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The Subcellular Localization of 17β-Hydroxysteroid Dehydrogenase Type 4 and its Interaction with Actin

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The porcine 17β-hydroxysteroid dehydrogenase type 4 is the key enzyme for the inactivation of estradiol. Its localization in peroxisomes was proven by immunogold electron microscopy. Interactions of the 17β-hydroxysteroid dehydrogenase with cytoskeletal proteins might be mandatory for a topical assignment of enzymatic activity to defined subcellular compartments.

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INTRODUCTION

The oxidation of estradiol to estrone by 17β-hydroxysteroid dehydrogenase type 4 (17β-HSD 4) protects cells against excess hormone. Estrone is subsequently hydroxylated at positions 6α- or 7α- by *P*450 monooxygenases. The oxidation of estradiol and the hydroxylations of estrone proceed with comparable kinetics which prevent the accumulation of estrone and allows for a shedding of the final products immediately after formation [1–3].

In 1978 Sierralta *et al.* [4] described an oxidative activity of a 17β-estradiol dehydrogenase associated with cytoplasmic structures. The purification of the enzyme required its solubilization from the particulate fraction of the homogenates by the nonionic detergent Brij 35 [5]. The extract was subjected to in-line chromatography over DEAE-Sepharose (adsorption of about 30% of applied protein, but no enzyme activity), Amberlite XAD 2 (adsorption of Brij 35) and lastly affinity chromatography on Blue Sepharose. The 17β-HSD 4 peak eluted from Blue Sepharose was subjected to a hydrophobic interaction chromatography on Butyl Sepharose producing two peaks with 17β-HSD 4 activity: a major peak of low hydrophobicity and a minor peak of very high hydrophobicity. Gel filtration and Mono S chromatography were the final purification steps.

The more hydrophilic major peak contained a single 32 kDa protein (17β-estradiol dehydrogenase = EDH) seen in SDS-PAGE after silver staining. The very hydrophobic fraction consisted of three bands of 32, 45 and 80 kDa. Amino acid sequencing revealed the nature of the bands: the 32 kDa-bands of both fractions were identical, the 45 kDa-band was actin and the 80 kDa protein contained amino acid sequences of the 32 kDa-enzyme, of actin but also of peptides unrelated to either. The composition of the unknown 80 kDa protein was obtained by cDNA cloning [6]. It turned out that a 3 kb mRNA codes for a primary translation product with a multi-domain structure unknown for other hydroxysteroid dehydrogenases [6, 7]. The 32 kDa EDH is a N-terminal peptide of 325 amino acids cut from the 80 kDa protein.

INTERACTIONS OF THE 32 kDa EDH WITH ACTIN

The presence of actin in the 80 kDa band was analyzed in electroeluted 80 kDa material exhaustively digested by endo-and exopeptidases. A covalent cross-link (γ-glutamyl-ε-lysine) of the 32 kDa enzyme and actin was identified [8], resulting in a ∼78 kDa complex which comigrates with the 80 kDa primary translation product of 17β-HSD 4 in SDS–PAGE. However, some of the actin and the 32 kDa EDH present in the very hydrophobic fraction interact non-covalently as demonstrated by the copurification of free

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Abbreviations: EDH, 32 kDa 17β-estradiol dehydrogenase; 17β-HSD, 17β-hydroxysteroid dehydrogenase; mab, monoclonal antibody; SCP2, sterol carrier protein 2.

actin and EDH [5]. Such interactions could be mediated by sequences of the 32 kDa enzyme showing homologies to several actin binding proteins [7]. The physiological meaning of covalent and non-covalent linkages with the cytoskeleton might be the positioning of the organelle-enclosed enzyme.

CYCLE-RELATED CHANGES IN EDH ACTIVITY AND OF SUBCELLULAR DISTRIBUTION

Immunofluorescence studies of pig endometrial glands showed that the staining pattern with antibodies against 17β-HSD 4 changes over the course of the estrus cycle [9]. Immunoreactivity and EDH-activity are low in the days shortly before and after ovulation (day 1). Fine, uniformly distributed dots of fluorescence become visible at day 4, when progesterone levels begin to rise. With the continuing rise of the progesterone concentration, the tagging concentrate at the basal site of the cells (days 11–15). The coalescence of immunofluorescent bright spots coincides with the maximum EDH-activity. With the sudden drop of the plasma progesterone concentration to preovulatory levels (after day 17/18) the staining becomes faint and diffuse again.

