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The Purification of 11β-Hydroxysteroid Dehydrogenase from Mouse Liver Microsomes

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11β-Hydroxysteroid dehydrogenase (11β-HSD) is a microsomal enzyme complex responsible for the interconversion of active 11-hydroxy glucocorticoids to inactive 11-oxo metabolites. It has long been controversially discussed whether 11-dehydrogenation and 11-oxoreduction are catalysed by a single bidirectional enzyme or if the 11β-HSD system comprises 2 kinetically distinct microsomal enzyme activities, 11-dehydrogenase and 11-oxoreductase. However, 11-oxoreduction of homogenously purified 11β-HSD could not be demonstrated under *in vitro* conditions until today. We have purified 11β-HSD from mouse liver microsomes to homogeneity by a purification method which affords a gentle membrane protein solubilization as well as providing a favourable detergent surrounding during the various chromatographic steps. Following 11-dehydrogenation of corticosterone and 11-oxoreduction of dehydrocorticosterone simultaneously throughout the entire purification procedure we could demonstrate that 11β-HSD retains both oxidative and reductive activities in almost the same ratio, which is also true for the homogenously purified enzyme. Deducing from the coincidentally increasing specific activities of 11-dehydrogenation and 11-oxoreduction the conclusion can be drawn that both activities reside within the same protein. Furthermore, in addition to NADP(H) also NAD(H) can serve as cosubstrate, which is mainly true for the oxidative direction. In conclusion, our results provide evidence that the oxidative and reductive behaviour of 11β-HSD can be explained by the concept of a unique, reversible oxidoreductase thus disproving the two enzyme theory.

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INTRODUCTION

11β-Hydroxysteroid dehydrogenase (11β-HSD) is a microsomal enzyme responsible for the conversion of the active glucocorticoid cortisol to the inactive metabolite cortisone (in the rat and mouse corticosterone to 11-dehydrocorticosterone) [1]. A physiological role for 11β-HSD has recently been demonstrated in the kidney, where it protects the mineralocorticoid (Type I) receptor from exposure to cortisol or corticosterone, thereby allowing preferential access for aldosterone [2, 3]. Deficiency of 11β-HSD, either congenital [4] or when the enzyme is inhibited by liquorice [5] or carbenoxolone [6], results in activation of mineralocorticoid receptors by cortisol or corticosterone with resultant sodium retention and hypertension.

11β-HSD is also found in glucocorticoid target tissues, notably the liver [7, 8] and preliminary studies indicate that it may regulate exposure of active glucocorticoids to the classical glucocorticoid (Type II) receptor.

The conversion of the 11β-hydroxycorticosteroids cortisol and corticosterone to the 11-dehydrocorticosteroids cortisone and dehydrocorticosterone is reversible *in vivo* under normal circumstances. It has long been controversially discussed, whether 11β-dehydrogenation and 11-oxoreduction are catalysed by a single bidirectional enzyme or by two distinct enzymes [9, 10]. Patients with AME (apparent mineralocorticoid excess) [4, 11] lack 11-dehydrogenase but express 11-reductase activity, consistent with the idea that these activities reside in different proteins. The two enzyme hypothesis was further supported by the findings that the rat hepatic 11β-HSD complex expresses different kinetic properties of the 11-dehydrogenation and 11-reduction components [10]. Another possible hypothesis of the 11β-HSD protein composition includes a single multifunctional enzyme that contains distinct dehydrogenation and reduction sites.

Lakshmi and Monder [12] have purified the corticosteroid 11-dehydrogenase component of the 11β-HSD

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complex from rat liver microsomes to homogeneity, which they showed to be devoid of any corticosteroid 11-reductase activity. They did not find evidence that rat liver contains a single enzyme which is able to catalyse both forward and reverse reaction. These authors have recently cloned the gene encoding rat hepatic 11β-HSD [13]. Although purified 11-dehydrogenase from rat liver had no 11-oxoreductase activity [12], expression of the 11-dehydrogenase cDNA in Chinese hamster ovary (CHO) cells, however, resulted in an enzyme with both dehydrogenase and reductase activities [14], casting doubt on the two enzyme theory.

