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REGULATION OF ARYLSULFATE SULFOTRANSFERASE FROM A HUMAN INTESTINAL BACTERIUM BY NUCLEOTIDES AND MAGNESIUM ION

LISA KONISHI-IMAMURA,[1] DONG-HYUN KIM,[2] MOTOAKI KOIZUMI[1] and KYOICHI KOBASHI[1]*

[1]*Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University, 2630 Sugitani, Toyama, Toyama 930–01, Japan*

[2]*College of Pharmacy, Kyung-Hee University, 1 Hoeki-Dong, Dong Dae Mun-ku, Seoul 131, Korea*

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Arylsulfate sulfotransferase (ASST) from a human intestinal bacterium stoichiometrically catalyzed the transfer of a sulfate group from phenylsulfate esters to phenolic compounds. Pentachlorophenol, one of the selective inhibitors of phenol sulfoconjugation in mammalian tissues, inhibited both phenol and tyramine sulfation by ASST. Nucleotide triphosphates such as ATP, GTP, UTP and CTP, and pyrophosphate inhibited the ASST activity, whereas Mg^{2+} and Mn^{2+} activated the enzyme and prevented its inhibition by ATP and pyrophosphate. Equimolar binding of [α-] and [γ-^{32}P]ATP to the enzyme showed that the enzyme protein was not phosphorylated, but bound ATP. These results suggest that nucleotide triphosphates and divalent cations are important modulators in the control of ASST activity.

KEY WORDS: Arylsulfate sulfotransferase, nucleotides, phenylsulfate esters, ATP

INTRODUCTION

Sulfate conjugation plays an important role in detoxification and excretion of endogenous and exogenous compounds,[1] the liver being the major organ involved in sulfate conjugation metabolism.[2] Phenol sulfotransferase (PST, EC 2.8.2.1), which catalyzes the transfer of a sulfate group from 3'-phosphoadenosine 5'-phosphosulfate (PAPS) to a phenolic acceptor substrate, has been purified from various mammalian organs.[3–5] Also, in human liver, three isozymes, steroid/bile acid sulfotransferase, monoamine sulfating PST (M-PST) and phenol sulfating PST (P-PST) have been demonstrated.[2] M- and P-PST have also been characterized in human platelets and

*Correspondence

Abbreviations: ASST, arylsulfate sulfotransferase; BSA, bovine serum albumin; CBB, Coomassie brilliant blue; MUS, 4-methylumbellyferylsulfate; PAPS, 3'-phosphoadenosine 5'-phosphosulfate; PNS, *p*-nitrophenylsulfate; PPi, pyrophosphate; PST, phenol sulfotransferase

brain, and shown to possess different substrate specificities and sensitivities to the inhibitor, dichloronitrophenol.[6] Kinetic studies of human brain M- and P-PST have demonstrated that the end product, 3'-phosphoadenosine 5'-phosphate (3', 5'-PAP), and the structural analog of PAPS, ATP, inhibit competitively the activity of the enzymes.[7] In addition, it has been suggested that the high- and low-molecular-weight endogenous inhibitors of PST are present in human red blood cells. The high-molecular-weight inhibitors have been investigated as PAPS-degrading enzymes[8] and the low-molecular-weight inhibitors are expected to be the structural analogues of PAPS such as ATP, ADP and 3', 5'-PAP.[9]

On the other hand, we previously reported a sulfotransferase from *Eubacterium* A-44, one of the predominant anaerobic bacteria in the human intestine.[10,11] This enzyme, which catalyzes the stoichiometric transfer of a sulfate group from phenyl sulfate esters to phenolic acceptors, is different from mammalian tissue PSTs. In addition, the specific activity of this bacterial enzyme is one thousand-fold higher than those of other PSTs. The enzyme catalyzes the transfer of a sulfate group to a specific position of various phenolic acceptors such as drugs, antibiotics, neurotransmitters, tannins, flavones and tyrosine residues of peptides.[12–16] Furthermore, the kinetic behavior of our bacterial enzyme, which proceeds by a ping-pong bi-bi mechanism, differs from that of PSTs from tissues such as liver, which show a rapid-equilibrium random bi-bi mechanism.[17,18] From these data, this enzyme is considered to be a new type of sulfotransferase, and has been named arylsulfate sulfotransferase (ASST, EC 2.8.2.22).[19] In the present study, we investigated the roles of nucleotides, pyrophosphate (PPi) and divalent cations in the mechanism of ASST activity regulation.

