DEVELOPMENT OF GLUCURONIDE PREPARATION SYSTEM FOR XENOBIOTIC METABOLITES USING GENETICALLY ENGINEERED BUDDING YEAST

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Glucuronidation, which is catalyzed by isoforms of UDP-glucuronosyltransferases (UGT), is the most common pathway for detoxification and elimination of hydrophobic xenobiotics occurring in tissues of most mammals. Because of their ubiquitous nature and high physiological significance, development of an efficient in vitro synthesis of glucuronides often becomes critical during studies of drug metabolism undertaken in the development of a new pharmaceutical product. Glucuronides have been obtained by chemical synthesis, by tedious isolation and purification of in vivo metabolites from biological samples of experimental animals, and by biosynthesis using hepatic microsomes as enzyme source. In order to synthesize the glucuronides as drug metabolites, we have now developed several mammalian CYP, UGT and UDP-glucose dehydrogenase (UGDH) coexpression systems in budding yeast.

We have previously constructed the expression system of several mammalian CYP and UGT isoforms in budding yeast cells, *Saccharomyces cerevisiae* AH22, to analyze the structure and function of these xenobiotic metabolizing enzymes. Yeast cells lack the ability of production of UDP-glucuronic acid (UDP-GlcUA) from UDP-glucose to synthesize the glucuronide in whole cells. To achieve the production of UDP-GlcUA for co-substrate of glucuronidation, UGDH gene deriver from rat or plant was introduced to yeast cell. Several CYP, UGT isoforms and UGDH were expressed in budding yeast using a multicopy plasmid vector and a genome integrated vector. Each enzyme expression in yeast was confirmed by Western blot analysis. Glucuronide formation in yeast cells was performed in reaction buffer containing 0.1M KPi (pH7.4) and 8% glucose. Using genetically engineered yeast strain containing human UGT1A6 and rat UGDH, glucuronide formation of 7-hydroxycoumarin (7HC) as a model substrate was examined. Most glucuronide of 7HC was found in reaction medium with time-dependent production, suggesting the functional expression of both enzymes and the presence of endogenous transport system for glucuronide in yeast. Optimization of the reaction conditions resulted in 95% conversion of 7HC into its gulucuronide. Compared with fission yeast system as host cell, budding yeast appears to be more competent for glucuronide formation. Mycophenolic acid with multiple glucuronidating sites was conjugated as UGT isoform-dependent formation, suggesting that the regiospecific glucuronides of several drugs could be obtained using UGT1A and 2B isoforms. In order to synthesize glucuronide from CYP-dependent metabolite during Phase I and II processes, rat CYP1A1 and yeast NADPH-P450 reductase were coexpressed with UGT in yeast. The resultant recombinant yeast cells with xenobiotic metabolizing enzymes was able to produce directly the glucuronide from 7-ethoxycoumarin via 7HC.

This coexpression system of mammalian CYP, UGT and UGDH in budding yeast would be a powerful tool for enzyme-assisted synthesis of various xenobiotic metabolites including glucuronides.

**Biography**

Shinichi Ikushiro, Ph.D., has been an Associate Professor of Department of Biotechnology, Faculty of Engineering, Toyama Prefectural University since 2005. He graduated from Faculty of Arts and Sciences, Hiroshima University and was a Research Associate of Faculty of Science, Himeji Institute of Technology, collaborated with Professor Takashi Iyanagi. (1992-2004). His research interests include structure and function of xenobiotic metabolizing enzymes, cytochrome P450 and UDP-glucuronosyltransferase.