Sulphoconjugation of steroids in porcine liver

Partial purification of the cytosolic sulphotransferases for pregnenolone and 5α-androst-16-en-3β-ol

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Steroid sulphotransferase activities for 5α-androst-16-en-3β-ol and pregnenolone in porcine liver cytosol have been assayed using 3'-phosphoadenosine-5'-phospho[^{35}S]sulphate as sulphate donor. 5α-Androst-16-en-3β-ol sulphotransferase activity was obtained from porcine liver cytosol by gel filtration chromatography; activity was linear with time up to about 5 min., the optimum pH was near 8.0 and optimum temperature 37°C. Pregnenolone sulphotransferase activity was partially purified from porcine liver cytosol using DEAE-cellulose chromatography with an ionic gradient of KCl. This enzyme activity was linear with time up to 10 min and had optimum pH and temperature of 8.0 and 37°C, respectively.

Liver *Porcine* *Sulphotransferase* *5α-Androst-16-en-3β-ol* *Sulphoconjugation* *16-Androstenes* *Pregnenolone*

1. INTRODUCTION

The steroid sulphates that are present in many mammalian tissues can serve as intermediates in biosynthesis and may act as sources of biologically active steroid hormones [1]. The cytosolic sulphotransferases of rabbit liver that catalyse the sulphation of DHA and oestrone have long been known [2]. Rat liver also contains a sulphotransferase [3] that accepts as substrates some 3β-hydroxysteroids, including DHA.

The testes of rats, pigs and man contain large quantities of steroid sulphates, in particular of 3β-hydroxysteroids, such as pregnenolone and DHA, formed from the free steroids by sulphotransferase activity [1,4]. In boar testes the principal 16-androstene sulphates are an-β sulphate [5,6] and an-α sulphate [5,6]; andien-β sulphate [5,7] is present to a lesser extent.

Although the physiological significance of steroid sulphates is still not entirely understood, there is accumulating evidence that they may be involved in the regulation of steroid-transforming enzymes in the testis and in other tissues [8]. Thus, in human testes, the C-17,20 lyase [9], the 3β-hydroxysteroid oxidoreductase/ 4,5-isomerase [10] and the 17β-hydroxysteroid oxidoreductase [11] are all affected in vitro by steroid sulphates. In the rat, the testicular sulphatase is affected [12] and androstanediol sulphates are known to control lutropin secretion in the immature animals [13].

In [14], we presented evidence for both microsomal and cytoplasmic sulphoconjugation of an-β in porcine liver. In view of the importance of the 16-androstenes in the pig as pheromones [15] and of the possible role of an-β sulphate in control of steroid metabolism [4], we have attempted the partial purification from porcine liver cytosol of the sulphotransferases for pregnenolone and an-β.

Abbreviations: DHA, 3β-hydroxy-5-androsten-17-one; pregnenolone, 3β-hydroxy-5-pregnen-20-one; an-α, 5α-androst-16-en-3α-ol; an-β, 5α-androst-16-en-3β-ol; andien-β, 5,16-androstadien-3β-ol

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2. MATERIALS AND METHODS

3′-Phosphoadenosine-5′-phospho[^{35}S]sulphate ([^{35}S]PAPS) (2.09 Ci/mmol) was purchased from New England Nuclear (Dreieich). To minimize decomposition, this was stored at −20°C in ethanol–water (1:1, v/v). [4–^{14}C]Pregnenolone (56.5 mCi/mmol) was from Amersham International (Buckinghamshire), and [5α,6α-^{3}H]an-β was synthesized and purified as in [14]. Unlabelled and labelled pregnenolone and an-β sulphates were synthesized as in [14].

Precoated silica gel plates for thin-layer chromatography (TLC) were purchased from Merck (supplied by Anderman and Co, East Molesey, Surrey). Sephacryl was obtained from Pharmacia Fine Chemicals AB (PO Box 175, S-1045, Uppsala) and DEAE-cellulose from Whatman Laboratory Sales (Maidstone, Kent).

