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ENZYMIC SYNTHESIS OF STEROID SULPHATES

XII. ISOLATION OF DEHYDROEPIANDROSTERONE SULPHOTRANSFERASE FROM HUMAN ADRENALS BY AFFINITY CHROMATOGRAPHY

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Summary

Steroid alcohol sulphotransferases (EC 2.8.2.—) have not previously been obtained in a pure state possibly because of their inherent instability. A rapid isolation procedure involving affinity chromatography was developed and initially applied to the isolation of dehydroepiandrosterone sulphotransferase from human adrenals, since the sulphate ester of this steroid is quantitatively (along with unconjugated cortisol) the most important secretory product of the human adrenal cortex.

By use of the coupled product of dehydroepiandrosterone-17-(*O*-carboxymethyl) oxime and AH Sepharose 4B, the enzyme was isolated in one step from an $(NH_4)_2SO_4$ cut derived from the cytosol of human adrenal glands. Enzyme activity, employing dehydroepiandrosterone or etiocholanolone as substrates, was associated with the major band revealed on acrylamide gel electrophoresis. One or two very minor bands, lacking enzyme activity, were also usually present. On sodium dodecyl sulphate gels, a band having a molecular weight of 34 500 was obtained. By sucrose gradient ultracentrifugation the active enzyme was found to have a molecular weight of 68 000 and thus contains two subunits. These appear to be identical, as judged by fingerprint data of the tryptic peptides and the amino acid composition.

Steroids other than dehydroepiandrosterone acted as substrates. The decreasing order of sulphurylation rates were: epiandrosterone, 1.6; androst-5-

Abbreviations: buffer 1: 0.05 M sodium phosphate buffer (pH 7.5) 0.1 mM dithiothreitol. P-Ado-P-SO_4: 3′-phosphoadenosine-5′-phosphosulphate. SDS; sodium dodecyl sulphate.

ene-3β,17α-diol, 1.4; dehydroepiandrosterone and pregnenolone, 1.0; etiocholanolone, 0.89; androst-5-ene-3β,17β-diol, 0.75; androsterone, 0.44; testosterone, 0.15; estradiol-17β, 0.17; 11-deoxycorticosterone, 0.10.

Complex wave-like curves were obtained when either substrate, i.e. dehydroepiandrosterone or 3′-phosphoadenosine-5′-phosphosulphate, was varied in the presence of a fixed concentration of the cosubstrate. In marked contrast, enzyme isolated from bovine liver using the same affinity gel, yielded normal Michaelis-Menten kinetics.

Introduction

The human adrenal gland secretes both dehydroepiandrosterone and dehydroepiandrosterone 3-sulphate [1—3]. Although the conversion of pregnenolone sulphate to dehydroepiandrosterone 3-sulphate has been demonstrated to occur in the gland, it is believed that the principle route of synthesis of dehydroepiandrosterone 3-sulphate is by way of direct sulphurylation of dehydroepiandrosterone [1]. The sulphotransferase responsible for this sulphurylation has not been isolated in a pure state. This appears to be due to the inherent instability of steroid alcohol sulphotransferases as a class [4]. Previous studies, carried out on partially purified preparations derived from human adrenals, showed that the enzyme had a molecular weight of approximately 70 000 but could exist in higher states of association [5]. Wave-like kinetics were obtained when dehydroepiandrosterone was the variable substrate [5], and this behaviour paralleled that exhibited by purified oestrogen sulphotransferase (3′-phosphoadenylylsulphate:oestrone 3-sulphotransferase, EC 2.8.2.4) [6,7]. The possible involvement of the sulphotransferase in regulating dehydroepiandrosterone 3-sulphate secretion from the human adrenal has been suggested [8].

Efforts to purify the human adrenal enzyme have been made in this laboratory using conventional techniques. Whilst the enzyme remains reasonably stable during ammonium sulphate fractionation, further purification by chromatography on ion-exchange columns leads to inactivation [9]. A rapid method of isolation was evidently needed and this suggested that use of affinity chromatography. We now wish to report the isolation of the enzyme from human adrenals, in substantially pure form, by the use of this technique.

