Natural Inhibitors in Urine Can Reduce β-Glucuronidase Performance and Drug Hydrolysis

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INTRODUCTION

Urine drug testing is the most common practice for monitoring the use of prescribed opioid drug medications. Screening and confirmatory assays benefit from the use of β-glucuronidases (βGUS) to hydrolyze glucuronidated drug metabolites. Endogenous compounds in clinical samples can compromise hydrolysis but have not yet been characterized in detail.

In this study, two recombinant βGUS, IMCSzyme RT and *Brachyspira pilosicoli* (BpGUS), are compared by two methods:

1. Inhibition assays utilizing 4-methylumbelliferyl-β-D-glucuronide (4-MUG) as the substrate and endogenous metabolites that could be found in urine.
2. Multiple patient samples using 3 different enzyme concentrations.

MATERIALS AND METHODS

- Enzyme activities were measured using a substrate-inhibitor cross titration assay where substrate and inhibitor concentrations varied while enzyme concentration remained constant. Hydrolysis rates were calculated and plotted as a function of substrate concentration and hydrolysis rate at each metabolite concentration.
- Opioid-positive urine specimens were obtained from a national testing laboratory. Patient specimens were buffered and hydrolyzed with IMCSzyme or BpGUS.
- Hydrolyzed urine samples were cleaned with solid phase extraction, solvent evaporated, reconstructed in methanol and diluted with mobile phase A (0.1% formic acid in water). 10 µL of sample was injected on a Thermo Scientific™ Vanquish™ UHPLC system coupled with a Thermo Scientific™ Endura™ Triple Quadrupole Mass Spectrometer using a Phenomenex Kinetex® 2.6 µm Phenyl-Hexyl 100 Å, 50 x 4.6 mm column. Mobile phase B was 0.1% formic acid in acetonitrile.

