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Formation of 2-Amino-3-methylimidazo[4,5-*f*]quinoline- and 2-Amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline-sulfamates by cDNA-expressed Mammalian Phenol Sulfotransferases

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In rat liver cytosol systems, 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) and 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) were converted into their sulfamates in the presence of 3′-phosphoadenosine 5′-phosphosulfate at rates of 51.2 and 50.7 pmol/min/mg cytosol in the male, and 23.7 and 22.5 pmol/min/mg cytosol in the female, respectively. IQ-sulfamate formation was low (0.24 pmol/min/mg cytosol) in human liver cytosols, and MeIQx-sulfamate was not detected (<0.1 pmol/min/mg cytosol). These results suggest only a minor contribution of IQ- and MeIQx-sulfamate formation to the detoxification of both heterocyclic amines in humans. Using sulfotransferase cDNA-expression systems, a rat ST1A1 arylsulfotransferase has been shown to catalyze the formation of the sulfamates, suggesting a role of the ST1A type of sulfotransferase in the N-sulfation of heterocyclic amines.

Key words: Pyrolysate carcinogen — Sulfation — N-Sulfation — Sulfotransferase — cDNA expression

Sulfate conjugation plays an important role in biotransformation of xenobiotics and endobiotics,[1,2] and is regarded as a detoxification process in most cases. This reaction is catalyzed by a number of cytosolic sulfotransferases in mammalian tissues.

More than ten heterocyclic amines found in cooked foods have been shown to be mutagenic to *Salmonella* and carcinogenic to experimental animals.[3] Among them, 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) and 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) were excreted to considerable extents (about 20% of administered dose) as the N-sulfates (sulfamates) in rats.[4,5] The sulfamates of IQ and MeIQx are not mutagenic to *Salmonella*, with or without metabolic activation systems.[4,5] Furthermore, the sulfamates are resistant to hydrolysis by arylsulfatase used for deconjugation of xenobiotic O-sulfates.[4,5] Thus, N-sulfation is considered as a detoxification pathway of IQ and MeIQx.

A number of sulfotransferases contained in mammalian livers have recently been characterized with the aid of molecular cloning techniques, as well as by protein purification.[6] The molecular forms of rat sulfotransferase catalyzing IQ- and MeIQx-sulfate formation, however, remained unidentified. Although MeIQx has been shown to undergo N-hydroxylation in liver microsomes of humans, the fate of ingested IQ and MeIQx is not well understood.[7] Thus, we have examined in the present study the capacity for N-sulfation of IQ and MeIQx using liver cytosols of rats and humans, and also cDNA-expressed sulfotransferases (rat ST1A1[8,9] and ST1C1,[10] and human ST1A2[6] and ST1A3[6]).

MATERIALS AND METHODS

Chemicals All the reagents used in this study were of the highest grade available. [^{35}S]3′-Phosphoadenosine 5′-phosphosulfate (PAPS, 2 Ci/mmol) was purchased from NEN Research Products (DuPont). IQ and MeIQx were supplied by the Japanese Chemical Carcinogen Repository for Cancer Research, Tokyo. IQ- and MeIQx-sulfamates were kindly supplied by Dr. R. J. Turesky (Nestle Co., Ltd.).

Preparation of liver cytosols of rats and humans Adult male and female Sprague-Dawley rats (9-week-old) were obtained from Japan Clea Co. Five Japanese liver samples were obtained from the Department of Pathology, School of Medicine, Keio University within 3 h of clinical death.[11,12] Case histories for the samples were as follows (age, sex and cause of death): 64-year-old female, duodenal ulcer; 58-year-old male, cancer of lachrymal gland (liver metastasis); 66-year-old male, renal failure (lung emphysema); 72-year-old female, unstable angina (pectoris); 62-year-old male, septicemia. Cytosols of rat and human livers were prepared as previously described.[9,10,13]

Expression of sulfotransferase cDNAs ST1A1, ST1C1 and ST1A3 cDNAs were each ligated with a mammalian

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expression vector p91023(B) and then transfected into COS-1 cells by electroporation using a Gene Pulser apparatus (Bio-Rad Laboratories, Richmond, CA).[9, 10] ST1A2, whose N-terminal eight amino acids were modified in the cDNA expression system, was expressed in *Escherichia coli* as previously described.[14] The cDNA-expressed cells were lysed by sonication and the lysate was centrifuged at 105,000*g* for 1 h. The supernatants were used as cytosols.

