Evolutionary analysis of 11β-hydroxysteroid dehydrogenase-type 1, -type 2, -type 3 and 17β-hydroxysteroid dehydrogenase-type 2 in fish

Michael E. Baker*

Department of Medicine-0693, University of California, San Diego 9500 Gilman Drive, La Jolla, CA 92093-0693, USA

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Abstract Steroid dehydrogenases regulate the access of active steroids to their receptors. In particular, 11β-hydroxysteroid dehydrogenase-type 1 (11β-HSD1) and 11β-HSD2 regulate the levels of glucocorticoids, such as cortisol, and 17β-HSD1 and 17β-HSD2 regulate the levels of androgens and estrogens. Human 11β-HSD1 and 11β-HSD2 are distant homologs, with less than 25% amino acid sequence identity, as are human 17β-HSD1 and 17β-HSD2. In contrast, human 11β-HSD2 and 17β-HSD2 are close homologs, with about 43% sequence identity. Until recently, deciphering early events in the evolution of 11β-HSD2 and 17β-HSD2 was difficult because only mammalian sequences were available. The completely sequenced Takifugu, Tetraodon and medaka genomes and the almost completed zebrafish genome provide an opportunity to investigate the evolution of 11β-HSD2, 17β-HSD2, and 11β-HSD1. Unexpectedly, a search of the Takifugu, Tetraodon and medaka genomes only found an ortholog to 11β-HSD2 and none to 17β-HSD2, while the zebrafish genome contains orthologs of both enzymes. This suggests that 17β-HSD2 was lost in teleosts after the divergence of zebrafish and medaka. Also unexpectedly, searches with 11β-HSD1 only identified several fish 11β-HSD3s, as well as an ortholog in Ciona, indicating that 11β-HSD3 is the ancestor of 11β-HSD1.

Keywords: 11β-Hydroxysteroid dehydrogenase-type 1; 11β-Hydroxysteroid dehydrogenase-type 2; 17β-Hydroxysteroid dehydrogenase-type 2; Evolution

1. Introduction

Enzymes that catalyze the synthesis and inactivation of steroids constitute an important mechanism for regulating the actions of steroids [1–6]. Of particular interest are 11β-hydroxysteroid dehydrogenase (11β-HSD), which regulates the levels of active glucocorticoids, and 17β-hydroxysteroid dehydrogenase (17β-HSD), which regulates the levels of active androgens and estrogens (Fig. 1). Although, in principle, each enzyme reaction is reversible, in vivo, two distinct 11β-HSDs are used to regulate glucocorticoids: 11β-HSD-type 1 (11β-HSD1), an NADPH-dependent reductase, which converts cortisone to cortisol, and 11β-HSD-2, an NAD^+-dependent oxidase, which converts cortisol to cortisone. 11β-HSD1 and 11β-HSD2 have less than 25% sequence identity, indicating that these two enzymes are not isoforms [7].

A similar mechanism is used to regulate estradiol levels in mammals. Although there are at least ten 17β-HSDs [2–4,6], two of these enzymes have functional similarities to 11β-HSD1 and 11β-HSD2. 17β-HSD1 is an NADPH-dependent reductase, catalyzing the conversion of estrone to estradiol; 17β-HSD2 is an NAD^+-dependent oxidase, catalyzing the oxidation of C17 alcohol to a ketone on estradiol and testosterone (Fig. 1). 17β-HSD1 and 17β-HSD2 have less than 25% sequence identity [8,9].

