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ENZYMIC SYNTHESIS OF STEROID SULPHATES

XIII. ISOLATION AND PROPERTIES OF DEHYDROEPIANDROSTERONE SULPHOTRANSFERASE FROM HUMAN FOETAL ADRENALS

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Summary

Human foetal adrenals have provided a rich source of steroid alcohol sulphotransferase (EC 2.8.2.-). The latter was isolated in pure form in one step by affinity chromatography of an $(NH_4)_2SO_4$ cut derived from the cytosol fraction of the glands. The yield was 6-fold higher than that obtained from adult human adrenals. General properties of the enzyme are given and it appears to be identical to that obtained previously from adult human adrenals (Adams, J.B. and McDonald, D. (1979) Biochim. Biophys. Acta 567, 144–153).

In a previous communication we reported the isolation of dehydroepiandrosterone sulphotransferase from adult human adrenal glands by means of affinity chromatography [1]. The enzyme was obtained in one step from an $(NH_4)_2SO_4$ cut derived from the cytosol fraction, and represented the first isolation of a steroid alcohol sulphotransferase in pure form. Oestrogen sulphotransferase (3′-phosphoadenylylsulphate : oestrone 3-sulphotransferase, EC 2.8.2.4), which has no activity towards steroid alcohols, has been isolated by conventional means [2,3]. Since human foetal adrenals contain a 5–6-fold higher concentration of steroid alcohol sulphotransferase compared to adult glands [4], this potentially rich source of the enzyme has now been investigated. We report the isolation of mg-quantities of pure enzyme which is apparently identical to that present in adult adrenals.

Abbreviations: DHEA, dehydroepiandrosterone; Paps, 3′-phosphoadenosine-5′-phosphosulphate.

Adrenals were obtained from 14—20 week-old foetuses after prostaglandin-induced termination of pregnancy. Tissue was removed and stored at −70°C for periods upto 5 months. Enzyme was isolated exactly as described previously for adult glands [1]. After final dialysis against 0.05 M Tris-HCl buffer (pH 7.5)/0.1 mM dithiothreitol/2% (v/v) propylene glycol, the preparation was concentrated by vacuum dialysis to give a protein concentration of approx. 1 mg/ml and stored in glass ampoules at −70°C. No significant difference in activity was observed after storage for 3.5 months.

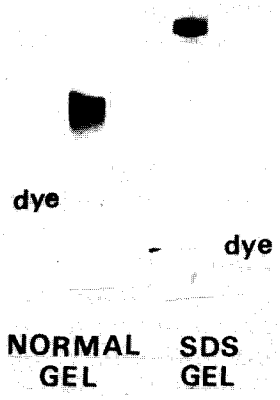
As shown in Table I, the enzyme was isolated in a yield of about 20%

TABLE I

ISOLATION OF ENZYME FROM FOETAL ADRENALS

The 35—55% saturated $(NH_4)_2SO_4$ fraction derived from the cytosol of pooled frozen foetal adrenals (11 g) was absorbed to, and eluted from, dehydroepiandrosterone-Sepharose 4B by dehydroepiandrosterone-containing buffer, as described previously employing adult adrenal tissue [1].

Stage	Protein (mg)	Specific activity (nmol/mg per min)	Units	Recovery %	Purification
Cytosol	511	0.55	281	100	—
$(NH_4)_2SO_4$ cut	217	0.83	180	64	1.5
Total buffer wash	216	0.01	2.2	0.8	—
Affinity gel eluant	3.0	20.1	60.3	21	36.6



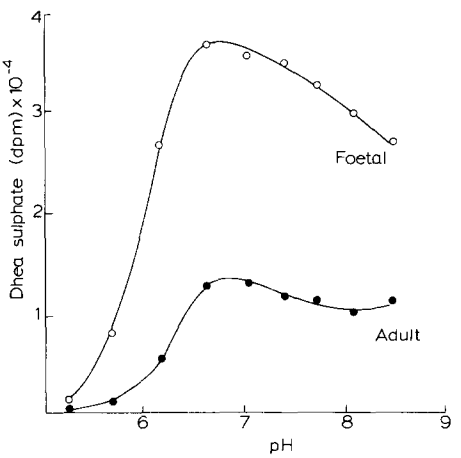


Fig. 1. Electrophoresis of foetal enzyme (60 μg protein) on a 6% polyacrylamide gel (normal gel) and on a 10% gel in the presence of SDS (30 μg protein). The behaviour fo the foetal enzyme was identical to that obtained previously with enzyme from adult adrenals [1].

