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The Hydrolysis of Oestrone Sulphate and Dehydroepiandrosterone Sulphate by Human Steroid Sulphatase Expressed in Transfected COS-1 Cells

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Whether the same or distinct steroid sulphatases (STS) are involved in the hydrolysis of alkyl and aryl steroid sulphates remains controversial. We have examined the ability of a placental steroid sulphatase to hydrolyse oestrone sulphate and/or dehydroepiandrosterone sulphate (DHA-S) by expressing the enzyme in COS-1 cells. Using either intact cells or broken cell preparations, the expressed sulphatase was found to hydrolyse both oestrone sulphate and DHA-S. The catalysis of oestrone sulphate and DHA-S by the expressed sulphatase was almost completely abolished by the steroid sulphatase inhibitor, oestrone-3-*O*-sulphamate. It is concluded that both alkyl and aryl steroid sulphates can be hydrolysed by the same steroid sulphatase.

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Steroid sulphates are important intermediates in the synthesis, transport and action of steroid hormones [1–4]. Conversion of oestrone sulphate to oestrone, and of dehydroepiandrosterone sulphate (DHA-S) to DHA, is thought to be an important source of biologically active steroids [5, 6]. Although the hydrolysis of DHA-S and oestrone sulphate involves the removal of a sulphate group, whether the hydrolysis of these conjugates is mediated by the same or different sulphatases remains controversial [7]. Mammalian aryl sulphatases are classified into three types: A, B and C. The microsomal aryl sulphatase C (steryl sulphate sulphohydrolase, EC 3.1.6.2.), which is responsible for the hydrolysis of sulphate esters of 3β-hydroxysteroids, has been purified using many different solubilization and isolation procedures. Using purified enzyme preparations, evidence has been reported to support the existence of distinct sulphatases for the hydrolysis of oestrone sulphate and DHA-S [8], and that the sulphatases hydrolysing oestrone sulphate and DHA-S are in fact the same enzyme [9, 10]. In addition, investigations of the physico-chemical or kinetic characteristics of steroid sulphatases in microsomal preparations of breast tumours or breast cancer cells have suggested that the same or distinct enzymes are responsible for the catalysis of oestrone sulphate and DHA-S [11–13]. A 2.4 kb cDNA for human placental steroid sulphatase has now been isolated, sequenced and shown to encode an enzymatically active protein [14, 15]. In this study we have transiently transfected this placental steroid sulphatase DNA into COS-1 cells and examined the ability of the expressed sulphatase to hydrolyse oestrone sulphate and DHA-S. In addition the ability of an irreversible steroid sulphatase inhibitor, oestrone-3-*O*-sulphamate [16], to inhibit the hydrolysis of oestrone sulphate and DHA-S by the expressed enzyme was also investigated.

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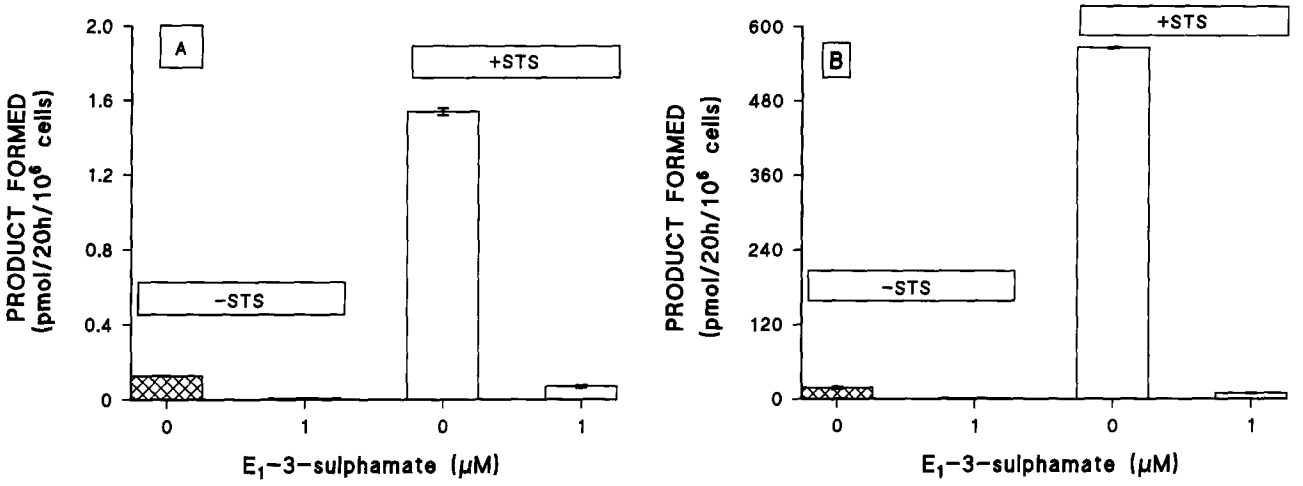