Scintillation-counting was performed using a Packard Tri-Carb scintillation spectrometer, model 3255. An aqueous scintillant was used which consisted of 2,5-diphenyloxazole (5 g) and 1,4-bis 2-(5-phenyloxazolyl)benzene (0.5 g), both dissolved in a mixture (2:1, v/v) of toluene:Triton X-100 (1 l). Counting efficiencies for ^{35}S, ^{3}H and ^{14}C were 98%, 55% and 88%, respectively.

2.1. *Preparation of tissues and assay of sulphotransferase activity*

Porcine liver was obtained from the abbatoir of Farmeat (Ashford, Kent), transported in ice to the laboratory and then homogenised using a Waring blender in Tris, 10 mmol/l; EDTA, 1 mmol/l; 2-mercaptoethanol, 3 mmol/l (pH 8.0). The homogenate (50%, w/v) was subjected to differential centrifugation at 4°C [16] using a Beckman L8 ultracentrifuge. The cytosolic fraction was re-spun at 176 000 × g_{max} for a further 40 min at 4°C. From this fraction, sulphotransferase activities for an-β and pregnenolone were obtained using gel filtration and ion-exchange chromatography (see sections 3 and 4).

Liver sulphotransferase activity was assayed as follows: the appropriate column fractions were incubated in Tris–HCl buffer (pH 8.0) in 50 μl final vol. with unlabelled steroid substrate (final conc. 0.1 μmol/l) and [^{35}S]PAPS (final conc. 3.5 μmol/l). Reactions were terminated after suitable intervals (see sections 3 and 4) by the addition of methanol (2 ml). A known quantity (1×10^4 dpm) of ^{3}H-labelled steroid sulphate was added at this time to enable recoveries to be assessed at the end of the analysis. The methanol was clarified by centrifugation at 2000 × g_{max} (Gallenkamp Junior centrifuge) for 2 min, transferred to clean tubes and the solvent removed under reduced pressure using a Büchi Rotovapor (Orme Scientific, Manchester). The residues were subjected to TLC in benzene–ethanol–butanone–water (3:3:3:1, by vol.) with authentic steroid sulphates run alongside on the same plate. The steroid sulphate region (R_F ~0.75, cf [14]) was removed, eluted with methanol (3 × 2 ml), and the solvent evaporated to dryness. The residues were redissolved in methanol (200 μl) and a portion (50 μl) taken for scintillation-counting of ^{35}S and ^{3}H. The former was then corrected for radioactive assay. All assays were performed in duplicate and control incubations, containing no steroid substrate, were used to correct for non-specific activity. Protein estimations were determined as in [17].

3. RESULTS

3.1. *An-β sulphotransferase activity*

Porcine liver cytosol, prepared as above, was subjected to gel filtration chromatography using a column (75 cm × 5 cm i.d.) containing Sephacryl

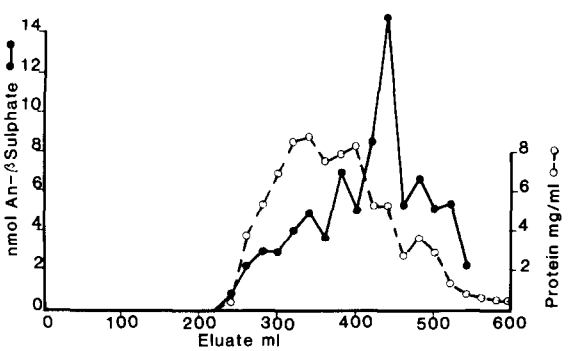

Fig. 1. Partial purification of porcine liver 5α-androst-16-en-3β-ol sulphotransferase activity. Porcine liver cytosol was applied to a column (75 cm × 5 cm i.d.) containing Sephacryl 200 and eluted with Tris–HCl buffer (pH 8.0). Fractions were collected and assayed for protein (○) and sulphotransferase activity (●), using 5α-[^{3}H]androst-16-en-3β-ol as substrate (0.1 μmol/l) in the presence of [^{35}S]PAPS (3.5 μmol/l) in a final volume of 50 μl. [^{3}H]steroid sulphate was isolated and purified as in section 2.

