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Purification and some characteristics of an oestrogen sulphotransferase from guinea pig adrenal gland and its non-identity with adrenal pregnenolone sulphotransferase

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An oestrogen sulphotransferase, active towards both oestrone and oestradiol, and of high specific activity, is present in cytosol prepared from adrenal glands of both sexes of English Shorthair and Hartley guinea pigs. The ovarian and testicular cytosolic activities of this enzyme are markedly low in comparison with the adrenal activity. The adrenal enzyme is distinct from an accompanying pregnenolone sulphotransferase as judged by f.p.l.c. gel filtration, chromatofocusing, and differences in activation brought about by the addition of thiol groups. The oestrogen sulphotransferase behaved as a 67 kDa protein on a Sephadex G100 column and as a 48 kDa protein on f.p.l.c. gel-filtration columns. Two forms of the enzyme with apparent pI values of 6.1 and 5.5 were eluted during f.p.l.c. chromatofocusing. Sequential salt fractionation, f.p.l.c. gel filtration and elution from an agarose–hexane–adenosine-3′,5′-diphosphate affinity gel has resulted in a preparation which, when resubmitted to f.p.l.c. gel filtration, yields a considerably purified oestrogen sulphotransferase. When submitted to SDS/polyacrylamide-gel electrophoresis under reducing conditions, a main protein band of 34–36 kDa is observed. It is suggested that the enzyme may exist as a dimer in the cytosol.

INTRODUCTION

During the past decade, evidence has accumulated to support the existence of a number of sulphotransferase enzymes, of varying affinities and specificities, whose substrates are steroids [1]. Certain of these enzymes, primarily oestrogen sulphotransferases, may act in tissues to limit the availability of free, biologically-active steroids, due to the production of sulphated forms, which do not by themselves bind to intracellular receptors [2]. The importance of these enzymes in this latter respect has been particularly noted in the mammalian intrauterine compartment [3–6]. Severe difficulties have been encountered in purifying and characterizing the poorly abundant oestrogen sulphotransferase(s) of intrauterine origin in small rodents including mouse [7,8] and guinea pig [9,10] during gestation. The reported presence of a hydroxysteroid sulphotransferase, which is particularly active toward pregnenolone [11], in the male Hartley guinea pig adrenal cortex, led us to enquire into the possible occurrence of an oestrogen sulphotransferase in that tissue, especially since the bovine adrenal gland contains an abundance of a stable enzyme of this type [12]. We present evidence for the existence of a highly active oestrogen sulphotransferase in the guinea pig adrenal cortex, and for its non-identity with the pregnenolone sulphotransferase mentioned above. The activity of similar enzymes in guinea pig gonads is very low when compared with that in the adrenal gland. Considerable purification and some characterization of the adrenal oestrogen sulphotransferase has been achieved.

EXPERIMENTAL

Materials

[6,7-^{3}H]Oestrone and [6,7-^{3}H]oestradiol (sp. radioactivity 46–48.3 Ci/mmol), as well as [7-^{3}H(n)]pregnenolone (sp. radioactivity 22.6 Ci/mmol), were purchased from New England Nuclear, Dorval, Quebec, Canada. They were repurified where necessary and stored for later use under established conditions [13,14]. Unlabelled steroids were purchased from Steraloids, Wilton, NH, U.S.A., or Sigma Chemical Co., St. Louis, MO, U.S.A. 3′-Phosphoadenosine 5′-phosphosulphate, synthesized by the method of Singer [15], was purchased from the Contracts Office, University of Dayton Research Institute, Dayton, OH, U.S.A. Sephadex G100 (40–120 μm) and agarose–hexane–adenosine-3′,5′-diphosphate were obtained from Pharmacia, Dorval, Quebec, Canada. H.p.l.c.-grade water (Caledon Laboratories, Georgetown, Ontario, Canada) was utilized for all f.p.l.c. procedures, and double-distilled water was used for all enzyme incubations. Scintillation fluid for isotope counting was xylene-based Formula 963 (New England Nuclear). All buffers were prepared at 21 °C, and those for use with column systems were filtered through 0.2 μm filters (Supelco Canada Ltd., Oakville, Ontario, Canada) and degassed. All other chemical reagents were of appropriate analytical grade.

