Characterization of a glutathione transferase from fungus *Trichoderma reesei*

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BIOMOLECULAR PRODUCTION (TECH)

Introduction

Fusarium gramianerum is one of the most threatening fungal pathogens in agriculture. In wheat, the fungus causes a disease called Fusarium Head Blight (FHB) (Fig. 1). The pathogen causes devastating annual yield losses, and the number of reported cases has increased due to climate change and the rise of improper farming practices (1). The fungus produces many trichothecene mycotoxins, the most important of which is deoxynivalenol (DON) (2).

Fungicides have been used to combat *F. gramianerum,* but their effectiveness has lessened due to a rise in resistance (3). Glutathione transferases (GSTs) are a superfamily of enzymes that detoxify a wide range of hydrophobic compounds. They have been studied as a possible solution for the accumulation of DON (4). A GST from *Trichoderma reesei* (*Tr*GST) has been shown to bind DON with high affinity as well as to detoxify it (Fig. 2).

The aim of this thesis is to further characterize *Tr*GST. The stability and binding kinetics of the enzyme were studied using mass photometry and isothermal titration calorimetry. Crystallization with DON and its adduct DON-13-GSH were performed to produce crystals for X-ray structure determination.

Figure 1. Healthy (green) and FHB infected wheat heads.

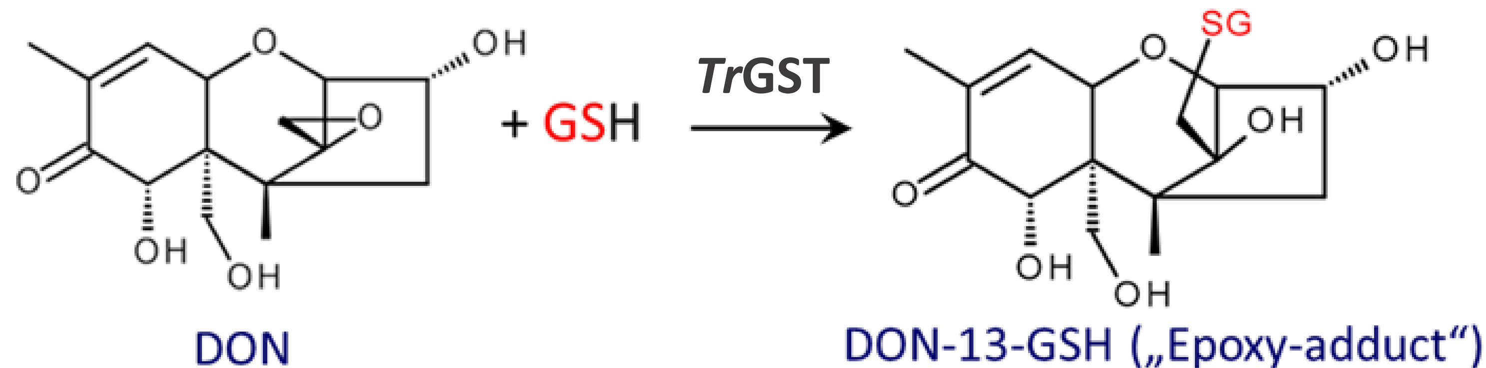

Figure 2. The detoxification reaction of DON in the presence of glutathione (GSH).

Materials and Methods

Stability measurements were done using Refeyn TwoMP Auto mass photometer.

Binding kinetics were measured using isothermal titration calorimetry (ITC). The instrument used was Malvern MicroCal Auto-iTC200.

Crystallization was performed using sitting-drop and hanging-drop methods. Sitting drop experiments were done using the Oryx4 crystallization robot by Douglas Instruments. Crystallization screens used for the experiments were JCSG, MIDAS, PACT Premier, and ShotGun (SG) 1 produced by Molecular Dimensions and INDEX produced by Hampton Research.

Hanging-drop experiments were done by hand.

Workflow is shown in figure 3.