200. The column was eluted with Tris–HCl buffer and fractions taken for sulphotransferase activity using [^{3}H]an-β as substrate (see section 2). Fig. 1 shows that peak activity was eluted in the 420–440 ml fractions. These were pooled, then divided into 1 ml portions and used as sources of an-β sulphotransferase for further studies. Storage at −20°C for up to two months resulted in no decrease in activity.

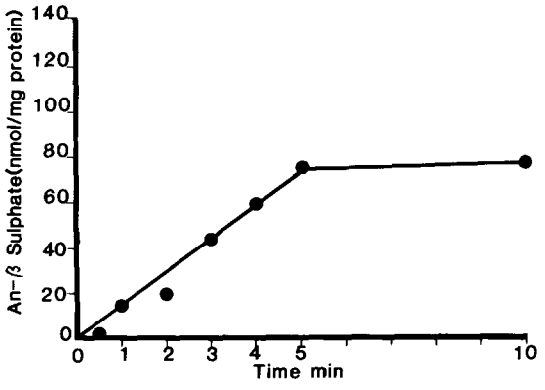

Fig. 2. Time course of porcine liver 5α-androst-16-en-3β-ol sulphotransferase activity. Portions of the fractions (420–440 ml) obtained from Sephacryl 200 chromatography (fig. 1) were incubated at 37°C for different periods of time in the presence of 5α-[^{3}H]androst-16-en-3β-ol and [^{35}S]PAPS, as described in fig. 1 and section 2.

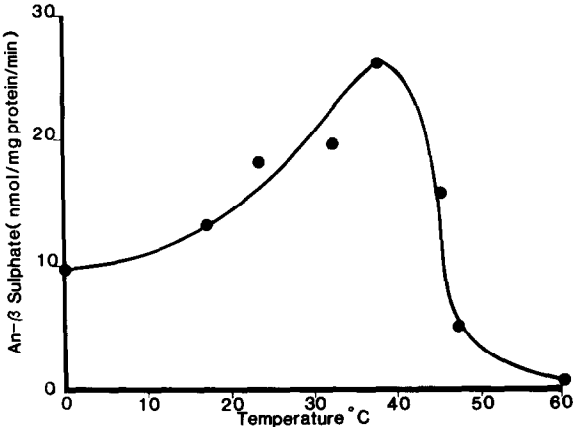

Fig. 3. Temperature dependence of porcine liver 5α-androst-16-en-3β-ol sulphotransferase activity. Sulphotransferase activity of fractions (420–440 ml) (fig. 1), with 5α-[^{3}H]androst-16-en-3β-ol as substrate, was measured by incubation with [^{35}S]PAPS at pH 8.0 in Tris–HCl buffer for 4 min at different temperatures.

The time course for this partially purified enzyme was studied by measuring the amount of [^{35}S]an-β sulphate formed with time. The activity was found to be linear up to ~5 min, after which a plateau was formed (fig. 2). Thus, for further studies, an incubation time of 4 min was used in standardized conditions. The optimum temperature was found to be close to 37°C (fig. 3) and the optimum pH around 8 (fig. 4).

3.2. *Pregnenolone sulphotransferase activity*

Porcine liver cytosol, prepared as above (see section 2), was applied to a DEAE-cellulose column (20 cm × 5 cm i.d.) and eluted with Tris–HCl buffer (pH 8.0) (1000 ml). Eluted fractions were assayed for activity using [^{14}C]pregnenolone as substrate in the presence of [^{35}S]PAPS (see section 2) but none was found. The ionic gradient of KCl was then commenced (fig. 5) and fractions (20 ml) were collected and assayed for protein and pregnenolone sulphotransferase activity. Peak activity emerged at 10 nmol KCl/l, with a smaller peak at 12.5 mmol/l. The material eluted as the former peak was divided into 1 ml fractions which were stored at −20°C without loss of activity for 2 months and were used as a source of pregnenolone sulphotransferase. The time course for this activity was shown to be linear up to at least 10 min (not shown), so that an incubation time of 10 min was used for estimation of optimum temperature (37°C) and pH (8.0) (not shown).

