Characterization and Expression of Hepatic Sulfotransferase Involved in the Metabolism of N-Substituted Aryl Compounds

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An aryl sulfotransferase, whose cDNA was isolated from the rat liver library, was found to catalyze bioactivation of minoxidil through N-O-sulfation and N-sulfation of a carcinogenic heterocyclic amine, IQ, by expression in COS-1 cells. cDNA of a human ortholog also was isolated and characterized as a major minoxidil-activating enzyme in human liver. Another group of aryl sulfotransferases catalyzing O-sulfation of carcinogenic N-hydroxyarylamines was separated from livers of rats and humans. These sulfotransferases have been shown to possess similar functional properties and also to relate immunologically with each other. Current understanding on the primary structure of these sulfotransferases also is discussed. — Environ Health Perspect 102(Suppl 6):99-103 (1994)

Key words: sulfotransferase, heterocyclic amine N-sulfation, N-hydroxyarylamine activation, growth hormone regulation of hepatic sulfation, primary structure

Introduction

Sulfation is a major metabolic pathway of xenobiotics and endobiotics and also is known to take part in the metabolic activation of toxic and carcinogenic N-substituted aryl compounds. These sulfations are catalyzed by a group of sulfotransferase in livers and other tissues. Extensive studies indicate that multiple forms of aryl (phenol) sulfotransferase with distinct catalytic and immunologic properties are contained in experimental animal species (1,2). Recent studies also have provided evidence that human liver contains multiple forms of aryl sulfotransferases (3).

Enormous progress has been made within the past 10 years for understanding the structure of drug-conjugating enzymes such as glutathione transferase, glucuronyltransferase, and acetyltransferase at molecular levels. The information on sulfotransferase, however, remained unclear. Therefore, we have conducted studies for a few years to obtain structural information on the primary structure of this enzyme by molecular cloning (4-6) as a first step to understand the exact role of this enzyme in drug and carcinogen activation. In this article, rat and human aryl sulfotransferases are characterized by their cDNA expression. In addition, properties and relationships of N-hydroxyarylamine sulfotransferases in rats and humans are shown.

Experimental Procedure

Chemicals used and methods for assays of sulfotransferase activity and covalent binding to DNA are the same as described previously (7,8). Minoxidil sulfation was determined by the method of Johnson and Baker (9). Isolation and characterization of sulfotransferase cDNAs were performed as described (6,10). Experimental details for animal treatments were done as described in our previous reports (11,12).

Isolation of Aryl Sulfotransferase cDNAs

As reported previously (6), we isolated a cDNA (PST-1) encoding a rat aryl sulfotransferase by the use of anti-aryl sulfotransferase antibodies. PST-1 consisted of 1028 nucleotides and contained an open reading frame of 873 nucleotides encoding a 291 amino acid protein. Another clone-related PST-1, but showing a longer nucleotide length, was isolated recently by further screening of a rat liver cDNA library with PST-1. The cDNA (PST-1v) contained a coding sequence identical to PST-1 but had extended 5'-flanking (12 base) and 3'-untranslated regions (196 base) (Figure 1).

Consistent with the isolation of two cDNAs with different sizes, two distinct hybridized bands were detected with 32P-labeled PST-1 at around 155 and 165 in Northern blots of rat RNAs. These bands were detected in livers and kidneys of both sexes and showed no clear sex-related difference. A hybridizable band also was detected in the RNA samples of brain, heart, and colon tissues, suggesting that ST1A1 encoded by PST-1 is expressed in various extrahepatic tissues.

Characterization of Phenol Sulfotransferase cDNAs

As shown in Figure 2, transfection of a construct containing PST-1 with normal (syn), but not opposite (anti), orientation resulted in the expression of a sulfotransferase (ST1A1) in COS-1 cells, ST1A1 migrated to a position identical with a major immunodetectable protein (32.5 kDa) in rat livers in SDS-polyacrylamide gel electrophoresis and Western blotting. ST1A1 constituted nearly 0.2% of total cytosolic protein in the transfected COS-1 cells, and this level corresponded roughly to half of the specific content (Western blot's intensity per milligram protein) in rat livers.

