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Characterization of equine GST A3-3 as a steroid isomerase

Helena Lindström[a], Shawna M. Peer[b], Nancy H. Ing[b,*], Bengt Mannervik[a,*]

[a] Department of Neurochemistry, Stockholm University, Arrhenius Laboratories, SE-10691 Stockholm, Sweden
[b] Department of Animal Science, Texas A&M University, 2471 TAMU, College Station, TX 77843-2471, USA

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ABSTRACT

Glutathione transferases (GSTs) comprise a superfamily of enzymes prominently involved in detoxication by making toxic electrophiles more polar and therefore more easily excretable. However some GSTs have developed alternative functions. Thus, a member of the Alpha class GSTs in pig and human tissues is involved in steroid hormone biosynthesis, catalyzing the obligatory double-bond isomerization of Δ^5-androstene-3,17-dione to Δ^4-androstene-3,17-dione and of Δ^5-pregnene-3,20-dione to Δ^4-pregnene-3,20-dione on the biosynthetic pathways to testosterone and progesterone. The human GST A3-3 is the most efficient steroid double-bond isomerase known so far in mammals. The current work extends discoveries of GST enzymes that act in the steroidogenic pathways in large mammals. The mRNA encoding the steroid isomerase GST A3-3 was cloned from testis of the horse (*Equus ferus caballus)*. The concentrations of GSTA3 mRNA were highest in hormone-producing organs such as ovary, testis and adrenal gland. EcaGST A3-3 produced in *E. coli* has been characterized and shown to have highly efficient steroid double-bond isomerase activity, exceeding its activities with conventional GST substrates. The enzyme now ranks as one of the most efficient steroid isomerases known in mammals and approaches the activity of the bacterial ketosteroid isomerase, one of the most efficient enzymes of all categories known today. The high efficiency and the tissue distribution of EcaGST A3-3 support the view that the enzyme plays a physiologically significant role in the biosynthesis of steroid hormones.

1. Introduction

Hydrophobic exogenous and endogenous electrophiles cause damage to mammalian organisms as they react with proteins, DNA and other essential cell components that feature exposed nucleophilic centers. Glutathione transferases (GSTs) comprise a superfamily of enzymes (EC 2.5.1.18) prominently involved in detoxication processes by conjugating the polar tripeptide glutathione (γ-Glu-Cys-Gly) to hydrophobic electrophiles, thereby forming polar conjugates more easily excretable from the organism [1–3]. However, certain enzymes from some GST classes have developed alternative functions. Examples include GST P1-1 that acts as modulator of protein kinases involved in cellular signaling [4–6] and GST S1-1, known as prostaglandin D synthase that catalyzes the conversion of prostaglandin H_2 into prostaglandin D_2 [7,8].

Among the alternative GST functions a role in steroid hormone biosynthesis has emerged for the human alpha-class enzyme GST A3-3 [9,10]. The enzyme is an efficient catalyst of the obligatory double-bond isomerization of Δ^5-androstene-3,17-dione (Δ^5-AD) to Δ^4-androstene-3,17-dione (Δ^4-AD) and of Δ^5-pregnene-3,20-dione (Δ^5-PD) to Δ^4-pregnene-3,20-dione (Δ^4-PD) in the biosynthetic pathways of testosterone and progesterone, respectively (Fig. 1). Human GST A3-3 (HsaGST A3-3) is prominently expressed in steroidogenic tissues indicating its physiological role, and experiments with cell lines supported this notion [11]. However, no direct support for the involvement of the enzyme in steroid hormone biosynthesis has been available at the organism level.

To extend the characterization of alpha-class GST enzymes involved in steroid hormone isomerization to other species, homologs of human GSTA3 mRNA were cloned from pig testis and ovary [12] and bovine ovary [13]. When the porcine SscGST A2-2 and the bovine BtaGST A1-1 proteins were expressed in vitro, however, they were considerably less active as steroid isomerases than HsaGST A3-3. Specifically, SscGST A2-2 was more active than BtaGST A1-1, but it had four to five-fold lower specific activity with Δ^5-AD and 19-fold lower specific activity with Δ^5-PD than HsaGST A3-3. It is notable that rodents utilize the Δ^4 pathway of steroidogenesis [14] and apparently lack any homolog with the high isomerase activity distinguishing the human GST A3-3 enzyme.

Recently, the search for alpha-class GST enzymes with steroid isomerase activities was extended to the horse. Equine GST A3-3 (EcaGST A3-3) was discovered and its mRNA cloned from stallion testis [15]. Testosterone levels in stallion serum were suppressed in parallel with

* Corresponding authors.
E-mail addresses: NING@cvm.tamu.edu (N.H. Ing), bengt.mannervik@neurochem.su.se (B. Mannervik).