The type 2 enzymes, 11β-HSD2 and 17β-HSD2, constitute an important mechanism for controlling access of active glucocorticoids [1,9–12] and androgens and estrogens [2–4,6,9] to their receptor. 11β-HSD2 also has an important role in the biological actions of aldosterone, the main mineralocorticoid in mammals, because cortisol and aldosterone have similar affinities for the mineralocorticoid receptor (MR) [13]. Since circulating concentrations of glucocorticoids are at least 100-fold higher than that of aldosterone, one would expect under normal physiological conditions that glucocorticoids occupy the MR and regulate gene transcription, instead of aldosterone. However, unlike cortisol and corticosterone, aldosterone is inert to 11β-HSD2. Thus, selective expression of 11β-HSD2 in target tissues for mineralocorticoids, such as the distal tubule of the kidney, allows aldosterone, but not glucocorticoids, to bind to the MR and regulate transcription of genes that characterize the mineralocorticoid response [10,11].

Although the two 11β-HSDs are distant homologs, as are the type 1 and type 2 17β-HSDs, human 11β-HSD2 and 17β-HSD2 are close homologs; their amino acid sequences are about 43% identical [1,7,9]. Until recently, little was known about early events in the evolution of 11β-HSD2 and 17β-HSD2, as well as 11β-HSD1 and 17β-HSD1, because only mammalian sequences were available. For example, did the common ancestor of 11β-/17β-HSD2 metabolize glucocorticoids, androgens, estrogens or a combination? Further complicating this question is that rainbow trout 11β-HSD2 oxidizes 11β-hydroxy-testosterone to 11-keto-testosterone (Fig. 1), as well oxidizing cortisol to cortisone [14]. 11-keto-testosterone is the active androgen in fish [15,16].

However, conditions have improved considerably for an analysis of the origins and evolution of steroid dehydrogenase due to the recent cloning of genes from fish for 17β-HSD1 [17,18] and 11β-HSD2 [14,19], the completion of two pufferfish genomes, *Takifugu rubripes* and *Tetraodon nigroviridis*, and the

* Fax: +1-619-543-7069.
E-mail address: mbaker@ucsd.edu.

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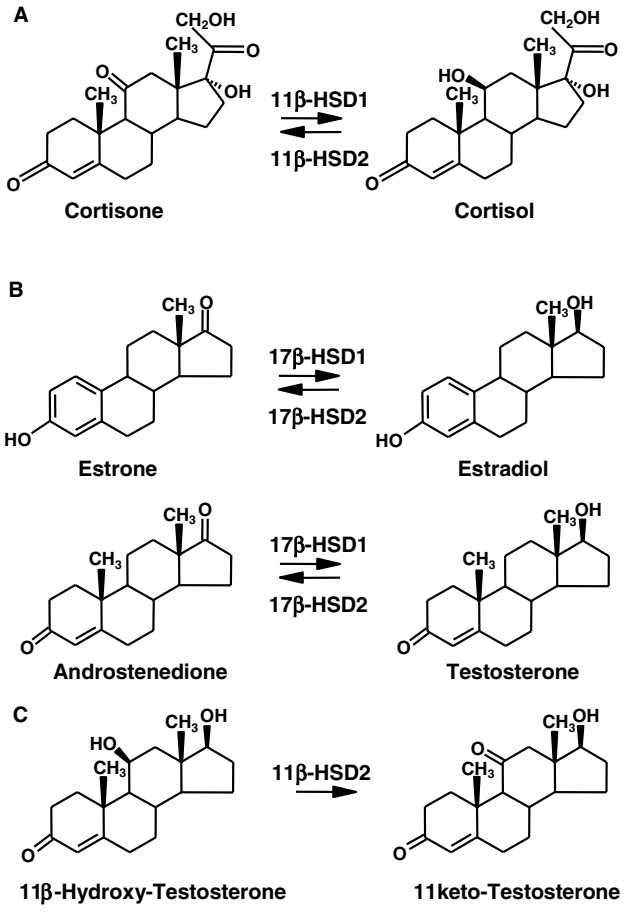

Fig. 1. Steroids metabolized by 11β-hydroxysteroid dehydrogenase-type 1 and -type 2 and 17β-hydroxysteroid dehydrogenase-type 1 and -type 2. (A) 11β-HSD1 reduces the C11 ketone on cortisone to yield cortisol. 11β-HSD2 oxidizes the C11 alcohol on cortisol. Cortisone is an inactive glucocorticoid. (B) Mammalian 17β-HSD1 reduces the C17 ketone on estrone to yield estradiol. 17β-HSD2 oxidizes the C17 alcohol on estradiol and testosterone to form estrone and androstenedione, respectively. Estrone and androstenedione are a weak estrogen and androgen, respectively. (C) Fish 11β-HSD2 oxidizes the C11 alcohol on testosterone to yield 11-ketotestosterone, an active androgen in fish.

medaka genome, and substantial progress in completing the zebrafish genome.

