STEROID SULFATE SULFATASE IN HUMAN BENIGN PROSTATIC HYPERPLASIA: CHARACTERIZATION AND QUANTIFICATION OF THE ENZYME IN EPITHELIUM AND STROMA

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Summary—Characteristics and activities of estrone sulfate (E1S) and dehydroepiandrosterone sulfate (DHAS) sulfatases were studied in epithelium and stroma of benign hyperplastic tissues from human prostates. Tissues were obtained by suprapubic prostatectomy, and epithelium and stroma were separated mechanically by standard techniques. The assay procedure comprised homogenization in Tris-buffer, incubation of the homogenate with [3H]E1S or [3H]DHAS, separation of free steroids from nonhydrolyzed steroid sulfates by extraction with ether, and their final quantification by LSC. The main results were: (1) The pH-optimum of the sulfatase was found at pH 7.0. (2) The highest specific sulfatase activity was found in the epithelium and was associated with its nuclear fraction. (3) Michaelis–Menten constants \( K_m \) were 8.7 ± 1.4 (7) and 4.3 ± 0.8 (5), maximum velocity rates \( V_{max} \) (nmol/h x mgDNA) were 47.4 ± 8.8 (7) and 8.4 ± 1.5 (5) for E1S and DHAS, respectively (means ± SEM (n)). (4) The enzymatic cleavage of E1-sulfate was competitively inhibited by DHA-sulfate and vice versa with inhibition constants \( K_i \) of 4.0 ± 0.5 (2) for E1S and 2.7 ± 0.4 (2) for DHAS. On the basis of these findings, possible roles of steroid sulfate-sulfatases in forming precursors of active androgens and estrogens from the high amounts of E1S and DHAS in blood are discussed.

INTRODUCTION

Steroid sulfates quantitatively dominate the circulating steroid pool in blood. In man, plasma levels of dehydroepiandrosterone sulfate (DHAS) as well as of estrone sulfate (E1S) by far exceed the concentrations of the respective free steroids. Particularly DHAS has the highest plasma concentration of all detectable steroid metabolites, thus exceeding the level of the nonconjugated DHA by a factor of about 500 [1]. Similarly, E1S is found in an about 20 fold higher concentration in plasma as compared to free E1 [2, 3]. Rather little is known about the biological importance of these steroid sulfates, except in placental estrogen biosynthesis. This tissue converts fetal DHAS to estradiol [4] using subsequently catalyzing activities of four enzymes, i.e. steroid sulfate-sulfatase, the 3β-hydroxysteroid dehydrogenase/4,5-isomerase complex, aromatase, and the 17β-hydroxysteroid dehydrogenase (17β-HSDH).

The role of estrogens in the induction or promotion of prostatic growth, particularly of benign prostatic hyperplasia (BPH), is still open to question [5–8]. Furthermore, controversy exists concerning the question whether androgens can be aromatized directly in BPH tissue [9–13]. In addition to descriptive findings in human prostates [14, 15], particularly animal studies support the hypothesis that estrogenic effects might be involved in the development of BPH [16–19]. Intraprostatic formation of estrogens via the aromatase, however, did not seem to us to be an important pathway in estrogen supply of the human prostate [13].

Besides the aromatase, two alternative pathways, both of which require the cleavage of a steroid sulfate and subsequent 17β-reduction, could form estrogenic compounds within prostatic tissue: The cleavage and 17β-reduction of E1S would result in the formation of estradiol, the most potent natural estrogen. Correspondingly, 5-androstene-3β,17β-diol could be formed from DHAS. This steroid has also been reported to bind to the estrogen receptor and to induce estrogen dependent processes [20–22]. 17β-reduction of E1 as well as of DHA in human BPH tissue has already been described [23].

In earlier studies, we [24] and others [6, 25] noticed that the tissue levels of DHA and E1 in human benign hyperplastic prostates exceed those found in plasma. This tissue-plasma gradient may be explained by the presence of steroid sulfate-sulfatase activity within the prostate which in cooperation with the 17β-HSDH could form hormonal active compounds from available steroid sulfates. These findings may point to another possible biological role of steroid sulfates and of their enzymatic cleavage by sulfatase. The presented study described localization and properties of this enzyme in human BPH tissue.

