Bacterial expression of two human aryl sulfotransferases

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Abstract

The effect of replacing a single codon in the N-terminal of human aryl sulfotransferase (HAST) 1 and 3 with one that is more commonly found in *E. coli* genes was assessed. The pKK233-2 *E. coli* expression vector was employed and the polymerase chain reaction (PCR) was used to introduce the 5′ nucleotide substitution, at the same time maintaining the fidelity of the amino acid sequence. The data indicates that this change had a minimal effect on protein production, subcellular localization or, in the case of HAST3, catalytic activity. In general, the pKK233-2 *E. coli* vector has been less than optimal for expressing human sulfotransferase cDNAs.

Keywords: Sulfotransferase; Expression; *Escherichia coli*

Introduction

Sulfate conjugation is an important pathway in the biotransformation of numerous xeno- and endobiotics such as drugs, hormones, bile acids and neurotransmitters. The reaction is catalysed by the sulfotransferases, a family of structurally and functionally related enzymes. To date, at least seven distinct human sulfotransferases have been characterised which differ in their primary sequence and substrate specificities. These include five aryl sulfotransferases, a hydroxysteroid sulfotransferase and an estrogen sulfotransferase [1]. In our laboratory, five human aryl sulfotransferase (HAST) cDNAs have been cloned (HAST1, HAST2, HAST3, HAST4 and HAST4v) [2, 3, 4]. HAST1 and HAST2 are thermostable forms of aryl sulfotransferases and sulfonate small phenolic compounds such as *p*-nitrophenol. The cDNAs which encode these proteins are identical in their coding sequence but differ in the 5′ untranslated regions [2, 3]. HAST3, a thermolabile form of aryl sulfotransferase is 93% identical in its coding sequence to HAST1 and HAST2. The monoamine dopamine is the preferred substrate of HAST3 [3, 5]. Recently we have cloned two cDNAs which we have termed HAST4 and HAST4v [4]. These allelic variants share 97 and 94% sequence homology with HAST1 and HAST3, respectively [4]. While *p*-nitrophenol is the preferred substrate for both HAST4 and HAST4v the latter has at least a 10-fold lower K_m for this substrate as well as for the co-factor 3′-phosphoadenosine-5′-phosphosulfate (PAPS) [4].

While multiple forms of human sulfotransferases have been shown to exist, the structural features that determine their substrate and co-factor (PAPS) preference are yet to be elucidated. To date no crystal structure is available for any sulfotransferases and this has limited our understanding of the structural and functional relationships of these enzymes. In order to fully characterise the different isoforms of sulfotransferases, structural studies are required, necessitating large amounts of functional protein. Purification of sulfotransferases from human tissues has a number of technical limitations, such as the availability of tissue in sufficient quantity, the inability to separate closely related forms and the differential stability of various forms. In the case of other xenobiotic metabolising enzymes, the use of heterologous expression systems has overcome many of these difficulties. The transient or stable expression of xenobiotic metabolising enzyme cDNAs in mammalian cells (COS cells, vaccinia virus systems or lymphoblastoid cell lines) has enabled important functional studies to be carried out on the proteins they encode. However, the major disadvantage of these systems is that the level of protein expression is too low to support serious structural studies. In contrast, the *Escherichia coli* expression system offers a higher potential enzyme yield [6]. Although bacteria do not usually carry out post-translational modification of proteins in the same manner as eukaryotes, this has not been a limitation with most xenobiotic metabolising enzymes.

The aim of the current study was to optimise an *E. coli* expression system for sulfotransferases to allow high level protein expression. Other investigators [7] have also used this system to express sulfotransferases but only a limited analysis of factors that influence the level of recombinant protein expression has been reported. Numerous studies conducted with other xenobiotic metabolising enzymes have attempted to maximise the levels of recombinant protein by manipulating factors thought to influence transcription and translation rate and efficiency, mRNA and protein stability, and host toxicity due to the expressed protein. In particular, modifications to the N-terminal nucleotide sequence have been shown to improve the yields of some recombinant proteins [7].

Materials and Methods

In this study, we assessed the effect on expression in *E. coli* of introducing a single mutation into the N-terminal sequence of HAST1 and HAST3 cDNAs thereby replacing an uncommon *E. coli* codon with one that is more commonly found in *E. coli* genes. PCR was used to introduce the 'preferred codon' mutation into a short fragment of the 5′ coding sequence. Two PCR primers were designed: the first (5′-AATGAGCTCAGGACCATGGAGCTGATCCAGGAC-3′) was designed to encode the common native HAST1 and HAST3 sequence and a 5′ *Nco*1 site; and the second (5′-AATGAGCTCAGGACCATGGAACTGATCCAGGAC-3′) was designed to introduce the preferred codon mutation but encoding the same amino acid sequence. Fragments containing the 3′ coding sequences of the sulfotransferases were obtained from the relevant cDNAs cloned into the mammalian expression vector pCMV5. The two fragments were then cloned into the bacterial expression vector pKK233-2 and used to transform DH5αF'IQ™ *E. coli* strain. Transformants were selected with ampicillin and used to inoculate liquid cultures. Protein expression was induced with isopropyl-*β*-ᴅ-thiogalactopyranoside (IPTG). Cells were harvested after 24 h, treated with lysozyme and sonicated, and subcellular fractions were prepared by differential centrifugation as described previously [8].