English Shorthair guinea pigs were bred and housed in our animal facility as outlined elsewhere [16]. Guinea pigs of the Hartley strain were purchased from Charles River, St. Constant, Quebec, Canada, housed as above, and allowed to become acclimatized in our animal quarters for at least 2 weeks before being used.

Preparation of tissue cytosol

Animals were killed by a single blow to the neck region as approved at that time by the Canadian Council for Animal Care. Tissues were rapidly removed, cleaned and homogenized by hand in a glass homogenizer on ice (1–2 g of tissue/10 ml of buffer) in 0.25 M-sucrose containing 10 mM-Tris/HCl, pH 7.6, 1 mM-EDTA, 10 mM-monothioglycerol and 0.01 % (w/v) NaN_3. Adrenal homogenates were prepared from each sex, utilizing 1–2 adult animals, and up to 18 animals of 3–10 days of age, per homogenate. When salt fractionation was performed, several of these homogenates, primarily from adults, which had been stored at −20 °C, were thawed and combined without regard for the sex of the animals. Ovaries from 2–3 animals and testes from 1–2 animals were each combined to prepare individual homogenates.

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Cytosols were prepared [10] by centrifuging at 105000 *g* for 60 min at 4 °C. Assay of enzyme activities and various column separation procedures were carried out on these cytosols, either in the fresh state (day of preparation) or following various periods of storage at −20 °C or at 0 °C. Protein was measured throughout the study by the method of Bradford [17], with bovine serum albumin (Bio-Rad) as standard.

Characterization of adrenal cytosolic sulphotransferases

When salt fractionation was employed, 12–34 ml portions of cytosol containing 86–189 mg of protein were maintained on ice with gentle stirring while slowly adding crystalline $(NH_4)_2SO_4$. The precipitate forming at 35% saturation, after 2 h of flocculation, was centrifuged at 12000 *g* for 20 min at 4 °C and rejected. Protein precipitating between 35% and 70% saturation, after 15 h of flocculation, was recovered by centrifugation as above and redissolved in 2 ml of 25 mM-Tris/HCl, pH 8, containing 0.25 M-sucrose, 1 mM-EDTA, 10 mM-monothioglycerol and 0.01% (w/v) NaN_3 (buffer A). Solutions prepared in this way, as well as samples of whole cytosol, were passed through 0.45 μm nylon filters (Micron Separations Inc., Westborough, MA, U.S.A.) before application to columns. Portions (0.5 ml) of whole cytosol, each containing approx. 1–2 mg of protein, were applied to a precalibrated 93.5 cm × 0.9 cm column of Sephadex G100 equilibrated with 0.15 M-NaCl containing 50 mM-Na_2HPO_4, 1 mM-EDTA, 10 mM-monothioglycerol, and 0.01% NaN_3 adjusted to pH 8 with HCl (buffer B) at 21 °C. The column was eluted with buffer B at 21 °C at a rate of 14 ml/h. Fractions (1 ml) were collected in tubes maintained at 0 °C. Samples (0.5 ml) of whole cytosol or of salt-fractionated material, containing 1–15 mg of protein, were subjected to gel filtration on Superose 12 f.p.l.c. columns attached to a Pharmacia f.p.l.c. system [9]. Selected fractions containing oestrogen sulphotransferase activity were combined, desalted using Pharmacia PD-10 columns [9], and applied to an affinity column containing 1–5 ml of agarose–hexane–adenosine-3′,5′-diphosphate gel equilibrated with buffer A. Elution was carried out with 2 mM-ADP [9]. Selected eluates from the latter step were concentrated to one-tenth of the original volume by centrifuging at 12000 *g* and 4 °C in Centricon 10 microconcentrators, cut-off limit 10 kDa (Amicon Canada Ltd., Oakville, Ontario, Canada), diluted with buffer A to decrease the ADP concentration at least 100-fold, and applied to a similar affinity column which was eluted with a linear gradient of ADP (50 ml each of buffer A and 7.5 mM-ADP in the same buffer). Chromatofocusing was performed as described in earlier publications [7,10].

