AN INVESTIGATION INTO THE USE OF 4-METHYLUMBELLIFERONE, p-NITROPHENOL AND 1-NAPHTHOL AS SUBSTRATES FOR RECOMBINANT HUMAN SULT1B1 EXPRESSED IN *E. coli.*

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ABSTRACT

It is well known that SULT1B1 acts on thyroid hormones but the range of exogenous substrates sulfated by SULT1B1 has not been widely investigated to date. We routinely use 4-methylumbelliferone and 1-naphthol as substrates for preparations of recombinant SULT1A1*1 and SULT1A3 respectively. While SULT1B1 has been shown to sulfate p-nitrophenol, an alternative substrate used by us for the assay of SULT1A1*1 activity, it is not known whether it can act on 4-methylumbelliferone or 1-naphthol.

We have adapted our assay procedures to determine whether 4-methylumbelliferone and 1-naphthol are substrates for SULT1B1 and to determine the kinetic parameters for the sulfation of 4-methylumbelliferone, 1-naphthol and p-nitrophenol by SULT1B1.

Recombinant human SULT1B1 was able to catalyse the sulfation of both 4-methylumbelliferone and 1-naphthol. Full kinetic analysis of the reactions was carried out and K_m values determined for the substrates.

INTRODUCTION

Sulfotransferases are important Phase II drug metabolising enzymes, catalysing the sulfation of xenobiotics, hormones and neurotransmitters. SULT1B1 is one of seven isoforms of the human sulfotransferase family, and is involved in the sulfation of a number of thyroid hormones *in vivo* (Wang et al 1998).

Although p-nitrophenol (pNP) has been reported as a substrate for SULT1B1 (Tabrett, C.A et al 2003), there is little information regarding other substrates for the *in vitro* assay of this enzyme. We have investigated two substrates, 1-naphthol and 4-methylumbelliferone (4MU), which we routinely use for the determination for SULT1A1*1 and SULT1A3 activity, along with pNP for use as suitable substrates for the assay of SULT1B1.

MATERIALS AND METHODS

CHEMICALS
1-naphthol and 1-naphthylsulfate were purchased from ICN Biomedicals Inc. HPLC Grade methanol was purchased from Fisher Scientific. All other chemicals were purchased from Sigma-Aldrich or VWR.

INCUBATIONS
Cytosol isolated from *E.coli* expressing human SULT1B1 (Cypex Ltd, Dundee) was incubated at 37°C in 50mM phosphate buffer (pH 7.4) containing 5mM $MgCl_2$, 10mM dithiothreitol, 20µM PAPS and substrate in amber tubes. Incubations were stopped by the addition of acetonitrile and centrifuged. Supernatants were analysed by HPLC.

RESULTS

All of the substrates were sulfated by SULT1B1 and, in each case, the formation of metabolite was found to be linear with respect to time for at least 5 minutes and with protein concentration over an appropriate range. See figs 1 and 2 for pNP, data for 4MU and 1-naphthol not shown.

Kinetic analysis of the sulfation of all three substrates was carried out. Data was fitted to a Hanes plot before direct determination by Michaelis-Menten plot. Kinetic parameters were obtained as follows:

p-nitrophenol (see figs 3 and 4)

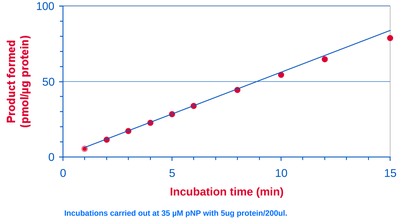
Km 44 µM, Vmax 4.5 pmol/min/µg protein

4-methylumbelliferone (see figs 5 and 6)

Km 35 µM, Vmax 13 pmol/min/µg protein

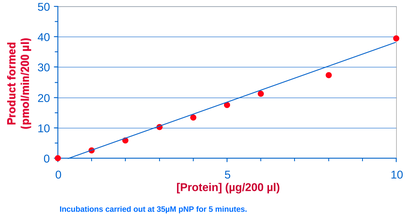
It was not possible to obtain Km and Vmax parameters for the sulfation of 1-naphthol, as it exhibited non Michaelis-Menten kinetics (fig 7). There was strong substrate inhibition present at concentrations above 2µM.