THE ORGANELLES CONTAINING 17β-HSD 4 ARE PEROXISOMES

The isolation of 17β-HSD 4 containing structures was accomplished by a careful homogenization procedure [10] and fractionation of the homogenate by differential centrifugation. Subsequent fractionation of the postmitochondrial supernatant by successive density gradient centrifugations (Percoll and sucrose) in vertical rotors resulted in an accumulation of the enzyme in fractions of 1.18 g/ml density [11]. Marker enzymes for plasma membrane, mitochondria, endoplasmic reticulum or lysosomes were not enriched in these fractions. Immunogold electron microscopy revealed that 17β-HSD 4 is localized in structures of 120–200 nm in diameter with a medium electrondense matrix surrounded by single membranes resembling peroxisomes. The same organelles could be observed in ultrathin sections of porcine uterine cells excluding an artificial formation *in vitro* [10, 12].

Several structural features of the 80 kDa primary translation product of 17β-HSD 4 suggested, that it might be a peroxisomal protein: (1) an AAP cleavage signal typical for peroxisomal precursor proteins is located between the N-terminal 32 kDa EDH and the central domain; (2) the central domain resembles peroxisomal enzymes for β-oxidation of fatty acids; (3) the

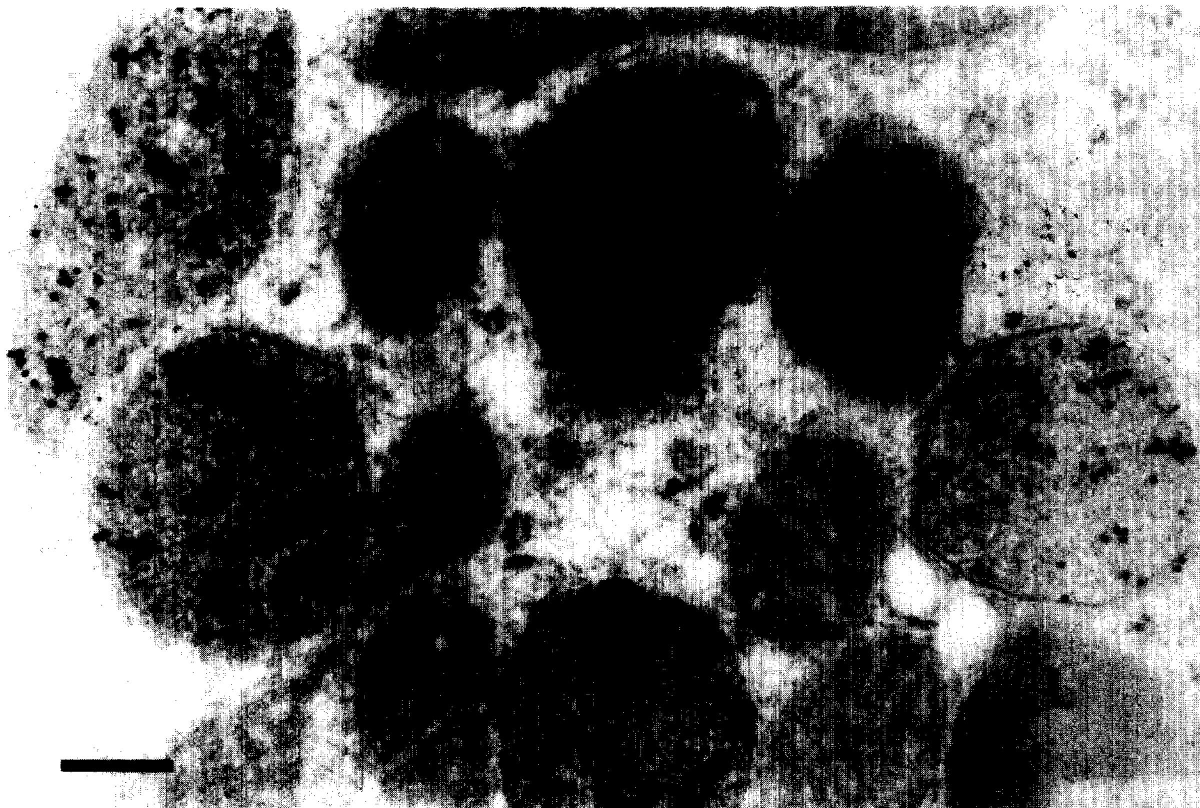

Fig. 1. Peroxisomal localization of 17β-HSD 4. Ultrathin section of LR Gold-embedded porcine kidney tissue were double labeled with mouse anti-EDH antibody F1 (15 μg/ml) followed by goat anti-mouse antibodies coated on 6 nm gold particles and rabbit anti-catalase antibodies (10 μg/ml) followed by goat anti-rabbit antibodies on 10 nm gold particles. Different shapes of peroxisomes can be seen. M, mitochondria; L, lysosomes; Bar = 200 nm.