Assay of formation of IQ- and MeIQx-sulfamates Sulfations of IQ and MeIQx were detected based on the radioactivity of the metabolites obtained with [^{35}S]PAPS as a sulfate donor after thin layer chromatography. A typical incubation mixture consisted of 8 m*M* potassium phosphate (pH 7.2), 1 m*M* DTT, various concentrations of a heterocyclic amine, [^{35}S]PAPS and 0.2–0.4 mg/ml of liver cytosol in a final volume of 10 μl. The reaction was initiated by the addition of [^{35}S]PAPS and terminated by the addition of 10 μl of acetonitrile after incubation of the reaction mixture for 20 min at 37°C. The reaction without a substrate was always included as a control. Portions (10 μl) of the reaction mixture were applied to a cellulose thin layer plate (Art. 5565, Merck). Metabolites on the chromatogram were developed with n-propanol:ammonia:water (6:3:1 for IQ-sulfamate detection or 6:4:2 for MeIQx-sulfamate detection). The radioactive spots, which were positive only in the presence of IQ or MeIQx, were scraped from the plates and quantified by liquid scintillation counting. The rates of the N-sulfations were calculated after subtraction of the respective control radioactivities, and were expressed as pmol of sulfamate formed per mg protein per min.

RESULTS

Formation of IQ- and MeIQx-sulfamates in liver cytosols of rats and humans Radioactive metabolites of IQ (Rf=0.51, system 1; Fig. 1) and MeIQx (Rf=0.40, system 2) were detected by thin layer chromatography after the reaction in the presence of mammalian liver cytosol, either IQ or MeIQx, and [^{35}S]PAPS. The radioactive products derived from IQ and MeIQx comigrated with authentic IQ- and MeIQx-sulfamates.[4, 15, 16] As described in Table I, IQ and MeIQx were N-sulfated in male rat liver cytosols at rates of 51.2±2.5 and 50.7±2.7 pmol/min/mg protein, respectively. The rates were two times higher in male rats than female rats. The rate of IQ-sulfamate formation in human liver cytosol was, however, low even at a high IQ concentration (500 μ*M*) (Table I). No sulfamation activity towards MeIQx was detected in human liver.

Formation of IQ- and MeIQx-sulfamates catalyzed by cDNA-expressed sulfotransferases To identify molecular forms of rat sulfotransferase involved in the formation of IQ- and MeIQx-sulfamates, we examined the capacities of two different aryl(phenol)sulfotransferases, ST1A1 and ST1C1, for the formation of IQ- and MeIQx-sulfamates using cDNA expression systems. As shown in Fig. 2A, ST1A1 catalyzed sulfamate formation of IQ and MeIQx at rates of 19.4±2.3 and 12.6±1.1 pmol/min/mg cytosolic protein, respectively. ST1C1 also biotransformed IQ and MeIQx to their sulfamates at rates of 2.8±0.1 and 4.2±0.9 pmol/min/mg cytosolic protein, respectively. No appreciable amounts of IQ- and MeIQx-sulfamates were formed in the presence of cytosols of cells expressing human ST1A2 and ST1A3 (<0.1 pmol/min/mg cytosol). The amounts of ST1A1 and ST1C1

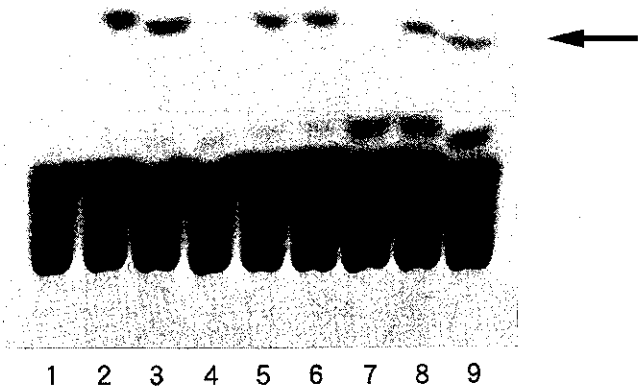

Fig. 1. Detection of IQ-sulfamate on cellulose thin layer chromatography. IQ (100 μ*M*) was incubated with cytosols of livers of male and female rats and of ST1A1-expressing COS-1 cells in the presence of 8 μ*M* [^{35}S]PAPS at 37°C for 20 min. IQ-sulfamate formed was detected on a cellulose thin layer plate by autoradiography as described in the text. The arrow indicates the position of IQ-sulfamate. 1–3, male rat; 4–6, female rat; 7–9, ST1A1-expressed cells; 1, 4 and 7, in the absence of IQ; 2, 3, 5, 6, 8 and 9, in the presence of IQ.