Fig 1. Linearity with time for SULT1B1 with p-nitrophenol as substrate



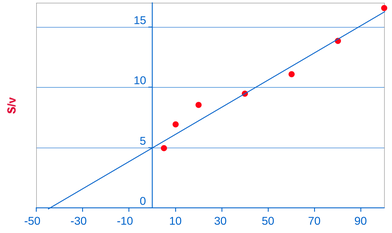
Incubations carried out at 35 µM pNP with 5ug protein/200ul.

Fig 2. Linearity with protein concentration for SULT1B1 with p-nitrophenol as substrate



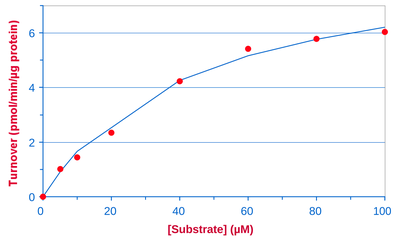
Incubations carried out at 35µM pNP for 5 minutes.

Fig 3. Hanes plot for pNP assay with SULT1B1



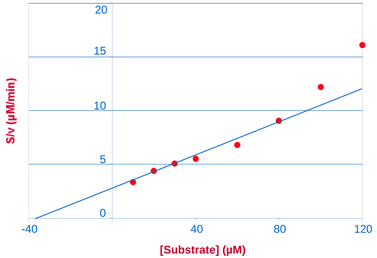
Incubations carried out with 4µg protein/200µl for 5 minutes.

Fig 4. Fit of data to Michaelis-Menten equation for pNP assay with SULT1B1



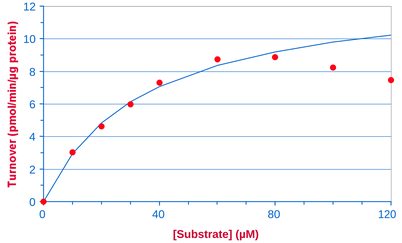
Incubations carried out with 4µg protein/200µl for 5 minutes.

Fig 5. Hanes plot for 4MU assay with SULT1B1



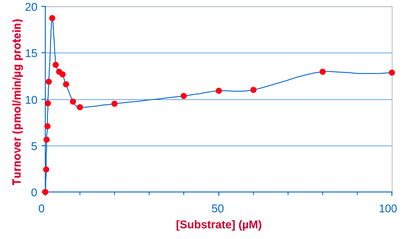
Incubations carried out with 4µg protein/200µl for 5 minutes.

Fig 6. Fit of data to Michaelis-Menten equation for 4MU assay with SULT1B1



Incubations carried out with 4µg protein/200µl for 5 minutes.

Fig 7. Plot of [S] against v for 1-naphthol assay with SULT1B1



Incubations carried out with 4µg protein/200µl for 5 minutes.

CONCLUSIONS

All three substrates were able to be sulfated by SULT1B1. For our purposes, 1-naphthol is not a suitable substrate as it exhibits non Michaelis-Menten kinetics.

Based on our results, we have chosen 4-methylumbelliferone as our substrate for SULT1B1, as with this substrate, it has a higher activity and a lower Km than with pNP. Although there was slight substrate inhibition present at concentrations of 4MU above 80µM, this does not affect our assays which are run at a far lower substrate concentration.

As the Km and Vmax values for SULT1A1*1 with 4MU differ from those for SULT1B1, it allows us to distinguish between the two enzymes (Average values for SULT1A1*1, Km : 1.3 µM, Vmax : 9.5 pmol/min/µg protein).

REFERENCES

1) Wang, J., Falany, C.N., (1998) Expression and characterization of a novel thyroid hormone-sulfating form of cytosolic transferases from human liver. *Mol pharmacol* 53: 274-282.

2)Tabrett, C.A., Coughtrie, M.W.H., Phenol sulfotransferase 1A1 activity in human liver: kinetic properties, interindividual variation and re-evaluation of the suitability of 4-nitrophenol as a probe substrate. *Biochemical Pharmacology* 66 (2003): 2089-2907.