Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Steroid Biochemistry & Molecular Biology 91 (2004) 185–190
The Journal of Steroid Biochemistry & Molecular Biology
www.elsevier.com/locate/jsbmb

Purification and characterization of 7β-hydroxysteroid dehydrogenase from rabbit liver microsomes

Hironori Shiotsuki, Yorio Maeda, Kazuo Chijiiwa*

Department of Surgery I, Miyazaki University School of Medicine 5200 Kihara, Kiyotake Miyazaki, 889–1692, Japan

Received 25 July 2003; accepted 9 April 2004

Abstract

7β-Hydroxysteroid dehydrogenase (7β-HSD), a specific enzyme active in the metabolization of 7β-hydroxycholesterol, was purified about 300-fold from male rabbit liver microsomes using ion exchange, hydroxylapatite, 2′5′ADP Sepharose 4B, and high-performance liquid chromatography on the basis of its catalytic activity.

The specific activity of the purified enzyme was 276 nmol/min/mg protein. The molecular weight of the purified enzyme was 34,000. The preferred coenzyme was β-$NADP^+$. The optimum pH for oxidation was around 7.7 in potassium phosphate buffer, and 11.0 in glycine–NaOH buffer. The purified enzyme catalyzed the synthesis of not only 7β-hydroxycholesterol but also corticosterone and hydrocortisone. Enzyme activities toward these three substrates accompanied all purification steps of 7β-HSD. The amino acid sequence of the N-terminal of the purified enzyme showed that 7β-HSD had sequence similarity to rabbit type I 11β-hydroxysteroid dehydrogenase (11β-HSD), indicating that 7β-HSD may belong to the rabbit type I 11β-HSD family and may play the same role in the metabolism of 11-hydroxysteroids and 7-hydroxysterols.
© 2004 Elsevier Ltd. All rights reserved.

Keywords: Rabbit; Liver microsomes; 7β-Hydroxycholesterol; 7β-Hydroxysteroid dehydrogenase; 11β-Hydroxysteroid dehydrogenase

1. Introduction

7-Oxycholesterol is thought to be an autooxidation product of cholesterol in mammals [1,2]. This sterol is found in human aorta, brain, kidney, liver and serum. Recently, many effects of 7-oxycholesterol in experimental animals and humans have been demonstrated [3–7]. However, its biological importance is still under investigation.

7α- and 7β-hydroxycholesterol are products of cholesterol. 7α-Hydroxycholesterol is an intermediate of the bile acid biosynthetic pathway and metabolized into bile acids [8–10]. On the other hand, the role and metabolic fate of 7β-hydroxycholesterol are not well defined. Björkhem et al. [11] have preliminarily reported the presence of 7β-hydroxysteroid dehydrogenase (7β-HSD) in a 20,000 g supernatant of rat liver that converted 7β-hydroxycholesterol to 7-ketocholesterol. Song et al. [12,13] reported purification of an enzyme from hamster liver microsomes that converts 7α-hydroxycholesterol into 7-ketocholesterol. They called the enzyme 7α-hydroxycholesterol dehydrogenase and reported that the purified enzyme catalyzed not only 7α-hydroxycholesterol but also 7β-hydroxycholesterol, corcicosterone and hydrocortisone with almost the same catalytic activity. They also reported that a partial N-terminal amino acid sequencing showed that this enzyme had a sequence similar to that of human 11β-hydroxysteroid dehydrogenase (11β-HSD). Recently, we reported a comparative study of the conversion of 7α- and 7β-hydroxycholesterol in various animals and demonstrated species differences and possibility of interconversion between these sterols [14]. While 7α-hydroxysteroid dehydrogenase (7α-HSD) was observed in hamster and chicken, 7β-HSD was observed in all vertebrates tested.

To clarify further the biological importance of the 7β-dehydrogenation of 7β-hydroxycholesterol, we here describe the purification and characterization of the enzyme from rabbit liver microsomes.

2. Materials and methods

2.1. Materials

7β-Hydroxycholesterol and 7-ketocholesterol were purchased from Steraloid (Wilton, NH). 7α-Hydroxy-4-cho-

* Corresponding author. Tel.: +81 985 85 2905; fax: +81 985 85 5814.
E-mail address: kazuochi@post.miyazaki-med.ac.jp (K. Chijiiwa).

