Purification of a 32.5 kDa monomeric sulfotransferase from rat liver with activity for bile acids and phenolic steroids

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Both bile acid and phenolic steroid sulfotransferase activities in rat liver cytosol have previously been identified in fractions corresponding to apparent molecular masses of 60–70 and 30–35 kDa. We purified the latter activity corresponding to a monomeric protein. Activity for bile acids and phenolic steroids co-eluted on sequential chromatography on Sephadex G-75 sf, Affigel blue, chromatofocusing and hydroxyapatite. The protein was homogeneous on SDS-PAGE (32.5 kDa).

Sulfotransferase *Bile acid* *Phenolic steroid* *(Rat liver cytosol)* *Protein purification*

1. INTRODUCTION

Sulfation of bile acids has been implicated as a possible protective mechanism against hepatotoxic monohydroxy bile acids. Bile acid sulfotransferase, an enzyme catalyzing the transfer of a sulfate group from 3′-phosphoadenosine 5′-phosphosulfate (PAPS) to bile acids, was originally identified in cytosol of rat liver and kidney [1,2] and has been partially purified from guinea pig liver [3] and human liver [4]. Much confusion has existed regarding the M_r of this enzyme although it is generally agreed upon that the subunit has an M_r of 30000–35000. We have previously reported that only monomeric and dimeric forms of bile acid sulfotransferase can be identified in rat liver cytosol [5]. Similar results have been reported by Barnes and Spenny [6]. However, when the M_r separation has been carried out by others as a late step in purification, much larger M_r values have been found consistent with only a tetrameric form in rat liver [1].

We have previously identified the Y′ fraction in rat liver cytosol. Various activities have been identified in this fraction including bile acid binding proteins (33 kDa) [7] which we recently identified as 3α-hydroxysteroid dehydrogenases [8] and sulfotransferase activity for phenolic steroids and bile acids [5]. The sulfotransferases were not purified to homogeneity and it was uncertain whether activity for both bile acids and phenolic steroids resides within the same protein.

2. MATERIALS AND METHODS

2.1. *Preparation of Y′ fraction*

The livers of male Sprague-Dawley rats weighing approx. 250 g were perfused in situ with 0.01 M sodium phosphate, 0.25 M sucrose buffer, pH 7.4, and removed. Homogenates (33%, w/v) were prepared in the same buffer and the supernatant (cytosol) was harvested after centrifugation at 100000 × *g* for 60 min [7]. Gel filtration was performed on Sephadex G-75 sf with 80 ml cytosol on a 5 × 100 cm column. Fractions corresponding to M_r 30000–35000 (Y′ fraction) were pooled for further purification.

2.2. *Sulfotransferase assay*

Sulfotransferase activity with taurolithocholate,

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glycolithocholate and estradiol as substrates was measured with the butanol extraction method described by Barnes et al. [9]. The incubation mixture (100 μl) consisted of 90 μM PAPS (Sigma, St. Louis, MO) with 0.2 μCi [^{35}S]PAPS (1.6 Ci/mmol, New England Nuclear, Boston, MA), 100 μM acceptor substrates, 5 mM $MgCl_2$ and 0.1 M sodium phosphate, pH 7.0. In some cases, the concentration of unlabeled PAPS was lowered to raise its specific activity. Incubations were performed for 30 min at 37°C and terminated by boiling. After dilution with 0.4 ml of 1 M NH_4OH, products were extracted with 1 ml butanol, washed with 0.45 ml of 1 M NH_4OH containing 0.1 M sodium phosphate buffer, pH 7.0, and an aliquot of butanol layer was counted for radioactivity. Taurolithocholate was used as substrate to identify peaks of enzyme activity during purification. However, to avoid any concern about the completeness of the butanol extraction of taurolithocholate-sulfate, final enrichments and recoveries were determined on pooled aliquots of each step using the less polar glycolithocholate as substrate.

2.3. *3α-Hydroxysteroid dehydrogenase assay*

3α-Hydroxysteroid dehydrogenase activity was measured by monitoring the production of NADPH as the increase in A_{340} at 37°C [10]. The incubation mixture (2 ml) consisted of 1.47 mM acenaphthenol, 250 μM NADP in 0.05 M glycine-NaOH buffer, pH 9.0.