MATERIALS AND METHODS

Materials

p-Nitrophenylsulfate (PNS) and bovine serum albumin (BSA) were purchased from Sigma Chemical (U.S.A.). Tyramine was from Nacalai Tesque, Inc. (Japan). ATP, GTP, ADP, PPi and pentachlorophenol were from Wako Pure Chemical Industries (Japan). GAM broth was from Nissui Seiyaku Co. Ltd. (Japan). UTP, CTP, UDP, CMP and UMP were from Boehringer Mannheim (Germany). [α-^{32}P]ATP and [γ-^{32}P]ATP were from New England Nuclear (U.S.A.). Bray's reagent was prepared according to the method of Bray.[20] All other chemicals were of analytical reagent grade.

Enzyme preparation

ASST obtained from *Eubacterium* A-44 was partially purified according to the procedure described previously.[17] Specific activity of the purified enzyme was 81.5 units per mg protein (91% purity based on the highest specific activity[11] of the homogeneous enzyme).

Activity assay

The assay method for ASST was undertaken according to our previous report.[11] PNS and tyramine were used as a control donor and acceptor, respectively. One unit of

enzyme activity was defined as the amount required to catalyze the formation of 1.0 μmol of *p*-nitrophenol per minute. Specific activity was defined in terms of units per mg protein.

Inhibition and activation

Nucleotides, PPi, metal chloride salts, EDTA and pentachlorophenol were dissolved in 0.1 M Tris-HCl buffer, pH 8.0, and the enzyme activity was assayed according to the standard assay method in the reaction mixture containing each compound at various concentrations.

Gel filtration of ATP-binding sulfotransferase

The ATP-binding reaction was performed for 180 min at 18°C under the following conditions: The reaction mixture (total volume, 80 μl) contained 50 μl of purified enzyme (18 μg) dissolved in 0.1 M Tris-HCl buffer (pH 7.0), 5 μl of [γ-^{32}P]ATP or [α-^{32}P]ATP (0.37 MBq, 111 TBq/mmol) and 20 μl of 2 mM nonradioactive ATP with or without 5 μl of 0.2 M $MgCl_2$. Each reaction mixture was made up to 0.5 ml with 50 mM Tris-HCl, pH 7.0, and then applied to a Sephadex G-50 coarse column (1.6 $\times$ 37 cm). The column was eluted with 50 mM Tris-HCl buffer, pH 7.0 (fraction volume, 2 ml). After Bray's reagent had been added to the eluted fractions (0.25 ml), the radioactivity was determined with a liquid scintillation counter (Aloka, LSC 671).

Protein determination

Protein concentrations were estimated by the method of Lowry *et al.*[21] using BSA as a standard.

RESULTS

Effect of pentachlorophenol on ASST activity

Mulder *et al.* have reported that pentachlorophenol inhibits rat liver sulfotransferase activity in the same manner as 2,6-dichloro-4-nitrophenol *in vitro*.[22] In the present study, we tested the effect of pentachlorophenol on ASST activity (Figure 1). The inhibitor concentration which gave 50% inhibition was 25.5 μM, using tyramine as an acceptor substrate.

Inhibition of ASST by nucleotides

Mammalian PST has been reported to be inhibited by 3'-phosphoadenosine 5'-phosphate, ADP and ATP.[9] The bacterial enzyme was found to be inhibited by nucleotide triphosphates (NTP) such as ATP, GTP, UTP and CTP, and PPi, as shown in Table 1, but not inhibited by nucleotide monophosphates (NMP), nucleotide diphosphates (NDP) and sodium phosphate at 5-fold higher concentrations of NTP. The inhibitory efficacies of NTPs were higher than those of phenylphosphate esters.[10]

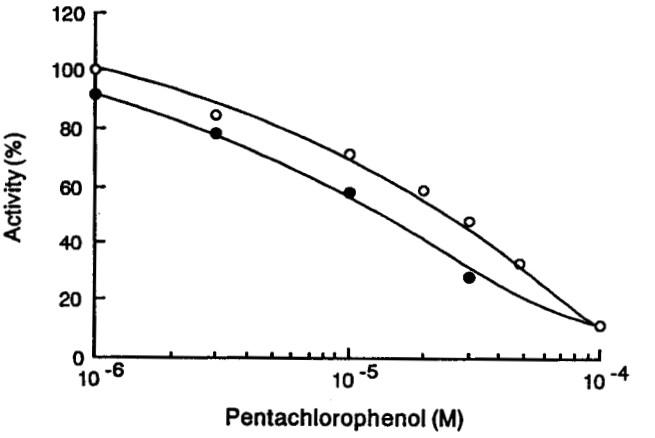

FIGURE 1 Inhibition of ASST activity by pentachlorophenol. ASST (0.04 unit) was incubated in the presence of various concentrations of pentachlorophenol using PNS and tyramine (○) or phenol (●) as substrates. Each value shows the mean of triplicate determinations.

