J. Biol. Macromol., **20** (1), 3-8 (2020)

Review

Library of drug metabolizing enzymes: applications for metabolic prediction and biosynthesis of human drug metabolites

Miyu Nishikawa

Department of Biotechnology, Faculty of Engineering, Toyama Prefectural University, 5180 Kurokawa, Imizu, Toyama 939-0398, Japan, TOPUBIO Research Co., Ltd, 5180 Kurokawa, Imizu, Toyama 939-0398, Japan

Received October 11, 2019; Accepted October 24, 2019

Drug metabolizing enzymes (DMEs) play a role in modifying drug activity and in modulating the clearance of target compounds such as endo- and xenobiotics, small molecule drugs, steroid hormones and dietary flavonoids. Thus, the evaluation of metabolites generated by DMEs is required for drug development, food science and environmental assessment. Here, we generated a library of DMEs expressed in yeast cells as a means of studying xenobiotic metabolism from early phase metabolic screening to structural determination and evaluation. In this review, advantages of the DME expression system are introduced as well as applications of the DME platform for studying drug metabolism.

Key words: Drug metabolizing enzymes, biotransformation,

Introduction

Drug metabolizing enzymes (DMEs) modify drug activity and influence the clearance of target compounds such as endo- and xenobiotics, small molecular drugs, dietary polyphenols and endogenous steroids. Metabolism by DMEs is mainly divided into two phases; oxidation, reduction and hydrolysis (phase I) followed by the transfer of hydrophilic groups (phase II). Cytochrome P450s (CYPs) are common phase I enzymes that catalyze the oxidation of a wide variety of compounds. Microsomal CYPs are often involved in the metabolism of xenobiotics. The reactions catalyzed by CYPs contribute 70% of the main metabolic pathways of pharmaceutical drugs [1]. Uridine 5'-diphospho-glucuronosyltransferase (UDP-glucuronosyltransferases, UGTs) are membrane-bound major phase II enzymes, which are mostly localized in the endoplasmic reticulum (ER) and catalyze the transfer of a glucuronic acid from UDP-glucuronic acid (UDP-GA) to a hydroxyl group of the substrate compound. Cytosolic sulfotransferases (SULTs) are also major phase II enzymes, but they utilize 3'-phosphoadenosine-5'-phosphosulfate (PAPS) as a universal sulfate donor and transfer a sulfate group to a hydroxyl moiety of the substrate.

DMEs comprise a gene superfamily, which enables DMEs to convert various types of compounds into metabolites. In human, more than 50 CYP, 19 functional UGT, and at least 13 SULT isoforms have been reported [2-7].

Usually, DMEs mediate biological inactivation and hydrophilization of the original compounds for elimination in urine or bile. Sometimes, however, chemical reactivity of the original compound is increased via metabolism mediated by DMEs, which often becomes a risk factor due

E-mail: m-nishikawa@pu-toyama.ac.jp

to enhanced toxicity. In mammals, acyl-glucuronidation is one of the major conjugation reactions of carboxylic acid containing drugs such as non-steroidal anti-inflammatory drugs (NSAIDs). The resulting acyl-glucuronides are unstable chemically reactive metabolites [8-10]. Acyl-glucuronides often covalently modify macromolecules leading to cytotoxicity. By contrast, several pharmaceutical drugs are specifically designed to be activated in the body by this reaction. For example, morphine-6-glucuronide, which is a major active metabolite of morphine, is formed from morphine by the enzyme UGT2B7 [11]. Morphine-6-glucuronide elicits a more potent analgesic effect than the parent compound morphine [12, 13].

Recently, DMEs have also become the focus of research in the field of food science. For example, polyphenolic compounds are a diverse group of phytochemicals that are commonly found in the human diet. A number of reports suggest phase II metabolites of polyphenols act as the delivery form in plasma [14-16].

The preparation of metabolites converted by DMEs can be a bottleneck in studying the metabolism of drugs or nutrients such as polyphenols. Moreover, it is often difficult to prepare authentic standards of the metabolites in the correct regio- or stereo-selective form by conventional organic synthesis. An enzymatic approach to generating these compounds is therefore an attractive alternative strategy. However, the requirement for expensive cofactors is a serious disadvantage for this enzymatic synthesis at an industrial scale.

