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Structural and functional characterisation of human sulfotransferases

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Abstract

The human aryl sulfotransferases HAST4 and HAST4v vary by only two amino acids but exhibit markedly different affinity towards the sulfonate acceptor *p*-nitrophenol and the sulfonate donor 3%-phosphoadenosine-5%-phosphosulfate (PAPS). To determine the importance of each of these amino acid differences, chimeric constructs were made of HAST4 and HAST4v. By attaching the last 120 amino acids of HAST4v to HAST4 (changing Thr235 to Asn235) we have been able to produce a protein that has a *K*m for PAPS similar to HAST4v. The reverse construct, HAST4v:4 produces a protein with a *K*m for PAPS similar to HAST4. These data suggests that the COOH-terminal of sulfotransferases is involved in co-factor binding.

Keywords: Human; Sulfotransferase; Active site; Structure; Function

Introduction

Sulfonation is an important metabolic pathway involved in the detoxification and:or activation of a range of endogenous compounds (e.g. monoamine neurotransmitters, thyroid and steroid hormones), drugs (e.g. minoxidil) and xenobiotics (e.g. aromatic amines and benzylic alcohols of polycyclic aromatic hydrocarbons) [1]. This reaction involves transfer of a sulfonate group from the 3%-phosphoadenosine- 5%-phosphosulfate (PAPS) to the acceptor substrate. In the case of xenobiotics, this reaction is catalysed by a group of cytosolic sulfotransferase enzymes (STs). However, it is important to recognise that a number of membrane bound STs also exist [2]. Based on amino acid alignments, the cytosolic sulfotransferases have been divided into three families: phenol, hydroxysteroid and flavonol STs [2]. In the laboratory, five human aryl (phenol) STs have been cloned: HAST1, HAST2, HAST3, HAST4 and HAST4v. HAST1, HAST4 and HAST4v were isolated from a liver cDNA library, whereas HAST2 and HAST3 were obtained from a brain cDNA library [3–5].

All of the human aryl STs share greater than 90% sequence homology at the amino acid level but vary markedly in their substrate preferences. For example, HAST1 and HAST3 are 93% identical at the amino acid level but their affinity (K_m) for *p*-nitrophenol is 0.6 and 2200 mM, respectively (Table 1) [6]. HAST4 metabolises *p*-nitrophenol with an intermediate affinity (K_m 74 mM) compared to HAST1 and HAST3 but it exhibits no activity towards dopamine as a substrate (Table 1) [5]. A variant of HAST4 (HAST4v) shows significantly higher affinity for *p*-nitrophenol and the co-factor PAPS than HAST4 (Table 1) [5]. These allelic variants differ by only two amino acids (HAST4: Thr-7 and Thr-235 and HAST4v: Ile-7 and Asn-235) [5]. Human aryl sulfotransferases, like many other xenobiotic metabolising enzymes (e.g., P450s), exhibit overlapping substrate specificities at high substrate concentrations which are probably not relevant from a physiological viewpoint (e.g., HAST1 has a K_m for dopamine of 345 mM). At the present time, except for the data on HAST4:HAST4v, there is no information available to explain the catalytic diversity of human aryl STs. It is still unclear what influence the amino acid sequence of each form of ST has on its distinct substrate specificities and the binding of the co-factor PAPS. A crystal structure for a ST is yet to be published and this has hindered the understanding of the structural and functional relationships of these enzymes. The recent cloning of an increasing number of sulfotransferases from a variety of species and analysis of their amino acid sequences has revealed at least four highly conserved regions [2,7]. A number of laboratories have now utilised this data to construct chimeric cDNAs and perform site-directed mutagenesis on these regions, as well as carry out affinity labelling to gain insight into the substrate and PAPS binding sites of guinea pig [8,9], rat [10] and plant [7,11] STs. It has been suggested that the PAPS binding region resides in a highly conserved

GXXGXXK motif at the C-terminal end of STs [8,9]. Site-directed mutagenesis within this region (GISGDWKN) in the guinea pig estrogen sulfotransferase markedly affected the K_m for PAPS and confirmed this part of the amino acid sequence to be a critical component of the active site [9]. Marsolais and Varin have determined by site-directed mutagenesis of the flavonol 3-sulfotransferase that Arg276, which is in close proximity to the suggested GXXGXXK motif, is crucial for PAPS binding [7]. This arginine residue is conserved amongst all the known cytosolic STs and supports the initial observation by Borchardt et al. of the importance of arginine residue(s) in the sulfonate transfer reaction [12,13]. Studies by Zheng et al. which have used an affinity labelling approach to study PAPS binding on a rat aryl sulfotransferase have found the amino acid sequence 63-Leu-Glu-Lys-Cys-Gly-Arg-68 in region I to be present at the PAPS binding site [10]. In the flavonol 3-sulfotransferase mutation of Lys59 (also in region I) has shown this amino acid to be critical for catalysis but not PAPS binding [7]. It is possible to speculate that within the three dimensional structure of STs, interactions between the different regions may be critical for determining both PAPS and substrate binding.