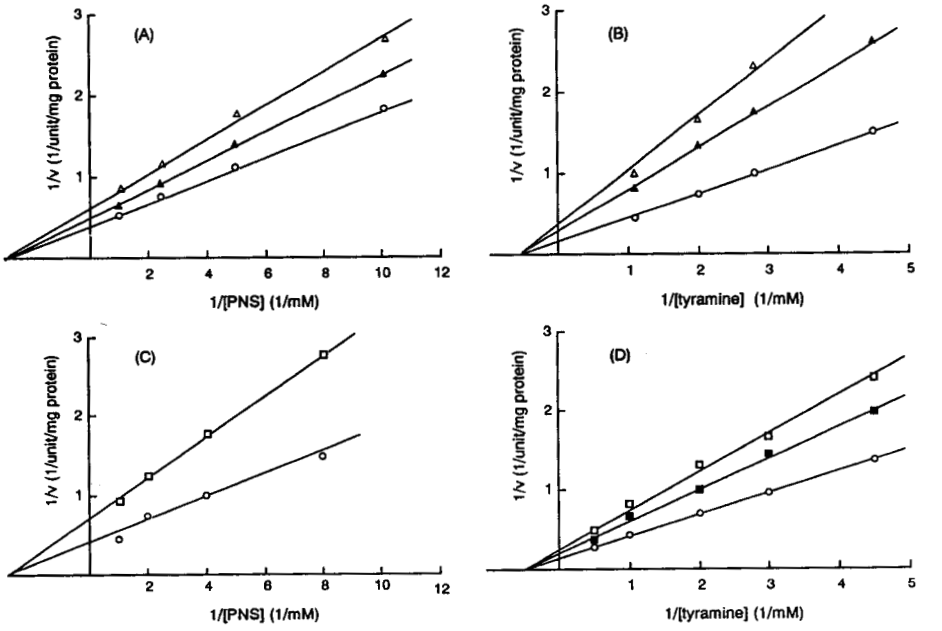

FIGURE 2 Lineweaver-Burk plot of inhibition of ASST. PPi for PNS (A), PPi for tyramine (B), ATP for PNS (C) and ATP for tyramine (D). Concentrations of inhibitor were as follows: ▲, 0.16 mM PPi; △, 0.80 mM PPi; ■, 0.1 mM ATP; □, 0.45 mM ATP; ○, none. Each value shows the mean of triplicate determinations.

TABLE 1
Effects of nucleotides and Mg^{2+} on the activity of ASST

Compounds		Activity (%)***
Nucleotides*	Mg^{2+}**	
ATP	–	38
GTP	–	38
UTP	–	40
CTP	–	69
NTPs	+	148–155
–	+	157
ADP**	–	97
GDP**	–	90
UDP**	–	90
AMP**	–	98
CMP**	–	105
Pi**	–	99
PPi	–	39
PPi	+	151

Each value shows the mean of triplicate determinations.
*Final concentration was 0.6 mM.
**Final concentration was 3 mM.
***The activity for PNS as a donor substrate and tyramine as an acceptor substrate (81.5 unit/mg protein) was taken as 100.

ATP and PPi inhibited the enzyme non-competitively (Figure 2), whereas phenylphosphate esters showed competitive inhibition (data not shown). K_i values of ATP and PPi for PNS (K_m = 0.1 mM) were 0.66 mM and 0.7 mM, respectively. K_i values of ATP and PPi for tyramine (K_m = 2.0 mM) were 0.45 mM and 0.2 mM, respectively.

Activation of ASST by divalent cations

ASST was activated by divalent cations, such as Mg^{2+} and Mn^{2+}, but not by other metal ions; in fact the latter were rather inhibitory (Table 2). Mg^{2+} was the best activator among the metal ions tested. Mg^{2+} protected against loss of the enzyme activity on storage (data not shown). In order to investigate the effect of metal ions on ATP or PPi inhibition, these metal ions were added at the same concentration as that of ATP or PPi in the assay mixture (Table 2). ATP and PPi inhibition was prevented

TABLE 2
Effects of various metal ions, ATP and their mixture on the activity of ASST

Metal ions**	Activity (%)*		
		plus ATP	plus PPi
Mg^{2+}	151	148	150
Mn^{2+}	123	133	129
Ca^{2+}	105	102	100
Cu^{2+}	50	28	35
Co^{2+}	98	142	141
Fe^{2+}	95	32	48
Ni^{2+}	67	97	93
Zn^{2+}	1	1	2
None	100	43	40

Each value shows the mean of triplicate determinations.