Enzyme assay

The standard assay for oestrogen sulphotransferase involved incubation of 0.1 μM-[^{3}H]oestrone in the presence of a non-limiting concentration of 3′-phosphoadenosine 5′-phosphosulphate under the conditions described elsewhere [5,10,18]. The steroid concentration was varied where necessary. Pregnenolone sulphotransferase activity was assayed as decribed in an earlier publication [18]. The effect of pH on enzyme activity was studied by employing buffer systems described elsewhere [10].

Polyacrylamide-gel electrophoresis

SDS/polyacrylamide slab gels, 0.75 mm or 1 mm in thickness, were run at 200 V under reducing conditions at 21 °C by the general procedure of Laemmli [19], utilizing the Bio-Rad Mini Protean II electrophoresis cell. Stacking and separating gels were respectively composed of 4% (w/v) and 12% (w/v) polyacrylamide. The respective buffers had pH values of 6.8 and 8.9. Low-molecular-mass protein kits from Bio-Rad or Pharmacia, composed of phosphorylase *b*, bovine serum albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor and lysozyme, were used as standards. Gels were stained with Coomassie Brilliant Blue R-250 and then with silver by the Bio-Rad silver stain kit.

RESULTS

The pH optimum of the oestrogen sulphotransferase under our experimental conditions was 8. The enzyme catalysing sulphation of pregnenolone exhibited a wide pH optimum, between 7 and 9. The rate of sulphation for both enzymes was linear with time of incubation for at least 20 min. In order to obtain a similar percentage sulphation from cytosols and gel-filtration fractions, 10–15 times more protein was necessary for pregnenolone than for oestrone at 0.1 μM substrate concentration. Oestrogen sulphotransferase activity in preparations without thiol groups in the homogenizing buffer was increased by about 40-fold by preincubating with 10 mM-monothioglycerol [5]. Under the same conditions the sulphation rate of pregnenolone was increased by less than 2-fold.

Table 1. Activities of oestrogen sulphotransferase in cytosols of guinea pig adrenal glands

Enzyme assays were performed as described in the Experimental section, using a steroid concentration of 0.1 μM. Oestrogen sulphotransferase was assayed using 1–2.5 μg of protein/incubation tube. This yielded about 30% sulphation/15 min. The number of individual adult animals employed is indicated (*n*). 'Adult' refers to animals of at least 5 months of age, or at least 500 g in weight. A single cytosol was prepared from the adrenal glands of 6–18 animals in each of the groups aged 3–10 and 14–19 days.

Animal strain	Sex	Age	*n*	Oestrogen sulphotransferase (nmol/min per mg of protein)	Oestrogen sulphotransferase (nmol/min per adrenal gland)
English Shorthair	Female	Adult	5	0.45–1.16	5.4–8.2
	Female	3–10 days	1	0.22	0.17
	Female	14–19 days	1	0.39	0.53
	Male	Adult	3	0.57–1.78	5.2–37.0
	Male	3–10 days	1	0.21	0.14
	Male	14–19 days	1	0.36	0.38
Hartley	Female	Adult	2	0.90, 0.93	4.9, 5.3
	Male	Adult	2	1.4, 1.6	7.6, 10.2