Materials and Methods

Estradiol-17β, 11-deoxycorticosterone, androst-5-ene-3β,17β-diol, etiocholanolone, pregnenolone, dehydroepiandrosterone and cholesterol esters were obtained from Sigma Chemical Co. Testosterone and epiandrosterone were Koch-Light products and androsterone was purchased from Ikapharm. Cholesterol was of Biochemical Grade obtained from British Drug Houses Ltd. Androst-5-ene-3β,17α-diol was kindly supplied by the M.R.C. Steroid Reference Collection, London.

[7(*n*)-^{3}H]Dehydroepiandrosterone, spec. act. 16.6 Ci/mmol and [7(*n*)-^{3}H]-cholesterol, spec. act. 9.3 Ci/mmol, were purchased from the Radiochemical Centre Amersham. *O*-Carboxymethylhydroxylamine hemihydrochloride was purchased from Calbiochem.

Dehydroepiandrosterone-17-(O-carboxymethyl) oxime. The method used was adopted from that of Erlanger et al. [10] for the preparation of testosterone 3-(*O*-carboxymethyl) oxime. 2 g dehydroepiandrosterone and 2.6 g *O*-carboxymethylhydroxylamine hemihydrochloride were dissolved in 500 ml ethanol, the solution made alkaline with 5 N NaOH and refluxed for 7 h. After concentrating to low volume, water was added and the mixture extracted with diethyl ether. The aqueous phase was acidified and the precipitate collected at the pump, washed with water and recrystallized from aqueous ethanol. Yield, 1.8 g. 3 β-Hydroxyandrost-5-en-17-(*O*-carboxymethyl) oxime had m.p. 217°C, absorption maximum in ethanol 222 nm, ξ 1290. (Found: C, 69.9; H, 8.5; N, 3.6; $C_{21}H_{31}NO_4$ required C, 69.8; H, 8.6; N, 3.9.)

Other materials were as described prevously [6].

Coupling of dehydroepiandrosterone-17-(O-carboxymethyl) oxime to AH Sepharose 4B. The method was based on that described by Cuatrecasas and Anfinsen [11]. 1 g AH Sepharose 4B was allowed to swell in 20 ml 0.5 M NaCl then washed on a Buchner with 200 ml 0.5 M NaCl and then with water. 100 mg dehydroepiandrosterone-17-(*O*-carboxymethyl) oxime was dissolved in 10 ml dimethylformamide and 2 ml water added. The Sepharose gel was then added, the pH was adjusted to 4.7 with 1 N HCl and 800 mg 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride introduced in portions with stirring. Stirring was continued overnight and the mixture washed on a Buchner funnel with 1 l 50% aqueous dimethylformamide. The gel was then packed into a small column and washed overnight with 1 l 80% aqueous methanol. This was followed by washing with 100 ml 0.05 M sodium phosphate buffer (pH 7.5), 0.1 mM dithiothreitol (buffer 1). Fresh batches of coupled affinity gel were made and washed by the above procedure immediately prior to enzyme isolation.

By the use of [^{3}H]dehydroepiandrosterone, 1.34 and 1.84 μmol dehydroepiandrosterone were found to be coupled/g of AH Sepharose 4B, in two separate experiments. This corresponds to 3.4—5.6% and 4.6—7.7%, respectively, of the available amino groups on the AH Sepharose 4B.

Affinity chromatography. A number of separate preparations were carried out on adrenal glands obtained from individual patients from the operating theatre. These glands were transported to the laboratory on ice and stored at −20°C for periods up to 3 weeks before processing. Tissue (usually 10—20 g) was homogenized with 3 vols. 0.05 M Tris-HCl buffer, 0.08 M KCl, 0.1 mM dithiothreitol, employing an Ultra-Turrax homogenizer. After centrifuging for 10 min at 10 000 × *g*, the supernatant was then spun at 100 000 × *g*. An $(NH_4)_2SO_4$ fraction, precipitating between 35% and 55% saturation, was prepared and dissolved in 10—15 ml of buffer 1. Affinity gel, prepared as above, was suspended in buffer 1 to give a final vol. of 5 ml. An aliquot of suspended gel was taken in the proportion of 0.05 ml/g adrenal tissue and stirred overnight at 4°C with the $(NH_4)_2SO_4$ fraction. The mixture was filtered on a small Buchner, the gel plus filter paper transferred back to the original flask and stirred for 10 min at 4°C with buffer 1 (10 ml) containing 2% (v/v) propylene glycol and filtered. The process was repeated three times. Gel and filter papers were then eluated by stirring at room temperature for 40 min with 150 ml buffer 1, 0.1 mM dehydroepiandrosterone, 2% (v/v) propylene glycol,