*The activity for PNS as a donor substrate, tyramine as an acceptor substrate and no addition of metal ion (28 unit/mg protein) was taken as 100.

**All metal ions were added as chloride salts at a final concentration of 0.6 mM. ATP and PPi were added at the same final concentration.

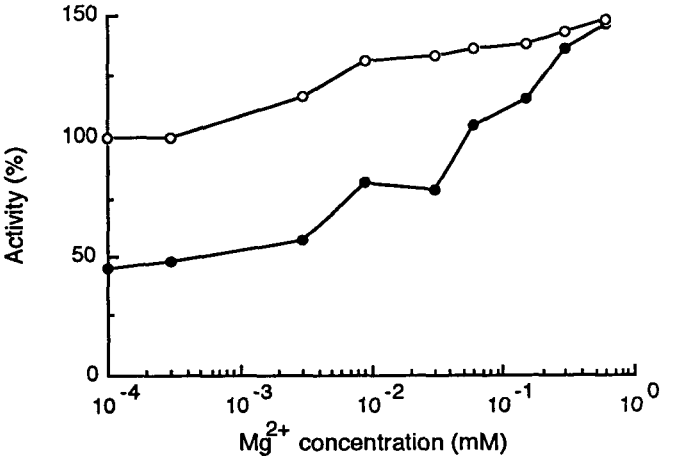

FIGURE 3 Effect of magnesium ion on ATP inhibition of ASST. ASST (0.4 unit) was preincubated for 10 min with (●) or without (○) 0.6 mM ATP. Then, ASST activities were measured in the presence of various concentrations of Mg^{2+}. Each value shows the mean of triplicate determinations.

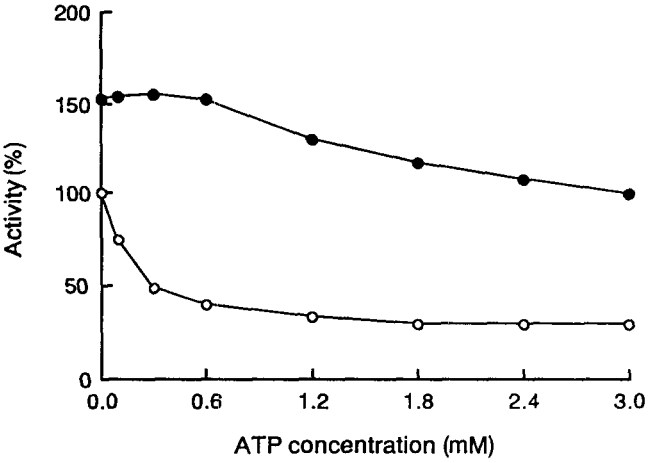

FIGURE 4 Inhibition of magnesium ion-activated enzyme by ATP. ASST (0.4 unit) was preincubated for 10 min with (●) or without (○) 0.6 mM Mg^{2+}. Then, ASST activities were measured in the presence of various concentrations of ATP. Each value shows the mean of triplicate determinations.

by the addition of divalent cations such as Mg^{2+}, Mn^{2+}, Ca^{2+}, Co^{2+} and Ni^{2+}. The prevention of ATP inhibition was dependent on the concentration of Mg^{2+} (Figure 3). The enzyme activity at 0.6 mM ATP was 43% in the absence of divalent cations but was restored to 100% by the addition of 0.06 mM Mg^{2+}, and further activated to 150%, by that of 0.6 mM Mg^{2+} which was the level obtained by the addition of 0.6 mM Mg^{2+} only. In addition, we examined the effect of ATP on activity of the 0.6 mM Mg^{2+}-activated enzyme. As shown in Figure 4, the enzyme activity was not affected by ATP at concentrations under 0.6 mM, which is the same concentration as that of Mg^{2+}. The activity was inhibited by ATP at higher concentrations, though the inhibitory efficacy was very low.

ATP-binding to the enzyme

In order to investigate the mechanism of ATP inhibition, the enzyme was incubated with [α-] and [γ-^{32}P]-labeled ATP under several conditions. These reaction mixtures were then analyzed by Sephadex G-50 coarse gel filtration. As shown in Figure 5, the enzyme fraction contained almost equal counts of radioactivity derived from both [α-] and [γ-^{32}P]ATP, suggesting the formation of an enzyme-ATP complex.