INHIBITOR ASSAYS WITH 4-MUG

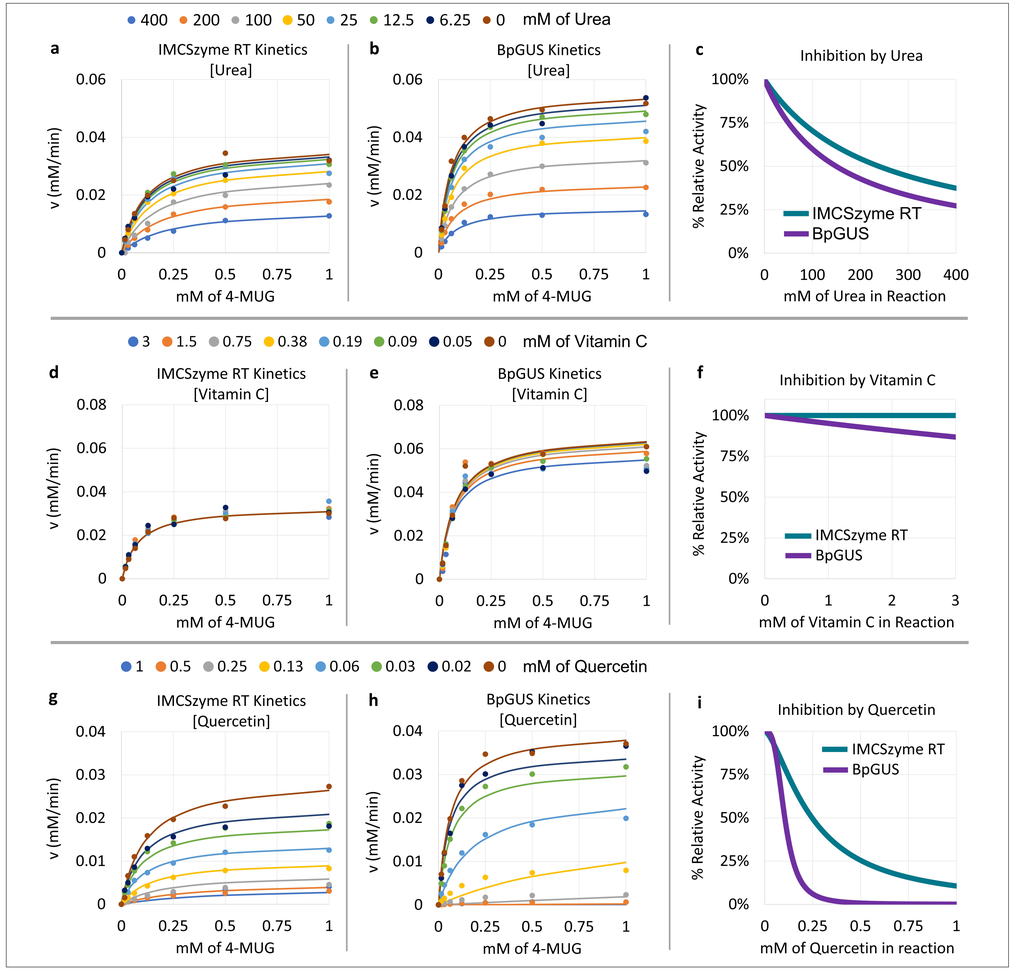

Figure 1. *Urea is a naturally occurring component in urine. 4-MUG hydrolysis shows that as urea concentration increase IMCSzyme RT and BpGUS activity decreases **(a, b, c)**. Vitamin C is an essential vitamin from fruits and vegetables. It does not impact IMCSzyme RT hydrolysis, and it minimally impacts BpGUS hydrolysis **(d, e, f)**. Quercetin is a flavonoid from fruits and vegetables, and it inhibited both IMCSzyme RT and BpGUS with effects more exaggerated on the latter **(g, h, i)**.*

INHIBITION IN PATIENT SAMPLES

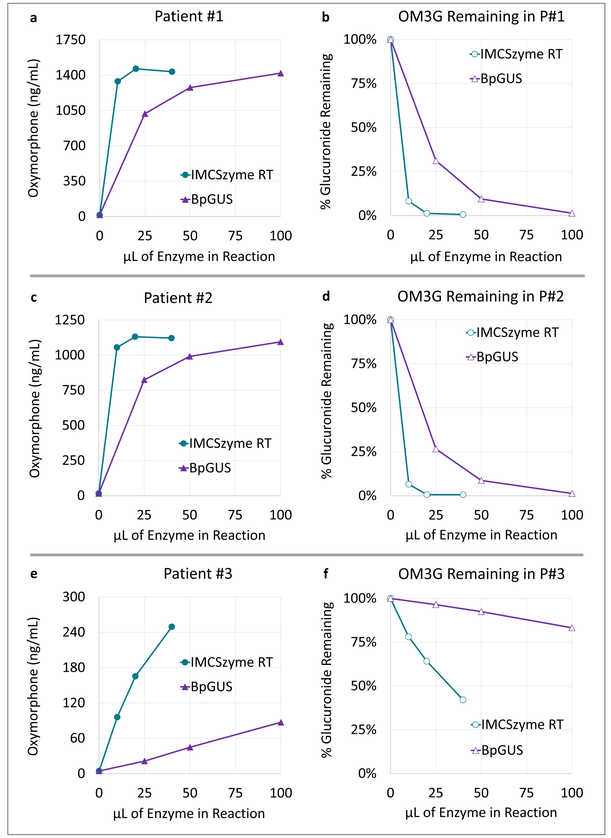

Figure 2. *Three different patient samples were hydrolyzed with either IMCSzyme RT or BpGUS with three different enzyme concentrations. Hydrolyzed oxymorphone (ng/mL) in each patient are on the left **(a, c, e)** and oxymorphone glucuronide (OM3G) remaining in each patient are on the right **(b, d, f)**. Hydrolysis is complete when there is < 20% OM3G remaining. Patient 1 and 2 completed oxymorphone hydrolysis with 10 µL of IMCSzyme RT and 50 µL of BpGUS **(a, b, c, d)**. Patient 3 exhibited inhibition and did not complete hydrolysis with IMCSzyme RT or BpGUS **(e, f)**.*

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CONCLUSION

- Endogenous metabolites in urine can reduce β-glucuronidase performance.
- IMCSzyme RT shows higher tolerance towards inhibitors found in patient samples.

REFERENCES

1. Taylor LL *et al.* J Anal Toxicol2017;41:407–11.
2. Sitasuwan P *et al.* J Anal Toxicol2019;43:221–7.