We previously constructed a heterologous DME expression system for CYP, UGT and SULT in the budding yeast *Saccharomyces cerevisiae* AH22 strain. In addition, biotransformation of the metabolites was successfully achieved using various substrate compounds without the requirement for exogenous cofactors [17, 18]. In this review, advantages of the recombinant DME expression system in yeast are described and several applications are introduced.

The DME expression system in yeast cells

Usually, metabolic reactions catalyzed by DMEs require cofactors. For example, NAD(P)H is an electron donor in many phase I xenobiotic metabolism reactions including those catalyzed by CYPs. NADPH-cytochrome P450 reductase (CPR), NADH-cytochrome b5 reductase, and cytochrome b5, which act as an electron transfer partner, is also essential for microsomal CYP mediated reactions [19]. To reconstitute this electron transfer system *in vitro*, β-NADH+, glucose-6-phosphate and glucose-6-phosphate dehydrogenase are often added to the reaction as a NADPH-generating system. UGT and SULT also require a more expensive cofactor UDP-GA or PAPS, which are donors of glucuronic acid in the UGT reaction and sulfate in the SULT reaction, respectively [20, 21].

To achieve enzymatic synthesis of xenobiotic metabolites without exogenous cofactors, we applied whole-cell dependent biosynthesis using the DME expression system in yeast. Mammalian DMEs, including some from human, were heterologously expressed in *Saccharomyces cerevisiae* AH22 cells. In order to construct the CYP expression system in yeast cells, Sakaki et al. used the CYP/CPR fusion protein and successfully biotransformed the substrate compounds to their corresponding metabolites [22, 23]. We also constructed a co-expression system of UGT with UDP-glucose dehydrogenase (UGDH) to supply endogenous UDP-GA. This was necessary because yeast strain AH22 lacks the native UGDH, which is the carboxyl dehydrogenase to produce UDP-GA (Fig. 1).

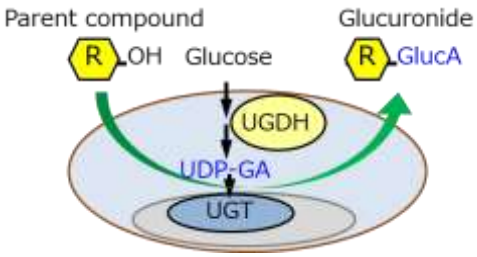

Fig. 1. Scheme of glucuronide biosynthesis by recombinant UGT and UGDH expressed in yeast.

Using this system, we successfully produced several hundred milligrams of glucuronide metabolites per liter without exogenous addition of UDP-GA [18]. As well as CYP and UGT, we also constructed a SULT expression system in yeast. Heterologous expression of SULT was sufficient for whole-cell dependent sulfoconjugation without exogenous addition of PAPS, suggesting that native PAPS can act as a sulfate donor for the recombinant SULT [17]. As well as studying the metabolism of drug candidates brought about by CYP mediated modification, it is also important to screen for the effect of other DMEs such as UGT. Thus, establishment of a DME platform will be invaluable for studying xenobiotic metabolism.

Several host cells have been used as a heterologous expression system of DMEs by different groups, which include prokaryotic microbes such as *E. coli* and S*almonella typhimurium*, insect cells and mammalian cell lines in addition to yeast [24-27]. In terms of large scale cultivation, prokaryotic microbes are attractive hosts for heterologous expression. However, functional expression of CYPs and UGTs in prokaryotic microbes is often difficult to achieve because these are membrane-bound enzymes mostly localized in the ER. Thus, we selected yeast cells *S. cerevisiae AH22* to construct the DME library, which includes membrane-bound enzymes. Using this host we successfully constructed an expression system for CYP, UGT and SULT. Moreover, for performing the reactions we can use stocks of recombinant yeast cells immediately after thawing, without the need to generate an enzyme preparation.

