Look4Grail

Looking for Glutathione Transferase substrates and ligands

Principle investigator: Arnaud Hecker, UMR Interactions Arbres/Micro-organismes (IAM) 1136

LabEx partners : Pascal Frey ; UMR Interactions Arbres/Micro-organismes (IAM) 1136

Collaborations : David Touboul (ICSN, Gif-dur-Yvette), Cédric Paris (ENSAIA)

Thematic actions concerned: WP1 WP2 WP3 WP4 Transversal

Abstract

Due to their inability to move, terrestrial photosynthetic organisms are subject to various environmental (biotic or abiotic) constraints that modify the cellular redox homeostasis. Molecules with antioxidant properties such as glutathione, ascorbate, carotenoids, tocopherols and other secondary/specialized metabolites are produced to counteract possible direct or indirect toxic effects of the reactive oxygen and/or nitrogen species that are notably produced in these conditions. The secondary metabolites may be paradoxically also toxic for plants themselves and they have thus developed a high-performance enzymatic arsenal to cope with these molecules. Among these enzymes, glutathione transferases (GSTs) constitute a large multigene family. The expansion of the GST gene family in plants may be related to their documented role in detoxification of toxic compounds (often referred to as xenobiotics) such as herbicides in annual species, but also to their involvement in various metabolic pathways, notably in the synthesis of secondary, defense metabolites. Moreover, several GSTs have been shown to interact with or modify flavonoids, oxygenated fatty acids or porphyrins. These functions rely either on the catalytic transformation of substrates, mostly GSH-conjugation and deglutathionylation reactions or to their so-called "ligandin" property dedicated to the transport or storage of various (uncatalyzed) ligands. Yet, the substrates/ligands and thus precise physiological functions of most GSTs are still unknown.

This research project called Look4Grail (Looking for Glutathione Transferase substrates and Ligands) is dedicated to identifying the ligands and substrates of a selection of poplar GSTs using *in vivo* and *in vitro* ligand/substrate fishing approaches coupled with metabolomic analyses and to the characterization of these biomolecular interactions at both biochemical and structural levels. These ligand fishing approaches have the potential to give new insights into GST function *in planta* as well as identifying novel classes of natural product inhibitors of enzymes of biotechnological interest such as GSTs.

Context —

Glutathione transferases (GST) 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 GST 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 GST 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 areas:
(1) Purification of targeted GSTs in the form of recombinant proteins and preparation of biological material:
This axis is devoted to the expression and purification of targeted GST after overproduction in *E. coli* bacteria, which will be used as bait 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 Institut de Chimie des Substances Naturelles (ICSN, https://icsn.cnrs.fr/recherche/cbsa/ms) and the Plateau d'Analyse Structurale et Métabolomique (PASM) of ENSAIA (Nancy).
(2) Identification of molecules interacting with targeted poplar GSTs:
This axis aims to identify and characterize molecules (ligands and/or substrates) interacting with selected poplar GST using *in vitro* and *in vivo* approaches.
(3) Biochemical and structural studies of the binding properties of molecules interacting with selected GST:
This axis aims to validate and describe at the molecular level the interactions between the selected GST and the ligands or substrates identified using 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, thesis co-supervisor).

Key results —
(1) The biological material required for the analyses was obtained (purification of GSTs and extraction of metabolites from leaves derived from kinetic infection of isolated poplar leaves by *Mlp*, as well as metabolites from various poplar tissues).
(2) Poplar extracts were analyzed by liquid chromatography-mass spectrometry in collaboration with ICSN and the PASM platform. Based on these analyses, a metabolic database of over 5,000 molecules was constructed. In particular, it shows the prevalence of flavonoids and terpenoids in poplar buds (over 30%), in contrast to fruit, for example (which contains just 8%). During *Mlp* infection of poplar leaves, the results obtained show little variation in the content of specialized metabolites, whatever the extraction method used (ethyl acetate and methanol 60%). Nevertheless, the proportion of around one hundred molecules changed significantly during *Mlp* infection. More detailed analyses are currently underway to determine the exact nature of these compounds.
(3) Experiments to identify molecules interacting with the GSTs of interest using molecule libraries or plant extracts concentrated in specialized metabolites have been initiated. Initial results show the specificity of the GSTs of interest for different extracts or classes of molecules. In particular, bud extracts appear to induce a strong response from GSTs. Isolation experiments using high-pressure liquid chromatography (HPLC) and co-crystallization of proteins (in conjunction with CRM2) with fractions containing the molecules of interest are currently underway. Using the databases obtained in Axis 2, it will be possible to determine the precise metabolites involved. The same type of experiment was carried out using extracts from the kinetics of *Mlp* infection of poplar leaves (methanoic extracts and ethyl acetate). GSTs identified as being differentially expressed during infection by previous transcriptomic analyses were brought into contact with the above extracts. No interactions were detected, validating the low molecular variation between the different extracts obtained.

Main conclusions including key points of discussion —
Having prepared the biological material required for metabolomic analyses and built a database of molecules, the corresponding metabolomic networks are now being established for a few poplar tissues (leaves, flowers, buds, fruit). These initial analyses have led to the simultaneous validation of extraction methods and molecule analysis methods. These data provide a good basis for the very short-term identification of molecules (substrates/ligands) interacting with the few targeted poplar GSTs that have been overexpressed in *E. coli* and then purified to homogeneity, and whose role(s) and function(s) we wish to clarify. Initial experiments to identify ligands/substrates from molecule libraries or poplar extracts that interact with the GSTs of interest tend to show that these enzymes interact preferentially with certain metabolite-enriched extracts and certain classes of metabolites.

Perspectives —
The interactions identified between targeted GSTs and metabolites (substrates or ligands) will be characterized biochemically and structurally using structural biology methods coupling original technologies such as SwitchSense, 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,...)
Morette, L., Levasseur, M., Touboul, D., Frey, P., Didierjean, C., Hecker, A. Vers l'élucidation du rôle des glutathion transférases GSTU19 et 20 de peuplier au cours de l'interaction hôte-pathogène entre le peuplier et le champignon *Melampsora larici-populina*. 15èmes journées scientifiques du Réseau Francophone de Métabolomique et Fluxomique (RFMF), 24 au 26 mai 2023, Perpignan.

Leveraging effect of the project—
The data obtained is likely to be of interest to all UMR 1136 IAM teams, since it will complement the data accumulated in recent years on the poplar-*Mlp* pathosystem. They are also likely to interest the scientific community in the field in understanding the biotic stress of poplar, a necessary prerequisite before considering means of combating this important biotic stress for poplar cultivation.