2.4. *Affigel blue chromatography*

Pooled Y′ fraction with added EDTA (final concentration 2 mM) was applied to an Affigel blue column (Biorad, 2.5 × 17 cm) which was equilibrated with 0.01 M sodium phosphate, 2 mM EDTA buffer, pH 7.4. After washing the column with 70 ml starting buffer followed by 200 ml starting buffer containing 0.55 M NaCl, the column was eluted with 500 ml of a linear Na gradient (0.55 to 2.5 M). A_{280}, sulfotransferase and 3α-hydroxysteroid dehydrogenase activities were measured in the fractions.

2.5. *Chromatofocusing*

The pooled fractions of enzyme activity from Affigel blue were concentrated by ultrafiltration with a PM-10 membrane and applied to a chromatofocusing column (PBE94, Pharmacia, Uppsala, 1 × 45 cm) which had been equilibrated with 0.025 M Bistris-HCl, pH 6.2. The column was eluted with 500 ml of a 1:10 dilution of polybuffer-HCl, pH 4.6. Fractions were collected into tubes containing 1 M sodium phosphate buffer, pH 8, so that the final pH values of the fractions were near 7. This neutralization was critical for stabilizing the sulfotransferase activities. A_{280}, pH and sulfotransferase and 3α-hydroxysteroid dehydrogenase activities were measured in the fractions.

2.6. *Hydroxyapatite chromatography*

The pooled fractions from chromatofocusing containing sulfotransferases were dialysed against 0.01 M potassium phosphate, pH 6.7, and applied to a hydroxyapatite column (HA-Ultrogel, LKB, 1 × 10 cm). The column was washed with 50 ml of the same buffer and then eluted with 80 ml of 0.01–0.2 M potassium phosphate, pH 6.7, and finally washed with 0.5 M buffer. A_{280} and sulfotransferase activities were measured in the fractions.

2.7. *SDS-polyacrylamide gel electrophoresis (SDS-PAGE)*

SDS-PAGE was performed according to Laemmli [11] with 12.5% gel, using M_r protein standards.

3. RESULTS

As we have previously reported [5], in gel filtration of rat liver cytosol sulfotransferase activity for taurolithocholate and estradiol was identified in fractions corresponding to molecular masses of 60–70 kDa and 30–35 kDa (not shown). The latter fractions (Y′), which also contain 3α-hydroxysteroid dehydrogenases, were pooled and further purified on Affigel blue (fig.1).

The bulk of activity of sulfotransferase for taurolithocholate and estradiol and 3α-hydroxysteroid dehydrogenase coeluted after the salt gradient. No enzyme activity was eluted after washing the column with 2.5 M NaCl. The pooled fractions were next subjected to chromatofocusing which separated the dehydrogenases and sulfotransferase (fig.2). Two peaks of 3α-hydroxysteroid dehydrogenase, eluting at pH 5.6

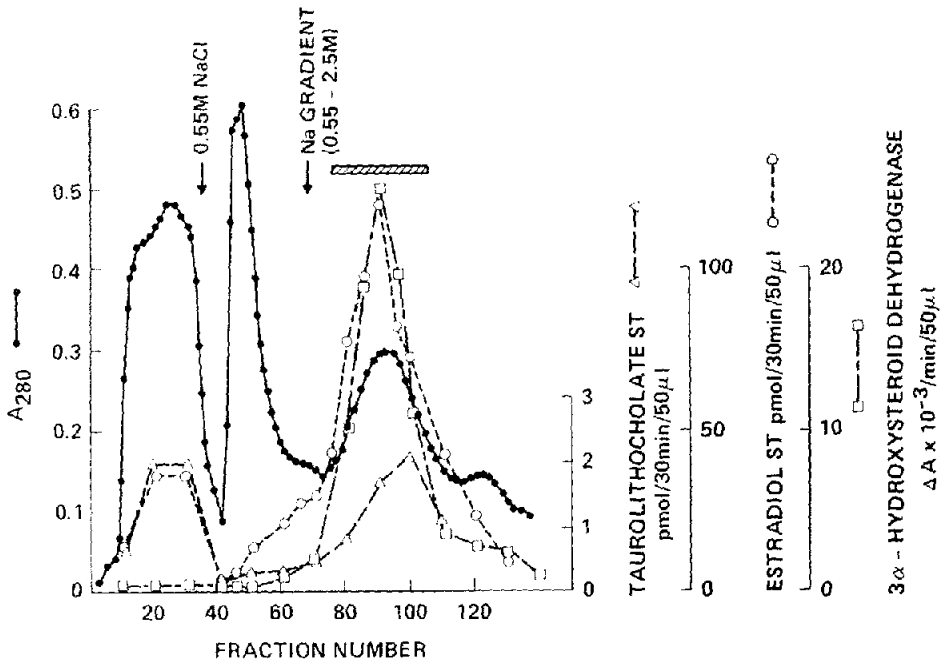

Fig.1. Affigel blue chromatography of Y′ fraction. Sulfotransferase activities were measured with 90 μM PAPS. See section 2 for details. Horizontal bar indicates fractions pooled for further purification.