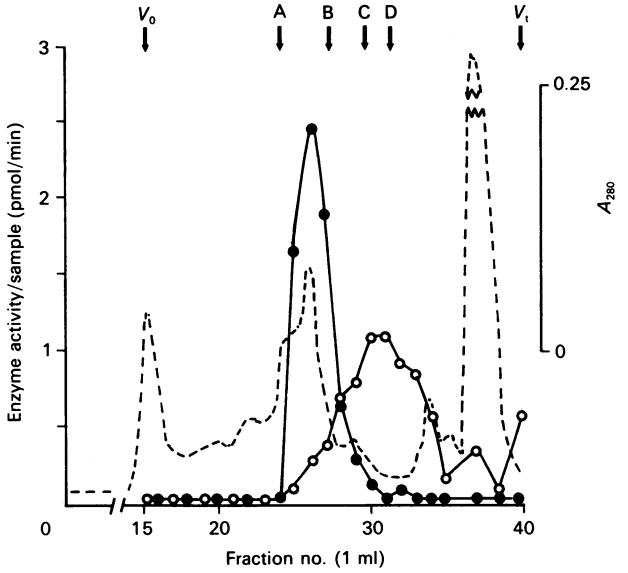

Fig. 1. Elution profile of adrenal oestrogen sulphotransferase and pregnenolone sulphotransferase obtained by f.p.l.c. gel filtration

A cytosol sample (0.5 ml; 0.78 mg of protein), prepared from the adrenal of an adult English Shorthair male guinea pig, was injected on to two Pharmacia Superose 12 HR 10/30 gel filtration columns (each 30 cm × 1 cm) in series, which had been equilibrated with buffer B at 0–4 °C, and pre-calibrated with Dextran Blue (V_0), BSA (67 kDa, A), ovalbumin (43 kDa, B), chymotrypsinogen A (25 kDa, C) and ribonuclease (13.7 kDa, D). V_t is the total column volume determined for the elution of acetone. Elution was performed utilizing buffer B at a flow rate of 30 ml/h at 0–4 °C, and 1.0 ml fractions were collected. Oestrogen sulphotransferase (●) and pregnenolone sulphotransferase (○) activities were assayed respectively in 25 μl and 300 μl samples of each fraction. Steroid substrate concn. was 0.1 μM. The protein elution profile (----) was obtained by scanning at 280 nm. Enzyme units refer to the activity/sample assayed, so that the pattern for pregnenolone sulphotransferase is clearly defined.

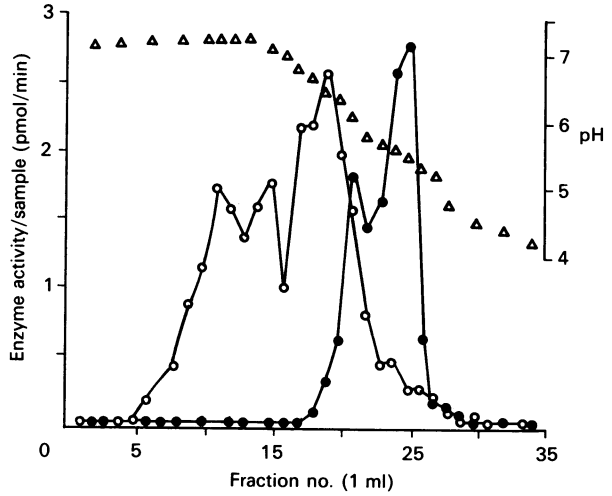

Fig. 2. Elution profile of adrenal oestrogen sulphotransferase and pregnenolone sulphotransferase obtained by f.p.l.c. chromatofocusing

A 1.4 ml volume of cytosol containing 3.36 mg of protein, prepared from adult English Shorthair female guinea pig adrenals, was mixed with 8.6 ml of start buffer (25 mM-Bistris containing 1 mM-EDTA/10 mM-monothioglycerol, adjusted to pH 7.1 with iminodiacetic acid at 21 °C). This was injected on to a Pharmacia Mono P HR 5/20 ion-exchange f.p.l.c. column (20 cm × 0.5 cm), pre-equilibrated with start buffer at 0–4 °C, at 30 ml/h. The column was eluted, at the same rate and temperature, with running buffer prepared by diluting 10 ml of Pharmacia polybuffer PB 74 to 100 ml with water and adjusting to pH 4.0 with iminodiacetic acid at 21 °C. The diluted buffer contained 1 mM-EDTA and 10 mM-monothioglycerol. Fractions of 1 ml were collected and their pH values (△) were measured at 4 °C, and each was then rapidly adjusted to pH 8 by the addition of 0.5 ml of 1 M-Tris/HCl, pH 8. The pH values shown for the gradient have been corrected to 21 °C. Oestrogen sulphotransferase (●) and pregnenolone sulphotransferase (○) activities were assayed respectively in 15 μl and 300 μl samples of each fraction. Steroid concn. was 0.1 μM. Enzyme units refer to the activity/sample assayed, so that the pattern for pregnenolone sulphotransferase is clearly defined.