Table 1
Computer-derived Michaelis–Menten parameters for the sulfonation of *p*-nitrophenol and dopamine by COS-cell expressed native and chimeric HASTs

Enzyme	*p*-Nitrophenol	Dopamine	PAPS
	K_m (μM)	K_m (μM)	K_m (μM)
HAST1	0.6	345	0.98
HAST3	2200	9.7	1.1
HAST4	73.7	ND	5.25
HAST4v	7.75	ND	0.203
HAST4/4v	10.4	ND	0.445
HAST4v/4	45.7	ND	4.91

K_m values for the HAST enzymes towards the co-substrate PAPS are also shown.
The substrate saturation curves were performed using (1) 0.5–50 μM *p*-nitrophenol for HAST4/4v and 0.5–200 μM for HAST4v/4 with a saturating concentration of PAPS (8 μM) or using (2) 0.05–5 μM PAPS for HAST4/4v and 0.05–10 μM for HAST4v/4 with a fixed concentration of 5 μM *p*-nitrophenol. All data represented in the table were from COS cells obtained in one transfection with the respective cDNAs as to avoid batch-to-batch variability and were assayed in duplicate.
ND: no detectable activity.

Chimeric constructs of HAST4 and HAST4v

As described earlier, HAST4 and HAST4v vary by only two amino acids but exhibit markedly different affinity towards the sulfonate acceptor *p*-nitrophenol and the sulfonate donor PAPS (Table 1). To determine the importance of each of these amino acid differences, chimeric constructs were made of HAST4 and HAST4v. This was done by using a common *Pml*I restriction site and a *Xba*I site in the poly linker of pCMV5. This enables us to exchange the last 120 amino acids of HAST4 with the same portion of HAST4v (HAST4:HAST4v construct) and similarly the last 120 amino acids of HAST4v with the same portion of HAST4 (HAST4v: HAST4 construct). When the above chimeric constructs were expressed in COS cells they encoded proteins on immunoblots of identical molecular weights to HAST4 and HAST4v. The Foldes and Meek assay [14] was used to determined the Michaelis–Menten parameters, K_m (Table 1) and V_{max}, of the native HAST4 and HAST4v for *p*-nitrophenol and PAPS, respectively, as well as the two chimeric constructs HAST4:HAST4v and HAST4v:HAST4. HAST4v has a higher affinity for both *p*-nitrophenol and PAPS compared to HAST4. For example, HAST4v has a 26-fold lower K_m than HAST4 for PAPS as the substrate. Since HAST4 and HAST4v vary by only two amino acids, it is suggestive that these amino acid changes reside close to the substrate and co-factor binding domains. However, the change at position 235 in HAST4v compared to HAST4 involves a conservative substitution of an Asn for a Thr. This change is in close proximity to the putative PAPS binding domain [3,8,9] and resides within a region that shows subtle differences in amino acid differences between HAST1, HAST3 and HAST4 proteins. From Table 1 it is apparent that by attaching the last 120 amino acids of HAST4v to HAST4 it was possible to produce a protein that has a K_m for PAPS similar

to HAST4v. The reverse construct, HAST4v:HAST4, where a Thr is at position 235, produces a protein with a K_m for PAPS similar to HAST4. These data suggests that the COOH-terminal of sulfotransferases is involved in co-factor binding. The presence of an Asn at position 235 in HAST4v and HAST4:HAST4v, compared to a Thr in HAST4 and HAST4v:HAST4, also affected the K_m for *p*-nitrophenol and these data like that of Driscoll et al. [9] supports the assumption that critical substrate binding elements also reside in an area where the co-factor PAPS binds. The above data confirm that critical amino acid changes can markedly alter the affinity of HAST proteins for both PAPS and *p*-nitrophenol. Further studies on structural and functional relationships between human sulfotransferases are currently under investigation.

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