Fig. 1. Hydrolysis of oestrone sulphate (A) or DHA-S (B) by intact monolayers of COS-1 cells transfected with pSVL-STS-DNA (+STS) or the plasmid in which the STS coding region was deleted (−STS). Enzyme activity was: assayed in the absence or presence of a steroid sulphatase inhibitor, oestrone-3-*O*-sulphamate (E_1-3-sulphamate), $n = 3$, means ± SD.

MATERIALS AND METHODS

Cell culture and transient transfection experiments

COS-1 cells were routinely cultured in DMEM (Flow Labs, Irvine, Strathclyde, Scotland) with the addition of 10% (v/v) stripped foetal calf serum. Cells were grown until they were approx. 60% confluent when they were harvested with 0.05% (w/v) trypsin and 0.02% (w/v) EDTA, and washed with phosphate-buffered saline (PBS). After suspension in PBS, cells (5×10^6 cells/ml) were transfected by electroporation with 20 μg pSVL-STS-DNA (kindly provided by Dr K. von Figura [15]) or the parent vector lacking the sulphatase coding region. Electroporation was carried out using a Biorad Gene pulser (Hemel Hempstead, Herts, England) set at: 450 V 250 μF, time constant 5 s. After electroporation, the cells were incubated for a further 10 min at 4°C before plating out in T25 cell culture flasks. The medium was removed after 24 h and replaced with fresh medium and cultured for a further 24 h before assaying for sulphatase activity.

Assay of oestrone sulphatase and DHA-sulphatase

The ability of the transfected COS-1 cells to hydrolyse oestrone sulphate or DHA-S was examined using intact cell monolayers and broken cell preparations, obtained by sonicating the cells for 3 periods of 5 s at 25 kHz. [6,7-^{3}H]Oestrone sulphate (60 Ci/mmol, N.E.N., Boston, MA, U.S.A.), [4-^{14}C]oestrone (52 mCi/mmol, N.E.N.), or [6,7-^{3}H]DHA-S (28.5 Ci/mmol, N.E.N.) and [4-^{14}C]DHA (51 mCi/mmol, N.E.N.) were used to measure sulphatase activities in intact monolayers over 20 h as described previously [17]. Briefly, intact monolayers of MCF-7 cells were washed with Earle's balanced salt solution and incubated at 37°C with either [^{3}H]oestrone sulphate (5 pmol, 7×10^5 dpm) or [^{3}H]DHA-S (2.5 nmol, 4×10^5 dpm) in serum-free medium. After incubation medium was removed from the cells and unconjugated steroids extracted into toluene. The mass of oestrone sulphate or DHA-S was calculated from the ^{3}H counts present in the organic phase, corrected for recovery, and the specific activity of the substrates used. Sulphatase activity in broken cell preparations was measured by incubating broken cell suspensions (0.3–0.5 mg protein) for 1–2 h with [^{3}H]oestrone sulphate (20 μM, 4×10^5 dpm) or [^{3}H]DHA-S (20 μM, 4×10^5 dpm).