followed by filtration. The process was repeated with a further 50 ml for 10 min. Combined filtrates were concentrated by vacuum dialysis to approx. 10 ml, dialysed overnight against 5—8 l of 0.05 M Tris-HCl buffer (pH 7.5), 0.1 mM dithiothretol, 2% (v/v) propylene glycol. Final concentration (to 0.5 ml) was achieved by vacuum dialysis using a collodion bag and the apparatus supplied by Sartorius, Göttingen, F.R.G. The preparation, containing 50—80 μg protein/g wet weight adrenal tissue, was redialysed against the Tris/thiol buffer and stored at 0° C under N_2. Activity was retained for at least 4 weeks.

Assay of enzyme activity. Unless otherwise stated, the incubation contained: 15 nmol P-Ado-P-SO_4, 10^5 dpm P-Ado-P-$^{35}SO_4$, 7.5 μmol Tris-HCl buffer (pH 7.5), 1.5 μmol $MgCl_2$, 4.5 nmol dehydroepiandrosterone added in 5 μl propylene glycol and enzyme in a total vol. of 0.15 ml. Controls contained propylene glycol alone. Incubation was carried out at 37° C for variable time intervals: these are given in the figure and table legends. Dehydroepiandrosterone 3-[^{35}S]sulphate was measured after precipitation of $^{35}SO_4^{2-}$ and P-Ado-P-$^{35}SO_4$ by Ba^{2+} and counting the labelled ester [^{35}S]sulphate in the supernatant [12]. Counts in the control were subtracted from those recorded in the incubation containing dehydroepiandrosterone. It was found that stock solutions of labelled and unlabelled P-Ado-P-SO_4 were stable for many months if stored in 50% aqueous ethanol solution (pH 9) at −20° C. Aliquots of these stock solutions were added to tubes and solvent removed in a stream of N_2 prior to addition of the other components.

Radioactivity measurements. This was carried out using a Triton/toluene phosphor system [13] and employing a computerized Packard 2650 instrument. Quench corrections were made by the external standard method.

Acrylamide gel electrophoresis. The method of Ornstein and Davis [14] employing 6% gels was used.

Sodium dodecyl sulphate (SDS) acrylamide gel electrophoresis. The method of Weber et al. [15] was used employing 10% gels containing 0.1% SDS. Samples were heated at 100° C for 2 min in 0.01 M sodium phosphate buffer (pH 7.0), 1% SDS, 1% β-mercaptoethanol. Coomassie brilliant blue was used for staining.

Amino acid analysis. Reduced and S-carboxymethylated [6] enzyme was hydrolysed for 24 h and subjected to amino acid analysis on a Beckman model 121-M instrument.

Microtryptic digestions and finger printing. Tryptic digestion was carried out on 180 μg of preparation H.S. previously reduced and S-carboxymethylated [16]. Peptides were separated on 20 × 20 cm cellulose thin-layer chromatography plates (Polygram Cel 300, Macherey Nagel). The first dimension was by electrophoresis at 750 V for 40 min in pyridine/acetic acid/water (1 : 10 : 89, by vol.), pH 3.5. The second dimension was by chromatography in *n*-butanol/pyridine/acetic acid/water (150 : 100 : 3 : 100, by vol.). Plates were washed by chromatography in *n*-butanol/pyridine/acetic acid/water (150 : 100 : 30 : 120, by vol.) prior to sample application. Peptides were visualized by spraying with: (i) 3% pyridine in acetone, (ii) 0.01% fluorescamine in acetone, (iii) 3% pyridine in acetone and viewing in ultraviolet light (Whittaker, R., personal communication).

Protein was measured by the method of Lowry et al. [17].

