

## Sulfation of Phenolic Antibiotics by Sulfotransferase Obtained from a Human Intestinal Bacterium

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Novel sulfotransferase which was isolated from *Eubacterium A-44*, a human intestinal bacterium, sulfated phenolic antibiotics, such as amoxicillin, ceftiofloxacin and cefoperazone. The  $K_m$  values of sulfotransferase for these antibiotics were 6.9, 4.3 and 22.2 mM, respectively. The  $V_{max}$  values were 8.3, 3.3 and 1.6  $\mu\text{mol}/\text{min}/\text{mg}$  protein. The optimal pH of the enzyme was 9.0, a weakly alkaline region. The antibacterial activity of amoxicillin was not altered by enzymic sulfation of the phenolic hydroxyl group.

**Keywords** sulfotransferase; phenolic antibiotics; *Eubacterium A-44*; intestinal bacterium; antibiotics; sulfation; minimum inhibitory concentration

Sulfoconjugation represents a major mechanism for detoxification of endogenous and exogenous compounds bearing phenolic functional groups.<sup>1,2</sup> Relating to the sulfoconjugation reaction, the liver and intestinal epithelial cells are generally regarded as the major organ of detoxification. Arylsulfotransferase which catalyzes the transfer of a sulfate group from 3'-phosphoadenosine 5'-phosphosulfate (PAPS) to a phenolic acceptor substrate was purified from guinea pig liver by Banerjee and Roy.<sup>3</sup> Thereafter, the presence of the enzyme has been demonstrated in various mammalian organs such as liver, brain, kidney and intestinal epithelial cells.<sup>4</sup>

Recently, a novel sulfotransferase was purified from *Eubacterium A-44*, a human intestinal bacterium, and catalyzed the sulfate group from only phenolic sulfate esters, but not from PAPS, to phenolic acceptor substrates.<sup>5,6</sup> In addition, modification of antibiotics with microorganisms, such as acetylation, phosphorylation, adenylation and cleavage of  $\beta$ -lactam of antibiotics is considered to be one of the significant inactivation processes of antibiotics.<sup>7</sup> However, sulfation of antibiotics has not been investigated by human intestinal flora. The present study was undertaken in an attempt to sulfate the phenolic hydroxyl group of antibiotics with intestinal bacterial sulfotransferase.

### Materials and Methods

**Materials** *p*-Nitrophenylsulfate (PNS) was purchased from Sigma Chemical Co. (U.S.A.). General anaerobic medium (GAM) broth was from Nissui Seiyaku Co., Ltd. (Japan). Antibiotics were kindly donated from Ms. S.-H. Yook, Korean National Institute of Health. Amoxicillin *O*-sulfate was synthesized by sulfation of amoxicillin with sulfotransferase of *Eubacterium A-44*. All other chemicals were of analytical reagent grade.

**Animals** Male rats (Wistar, body weight 180—220 g) and male mice (ddy, 20—30 g) were maintained on pellet food (Nippon Clea CE-2) and tap water *ad lib.* as one group of five to ten. The animals were placed in the metabolic cage that permitted separate collection of urine and feces. The feces samples were made up to a known volume with distilled water and homogenized.

**Assay of the Sulfotransferase Activity** The assay mixture (total volume of 0.63 ml) contained 0.29 ml of tyramine (occasionally other acceptors), 0.03 ml of 50 mM PNS, 0.21 ml of 0.1 M Tris-HCl buffer, pH 8.0, and 0.1 ml of the enzyme. After incubation of the assay mixture at 37°C for 15 min, the absorbance at 405 nm,  $\lambda_{max}$  of *p*-nitrophenol, was measured. One unit of enzyme activity was defined as the amount required to catalyze the formation of 1  $\mu\text{mol}$  of product per min under the standard assay conditions. Specific activity was defined in term of units per mg protein.

**Purification of the Sulfotransferase from *Eubacterium A-44*** The bacterium, *Eubacterium A-44*, isolated from human feces was precultured in a GAM broth and the precultured medium (50 ml) was inoculated in 21 of GAM broth containing 1 mM PNS as an inducer<sup>9</sup> and cultured at 37°C for 16—20 h in an anaerobic box. The cultured medium was centrifuged at 7000 rpm for 20 min at 4°C. The harvested cells were washed twice with saline. The resulting precipitate was suspended in 0.1 M acetate buffer, pH 5.5, and disrupted by a sonicator (Heat Systems-Ultrasonics, U.S.A.). The sonicated solution was centrifuged at 13000 rpm for 30 min at 4°C. In order to purify the enzyme, ammonium sulfate fractionation, diethylaminoethyl (DEAE)-cellulose, hydroxyapatite and Sephacryl S-300 fine column chromatography were carried out according to the previous method.<sup>5</sup> The purified enzyme was 86.4 units/min/mg protein.

**Assay of Antibacterial Activity** The antibacterial activity was assayed on Mueller-Hinton agar plates which contained amoxicillin and amoxicillin *O*-sulfate at various concentrations. *Escherichia coli* NIHJ or *Staphylococcus aureus* ATCC 6538p ( $2 \times 10^5$  cell) were inoculated in the agar plates and cultured at 37°C for 24 h. Minimum inhibitory concentrations (MIC) of test compounds were measured.

**Determination of Protein** Protein was determined by the method of Lowry *et al.*<sup>9</sup> with bovine serum albumin as the standard.