0960-0760/$ – see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jsbmb.2004.04.005

lesten-3-one was synthesized according to the method described by Shimasue [15]. DEAE Sepharose and 2′5′ ADP Sepharose 4B were purchased from Pharmacia (Uppsala, Sweden). Hexane, isopropanol and methanol of high-performance liquid chromatography (HPLC) grade, β-$NADP^+$ and glycerol were purchased from Wako Pure Chemical (Osaka, Japan). Other reagents were of the highest grade commercially available. Hydroxylapatite was obtained from Bio-Rad (Hercules, CA).

2.2. *Purification of the enzyme*

A male New Zealand white rabbit weighing 2.0–2.5 kg was sacrificed under diethyl ether anesthesia. The liver was quickly removed, perfused with 0.9% NaCl and then homogenized with 4 volumes of 0.25 M sucrose containing 5 mM Tris–HCl and 1 mM EDTA, pH 7.4. The homogenate was centrifuged at 9000 × *g* for 15 min in the cold. The supernatant was centrifuged at 105,000 × *g* for 60 min. The precipitate was washed and recentrifuged at 105,000 × *g* for 60 min.

The microsomal precipitate was suspended in 100 mM Tris–HCl buffer, pH 7.4, containing 20% glycerol, 1 mM EDTA, and 0.5 mM dithiothreitol (DTT). The following procedures were carried out at 4 °C except for HPLC, which was performed at room temperature. All buffer solutions used in the following purification procedures contain 20% glycerol, 1 mM EDTA, and 0.5 mM DTT unless otherwise stated.

The microsomal pellet was solubilized with 1% (w/v) sodium deoxycholate. The solubilized preparation was fractionated with 50% polyethylene glycol (PEG). A fraction precipitated with 8%–16% PEG was re-dissolved in the 100 mM Tris–HCl buffer (pH 7.4), containing 0.5% sodium deoxycholate. The re-dissolved solution was dialyzed against 100 mM Tris–HCl (pH 7.4) containing 0.2% sodium deoxycholate. The dialyzate was subjected to Phenyl Sepharose CL4B column chromatography (2.5 cm × 10 cm). The column was washed with the same buffer and eluted with 100 mM Tris–HCl (pH 7.4), containing 1% Lubrol PX. The fractions showing enzyme activity were collected and dialyzed against 20 mM Tris–HCl (pH 8.0) containing 0.02% Lubrol PX. The dialysate was subjected to a DEAE Sepharose CL6B column (2.5 cm × 5 cm) equilibrated with the same buffer used for dialysis. The column was washed with the equilibration buffer and eluted with a linear gradient of 0–0.5M sodium chloride in the equilibrated buffer. The fraction showing enzyme activity were pooled and dialyzed against 20 mM potassium phosphate buffer (pH 7.4) containing 0.02% Lubrol PX. The dialyzate was applied to a hydroxylapatite column (2.5 cm × 2.0 cm) equilibrated with the same buffer used for dialysis and a stepwise elution was performed with 40, 100, 200 and 400 mM potassium phosphate buffer (pH 7.4). The fraction showing enzyme activity was pooled and dialyzed against 20 mM potassium phosphate buffer (pH 7.4), containing 0.02% Lubrol PX. The dialysate was subjected to column chromatography (1.5 cm × 1.5 cm). The column was washed with the equilibration buffer and eluted with 2 M sodium chloride in the equilibrated buffer. The fraction with the enzyme activity was collected and dialyzed against 20 mM Tris–HCl (pH 8.0) containing 0.4% Lubrol PX. Aliquots were injected into a TSK-gel DEAE-5PW column (0.75 cm × 7.5 cm) equilibrated with the same buffer used for dialysis. The column was eluted with a linear gradient of 0–0.5M sodium chloride in the equilibrated buffer. The flow rate of the anion exchange column chromatography was 0.5 ml/min, and effluents were monitored at 280 nm. The fractions with enzyme activity were collected and concentrated with a centricon. After concentration, the samples were stored at −20 °C.