The oestrogen sulphotransferase activities in adrenal cytosols are shown in Table 1. Adrenal activity was already present in both sexes at 3–10 days of age, and it increased with animal age and adrenal gland size. The ratio of oestrogen sulphotransferase to pregnenolone sulphotransferase activity (results not shown) ranged between 10 and 19 for adults, regardless of sex, when assayed with 0.1 μM-steroid substrate. This ratio appeared to be higher in immature animals of both sexes than in adults. Both enzyme activities were low, or possibly absent, in gonads as compared with the adrenal gland. Freshly prepared adrenal cytosols lost no oestrogen sulphotransferase activity during storage at −20 °C for periods of up to 5 months. Fresh cytosols stored at 0 °C retained 85% of their activity after 20 days and 50% after 50 days. When adrenal cytosol was submitted to gel filtration on Sephadex G100, oestrogen sulphotransferase and pregnenolone sulphotransferase were eluted together in the approximate position of BSA. F.p.l.c. gel filtration of salt-fractionated or whole cytosol yielded elution patterns of the type presented in Fig. 1. Oestrogen sulphotransferase appeared as a single peak corresponding to about 46–48 kDa, regardless of animal strain or sex. Pregnenolone sulphotransferase was primarily eluted in later fractions (Fig. 1). The behaviour of both enzymes during f.p.l.c. gel filtration suggested interaction with the gel when compared with the results of Sephadex gel filtration. This was particularly evident for the pregnenolone sulphotransferase, where activity was even detectable in the fraction corresponding to total column volume. The pattern of elution was independent of whether salt-fractionated protein or whole cytosol was run on the columns.

Chromatofocusing of adrenal cytosol in a pH gradient of 7.1–4.0 resulted in the elution pattern seen in Fig. 2. Considerable pregnenolone sulphotransferase activity was eluted in the start buffer, indicating a pI of greater than 7.1. Another peak was eluted at about pH 6.5. Oestrogen sulphotransferase separated partially into two forms, corresponding to apparent pI values of 6.1 and 5.5.

Fractions collected during f.p.l.c. gel filtration (as shown in Fig. 1) which were enriched in oestrogen sulphotransferase exhibited increased specific activities after elution with 2 mM-ADP from the affinity column. Preparations obtained in this way, when transferred to a second affinity column, were eluted by a concentration gradient of ADP as shown in Fig. 3. A clear separation of the two sulphotransferases was not achieved, but the later fractions possessed a higher ratio of oestrogen sulphotransferase to pregnenolone sulphotransferase, suggesting contamination by the latter. This second affinity column yielded no further increase in specific activity of the oestrogen sulphotransferase. Fractions from the gradient column were stored at −20 °C for 2–3 weeks without loss of activity. A typical example of the stepwise purification achieved for oestrogen sulphotransferase is shown in Table 2. F.p.l.c. gel filtration of enzyme recovered from the affinity column stage, either gradient or stepwise, resulted in a well-defined A_{280} peak which co-eluted

with oestrogen sulphotransferase activity. In addition, two further peaks of apparently small molecular size, each devoid of enzyme activity, were eluted (results not shown in diagrammatic form). Collection and repeated f.p.l.c. gel filtration of the A_{280} peak associated with oestrogen sulphotransferase activity resulted in the elution pattern shown in Fig. 4. This step was not accompanied by a further increase in enzyme specific activity, but partial structural alteration was evident. A product with absorbance at 280 nm and containing protein measurable by the Bradford method, but possessing no enzyme activity, was eluted slightly beyond the total column volume fraction (Fig. 4).