### Results

**Activities of Sulfotransferase and Sulfatase from Intestinal Contents** In preliminary experiments, sulfotransferase activity of human, rat and mouse feces using PNS as a donor substrate and tyramine as an acceptor substrate were found to be  $15.4 \pm 4.32$  ( $n=6$ ),  $7.3 \pm 3.45$  ( $n=10$ ) and  $54.1 \pm 21.5$  ( $n=8$ )  $\mu\text{mol}/\text{h}/\text{g}$  wet feces, respectively. In addition, sulfatase activity of human, rat and mouse feces using PNS as substrate were  $1.2 \pm 1.17$ ,  $0.15 \pm 0.11$  and  $7.9 \pm 1.4$  ( $n=4$ )  $\mu\text{mol}/\text{h}/\text{g}$  wet feces, respectively. The sulfotransferase activity was markedly higher than sulfatase activity in all feces of human, rat and mouse. These activities of rat and mouse were markedly decreased by the oral administration of an antibiotic mixture (chloramphenicol 17.5 mg, nystatin 5000 units, streptomycin 20 mg, erythromycin 10 mg and penicillin 2000 units per d per head) once a day for 3 d to 5% of the control values of conventional rat and mouse, but these activities were completely restored one month after the cease of the administration of the antibiotic mixture.

**Sulfation of Antibiotics by Purified Bacterial Sulfotransferase** In order to investigate sulfoconjugation of phenolic antibiotics in human intestine, we assayed *in vitro* acceptor specificity of various phenolic antibiotics using PNS as a donor substrate by sulfotransferase purified from *Eubacterium A-44*, an anaerobic bacterium obtained from human intestinal flora. Amoxicillin, cefadroxil and

TABLE I. Acceptor Substrate Specificity

Substrate	Activity (%)
Tyramine	100 <sup>a)</sup>
Phenol	101
<i>p</i> -Cresol	171
Amoxicillin	7.3
Cefadroxil	3.2
Cefoperazone	1.1
Tetracycline	0
Oxytetracycline	0

a) Specific activity of the enzyme used was 86.4  $\mu\text{mol}/\text{min}/\text{mg}$  protein, using PNS as a donor and tyramine as an acceptor, which was taken as 100%.

TABLE II.  $K_m$  and  $V_{\text{max}}$  Values of Sulfotransferase for Antibiotics

Antibiotics	$K_m$ (mM)	$V_{\text{max}}$ ( $\mu\text{mol}/\text{min}/\text{mg}$ )
Amoxicillin	6.9	8.1
Cefadroxil	4.3	3.3
Cefoperazone	22.2	1.6
Tyramine	3.5	99.3

cefoperazone were slowly sulfated in descending order by the enzyme, but tetracycline and oxytetracycline were not sulfated at all. Cresol and phenol, which are known to be produced in the lower parts of human intestine by bacterial enzymes, were good substrates as acceptors (Table I).

**$K_m$  and  $V_{\text{max}}$  Values of Bacterial Sulfotransferase for Antibiotics** An apparent  $K_m$  value for PNS using tyramine as an acceptor substrate and that for tyramine using PNS as a donor substrate were determined to be 0.1 and 3.5 mM, respectively.<sup>5)</sup> Lineweaver-Burk plots of sulfotransferase for amoxicillin, cefadroxil and cefoperazone were obtained (data not shown). The  $K_m$  and  $V_{\text{max}}$  values of the enzyme for these antibiotics and tyramine were summarized in Table II.

**Optimal pH** The pH activity profiles of the enzyme using PNS as a donor substrate amoxicillin, cefadroxil and cefoperazone as acceptor substrates were examined under the same conditions. The optimal pH of the enzyme was found to be 9.0 in all cases.

**Antibacterial Activity** MICs of amoxicillin for *S. aureus* and *E. coli* were 1.3 and 50  $\mu\text{g}/\text{ml}$ , respectively. Those of amoxicillin *O*-sulfate were 1.3 and 50  $\mu\text{g}/\text{ml}$ , respectively. The sulfated antibiotics were stable with the incubation at 37°C for 1 d in the control assay medium, and no sulfatase and sulfotransferase activities were de-

tected in both test bacteria. Therefore, antibacterial activity of amoxicillin was not affected by sulfation of the phenolic hydroxyl group.

## Discussion

The sulfotransferase which was produced by intestinal bacteria of human, rat and mouse was diminished by the oral administration of antibiotics. In addition, phenolic antibiotics may be sulfated and lost in antibacterial activity by bacterial sulfotransferase. The enzyme actually sulfated *in vitro* some of the antibiotics though at a slow rate of sulfation. The  $K_m$  and  $V_{\text{max}}$  values for these antibiotics are shown in Table II. The transformed antibiotics, antibiotic sulfate esters, are more hydrophilic than mother antibiotics. Absorption of the transformed antibiotics *via* intestinal epithelial membrane may be different from that of the original antibiotics. These results suggest that the pharmacological action of antibiotics may be affected by sulfotransferase of intestinal bacteria. However, antibacterial activities of amoxicillin and amoxicillin *O*-sulfate were the same *in vitro*.

The bacterial sulfotransferase differs from the known mammalian enzymes in the substrate specificity. That is, the bacterial sulfotransferase catalyzes the transfer reaction of a sulfate group from phenolic sulfate esters, but not from PAPS which is a donor substrate of tissue sulfotransferase, to other phenolic compounds. It is considered that the sulfate groups are skipingly transferred from phenolic sulfate esters to other phenols in intestine by the bacterial sulfotransferase and that intestinal flora may have important roles in metabolism and the detoxification of phenolic compounds.

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