A possible argumentation for these conflicting findings is that 11-oxoreductase activity of 11β-HSD from rat liver microsomes is more labile than 11-dehydrogenase, which may be successively destroyed during the purification procedure. Consequently, until today 11-oxoreductase activity of homogenously purified 11β-HSD could not be demonstrated under *in vitro* conditions.

The aim of our study was the purification of 11β-HSD from mouse liver microsomes in order to promote the elucidation of the current discussion of whether 11β-HSD activity is the resultant of the action of two separate, interdependent enzymes, 11-dehydrogenase and 11-oxoreductase, or whether this microsomal enzyme is a unique, bidirectional oxidoreductase.

In order to preserve 11-oxoreducing activity of 11β-HSD during the purification procedure we developed a new purification method which affords a gentle membrane protein solubilization as well as providing a favourable detergent surrounding during the subsequent chromatographic steps. On the other hand, we chose mouse liver microsomes as the biological source of 11β-HSD, because mouse liver 11β-HSD might possibly be more stable and less sensitive in the purified state within the artificial detergent surrounding.

EXPERIMENTAL

Animals

Female NMRI mice (25–30 g) were used for the experiments. Animals were raised in our institute and were kept on a standard laboratory pellet diet and water *ad libitum* at a fixed 12-h light–dark cycle starting at 06:00 h. Mice were not fasted before killing.

Chemicals

Glucocorticoids (cortisol, cortisone, corticosterone and 11-dehydrocorticosterone) were purchased from Sigma Chemie GmbH (Munich, Germany). Enzymatic tests were performed using NAD^+, NADH, $NADP^+$ and NADPH from Boehringer Mannheim (Mannheim, Germany). For HPLC acetonitrile of HPLC-grade from E. Merck (Darmstadt, Germany) was used. Enzyme purification was carried out with octyl–sepharose CL-4B and red-sepharose A from Pharmacia Fine Chemicals (Uppsala, Sweden) and DEAE–cellulose from Sigma. The detergents listed below were supplied from the following companies: Emulgen 913 (Kao-Atlas Co., Tokyo, Japan), sodium cholate and Triton X-100 (E. Merck). The low molecular weight markers were obtained from Sigma. All other chemicals used in the experiments were reagent grade and were obtained from commercial suppliers.

Preparation of liver microsomes

The animals were killed by means of dislocation of the cervical spinal cord. After perfusing the livers with an ice cold isotonic solution of KCl they were homogenized in 4 vol of 20 mM Tris–HCl buffer, pH 7.4, containing 250 mM sucrose, 1 mM EDTA and 1 mM phenylmethanesulphonylfluoride (PMSF) using a glass–Teflon Potter-Elvehjem homogenizer. The homogenate was centrifuged at 600 ***g*** for 10 min and at 10,000 ***g*** for 10 min to sediment nuclei, cell debris and mitochondria. The supernatant at this stage was centrifuged at 170,000 ***g*** for 1 h to sediment the microsomes. The microsomal pellet was resuspended and washed with 0.15 M KCl to remove glycogen and then resuspended in the homogenization buffer without PMSF finally yielding a protein concentration of about 20 mg/ml.

Purification of the enzyme

Enzyme solubilization. For solubilization of membrane associated proteins the microsomal suspension was diluted with an equal volume of a 10 mM sodium phosphate buffer, pH 7.2, containing 1 mM EDTA, 1 M NaCl, 40% glycerol (w/v) and 0.4% (w/v) of the nonionic detergent Emulgen 913. The solution was gently stirred for 45 min and subsequently centrifuged at 210,000 ***g*** for 60 min. The supernatant was adjusted to 0.4% (w/v) of sodium cholate before being applied to the octyl–sepharose CL-4B column.