When various preparations obtained during enzyme purification were submitted to SDS/PAGE, the electrophoretic patterns shown in Fig. 5 were obtained. Proteins eluted by ADP from the first affinity column contained strongly staining bands in the region of 35 kDa. Fractions eluted from the gradient affinity column step also contained protein of this size. The preparation with most pregnenolone sulphotransferase contamination (see Fig. 3) exhibited a weak band of slightly lower molecular mass than the main band. Each fraction eluted from the affinity column steps also contained 16–18 kDa proteins. The oestrogen sulphotransferase preparation which was recovered from the final f.p.l.c. gel filtration step (see Fig. 4) consisted of a protein estimated to be in the range 34–36 kDa. Although not shown here, electrophoresis of individual fractions obtained by

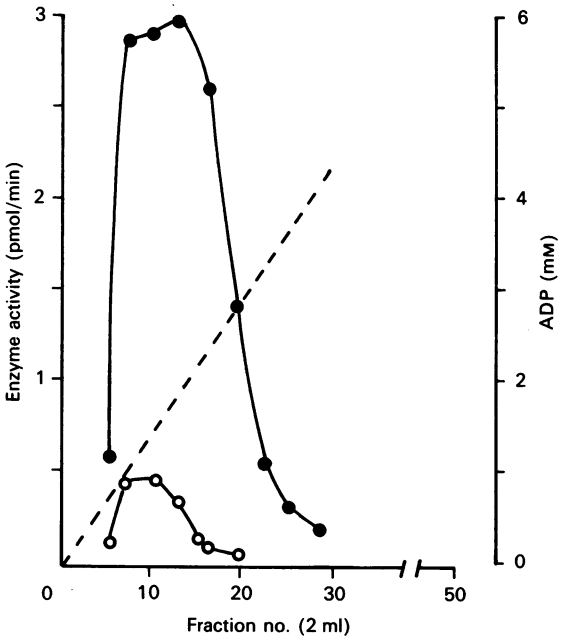

Fig. 3. Affinity chromatography of partially purified adrenal oestrogen sulphotransferase and residual pregnenolone sulphotransferase on a column of agarose–hexane–adenosine-3′,5′-diphosphate gel

Cytosol prepared from the combined adrenals of six animals, containing 189 mg of protein in 34 ml, was submitted to $(NH_4)_2SO_4$ fractionation, f.p.l.c. gel filtration, desalting and stepwise elution with 2 mM-ADP in buffer A as described in the Experimental section. The enzyme recovered was successively concentrated and diluted with buffer A twice, applied to a 4 ml agarose–hexane–adenosine-3′,5′-diphosphate gel in a 9 cm × 1 cm column, washed with 50 ml of this buffer and eluted by a gradient (----) consisting of 50 ml each of buffer A and 7.5 mM-ADP in the same buffer. Fractions of 2 ml were collected at 4 °C. Oestrogen sulphotransferase (●) and pregnenolone sulphotransferase (○) activities were respectively assayed in 25 μl and 100 μl samples of each fraction, at a steroid concn. of 0.1 μM. Enzyme units refer to the activity corrected for the difference in the sample volumes for each enzyme. Fractions 6–13 and 14–19 were separately combined and shown to possess oestrogen/pregnenolone sulphotransferase activity ratios of 23:1 and 49:1 respectively.

