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Steroid sulfatase, arylsulfatases A and B, galactose-6-sulfatase, and iduronate sulfatase in mammary cells and effects of sulfated and non-sulfated estrogens on sulfatase activity

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Abstract

Sulfatase enzymes have important roles in metabolism of steroid hormones and of glycosaminoglycans (GAGs). The activity of five sulfatase enzymes, including steroid sulfatase (STS; arylsulfatase C), arylsulfatase A (ASA; cerebroside sulfatase), arylsulfatase B (ASB; *N*-acetylgalactosamine-4-sulfatase), galactose-6-sulfatase (GALNS), and iduronate-2-sulfatase (IDS), was compared in six different mammary cell lines, including the malignant mammary cell lines MCF7, T47D, and HCC1937, the MCF10A cell line which is associated with fibrocystic disease, and in primary epithelial and myoepithelial cell lines established from reduction mammoplasty. The effects of estrogen hormones, including estrone, estradiol, estrone 3-sulfate, and estradiol sulfate on activity of these sulfatases were determined. The malignant cell lines MCF7 and T47D had markedly less activity of STS, ASB, ASA, and GAL6S, but not IDS. The primary myoepithelial cells had highest activity of STS and ASB, and the normal epithelial cells had highest activity of GALNS and ASA. Greater declines in sulfatase activity occurred in response to estrone and estradiol than sulfated estrogens. The study findings demonstrated marked variation in sulfatase activity and in effects of exogenous estrogens on sulfatase activity among the different mammary cell types.

Keywords: Sulfatase; Steroid sulfatase; Estrogen; Estradiol; Estrone sulfate; Estrone; Arylsulfatase B; Arylsulfatase A; Iduronate sulfatase; Galactose-6-sulfatase; Glycosaminoglycan; Peptidoglycan; Hormone replacement therapy

1. Introduction

Steroid sulfatase (STS) is a member of the family of highly conserved sulfatase enzymes that are required to remove specific sulfate residues. Steroid sulfatase is required for the desulfation of the sulfated steroids, including estrone 3-sulfate, cholesterol sulfate, and dehydroepiandrosterone sulfate, and activity of STS leads to the generation of active steroid hormones from inactive sulfated precursors. Changes in STS activity have an important role in mammary cells, since the formation of estrone from estrone 3-sulfate by the action of STS is quantitatively more important than production of estrone from androstenedione by aromatase. *In vitro* STS activity is reported to be 1000 times or more higher than aromatase activity in most breast tissues. Also, serum levels of estrone sulfate (E1S) are as much as 10 times higher than those of unconjugated estrone and estradiol, and the half-life of E1S is much longer than the half-life of unconjugated estrogen, suggesting an important regulatory role for STS in estrogen metabolism [1–5].

Intracellularly, steroid sulfatase is localized in the rough endoplasmic reticulum, whereas arylsulfatases A and B, galactose-6-sulfatase, and iduronate-2-sulfatase are found predominantly in endosomes and lysosomes. The sulfatase hormones acquire activity following post-translational modification, leading to the conversion of a conserved cysteine residue to a formyl glycine. Activated sulfatases can remove

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sulfate groups from glycosaminoglycans (GAGs), including chondroitin sulfate, heparin, heparan sulfate, keratan sulfate, and dermatan sulfate [6–8]. The proteoglycans with which the GAGs are associated are critical for cell–cell and cell–matrix interactions [9].

Arylsulfatase A (ASA; cerebroside sulfatase) and arylsulfatase B (ASB; *N*-acetylgalactosamine-4-sulfatase) have been reported to vary during the ovulatory cycle. In premenopausal women, ASA activity was found to be highest during the ovulatory phase and lowest during the early follicular phase. In contrast, in postmenopausal women, the level did not fluctuate and was similar to that found during the early follicular phase. Studies in rabbits demonstrated that estrogen exerted an inducing effect on ASA, whereas progesterone was inhibitory. ASA activity increased during the follicular phase and decreased during the luteal phase in the rabbit endometrium. In contrast, ASB activity decreased during the follicular phase and increased during the luteal phase. The level of ASA activity was greater than that of ASB [10–12].

In males, human seminal plasma has significantly lower activity of arylsulfatase in infertile men than normal subjects [13]. Also, ASB was detected in both seminal plasma and extracellular fluids of the human testis. In veterinary studies, ASB was found to be secreted by the seminal vesicles of boars [14]. Thus, arylsulfatases may have important roles in male, as well as female reproduction.