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†Part of doctoral thesis.
EXPERIMENTAL

Chemicals

[6,7-3H]E1S (52 Ci/mmol), [7-3H]DHAS (23 Ci/mmol), [4,14C]E1 (55 mCi/mmol), [4-14C]DHA (50 mCi/mmol), and liquid scintillation cocktail (Biofluor®) were obtained from New England Nuclear (Dreieich). Nonlabelled steroid sulfates were purchased from Serva (Heidelberg). Purity of the radiolabelled steroid sulfates was checked by TLC (Silica gel G, ethyl acetate-

Nuclear (Dreieich). Nonlabelled steroid sulfates were correspondingly removed by ether extraction. Steroid sulfate concentrations of the remaining aqueous solutions were subsequently determined by one of the following procedures: DHAS was measured by RIA using a commercially available assay system (Coat-A-Count DHEA-SO4, 121 Kit, Diagnostic Products Corp, Los Angeles, Calif.) according to the recommendations of the manufacturer. E1S was hydrolyzed, and the free E1 was quantified by fluorescence spectroscopy using standard techniques [26].

Tissues

Prostates (weighing 30-160 g, mean 52 g) were obtained from 11 patients (age 56-82 yrs, mean 70 yrs) by suprapubic prostatectomy. After extirpation, the tissue was immediately placed on ice. One part was taken for histological examination, which confirmed the clinical diagnosis of BPH in all of the cases. The remainder of the organ was rapidly transported to the laboratory, cut into pieces of about 3 mm diameter, chilled with liquid nitrogen, and stored frozen at -20°C until further processing.

Preparation of tissue subfractions

All procedures were carried out at 0-4°C. Mechanical separation of epithelium and stroma essentially followed the method of Cowan et al. [27] as modified by Krieg et al. [14]. In brief, frozen pieces of tissue were mixed with 4 volumes of Tris-buffer, shired in a Bühler rotating edge-homogenizer (two periods of 30 and 15 s, respectively, with a cooling interval of 30 s) and the resulting tissue mash was filtered with gentle suction through 150-μm nylon gauze. From the filtrate, the epithelium was pelleted by centrifugation at 1000 g, taken up in 4 vols (wt/vol) of Tris-buffer, and homogenized in a Dounce apparatus with a tight-fitting pestle. This homogenate was frozen in liquid nitrogen and stored at -20°C. The material remaining on the nylon sieve was also taken up in Tris-buffer, shired further with an Ultraturrax and filtered again with strong suction. The retained stroma was washed with Tris-buffer, chilled with liquid nitrogen, pulverized, and was finally homogenized with 4 vols of Tris-buffer in a Dounce apparatus.

For preparation of subcellular fractions by differential centrifugation, epithelium was homogenized in Tris-buffer containing 0.25 M sucrose. The 800 g and 800-100,000 g pellets (both resuspended in Tris-buffer) and the 100,000 g supernatant of this homogenate represented the nuclear, mitochondrial + microsomal, and cytosolic fractions, respectively. Nuclei were further purified as described previously [28]. Before assay of sulfatase, the purified nuclei were finally washed with Tris-buffer in order to reduce the sucrose contamination.

Determination of enzyme activities

Samples were allowed to thaw at 4°C, and epithelium homogenates were further diluted with 1 or 3 vols of Tris-buffer (final dilutions: 1 + 9 and 1 + 19) for assay of DHAS or E1S sulfatase, respectively. Reaction mixtures of totally 400 μl in Pyrex® glass tubes with screw caps and Teflon® seals consisted of 200-μl portions of these diluted homogenates and 100 μl of each substrate, radio-labelled and nonlabelled, both dissolved in Tris-buffer. Substrate concentrations ranged 0.2-75.4 μM for E1S and 0.2-85.5 μM for DHAS with 1,1,1-diprop of the respective tritiated steroid sulfate. Reactions were started by addition of the homogenate and immediate transfer into a shaking waterbath. After incubation at 37°C for 30 min, 10-ml portions of ether were added together with 1000 dpm of either [14C]E1 or [14C]DHA in 100 μl of Tris-buffer in order to stop the reaction, to extract the formed free steroid by vigorous shaking for 2 min, and to determine the recovery of this extraction process. Separation of the two phases by freezing was performed as previously described in detail [29], and the ether phases were decanted into scintillation vials. After evaporation of the solvent, 10-ml portions of Biofluor® were added, the vials were shaken, and analyzed for 3H and 14C content in a Packard Tri-Carb® 460C liquid scintillation counter with quench correction by external standardization.