Results

Isolation of enzyme

Preliminary experiments were carried out using higher ratios of dehydroepiandrosterone-Sepharose 4B gel to protein than subsequently adopted. Such preparations contained some 50% of contaminating proteins, possibly arising from non-specific absorption onto the spacer arm of the affinity gel. However, by limiting the amount of affinity gel employed, preparations containing a protein possessing dehydroepiandrsterone sulphotransferase activity, and comprising some 95% of the total protein present, could be routinely isolated. Activity was associated with the major band revealed on acrylamide gel electrophoresis, when either dehydroepiandrosterone or etiocholanolone were used as substrates (Fig. 1).

As shown in Table I, the enzyme was isolated in a yield of about 20%, starting with the adrenal cytosol fraction.

Molecular properties

The enzyme was characterised by its high electrophoresis mobility on acrylamide gels, travelling just behind bovine serum albumin used as a reference (Fig. 2). On SDS gels, a major polypeptide was found which had a molecular weight of 34 500, as determined by calibration with reference proteins (Fig. 2).

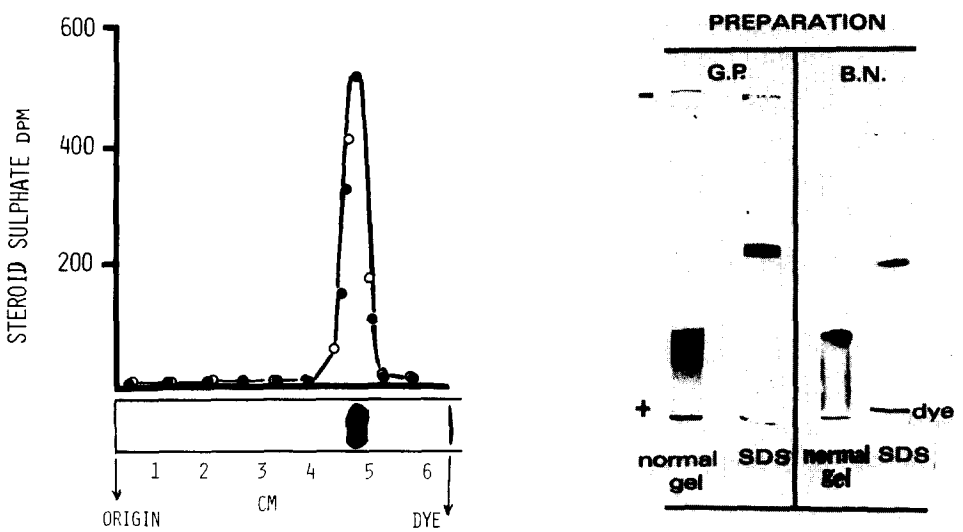

Fig. 1. Coincidence of enzyme activity and protein following acylamide gel electrophoresis. Three gels were run: one containing 35 μg (preparation H.S.) for protein staining and the others containing 7 μg protein for assaying activity. In addition to the major band, two other minor protein bands were present which are not revealed on the photograph (see Fig. 2). Selected 1 mm segments of unstained gel were macerated in 0.2 ml of 0.05 M Tris-HCl buffer, pH 7.5. Steroid (dehydroepiandrosterone (•) or etiocholanolone (○)) was added to give a final concentration of 25 μM, followed by P-Ado-P-$^{35}SO_4$ (3 pmol; 24 · 10^4 dpm). Incubation was carried out for 1 h at 37°C and steroid [^{35}S]sulphate estimated by the Wengle method [12].

Fig. 2. Polyacrylamide and SDS-polyacrylamide gel electrophoresis of two separate enzyme preparations. For preparation G.P., 70 μg protein was loaded onto the gel and for preparation B.N., 35 μg was employed. The subunit molecular weight was determined by comparison with reference proteins.

TABLE I

ISOLATION OF ENZYME FROM HUMAN ADRENAL TISSUE

The 35—55% saturated $(NH_4)_2SO_4$ fraction derived from the cytosol of human adrenal tissue (18 g) was absorbed to, and eluted from, dehydroepiandrosterone-Sepharose 4B by dehydroepiandrosterone-containing buffer. Assays were performed using P-Ado-P-$^{35}SO_4$ with an incubation time of 10 min.

