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FUNCTIONS OF 17ß-HYDROXYSTEROID DEHYDROGENASE TYPE 4

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Abstract
Five types of 17β-hydroxysteroid dehydrogenases catalyzing the conversion of estrogens and androgens at position C17 have been identified so far. The porcine peroxisomal 17β-hydroxysteroid dehydrogenase type 4 (17ß-HSD 4) catalyzes the oxidation of estradiol with high preference over the reduction of estrone. The 17ß-HSD 4 reveals only 25% amino acid similarity with 17ß-HSD 1, 2, 3 and 5 enzymes. The highest levels of 17ß-HSD 4 mRNA transcription and specific activity are found in liver and kidney followed by ovary and testes. In porcine gonads the immunofluorescence assigned the 17ß-HSD 4 to granulosa cells, Leydig and Sertoli cells. A 2.9 kb mRNA codes for an 80 kDa (737 amino acids) protein featuring domains which are not present in the other 17βHSDs. Although five Asn-Xaa-Ser/Thr (Xaa - unspecified amino acid) sites are found in the 80 kDa protein the enzyme is not glycosylated.The 80 kDa protein is N-terminally cleaved to a 32 kDa enzymatically active fragment. Both the 80 kDa and the N-terminal 32 kDa (amino acids 1-323) protein are the first enzymes that are able to perform the dehydrogenase reaction not only with steroids at the C17 position but also with 3-hydroxyacyl-CoA. The central part of the 80 kDa protein (amino acids 324-596) catalyzes the 2-enoyl-acyl-CoA hydratase reaction with high efficiency. The C-terminal part of the 80 kDa protein (amino acids 597-737) facilitates the transfer of 7-dehydrocholesterol and phosphatidylcholine between membranes *in vitro*. The unique multidomain structure of the 80 kDa protein permits the catalysis of several reactions so far thought to be performed by complexes of different enzymes.

Abbreviations

aa - amino acid, bp - base pairs, EDH - 32 kDa N-terminal fragment of porcine 17ß-estradiol dehydrogenase, E_2 - 17ß-estradiol, E_1 - estrone, HSD - 17ß-hydroxysteroid dehydrogenase, hHSD 4 -human 17ß-hydroxysteroid dehydrogenase type 4, pHSD 4 - porcine 80 kDa primary transcript of 17ß-hydroxysteroid dehydrogenase 4, GST - glutathione-S-transferase, VHF - very hydrophobic fraction

Several dehydrogenases convert steroids at the position C17

Biological potency of estrogens and androgens is regulated by conversions at position C17 by 17ß-hydroxysteroid dehydrogenases (HSD). Several enzymes with close substrate specificities are participating in that process. The identification and characterization of individual human HSDs was limited by scanty amounts of tissue available for purification. However, analyses performed with homogenates or with subcellular fractions allowed the kinetical differentiation of several enzymes such as the soluble 17ß-hydroxysteroid oxidoreductase of placenta or the structure-associated 17ß-estradiol dehydrogenase of uterus epithelium [1-3]. Before molecular biology techniques became widespread the readily available human placenta allowed the purification of the first human 17ß-hydroxysteroid dehydrogenase (HSD 1) which became a model for studies of steroid converting enzymes. After cloning and elucidation of the gene structure [4,5] it represents the best characterized human 34 kDa steroid dehydrogenase interconverting 17ß-estradiol and estrone with similar velocities. Detailed kinetical studies [6] have shown that placenta expresses additional HSDs and one of them, namely HSD 2, was cloned recently [7] (Table 1). This enzyme is a 43 kDa microsomal dehydrogenase revealing a two-fold higher rate of oxidation than reduction for both estrogens and androgens. A further enzyme, the HSD 3 is most abundant in testes and represents a transmembrane microsomal 35 kDa protein with a strong preference for the reduction of androgens [8]. The recently cloned mouse and human 17ß-HSDs type 5 have not yet been fully characterized [9,10]. The complexity of the 17ß-hydroxysteroid dehydrogenases was reviewed recently [11-14].