Inhibition studies

To examine whether the expressed sulphatase in COS-1 cells could be inhibited by oestrone-3-*O*-sulphamate [16], intact cells were treated with the inhibitor (1 μM) for 20 h in the presence of each substrate. For assays employing broken cell preparations, oestrone-3-*O*-sulphamate was used at concentrations of 1 and 10 μM.

All experiments were carried out in triplicate and the results are expressed as means ± SDs.

RESULTS

The ability of intact COS-1 cells to hydrolyse oestrone sulphate or DHA-S in either non-transfected cells or cells transfected with the parent expression vector was very low, but detectable. In contrast, when the steroid sulphatase was transfected into cells then they efficiently hydrolysed oestrone sulphate and DHA-S with an approximate 20-fold increase in activity compared with control cells [Fig. 1(A and B)]. The substrates were used at physiological concentrations (2 nM and 2 μM for oestrone sulphate and DHA-S, respectively) for the assay of steroid sulphatase activity in intact cells. In cells transfected with

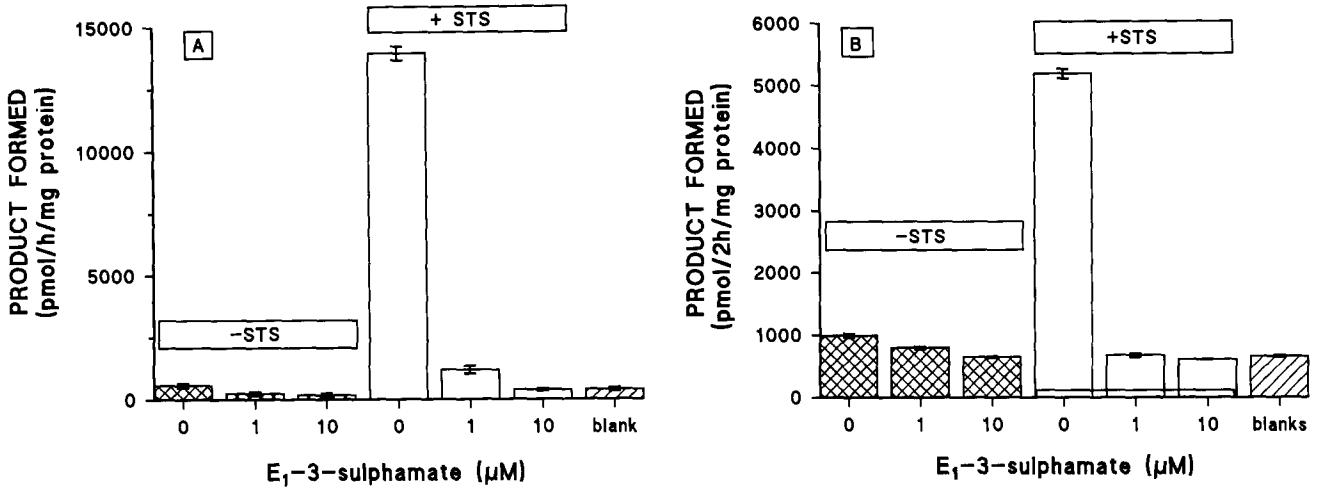

Fig. 2. Hydrolysis of oestrone sulphate (A) and DHA-S (B) by broken cell preparations of COS-1 cells transfected with pSVL-STS-DNA (+STS) or the plasmid in which the STS coding region was deleted (−STS). Enzyme activity was assayed in the absence or presence of the steroid sulphatase inhibitor, oestrone-3-*O*-sulphamate (E_1-3-sulphamate), $n = 3$, means ± SD. Blanks are incubations of substrate in the absence of cells.

the parent vector or STS-DNA the products formed represented 7.4 and 98% of the starting substrate concentration for oestrone sulphate and 5.6 and 59.6% for DHA-S.

The hydrolysis of oestrone sulphate and DHA-S was almost completely (>95%) inhibited by oestrone-3-*O*-sulphamate [Fig. 1(A and B)]. Sulphatase activities in cells transfected with the parent expression vector were also reduced by this inhibitor, confirming the presence of some basal sulphatase activity in these cells.

