Supporting Information

Sulfotransferase 1A1 Substrate Selectivity - A Molecular Clamp Mechanism

Ian Cook, Ting Wang and Thomas S. Leyh

Department of Microbiology and Immunology, Albert Einstein College of Medicine, 1300 Morris Park Ave, Bronx, New York 10461-1926

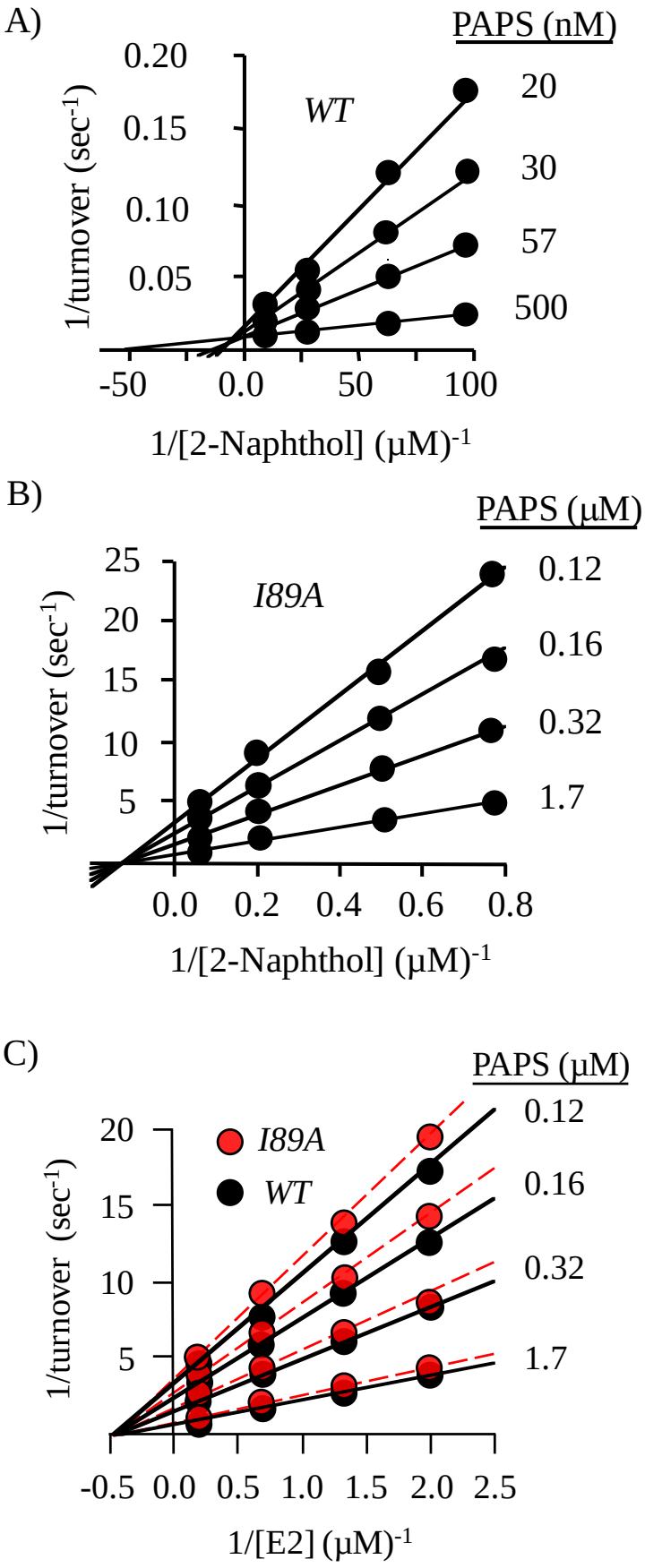
Corresponding Author:

Thomas Leyh, Ph. D.

