RECOMBINANT CORE PROTEINS OF POLYPHENOL OXIDASES ON EXAMPLE OF AURONE SYNTHASE

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INTRODUCTION

Aurone synthases belong to the family of polyphenol oxidases (PPOs), which contain a dinuclear (type III) copper center. PPOs catalyze the *o*-hydroxylation and the oxidation of phenolic compounds and are found in almost all plants[1]. Even though PPOs are involved in enzymatic browning reactions of e.g. fruits and vegetables, their involvement in the secondary plant metabolism, except for few cases, still remains unclear[2]. Plant PPOs are expressed as latent (inactive) pro-enzymes (~ 60 kDa) in which the catalytically active domain (~ 40 kDa) is shielded by its C-terminal domain (~ 20 kDa)[3]. However, the latent pro-enzymes can be activated *in vitro* by sodium dodecyl sulfate (SDS)[4]. During the maturation, the C-terminal domain is cleaved proteolytically, resulting in active enzyme[1]. Aurone synthases are specialized plant PPO involved in the formation of the yellow coloured flower pigments aurones from chalcones[5] (Fig. 1). In petals of *Coreopsis grandiflora* two candidate aurone synthases, *AUS1* and *AUS2*, are found to be expressed[6]. As the natural substrates of these PPOs are known the availability of fully active recombinantly expressed aurone synthases is of high importance in order to get insights into the substrate specificity of plant PPOs on a molecular level.

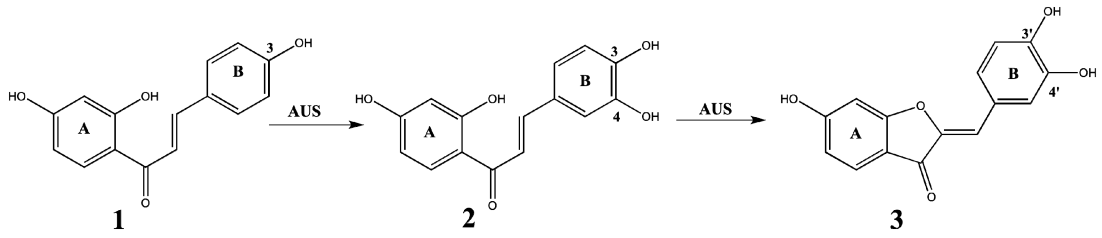

Figure 1: Excerpt of the secondary metabolism leading from the chalcone isoliquiritigenin **(1)** to the aurone sulfuretin **(3)** via butein **(2)**

EXPERIMENTAL

The aurone synthases *AUS1* and *AUS2* were cloned into the pGEX-6P-1 expression vector in both variants, to obtain (i) the full-length latent pro-enzyme as well as (ii) the shorter active enzyme. The *AUS* variants were expressed as fusion proteins containing glutathione S-transferase (GST) at the N-terminus. The protein purification was performed by fast protein liquid chromatography (FPLC) using affinity chromatography and ion exchange chromatography and confirmed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The GST-tag of the fusion protein was removed proteolytically using PreScission™ protease. The substrate specificity of the purified enzymes was examined using high-performance liquid chromatography (HPLC) and UV/VIS spectroscopy.

RESULTS AND DISCUSSION

To date, we successfully expressed the latent pro-enzymes as soluble proteins. A high purity of the aurone synthases was achieved by affinity and ion exchange chromatography as confirmed by SDS-PAGE. After *in vitro* activation with SDS, both isoenzymes showed the expected enzymatic activity (Fig. 2) revealing that the proteins are correctly folded and functionally active.

Also the core sequences of *AUS1* and *AUS2* were expressed as soluble fusion proteins, as verified by SDS-PAGE. Furthermore, the proteins remained soluble after proteolytic removal of the GST-tag, however, they did not show any enzymatic activity, neither with nor without SDS. These results probably indicate that the proteins are folded correctly, but the incorporation of the copper atoms within the active site was not successful. An incubation with copper also did not result in enzymatic activity. Therefore, further experiments are necessary in order to obtain aurone synthases in their active forms.

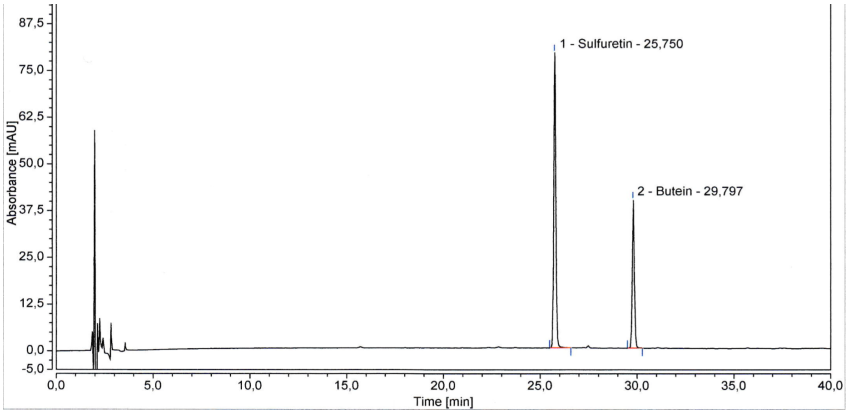

Figure 2: Chromatogram of an enzymatic assay of an active aurone synthase e.g. *AUS1* (substrate: butein; product: sulfuretin)

CONCLUSION

This work evidenced that it is possible to recombinantly express latent *PPO*s, which were functionally active after *in vitro* activation. However, the production of the catalytically active main core resulted in soluble but inactive protein, maybe due to a lack in copper incorporation.

A successful *in vitro* incorporation of the copper atoms within the active site would enable us to investigate the substrate specificity of plant polyphenol oxidases. Furthermore, the co-crystallization of aurone synthase (loaded with copper and/or zinc) with substrates and inhibitors will provide novel insights into the mechanism of plant PPOs.

ACKNOWLEDGMENT

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