Screening assay using DME panels

Predicting the metabolic fate of a candidate compound is an important step during the drug development process. More recently, a similar question is often also addressed in the area of food science research. We have constructed 19 CYP, 35 UGT, and 10 SULT isoforms of mammalian origin, some of which are human. This variation enables us not only to examine the enzymes that contribute to the metabolism but also to compare the metabolism between human and several experimental animals. We then performed screening assays of drugs and dietary polyphenols using the panel of DMEs. Specifically, the library includes a variety of DME isoforms from human, mouse, rat, rabbit and pig. In UGT screening of mefenamic acid and flufenamic acid, human UGT1A7, 1A8, 1A9 and 1A10 showed relatively high levels of activity, whereas the activity of UGT2B family enzymes was quite low. In contrast, rat and mouse Ugt2b family enzymes showed high activity toward these compounds (Fig. 2). In human SULT screening of polyphenols, SULT1E1 showed a low level of regio-selectivity toward resveratrol and quercetin (Fig. 3) [17].

Scale up for the preparation of metabolites

Using a variety of enzymes can also increase the success rate of metabolite preparation. For example, rat Ugt2b1 is a useful isozyme to prepare acyl-glucuronides from several drug compounds (Table 1). These glucuronides are also synthesized by human UGTs, but the yield was significantly lower. The evaluation of acyl-glucuronides is required by FDA guidelines

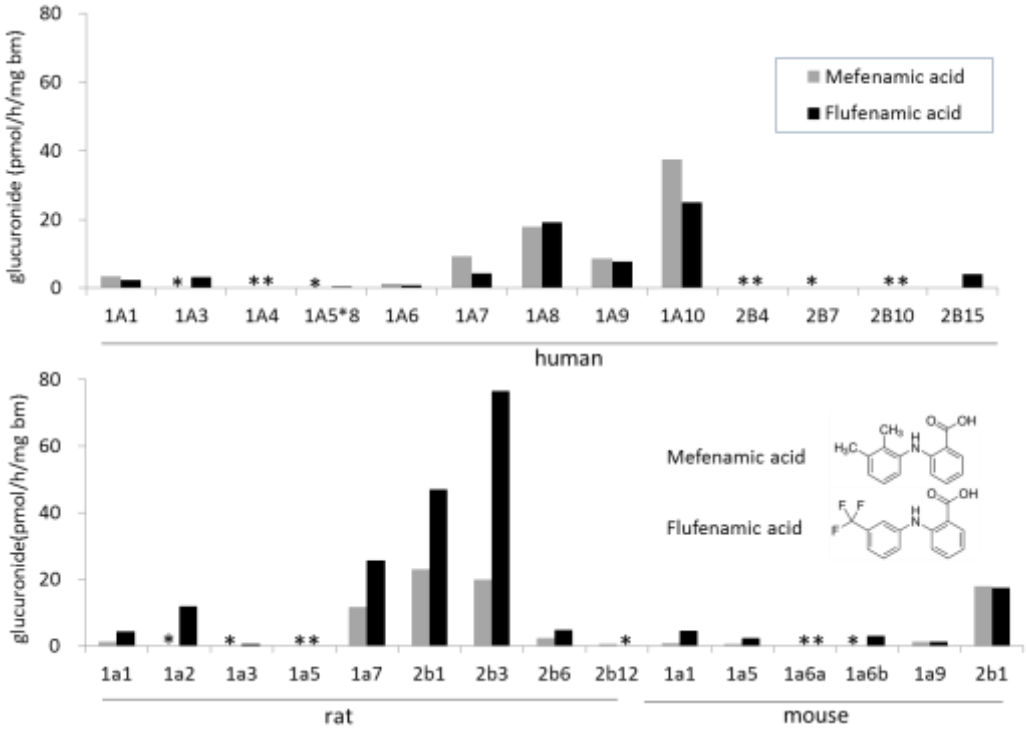

Fig.2: Whole-cell UGT screening of mefenamic acid and flufenamic acid.

*: Not-detected by HPLC-UV., bm: recombinant UGT biomass

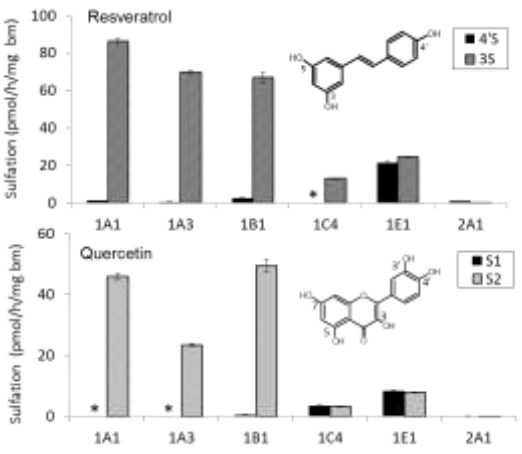

Fig. 3. Whole-cell human SULT screening of resveratrol and quercetin.