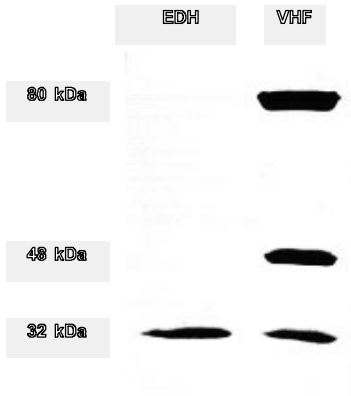
The oxidative 17ß-HSD activity found in human uterus endometrium could not be unequivocally ascribed to the known enzymes [3]. Attempts to isolate the endometrial 17ß-HSD from the particulate fraction of homogenates [15] resulted in 40-fold enrichment. However, because of difficulties in collecting and paucity of starting material the enriched fractions were not applied to amino acid sequencing and antibody production. Entenmann et al. discovered in porcine endometrium oxidative activity for 17ß-estradiol [16]. This microsomal activity revealed comparable kinetical parameters (NAD^+-dependency, K_m less than 1 μM). The parameters suggested a role in the inactivation of hormones.

Table 1. Human 17ß-hydroxysteroid dehydrogenases

enzyme	tissue	subcellular localization	mass kDa	best substrate	K_m µM	catalysis *in vivo*	ref.
17ß-HSD 1	placenta ovary	soluble	34	estrone estradiol	8.6 5.9	reduction	[4]
17ß-HSD 2	placenta liver	microsomal	43	testosterone estradiol	0.4 0.2	oxidation	[7]
17ß-HSD 3	testis	microsomal	35	androstenedione estrone	0.5 0.5	reduction	[8]
17ß-HSD 4	liver kidney	peroxisomal	80	estradiol androstenediol	0.2 0.4	oxidation	[17]
17ß-HSD 5	liver testis	microsomal	34	androstenedione	-	reduction	[10]

Purification of the porcine 17ß-estradiol dehydrogenase 4

Porcine uterus could be easily collected at a preparative scale. The separation of epithelial layer from the myometrium was achieved by curretage [18]. The most critical step of the purification turned out to be the solubilization of 17ß-estradiol dehydrogenase activity from the particulate fraction (38,000 x g) pellets of homogenates as activity was either deteriorated or not extracted [19]. The best results were obtained with the nonionic detergent Brij 35 with which 6% of enzyme activity was solubilized. Extracts were applied to DEAE-Sepharose, depleted of free detergent on Amberlite XAD-2 and further purified by affinity chromatography on Blue Sepharose [20]. In epithelial cells the proportion of oxidation to reduction at optimal assay conditions is 30:1 and remains unchanged until the affinity chromatography step. However, the activity of the 17ß-estradiol dehydrogenase was eluted with 0.7 M KCl while the estrone reductase activity (free of dehydrogenase) required 1.8 M KCl for the elution. Therefore, oxidation and reduction of steroids in porcine uterus epithelium is performed by two distinct enzymes. This has already been suggested by Bogovich and Payne for the testosterone/androstenedione transformations in rat testes [21]. Because of the separation of a genuine reductase the rate of oxidation to reduction increased to 360:1 for 17ß-HSD rich fraction. Further purification on Butyl Sepharose resulted in two products rich in 17ß-estradiol dehydrogenase activity: a major moderate hydrophobic fraction (EDH) and a minor very hydrophobic fraction (VHF). They were processed in parallel by gel filtration and ion-exchange chromatography on Mono S (Fig. 1). The EDH fraction was purified to homogeneity (4081-fold enrichment) and



revealed a single band at 32 kDa in the denaturing SDS-PAGE. The VHF was a mixture of proteins of 32, 45 and 80 kDa (2402-fold enrichment).

Fig. 1. Purified porcine 17ß-HSD 4

VHF is a mixture of a 80 kDa protein, its cleavage products of 32 and 48 kDa and actin [20].

Characterization of EDH and VHF

Both purification products, the EDH and the VHF, show the same K_m for steroids and cofactors as those measured with two-substrate kinetics in the particulate fraction of homogenates of porcine uterus epithelium (Table 2). They reveal an ordered mechanism of reaction with the cofactor binding first [20,22]. EDH and VHF share as well the substrate specificity, which is highest for 17ß-estradiol and, unexpectedly, comparably high for Δ5-androstene-3ß,17ß-diol (K_m = 0.2 µM). Other androgens or progestagens are not converted.

