Microbial Cell Factories

Poster Presentation

Heterologous expression of isotopically labeled *Trichoderma reesei* tyrosinase 2 in *Pichia pastoris*

Ann Westerholm-Parvinen*, Maija-Liisa Mattinen, Emilia Selinheimo and Markku Saloheimo

Address: VTT Biotechnology, P.O. Box 1000, FIN-02044 VTT, Finland
* Corresponding author

from The 4th Recombinant Protein Production Meeting: a comparative view on host physiology
Barcelona, Spain. 21–23 September 2006

Published: 10 October 2006

Microbial Cell Factories 2006, **5**(Suppl 1):P65 doi:10.1186/1475-2859-5-S1-P65

Background

Tyrosinase (EC 1.14.18.1) is a copper-containing oxidase that is widely distributed in mammals, invertebrates, plants and microorganisms. In mammals the enzyme is essential for the formation of melanin pigments, whereas tyrosinases in fruit and vegetables are related to the browning reaction that occurs upon bruising and long term storage. Tyrosinase is of great interest for many applications in the field of medicine, biotechnology and food engineering. It is a promising target enzyme for prodrug activations in melanomas and in biotechnological applications including crosslinking of protein matrices. It is of great importance to find ligands and inhibitors for tyrosinase. Structural studies and screening for ligands and inhibitors can be carried out using NMR spectroscopy with isotopically labeled tyrosinase. Therefore, we cloned a novel tyrosinase from *Trichoderma reesei* and expressed it heterologously in the methylotrophic yeast *Pichia pastoris*.

Results

A novel tyrosinase, tyrosinase 2 (TYR2), was cloned from *Trichoderma reesei*. The cDNA sequence was expressed under the control of the AOX1 promoter in the *Pichia pastoris* X-33 strain. The *Saccharomyces cerevisiae* α-MF prepro sequence was used for secretion and an N-terminal His_6-tag was fused to the tyrosinase to facilitate the detection and purification of the recombinant protein. Heterologous expression was carried out in shake flask cultivations and the enzymatic activity was measured directly on the culture medium, using L-Dopa as a substrate. A protein of approximately 45 kDa was detected by Western blot with antibodies against the His-tag. The full length of TYR2 has a predicted MW of 60.4 kDa. When TYR2 is homologously over-expressed in *Trichoderma reesei*, the C-terminal is cleaved and the mature protein has a MW of 43.5 kDa. Thus, it seems probable that *Pichia pastoris* processes the C-terminal correctly. Extensive optimisation of the expression in shake flasks was carried out as the stable isotope labels are costly. Different temperatures, different $CuSO_4$ and NH_4SO_4 concentrations and different shake flasks were tested. The expression level of recombinant TYR2 was increased tenfold as a result of the optimisation. Metabolic ^{15}N-labeling of TYR2 was carried out with $^{15}NH_4SO_4$ in minimal medium to assess its suitability for investigations by NMR spectroscopy. Initial 3D heteronuclear ^{1}H-^{15}N HSQC NMR spectrum of TYR2 showed signals with chemical shifts typical of folded proteins.

Conclusion

The *Trichoderma reesei* tyrosinase 2 was successfully expressed and uniformly ^{15}N-labeled in the yeast *Pichia pastoris*. This methylotrophic yeast is a suitable expression system for the production of recombinant proteins for NMR studies as it is cost-effective and possesses the ability to perform many of the posttranslational modifications of higher eukaryotes.