Fig. 2. Comparison of pH profiles obtained with foetal and adult enzymes. Tris/maleate/NaOH buffer was employed throughout. Approximately three times as much foetal enzyme was used compared with the adult enzyme. Assays were carried out using 3′-phosphoadenosine 5′-phospho[^{35}S]sulphate and unlabelled DHEA [1].

starting from the cytosolic fraction. These results are similar to those obtained with adult adrenals, although the foetal tissue is a much richer source of enzyme, as seen from the specific activity in the cytosol, and the 6-fold higher yield obtained per g tissue. Some inactivation of the enzyme must occur on the basis of the apparent overall purification data.

Fig. 1 shows the electrophoretic behaviour of the enzyme on normal polyacrylamide gels and on SDS-polyacrylamide gels. The foetal enzyme behaved identically to the adult enzyme in possessing a very high electrophoretic mobility on normal gels and yielding a single band of molecular weight 34 000 on SDS-polyacrylamide gels. On ultracentrifugation in sucrose gradients, a single enzymically active zone was evident in the position of reference haemoglobin. This behaviour was again identical to the adult enzyme and is consistent with an normally active enzyme species having a molecular weight of 68 000 composed of two subunits [1].

A pH optimum of about 6.8 was obtained using Tris/maleate/NaOH buffer and the shape of the curve was very similar to that given by adult enzyme (Fig. 2). From Table II, it can be seen that the specificity of the foetal enzyme closely resembles that of the adult. Data offering an explanation for the wide specificity exhibited by the enzyme will be published separately.

Finally the unusual wave-like kinetics, which are a unique feature of the adult enzyme, are also exhibited by the foetal enzyme (Fig. 3). In common with the adult enzyme, this occurs when either substrate is varied in the presence of a fixed concentration of cosubstrate.

We conclude that the steroid alcohol sulphotransferase of human foetal adrenals is very probably identical to that in the adult gland and that foetal tissue should provide an excellent source of this enzyme to enable further studies to be made of its properties.

TABLE II

ENZYME SPECIFICITY

Relative rates of sulphurylation of various steroids are given with dehydroepiandrosterone set at 1.0. Rates given for the enzyme obtained from adult adrenals are generally the means of four separate enzyme preparations, but some additional steroids have been included with two preparations only and these are indicated. All steroids were assayed at a concentration of 33 μM (see Ref. 1). Adult sulphurylation rate, mean ± S.D.

Steroid	Sulphurylation rate		
	Adult		Foetal
3β-Hydroxyandrost-5-en-17-one (dehydroepiandrosterone)	1.0		1.0
Androst-5-ene-3β,17α-diol	1.39	0.27	1.22
Androst-5-ene-3β,17β-diol	0.98	0.19	0.94
3α-Hydroxy-5α-androstan-17-one (androsterone)	0.50	0.16	0.41
3β-Hydroxy-5α-androstan-17-one (epiandrosterone)	1.21	0.38	0.90
3α-Hydroxy-5β-androstan-17-one (aetiocholanolone)	0.94	0.33	0.74
3β-Hydroxypregn-5-en-20-one (pregnenolone)	1.09	0.13	0.77
17β-Hydroxyandrost-4-en-3-one (testosterone)	0.20	0.10	0.11
17α-Hydroxyandrost-4-en-3-one (epitestosterone)	1.16	0.05	1.29
21-Hydroxypregn-4-ene-3,20-dione (11-deoxycorticosterone)	0.14	0.07	0.13
Oestra-1,3,5(10)-triene-3,17β-diol (17β-oestradiol)	0.19	0.02	0.23
Pregn-4-ene-11β,21-diol-3,20-dione (cortisol)	0.01 (2)*		0.01
Oestra-1,3,5(10)-triene-3,17α-diol (17α-oestradiol)	1.17 (2)		1.24
Oestra-1,3,5(10)-triene-3,17β-diol-3 methyl ether (17β-oestradiol-3 methyl ether)	0.08 (2)		0.09
Oestra-1,3,5(10)-trien-3-ol-17-one (oestrone)	0.11 (2)		0.23

*Mean of two preparations.

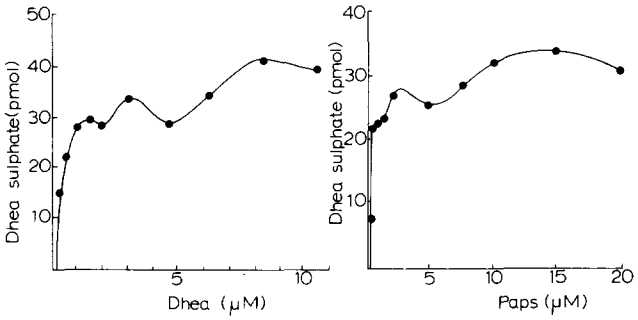


Fig. 3. Kinetics obtained by varying DHEA in the presence of a fixed concentration of PAPS (50 μM), or varying the latter at a fixed concentration of DHEA (20 μM). Assays were carried out using [7(n)-^{3}H]DHEA followed by separation of the labelled steroid sulphate by thin-layer chromatography, as described previously [1].

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