POLYUGTS: NEW ENZYMES FOR BIOCATALYTIC SYNTHESIS OF GLUCURONIDES

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Abstract

Glucuronidation is a major route of metabolism of drugs, resulting in increased hydrophilicity to facilitate elimination of drugs from the body. Whilst rarely pharmacologically active, major glucuronides of drugs may need to be evaluated for interactions with transporters and other xenobiotic enzymes such as cytochrome P450s, and, subject to the level of conversion from the parent drug, they may need to be quantified in clinical samples for bioanalysis, and investigated in other studies e.g. drug-drug interactions.

Here we present new microbially-derived UGTs for making glucuronides. The enzymes, called PolyUGTs, were cloned from filamentous bacteria already known to glucuronidate drugs, expressed in *E.coli* and purified by affinity chromatography. Eleven of the isoforms were incorporated into a screening kit with UDPGA, and assessed against a substrate panel known to form glucuronides. PolyUGT enzymes mimic human UGTs in making *O*-, acyl and some *N*-glucuronides of drugs. The poster explores the application of PolyUGT enzymes to synthesize glucuronides, illustrated by the production of major and minor *O*-glucuronides of ezetimibe, acyl glucuronide of zomepirac , and the *N*-carbamoyl glucuronide of lorcaserin.

About PolyUGTs enzymes

PolyUGT enzymes are new UDP-glucuronosyl-transferases (UGTs) cloned from selected bacteria in Hypha's strain collection. Through genome mining analysis, a total of 702 putative glycosyltransferases (GTs) were identified, including 47 putative UGTs from 11 bacterial strains. The purified enzymes are potent and some PolyUGTs remained highly active in the micromolar range and capable of fully glucuronidating drug compounds.

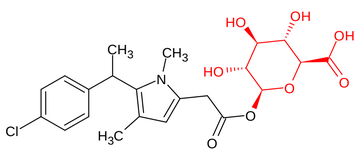
The PolyUGT enzymes were assessed against a substrate panel known to produce glucuronide products. They are inverting UGT enzymes and produce the same human glucuronide product in the β-configuration.

The best performing enzymes were further developed and incorporated into a PolyUGT metabolite screening kit.

The PolyUGT screening kit contains 11 PolyUGT isoforms (gold), UDPGA cofactor (black), positive control substrate (silver) and extra enzyme (gold), pH reduction vial (pink) and formulation reagent red).

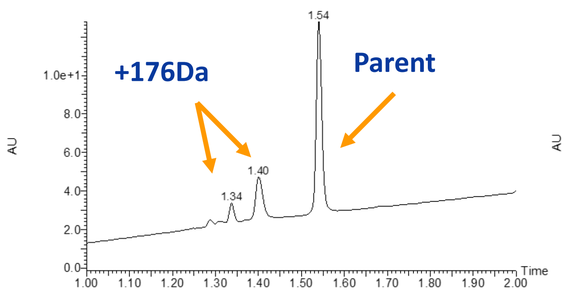
Biocatalytic synthesis of acyl glucuronides

Lowering incubation pH to reduce migration isomers

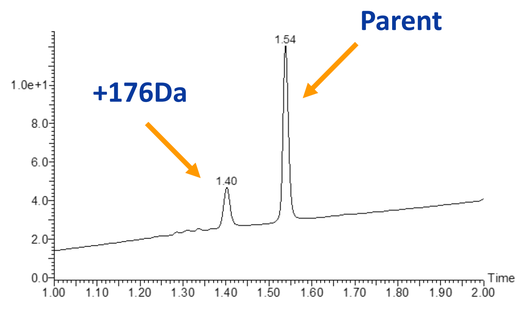


Zomepirac acyl glucuronide

Zomepirac is a nonsteroidal anti-inflammatory drug that was withdrawn from the market due to formation of a reactive unstable acyl-glucuronide, responsible for the adverse effects of drug through irreversible protein binding and immune-mediated toxicity.



Incubations at pH 7.5 - 28.6% conversion

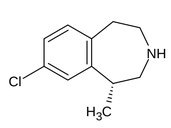


Incubations at pH 6.5 - 23% conversion

Incubation pH can effect the extent of acyl ring migration of some acyl glucuronides. Lowering the pH from 7.4 to pH 6.5 using the pH reduction vial, reduced formation of acyl migration isomers during the reaction of zomepirac with PolyUGT 203.

