *in vivo* approaches. This axis will rely on the expertise of the "Stress response and redox regulation" team of the UMR IAM for the *in vitro* and *in vivo* fishing approaches and on the "Mass spectrometry" team of the Institute of Chemistry of Natural Substances (ICSN) for the complex and time-consuming step of metabolite identification.

(3) Biochemical and structural studies of the binding properties of the molecules interacting with the selected GST
This axis aims at validating and describing at the molecular level the interactions between the selected GSTs and the identified ligands or substrates by biochemical and structural approaches. This work will be carried out in

collaboration with the ASIA platform (<http://a2f.univ-lorraine.fr/en/plateformes-anglais/>) and Claude Didierjean (Univ. Lorraine, CRM2, PhD co-director).

Key results — (presented as separated bullet points)

The results obtained can be divided along the same lines as above:

- (1) The biological material necessary for the realization of the analyses was obtained (purification of GSTs and extraction of metabolites from leaves resulting from kinetic infection of isolated poplar leaves by *Mlp*).
- (2) A collaboration with the ICSN allowing to obtain a metabolic database built from extracts coming from various poplar organs (leaves, flowers, buds, fruits) was set up and led to the establishment of a database of molecules and metabolomic maps.
- (3) Experiments to identify molecules interacting with GSTs of interest from molecule libraries or plant extracts concentrated in specialized metabolites have been initiated. The first results obtained show a specificity of the GSTs of interest for different extracts or classes of molecules.

Main conclusions including key points of discussion —

After having prepared the biological material necessary for metabolomic analyses, a database of molecules was constituted and the corresponding metabolomic networks established for some poplar tissues (leaves, flowers, buds, fruits). These first analyses led to the simultaneous validation of extraction methods and molecule analysis methods. These data constitute a good basis for the identification of molecules (substrates/ligands) interacting with the few targeted poplar GSTs that were overexpressed in *E. coli* and then purified to homogeneity and whose role(s) and function(s) we wish to clarify. The first experiments to identify ligands/substrates from molecule libraries or poplar extracts interacting with the GSTs of interest tend to show that these enzymes interact preferentially with certain extracts enriched in metabolites and certain classes of metabolites.

Perspectives —

In the very short term, it is envisaged to apply the different approaches discussed above to extracts from poplar leaves infected by the *Mlp* fungus (collaboration with Pascal Frey, IAM) and to GSTs encoded by genes whose expression is strongly regulated during the host-pathogen interaction in poplar. In the longer term, the interactions identified between targeted GSTs and metabolites (substrates or ligands) will be characterized biochemically and structurally via structural biology methods coupling original technologies such as the SwitchSense technology available on the ASIA platform (<https://a2f.univ-lorraine.fr/asia/>).

Valorization — (scientific: publications, book chapter, presentation at conferences,...); economic: Soleau envelope, patent, license,...; distribution: press release, interview,...)

We thus plan to exploit and publish these results in leading peer-reviewed scientific journals in the fields of general biochemistry and/or plant biology and/or to present them at national and international conferences as oral communications and/or posters.

Leveraging effect of the project—

The data that will be obtained will be likely to interest all the teams of the UMR 1136 IAM since they will complete the data accumulated these last years on the poplar-*Mlp* pathosystem. They are also likely to interest the scientific community in the field in the understanding of the biotic stress of poplar, a necessary prerequisite before considering means of control against this important biotic stress for poplar.