Identification and localization of soluble sulfotransferases in the human gastrointestinal tract

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Supplementary Data

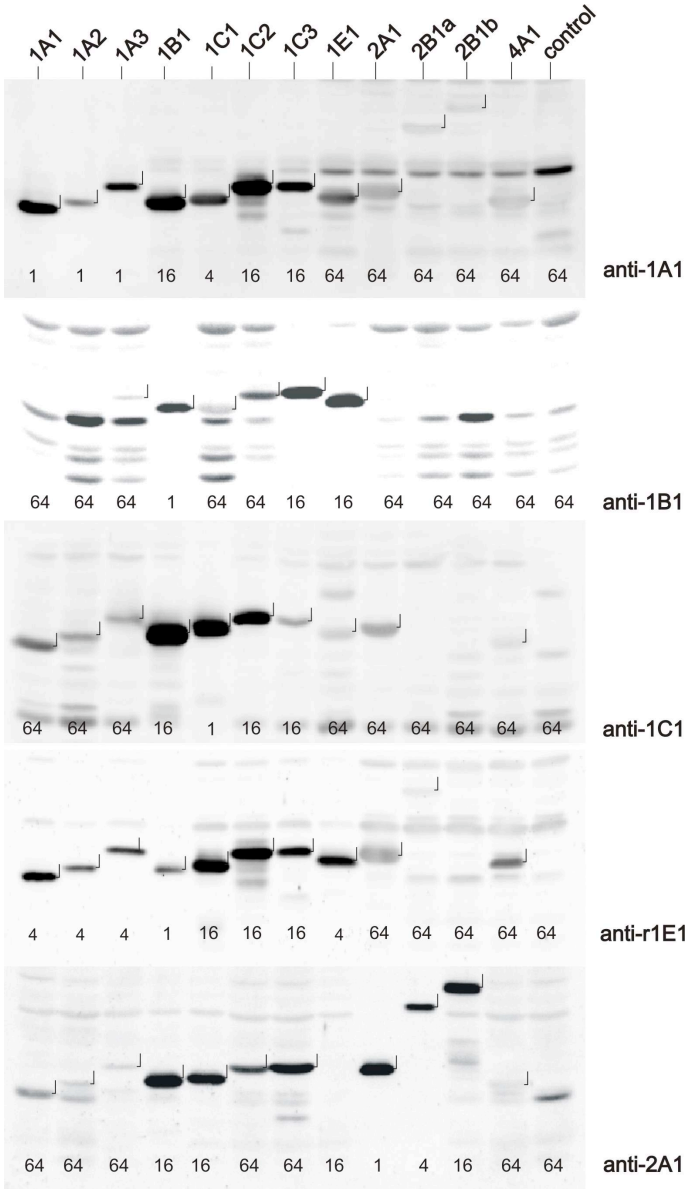

Figure 1 Immunodetection of human SULT forms, individually expressed in *S. typhimurium* TA1538, using various antisera

Bacterial cytosolic preparations were electrophoresed, transferred to nitrocellulose membranes, and then probed with the indicated native antisera (without any immunoabsorption to enhance specificity). Based on the results of initial dose-finding experiments (not shown), 1, 4, 16 or 64 µg of protein of the SULT-expressing strains was used. An amount of 64 µg was always employed for the untransformed strain ("control"). Dashes indicate the position of the corresponding SULT protein.

Table 1 Electrophoretic mobility and immunodetection of human SULT forms

Various amounts of cytosolic fractions of *S. typhimurium* strains expressing the indicated human SULT form were electrophoresed, blotted and probed with the indicated antisera (AS). The electrophoretic mobility is expressed as apparent molecular mass (M_{app}). Representative blots are shown in Figure 6. In order to produce a strong signal, ≤1 µg [+++], 2 – 4 µg [++] or 8 – 16 µg [+] bacterial protein was required. In the other cases, immunoreactivity was weak [(+)] or indetectable [-].

		Immunoreactivity				
SULT form	M_{app}, kDa	Anti-1A1-AS	Anti-1B1-AS	Anti-1C1-AS	Anti-r1E1-AS	Anti-2A1-AS
1A1	32	+++	-	(+)	++	(+)
1A2	32.5	++	-	(+)	++	(+)
1A3	34	+++	-	(+)	++	-
1B1	32.5	+ *	+++	+ *	++	+
1C1	33	++	(+)	+++	+	+
1C2	34	+	(+)	+	+	(+)
1C3	34	+	+	(+)	+	(+)
1E1	33.5	(+)	+	(+)	++	(+)
2A1	34	(+)	(+)	(+)	(+)	+++
2B1a	40	-	-	-	-	++
2B1b	42	-	-	-	-	+
4A1	32	-	-	-	(+)	-

* Cross-reactivity of anti-1A1- and anti-1C1-antisera with SULT1B1 was abolished by pre-incubating with SULT1B1-sepharose.