Table I. Formation of IQ- and MeIQx-sulfamates in Liver Cytosols of Rats and Humans

	Rat[a]		Human[a]
	Male	Female	
	(pmol/min/mg cytosolic protein)		
IQ	51.2±2.5**	23.7±2.9	0.24±0.06
MeIQx	50.7±2.7*	22.5±6.7	<0.1

Sulfamate formation of IQ and MeIQx was determined at a concentration of 100 μ*M* for rat liver cytosol or 500 μ*M* for human liver cytosol.

a) Four male and three female rats (mean±SD). Significant differences between male and female rats are indicated (* P <0.02, ** P<0.01). Five human individuals (mean±SD).

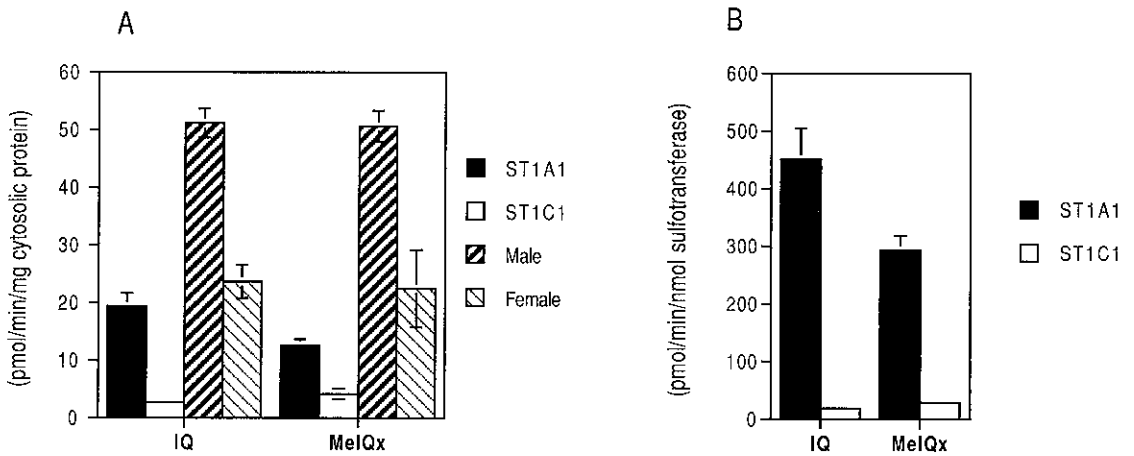

Fig. 2. Formation of IQ- and MeIQx-sulfamates in cytosols of ST1A1- or ST1C1-expressing COS-1 cells and of rat livers. Formation of IQ- and MeIQx-sulfamates was examined at a concentration of 100 μM in the presence of 25 μM [^{35}S]PAPS. Activities were expressed as pmol/min/mg cytosolic protein (A) and as pmol/min/nmol sulfotransferase (B), taking the expressed levels of ST1A1 and ST1C1 in cells into consideration.