N-carbamoyl glucuronidation

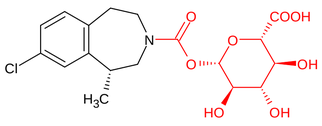
Lorcaserin is a weight loss drug that activates serotonin receptors to reduce appetite, however it was withdrawn from the market due to increased occurrence of cancer. Lorcaserin *N*-carbamoyl glucuronide was successfully produced by several PolyUGT enzymes by recreating conditions described by Gunduz *et al*. Five PolyUGTs could form the *N*-carbamoyl glucuronide in the presence of bicarbonate buffer alone, achieving a maximum of 15% conversion, compared to 18% observed with bicarbonate buffer and CO_2.



Lorcaserin

UGT2B7, 2B15, 2B17

15% conversion
PolyUGTs 212 & 213
Bicarbonate buffer

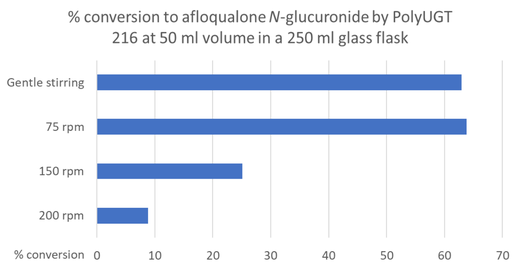


Lorcaserin *N*-carbamoyl glucuronide

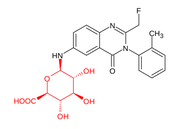
PolyUGT enzyme scale-up factors

Gentle mixing is critical for optimum performance

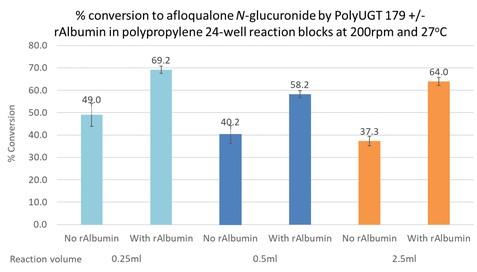
- Scale-up of reactions are influenced by a number of factors depending on the substrate and isoform. Vigorous shaking or agitation has a negative effect.
- Shaking - the best format is to incubate the reaction in a glass flask with gentle swirling or a slowly rotating stirrer bar.



Reducing non-specific binding with addition of albumin



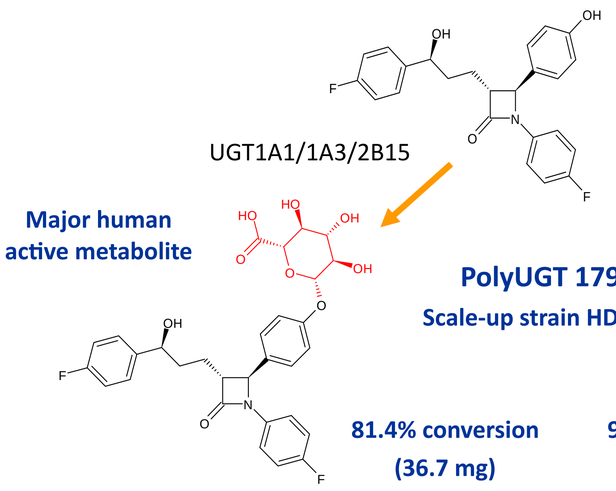
- Addition of rAlbumin (recombinant bovine serum albumin), standard BSA or HSA at 0.1 mg/ml in the reaction can help to prevent any non-specific binding of the UGT protein to reaction vessels, especially where plastic is being used.



Increasing yields with substrate and UDPGA optimization

- An increase in the substrate concentration from the 100 mg/L used in the screening reaction can result in greater yields, especially when coupled to an increase in the concentration of UDPGA from the 5mM used in the screen.
- This can sometimes result in over metabolism to e.g. to diglucuronides.

Live cell scale-up of *O*-glucuronidation reaction



Ezetimibe (50 mg in 0.5L reaction)

UGT1A1/1A3/2B15

UGT2B7

Major human active metabolite

PolyUGT 179
Scale-up strain HD038

81.4% conversion (36.7 mg)

9.1% conversion (3.7 mg)

Both glucuronides were scaled-up using a *Streptomyces* strain into which PolyUGT 179 had been cloned, generating 36.7 mg of the major metabolite at an isolated yield of 81.4%.

References

Gunduz M, Argikar UA, Baeschlin D, Ferreira S, Hosagrahara V, Harriman S. Identification of a novel N-carbamoyl glucuronide: in vitro, in vivo, and mechanistic studies. Drug Metab Dispos. 2010 Mar;38(3):361-7. doi: 10.1124/dmd.109.030650.

About Hypha: **Hypha Discovery is a specialist CRO with expertise in the scalable synthesis, purification and identification of drug metabolites. Using our biocatalytic and organic chemistry capabilities, we also make oxidized derivatives of lead compounds and API degradation products. Hypha also has a wealth of experience in the production, purification and structure elucidation of natural products. We work with pharmaceutical and agrochemical companies globally.**

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