Table 2. Kinetic parameters of porcine 17ß-estradiol dehydrogenase 4

parameter	$E_2 \rightarrow E_1$	$E_2 \leftarrow E_1$
optimal pH	7.8	6.6
K_m for steroid	0.22 µM	1.10 mM
best cofactor	NAD^+	NADPH
K_m for cofactor	44 mM	21 mM

The molecular mass was estimated in denaturing SDS-PAGE and under native conditions by gel filtration and density gradient centrifugation [20]. The 32 kDa protein has a capability of forming dimers with an apparent molecular weight of 75 kDa. The interactions in the VHF are more complex. A homodimer of two 80 kDa proteins may

correspond to a low molecular mass form (170 kDa) and a heterocomplex including 32, 45 and 80 kDa proteins to a high molecular mass form (240 kDa) observed in density gradients. Identification of VHF components was a prerequisite for understanding the intrinsic interactions. It was achieved by investigations with antibodies and by amino acid sequencing. Goat and rabbit polyclonal antibodies raised against 32 kDa EDH reacted with the 32 and 80 kDa protein of VHF. The mouse monoclonal antibody (mab) W1 recognized only the 32 kDa protein and the mab F1 both the 32 and 80 kDa proteins [20,23]. Neither antibody reacted with the 45 kDa protein. The identity of the latter band with ß-actin was shown by amino acid sequencing and by western blots. The 80 kDa band of VHF revealed the presence of peptides known from the sequence of actin, the 32 kDa protein and some unassigned sequences [24]. The nature of the 80 kDa protein remained unclarified until its molecular cloning. The 32 kDa protein was identical in both purification products EDH and VHF and represented a new 17ß-estradiol dehydrogenase addressed as 17ß-HSD 4.

Processing of the porcine 80 kDa protein

The single open reading frame of the cDNA codes for an 80 kDa protein which undergoes a complex processing. It is N-terminally cleaved, probably after the sequence Ala320-Ala-Pro-Ser324, to a 32 kDa fragment representing EDH [25]. The comparison of specific activities of the purified EDH and VHF suggest that the primary translation product is enzymatically active. The EDH may reversibly interact with actin and some of the enzyme molecules are covalently crosslinked with actin through side groups of γ-glutamyl and ε-lysyl amino acids forming a 78 kDa complex [24]. The 78 kDa actin-EDH complex and the 80 kDa primary translation product are not resolved by SDS-PAGE. The comigration explains the detection of actin in the 80 kDa band of western blots [26]. The interactions forming homodimers of EDH and heterocomplexes of EDH, 80 kDa protein and actin must be of a nature withstanding at least in part the conditions of solubilization from the particulate fraction and further purification steps. Such partial dissipation leads to isolation of two fractions representing EDH (homogenous 32 kDa protein) and the VHF (EDH, actin, EDH-γ-glutamyl-ε-lysyl-actin and primary translation product).

Molecular cloning of the porcine and human 17ß-estradiol dehydrogenases type 4

With degenerated PCR-primers, designed according to partial amino acid (aa) sequence of the 32 kDa EDH, a fragment of 405 base pairs (bp) was amplified from porcine endometrium cDNA. It had a single open reading frame coding for an amino acid sequence

which was identical to the 32 kDa EDH as confirmed by Edman degradation. Using this fragment as a probe a 2.9 kb cDNA was isolated from a porcine λZAP kidney cDNA library. The sequence was later confirmed in porcine uterus [26]. The cDNA coded in a single open reading frame for a protein of 80 kDa (named pHSD 4) consisting of 737 aa and was not similar to any known steroid dehydrogenases [27,28]. About 70% of its amino acid sequence was already known from peptides of the 32 and 80 kDa proteins. Screening of human cDNA λgt11 libraries of liver and placenta was performed with a fragment corresponding to nucleotides 271 to 683 of the porcine enzyme. A 3 kb cDNA was identified which coded for a 80 kDa protein of 736 aa (addressed as hHSD 4) representing the human counterpart of the porcine enzyme [17]. Ortholog proteins were recently cloned in mouse [29] and rat [30].

Tissue distribution of porcine and human 17ß-HSD 4

In the studied porcine tissues the oxidation of 17ß-estradiol is predominating over the reduction. Highest pHSD4 concentrations are found in liver and kidney followed by uterus, lung, ovary and testes (Table 3).