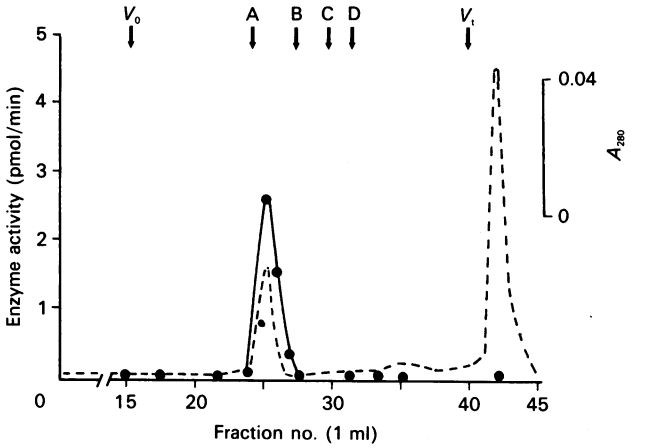

Fig. 4. F.p.l.c. gel filtration of pre-purified adrenal oestrogen sulphotransferase

Combined fractions, containing 96 μg of protein, from the affinity column stage described in Fig. 3, were concentrated and resubmitted to f.p.l.c. gel filtration as described in the Experimental section. The peak containing oestrogen sulphotransferase activity was collected, concentrated and rerun in the same gel filtration system to yield the profile shown here. Oestrogen sulphotransferase (●) was assayed in 20 μl samples as described in the Experimental section, with 0.1 μM-oestrone as substrate. Scanning of the eluate was performed at 280 nm (----).

Table 2. Stepwise purification of guinea pig adrenal oestrogen sulphotransferase

A cytosol prepared from the adrenals of six adult animals was submitted to $(NH_4)_2SO_4$ fractionation, f.p.l.c. gel filtration and stepwise elution from the affinity column as explained in the Experimental section. Enzyme activity was measured in cytosol, gel filtration fractions and affinity column fractions respectively by incubating 1.5 μg, 0.5 μg and 40 ng of protein. Enzyme activity is not shown for the salt-fractionated pellet because of interference due to $(NH_4)_2SO_4$.

Purification step	Total protein (mg)	Total units (nmol/min)	Specific activity (nmol/min per mg)	Fold increase in sp. activity
Cytosol	189	221	1.17	–
Salt fractionation	71	–	–	–
F.p.l.c. gel filtration	31	96	3.1	2.6
Affinity column	1.36	37.3	27.4	23.4

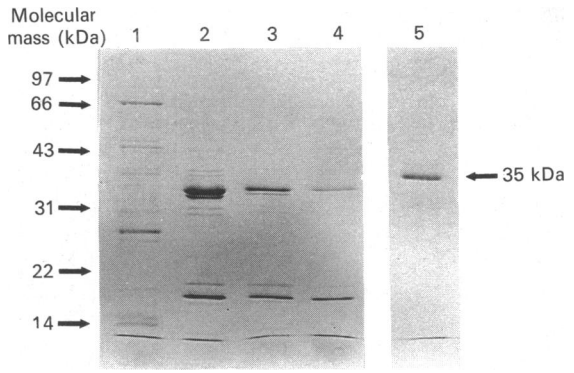

Fig. 5. SDS/PAGE of oestrogen sulphotransferase preparations

Gels, 1 mm in thickness, were run under reducing conditions as described in the Experimental section. Lane 1 contains protein which did not bind to the affinity column. Lane 2 contains protein eluted stepwise by 2 mM-ADP from the affinity column. Lane 3 contains protein in combined fractions 6–13 from the gradient-eluted affinity column (see Fig. 3). Lane 4 contains protein in combined fractions 14–19 from the gradient column. Lane 5 contains protein in the oestrogen sulphotransferase peak obtained by final f.p.l.c. gel filtration (see Fig. 4). Amounts of protein applied were as follows: lane 1, 2 μg, lanes 2–5, 0.5–1 μg. Note that lane 5 is from a gel run photographed separately from those of lanes 1–4. It was calibrated with standard proteins run concurrently.

chromatofocusing indicated enrichment of a protein of this same size wherever active oestrogen sulphotransferase was detectable.

