Phosphatase-Coupled Sulfotransferase Assay

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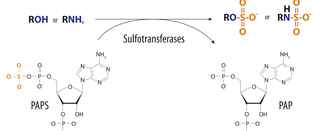
ABSTRACT

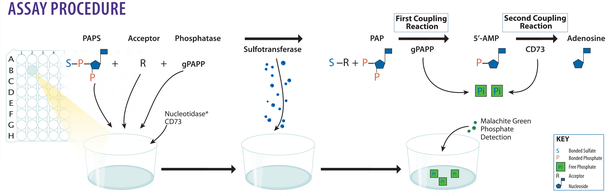
Sulfotransferases are a large group of enzymes that transfer sulfate from the donor substrate 3′-phosphoadenosine-5′-phosphosulfate (PAPS) to various acceptor substrates, generating 3′-phosphoadenosine-5′-phosphate (PAP) as a by-product. A universal phosphatase-coupled sulfotransferase assay is described here. In this method, Golgi-resident PAP-specific 3′-phosphatase (gPAPP) is used to release the 3′-phosphate from PAP, generating 5′-adenosine monophosphate (5′-AMP). In addition, CD73, a 5′-nucleotidase, can be used to release the 5′-phosphate from 5′-AMP to increase the assay sensitivity by two-fold. The released phosphate is then detected using Malachite Green phosphate detection reagents. This assay eliminates the need for both radioisotope labeling and substrate-product separation, and is high-throughput compatible. The assay allows for the kinetic analysis of all sulfotransferases that use PAPS as a donor substrate. Assay examples are given for the carbohydrate specific sulfotransferases CHST3 and CHST10, and the cytosolic sulfotransferases SULT1C4 and SULT1A1.

METHODS

SULFOTRANSFERASE REACTION



ASSAY PROCEDURE



STEP 1 In a 96-well plate, prepare a reaction mix containing the donor (PAPS), acceptor substrate, and coupling phosphatase (gPAPP). *The addition of the nucleotidase CD73 is optional. CD73 can increase phosphate release by two-fold.

STEP 2 Initiate the reaction by adding a specific sulfotransferase.

STEP 3 Stop the reaction and develop the color using Malachite Green Phosphate detection reagents (Catalog # DY996). Read the absorbance at 620 nm with a plate reader.

Reaction Buffer: 25 mM Tris and 15 mM $MgCl_2$ at pH 7.5.

FIGURE 1

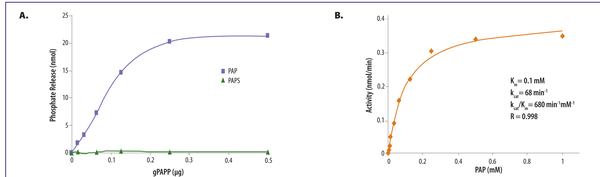


FIGURE 1 gPAPP is a PAP-specific Phosphatase. (**A**) Equal amounts of PAP (purple line) and PAPS (green line) were incubated (separately) with increasing concentrations of gPAPP for 20 minutes at 37 °C. Phosphate release is plotted versus enzyme dose. gPAPP selectively removes a phosphate group from PAP but not PAPS. (**B**) gPAPP (20 ng) enzyme activity is plotted versus substrate PAP.

TABLE 1 gPAPP Kinetics.*

	K_m (mM)	K_d (mM)	K_i (mM)	V_{max} (pmol/min/µg)	k_{cat} (min^{-1})	k_{cat}/K_m ($min^{-1}mM^{-1}$)
PAP	0.098			19,863	786	8,020
PAPS				132	5.23	NA
Mg^{2+}		6.1				
Na^+			17.2			

*The enzyme kinetics of gPAPP was determined using PAP and PAPS as substrates. Mg^{2+} is a cofactor. Na^+ is an inhibitor.

Reaction Buffer: 25 mM Tris and 20 mM Mg^{2+}, pH 7.5

FIGURE 2

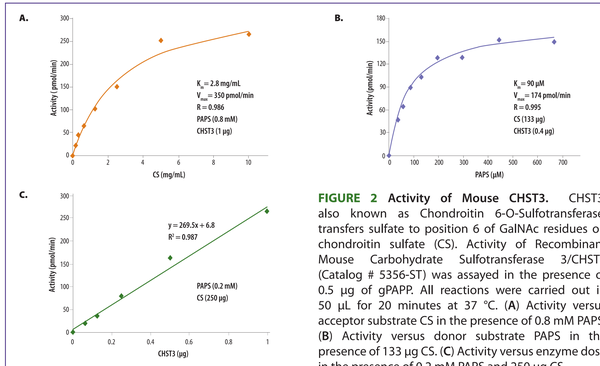


FIGURE 2 Activity of Mouse CHST3. CHST3, also known as Chondroitin 6-O-Sulfotransferase, transfers sulfate to position 6 of GalNAc residues on chondroitin sulfate (CS). Activity of Recombinant Mouse Carbohydrate Sulfotransferase 3/CHST3 (Catalog # 5356-ST) was assayed in the presence of 0.5 µg of gPAPP. All reactions were carried out in 50 µL for 20 minutes at 37 °C. (**A**) Activity versus acceptor substrate CS in the presence of 0.8 mM PAPS. (**B**) Activity versus donor substrate PAPS in the presence of 133 µg CS. (**C**) Activity versus enzyme dose in the presence of 0.2 mM PAPS and 250 µg CS.