Stage	Protein (mg)	Specific activity (nmol/mg per min)	Units	Recovery (%)	Purification
Cytosol	1050	0.03	31.5	100	—
$(NH_4)_2SO_4$ cut	195	0.146	28.5	91	5
Affinity gel eluant	0.8	7.3	6.2	20	245

Identical results were obtained with three separate enzyme preparations. Upon sucrose gradient ultracentrifugation, carried out as described previously [5], a single symmetrical zone of enzyme activity was observed and the molecular weight was determined to be 68 000 by comparison with reference hemoglobin. Thus the enzyme would normally appear to be comprised of two subunits. The latter are apparently identical, since the fingerprint of a microtryptic digest revealed some 30 spots and, by amino acid analysis, 35 basic residues were found/polypeptide of molecular weight 35 000.

Specificity

Relative rates of sulphurylation for a group of steroids are shown in Table II. Such results stand in marked contrast to estrogen sulphotransferase which is specific for natural estrogens and will not sulphurylate steroid alcohols [7,19].

Kinetic properties

Reaction rate remained linear for a period of some 30 min. In kinetic studies, rates were measured using [^{3}H]dehydroepiandrosterone of known

TABLE II

ENZYME SPECIFICITY

Steroids were compared at a final concentration of 33 μM. Assay condition as in Materials and Methods with an incubation time of 1.5 h and 13 μg enzyme protein (preparation G.P.). Assays carried out in the absence of steroid (propylene glycol alone) corresponded to only 1% of values obtained with 33 μM dehydroepiandrosterone (DHEA).

Steroid	Sulphurylation (DHEA = 1.0)
3β-Hydroxy-5α-androstan-17-one (epiandrosterone)	1.6
Androst-5-ene-3β,17α-diol	1.4
3β-Hydroxyandrost-5-en-17-one (DHEA)	1.0
3β-Hydroxypregn-5-en-20-one (pregnenolene)	1.0
3α-Hydroxy-5β-androstan-17-one (etiocholanolone)	0.89
Androst-5-ene-3β,17β-diol	0.75
3α-Hydroxy-5α-androstan-17-one (androsterone)	0.44
17β-Hydroxyandrost-4-en-3-one (testosterone)	0.15
Estra-1,3,5 (10)-triene-3,17β-diol (estradiol-17β)	0.17
21-Hydroxypregn-4-ene-3,20-dione	0.10

specific activity. The [^{3}H]dehydroepiandrosterone 3-sulphate was separated from unreacted [^{3}H]dehydroepiandrosterone by thin-layer chromatography and counted. Details are given figure legends. Fig. 3 shows the effect of varying dehydroepiandrosterone at constant P-Ado-P-SO_4. The wave-like nature of the response is then similar to that obtained previously using partially purified human adrenal preparations [5]. The position of the peaks and troughs was identical in repeated experiments using the same enzyme preparation and the same enzyme concentration. However, when this concentration was decreased, whilst the position of the peaks and troughs remained the same up to a dehydroepiandrosterone concentration of 4 μM, the shape of the curve altered at steroid concentrations above this value. Separate enzyme preparations yielded very similar velocity-substrate profiles, but small changes were apparent in the position of the peaks and troughs at dehydroepiandrosterone concentrations above 4 μM. Such properties of the enzyme are under investigation and results will be reported at a later date. The close resemblance to the velocity-substrate curve given by purified estrogen sulphotransferase [18], when estrogen was varied over a similar concentration range to that employed for dehydroepiandrosterone in Fig. 3, was apparent.