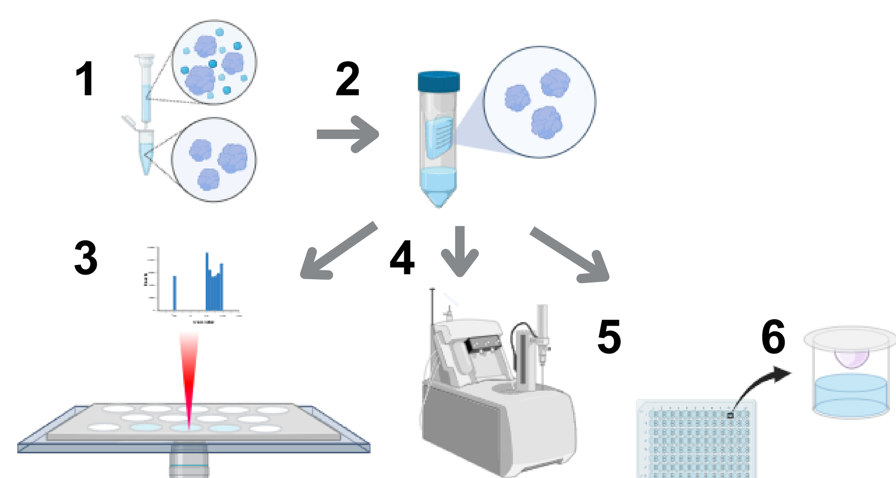

Figure 3. A visualization of the workflow. 1. Protein purification 2. Concentration of the protein 3. Mass photometry 4. Isothermal titration calorimetry 5. Sitting drop experiments 6. Hanging drop experiments. Created using BioRender.com.

Acknowledgements

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Results

Mass photometry measurements (Fig. 4) showed that *Tr*GST naturally forms a characteristic dimeric structure in solution. However, with time, the molecular mass of the samples were lowered indicating potential degradation of the protein.

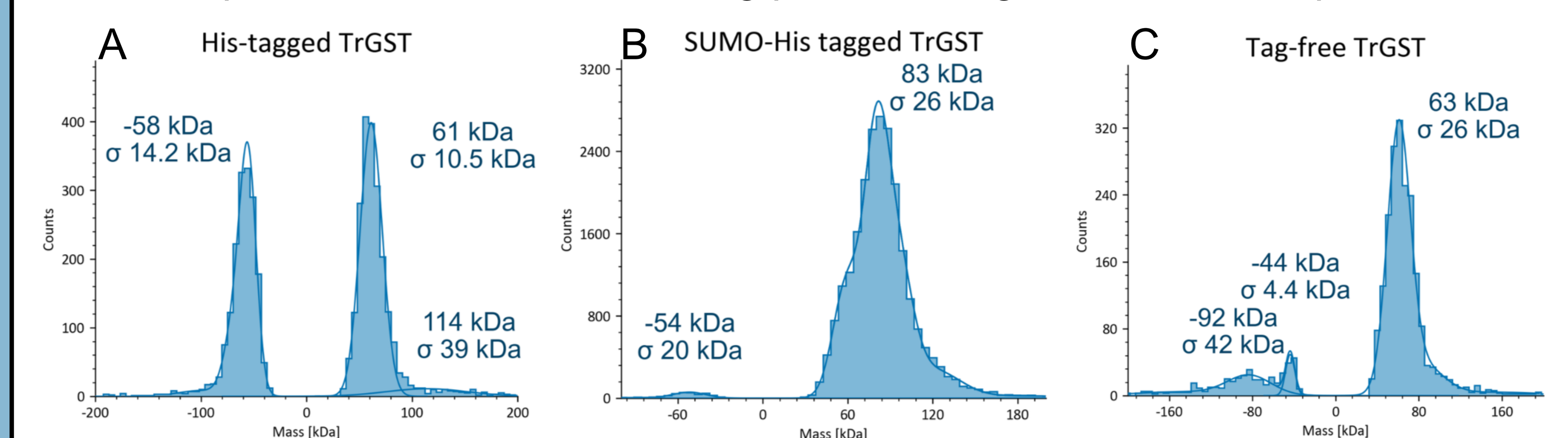

Figure 4. Mass photometry measurements for the purified proteins containing a (A) His-tag, a (B) His-SUMO-tag, and (C) tag-free from tag.

The ITC experiments showed that *Tr*GST binds DON with and without GSH. The binding affinity for the *Tr*GST-DON titration was 3.6 μM and for the *Tr*GST-GSH-DON titration 0.25 μM. ITC also suggested potential conformational changes in the enzyme during DON binding in the presence of GSH (Fig. 5).