The research protocol was approved by the animal study committees at Miyazaki Medical College (2002–16).

2.3. *Enzyme assay*

7β-Hydroxysteroid dehydrogenase activity was measured as described previously [14]. A typical assay mixture contained 0.1 M of potassium phosphate buffer (pH 7.4), 1 mM EDTA, 0.5 μmol of $NADP^+$ and 20 nmol of 7β-hydroxycholesterol dissolved in 10 μl of isopropanol in a final volume of 1.0 ml. Incubation was carried out at 37 °C for 5 min and stopped by addition of 1 ml of methanol. Steroids were extracted with 5 ml of *n*-hexane. An aliquot of the extract was evaporated, dissolved in isopropanol and injected into a high-performance liquid chromatograph produced by Shimadzu (Kyoto, Japan, Model LC-8A) and equipped with an integration system (Shimadzu; Model Chromatopack C-R3A). The HPLC column (Wakosil 5SIL, 4.6 mm × 250 mm) was obtained from Wako Pure Chemical Industries.

Oxidation enzyme activities at C-11 of hydrocortisone and corticosterone were determined by measuring the rate of conversion in the presence of β-$NADP^+$. A typical incubation mixture contained 0.1 M of potassium phosphate buffer (pH 7.4), 1 mM EDTA, 0.5 μmol of β–$NADP^+$ and 20 nmol of hydrocortisone or corticosterone dissolved in 10 μl of methanol in a final volume of 1.0 ml. Incubation was carried out at 37 °C for 5 min and steroids were extracted with 5 ml of ethyl acetate. An aliquot of the extract was evaporated, dissolved in methanol and injected into the HPLC. The column used was a C18 column (Wako l, 4.6 mm × 150 mm), and the solvent mixture was methanol/water (50/50, v/v). Effluents were monitored at 240 nm.

2.4. *Other methods*

Protein concentration was determined by the method of Lowry et al. [16]. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method of Laemmli [17], and proteins were visualized by silver staining.

2.5. Amino acid sequence analysis

Partial N-terminal amino acid sequence analysis was performed. The purified enzyme was subjected to SDS-PAGE and transferred to a polyvinylidene difluoride membrane using transfer buffer, containing 10 mM CAPS and 10% (v/v) methanol. Transferred protein was stained with 0.02% Coomassie brilliant blue. The band of protein was cut out and sequenced using an Hewlett–Packard G1005A Protein Sequencer System.

3. Results

Table 1 summarizes the purification of 7β-HSD from male rabbit liver microsomes. The activity of the enzyme was decreased significantly after solubilization of microsomes with sodium cholate (data not shown). On the other hand, a significantly increased activity (approximately three times that of the microsomal fraction) was observed when sodium deoxycholate was used as the detergent. Over 60% of the total enzyme activity was retained in the fraction eluted between 8% and 16% of PEG 6000. The enzyme activity was observed in the non-bound fractions of the DEAE chromatography. The fractions eluted by 200 and 400 mM of potassium phosphate buffer (pH 7.4) had high enzyme activities. The fractions of a HA column eluted with 400 mM potassium phosphate buffer were applied to a 2′5′ ADP Sepharose 4B column. The enzyme was eluted with the 20 mM potassium phosphate buffer (pH 7.4), containing 2 M NaCl. The enzyme was further purified using HPLC equipped with an anion-exchange column (DEAE-5PW).

SDS-polyacrylamide gel electrophoretograms of the fractions separated by HPLC are shown in Fig. 1. The molecular weight of the purified enzyme from rabbits was 34,000. Table 2 shows the substrate specificity of the purified enzyme. Cholesterol, 7α-hydroxycholesterol, 25-hydroxycholesterol, pregnenolone, dehydroepiandrosterone,

Table 1
Purification steps for rabbit liver microsomal 7β-hydroxysteroid dehydrogenase

Fraction	Total protein (mg)	Total activity (nmol/min)	Yield (%)	Specific activity (nmol/min/mg protein)
Microsome	2520	2368.8	100	0.94
Solubilization	2544	7248.5	306.6	2.885
PEG (8%–16%)	936	5083.4	214.6	5.43
Phenyl Sepharose				
Non-bound	203.4	2359.3	99.6	11.60
DEAE				
Non-bound	82.7	2096.4	88.5	25.3
HA				
Elution with 400 mM	6.2	824.6	34.8	133.0
HPLC (DEAE 5PW)	1.7	469.4	19.8	276.1