With this in mind, we searched databases containing Takifugu, Tetraodon, medaka and zebrafish genes with human and fish 11β-HSD2 and human 17β-HSD2 and 11β-HSD1 for orthologs. As reported here, we find an ortholog of 17β-HSD2 in zebrafish, but not in Takifugu, Tetraodon or medaka. Moreover, searches with human 11β-HSD1 did not find an ortholog in Takifugu, Tetraodon, medaka or zebrafish. Instead, these fish contain a gene that is closest to a recently cloned human short chain dehydrogenase/reductase-10b (SDR10b) (Huang et al. GenBank Accession No. AAP42286). This enzyme in chicken and fish has been called 11β-HSD3 by Huang et al., because 11β-HSD3 is about 48% identical to that of 11β-HSD1. This indicates that although the two enzymes are close, they are not isoforms and likely have some differences in substrate specificity. We also find that Ciona contains an ortholog to 11β-HSD3, indicating that 11β-HSD3 is the ancestor of 11β-HSD1. We propose that 11β-HSD1 arose after the divergence of ray-finned fish and lobe-finned fish and that pufferfish, medaka and other ray-finned fish have lost 17β-HSD2. In these fish, other enzymes [2–4,6] oxidize the C17 alcohol on androgens and estrogens.

2. Materials and methods

11β-HSD2 sequences of trout (BAC76709), tilapia (AAO42610), eel (BAC67576) and human (P80365), 17β-HSD2 of human (P37059) and 11β-HSD1 of human (DXHUBH), and frog (AAH54291) were extracted from GenBank (www.ncbi.nlm.nih.gov/entrez). BLAST [20] was used to extract Takifugu 11β-HSD2 (FuguGenscan_1031) from (www.ncbi.nlm.nih.gov/BLAST/Genome/fugu.html, zebrafish 11β-HSD2 (TC168653) from the TIGR database (www.tigr.org/tdb/tgi/zgi), Tetraodon 11β-HSD2 (47214187) from GenBank and chicken 11β HSD1 (Contig65.205.1.2284.869.2108), 11β-HSD2 (Contig100.101.1.33711.25364.32850) and 17β-HSD2 (Contig156.8.1.9857.139.7822) from Ensembl (http://www.ensembl.org/).

A search of the June 1, 2004 release of the zebrafish database in TIGR found an ortholog to 17β-HSD2 (TC245819). The protein and nucleotide sequences of zebrafish and human 17β-HSD2 were used to search Takifugu, Tetraodon (http://www.genoscope.cns.fr/externe/English/corps_anglais.html) and medaka (http://www.dolphin.lab.nig.ac.jp/medaka/index.php) databases. The highest scoring matches were found to be fish 11β-HSD2 sequences. This conclusion was reached after a two-step analysis. First, a BLAST search with 17β-HSD2 of Takifugu, Tetraodon and medaka genomes retrieved the highest scoring protein. Then, the non-redundant database GenBank was ''back-searched'' with the high scoring protein, which in all cases, was most similar to 11β-HSD2.