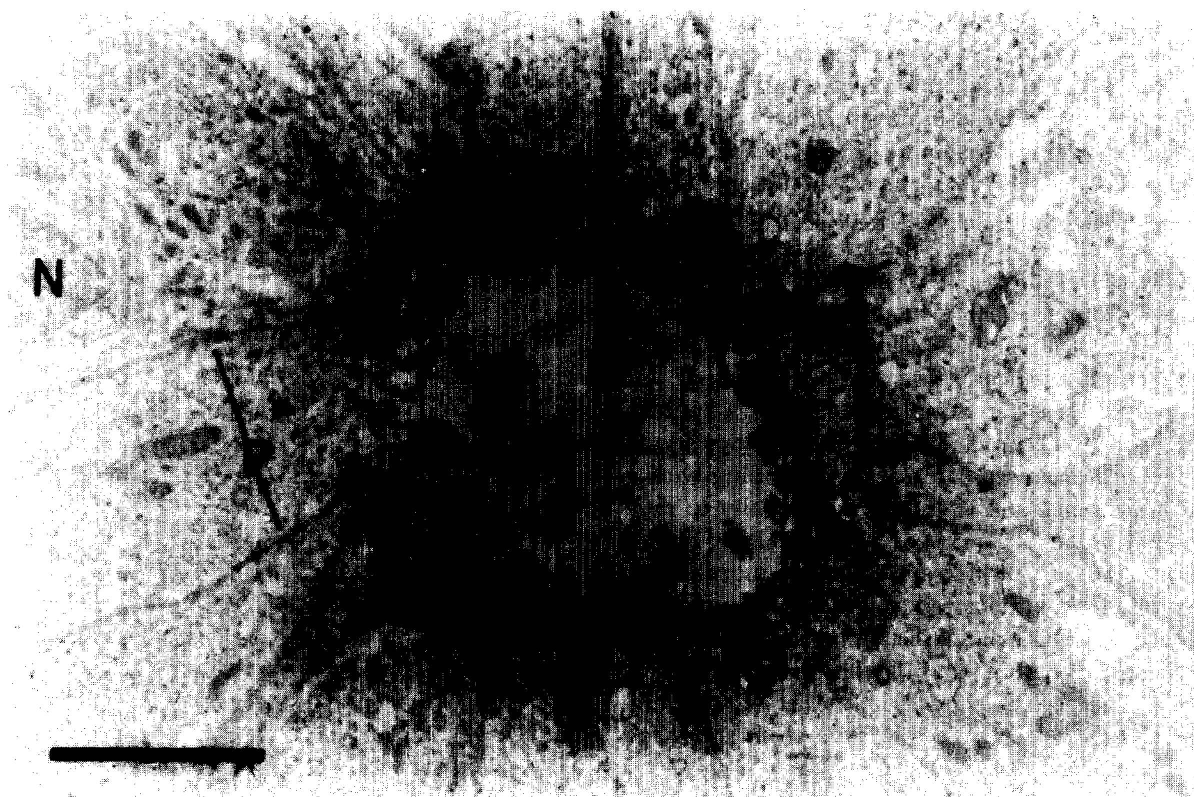

Fig. 2. Section of porcine endometrial gland labelled with antibody against actin. Mouse mab C4 anti-actin antibody (2,5 μg/ml) was detected by secondary goat anti-mouse antibodies attached to 10 nm gold. The tagging is most intense at the apical sides of the cells. C, cillia; P, plasma membrane; N, nucleus; Bar = 2 μm.

C-terminal domain features homologies with the peroxisomal sterol carrier protein 2 including; (4) the peroxisomal target signal AKI.

The localization was proven by double-labeling immunogold electron microscopy studies. Porcine 17β-HSD 4 was labeled with monoclonal mouse antibodies (mab) F1 [5] and peroxisomes were identified by polyclonal rabbit antibodies against the peroxisomal enzymes catalase and acyl-CoA oxidase. Secondary reagents were 6 nm or 10 nm gold clusters coated with goat anti-mouse or goat anti-rabbit IgG, respectively. Tissues studied were: the estradiol target organ uterus, kidney (Fig. 1) which exhibits high EDH activity and liver as the classical peroxisome-containing organ. The taggings of 17β-HSD 4 and peroxisomal marker enzymes were found in the same organelles showing that 17β-HSD 4 is indeed localized in peroxisomes in all tissues studied [13].

An indirect participation of peroxisomes in steroid synthesis was first presumed by Reddy and Svoboda in 1972 [14]. Stereological studies showed that the volumes of peroxisomes and of smooth endoplasmic reticulum correlate with the rate of testosterone synthesis in rat Leydig cells. The LH-stimulated testosterone production was accompanied by elevated contents of sterol carrier protein 2 in peroxisomes [15]. This protein is usually assigned to peroxisomes but was found to stimulate the mitochondrial steroidogenesis. In patients with peroxisomal dysfunction the SCP2-content is usually lower than in healthy subjects. The patients also suffer from deficiencies in steriodogenesis [16]. However, neither steroid-synthesizing nor metabolizing enzymes have previously been localized in peroxisomes. The porcine 17β-HSD 4 is the first hormone-inactivating enzyme assigned to these organelles. The detection of a steroid metabolizing enzyme in peroxisomes might help to clarify the relationship of these disorders.

INTERACTION OF PIG UTERINE GLAND PEROXISOMES AND ACTIN

The identification of a system in which peroxisomes change their localization according to metabolic conditions might help to understand the mechanisms of peroxisomal positioning. The 17β-HSD 4 exhibits interactions with actin (both non-covalent and covalent). The covalent, the non-covalent EDH–actin complexes and the 80 kDa protein are only solubilized by detergent containing extractants, which points to an association with membranes. In that case the enzyme-bound actin could extend into the cytoplasm. However, neither actin molecules around the organelles nor actin tracks proceeding from the organelles have been observed in immunoelectron microscopy. The vast amount of taggings with mouse mab C4 against actin

is localized in the region of the terminal webb (Fig. 2) and only a very small amount can be seen in other cell parts. A few 17β-HSD 4 containing organelles are also labeled (Fig. 3). However, in most of the peroxisomes no anti-actin taggings are found (Fig. 4). The lack of staining could be due to the fact that the amount of EDH-connected actin is below the detection limit in ultrathin sections or because the antigene epitopes are masked by the binding of EDH.

The actin found in peroxisomes (Fig. 3) could contribute to the establishment of internal matrix structures and morphological changes of organelles. It has been reported that peroxisomes can build a tubular network [17]. In cultured cells their morphology seems to change with the cultivation time, beginning with a tubular appearance and changing sequentially to randomly distributed separate round organelles [18]. A transition between tubular and globular morphology was reported from various cell compartments including endosomes, lysosomes and the Golgi apparatus [19]. Little is known about the regulation of structural changes of the peroxisomes. The finding of an actin-bound peroxisomal enzyme might be stimulatory for future research.

Fig. 3. Double labeling of glandular epithelium of porcine endometrium with anti-actin and anti-EDH antibodies. Mouse anti-actin mab C4 (2,5 μg/ml, detected by secondary goat anti-mouse antibodies attached to 10 nm gold) associated with the plasma membrane (arrowheads) and peroxisomes (arrow) which were identified with polyclonal rabbit serum against EDH, dilution 1:100 and detected by goat anti-rabbit IgG attached to 6 nm gold. N, nucleus; Bar = 200 nm.

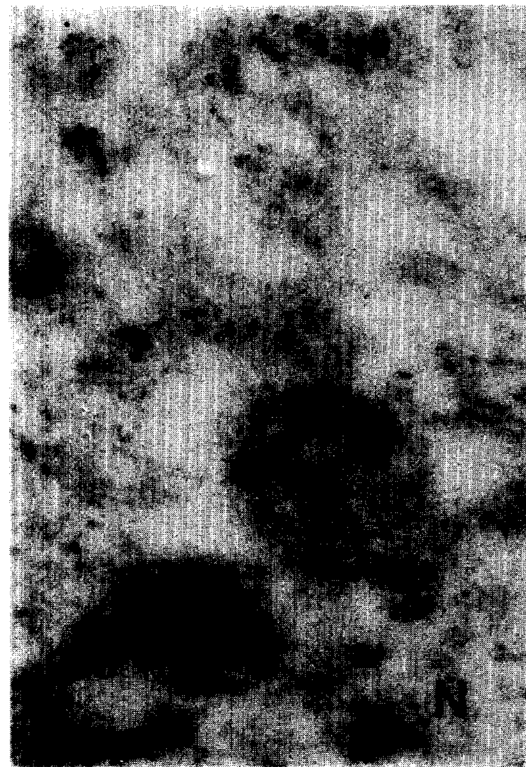

Fig. 4. Double labeling with anti-actin and anti-EDH antibodies of a glandular epithelial cell of porcine endometrium. Tagging of actin with mab C4 (2,5 μg/ml, detected by goat anti-mouse IgG attached to 6 nm gold). An association with peroxisomes (identified by rabbit anti-EDH serum, dilution 1:100) is not seen. 10 nm gold coated with goat anti-rabbit IgG was used for the detection of the EDH antibodies. N, nucleus; Bar = 200 nm.

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