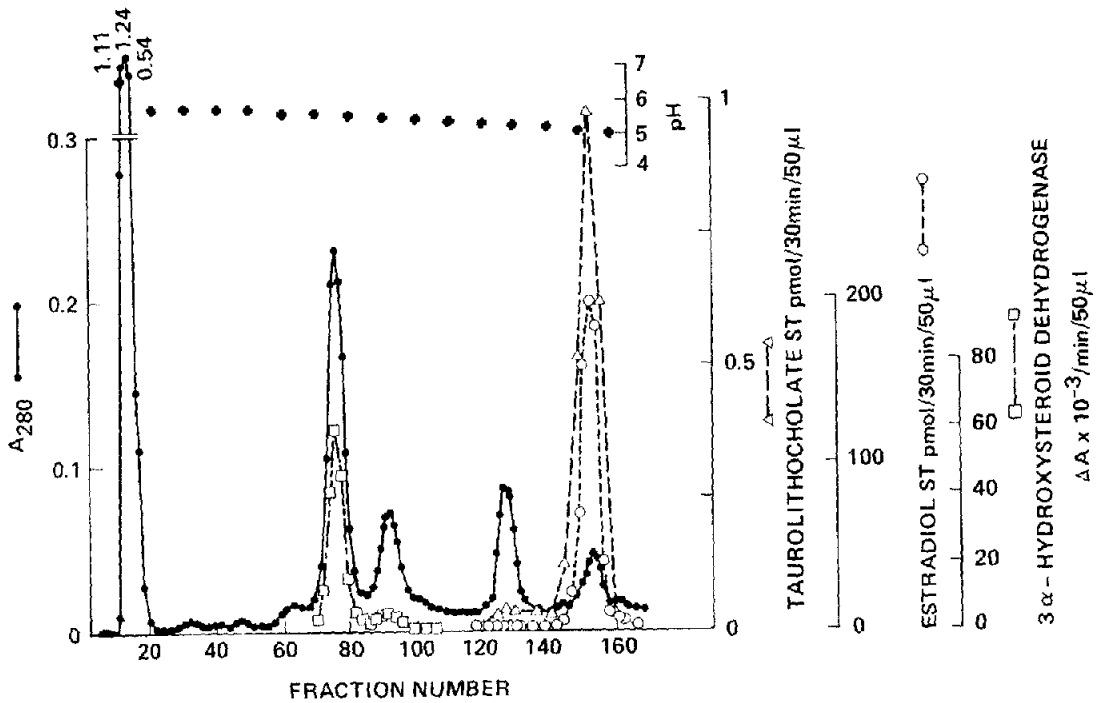

Fig.2. Chromatofocusing of pooled sulfotransferase and 3α-hydroxysteroid dehydrogenase from Affigel blue. Sulfotransferase activities were measured with 90 μM PAPS for estradiol and with 2 μM PAPS for taurolithocholate. See section 2 for details. Fractions 148–156 were pooled for further purification.

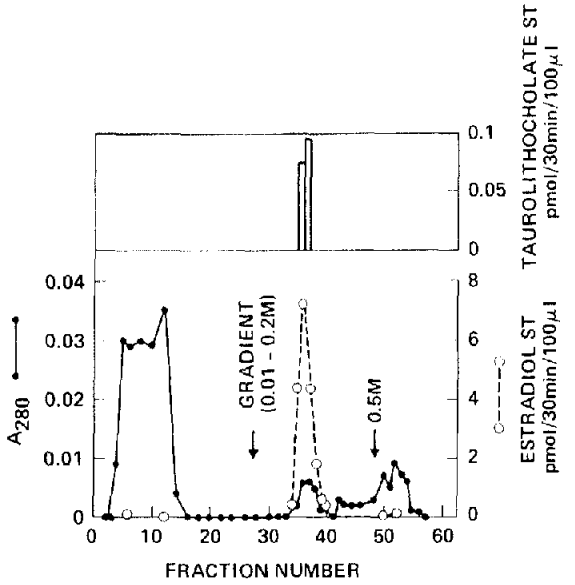

Fig.3. Hydroxyapatite chromatography of pooled sulfotransferase from chromatofocusing. Sulfotransferase activities were measured with 2 μM PAPS. Taurolithocholate sulfotransferase activity (upper panel) was only detected in the peak corresponding to estradiol sulfotransferase activity. See section 2 for details. Fractions 33–40 were pooled for SDS-PAGE.