In order to measure sulfatase activities as functions of the pH, this procedure was slightly modified. The tissue was homogenized in 155 mM sodium chloride, which was also used to dissolve the tritiated as well as the nonlabelled substrates. Tris/EDTA/phosphate-buffers (25 mM Tris, 25 mM sodium phosphate, 5 mM EDTA, 25 mM magnesium chloride) were adjusted to various pH values between 4.0 and 9.0 by addition of either sodium hydroxide solution or hydrochloric acid. Reaction mixtures of totally 700 μl were composed of 400-μl portions of these buffers, 200-μl portions of the homogenate prepared in saline, and another 100 μl of 155 mM saline with dissolved
Steroid sulfate sulfatase labelled and nonlabelled substrate. All assays were run in duplicate with parallel incubation of heat-denaturated samples (95°C, 30 min) for blank correction. For substrate saturation curves, series of experiments with 10 different substrate concentrations were performed. \( K_m \) and \( V_{max} \) values were read from double-reciprocal plots according to Lineweaver and Burk [30] with regression lines calculated by the method of least squares. Inhibition constants \( K_i \) for competitive inhibition of E1S hydrolysis by DHAS and vice versa were determined according to the method of Dixon [31].

Miscellaneous

Protein and DNA were quantified by the Biuret reaction and the modified Burton reaction as described previously [29, 32]. Statistical comparisons were performed using the two-tailed t-test.

RESULTS

Evaluation of optimal incubation conditions

Cleavage of E1S and DHAS were investigated as functions of substrate and enzyme concentration, incubation time, temperature, and pH. Due to the high enzyme activity in this compartment, all optimization experiments were performed with isolated epithelium, which was diluted 1 + 9 for assays with DHAS and 1 + 19 for assays with E1S. With these amounts of tissue, acceptable conversion rates between 5% and 20% were obtained with substrate concentrations in the range 0.2-90 \( \mu \)M. Linearity between incubation time (Fig. 1) as well as amount of enzyme (Fig. 2) on the one hand and the velocity of the reaction on the other hand could be demonstrated up to 60 min and 300 \( \mu \)l of the diluted epithelial homogenate, respectively. With 30 min and 200 \( \mu \)l of homogenate, standard assay conditions were chosen within these ranges of linearity. For both substrates, variation of the incubation temperature (Fig. 3) and of the buffer pH (Fig. 4) revealed maximal enzyme activities at 60°C and pH 7.0, respectively. The sulfatase was found to be more resistant to an alkaline than to an acidic milieu. For the standard assay conditions, physiological values of 37°C and pH 7.4 were chosen.

Quality control

The assay procedure revealed acceptable characteristics with respect to specificity, sensitivity, and preci-
Table 1. Subcellular distribution of steroid sulfate-sulfatase in epithelium of human BPH tissue as measured by differential centrifugation. Sulfatase activities were measured by one-point assays with 30 μM of the respective substrate and are given as percent of the initial activity detectable in unfractionated epithelial homogenate. Means of two determinations.

<table>
<thead>
<tr>
<th>Subcellular Fraction</th>
<th>EI S</th>
<th>DHAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole epithelium</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>800 g fraction</td>
<td>108%</td>
<td>90%</td>
</tr>
<tr>
<td>Purified nuclei</td>
<td>89%</td>
<td>61%</td>
</tr>
<tr>
<td>Mitochondrial + microsomal</td>
<td>1.5%</td>
<td>2.4%</td>
</tr>
<tr>
<td>Cytosol</td>
<td>1.3%</td>
<td>2.1%</td>
</tr>
</tbody>
</table>

Recovery rates of EI and DHAS during the separation of free steroids from their nonhydrolyzed sulfates were about 94%, and no nonhydrolyzed steroid sulfates could be detected in the organic phase. The results were not influenced by other enzymes present in prostatic tissue: Products of secondary metabolic modification of the formed free steroids, e.g. the 17β-reduction products 17β-estradiol and 5-androstene-3β,17β-diol were simultaneously extracted and were consequently included in the account of sulfatase activity. Under the standard assay conditions, blanks were found to be in the order of 1–2%, and conversion rates of 3% were considered to be the detection limit. Coefficients of variation were found below 10% for intra-assay as well as inter-assay precision.

Localization and characterization of the enzyme

Related to the amount of tissue, sulfatase activities measured in isolated epithelium by far exceeded activities detected in stromal preparations. In two independent experiments, enzyme activities in stroma did not significantly differ from values which had to be expected from the contamination of these preparations by residual epithelium. Therefore, our further experiments were focused on the epithelial activity.