*: Not-detected by HPLC-UV., bm: recombinant SULT biomass

in some cases [28]. Thus, recombinant rat UGT2B1 is often used for glucuronide preparation.

Dietary polyphenols preferably undergo metabolism by UGTs and SULTs. Polyphenols have more target sites for DMEs than pharmaceutical drugs because of the phenolic hydroxyl groups. Thus, an enzymatic approach is preferable in terms of both regio- and stereo-selectivity. We successfully prepared glucuronides and sulfoconjugates with high levels of purity (>95% by HPLC-UV) and determined their chemical structure by NMR. Quercetin (Q) is a major dietary polyphenol that elicits various biological activities including anti-inflammation, anti-oxidant and anti-obesity. It has been reported that Q-3-O-glucuronide is a major metabolite in human, but other regio-isomers of glucuronide are also generated by human UGTs [29, 30]. We prepared four regio-isomers of quercetin-O-glucuronides (-3, -7, -3' and -4' position) and are currently evaluating the chemical properties and biological activities of these regio-isomers *in vitro* and *in vivo* (unpublished data).

Table 1. Productivity of acyl-glucuronide in scale-up reaction.

Substrate	UGT source	Yield (mg/L/day)
Mefenamic acid	rat 2b1	60
Flufenamic acid	rat 2b1	132
Diclofenac	rat 2b1	260
Zomepirac	porcine 1A3	47

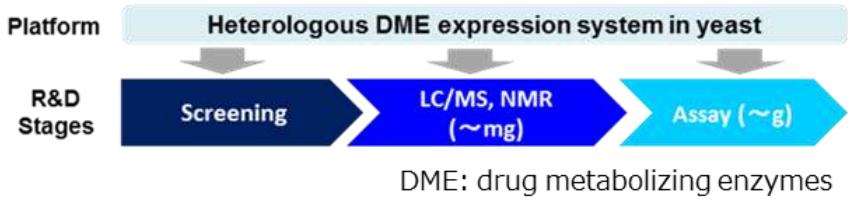

Fig. 4 Application of the recombinant DME platform during drug development.

Conclusion.

The DME platform can be used to study the metabolism of drugs or nutrients. The technology is useful for screening to large scale preparation of analytical standards, structure determination and even for their evaluation *in vitro* and *in vivo* (Fig. 4). Furthermore, the DME platform might also be useful in the area of analytical science and in the evaluation of environmental pollutants.

We are currently constructing additional expression systems for DMEs, which will aid the detailed study of metabolism.

References

1. Williams, J. A., Hyland, R., Jones, B. C., Smith, D. A., Hurst, S., Goosen, T. C., Peterkin V., Koup J. R., Ball, S. E. (2004) *Drug Metab. Dispos.* **32**, 1201-1208.
2. Nelson, D. R., Zeldin, D. C., Hoffman, S. M, Maltais L. J., Wain, H. M., Nebert, D. W. (2004) *Pharmacogenetics.* **14**, 1-18.
3. King, C. D., Rios, G. R., Green, M. D., Tephly, T. R. (2000) *Curr. Drug Metab.* **1**, 143-161.
4. Radominska-Pandya, A., Bratton, S., Little, J. M. (2005) *Curr. Drug Metab*. **6**, 141-160.
5. Blanchard, R. L., Freimuth, R. R., Buck, J., Weinshilboum, R. M., Coughtrie, M. W. (2004) *Pharmacogenetics*. **14**, 199–211.
6. Freimuth. R. R., Wiepert, M., Chute, C. G., Wieben, E. D., Weinshilboum, R. M. (2004) *Pharmacogenomics J.* **4**, 54–65.
7. Hildebrandt, M. A., Carrington, D. P., Thomae, B. A., Eckloff, B. W., Schaid, D. J., Yee, V. C., Weinshilboum, R. M. Wieben, E. D. (2007) *Pharmacogenomics J.* **7**, 133–143.
8. Iwamura, A., Nakajima, M., Oda, S., Yokoi, T. (2017) *Drug Metab. Pharmacokinet.* **32**, 2-11.
9. Regan SL, Maggs JL, Hammond TG, Lambert C, Williams DP, Park BK. (2010) *Biopharm. Drug Dispos*. 31, 367-395.
10. Bailey, M. J., Dickinson, R. G. (1996) *Chem. Res. Toxicol.* **9**, 659-966.
11. Coffman, B. L., Rios, G. R., King, C. D., Tephly, T. R. (1997) *Drug Metab. Dispos.* **25**, 1-4.
12. Sverrisdóttir, E., Lund, T. M., Olesen, A. E., Drewes, A. M., Christrup, L. L., Kreilgaard, M. (2015) *Eur. J. Pharm. Sci.* **74**, 45-62.
13. De Gregori, S., De Gregori, M., Ranzani G. N., Allegri, M., Minella, C., Regazzi, M.