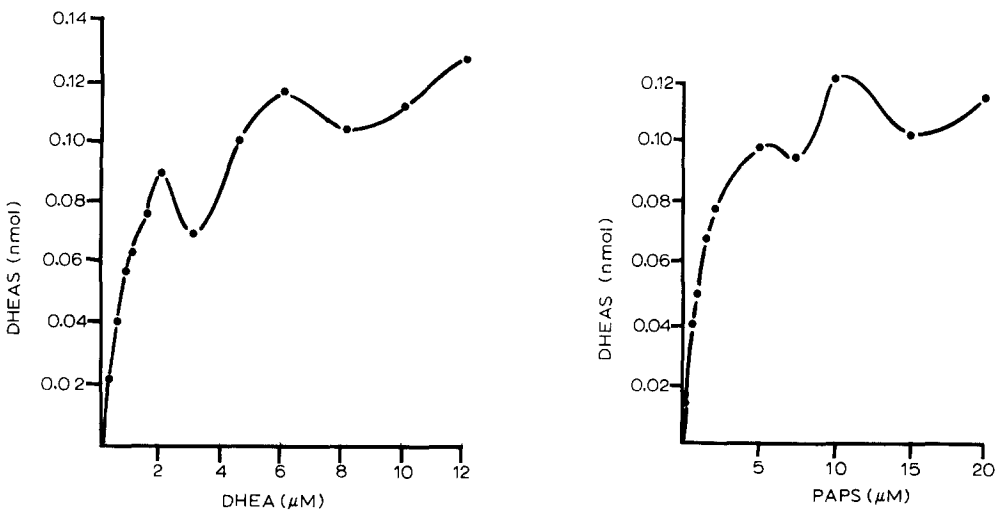

Fig. 3. Dehydroepiandrosterone 3-sulphate (DHEAS) formation with dehydroepiandrosterone (DHEA) as variable substrate. Each tube contained 7.5 nmol P-Ado-P-SO_4, 1.5 μmol $MgCl_2$, 7.5 μmol Tris-HCl buffer, pH 7.5, 15 nmol dithiothreitol, $4.7 \cdot 10^5$ dpm [7(n)-^{3}H]dehydroepiandrosterone, unlabelled dehydroepiandrosterone at the concentrations stated and enzyme (1.8 μg of preparation H.S.) in a total vol. of 0.15 ml. Incubation was for 15 min at 37° C and reaction was stopped by placing tubes in a boiling water bath for 1 min. 20 μg carrier dehydroepiandrosterone 3-sulphate was added and a 0.05-ml aliquot applied to a 20 × 20 cm silica gel thin-layer chromatography plate (Merck, 0.25 mm thickness) along with markers of dehydroepiandrosterone and dehydroepiandrosterone 3-sulphate. Development was carried out with benzene/methyl ethyl ketone/ethanol/water (3 : 3 : 3 : 1, by vol.) then with cyclohexane/ethyl acetate (1 : 1, v/v). The reference substances were revealed by spraying with $SbCl_3$ and warming. Dehydroepiandrosterone was well separated from dehydroepiandrosterone by this procedure and these areas were scraped off and eluted with ethanol (3 times 3 ml). The combined eluates were evaporated to dryness in vials and counted.

Fig. 4. Dehydroepiandrosterone 3-sulphate (DHEAS) formation with P-Ado-P-SO_4 (PAPS) as variable substrate. Assay as in Fig. 4, employing 20 μM dehydroepiandrosterone (DHEA) ($1.2 \cdot 10^6$ dpm).

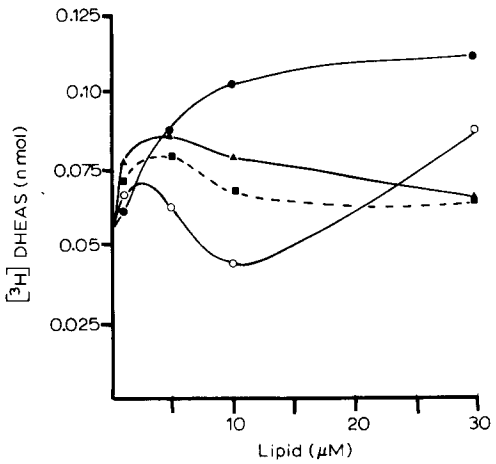

Fig. 5. Effect of lipids on enzyme activity. Each tube contained 14 nmol P-Ado-P-SO_4, 1.5 μmol $MgCl_2$, 7.5 μmol Tris-HCl buffer (pH 7.5), 15 nmol dithiothreitol, 1 nmol dehydroepiandrosterone ($6.6 \cdot 10^5$ dpm) and enzyme (2 μg of preparation B.N.) in a total vol. of 0.15 ml. Cholesterol and glyceryl trioleate were added from propylene glycol stock solutions (5 μl). Cholesteryl oleate and stearate were added from ethanol and ether stock solutions, respectively, to tubes containing propylene glycol (5 μl) and the volatile solvents removed in a stream of N_2. Incubation was carried out for 20 min at 37°C and labelled [^{3}H]dehydroepiandrosterone sulphate ([^{3}H]DHEAS) isolated and assayed as in the legend to Fig. 3. ●———●, Cholesterol; ○———○, cholesteryl oleate; ▲———▲, cholesteryl stearate; ■- - - - -■, glyceryl trioleate.