Phone: 718-430-2857

Fax: 718-430-8711

E-mail: tom.leyh@einstein.yu.edu


A)
PAPS (nM)
0.20
0.15
0.10
0.05
1/turnover (sec-1)
WT
20
30
57
500
-50
0.0
50
100
1/[2-Naphthol] (µM)-1
B)
PAPS (µM)
25
20
15
10
5
1/turnover (sec-1)
I89A
0.12
0.16
0.32
1.7
0.0
0.2
0.4
0.6
0.8
1/[2-Naphthol] (µM)-1
C)
PAPS (µM)
20
15
10
5
1/turnover (sec-1)
I89A
WT
0.12
0.16
0.32
1.7
-0.5
0.0
0.5
1.0
1.5
2.0
2.5
1/[E2] (µM)-1

S.1. *The Effect of Lower Lip Mutation (I89A) on the Initial-Rate Parameters of Positive and Neutral Synergy Subsrates.* **A.)** ***An initial-rate study of 2-Nap sulfonation by SULT1A1.*** Sulfonation was measured by monitoring transfer of sulfuryl moiety from [^{35}S]-PAPS to 2-Nap. Reaction conditions were as follows: SULT1A1 (5.0 nM, dimer), 2-Nap (0.010 – 0.25 μM), [^{35}S]-PAPS (20 – 500 nM, SA = 4.3 Ci mmol^{-1}), $MgCl_2$ (5.0 mM), $NaPO_4$ (50 mM), pH 7.2, 25 ± 2 °C. Reactions were initiated by addition of [^{35}S]-PAPS and quenched as described in *Materials and Methods*. Radiolabelled reactants were separated using anion exchange TLC and quantitated using STORM imaging. **B.)** ***An initial-rate study of 2-Nap sulfonation by I89A SULT1A1.*** Reaction conditions were identical to *A* except: 2-Nap (0.50 - 13 μM) and [^{35}S]-PAPS (0.17 – 1.7 μM). **C.)** ***An initial-rate study of E2 sulfonation by wild type (black) and I89A (red) SULT1A1.*** E2 sulfonation was measured by monitoring the conversion of [^{3}H]-E2 (SA = 15 Ci mmol^{-1}) to [^{3}H]-E2-sulfate. Reaction conditions were similar to *B* except: [^{3}H]-E2 (0.30 -7.5 μM), PAPS (0.17 – 1.7 μM). Products were separated using chloroform extraction and quantitated by liquid scintillation spectroscopy. Initial rates were determined at the 16 conditions defined by a 4 x 4 concentration matrix in which donor and acceptor are varied from 0.20 – 5.0 x K_m in four, equal increments in double-reciprocal space. Velocities, determined in duplicate, were obtained from least-squares fitting of four-point progress curves. In each case, the maximum product formed was less than 5% of the product formed at the endpoint of the reaction. The lines through the points represent the global best-fit of the data using the sequential Bi-Bi model of Cleland (SEQUENO (*1*)).

GROMACS trajectories of SULT1A1 ternary complexes. Movies 1-4 show 1.0 ns, GROMACS trajectories of wild-type SULT1A1 ternary complexes (E•PAPS•acceptor) with Acet, E2, NAP, or DHEA, respectively. The fifth movie predicts the behavior of 2-Nap in the ternary complex of the I89A mutant. The GROMACS protocols are described in *Methods*. Movies were constructed using Pymol from the 251 sets of structural coordinates taken from the 1.0 ns trajectories at successive 4.0 ps intervals. Water, Na^+, Cl^-, and hydrogen were removed for clarity. Secondary structures were assigned manually using the SULT1A1·PAP structure (4GRA (*2*)) as a template. The protein backbone is rendered in cartoon and the active-site His, PAPS, and acceptors are shown in stick. Dotted lines indicate the position of the nucleophilic hydroxyl relative to the sulfuryl group and active-site His. Phe81 and Phe84 are shown in stick and surface. Images were loaded into Windows Movie Creater and published in Quick Time format.

References

1. Cleland, W. W. (1979) Statistical analysis of enzyme kinetic data, *Methods Enzymol 63*, 103-138.
2. Cook, I., Wang, T., Almo, S. C., Kim, J., Falany, C. N., and Leyh, T. S. (2013) The gate that governs sulfotransferase selectivity, *Biochemistry 52*, 415-424.