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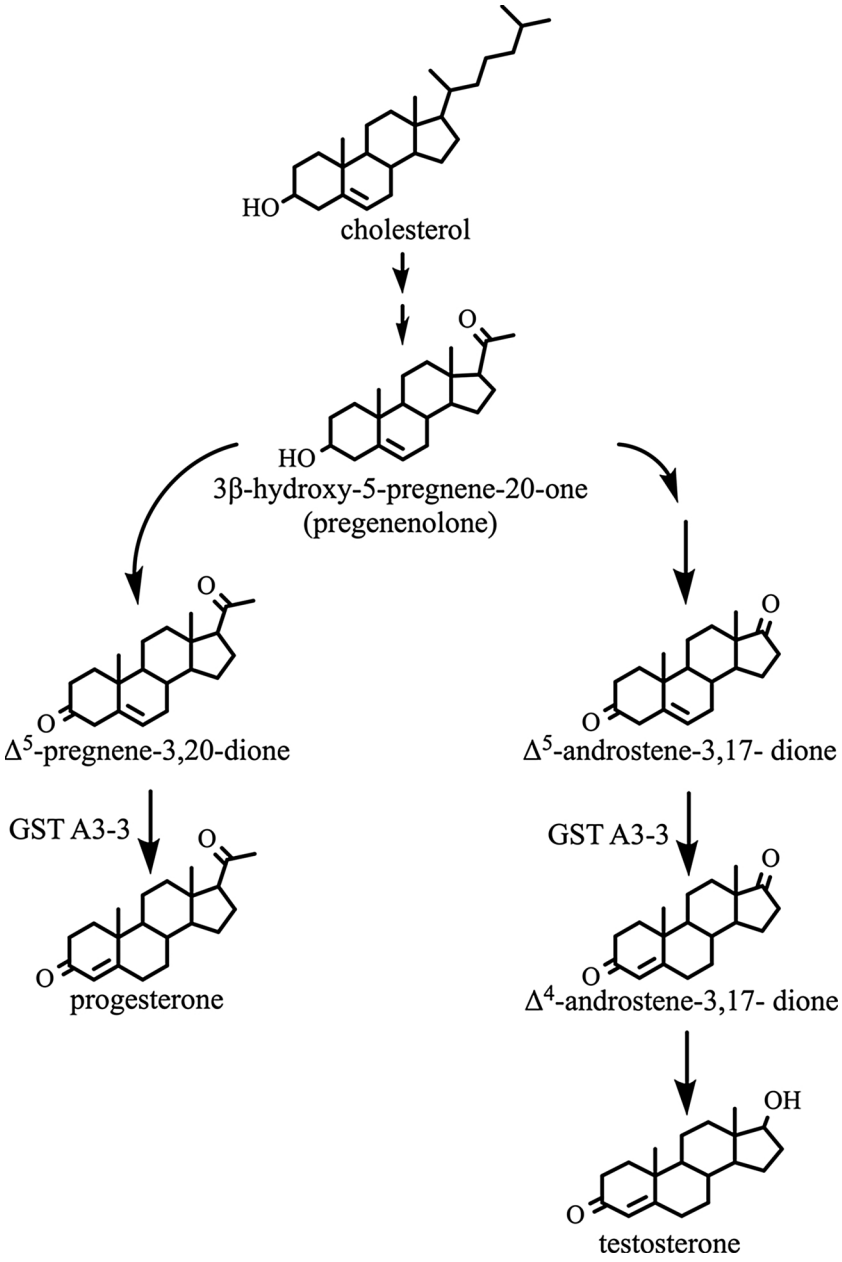

Fig. 1. Outline of the biosynthetic pathway of steroidogenesis leading to progesterone and testosterone indicating steps efficiently catalyzed by GST A3-3.

EcaGSTA3 mRNA concentrations in testes within 12 h of treatment with dexamethasone, a synthetic glucocorticoid. In addition, the Δ^5-AD isomerase activity in cytosolic extracts from testes of dexamethasone-treated stallions in vitro decreased by 50% compared to testis extracts from control stallions. This direct pharmacological evidence suggested that the horse may have an alpha-class GST enzyme with similar high steroid isomerase activity as HsaGST A3-3.

In the present investigation the steroid isomerase EcaGST A3-3 from E*quus ferus caballus* has been characterized and shown to be an enzyme efficiently catalyzing the double-bond isomerization of Δ^5-AD to Δ^4-AD and Δ^5-PD to Δ^4-PD. The catalytic efficiency of the equine enzyme matches or exceeds the high activity of the counterpart HsaGST A3-3 from *Homo sapiens*, previously considered the most efficient mammalian steroid isomerase [10]. Comparisons were also made with porcine SscGST A1-1 and SscGST A2-2 from *Sus scrofa domesticus* [12] and bovine BtaGST A1-1 from *Bos taurus* [13].

2. Results

2.1. Expression of mRNA from the equine GSTA3 gene

The horse genome contains several alpha-class GST (*GSTA*) genes, and in order to distinguish the different transcripts it was necessary to determine their sequences and quantify them by RT-PCR, as previously done for the human GSTA3 mRNA [16]. As demonstrated in the case of the human gene [9,10,17] several shorter splice variants were noted in addition to the equine full length mRNA. The concentration of equine GST A3-3 mRNA was measured by quantitative RT-PCR in 15 different tissues from adult horses. The tissues included the adrenal gland, cerebrum, heart, hypothalamus, kidney cortex, liver, lung, mammary gland, ovary, skeletal muscle, small intestine, spleen, urinary bladder, and uterus. Fig. 2 presents the results, with all values normalized relative to the GSTA3 mRNA concentrations in the mammary gland. As expected, the highest levels of GSTA3 gene expression were noted in the steroidogenic tissues ovary, adrenal gland, and testis (Fig. 2a); the lowest levels were several orders below those, such as in mammary