Expressed-ST1A1 catalyzed sulfation of simple phenols such as p-nitrophenol and 1-naphthol in the presence of PAPS (Table 1). The rate of sulfation of p-nitrophenol was 2- to 3-fold higher at pH 5.5 than at pH 7.4. A similar phenomenon also was observed in hepatic cytosols of rats. The
Table 1. Sulfating activities of STIAI expressed in COS-1 cells.

| Cytosol | Minoxidil Activity, pmol/mg protein/min* | p-Nitrophenol | 1-Naphthol | N-OH-AAF |
|---------|----------------------------------------|----------------|------------|----------|
| Male liver | 590 | 480 | 2790 | 3700 | 380 |
| COS-1/PST-1 (syn) | 170 | 80 | 214 | 740 | 6 |
| COS-1/PST-1 (anti) | < 7 days | < 5 days | < 5 days | < 10 days | < 3 days |

*Activities were determined at pH 7.4 for sulfations of minoxidil and p-nitrophenol, and at pH 5.5 for sulfation of 1-naphthol, N-hydroxy-2-acetylaminoanthracene (N-OH-AAF), and p-nitrophenol. **PST-1 is a cDNA clone of STIA1. *Syn and anti indicate introduction of PST-1 in normal and opposite directions, respectively. **Below the limits of detection.

Figure 1. Nucleotide sequences of PST-1 and a variant clone (PST-1v) encoding STIA1. Only the 5' and 3' ends of both cDNA sequences are shown. Initiation and termination codons, ATG and TGA, are indicated in bold letters and asterisks, respectively. Complete identity was observed on their amino acid-coding sequences.

Figure 2. Western blots of STIA1 expressed in COS-1 cells. Syn and anti indicate introduction of PST-1 in normal and opposite directions, respectively.

Figure 3. Chemical structures of aryl sulfotransferase substrates.

Figure 4. Lineweaver-Burk plots of minoxidil sulfation in cytosols of COS-1 cells, male and female rat livers. Minoxidil sulfation was determined using a concentration range of 62.5 to 4000 μM. K_m for minoxidil was determined as 560 μM in COS-1, 1550 μM in male rat, and 470 μM in female rat systems.
of their in vivo metabolism showed that heterocyclic amines with an aminooxaarene moiety were excreted to considerable extents as the N-sulfates (sulfamates) (16,17). Therefore, we examined N-sulfation of typical azaarenes and carbolines using liver cytosols of rats and humans as well as cytosols of ST1A1-expressed COS-1 cells. As described in Table 2, all the heterocyclic amines examined were N-sulfated in rat liver cytosol systems. Extents of sulfation were the highest on 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) in hepatic cytosols of both sexes. N-Sulfations of IQ and MelQx showed male dominance, while N-sulfations of three other amines were higher in females than males. ST1A1 catalyzed N-sulfation of IQ and MelQx at comparable rates as a sulfotransferase in rat livers but was devoid of detectable activity for other heterocyclic amine substrates. Human liver cytosol also catalyzed N-sulfation of IQ at low rates, but showed no detectable activity on other amines. These results indicate that plural forms, ST1A1 and another form of sulfotransferase (likely to be a female-dominant form), catalyzed N-sulfation of aryldimines in rat livers.

Using ST1A1 cDNA (PST-1), a cDNA library of a human liver was screened to isolate a human sulfotransferase cDNA related to ST1A1. A clone with a 1.0-Kb insert was isolated and found to contain information of a human sulfotransferase (ST1A2). The isolated cDNA covered almost the entire coding sequence and showed a 77% homology to ST1A1. To assess the cDNA encoding a functionally active enzyme, the cDNA was expressed in Escherichia coli using an ATG-containing vector. In Western blots with anti-rat sulfotransferase antibodies, both the expressed protein and human cytosol were immunostained at an identical mobility. Similar to the rat counterpart, expressed-ST1A2 catalyzed sulfation of minoxidil and p-nitrophenol. Levels of immunodetectable ST1A protein in human livers varied more than 10-fold and showed a good correlation with the extents of minoxidil sulfation in individual human cytosols (r = 0.80). These results suggest that ST1A2 encodes a major sulfotransferase catalyzing minoxidil bioactivation in humans.