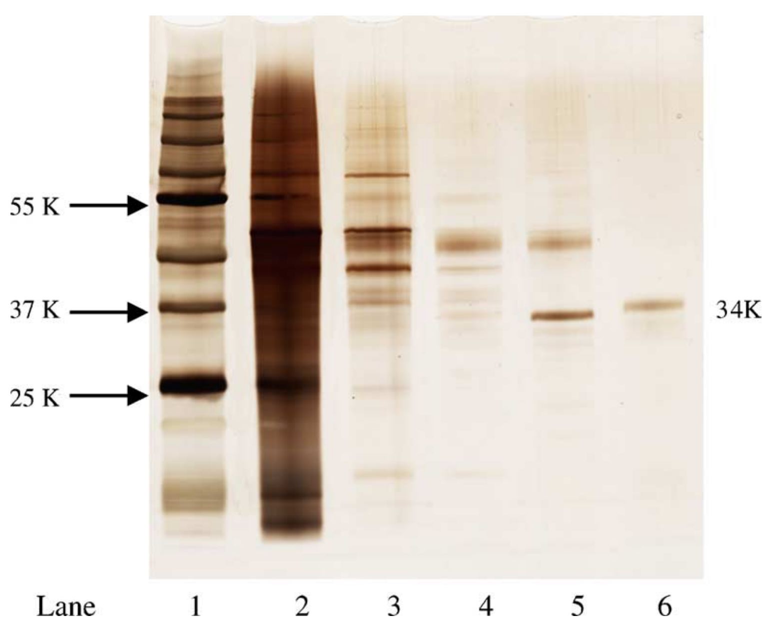

Fig. 1. SDS-PAGE of the purification steps of rabbit liver microsomal 7β-HSD. Lane 1: standard; lane 2: protein profile of rabbit liver microsomes; lane 3: PEG 8%–16% fraction; lane 4: after DEAE; lane 5: after HA; lane 6: after DEAE 5PW. Protein bands were visualized by silver staining.

Table 2
Substrate specificity of 7β-hydroxysteroid dehydrogenase

Substrate	Product formed (nmol of product/min/mg protein)
5-Cholesten-3β-ol	ND[a]
5-Cholesten-3β,7α-diol	ND
5-Cholesten-3β,7β-diol	267.9
3α,7α-Dihydroxycholanoic acid	ND
3a,7p-Dihydroxycholanoic acid	ND
Cortisol	534.4
Corticosterone	477.7

[a] Not detected.

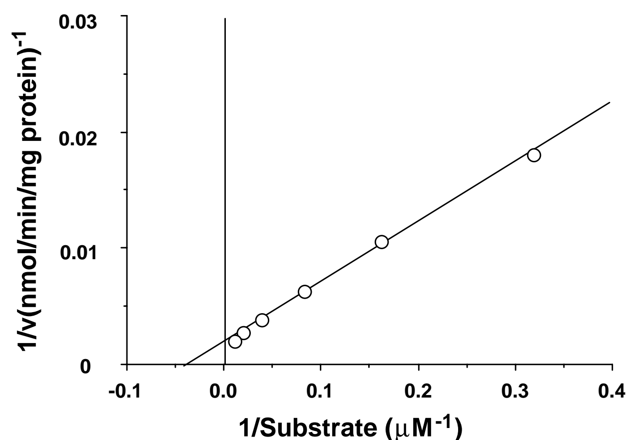

Fig. 2. Lineweaver–Burk plot. One microgram of purified enzyme was incubated with various amounts of substrate and the products were determined by HPLC as described in Section 2.

Table 3
N-terminal amino acid sequence of purified rabbit hepatic 7β-HSD

Rabbit 7β-HSD	AFMKKYLLPLLGLFLAYYY
Rabbit 11β-HSD	AFMKKYLLPLLGLFLAYYYYSA (Ozols, 1995)

chenodoxycholic acid and ursodeoxycholic acid were not oxidized by the purified enzyme. A Lineweaver-Burk plot is shown in Fig. 2. The V_{max} and K_m were 512.8 nmol/min/mg of protein and 2.63 μM, respectively. Enzyme activities of each purification step are shown in Fig. 3. Enzyme activities toward the three substrates accompanied each other at all purification steps.