The ability of the expressed steroid sulphatase to hydrolyse oestrone sulphate (20 μmol/l) and DHA-S (20 μmol/l) was also examined using broken cell preparations [Fig. 2(A and B)]. As found using intact cells, these preparations were able to efficiently hydrolyse the conversion of both steroid sulphates. Using broken cell preparations, the products formed with cells transfected with the parent expression vector or STS-DNA represented 1.1 and 34.8% of the initial substrate for oestrone sulphate and 1.1 and 8.7% for DHA-S. The hydrolysis of both steroid sulphates by the broken cell preparations was effectively inhibited by oestrone-3-*O*-sulphamate.

DISCUSSION

The existence of distinct enzymes for the catalysis of oestrone sulphate and DHA-S, based on investigations employing either tissue or cell microsomes or enzyme preparations purified to apparent homogeneity, is controversial. Dao and Libby [11] originally reported that a greater proportion of breast tumours were able to hydrolyse oestrone sulphate than DHA-S, suggesting the presence of distinct sulphatases. In another similar investigation, however, the two sulphatase activities were found to be consistently present in breast tumours [12]. Based upon the different sensitivities of oestrone sulphatase and DHA-sulphatase to heat, ethanol precipitation, inhibitor sensitivities and inhibitor kinetics, MacIndoe *et al.* [13] concluded that separate enzymes were involved in the hydrolysis of oestrone sulphate and DHA-S.

Many different methods have been used to isolate steroid sulphatase from human placenta and the isolation of separate enzymes for the hydrolysis of alkyl and aryl sulphates has been reported [8]. From most recent studies, however, although some variations in the molecular weight of the purified steroid sulphatase have been observed (from 60–72 kDa), a consensus is emerging that only one enzyme is involved in the hydrolysis of oestrone sulphate and DHA-S [9, 13, 18]. Additional support for only one enzyme being involved in the hydrolysis of these steroid sulphates comes from studies of placental sulphatase deficiency and X-linked icthyosis. This defect is thought to be due to a deletion of the steroid sulphatase gene which results in an impairment not only of the hydrolysis of DHA-S and cholesterol sulphate, but also oestrone sulphate [14, 19].

The results from the present investigations, in which placental steroid sulphatase DNA was transiently transfected into COS-1 cells, demonstrates that one enzyme is capable of hydrolysing both oestrone and DHA sulphates. The hydrolysis of oestrone sulphate (1550 fmol/20 h/10^6 cells) and DHA-S (580 pmol/20 h/10^6 cells) in STS-transfected COS-1 cells was considerably higher than in other cell lines previously investigated, due to over-expression of the STS gene. In MCF-7 cells, for example, conversion of oestrone sulphate to oestrone and DHA-S to DHA is 100–120 fmol/20 h/10^6 cells and 30–35 pmol/20 h/10^6

cells, respectively. In MCF-7 cells sulphatase activity is completely inhibited by the inhibitor oestrone-3-*O*-sulphamate, at a concentration of 1 nM, compared with the much higher concentration (1 μM) required to inhibit the increased sulphatase activity in STS-transfected COS-1 cells in the present study.

As the hydrolysis of oestrone sulphate and DHA-S, by conversion to DHA and then to androstenediol, may be a major source of oestrogenic hormones to support the growth of hormone dependent tumours, a number of steroid sulphatase inhibitors are currently being developed [20, 21]. One such inhibitor, oestrone-3-*O*-sulphamate, as used in the present study, was found to almost completely inhibit the hydrolysis of both oestrone sulphate and DHA-S in sulphatase transfected COS-1 cells. This inhibitor may therefore be of use in reducing tissue and plasma concentrations of oestrogen and androstenediol in women with breast or endometrial cancer.

In conclusion, the results from this study in which steroid sulphatase was transiently expressed in COS-1 cells have revealed that one enzyme can catalyse the conversion of both oestrone sulphate and DHA-S. This finding therefore provides further evidence for only one enzyme being responsible for the hydrolysis of both alkyl and aryl steroid sulphates.

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