Since sulfate has been identified as a negative regulator of sulfatase, changes in activity of one sulfatase may impact on activity of other sulfatases. Also, phosphate has been identified as an inhibitor of sulfatase activity [15,16].

With this background in mind, we have studied the activity of five human sulfatases, including STS, ASB, ASA, GALNS, and iduronate-2-sulfatase (IDS) in malignant and normal mammary cells in tissue culture. We have determined the effects of exposure to four estrogenic hormones [estrone (E1), estradiol (E2), estrone 3-sulfate (E1S), and estradiol sulfate (E2S)] on sulfatase activity in MCF7, T-47D, HCC1937, and MCF10A cell lines, and in non-malignant epithelial (EC) and myoepithelial (MEC) cells from primary cell cultures. Since the majority of human hormonal replacement therapy (HRT) trials have used sulfated estrogens, such as premarin, we have tested for differences in effects of sulfated versus non-sulfated hormones on sulfatase activity in these mammary cells. Our previous work demonstrated markedly increased activity of STS in normal MEC compared to malignant MCF7 cells, and marked inhibition of STS activity in the MCF7 cells by estradiol [17]. The potent inhibitory effect of estradiol on estrogen sulfatase activity and, thereby, on formation of estradiol in T47D and MCF7 cells has been demonstrated previously by other investigators, as well [18]. This report extends the previous observations, by comparing the activity of five different sulfatases in six mammary cell types and measuring the effects of exposure to sulfated versus non-sulfated hormones on sulfatase enzyme activity.

2. Materials and methods

2.1. Cell lines and growth conditions

Malignant cell lines MCF7, T47D, and HCC1937 and the fibrocystic disease cell line MCF10A were obtained from ATCC (Manassas, VA, USA), and specifications for media and growth conditions were followed. Mammary cells were plated on six-well uncoated plates in a 5% CO_2 environment at 37 °C. Cells reached 80% confluence in about 1 week and were harvested by scraping. MCF7 cells were grown in phenol-red free RPMI 1640 with 10% FBS and penicillin (100 U/ml) and streptomycin (100 μg/ml) antibiotics. T47D and HCC1937 malignant mammary cell lines were grown in RPMI 1640 medium with 2 mM L-glutamine, 1.5 g/l sodium bicarbonate, 4.5 g/l glucose, 10 mM HEPES, and 1.0 mM sodium pyruvate, 10% FBS and penicillin–streptomycin antibiotics. Human recombinant insulin 0.2 U/ml was added to the media for the T47D cells. MCF10A cells were grown in serum-free, phenol-red free Mammary Epithelial Growth Media (MEGM; Cambrex, NJ, USA), supplemented with hydrocortisone, bovine pituitary extract hepidermal growth factor, insulin, gentamicin/amphotericin-B, and 100 ng/ml cholera toxin. Human myoepithelial cells and epithelial cells were cultivated from mammary tissue obtained from reduction mammoplasty in accord with a protocol approved by the Institutional Review Board of the University of Illinois at Chicago. Myoepithelial cells (MEC) were grown in Ham's F-12 media supplemented with 10% FBS. Methods used to obtain MEC from reduction mammoplasty have been described previously [19]. Primary human mammary epithelial (EP) cells were separated from the MEC by selective use of media and by growth on a Matrigel (Sigma) substrate. The EP cells were grown using MEGM without cholera toxin. The MCF7 and T47D cells are positive for estrogen receptors, and the HCC1937 and MCF10A cells are negative for estrogen receptors (ATCC, Manassas, VA) [20]. Protein determinations of the cell lysate and the media were performed using a standard BCA protein assay kit (Pierce). Photomicrographs were taken of the primary epithelial cells in culture using the Moticam 2000 camera attachment to the Motic AE31 inverted microscope. MEC were grown directly on four-compartment slides and stained for α-smooth muscle specific antibody (SMSA, Sigma) using standard methods for immunostaining [19]. Fig. 1, Panel A demonstrates the normal primary epithelial cells and Panel B the primary MEC stained for SMSA.

2.2. Sulfatase assays

Determinations of activity of five sulfatases were performed in MCF7, MCF10A, T47D, and HCC1937 cell lines, as well as MEC and EP cells. Measurements were made from cell lysates of cultures grown to 80% confluence. Similar methods were used for each enzyme assay in the different cell types. Assays were performed using triplicate biological samples with technical replicates of each measurement (Fig. 2).