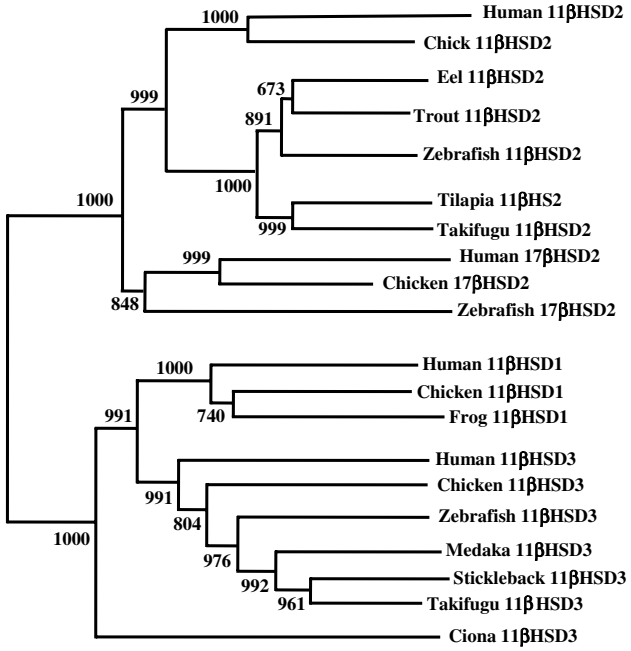

Fig. 2. Phylogenetic analysis of 11β-hydroxysteroid dehydrogenase-type 1 and -type 2 and 17β-hydroxysteroid dehydrogenase-type 2. The Clustal X program [21] (ftp://ftp-igbmc.u-strasbg.fr/pub/ClustalX) with 1000 bootstrap trials was used to construct the phylogenetic tree. Branch lengths are proportional to the distances between each protein. Bootstrap values are the number of trials that this cluster was found in 1000 trials. There are six alternatively spliced isoforms of 11β-HSD3 (SDR10) in GenBank. Only SDR10-isoform b (286 residues) and SDR10-isoform e (315 residues) contain a sequence that overlaps human 11β-HSD1 (data not shown).

A BLAST search with human 11β-HSD1 found Takifugu (SINFRUP00000071282) and zebrafish (AAH52103), which were then found to be closest to human SDR10b (AAP42286), one of several spliced variants (see legend to Fig. 2), which Huang et al. describe as similar to 11β-HSD1, and which we will call 11β-HSD3 because this is the name used by Huang et al. for orthologs in other species. Further searches of GenBank found 11β-HSD3 from chicken (AAS89255), medaka (AAS89258), three spined stickleback (AAS89257), Tetraodon (CAG01365) and Ciona (AK116129). The available sequences of Tetraodon 11β-HSD2 (272 amino acids) and 11β-HSD3 (207 amino acids) are incomplete. Thus, they were not included in the phylogenetic tree.

Phylogenetic analysis was done with Clustal X [21].

3. Results

3.1. Phylogenetic analysis of 11β-HSD1, 11β-HSD2, 11β-HSD3 and 17β-HSD2

The phylogenetic relationship of 11β-HSD1, 11β-HSD2, 11β-HSD3 and 17β-HSD2 to each other is shown in Fig. 2. 11β-HSD1 and 11β-HSD3 cluster together, similar to clustering of 11β-HSD2 and 17β-HSD2.

The distances between branches in Fig. 2 indicate that fish 11β-HSD2 and 11β-HSD3 are close to their fish orthologs. This is consistent with pairwise sequence comparisons, which indicate that each fish ortholog is from 55% to 75% identical to the other fish orthologs (data not shown). Based on this similarity, we expect that BLAST searches with zebrafish 17β HSD2 of Takifugu, Tetraodon and medaka genomes would find a sequence with at least 50% identity, if a 17β HSD2 ortholog is present in these genomes.

3.2. Takifugu, Tetraodon and medaka do not contain a 17β-HSD2 ortholog

Several fish contain 11β-HSD2 [14,19] (Fig. 2). BLAST analysis with human 17β-HSD2 of the TIGR zebrafish EST database found a 17β-HSD2 ortholog. However, to our surprise, a BLAST search of Takifugu, Tetraodon and medaka genomes with human and zebrafish 17β-HSD2 did not find a 17β-HSD2. All high scoring sequences in the search were found to be 11β-HSD2 orthologs, upon back-searching of GenBank.

