sulfonation not only of the alicyclic amines but also dehydroepiandrosterone. However, it had little sulfonating activity toward 2-naphthol, desipramine and aniline. The apparent kinetic parameters obtained for the enzymatic sulfonation of PTHP were as follows: K_m 382 μM and V_{max} 278 nmol/mg of protein/min.

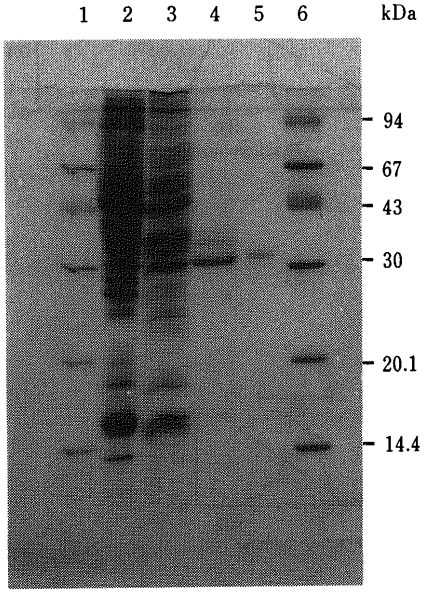

Fig. 2. SDS-PAGE of Purified Alicyclic Amine *N*-Sulfotransferase

Lanes 1 and 6 contain the molecular mass standard proteins, including phosphorylase b (94000), bovine serum albumin (67000), ovalbumin (43000), carbonic anhydrase (30000), soybean trypsin inhibitor (20100) and α-lactoalbumin (14400). Lane 2 contains the protein of female rat liver cytosol. Lanes 3 and 4 contain the active alicyclic amine *N*-sulfotransferase fraction recovered DE-52 and PAP-agarose chromatography, respectively. Lane 5 contains the purified alicyclic amine *N*-sulfotransferase. Staining was performed with Coomassie blue.

TABLE II. Substrate Specificity of Purified NST-1

Substrate	Concentration (mM)	Activity of NST-1 (nmol/mg protein/min)	
		pH 7.4	pH 10
PTHP	2.0	44	212
Tiaramide	5.0	120	165
DETR	5.0	18	77
Desipramine	0.5	N.D.	3.7
Aniline	5.0	N.D.	N.D.
2-Naphthol	0.5	5.1	4.8
Dehydroepiandrosterone	0.05	391	368

The activity was assayed with 100 mM phosphate buffer (pH 7.4) or 100 mM glycine–NaOH buffer (pH 10) containing 0.2 mM PAPS and the indicated concentration of substrates. N.D.; not detected (<0.4 nmol/mg protein/min).

NH_2-Terminal Amino Acid Sequence The results of the NH_2-terminal amino acid sequence analysis of NST-1 is shown in Fig. 3. The NH_2-terminal amino acid sequence of NST-1 exhibited a high homology with those of STa[14] and BAST I.[15] The sequence differed by two (2nd and 12th) and by four (2nd, 12th, 17th and 23rd) amino acids from those of STa and BAST I, respectively.

Sex Difference in Hepatic Level of NST-1 Immunoblot analysis of male and female rat liver cytosol was performed using rabbit polyclonal antibodies raised against purified NST-1, in order to obtain direct evidence as to whether the enzyme played an important role in the previously demonstrated sex difference (female≫male). The male and female rat liver cytosols contained a M.W. 30500 protein, corresponding to NST-1, and there was a marked difference in the cytosolic levels of NST-1 between female and male rat livers (female≫male) (Fig. 4). Other immunoreactive proteins were also observed in both cytosols.

DISCUSSION

We report here the purification of a form of female rat liver sulfotransferase conjugating PTHP. Purification of an alicyclic amine *N*-sulfotransferase was performed by a three-step procedure. NST-1 had a higher affinity towards PAP-agarose and hydroxylapatite-HPLC than the other alicyclic amine *N*-ST isozymes (Fig. 1).

NST-1 catalyzed sulfonation not only of the alicyclic amines but also the endogenous steroid dehydroepiandrosterone, which is a typical substrate for rat liver hydroxysteroid ST.[1,2] NST-1 had little sulfonating activity towards 2-naphthol, which is a typical substrate for rat liver aryl ST.[1,2] The aryl STs have been demonstrated to be unable to catalyze sulfonation of endogeneous steroids.[1,2] Therefore, NST-1 could be an isozyme of hydroxysteroid ST. Thus, this study provides the first direct evidence that the hydroxysteroid ST participates in enzymatic alicyclic amine *N*-sulfonation. The purified enzyme exhibited no activity toward an alkylamine (desipramine) and an arylamine (aniline). These amines might be catalyzed by other isozymes. Recently, an amine *N*-sulfotransferase sulfonating 2-naphthylamine was purified from guinea pig liver.[4] However, this purified enzyme catalyzed not only of arylamines but also alicyclic amines. These results suggest that this purified enzyme has different characteristics from NST-1.

Recently, Ogura *et al.* reported the N-terminal amino

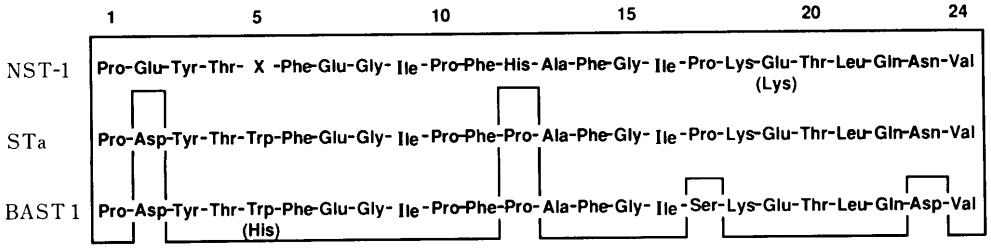

Fig. 3. Comparison of NH_2-Terminal Amino Acid Sequences of NST-1, STa and BAST I

In the sequence of NST-1, "X" indicated an unidentified amino acid. The sequences of STa and BAST I have been reported by Ogura *et al.* and Barnes *et al.*, respectively.[14,15]