(2012) *Metab Brain Dis.* **27**, 1-5.

14. Kunihiro, A. G., Luis, P. B., Brickey, J. A., Frye, J. B., Chow, H. S., Schneider, C., Funk, J. L. (2019) *J. Nat. Prod*. **82**, 500-509.
15. Terao, J., Murota, K., Kawai, Y. (2011) *Food Funct.* **2**, 11-17.
16. Patel, K. R., Andreadi, C., Britton, R. G., Horner-Glister, E., Karmokar, A., Sale, S., Brown, V. A., Brenner, D. E., Singh, R., Steward, W. P., Gescher, A. J., Brown, K.. (2013) *Sci. Transl. Med.* **5**, 205ra133.
17. Nishikawa, M., Masuyama, Y., Nunome, M., Yasuda, K., Sakaki, T., Ikushiro, S. (2018) *Appl. Microbiol Biotechnol*. **102**, 723-732.
18. Ikushiro, S., Nishikawa, M., Masuyama, Y., Shouji, T., Fujii, M., Hamada, M., Nakajima, N., Finel, M., Yasuda, K., Kamakura, M., Sakaki, T. (2016) *Mol. Pharm*. **13**, 2274-2282.
19. Nebert, D. W., Gonzalez, F. J. (1987). *Annu. Rev. Biochem.*. **56**, 945–993
20. Tukey, R. H., Strassburg, C. P. (2000) *Annu. Rev. Pharmacol. Toxicol.* **40**, 581–616.
21. Coughtrie, M. W. (2016) *ChemBiol. Interact.* **259**, 2–7.
22. Sakaki, T., Shibata, M., Yabusaki, Y., Murakami, H., Ohkawa, H. (1990) *DNA Cell Biol.* **9**, 603-614.
23. Inouye, K., Kondo, S., Yamamura, M., Nakanishi, D., Sakaki, T. (2001) *Biochem. Biophys. Res. Commun*. **280**, 1346-1351.
24. Radominska-Pandya, A., Bratton, S., Little, J. M. (2005) *Curr. Drug Metab*. **6**, 141-160.
25. Fujita, K., Mogami, A., Hayashi, A., Kamataki, T. (2000) *Life Sci.* **66**, 1955-1967.
26. Ouzzine, M., Pillot, T., Fournel-Gigleux, S., Magdalou, J., Burchell, B., Siest, G. (1994) *Arch. Biochem. Biophys.* **310**, 196-204.
27. Barnes, H. J., Arlotto, M. P., Waterman, M. R. (1991) *Proc. Natl. Acad. Sci.* **88**, 5597-5601.
28. U.S. Department of Health and Human Services. (2016) Food and Drug Administration. Center for Drug Evaluation and Research. Safety Testing of Drug Metabolites Guidance for Industry. Revision 1.
29. Day, A. J., Mellon, F., Barron, D., Sarrazin, G., Morgan, M. R., Williamson, G. (2006) *Br J Nut*r. **96**, 107-16.
30. Mullen, W., Edwards, C. A., Crozier, A. (2001) *Free Radic. Res*. **35**, 941-952.

Communicated by Yasukawa Kiyoshi