Table 3. Distribution of 17ß-estradiol dehydrogenase activity in porcine tissues

tissue	$E_2 \rightarrow E_1$ µU/mg	$E_2 \leftarrow E_1$ µU/mg	immunocytochemistry
liver	687.5	5.6	hepatocytes
kidney	336.4	0.7	epithelium of proximal tubuli
ovary	293.9	1.5	granulosa cells
lung	185.4	0.8	bronchial epithelium
testes	69.2	1.8	Leydig cells
uterus	30.1	1.2	luminal and glandular epithelium
skeletal muscle	10.9	ND	myocytes
prostate	0.8	0.1	ND
blood erythrocytes	ND	ND	ND

Activities were measured in the particulate fractions of homogenates in tissue from boars or gilts in the luteal phase of the ovarian cycle [20]. Immunohistology was performed with rehydrated paraffin sections using monoclonal antibody F1-peroxidase conjugates as described [31], ND - not detected.

A slightly different expression pattern was seen in human tissues. The highest mRNA level of human 17ß-HSD 4 was observed in liver, followed by heart, prostate and testis. Moderate expression occurred in lung, skeletal muscle, kidney, pancreas, thymus,

ovary, intestine and term placenta. Weak signals were observed in brain, spleen, colon and lymphocytes. In all cases only a single band at 3 kb was discernible [17]. The wide distribution of 17ß-HSD 4 may in part explain oxidative activities measured in human tissues [32]. The expression of 17ß-HSD 4 is in contrast with that of 17ß-HSD 1 and 2 predominantly seen in placenta. Several human cancer cell lines also express 17ß-HSD 4. Estrogen receptor positive mammary cell line T47D express more hHSD 4 mRNA transcript than BT-20, MDA-MB-453 and MDA-MB-231 which are estrogen receptor negative. The megakaryotic cell line DAMI reveals a very high levels of hHSD 4 mRNA while less is present in hepatocellular carcinoma HEP-G2 and early embryonic Tera-1 cell lines [17].

Subcellular distribution of pHSD 4

Immunocytochemical and immunofluorescence studies in porcine uterus restricted the pHSD 4 to luminal and glandular epithelium [31] similar to analyses of human endometrium [33,34]. However, the staining was not diffuse in the cytoplasm but showed punctated appearance. The intensity of the mab F1-peroxidase staining followed the changes of porcine EDH activity. It raised 4-fold after day 5 of the ovarian cycle and rapidly decreased after day 17 similar to the levels of progesterone. At day 4 faint spots of fluorescence appeared in the cytoplasm of gladular epithelium. The spots accumulated at the cell basis between days 11 through 17 (luteal phase) and disappeared within one day. The pattern of immunofluorescence staining suggested that pHSD 4 is localized in vesicles. The latter have been isolated from porcine uterus epithelium homogenates by sequential density gradients of isopycnic 30% Percoll and linear 0.3 - 2 M sucrose in vertical rotors [23,35,36]. The vesicles harboring the 17ß-HSD activity equilibrated at the density of 1.18 g/ml, were 120 - 200 nm in diameter, revealed a moderate electron dense matrix bounded by a single membrane and were morphologically and enzymatically distinct from mitochondria, lysosomes, fragments of plasma membrane, endoplasmic reticulum and the Golgi apparatus. In immunogold electron microscopy the labelling with mab F1 (recognizing the 32 kDa EDH and the 80 kDa protein) and W1 (reacting with EDH only) confirmed that all forms of the enzyme are present in the same vesicles, both in tissue and in the isolated fraction [36].

Several clues pointed to the identity of EDH containing vesicles as peroxisomes: (1) the morphology and density is similar to that of peroxisomes, (2) the 80 kDa primary transcript features the peroxisomal targeting signal Ala-Lys-Ile and a putative recognition sequence (Ala-Ala-Pro) for a protease processing peroxisomal proteins [37] and (3) the 80

kDa protein is similar to enzymes participating in peroxisomal ß-oxidation of fatty acids. Indeed, typical peroxisomal markers such as catalase and acyl-CoA oxidase colocalized with pHSD 4 in immunogold labelling studies in uterus, kidney and liver [38,39]. Therefore, peroxisomes participate in the inactivation of steroids.

Features of the amino acid sequence of a new type of 17ß-estradiol dehydrogenases

The amino acid sequences of porcine and human 80 kDa HSD 4 are very similar (84%). They reveal a three-domain structure (Fig. 2) unknown for other dehydrogenases [40].

