A COMPARATIVE APPROACH TO RECOMBINANTLY PRODUCE THE PLANT ENZYME HORSERADISH PEROXIDASE IN *ESCHERICHIA COLI*

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INTRODUCTION

Horseradish peroxidase (HRP, EC 1.11.1.7, Figure 1) represents an important heme-containing oxidoreductase that catalyses the oxidation of a variety of organic and inorganic substrates using hydrogen peroxide as oxidizing agent (H_2O_2). HRP is used in many biotechnological and medical applications, such as immunoassays (Figure 2) or targeted cancer treatment[3, 4]. Currently, HRP is still isolated from plant, though linked to several disadvantages. These comprise a quite expensive isolation and purification procedure, low production yields as well as the fact that final preparations describe a mixture of heterogeneously glycosylated HRP isoenzymes rather than a well-defined enzyme preparation[4, 5].

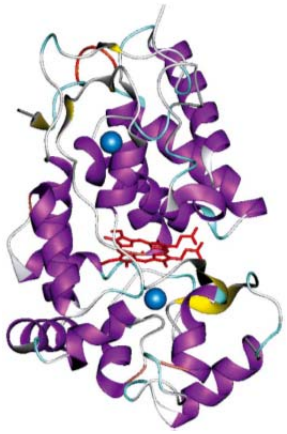

Figure 1. Three-dimensional image of the X-ray crystal structure of HRP isoenzyme C. The heme group (center region) is located between the two domains which each contain one Ca2+-ion (spheres)[1].

Thus, recombinant expression of HRP in the bacterium *Escherichia coli* was investigated to overcome these hurdles. However, production of HRP resulted in the formation of insoluble inclusion bodies (IBs) in the cytoplasm of *E. coli*, which have to be refolded to give active HRP. Up to now, obtained refolding yields are quite low, giving a final concentration of only 10 mg HRP per litre cultivation broth[6]. Alternatively, attempts were made to produce active HRP by translocation into the periplasm of *E. coli*. Although production was successful, obtained final yields did not exceed 0.5 $mg \cdot L^{-1}$ cultivation broth[6]. Consequently production of HRP in *E. coli* is currently not competitive and traditional isolation from plant still prevails.

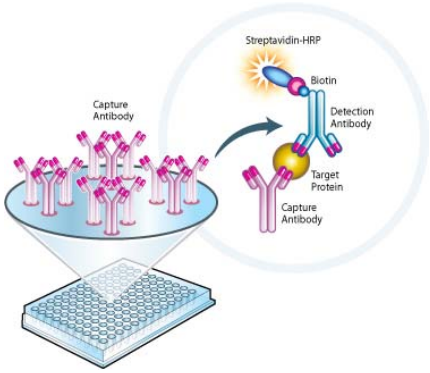

Figure 2. Visualization of HRP-conjugated antibodies used for immunoassays. (Conjugation is performed by Biotin-Streptavidin interaction.)[2]

EXPERIMENTAL APPROACH

In this study, we revisited the production of HRP in *E. coli* and investigated and compared both strategies, A) the production of HRP as IBs and subsequent refolding, as well as B) the production of active HRP in the periplasm. The latter strategy was examined by an integrated approach, investigating various variables along the process by performance of two design of experiments (DoE). An overview graphic of the experimental principle is shown in Figure 3.

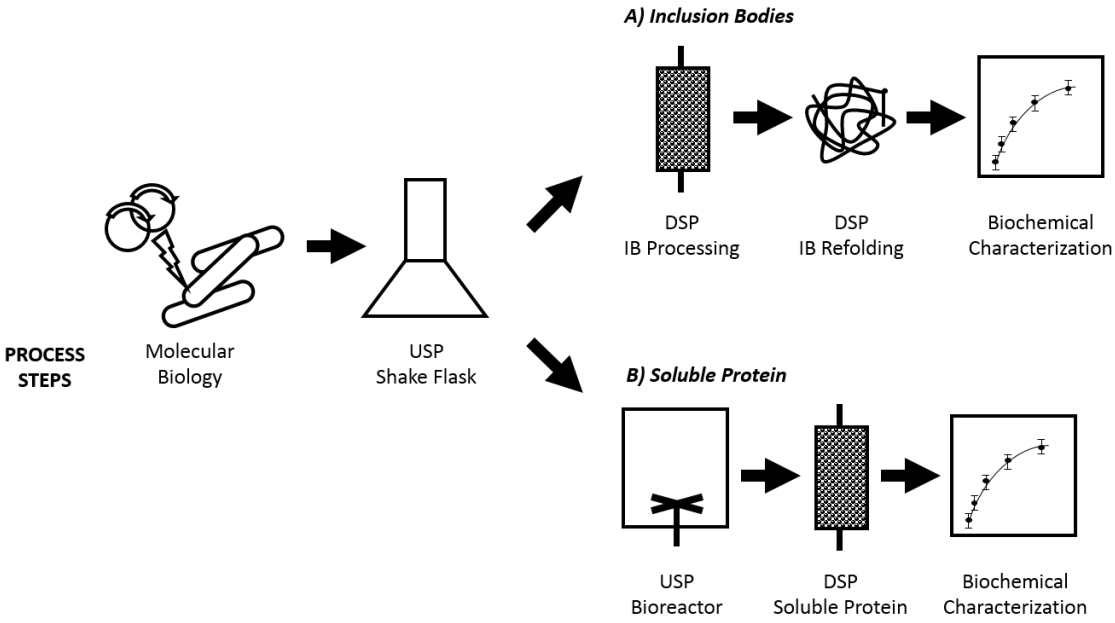

Figure 3. Overview – Experimental principle of the study

RESULTS AND DISCUSSION

In fact, we were able to produce HRP in *E. coli* via both strategies. On the one hand we obtained a refolding yield of 10 % from IBs resulting in a final production yield of 100 mg active HRP per litre cultivation broth, and on the other hand we were able to produce 48 mg active enzyme per litre cultivation broth in the periplasm (both titres are based on a biomass concentration of 60 g DCW·L^{-1} cultivation broth). Regarding biochemical properties, catalytic activity and thermal stability of soluble HRP were highly reduced, which may be caused by the impact of the fused DsbA protein, needed for translocation into the periplasm. Refolded HRP showed comparable substrate affinity, but a 9-fold reduced catalytic activity and 2-fold reduced thermal stability compared to plant HRP. However, the reduced kinetic properties can be compensated by protein engineering.

CONCLUSION

In conclusion, the combination of both production strategies describes a promising toolbox for HRP engineering and production. Thereby, HRP can be engineered by directed evolution or semi-rational protein design and expressed in the periplasm of *E. coli* allowing straight forward screening for improved variants, which are finally produced as IB in high amounts and subsequently refolded.

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