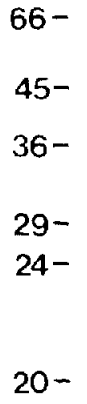

Fig.4. SDS-PAGE of sulfotransferase from hydroxyapatite. 3.5 μg protein was used. Numbers correspond to molecular mass standards (kDa).

and 5.5, correspond to the previously identified bile acid binders I and II, respectively [7]. Sulfotransferase activity for taurolithocholate and estradiol co-eluted at pH 5.1. The sulfotransferase was further purified on hydroxyapatite column (fig.3). Sulfotransferase activity for taurolithocholate and estradiol co-eluted after initiating the salt gradient. The purified protein was homogeneous (M_r 32500) on SDS-PAGE (fig.4). Table 1 summarizes the purification procedure. Poor recovery reflects the marked instability of this enzyme. Although addition of 20% glycerol in each step improved stability, the resolution was markedly worsened precluding purification to homogeneity.

Table 1

Purification of sulfotransferase

	Estradiol sulfotransferase			Glycolithocholate sulfotransferase	
	Protein (mg)	Spec. act. (pmol/min per mg protein)	Total activity (pmol/min)	Spec. act. (pmol/min per mg protein)	Total activity (pmol/min)
Sephadex G-75 sf	160	290	46400	9.3	1485
Affigel blue	28	294	8230 (17.7%)	17.3	483 (32.5%)
Chromatofocusing	1.3	523	680 (1.5%)	42.5	55.2 (3.7%)
Hydroxyapatite	0.16	831	133 (0.3%)	93.8	15.0 (1.0%)

Estradiol (100 μM) or glycolithocholate (100 μM) and [^{35}S]PAPS (90 μM) were incubated with protein for 30 min and sulfated product measured according to Barnes et al. [9]. Values in parentheses indicate recoveries

4. DISCUSSION

We previously identified bile acid and phenolic steroid sulfotransferase activity in the Y′ fraction (M_r 30000–35000) but were unable to determine if both activities reside within the same protein. We now report the first successful purification of this protein to homogeneity. Our approach beginning with molecular sieving, rather than ending the purification scheme with it, permitted the identification of a monomeric form. The enzyme exists in rat liver cytosol as a monomeric form (32.5 kDa) and exhibits activity with both bile acids and phenolic steroids.

Due to marked instability of the enzyme and poor recovery, we cannot exclude the possibility that other monomeric forms of sulfotransferase exist with distinct specificities for bile acids vs steroids. However, we found only one peak of enzyme activity with both substrates at each step in purification. Thus, although we could have lost other forms, we successfully isolated one form with activity for both bile acids and estradiol.

Since the sulfotransferase activity for both bile acids and phenolic steroids also elute in gel filtration at M_r 60000–70000, it is likely that dimeric form(s) of this enzyme also are present in rat liver cytosol. Future work will determine if these dimeric forms are homodimers of the monomeric form we have purified.

REFERENCES

[1] Chen, L.J., Bolt, R.J. and Admirand, W.H. (1977) Biochim. Biophys. Acta 480, 219–227.
[2] Chen, L.J., Imperato, T.J. and Bolt, R.J. (1978) Biochim. Biophys. Acta 522, 443–451.
[3] Chen, L.J. (1982) Biochim. Biophys. Acta 717, 316–321.
[4] Chen, L.J. and Segel, I.H. (1985) Arch. Biochem. Biophys. 241, 371–379.
[5] Sugiyama, Y., Stolz, A., Sugimoto, M., Kuhlenkamp, J., Yamada, T. and Kaplowitz, N. (1984) Biochem. J. 224, 947–953.
[6] Barnes, S. and Spenney, J.G. (1982) Biochim. Biophys. Acta 704, 353–360.
[7] Sugiyama, Y., Yamada, T. and Kaplowitz, N. (1983) J. Biol. Chem. 258, 3602–3607.
[8] Stolz, A., Takikawa, H., Kuhlenkamp, J., Sugiyama, Y. and Kaplowitz, N. (1985) Hepatology 5, 1007.
[9] Barnes, S., Waldrop, R. and Neighbors, S. (1983) Anal. Biochem. 133, 470–475.
[10] Worner, W. and Oesch, F. (1984) FEBS Lett. 170, 263–267.
[11] Laemmli, U.K. (1970) Nature 227, 680–685.