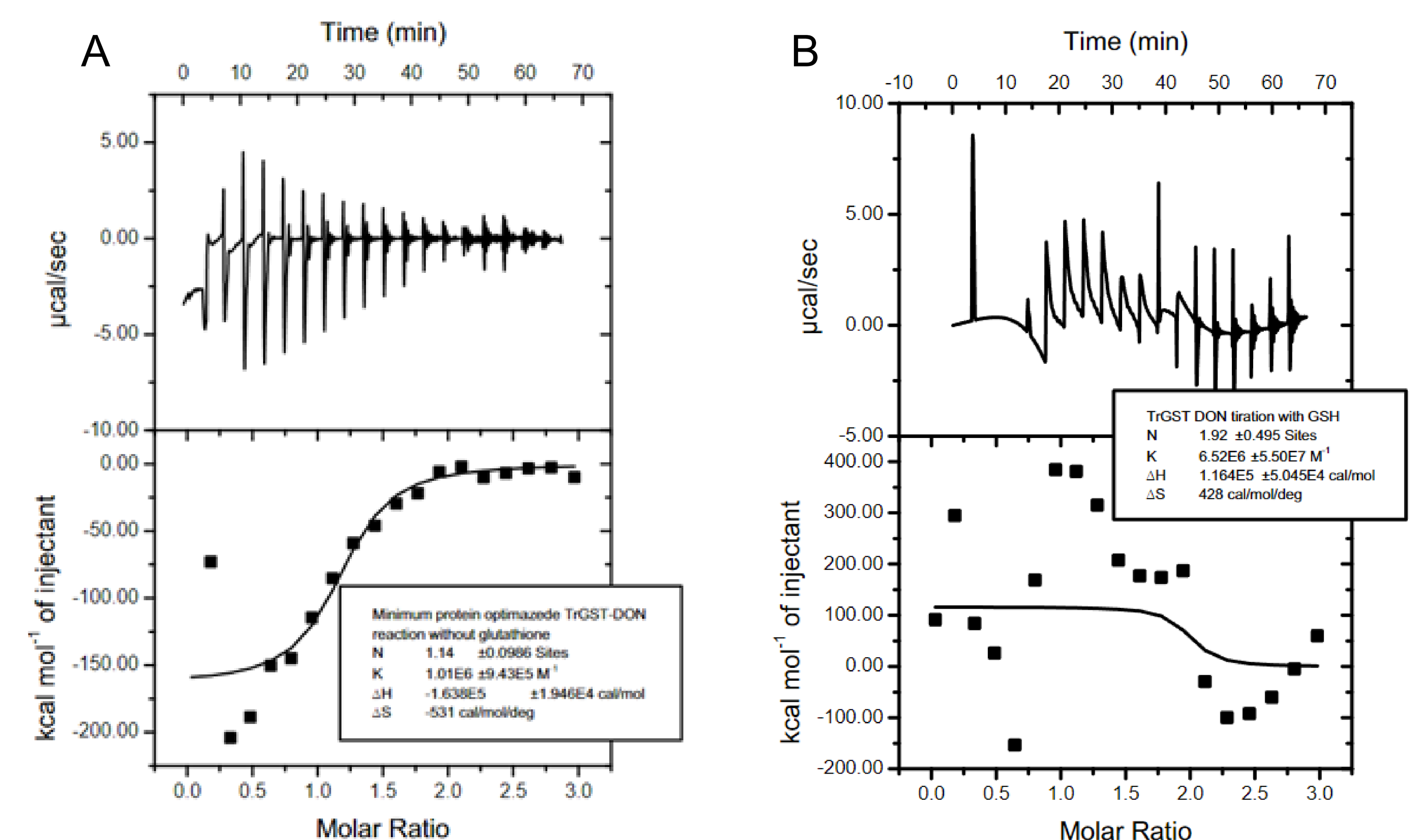

Figure 5. Graphs of ITC measurements. A. *Tr*GST-DON titration and B. *Tr*GST-GSH-DON titration.

The crystallization experiments produced microneedles in sitting-drop and hanging-drop experiments (Fig. 6).