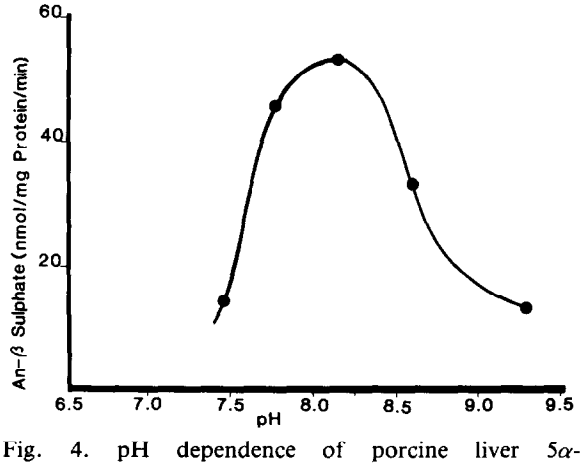

Fig. 4. pH dependence of porcine liver 5α-androst-16-en-3β-ol sulphotransferase activity. Sulphotransferase activity was assayed at 37°C at different pH values as described in section 2 and fig. 3.

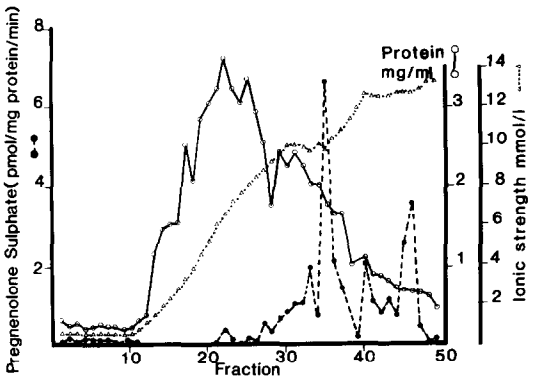

Fig. 5. Partial purification of porcine liver pregnenolone sulpotransferase activity. Porcine liver cytosol (prepared as in section 2) was applied to a DEAE-cellulose column (20 cm × 5 cm i.d.) and eluted with Tris–HCl buffer (pH 8.0) (1000 ml), after which the KCl gradient (Δ) was commenced. Fractions (20 ml) were collected and assayed for protein (○) and sulphotransferase activity (●), at pH 8.0 and 37°C as described in fig. 3, except that [4-^{14}C]pregnenolone was used as substrate.

4. DISCUSSION

In [14] we presented evidence for the enzymic formation of an-β sulphate in porcine liver cytosol and microsomal fractions. In those experiments ATP was incubated with the free steroid and the appropriate tissue fraction so that we had to assume the presence of the enzymes involved in the formation of PAPS [18] before this could be utilised in steroid sulphate formation. Here, [^{35}S]PAPS has been used as a sulphate donor so that we have been able to study directly the sulphation of two steroids, pregnenolone and an-β. Further, the fact that ^{35}S has been incorporated into the product provides evidence that we have studied the formation of steroid sulphoconjugates rather than any other conjugate.

It is becoming clear that porcine liver contains a number of sulphotransferases that catalyse the sulphoconjugation of pregnenolone and DHA [4], as well as microsomal and cytoplasmic counterparts that utilise the 16-androstene, an-β as substrate. The temperature and pH optima are all close to 37°C and pH 8.0, although the microsomal an-β sulphotransferase has an optimum pH of 7.4 [14].

A number of different studies using different tissues such as the testis [9–12], liver [4] and placenta [19] have indicated that steroid sulphotransferases are subject to control by endogenous steroids, or that steroid sulphates may control sulphatase activity [1,8]. It is here that it may be possible to study the sulphotransferases of porcine liver in more detail.

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