Table 2 Influence of the codon 213 Arg/His genotype on the level of SULT1A1 protein in colon and rectum mucosa

Values refer to the same SULT1A1 protein determinations as summarized in Table 4. One rectal sample could not be genotyped as it had been used up.

Tissue	Genotype	n	ng SULT1A1 / mg cytosolic protein	
			Mean	Range
Colon	Arg/Arg	14	171	58 – 270
	His/His	5	151	125 – 250
	His/Arg	9	208	44 – 480
Rectum	Arg/Arg	5	173	85 – 220
	His/His	1	100	-
	His/Arg	4	243	90 – 320

Table 3 Levels of SULT proteins in cytosolic fractions of human gastrointestinal mucosa and liver

SULT levels were determined semi-quantitatively using inclusion bodies and cytosolic fractions from recombinant bacteria as standards. This method may involve inaccuracies in the absolute value (we estimate by a factor of up to 3), but should be more accurate for comparison of the same protein in different samples (within rows). Values are means and, in parentheses, ranges of n samples (indicated in the column heading). -, below the limit of detection (75 ng/mg for SULT1A2 and 1A3, 3 ng/mg for SULT1C1, 10 ng/mg for SULT2A1, and 6 ng/mg for SULT1E1).

	ng SULT / mg cytosolic protein						
SULT form [*]	Rectum (n = 11)	Colon (n = 28)	Cecum (n = 1)	Jejunum (n = 1)	Ileum (n = 4)	Stomach (n = 1)	Liver (n = 1)
1A1	210 (85 – 320)	180 (44 – 480)	150	75	1200 (990 – 1600)	75	800 [#]
1A2	-	- [†]	75	-	- [†]	-	75 [#]
1A3	310 (130 – 480)	320 (100 – 880)	300	300	1500 (1200 – 1700)	150	- [#]
1B1	130 (50 – 230)	120 (28 – 270)	100	50	420 (110 – 530)	50	25 [#]
1C1	-	- [†]	-	-	- [‡]	25	-
1E1	-	-	6	12	50 (25 – 100)	-	100
2A1	-	-	25	200	150 (50 – 300)	-	1000

[*] The remaining forms were not detected in the gastrointestinal tract and liver, but the limit of detection was relatively high (100 ng/mg for SULT2B1a and ≥ 400 ng/mg for SULT1C2, 1C3, 2B1b and 4A1).

[†] Two samples showed immunoreactivity at the limit of detection.

[‡] One sample showed immunoreactivity at the limit of detection.

[#] Using a similar protocol we recently analyzed three other liver samples for expression of these SULT proteins. Levels (ng / mg cytosolic protein) amounted to 700-2500 for SULT1A1, 100-300 for SULT1A2, 0 for SULT1A3 and 100-500 for SULT1B1 [19].

Table 4 SULT activities towards estradiol (20 nM), characteristic for SULT1E1, and DHEA (3 µM), characteristic for SULT2A1, in cytosolic preparations from human tissues

Gastrointestinal samples were prepared from the mucosa. Colon pool was generated by mixing equal amounts of protein from 8 samples of colon mucosa homozygous for SULT1A1*213Arg. SULT pool was generated by mixing soluble fractions of *E. coli* BL21-SULT1A1*213Arg, -1B1 and -1A3 with cytosolic protein of SULT-deficient Chinese hamster V79 cells so that its SULT levels and patterns (determined by immunoblotting) reflected the colon pool. Values are means ± SE of two enzyme activity measurements.

	SULT activity, pmol · mg^{-1} · min^{-1}	
Sample	Estradiol	DHEA
SULT pool (1A1, 1A3 and 1B1)	0.11 ± 0.04	not detected *
Rectum †	0.07 ± 0.01	not detected *
Colon pool	0.04 ± 0.01	not detected *
Cecum †	0.6 ± 0.1	10 ± 4
Ileum		
Sample 1	3.3 ± 0.4	59 ± 9
Sample 2	2.3 ± 0.2	22 ± 4
Sample 3	3.8 ± 0.8	121 ± 17
Sample 4	8.6 ± 0.7	35 ± 2
Jejunum †	2.0 ± 0.2	81 ± 13
Stomach †	0.13 ± 0.03	not detected *
Liver †	8.7 ± 0.1	372 ± 9

* Limit of detection was 5 pmol · mg^{-1} · min^{-1}.

† A single sample was analyzed for this tissue.