3.3. Fish contain 11β-HSD3, an ancestor of 11β-HSD1

BLAST searches with 11β-HSD1 of the Takifugu, Tetraodon, medaka and zebrafish genomes and GenBank identified 11β-HSD3, which is closest to human SDR10b (11β-HSD3), and which Huang et al. noted is close to 11β-HSD1. Moreover, GenBank contains a Ciona protein that is about 40% identical (data not shown) to other 11β-HSD3s, with which it clusters in the phylogenetic tree (Fig. 2).

4. Discussion

The biological roles of 11β-HSD2 in fish are complex. 11β-HSD2 is expressed in fish liver, kidney, brain and reproductive organs [14,19]. Kusakabe et al. [14] and Jiang et al. [19] showed that fish 11β-HSD2 oxidizes cortisol to cortisone. Thus, fish 11β-HSD2 has an activity similar to that of mammalian 11β-HSD2 in preventing cortisol activation of the GR, which mediates the stress response [22].

Also, 11β-HSD2 catalyzes the conversion of 11β-hydroxytestosterone to 11-keto-testosterone, a fish androgen, consistent with the presence of 11β-HSD2 in gonads. In mammals, testosterone and dihydrotestosterone are the main androgens. Thus, mammalian 11β-HSD2 does not have the same direct role as fish 11β-HSD2 in regulating androgen action. However, glucocorticoids inhibit androgen synthesis in mammalian testes [23]. Indeed, induction of 11β-HSD2 expression in Leydig cells during the onset of puberty is important for proper male reproductive development. Fish 11β-HSD2 is expressed in testis [14,19], where it can both inactivate cortisol and synthesize 11-keto-testosterone.

The role of fish 11β-HSD2 in regulating steroid access to the MR is not well understood because it is not clear which steroid(s) regulate mineralocorticoid action in fish [24–26]. Aldosterone, the main mineralocorticoid in land animals, does not appear to be present in fish [22,24,27]. Cortisol regulates ion transport in fish [22,24,25,27] and has about the same affinity for fish MR as aldosterone [24,25]. However, other corticosteroids with a C21 hydroxyl group also bind fish MR [24], which is also true for mammalian MR [13,28,29]. Further studies are needed to clarify the role of 11β-HSD2 in transactivation of fish MR by cortisol and other corticosteroids.

Phylogenetic analysis indicates that 11β-HSD3 is the fish ancestor of 11β-HSD1. Human 11β-HSD3 and 11β-HSD1 are a little closer to each other than are human 11β-HSD2 and 17β-HSD2, which differ in substrate specificity [9]. 11β-HSD3 is likely to metabolize different steroids in mammals, fish and Ciona. Elucidating their catalytic activity is likely to reveal another mechanism for regulating adrenal and sex steroid concentrations in vertebrates. It may also clarify whether 11β-HSD1 is important in the transition from fish to land animals, perhaps by providing a more specific mechanism for synthesis of cortisol.

BLAST analysis indicates that Takifugu, Tetraodon and medaka lack an ortholog to 17β-HSD2 and 11β-HSD1. Zebrafish belongs to the Cypriniformes order, which is near the base of ray-finned fish. Medaka and puffer fish belong to the Beloniformes and Tetraodontiformes orders, respectively, which arose after Cypriniformes [30]. This suggests that 17β-HSD2 was lost after the separation of zebrafish and medaka, with one of the other 17β-HSDs [2–4,6] assuming the role of the type 2 enzyme.

We have considered the possibility that Takifugu, Tetraodon and medaka genomes are incomplete and even partial sequences of orthologs to 17β-HSD2 and 11β-HSD1 are missing. However, this would require three different genomes to lack an EST for 17β-HSD2 and 11β-HSD1, which we think is unlikely.

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