The amino acid sequence of the N-terminal of the purified enzyme is shown in Table 3. As shown in the Table 3, the sequences of the enzyme were similar to those of the rabbit type I 11β-hydroxysteroid dehydrogenase reported.

4. Discussion

7β-Hydroxysteroid dehydrogenase is a hepatic microsomal enzyme that catalyzes the conversion of 7β-hydroxycholesterol to 7-ketocholesterol. This reaction has been described in the literature since 1968 [11], but details of

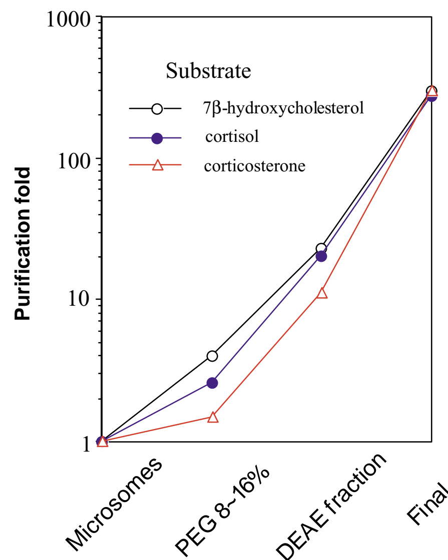

Fig. 3. Yield of each purification step. Proteins of each step were incubated with three different substrates and determined by HPLC as described in Section 2.

the enzyme have not been investigated to date. Recently, we studied 7-hydroxysteroid dehydration in several animals and demonstrated that 7β-dehydration occurs in many vertebrates, including humans [14]. On the other hand, 7α-dehydrolation occurs in hamster and chicken liver microsomes. In 1998, Song et al. [13] reported the purification of an enzyme from hamster liver microsomes that catalyzed the conversion of 7α-hydroxycholesterol to 7-ketocholesterol and that the apparent molecular weight of the purified enzyme was 34,000. They described the enzyme as a 7α-hydroxycholesterol dehydrogenase and demonstrated that the purified enzyme catalyzed the conversion of not only 7α-hydroxycholesterol but also 7β-hydroxycholesterol. On the other hand, according to our results, microsomes prepared from rabbit, guinea pig and rat catalyzed the conversion of 7β-hydroxycholesterol, but not 7α-hydroxycholesterol. From their reports regarding the partial N-terminal amino acid sequence [13], 7α-hydroxycholesterol dehydrogenase has a sequence similar to that of human 11β-HSD.

In this experiment, we purified rabbit liver microsomes and demonstrated that the purified enzyme catalyzed the formation of 7β-hydroxycholesterol, hydrocortisone and corticosterone but not 7α-hydroxycholesterol. The result suggests a species difference between rabbit and hamster in the substrate specificity of this enzyme and also suggests that the purified enzyme is different from the enzymes that catalyze the oxidoreduction of bile acids [18,19]. During the enzyme purification procedure, we determined that the specific enzyme activities toward these three substrates (7β-hydroxycholesterol, hydrocortisone and hydroxycorticosterone) were elevated simultaneously. In the present study, we did not test conversion between 7α-,

7β-dehydroepiandrosterone (DHEA) and 7-oxo-DHEA. However, recently, Robinzon et al. reported that interconversion of 7-oxo-DHEA and 7-OH-DHEA isomers is catalyzed by 11β-HSDI in both liver and kidney [20]. A partial N-terminal amino acid sequence of 7β-HSD was similar to that of rabbit type I 11β-HSD. These results suggest that rabbit 7β-HSD may belong to the family of rabbit type I 11β-HSD. To clarify its clinical importance, further study (for example, serum or tissue concentrations of those steroids) is needed.

