RECOMBINANT PRODUCTION AND PURIFICATION OF CHALCONE-3-HYDROXYLASE (CH3H) FROM YEAST

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INTRODUCTION

Cytochrome P450ies make up one of the largest and most diverse known protein family and are present in all kingdoms of life. Their name results from a characteristic absorption band at 450 nm which they exhibit when complexed with carbon monoxide. P450ies are responsible for catalysis of essential reactions including carbon source assimilation, hormone-synthesis, generation of structural compounds, carcinogenesis and degradation of xenobiotics [1]. Due to their ability to hydroxylate complex carbohydrates very specifically, attainment of high amounts of P450ies is also relevant in regard to biotransformation and industrial applications [2]. Isolation of P450ies from native plant or animal tissues often results in humble yields which is why recombinant production is a striking alternative. However, eukaryotic P450ies are in most cases membrane bound which makes recombinant production and subsequent purification challenging [3]. As a result there is also only little known about the structure and substrate conversion mechanisms of membrane bound P450ies.

EXPERIMENTS AND EXAMINATIONS

We are currently working on the recombinant expression and subsequent purification as well as characterization of chalcone-3-hydroxylase, a membrane bound P450 enzyme. CH3H is involved in the biosynthesis of anthochlor pigments in plants, which are important for yellow colorization, formation of UV-honey guides and exhibit health beneficial effects [4-6].

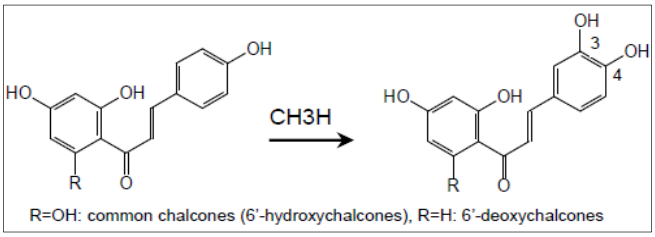

Figure 1: Reaction catalysed by CH3H

In order to shed light on the substrate conversion mechanisms and to elucidate the stringent substrate-specificity the enzyme needs to first be produced in large quantities. Yeasts were chosen as production host as they are able to both perform post translational modifications and quickly grow on inexpensive media up to high cell densities [7]. Subsequent attainment of CH3H involves cell disruption, solubilization of the membrane as well as isolation and purification of the protein.

This project involves bioprocess engineering for establishment of an up- and downstream process in order to attain high amounts of purified CH3H. Up- and downstream process development is the main focus of the integrated bioprocess development working group. Characterization of substrate specificity will then be performed in the division of photochemistry. Further disciplines involved include protein crystallography for structure elucidation and analytical chemistry for increased process understanding in up- and downstream process development.

RESULTS AND DISCUSSION

Recombinant CH3H was actively produced with a strep tag in *S. cerevisiae* strain INVSc1 under control of the CUP1 promoter, where expression of the enzyme is induced by addition of copper

sulfate. The producing strain was characterized in terms of uptake and production rates as well as yields and growth rate. Subsequently it was physiologically compared to the wild type strain.

A downstream process for purification of CH3H was established: After cell disruption, the membrane was solubilized in different detergents with varying concentrations to screen for suitable conditions. The enzyme was first purified using affinity chromatography and then the suitability of detergents for solubilization was evaluated by size exclusion chromatography. Based on the obtained chromatograms, the most appropriate detergent was selected and applied for large-scale purification.

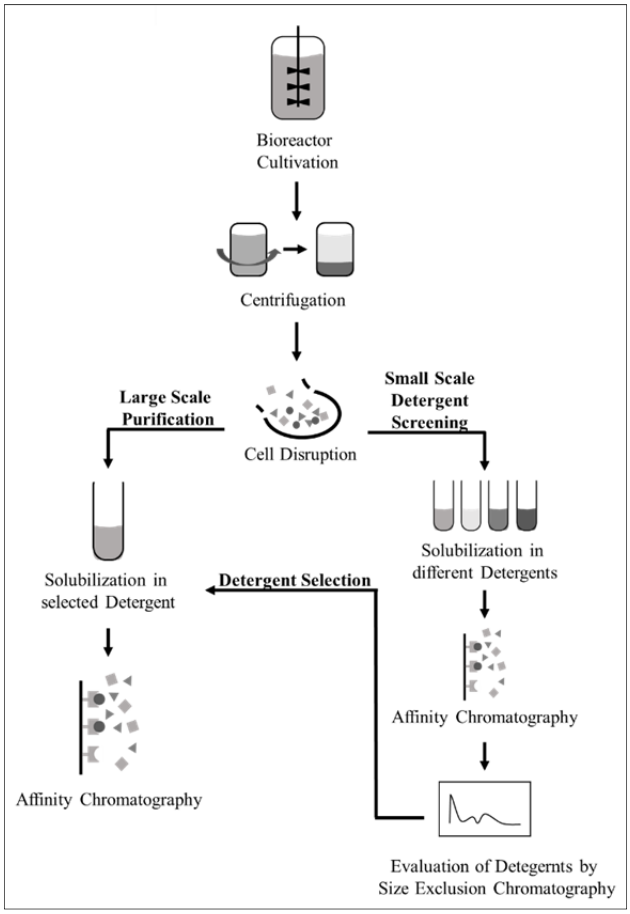

Figure 2: Scheme of cultivation and experimental design for establishment of the downstream process.

CONCLUSION

CH3H was actively expressed in *S. cerevisiae* and a downstream process for large scale purification was established.

Future experiments will involve investigations regarding substrate specificity and crystallization to elucidate the structure of the enzyme, and through correlation of both results shed light on the reaction mechanism. Furthermore protein engineering is ongoing to develop a soluble version of CH3H for facilitated downstream processing.

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