FIGURE 3

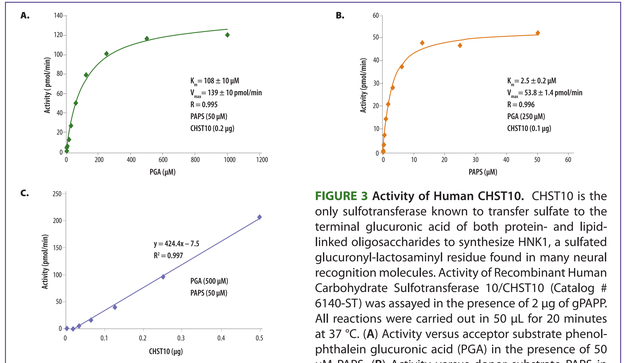


FIGURE 3 Activity of Human CHST10. CHST10 is the only sulfotransferase known to transfer sulfate to the terminal glucuronic acid of both protein- and lipid-linked oligosaccharides to synthesize HNK1, a sulfated glucuronyl-lactosaminyl residue found in many neural recognition molecules. Activity of Recombinant Human Carbohydrate Sulfotransferase 10/CHST10 (Catalog # 6140-ST) was assayed in the presence of 2 µg of gPAPP. All reactions were carried out in 50 µL for 20 minutes at 37 °C. (**A**) Activity versus acceptor substrate phenol-phthalein glucuronic acid (PGA) in the presence of 50 µM PAPS. (**B**) Activity versus donor substrate PAPS in the presence of 250 µM PGA. (**C**) Activity versus enzyme dose in the presence of 50 µM PAPS and 500 µM PGA.

FIGURE 4

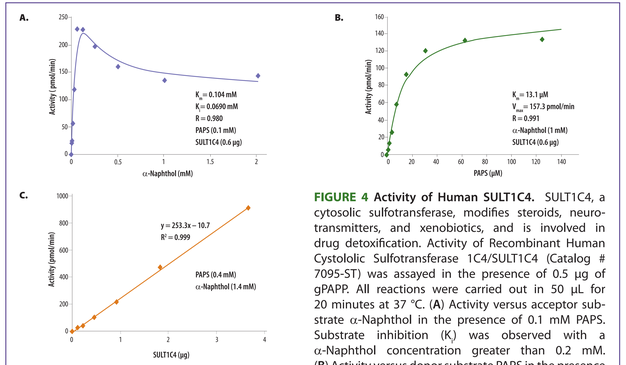


FIGURE 4 Activity of Human SULT1C4. SULT1C4, a cytosolic sulfotransferase, modifies steroids, neuro-transmitters, and xenobiotics, and is involved in drug detoxification. Activity of Recombinant Human Cystolic Sulfotransferase 1C4/SULT1C4 (Catalog # 7095-ST) was assayed in the presence of 0.5 µg of gPAPP. All reactions were carried out in 50 µL for 20 minutes at 37 °C. (**A**) Activity versus acceptor substrate α-Naphthol in the presence of 0.1 mM PAPS. Substrate inhibition (K_i) was observed with a α-Naphthol concentration greater than 0.2 mM. (**B**) Activity versus donor substrate PAPS in the presence of 1 mM α-Naphthol. (**C**) Activity versus enzyme dose in the presence of 0.4 mM PAPS and 1.4 mM α-Naphthol.

FIGURE 5

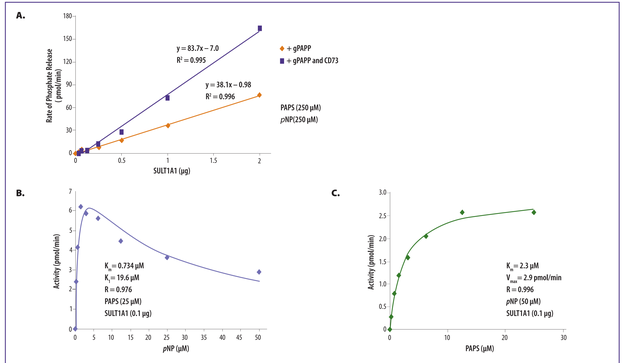


FIGURE 5 Activity of Human SULT1A1. SULT1A1, a cytosolic sulfotransferase involved in drug detoxification, functions optimally at very low substrate levels, therefore, a highly sensitive assay is required to determine K_m. Activity of Recombinant Human Cystolic Sulfotransferase 1A1/SULT1A1 (Catalog # 5546-ST) was assayed in the presence of 2 µg of gPAPP, and in some instances CD73 to increase sensitivity. All reactions were carried out in 200 µL for 20 minutes at 37 °C. (**A**) Phosphate release versus enzyme dose in the presence of 250 µM PAPS and 250 µM pNP. The reaction was carried out in both the absence (orange line) and presence (purple line) of 0.2 µg CD73. (**B**) Activity versus acceptor substrate *p*NP in the presence of 25 µM PAPS. Substrate inhibition (K_i) was observed with a *p*NP concentration greater than 3 mM. (**C**) Activity versus donor substrate PAPS in the presence of 50 µM *p*NP.

CONCLUSIONS

- This phosphatase-coupled assay is universal to all sulfotransferases that use PAPS as the donor substrate.
- The assay is quantitative and allows accurate kinetic analysis.
- It is a convenient and cost effective assay because no radioisotope and no separation of products and substrates are required.
- The assay can be performed in a 96-well plate and provides a platform for high-throughput drug screening.

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