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Review

Human xanthine oxidase recombinant in *E. coli*: A whole cell catalyst for preparative drug metabolite synthesis

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ABSTRACT

Human xanthine oxidoreductase (XOR), which is responsible for the final steps of the purine metabolism pathway and involved in oxidative drug metabolism, was successfully expressed in *Escherichia coli* BL21(DE3) Gold. Recombinant human (rh) XOR yielded higher productivity with the gene sequence optimized for expression in *E.coli* than with the native gene sequence. Induction of XOR expression with lactose or IPTG resulted in complete loss of activity whereas shake flasks cultures using media rather poor in nutrients resulted in functional XOR expression in the stationary phase. LB medium was used for a 25 L fermentation in fed-batch mode, which led to a 5 fold increase of the enzyme productivity when compared to cultivation in shake flasks. Quinazoline was used as a substrate on the semi-preparative scale using an optimized whole cell biotransformation protocol, yielding 73 mg of the isolated product, 4-quinazolinone, from 104 mg of starting material.

Contents

Abbreviations: XOR, xanthine oxidoreductase; AO, aldehyde oxidase; r, recombinant; h, human.

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1. Introduction

The metabolic pathways to transform and excrete a drug are complex and often use more than one enzyme to reach the final product (Coleman, 2010). The reactions by which a drug is metabolized can be divided in two phases: Phase I reactions are responsible for introducing or uncovering a functional group, and phase II reactions transform the products of phase I metabolism mainly into highly water soluble compounds (Skett and Gibson, 2001). One of the most important reactions of phase I metabolism is oxidation. Most drugs are metabolized by the microsomal mixed-function oxidase system consisting of cytochrome P450 (CYP) and NADPH-cytochrome P450 reductase (Skett and Gibson, 2001). Besides CYPs, there are several other important drug metabolizing enzymes in the human body, such as aldehyde oxidase (AO) (Garattini and Terao, 2011, 2012) and xanthine oxidoreductase (XOR) (Skett and Gibson, 2001).

Both enzymes, AO and XOR, are molybdo-flavoenzymes (MFEs), and are characterized not only by the presence of a molybdenum cofactor (MoCo) but also by a flavin in the active site (Xia et al., 1999). Mammalian MFEs consist of two identical subunits of 150 kDa each, that operate independently. Each unit has an *N*-terminal domain of approximately 20 kDa where the two 2Fe/2S redox centers are located, followed by the flavin binding domain of 40 kDa. The MoCo as well as the substrate binding site are located in the C-terminal domain of 85 kDa (Garattini et al., 2003). In mammals, XOR is responsible for the last steps of the purine metabolism, as it converts hypoxanthine to xanthine and xanthine further to uric acid. XOR has been associated with cases of ischemia (McCord, 1985) and described as an activator of pro-drugs (Pritsos, 2000).

The fact that XOR is mostly found in the small intestine and liver gave rise to the idea that this enzyme could be responsible for detoxifying the body from mainly nitrogen containing polar aromatic compounds. Krenitsky et al. (1986) believed that even poor substrates for XOR would be completely metabolized by this enzyme, considering its relatively high abundance in the human body. XOR has been described to oxidize several types of substrates, from pyridines, pyrazines, purines, pyrimidines, quinolones to pterines (Krenitsky et al., 1986). It can also oxidize drugs such as 6-deoxyacyclovir (Krenitsky et al., 1984), 6-mercaptopurine (Bergmann and Ungar, 1960), famciclovir (Kitamura et al., 2006), allopurinol (Kitamura et al., 2006; Pacher et al., 2006), pyrazinamide (Yamamoto et al., 1987), 5-fluorouracil (Kitamura et al., 2006), and quinazoline (McCormack et al., 1978).

Recombinant human drug metabolizing enzymes have proven to be useful catalysts for the production of drug metabolites (Schroer et al., 2010), which are needed in pharmaceutical research for structural characterization and testing for biological as well as toxicological activity. The expression of recombinant human AO in *Escherichia coli* and its application for drug metabolite synthesis on a 200 mg scale has recently been described (Rodrigues et al., 2014). Recombinant human XOR, however, has not yet been exploited for this purpose.

There are three major families of molybdenum cofactor containing enzymes in *E. coli* as shown by Kisker and Schindelin (1997), based on the type of cofactor bound: the xanthine oxidase family, the sulfite oxidase family and the DMSO reductase family. The enzymes of the xanthine oxidase family are involved in two-electron transfer hydroxylation and oxo-transfer reactions with water as the source of oxygen (Iobbi-Nivol and Leimkühler, 2013).

The scope of this study was to express human XOR in *E. coli* to obtain a whole cell catalyst with high activity and the application of the recombinant biocatalyst for the preparative synthesis of oxidized products, particularly drug metabolites.

2. Materials and methods

2.1. Materials

Xanthine, hypoxanthine and uric acid were purchased from Fluka Chemie GmbH (St.Loius, USA), quinazoline, allopurinol and menadione from Sigma-Aldrich GmbH (St.Louis, USA). LB medium, LB-Agar, both as a ready-made powder, yeast extract and tryptone were provided by Becton, Dickinson and Company (Franklin Lakes, USA) and peptone from casein from Merck KGaA (Darmstadt, Germany). The organic solvents were all provided by Merck KGaA, except for DMSO, which was from Sigma Aldrich GmbH. Isolute HM-N was purchased from International Sorbent Technology Ltd. (Mid Glam, UK). All other chemicals were purchased from Sigma, Aldrich, Fluka Chemie, Merck KGaA and Fischer Scientific (Waltham, USA), and were of analytical grade. A LiChroprep RP18 column was purchased from Merck KGaA and a Nucloeodur 100-10 C18ec column from Macherey-Nagel (Düren, Germany). An HPLC-DAD 1260 Infinity system from Agilent Technologies (Santa Clara, USA) or a UPLC-MS/DAD Acquity UPLC system from Waters (Milford, USA), equipped with a Waters Acquity UPLC PDA photodiode array detector and a Waters Acquity SQD Single Quadrupole Detector for mass spectroscopy were used for substrate and product detection. The NMR spectrum of 4-quinazolinone was recorded at 300 K on a Bruker AV-III-600 NMR spectrometer equipped with a 1.7 mm TCI Cryoprobe.

2.2. Cloning of XOR

The gene (NCBI accession number NM_000376) coding for human Xanthine dehydrogenase/oxidase (NCBI accession number NP_000370.2) was ordered at imaGenes (Berlin, Germany). Additionally, the gene sequence was optimized for expression in *E. coli* and ordered at GenScript (New Jersey, USA). The sequence can be found in the Supporting information. Plasmid DNA was retrieved according to the protocols delivered with the genes and used as templates for gene amplification using Phusion® High-Fidelity DNA