When P-Ado-P-SO_4 was the variable substrate (Fig. 4), then once again the curve was complex but the amplitude of the wave-like function was not as large as in the former case. The first 6 points, when plotted in double reciprocal form, were approximately linear and give an apparent K_m for P-Ado-P-SO_4 of 1 μM.

It was reported previously that cholesterol was sulphurylated at low rate, compared to that of dehydroepiandrosterone, by cytosol fractions of human adrenal glands [5]. This was confirmed in our current experiments by using [^{3}H]cholesterol incubated with P-Ado-P-SO_4 and an $(NH_4)_2SO_4$ cut from human adrenal cytosol. The labelled cholesteryl sulphate was separated from cholesterol by chromatography on a column of Sephadex LH-20. Fig. 5 shows the influence of cholesterol, cholesteryl esters of fatty acids, and glyceryl trioleate, on the formation of [^{3}H]dehydroepiandrosterone 3-sulphate from [^{3}H]dehydroandrosterone and unlabelled P-Ado-P-SO_4. The [^{3}H]dehydroepiandrosterone 3-sulphate was separated by thin-layer chromatography. It can be seen that these lipids at low concentrations can stimulate the activity of the enzyme.

Discussion

By use of the affinity gel obtained by amide linkage of dehydroepiandrosterone-17-(*O*-carboxymethyl) oxime to AH Sepharose 4B, dehydroepiandrosterone sulphotransferase has been isolated relatively simply from human adrenal preparations. Apart from estrogen sulphotransferase [6,7], this is the first steroid sulphotransferase to be isolated in essentially pure form. Based on a subunit molecular weight of 34 500, then a dimer composed of two identical

subunits, and having molecular weight of 69 000, is in good agreement with both the present and earlier data which show that the active enzyme has a molecular weight of 65 000—70 000 [5].

Upon acrylamide gel electrophoresis, activity towards dehydroepiandrosterone and etiocholanolone as substrates coincided with the position of the protein revealed by staining (Fig. 1). In other acrylamide gel experiments, activity using estradiol-17β as substrate was also coincident with the former steroids. The data in Table II will then be discussed assuming that a single protein species is responsible for the sulphurylation of the group of steroids studied. Due to the complexity of the velocity-substrate plots, it was not possible to calculate K_m and V values. It would appear that the equatorial hydroxyl group in 3β-hydroxy-5α-androstan-17-one (epiandrosterone) is sulphurylated at the maximum rate and a marked decrease in rate occurs with the axial hydroxyl group in 3α-hydroxy-5α-androstan-17-one (androsterone). It is surprising that etiocholanolone is such a good substrate considering the alteration in shape of the steroid resulting from *cis*-fusion of rings A and B. The fact that the 3α-hydroxyl group in etiocholanolone is equatorial, may be of some significance. In considering the androst-5-enes, the influence of the oxygen function on position-17 becomes apparent and could suggest that hydrogen bonding may occur on the α-face at this position. However, there remains the possibility that these various substrate may present themselves to the sulphurylation site at either the 3- or 17-positions.

One of the most challenging features of both oestrogen sulphotransferase and partially purified dehydroepiandrosterone sulphotransferase has been the wave-like kinetics exhibited when the steroid is the variable substrate [5,6]. Oestrogen sulphotransferase exists in a number of isoenzyme forms and each of these exhibits such wave-like kinetics [6]. The wave-like feature is again found in the dehydroepiandrosterone sulphotransferase obtained by affinity chromatography. However, in contrast to estrogen sulphotransferase which gives normal Michaelis-Menten kinetics when estrogen is fixed and P-Ado-P-SO_4 the variable substrate, dehydroepiandrosterone sulphotransferase yields complex curves under these conditions (Fig. 4). In this regard the influence of cholesterol and other lipids on the system is revealing. Whilst cholesterol itself is a poor substrate for the enzyme [5], variable concentrations of unlabelled cholesterol and cholesterol esters, can affect the rate of sulphurylation of a fixed concentration of [^{3}H]dehydroepiandrosterone (Fig. 5). Some resemblance to the wave-like response obtained by varying the substrate dehydroepiandrosterone (Fig. 3) can be seen, and could suggest that such effects are due to binding of either the steroid substrate, or lipids, at second (allosteric?) sites. Such results are in marked contrast to the behaviour of enzyme isolated from bovine liver using the same affinity gel. Normal Michaelis-Menten kinetics were obtained with both dehydroepiandrosterone and P-Ado-P-SO_4, and cholesterol was without effect on the system. It should be mentioned that the effects of increasing concentrations of cholesterol on the rate of sulphurylation of [^{3}H]dehydroepiandrosterone have not been consistent using different preparations of adrenal enzyme and could possibly reflect varying levels of lipid already bound to the enzyme (cf. bovine serum albumin). This aspect will be investigated in more detail in the future. The