Fig 2. Similarities of amino acid sequences

(complete in printed version)

FOX2 - multifunctional enzyme of *Saccharomyces cerevisiae*, SCAD - short chain alcohol dehydrogenase, SCP2 -sterol carrier protein 2, SCPx - sterol carrier protein X, % identity is given along the arrows

About 300 aa of the N-terminal sequence have low overall similarity of 27% to the short chain alcohol dehydrogenase (SCAD) family. Similarity to the human HSD 1, 2 and 3, which consist of a single domain of the short chain dehydrogenase type, is less than 25%. Exceptionally high homology (50%) is observed to the first domain of the multifunctional fatty acid hydratase-dehydrogenase-epimerase of *Candida tropicalis* [41] and the FOX2 enzyme of *Saccharomyces cerevisiae* [42].

The copurification of EDH and actin suggested the possibility of specific interactions. The EDH sequence (aa 73 -119) contains a motif similar to that of aldolase [25,43] and to those of SH3-domains which are reported to participate in reversible actin binding [44]. The proteolytic cleavage of 80 kDa protein releases the complete SCAD domain.

Amino acid sequences of human, porcine and mouse 80 kDa proteins reveal several potential glycosylation sites (Asn-Xaa-Ser/Thr, Xaa - unspecified amino acid): e.g 6 in the porcine and 3 in the human HSD 4 [17,26,29]. Except for Asn401 (porcine) or Asn400 (human) they are localized in the N-terminal parts. Since the 17ß-HSD 4 is a peroxisomal enzyme and glycosylated proteins have not yet been described in these organelles we explored potential modifications. In the porcine protein 5 glycosylation sites are present in the SCAD domain and one is located in the domain similar to fatty acid hydratases. Amino acid sequencing has shown that 4 of the 5 N-terminal sites are not posttranslationally modified [26]. Further information has been provided by lectin staining. The 32 kDa 17ß-HSD 4 was either purified from kidney (native enzyme) or as a recombinant GST fusion protein from *E.coli* (glycosylation excluded). No labelling was seen with biotinylated lectins isolated from *Triticum vulgaris*, *Phaseolus vulgaris* and *Abrus preactoriusor*. However, lectins of *Tetragonolobus purpureas* (strong labelling), *Glycine max* and *Helix pomatia* (both weak signal) have shown affinity for both proteins and therefore must represent unspecific binding. Results suggest that the 17ß-HSD 4 is not glycosylated.

The central aa 343-607 domain of pHSD 4 and the aa 343-606 domain of hHSD 4 are 40% identical (Fig. 2) with the C-terminal domains of fatty acid hydratase/dehydrogenase of *Saccharomyces cerevisiae* [42] and *Candida tropicalis* [41]. Although the relationship of ß-oxidation enzymes to the members of the short chain alcohol dehydrogenase family has been reported [45] the high resemblance of the 80 kDa proteins to multifunctional enzymes is unique and suggests a common ancestor.

The C-terminal segments of 17ß-HSD 4 are 39% similar to the sterol carrier protein 2 (SCP2) [46], including the peroxisome-targeting signal [47] Ala-Lys-Ile (pHSD 4) and Ala-Lys-Leu (hHSD 4). At present it is not known if any cleavage between the central and SCP2 domains is taking place. The peroxisomal 13 kDa SCP2 protein was reported to participate in the transfer of lipids and cholesterol which is important for the shuttling of sterols during steroid synthesis [37,46,48]. The organisation of the central and C-terminal domain in the porcine and human HSD 4 is similar to the structural pattern of the 60 kDa SCPx protein. The latter reveals an N-terminal homology to 3-ketoacyl CoA thiolase (ß-oxidation enzyme), a C-terminal similarity to SCP2 and is not proteolytically cleaved between the two domains [49]. However, it was recently demonstrated that SCP2 and SCPx are expressed from a single gene via alternative transcription initiation from two distinct promoters [50].

Simultaneous occurrence of domains responsible for steroid metabolism, ß-oxidation and sterol transport in a single protein has not yet been observed. The 17ß-HSD 4 proteins are the first of such type.