### Characterization of N-Hydroxyarylamine Sulphotransferase

Arylamines produce mostly tumors in livers of experimental animals. The incidence is often higher in male rats than in females (18–20). Studies from several laboratories established the major role of male-dominant sulfation on the occurrence of the sex-related difference in the arylamine-induced liver carcinogenesis in this species (20–22). The enzymes mediating this activation also have been isolated from several laboratories (8,23,24). Exact identification of the isolated proteins is, however, not yet performed, because of the instability of purified proteins.

We purified two distinct but immunologically related forms of sulfotransferases (HAST I and HAST II) from livers of adult male rats (8). Both forms catalyze sulfations of p-nitrophenol and N-hydroxy-2-acetylaminofluorene, and mediated PAPS-dependent DNA binding of N-hydroxy-2-acetylaminofluorene in the reconstituted system. As shown in Figure 5, immunodetectable amounts of HASTs were 3- to 4-fold higher in male than in female rats and decreased to a level of intact female rats with castration of male animals. Treatment of castrated male or female rats with 10 mg/kg of testosterone propionate for 1 week increased hepatic content of HASTs. HAST levels also varied during development. The contents were low and did not differ between the sexes until 4 weeks of age, but the sex-related difference became clear at around 5 weeks of age (Figure 6). These results are in accordance with data reported on cytosolic-sulfating activities of N-hydroxy-2-acetylaminofluorene (20,25), and confirmed the identity of HASTs as N-hydroxyarylamine-activating sulfotransferases. On the cause of the sex-related and developmental changes in hepatic contents of HASTs in rat livers,
we showed that hepatic levels of HASTs are under the influence of pituitary growth hormone and thyroid hormone using hypophysectomized rats (8,26). As shown in Figure 7, levels of both hepatic sulfaftion of N-hydroxy-2-acetylaminoazobenzene and content of HAST changed concordantly with each other, depending on growth hormone levels and administration mode. Both the levels of sulfafting activity and HAST content decreased to less than one-third after hypophysectomy of male rats, which was comparable to that of intact female rats. Intermittent injection of growth hormone twice a day for 7 days, which mimicked the male secretory pattern, to hypophysectomized rats increased both levels, while supplement of growth hormone by constant infusion, to mimic the female pattern, had only trivial effect. These results indicate that secretory profile and trough levels of plasma growth hormone are the main determinant of HASTs in rat livers.

Interestingly, Griffin et al. (27) observed in 1955 the association of a pituitary factor on liver carcinogenicity of arylamines in rats. In the study, arylamine-induced liver tumorigenesis was decreased in hypophysectomized rats, which is in good agreement with decreased levels of HAST in that condition. We know now that phase I enzymes such as P450 also are influenced by pituitary growth hormone (28,29). Therefore, we also should consider several factors other than sulfaftion. But lowered levels of hepatic sulfaftion may account in part for the decreased sensitivity to carcinogenic arylamines in hypophysectomized rats.

Pentachlorophenol (PCP) is known to inhibit sulfaftation-mediated activation of N-hydroxyarylamines in experimental animals (30). Both sulfaftion of N-hydroxy-2-acetylaminoazobenzene and PAPS-dependent DNA binding of N-hydroxy-2-aminoazobenzene were inhibited efficiently by the addition of ~20 μM PCP in human cytosolic systems (7). In addition, cytosolic p-nitrophenol sulfafting activities were separated into two fractions by DEAE-HPLC with a NaCl gradient elution of human liver cytosols. DNA binding of N-hydroxy-2-aminoazobenzene was not detectable in the first fraction, but comigrated in the second fraction, which contained a protein showing an intense band around 33 kDa in Western blots using a rat HAST antibody. These results suggest that sulfaftion of N-hydroxyarylamines in human livers is likely to be catalyzed by an enzyme related to rat HASTs. We have isolated several different cDNA clones of rat sulfaftransferases. In Northern blots, a cDNA containing information of a new form, STIC1, hybridized specifically with mRNA in livers of adult male but not female rats. These data are consistent with sex-specific and age-dependent appearance of HAST protein in rat livers. We are currently identifying STIC1 properties.

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