DISCUSSION

Sulfation has been widely accepted as one of the detoxification pathways of xenobiotic phenolic compounds, the reaction being catalyzed by tissue PST. However, we have discovered a different type of sulfotransferase from a human intestinal-predominant

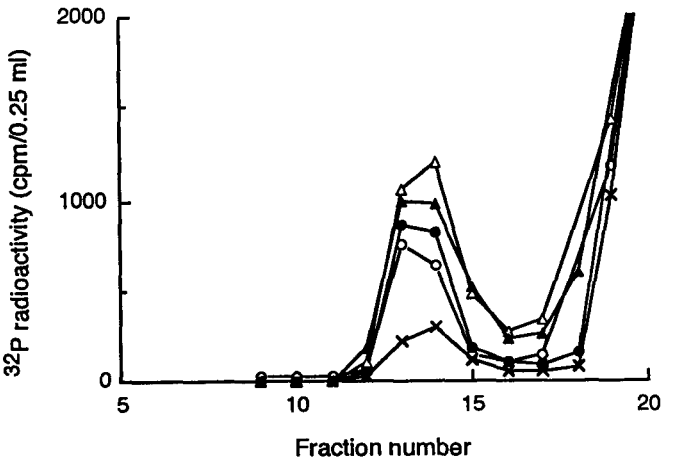

FIGURE 5 Gel filtration of the enzyme incubated with [γ-] or [α-^{32}P]ATP. Conditions: ○, [γ-^{32}P]ATP; △, [γ-^{32}P]ATP and Mg^{2+}; ●, [α-^{32}P]ATP; ■, [α-^{32}P]ATP and Mg^{2+}; X, [α-^{32}P]ATP and the enzyme being preincubated with nonradioactive ATP. The reaction conditions are described in "MATERIALS AND METHODS".

bacterium, *Eubacterium* A-44, which requires phenylsulfate as a donor substrate.[10,11] In addition, a crude extract from the bacterium did not show any arylsulfatase activity when PNS was used as a substrate.[23] In the present study, we investigated the regulatory mechanism of this enzyme using nucleotides as allosteric effectors.

Pentachlorophenol inhibited the activity of ASST, but, this inhibition was much less effective on the bacterial sulfotransferase than on rat liver sulfotransferase.[22] Also, the effect of pentachlorophenol on ASST is quite different from that on human platelet P-PST and M-PST.[6] In the latter cases, 10^{-5} M pentachlorophenol inhibited phenol-conjugating activity by 100%, but inhibited dopamine-conjugating activity by only 20%. Percentages of ASST inhibition at 10^{-5} M pentachlorophenol, using tyramine and phenol as acceptors, were 25% and 40%, respectively. In the case of ASST, phenol sulfation was not selectively inhibited by pentachlorophenol in comparison with that of tyramine. Therefore, it was considered that the activities of tissue PST and bacterial ASST might be regulated in a different manner.

The allosteric interaction of nucleotides with several enzymes related to the energy-producing system regulates the activity through an inhibition or stimulation mechanism.[24–27] We have already reported that ASST is activated by divalent cations, Mg^{2+} and Mn^{2+}, and inhibited by EDTA.[11] In the present study, we found that NTPs and PPi were inhibitors of this bacterial ASST, but NTP-Mg^{2+} and PPi-Mg^{2+} were activators of the enzyme. Mg^{2+} prevented the enzyme inhibition by ATP at the equimolar concentrations (Figure 3), which suggests that the ATP-Mg^{2+} complex is not inhibitory on ASST.

As shown in Figure 5, almost equal amounts of radioactivity from [α-^{32}P] and [γ-^{32}P]ATP were incorporated in the enzyme, showing that the protein was not phosphorylated, but bound ATP molecule itself. However, the binding of ATP to the enzyme was not so significantly affected by Mg^{2+}. The relation between the inhibitory effects of ATP on ASST activity and binding of ATP to the enzyme has not been elucidated in the present study, but suggests that nucleotide triphosphates and divalent cations are important modulators in the control of ASST activity in the bacterium.

On the other hand, sulfation of proteins and peptides has been considered to play a role in their secretion by eukaryotic cells.[28] Since ASST is a primary product in *Eubacterium* A-44, ATP and Mg^{2+} may be regulators of sulfation of the tyrosine residues of proteins, and thus of secretion of some proteins from the bacterium.

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