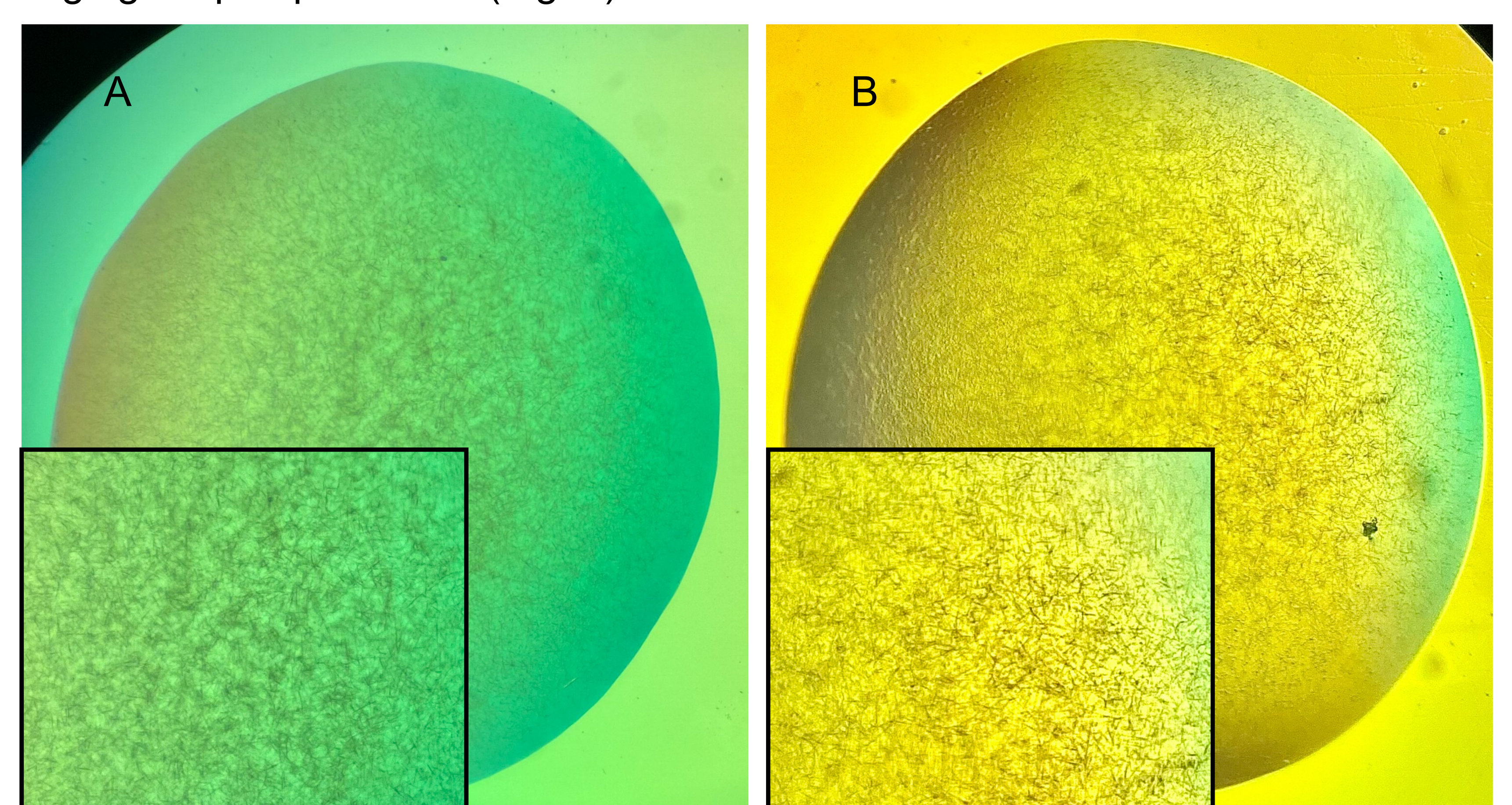

Figure 6. Microneedles produced in (A) *Tr*GST - DON and (B) *Tr*GST - DON - 13 - GSH in hanging-drop crystallization experiments.

Conclusions

- *Tr*GST exhibits the characteristics of fungal specific class A GST.
  - It naturally forms a dimeric structure.
  - The enzyme binds GSH and DON simultaneously. In the presence of GSH, there is a potential conformational change in the enzyme upon DON binding.
- The enzyme produces microneedles in crystallization experiments suggesting that further optimization is needed.

Key References

1. Wang, H., Sun, S., Ge, W., Zhao, L., Hou, B., Wang, K., et al. (2020). Horizontal gene transfer of Fhb7 from fungus underlies Fusarium head blight resistance in wheat. Science. doi: 10.1126/science.aba5435
2. Pestka, J. J. (2010). Deoxynivalenol: mechanisms of action, human exposure, and toxicological relevance. Archives of toxicology 84, 663–679. doi: 10.1007/s00204-010-0579-8
3. Chen, Y., Kistler, H. C., and Ma, Z. (2019). Trichothecene Mycotoxins: Biosynthesis, Regulation, and Management. Annu Rev Phytopathology, 57, 15–39. doi: 10.1146/annurev-phyto-082718-100318.
4. Morel, M., Ngadin, A., Droux, M., Jacquot, J.-P., and Gelhaye, E. (2009) The fungal glutathione S-transferase system. Evidence of new classes in the wood-degrading basidiomycete Phanerochaete chrysosporium. Cellular and Molecular Life Sciences, 66: 3711 – 3725. doi: 10.1007/s00018-009-0104-5