Multifunctionality of 17ß-HSD 4

In order to check for the presence of activities predicted by amino acid similarities of the 80 kDa protein (Fig. 2) its three domains were expressed separately as GST fusion proteins. Essentially pure fusion proteins were obtained after a single passage through glutathione-agarose. Cleavage of the fusion proteins on the glutathione-agarose column with thrombin released single peptides (Fig. 3). The authenticity of the recombinant proteins was checked by DNA sequencing and by N-terminal amino acid sequencing of the first 10 residues.

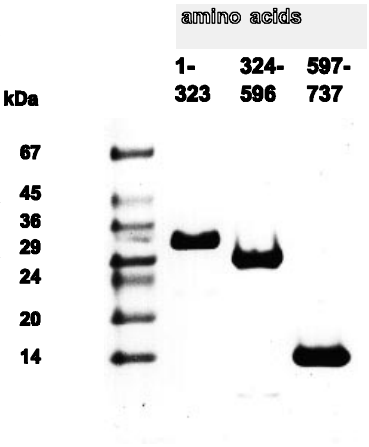


Fig. 3. SDS-PAGE and Coomassie blue staining of expressed domains of 17ß HSD 4

The domains were expressed as GST fusion proteins in *E.coli* , bound to glutathione agarose, cleaved with thrombin and eluted [40]

The N-terminal domain (aa 1-323) catalyzed both the 17ß-hydroxysteroid- and the 3-hydroxyacyl-CoA dehydrogenase reactions (Table 3). Kinetic parameters (K_m, V_{max}) of the expressed full length 80 kDa protein were close to those observed for the single expressed domains or for the native purified enzyme (EDH or VHF) [40].

Table 3. Kinetic parameters of domains of porcine 17ß-HSD 4

Sample	17ß-hydroxysteroid dehydrogenase		fatty acid-CoA hydratase		fatty acid-CoA dehydrogenase	
	K_m µM	V_{max} nmol x l^{-1} x min^{-1} x mg. $prot.^{-1}$	K_m µM	V_{max} µmol x l^{-1} x min^{-1} x mg. $prot.^{-1}$	K_m µM	V_{max} µmol x l^{-1} x min^{-1} x mg. $prot.^{-1}$
Purified 32 kDa	0.2	0.15	ND	ND	ND	ND
Recombinant 32 kDa	0.3	0.14	ND	ND	ND	ND
VHF	0.3	0.19	34.0	4.44	34.8	3.31
Recombinant 80 kDa	0.4	0.11	37.1	3.92	35.3	2.91
Recombinant central domain	ND	ND	34.7	1.36	21.9	1.25

Kinetic parameters of 17ß-HSD 4 were assayed with estradiol, fatty acid-CoA hydratase with crotonyl-CoA and fatty acid-CoA dehydrogenase with acetoacetyl-CoA [40]. Recombinant 32 kDa and 80 kDa proteins were assayed in homogenates of transfected cells and were corrected for background conversion [51]. The recombinant central domain was characterized after purification on gluthatione-agarose and thrombin cleveage, ND - activity not detected.

This was the first observation of an enzyme performing the dehydrogenase activity not only with steroids but also with 3-hydroxyacyl-CoA-derivates of fatty acids. Furthermore the central domain (aa 324 - 596) was responsible for the 2-enoyl-acyl-CoA hydratase activity (Fig. 5)

Fig. 5. Hydratase and dehydrogenase activities of 17ß-HSD 4.

The reactions are catalyzed by the N-terminal and central domains, respectively.

Estimated K_m-values for the hydratase and fatty acid dehydrogenase are similar to those of other enzymes of ß-oxidation of fatty acids [52,53]. Both K_m for steroids and fatty acids are in the physiological range.

As suggested by the similarity to SCP2 we investigated the recombinant C-terminal domain and the purified native enzyme for the ability to transfer 7-dehydrocholesterol and phosphatidylcholine. The specific transfer activities of the VHF and 80 kDa protein are close to those of SCPx [54]. More direct evidence for the involvement of the SCP2-related domain in the sterol and lipid transfer was obtained with the porcine recombinant peptide. Both the GST-SCP2 fusion product and the SCP2 domain were acitve in transfer. The purified porcine SCP2 peptide increases the transfer of 7-dehydrocholesterol and phosphatidylcholine 147- and 158-fold over the control levels (transfer by BSA), respectively (Fig. 6).