During cell fractionation experiments by differential centrifugation, recovery of sulfatase activity from step to step exactly paralleled the recovery of DNA. The bulk of the enzyme activity initially measured in epithelial homogenate was found associated with the 800 g pellet and later with the fraction of purified nuclei, whereas only trace amounts of sulfatase activity could be detected in the mitochondrial + microsomal and cytosolic fractions (Table 1). These findings indicate a nuclear or perinuclear localization of the sulfatase. Additionally, these experiments revealed the enzyme’s sensitivity to high osmolarity: the presence of 1.0 M sucrose reduced conversion rates by 50%. Concentrations in the reaction mixture up to 0.4 M did not influence the activity (data not shown).

Maximum velocities (V_max) and Michaelis–Menten constants (K_m) of the EI S as well as DHAS cleavage determined by saturation analysis (Fig. 5) in purified epithelium of several prostates are given in Table 2. In contrast to the similar K_m values in the μM range, all samples demonstrated 8-fold higher V_max values for hydrolysis of EI S as compared to DHAS.

DHAS competitively inhibited the cleavage of EI S (Fig. 6) and vice versa. The inhibition constants (K_i) of both sulfates were found in the order of their K_m values (Table 2).

DISCUSSION

Our kinetic data concerning DHAS sulfatase activity in human BPH tissue support earlier data of
Farnsworth [33]. He found a $K_a$ of 5 $\mu$M, which is very similar to the results of our experiments. This author also concluded from measurements in predominantly epithelial and stromal tissue specimens that the enzyme in question is mainly located in the former compartment of BPH, as we do from our measurements in the separated tissue fractions.

EIS sulfatase activity in whole human BPH tissue has been detected earlier by Carlström et al. [34]. Although no $K_m$ values were given, these investigators stated a severalfold higher activity in prostatic tissue than in muscle tissue.

We assume that both activities are properties of one enzyme. Similar $K_m$ values, a high degree of correlation between individual $V_{max}$ values, the similar subcellular localization, and the inhibition data favour this assumption. These data, along with the pH-optimum, the thermal stability, and the higher reaction rates for the substrate EIS as compared to DHAS are features of enzymes detected in other human organs, e.g. the endometrium [35], liver [36], lung [37], fetal membranes and decidua [38], but differ with respect to the substrate specificity from those detected in amnion tissue [39], which cleave only EIS.

Intraprostatic cleavage of steroid sulfates yielding active estrogenic compounds may be important in regulating the intensity of estrogenic stimulation and the growth of the prostate, and finally may also be involved in the growth control of the diseased prostate, i.e. BPH. Our study clearly demonstrates the major prerequisite of this pathway, the presence of the respective enzyme apparatus, i.e. the steroid sulfatase sulfatase. The estimation of its biological significance and of its involvement in the intracellular formation of free steroids, however, requires further resolution of two problems:

—From the cellular localization of steroid sulfate sulfatase, it can be concluded that the enzyme is not an important secretory protein of the prostate. This means, that the relatively polar substrates have to pass the cell membrane before cleavage. A transport mechanism for these compounds is not known.

—in view of the high metabolic activity of the steroid sulfate sulfatase, the high DHAS and EIS levels in plasma, and a plasma contamination of prostatic tissue in the range of 10% [40], care has to be taken in the interpretation of prostatic unconjugated DHA and E1 concentrations. During homogenization of the tissue, intracellular sulfatase could cleave extracellular sulfates to an unknown extent and artificially increase concentrations of unconjugated steroids, unless special precautions have been taken. Therefore, it might be difficult to discriminate between in vivo alterations in unconjugated DHA and E1 levels and artefacts.

Experiments from which the biological importance of the sulfatase pathway can be derived have been performed in mammary carcinoma. These tumors also possess steroid sulfate sulfatase. Vignon et al. [41] have shown that the behaviour of E1S in cultured breast cancer cells is similar to that of the unconjugated steroid. Santner et al. [42] have compared the relative importance of the sulfatase and aromatase pathways in the intratissular production of estrone in mammary carcinomas, and they came to the conclusion that the sulfatase pathway might be more important in these tissues although both seem to contribute to estrone formation. In view of low or even absent aromatase activity in the prostate [13], the sulfatase pathway might be even more important in this organ than in mammary cancer for stimulation of estrogen dependent processes.

REFERENCES

14. Krieg M., Klötzel G., Kaufmann J. and Voigt K. D.:


