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TESTICULAR 17β-HYDROXYSTEROID DEHYDROGENASE: ITS DISTRIBUTION, PURIFICATION AND PROPERTIES

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SUMMARY

Testicular 17β-hydroxysteroid dehydrogenase was localized intracellularly in the agranular microsomal fraction among the organelles of the interstital tissue. The microsomal 17β-hydroxysteroid dehydrogenase of rat testes was not completely solubilized by several available procedures, such as treatments with detergents, phospholipases, organic solvents, sonication, freezing and thawing, etc. Recently, however, the 17β-hydroxysteroid dehydrogenase of porcine testes, which was also present in the microsomal fraction was solubilized by sonication, and partially by freezing and thawing. The solubilized dehydrogenase of the porcine testes was purified by a fractional ammonium sulfate precipitation, and column chromatographies through Sephadex G-100, DEAE-cellulose, and Bio-Gel P-100, and finally, an apparent homogeneous preparation was obtained. Using the enzyme preparation obtained at the last step of purification, its molecular weight and radius, optimal temperature and pH, cofactor requirement, substrate preference, stoichiometry, stereochemical relationship between the substrate and NADPH through the oxido-reduction, and its activity as transhydrogenase from NADPH to NAD^+ were examined.

INTRODUCTION

In relation to our investigation of androgen biosynthesis *in vitro* [1, 2], an enzyme or 17β-hydroxysteroid dehydrogenase which was related to an obligatory step of testosterone formation was recently purified to a nearly homogeneous state. In this communication, we briefly present our experimental results on the intracellular distribution of this enzyme, solubilization of it from the particular organella, purification of the enzyme and then characteristics of the purified enzyme preparation.

RESULTS AND DISCUSSION

Function of testicular 17β-hydroxysteroid dehydrogenase

In the testis, testosterone is produced from pregnenolone through progesterone, 17α-hydroxyprogesterone and androstenedione (Δ^4-pathway) and also through 17α-hydroxypregnenolone, dehydro*epi*androsterone and 5-androstene-3β,17β-diol (Δ^5-pathway). In both biosynthetic pathways, 17β-hydroxy steroid dehydrogenase plays an important and essential role in testosterone formation, thus in the Δ^4-pathway, testosterone is produced from androstenedione by this enzyme, while, in the Δ^5-pathway, dehydro*epi*androsterone is converted to 5-androstenediol which is consequently converted to testosterone [3]. As a dehydrogenase, the 17β-hydroxysteroid dehydrogenase (E.C. 1.1.1.51) is directly concerned with the oxidation-reduction of androstenedione and testosterone or dehydro*epi*androsterone and 5-androstene-3β,17β-diol.

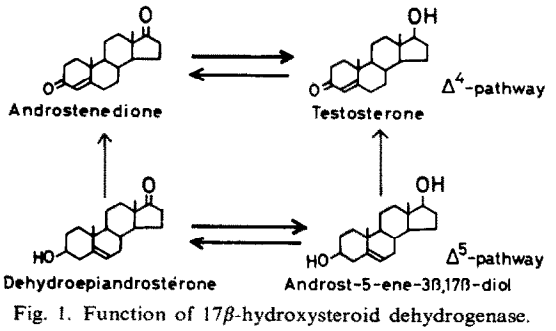

Fig. 1. Function of 17β-hydroxysteroid dehydrogenase.

Intercellular distribution of the 17β-hydroxysteroid dehydrogenase in testicular tissue

Histochemically, in the testes of man, newt, cockerel, cat, dog and monkey, the 17β-hydroxysteroid dehydrogenase is localized in the interstitial tissue or Leydig cells [4], while, in the rat testes, this enzyme activity is hardly found in this tissue [5]. By manual separation of the interstitial tissue from the seminiferous tubules of rat testes, the dehydrogenase activity per unit weight of protein in the interstitial tissue was about 2–3 times higher than that of the seminiferous tubules [6]. After X-ray irradiation locally at the testes of adult rat *in vivo*, 17β-hydroxysteroid dehydrogenase activity remained in the irradiated testis, whereas no spermatogenesis was observed, suggesting that this enzyme was localized into the radio-resistant cells of the testicular tissue [7]. From the above results, the major activity of the dehydrogenase was concentrated in the interstitial tissue [8].

Intracellular distribution of the 17β-hydroxysteroid dehydrogenase

The organelle and cytosol fractions which constituted the cellular structure were prepared by a conventional differential centrifugation from rat testicular

Table 1. Intracellular distribution of 17β-hydroxysteroid dehydrogenase in the testis of various animals

Species	Relative enzyme activity* Mitochondria	Microsomes	Cytosol	
Man	3	100	0	[9]
Mouse	5	100	18	[10]
Guinea-pig	28	100	2	[11]
Pig	33	100	35	[12]
Rat	34	100	1	[13]
Dog	20	100	10	[14]
Cat	—	100	7	[15]
Chicken	47	100	45	[16]
Rainbow trout	54	100	23	[17]

* Expressed as the specific activity of mitochondrial and cytosol fractions relative to those of the microsomal fractions.

homogenates, and then each organelle fraction was morphologically confirmed under an electron microscope. This enzyme was localized mainly in the microsomal fraction (10,000–105,000 *g* precipitate) of several species, as shown in Table 1.

Furthermore, by a discontinuous sucrose density centrifugation of the microsomal fraction in the presence of CsCl, this fraction was divided into the rough- and smooth-surfaced microsomal fraction which were respectively derived from agranular and granular endoplasmic reticulum. The validity of this separation procedure was established by an abundance of ribosomes in the former fraction under an electron-microscope, and by a significantly higher content of RNA in the former fractions [18]. The 17β-hydroxysteroid dehydrogenase activity was found to be concentrated in the smooth-surfaced microsomal fraction (Table 2), indicating that the site of testosterone production from androstenedione was the agranular endoplasmic reticulum of the testicular interstitial cells [8, 18].

Solubilization of the 17β-hydroxysteroid dehydrogenase from the testicular microsomes

Several attempts to solubilize the enzyme from rat testicular microsomes were made for years in our laboratory, but the enzyme could not be completely solubilized by the following methods [19]:
(1) Sonication with 10- and 20-K cycles. (2) Treatment with ionic and non-ionic detergents. (3) Treatment with n-butanol and other organic solvents at low temperature. (4) Repeated freezing and thawing. (5) Treatment with several kinds of phospholipases. (6) Combination of two of the above methods.

Table 2. Intermicrosomal distribution of rat testicular 17β-hydroxysteroid dehydrogenase

Microsomes	Specific enzyme activity*	Enzyme activity /gland†	RNA content‡
Smooth-surfaced	3·3	9·4	0·14
Rough-surfaced	1·3	2·1	0·42

* nmole product/mg protein.
† nmole product/testes.
‡ RNA mg/mg protein.

On the other hand, in case of porcine testes, the 17β-hydroxysteroid dehydrogenase which was also present in the microsomal fraction was solubilized by sonication (Sonic Dismembrator 300, Artek, Farmingdale, N.Y.), under temperature-regulated conditions (0–5°C) [12]. The solubilization of the enzyme was confirmed by the fact that the major activity of the 17β-hydroxysteroid dehydrogenase was observed in the supernatant fluid after centrifugation at 125,000 *g* for 60 min after the sonication, and also that the enzyme was precipitated by ammonium sulfate and the precipitate became soluble again in the absence of the salt. Later, we found that significant activity of the 17β-hydroxysteroid dehydrogenase was detected in the supernatant fluid at 105,000 *g*, when the frozen testes of pig was employed as a starting material for purification. The microsomal enzyme of porcine origin was also partially solubilized by freezing and thawing [20].

Purification of porcine testicular 17β-hydroxysteroid dehydrogenase

As the details of the purification procedures and results were described in our recent publication [12], the summarized results are shown as Table 3. Recently, in order to obtain the dehydrogenase from the same material on a larger scale, the purification procedure has been changed from that described above to include an affinity chromatographic technique, [2], and other modifications.

Properties of the purified 17β-hydroxysteroid dehydrogenase

The properties of the purified dehydrogenase are summarized as follows [12]:

(1) Purity: 1. Apparent homogeneity observed on polyacrylamide disc electrophoresis. 2. Single spot on thin layer chromatography.

(2) Stability: No loss of the activity at −20°C for 6 months when purified.

(3) Molecular weight: 35,500 by gel filtration column chromatography. 33,200 by thin layer chromatography.

(4) Molecular radius: 2·37 nm.

(5) Sedimentation behavior: 5·3 S, Symmetrical single peak at 32,500 *g* for 16·5 h.

(6) Substrate specificity: Similar preference for androstenedione, estrone and dehydro*epi*androsterone in their reduction by the enzyme.

(7) K_m: 40 μM for androstenedione and 8·3 μM for NADPH.

(8) Optimal temperature: 50°C in phosphate buffer at pH 7·4.

(9) Optimal pH: 6·5 to 7·5 at 37°C, 8·5 at 50°C.

(10 Cofactor preference: NADPH and its oxidized form [22].

(11) Stoichiometry: equimolar relationship between oxidation of NADPH and testosterone production from androstenedione [22].

Table 3. Summarized data on purification of 17β-hydroxysteroid dehydrogenase of porcine testes [12]

Purification step	Protein content (mg)	Total enzyme activity (nmole/min)	Specific enzyme activity (nmole/min/mg protein)	Purification factor
1. Microsomal fraction (10,000–105,000 × g precipitates)	27,000	970	0·036	1
2. 125,000 × g Supernatant fluid of the sonicated microsomes	2,800	930	0·328	9
3. Precipitate by ammonium sulfate (40–75%)	1,500	530	0·355	10
4. Sephadex G-100 Gel filtration (Fraction 17–22)	87	550	6·32	176
5. DEAE-cellulose column chromatography (Fraction 46–48	4·18	190	45·2	1260
6. Bio-Gel P-100 Gel filtration* (Fraction 66–77)	0·469	32	67·7	1880

* A part (5·0 ml) of the enzyme solution from step 5 (total volume 12 ml) was applied to Bio-Gel P-100 column.

Relationship of the 17β-hydroxysteroid dehydrogenase to NADPH

The following relationships were examined, and the results are to be published shortly elsewhere [22]. Besides the cofactor preference and stoichiometric study mentioned above, the stereospecificity of hydrogen transfer from NADPH to androstenedione by the enzyme has been studied, and no transhydrogenation activity of this enzyme between NADPH and NAD^+ was found.

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