11β-Hydroxysteroid dehydrogenase is a microsomal enzyme that is widely distributed in various tissues and responsible for the oxidation of 11-hydroxy- to 11-ketosteroids. Two tissue-specific isoforms with different functions have been described [21]. Type I 11β-HSD is widely distributed, with high levels in liver and kidney [22,23], while type II 11β-HSD is mainly present in kidney and placenta [24–26]. Recently, it was shown that the type I 11β-HSD plays an important role in xenobiotic carbonyl reduction [27–29]. In our experiment, reduction of 7β-hydroxycholesterol was demonstrated. What is this microsomal 7β-HSD and how does it function physiologically? Corticosterone and hydrocortisone are important endogenous hormones essential for water and electrolytes metabolism. 7β-Hydroxycholesterol is not a very active metabolite in the body, but it is a member of the oxycholesterols. Therefore, it should be clarified which 7-oxocholesterol (7-ketocholesterol or 7β-hydroxycholesterol) is the dominant product and which is less toxic (or harmful) in the lipid metabolism in the body.

In conclusion, we purified 7β-hydroxysteroid dehydrogenase from rabbit liver rmicrosomes and demonstrated an amino acid sequence similarity to that of the rabbit type I 11β-hydroxysteroid dehydrogenase.

References

[1] L.L. Smith, In: Cholesterol Autooxidation, Plenum Press, New York, 1981.

[2] L.L. Smith, Review of progress in sterol oxidations: 1987–1995, Lipids 31 (1996) 453–487.

[3] N. Tamasawa, A. Tamasawa, K. Takebe, M. Hayakari, The effect of dietary 7-ketocholesterol, inhibitor of sterol synthesis, on hepatic microsomal cholesterol 7α-hydroxylase activity in rat, Biochim. Biophys. Acta 1214 (1994) 20–26.

[4] A.A. Kandutsch, H.W. Chen, Inhibition of cholesterol synthesis by oxygenated sterols, Lipids 13 (1978) 704–707.

[5] J.M. Hayden, L. Brachova, K. Higgins, L. Obermiller, A. Sevanian, S. Khandrika, P.D. Reaven, Induction of monocyte differentiation and foam cell formation in vitro by 7-ketocholesterol, J. Lipid Res. 43 (2002) 26–35.

[6] A. Meynier, J. Lherminier, J. Demaison-Meloche, C. Ginies, A. Grandgirard, L. Demaison, Effects of dietary oxysterols on coronary arteries in hyperlipidaemic hamsters, Br. J. Nutr. 87 (2002) 447–458.

[7] Y. Miyashita, H. Ozaki, N. Koide, M. Otsuka, T. Oyama, Y. Itoh, T. Mastuzaka, K. Shirai, Oxysterol-induced apoptosis of vascular smooth muscle cells is reduced by HMG-CoA reductase inhibitor, pravastatin, J. Atheroscler. Thromb. 9 (2002) 65–71.

[8] H.R. Hutton, G.S. Boyd, The metabolism of cholest-5-en-3β,7α-diol by rat-liver cell fraction, Biochim. Biophys. Acta 116 (1966) 336–361.

[9] I. Björkhem, On the mechanism of the enzymatic conversion of cholest-5-ene-3β,7α-diol into 7α-hydroxycholest-4-en-3-one, Eur. J. Biochem. 8 (1968) 337–344.

[10] I. Björkhem, Mechanism of bileaacid synthesis in mammalian liver, In: H. Danielsson, J. Sjvalls (Eds.), Comprehensive Biochemistry, Elsevier, Amsterdam, 1986, pp. 231–278.

[11] I. Björkhem, K. Einarsson, G. Johansson, Formation and metabolism of 3β-hydroxycholest-5-en-7-one and cholest-5-ene-3β, 7β-diol, Acta Chem. Scand. 22 (1968) 1595–1605.

[12] W. Song, M. Pierce, Y. Saeki, R.N. Redinger, R.A. Prough, Endogeneous 7-oxocholesterol is an enzymatic product: characterization of 7α-hydroxycholesterol dehydrogenase activity of hamster liver microsomes, Arch. Biochem. Biophys. 328 (1996) 272–282.

[13] W. Song, C. Jiaming, L. William, N.R. Richard, A.P. Russel, Purification and characterization of hamster liver microsomal 7α-hydroxycholesterol dehydrogenase, J. Biol. Chem. 273 (1998) 16223–16228.