studies of Bardsley and Childs [20] are very relevant to this problem. They have predicted that wave-like velocity-substrate curves can occur in enzymes having multiple interacting sites. The complexity of such curves is governed by the degree of the polynomial equation responsible and curves shown by estrogen sulphotransferase [6,18], and dehydroepiandrosterone sulphotransferase (Fig. 3), would be of degree 4 or 5.

From the more physiological point of view, the fact that cholesterol, and perhaps other steroids or even lipids, may increase dehydroepiandrosterone 3-sulphate synthesis, is of particular interest. ACTH appears to stimulate free dehydroepiandrosterone secretion from the human adrenal more than dehydroepiandrosterone 3-sulphate [2]. The sulphurylation step may then be normally rate-limiting for secretion of dehydroepiandrosterone 3-sulphate. In turn, this opens up the question of a separate control of dehydroepiandrosterone 3-sulphate secretion via the sulphotransferase.

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References

1 Roberts, K.D. and Liebermans, S. (1970) in Chemical and Biological Aspects of Steroid Conjugation (Bernstein, S. and Solomon, S., eds.), pp. 219—290, Springer Verlag, New York
2 Nieschlag, E., Loriaux, D.L., Kirschner, M. and Lipsett, M.B. (1973) J. Endocrinol. 57, 123—134
3 Douss, T.W., Skinner, S.J.M. and Couch, R.A.F. (1975) J. Endocrinol. 66, 1—12
4 Roy, A.B. and Banerjee, R.K. (1967) Proceedings of the Second International Congress Series on Hormonal Steroids, pp. 397—403, Exerpta Medica International Congress Series, No. 132
5 Adams, J.B. and Edwards, A.M. (1968) Biochim. Biophys. Acta 167, 122—140
6 Adams, J.B., Ellyard, R.K. and Low, J. (1974) Biochim. Biophys. Acta 370, 160—188
7 Adams, J.B. and Low, J. (1974) Biochim. Biophys. Acta 370, 189—196
8 Adams, J.B. (1977) Cancer 40, 325—333
9 Low, J. (1973) Ph.D. Thesis, University of New South Wales
10 Erlanger, B.F., Borek, F., Beiser, S.M. and Lieberman, S. (1957) J. Biol. Chem. 228, 713—727
11 Cuatrecasas, P. and Anfinsen, C.B. (1971) Methods Enzymol. 22, 345—385
12 Wengle, B. (1964) Acta Chem. Scand. 18, 65—76
13 Adams, J.B. and Poulos, A. (1967) Biochim. Biophys. Acta 146, 493—508
14 Ornstein, L. and Davis, B.J. (1962) Disc Electrophoresis, Distillation Products Industries, Rochester, NY
15 Weber, K., Pringle, J.R. and Osborn, M. (1972) Methods Enzymol. 26, 3—27
16 Moss, B.A. (1978) in Research Methods in Immunology (Lefkovits, I. and Pernis, B., eds.), Academic Press, New York, in press
17 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.T. (1951) J. Biol. Chem. 193, 265—275
18 Adams, J.B., Dodsworth, I. and Jackson, D.E. (1975) Biochim. Biophys. Acta 384, 423—429
19 Rozhin, J., Soderstrom, R.L. and Brooks, S.C. (1974) J. Biol. Chem. 249, 2079—2087
20 Bardsley, W.G. and Childs, R.E. (1975) Biochem. J. 149, 313—328