An immunoblot of subcellular fractions prepared from a typical expression trial are seen in Fig. 1. HAST1 and HAST3 proteins were detected in the $10\,000 \times g$ pellet (containing cellular debris and inclusion bodies), $180\,000 \times g$ pellet (intracellular membranes) and $180\,000 \times g$ supernatant (soluble fraction). The *E. coli*-expressed HAST1 and HAST3 proteins demonstrated similar electrophoretic migration to the COS cell-expressed HAST1 and HAST3 proteins (Fig. 1) which migrate with molecular weights of 32 and 34 kDa, respectively [5]. The co-migration of the *E. coli* and COS cell-expressed HAST1 and HAST3 supports the idea that the sulfotransferases do not undergo any post-translational modifications in mammalian systems that would distinguish them from the *E. coli*-expressed proteins. An interesting finding was the high levels of expression observed in controls cultured in the absence of the inducer IPTG (Fig. 1). This suggests that expression controlled from the *trc* promoter was not completely repressed in the absence of inducer despite the use of the DH5αF'IQ™ strain of *E. coli* which overproduces the *lac*I^q repressor. Little difference was observed on visible inspection of blots between the expression levels of native and modified HAST proteins suggesting that overall protein expression was not significantly improved by modifying the N-terminal sequence at the second amino acid, however, no attempt was made to quantify protein levels. Similarly, no visible difference was observed between the subcellular localisation of expressed proteins. While N-terminal modifications have been shown to be important in enhancing P450 expression in *E. coli*, particular modifications have failed to produce consistent effects [8]. The present study involved only a single mutation at the second position and it is possible that further mutations in this region may alter expression levels. In more recent studies we have found that the use of the expression vector pET-3a which is controlled by the T7 RNA polymerase promoter [9], facilitated the production of significantly higher levels of both HAST1 and HAST3 protein when expressed in BL21(DE3) *E. coli* cells (unpublished results).
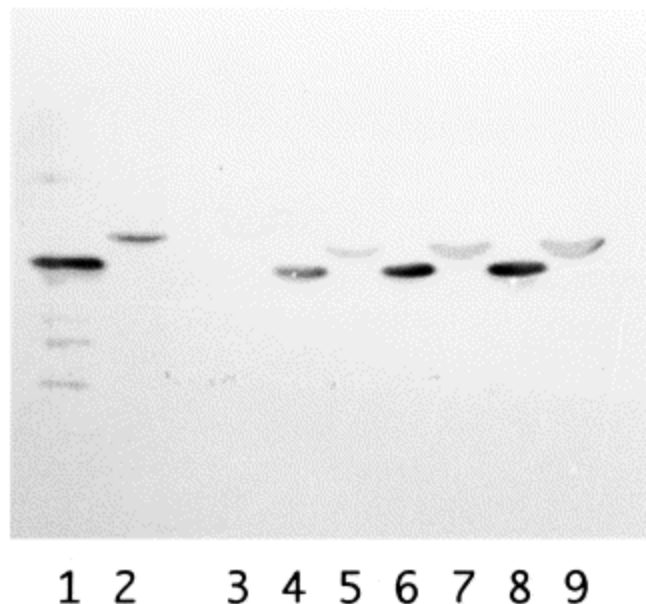
Fig. 1. Immunoblot of *E. coli*-expressed HAST1 and HAST3. Bacteria were cultured for 24 h at 30°C with 1 mM IPTG and subcellular fractions were prepared as described previously [8]. Lanes 1 and 2, 50 μg of total protein from COS cells transfected with pCMV5/HAST1 and pCMV5/HAST3, respectively [2, 3]. Lane 3–9, cytosol from *E. coli* cells transformed with: lane 3, pKK233-2 (154 μg); lanes 4 and 5, pKK233-2/HAST1 (native sequence (NS); 53 μg) and pKK233-2/HAST3 (NS; 131 μg) which were cultured in the absence of IPTG; lane 6, pKK233-2/HAST1 (NS; 58 μg); lane 7, pKK233-2/HAST3 (NS; 196 μg); lane 8, pKK233-2/HAST1 (preferred codon (PC); 38 μg); lane 9, pKK233-2/HAST3 (PC; 106 μg).

The functional characteristics of *E. coli*-expressed HAST1 and HAST3 proteins were determined towards their model substrates *p*-nitrophenol and dopamine, respectively, using a modified procedure of Foldes and Meek [10, 11].

Results

In the case of *E. coli*-expressed HAST3 (native protein) the K_m for dopamine sulfonation was similar to COS cell-expressed HAST3 (Table 1). However, HAST3 protein containing the preferred sequence showed a marginally lower K_m and an approximately 3-fold higher V_{max}. The higher V_{max} may have reflected an increase in active enzyme expression in the preferred codon construct. The determination of *E. coli*-expressed HAST1 activity towards its model substrate, *p*-nitrophenol, was complicated by high background activity observed in the absence of substrate (data not shown). These studies suggested the presence of an endogenous substrate in bacterial cytosol, possibly a small phenolic compound which may exhibit a high affinity for HAST1. This background activity complicated functional characterisation of *E. coli*-expressed HAST1 protein and further studies with this form may require purification of the expressed protein.

Table 1. Michaelis–Menten parameters for the sulfonation of dopamine by *E. coli*-expressed HAST3 and COS cell-expressed HAST3

Enzyme	K_m (μM)	V_{max} (nmol/ng per min)
HAST3 (NS)	11.1±0.8	2.5±0.06
HAST3 (PS)	3.4±0.3	6.6±0.2
HAST3 (COS)	9.7	2.8

NS, native sequence; PS, preferred sequence. Activity was measured using a modified procedure of Foldes and Meek [10,11]. All data in the above table were from *E. coli* (180 000×*g* supernatant) obtained in one expression experiment so as to avoid batch-to-batch variability and were assayed in duplicate. K_m and V_{max} values were determined using the Prism II™ program (Graph-Pad Software, San Diego, CA) and are shown as the mean±S.E.M. The values for COS cell-expressed HAST3 are from [4].

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