[14] Y. Maeda, H. Nagatomo, F. Uchiyama, J. Nagatomo, M. Yamada, H. Shiotsuki, Y. Ohta, S. Sato, M.-H. Kai, K.-H. Kondo, S. Higashi, T. Setoguchi, A comparative study of the conversion of 7-hydroxycholesterol in rabbit, guinea pig, rat, hamster, and chicken, Steroids 67 (2002) 703–708.

[15] A. Shimasue, The action of cholesterol: Oxygen oxidoreductase on cholest-5-ene-3β, 7α-diol and the enzymatic preparation of the labeled cholest-4-en 7α-ol-3-one, Hiroshima J. Med. 23 (1974) 265–272.

[16] O.H. Lowry, N.J. Rosenbrough, A.L. Farr, R.J. Randall, Protein measurement with the folin phenol reagent, J. Biol. Chem. 193 (1951) 265–275.

[17] U.K. Laemmli, Cleavage of structural proteins during the assembly of the head of bacteriophage T_4, Nature 227 (1970) 680–685.

[18] Y. Amuro, W. Yamade, T. Nakano, E. Hayashi, T. Hada, K. Higashino, Reduction of 7-ketolithocholic acid to chenodeoxycholic acid by rat liver preparations in vitro, Biochim. Biophys. Acta 841 (1985) 229–231.

[19] Y. Amuro, W. Yamade, K. Kudo, T. Yamamoto, K. Hada, K. Higashino, Reduction of 7-ketolithocholic acid by human liver enzyme preparations in vitro, Am. J. Physiol. 256 (1989) G67–71.

[20] B. Robinzon, K.K. Michael, S.L. Ripp, S.J. Winters, R.A. Prough, Glucocorticoid inhibit interconversion of 7-hydroxy and 7-oxo metabolites of dehydroepiandrosterone: a role for 11β-hydroxysteroid dehydrogenases? Arch. Biochem. Biophys. 412 (2003) 251–258.

[21] C. Monder, C.H.L. Shackleton, 11 beta-hydroxysteroid dehydrogenase: fact or fancy? Steroids 44 (1984) 383–417.

[22] V. Lakshmi, C. Monder, Purification and characterization of the corticosteroid 11 beta-dehydrogenase component of the rat liver 11 beta-hydroxysteroid dehydrogenase complex, Endocrinology 123 (1988) 2390–2398.

[23] J. Ozols, Luminal orientation and post-translational modification of liver microsomal 11β-hydroxysteroid dehydrogenase, J. Biol. Chem. 270 (1995) 2305–2312.

[24] E. Rusvai, A. Naray-Fejes-Toth, A new isoform of 11 beta-hydroxysteroid dehydrogenase in aldosterone target cells, J. Biol. Chem. 268 (1993) 10717–10720.

[25] K. Yang, M. Yu, Evidence for distinct isoforms of 11 beta-hydroxysteroid dehydrogenase in the ovine liver and kidney, J. Steroid Biochem. Mol. Biol. 49 (1994) 245–250.

[26] R.W. Brown, R.K.E. Chapman, C.R. Edwards, J.R. Seckle, Human placental 11 beta-hydroxysteroid dehydrogenase: evidence for and partial purification of a distinct NAD-dependent isoform, Endocrinology 132 (1993) 2614–2621.

[27] E. Maser, G. Bannenberg, 11 beta-hydroxysteroid dehydrogenase mediates reductive metabolism of xenobiotic carbonyl compounds, Biochem. Pharmacol. 47 (1994) 1805–1812.

[28] E. Maser, E. Richter, J. Friebertshauser, The identification of 11 beta-hydroxysteroid dehydrogenase as carbonyl reductase of the tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, Eur. J. Biochem. 238 (1996) 484–489.

[29] E. Maser, U.C.T. Oppermann, The 11β-hydroxysteroid dehydrogenase system, a determinant of glucocorcicoid and mineralcorticoid action Role of type-I 11β-hydroxysteroid dehydrogenase in detoxication processes, Eur. J. Biochem. 249 (1997) 365–369.