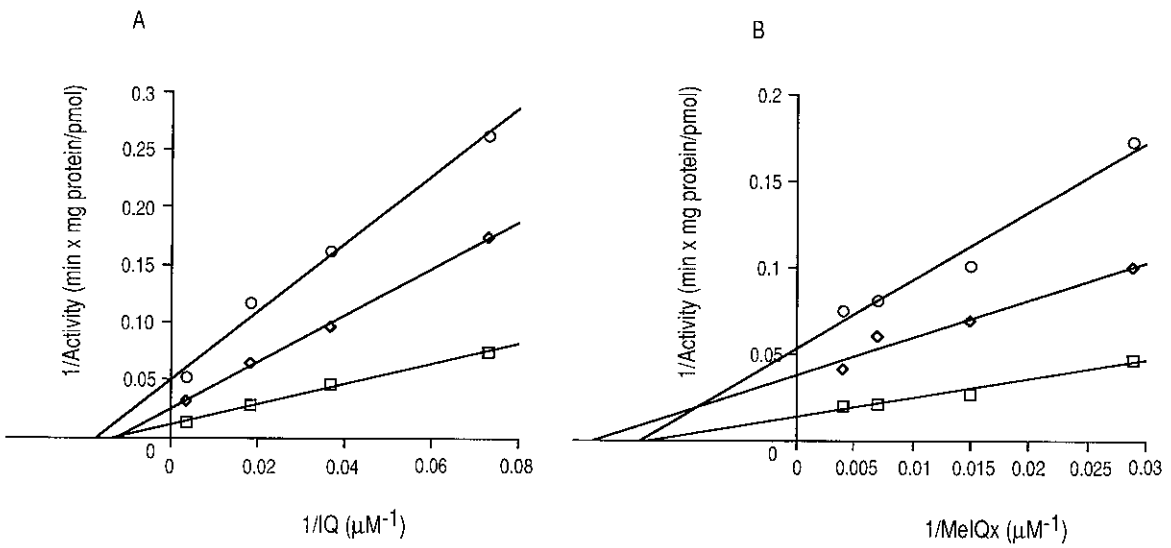

Fig. 3. Lineweaver-Burk plots of formation of IQ (A)- and MeIQx (B)-sulfamates catalyzed by cytosols of ST1A1-expressing cells and rat livers. The heterocyclic amine substrates were incubated with cytosols of ST1A1-expressed cells (○) and livers of male (□) and female (◇) rats at various concentrations of the substrates and 20 μM [^{35}S]PAPS. The sulfamate-forming activities were calculated in duplicate determinations. Deviations were within 7% of the mean.

expressed in COS-1 cells were determined as 0.042 and 0.143 nmol/mg cytosol using a [^{35}S]methionine labeling method.[14] Thus, the specific activities for the formation of sulfamates were calculated as shown in Fig. 2B. Formations of IQ- and MeIQx-sulfamates were 30- and 10-fold higher with ST1A1 (458±54 and 300±35 pmol/min/nmol ST1A1, respectively) than with ST1C1 (19±1 and 29±6 pmol/min/nmol ST1C1, respectively).

Kinetics of formation of IQ- and MeIQx-sulfamates catalyzed by cytosols of rat liver and ST1A1-expressing COS-1 cells Using the data obtained from experiments at different substrate concentrations (12.5–200 μM), K_m values for IQ sulfamate formation were calculated with liver cytosols of rats and ST1A1-expressing cell cytosol from the Lineweaver-Burk plots shown in Fig. 3A. Similar apparent K_m values for IQ were obtained in cytosols

of ST1A1-expressing cells (58 μM) and livers of male (80 μM) and female (85 μM) rats. Apparent K_m values for PAPS for IQ N-sulfation were 3.4 μM, 3.6 μM and 3.6 μM with ST1A1-expressing cell cytosol, and male and female rat liver cytosols, respectively (data not shown). Apparent K_m values for MeIQx for MeIQx-sulfamate formation were also similar in cytosols of ST1A1-expressing cells (75 μM) and livers of male (77 μM) and female (57 μM) rats (Fig. 2B).

DISCUSSION

IQ and MeIQx, given to rats intraperitoneally or *per os*, were excreted to considerable extents as the sulfamates.[4, 5, 17, 18] In accordance with previous *in vivo* data, IQ and MeIQx were N-sulfated to produce the sulfamates in hepatic cytosol systems of rats with PAPS as a sulfate donor (Table I). Formation of IQ- and MeIQx-sulfamates was low in liver cytosols of humans as compared to those of male rats (Table I).