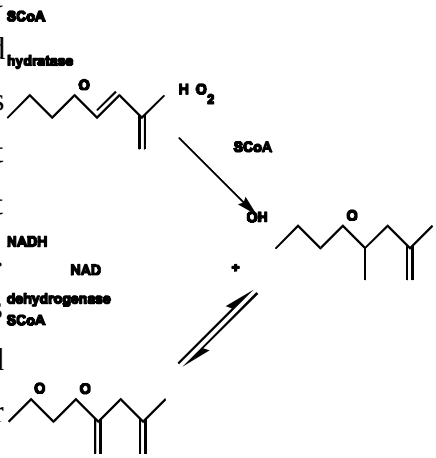


Fig. 6. Transport of cholesterol by 17ß-HSD 4
(availèable in printed version)
hSCP2 - human SCP2, hSCPX - human SCPX, GST-hSCP2 - fusion protein of glutation-S-transferase and hSCP2, pSCP2 - C-terminal domain of 17ß-HSD 4, control: BSA - bovine serum albumin [54].

Physiological significance

The high degree of resemblance in the amino acid sequence of porcine and human HSD 4 makes similarities in their enzymic properties likely. Table 1 compares the HSD 1 - 5 and depicts differences in catalytic parameters, posttranslational processing and subcellular localization. The catalytical property of porcine 32 kDa EDH revealing the virtually uni-directional oxidative activity, clearly define it as a steroid inactivating enzyme [55], since it produces estrone which shows little affinity to estradiol receptor. The ovarian cycle-triggered positioning of peroxisomes in glandular epithelium suggests the participation of the cytoskeleton in the regulation of hormone levels. Interactions of EDH with actin are also indicatory for that. The conversion of 17ß-estradiol to estrone might be complemented by hydroxylations in positions 6α or 7α [56] and producing steroids devoid of estradiol receptor affinity and permitting fast release from cells after formation. The V_{max} and K_m values for EDH are similar to those for estrone hydroxylases [57] and allows the metabolic conversion 17ß-estradiol → estrone → 6α / 7α-hydroxy-estrone without rate-limiting steps.

Our discovery of the 17ß-HSD 4 in peroxisomes stimulated the discussions about the possible role of peroxisomes in steroid metabolism [38]. The enzyme has yet unseen ability to be stimulated by both (1) progestins [58], which is a common feature of other 17ß-HSDs [59,60], and (2) peroxisomal activators such as WY14,643 [30,61] or clofibrate (M. Markus, J. Adamski unpublished results). The stimulation produces a tissue-specific response: progestins induce 17ß-HSD 4 about 10-fold in uterus epithelium but not in liver whereas a clofibrate treatment has no effects on uterus but results in 3-fold induction in the liver. It is unknown whether the differences are caused by local concentrations of receptors, modulation of receptor action by spatially restricted factors or alternated promoter usage similar to that observed for the estrogen receptor in osteoblasts and breast tissue [62]. The 17ß-HSD 4 inactivates D5-androstene-3ß, 17ß-diol to dehydroepiandrosterone (DHEA), a

known peroxisomal proliferator [63]. Although both DHEA and clofibrate induce peroxisomes they have opposite effects on the concentrations of triglycerides and cholesterol in blood. DHEA increases tthe levels of lipids while clofibrate acts as a hyperlipidemic drug. Decreased expression of enzymes which inactivate estradiol including Cyp2C11, and the reported increased expression of aromatase (converting testosterone to estradiol) may explain why male rats exposed to diverse peroxisomal proliferators have higher serum estradiol levels. These higher estradiol levels in male rats have been thought to be mechanistically linked to Leydig cell hyperplasia and adenomas. Increased conversion of estradiol to the less active estrone by 17ß-HSD 4 induction may explain how exposure to the di-(2-ethylhexyl)-phthalate leads to decreases in serum estradiol levels and suppression of ovulation in female rats [61,64].

The possible competition between fatty acids and steroids for the active center of enzyme would be a new aspect of enzymatic regulation. Also the functionality of the multi-domain structure (17ß-HSD 4 + hydratase + SCP2) is unknown. Nevertheless, the high level of conservation of amino acid sequence (85% identity) between human, mouse, rat and porcine 17ß-HSD 4 suggests an essential function of this type of protein.

Acknowledgements

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