Our most highly purified oestrogen sulphotransferase preparations catalysed sulphation of both oestrone and oestradiol over a steroid concentration range of 10 nM–1 μM, and displayed non-Michaelis–Menten kinetics of a type already described for certain oestrogen sulphotransferases [10,12]. On the other hand, kinetics approximating Michaelis–Menten behaviour were observed with crude cytosolic preparations. The purified preparations exhibited pregnenolone sulphotransferase activity amounting to 0.2–2 % of the oestrogen sulphotransferase activity with 0.1 μM-steroid as substrate.

DISCUSSION

There are at least two adrenal sulphotransferases, each of which acts upon steroidal structures, in Hartley and English Shorthair guinea pigs. One, already referred to by others [11], primarily utilizes pregnenolone as substrate. A distinct oestrogen sulphotransferase is also present, the cytosolic specific activity of which is considerably higher than that of similar enzymes in various tissues of small rodents [5,6]. The high activity in adrenal gland contrasts sharply with that in guinea pig gonads and raises a question as to the possible biological role of the adrenal enzyme. The high activity in both sexes is also perplexing. Nevertheless, valuable information about enzyme function and structure has been obtained for the bovine adrenal oestrogen sulphotransferase without proof of its biological importance [12,20]. Also, our data suggest that the guinea pig adrenal enzyme may be more stable than those of mouse placenta [7] and guinea pig chorion [10], and that it may therefore be useful for the study of enzyme structure and control of activity. However, the relatively pure adrenal oestrogen sulphotransferase does undergo alteration during repeated gel filtration. An inactive product formed from it presumably interacts with the gel of the Superose 12 columns, possibly via certain hydrophobic sites. The identity of this product is unknown, but if it is formed during incubation of our purest enzyme preparations it might account for the complex kinetics which we find.

Our findings suggest that the guinea pig adrenal oestrogen sulphotransferase consists of a 34–36 kDa protein which may, according to its behaviour on Sephadex G100, form a dimer in the cytosol. We have no evidence that the subunits are identical, and during various steps of our purification scheme a doublet can be seen in the region of 34–36 kDa on electrophoretic gels. The increase in specific activity achieved for the oestrogen sulphotransferase (Table 2) appears modest, but gel electrophoresis indicates considerable purity (Fig. 5). Oestrogen sulphotransferases from bovine adrenal gland and placenta are reported to be identical [21]. Cloning experiments [22] indicate the bovine placental enzyme to be a 34.6 kDa protein which may exist as a dimer. Another publication reports a molecular mass of 31 kDa for an unstable oestrogen sulphotransferase from porcine uterus [23]. It has also been demonstrated that the corresponding enzyme in mouse placenta and uterus behaves as if it were a 29–30 kDa protein [7,8]. The presence of two forms of guinea pig adrenal oestrogen sulphotransferase during chromatofocusing parallels information obtained for the mouse placental enzyme [7] and for the guinea pig chorionic enzyme [10]. Two forms of the bovine placental enzyme, separable by ion exchange, have also been described [24], and their presence has been attributed to partial deamidation.

The striking activation of guinea pig adrenal oestrogen sulphotransferase by thiol groups is similar to that already observed for the corresponding enzyme in guinea pig chorion and uterus [5] and in mouse placenta and uterus [6]. This may be due to the presence of a thiol group in or near the active site of these enzymes, as described for the bovine adrenal oestrogen sulphotransferase [12]. Interestingly, the pregnenolone sulphotransferase of guinea pig adrenal gland is minimally activated by thiol groups. This might indicate a basic difference in structure of the active sites and consequently a possible difference in the molecular mechanism of catalytic sulphation.

It will be noted that a trace of activity toward pregnenolone persists in our most highly purified preparations. Based upon the variability of this activity in different preparations, and upon a difference in elution pattern of the two activities from the affinity column (Fig. 3), we tend to view the activity toward pregnenolone as residual contamination. However, because of the very low pregnenolone sulphotransferase activity remaining, we are unable to state that the oestrogen sulphotransferase is itself totally without activity toward pregnenolone.

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