N-Acetylation is known to be a major metabolic pathway for carcinogenic arylamines such as 2-aminofluorene, 4-aminoazobenzene, benzidine and 4-aminobiphenyl.[19–21] However, IQ was scarcely N-acetylated in the presence of rat liver cytosol.[22] As an alternative metabolic pathway for IQ, IQ-sulfamate formation was catalyzed by rat liver cytosols (Table I). In addition, a rat sulfotransferase, ST1A1, has been shown to catalyze the formation of IQ- and MeIQx-sulfamates (458 and 300 pmol/min/nmol ST1A1, respectively) at rates 30- and 10-fold higher than those of another rat sulfotransferase, ST1C1. ST1A1 is expressed in livers of male and female rats at similar levels (0.1 nmol/mg cytosol).[10, 14] ST1C1 was also found in males at about 0.1 nmol/mg cytosol, but only a trace amount was present in female rats. These data suggest a potential role of ST1A1 in the formation of IQ- and MeIQx-sulfamates in rat liver. The sulfamation of IQ and MeIQx (100 μM each) in ST1A1-expressing cells and in rat livers was inhibited by more than 90% in the presence of 1 μM pentachlorophenol, a typical arylsulfotransferase inhibitor,[23] but not by 100 μM dehydroepiandrosterone, a typical substrate for hydroxysteroid sulfotransferase (data not shown). These results support the idea that an arylsulfotransferase, ST1A1, plays an important role in the formation of IQ- and MeIQx-sulfamates in livers of rats of both sexes. In our previous paper, another form of arylsulfotransferase, ST1C1, was shown to be the main enzyme catalyzing activation of carcinogenic N-hydroxyarylamine(amide)s.[10] ST1A1, in contrast to ST1C1, showed only a marginal activity in the covalent binding of N-hydroxy-2-acetylaminofluorene.[10] Thus, forms of arylsulfotransferase have been shown to mediate both detoxification and activation of carcinogenic arylamines.

Microsomal N-hydroxylations of IQ and MeIQx catalyzed by CYP1A2, CYP1A1 and CYP2C11 were shown to be the initial steps in the activation pathway for IQ and MeIQx in rats.[24–26] On the other hand, cytochrome P450s were also suggested to play a role in detoxification of IQ and MeIQx through hydroxylation at the C-5 position. The hydroxylated metabolites underwent successive sulfate conjugation to accomplish detoxification.[14, 27–29] Thus, sulfating enzymes play an important role in detoxification of carcinogenic heterocyclic amines through N- and O-sulfation.

IQ produced hepatocellular carcinoma in cynomologus monkeys, raising the possibility of carcinogenicity of IQ in humans.[30] N-Hydroxylation has been shown to be a major microsomal oxidation product of MeIQx in human livers.[7] As compared to rat liver cytosol, the rate of PAPS-dependent binding of N-hydroxy-IQ to DNA was low in human liver cytosol.[13] In monkey liver cytosol systems, N-hydroxy-IQ was shown to undergo acetyltransferase-mediated activation.[31] We have recently reported sulfotransferase-mediated activation of N-OH-PhIP using expression systems of cDNAs encoding human sulfotransferases, ST1A2 and ST1A3.[14] Formation of IQ- and MeIQx-sulfamates for the detoxification of IQ and MeIQx was low in human liver cytosols as compared to rat liver cytosols (Table I). No appreciable amounts of IQ- and MeIQx-sulfamates were formed in the presence of cytosols of ST1A2- and ST1A3-expressing *E. coli* and COS-1 cells, respectively (< 0.1 pmol/min/mg cytosol). Since the expressed levels of ST1A2 and ST1A3 were determined to be 0.121 and 0.110 nmol sulfotransferase/mg cytosolic protein,[14] the rates of formation of IQ- and MeIQx-sulfamates by ST1A2 and ST1A3 were less than 0.8 and 0.9 pmol/min/nmol sulfotransferase expressed. These results suggest that formation of IQ- and MeIQx-sulfamates is not a major detoxification pathway for these food-derived carcinogens in humans. Ring hydroxylation and successive conjugation reactions may play a role in the detoxification of IQ and MeIQx in humans.

Human ST1A2 and ST1A3 cDNAs were isolated using rat ST1A1 cDNA as the probe. Amino acid sequences of ST1A2 and ST1A3 showed 77 and 78% similarity to that of ST1A1, respectively. Thus, ST1A-type sulfotransferases are present in rat and human livers. In the present study, formation of IQ- and MeIQx-sulfamates was catalyzed by ST1A1, but not ST1A2 and ST1A3. On the contrary, ST1A2 and ST1A3 catalyzed activation of N-OH-PhIP at rates several-fold higher than did ST1A1.[14] Interestingly, substrate preference is considerably different between the rat and human forms of sulfotransferase, even though they show ca. 80% similarities in their amino acid sequences.

In conclusion, rat phenol(aryl)sulfotransferase, ST1A1, has a potential major role in the detoxification of IQ and MeIQx through sulfamate formation in rat liver. ST1C1, a major N-hydroxyarylamine(amide)s activating enzyme in rat liver, showed only marginal sulfamate-forming activity toward IQ and MeIQx. Formation of IQ- and MeIQx-sulfamates was not efficiently catalyzed by human liver cytosol.

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