Octyl–sepharose chromatography. In order to separate the enzyme from microsomal monooxygenase components a hydrophobic interaction chromatography according to Kling *et al.* [15] was performed. The following buffers were used: Buffer A: 10 mM sodium phosphate, 1 mM EDTA, 500 mM NaCl, 20% (w/v) glycerol, 0.5% (w/v) sodium desoxycholate, pH 7.4; Buffer B: 10 mM sodium phosphate, 1 mM EDTA, 400 mM NaCl, 20% (w/v) glycerol, 0.4% (w/v) sodium cholate, 0.1% (w/v) Emulgen 913, pH 7.4; Buffer C: 10 mM sodium phosphate, 1 mM EDTA, 20% (w/v) glycerol, 2% (w/v) Emulgen 913, pH 7.4.

Solubilized microsomes (maximum 24 ml) were applied to the octyl–sepharose CL-4B column (1.8 × 25 cm) previously equilibrated with 300 ml of buffer A. Elution was performed with buffer A until the end of peak 2, then with buffer B until the end of peak 3 followed by buffer C finally eluting peak 4. The elution profile was monitored measuring the absorbance of the fractions at the wavelength of 417 nm. The column flow rate was 84 ml/h and the volume per fraction 5 ml. Enzyme activity coincides only with peak 3, the fractions of which were collected, concentrated through an Amicon PM-10 membrane to about 20 ml and

dialysed overnight against 5 mM sodium phosphate buffer, pH 7.4.

DEAE–cellulose chromatography. The dialysed enzyme solution was applied to a column (1.6 × 20 cm) packed with DEAE–cellulose and previously equilibrated with 5 mM sodium phosphate buffer, pH 7.4. The column was washed with the equilibration buffer and the adsorbed enzyme was then eluted with a 40 mM phosphate buffer, pH 7.4, at a flow rate of 36 ml/h and fraction volumes of 3 ml. Enzymatically active fractions were pooled, concentrated through an Amicon PM-10 membrane to about 2–3 ml and supplemented with glycerol to a final concentration of 30%.

Red sepharose chromatography. Fractions from DEAE–cellulose chromatography were directly applied on a red sepharose A column (1.2 × 5 cm), previously equilibrated with a 10 mM phosphate buffer, pH 7.4. The column was rinsed successively with the equilibration buffer, then with the equilibration buffer containing 0.8 mM NaCl and 2 mM $NADP^+$ and then with the same buffer containing 1 M NaCl and 2 mM $NADP^+$. The enzyme was finally eluted with a 10 mM phosphate buffer, pH 7.4, containing 1 M NaCl, 2 mM $NADP^+$ and 0.1% Emulgen 913. The fractions with high enzyme activity were pooled, concentrated through an Amicon PM-10 membrane to about 2–3 ml and stored in 0.2 ml aliquots at −70°C.

Throughout the purification the temperature was kept at 4°C.

Enzyme assays

Assay of 11β-HSD dehydrogenation activity during the purification procedure was performed by preincubating 20 µl of 50 mM sodium phosphate buffer, pH 9, 10 µl of $NADP^+$ (final concentration 3.2 mM) and 10 µl of corticosterone (final concentration 1 mM) for 3 min at 37°C. Corticosterone was dissolved in 50% ethanol. Control experiments were determined in the presence of various quantities of the solvent which did not influence enzyme activity up to an ethanol concentration of 10%. The reaction was started by adding 10 µl of enzyme solution. After 30 min incubation time the reaction was stopped and corticosterone as well as its oxidized metabolite dehydrocorticosterone were extracted by adding 150 µl of ice cold acetonitrile. The samples were centrifuged for 6 min at 8000 *g* in the cold and 20 µl of the supernatant served for the determination of glucocorticoids by HPLC analysis. Control experiments without biological material were performed to determine nonenzymatic substrate conversion. Specific activities are expressed as µmol of dehydrocorticosterone formed per mg of protein within 30 min.

Assay of 11β-HSD 11-oxoreducing activity during the purification was performed by preincubating 20 µl of 50 mM sodium phosphate buffer, pH 7.4, 10 µl of NADPH (final concentration 3.2 mM) and 10 µl of dehydrocorticosterone (final concentration 1 mM). Incubation conditions and metabolite detection were the same as described under 11β-HSD dehydrogenation activity, except that incubation periods were 2 h. Specific activities are expressed as nanomoles of corticosterone formed per mg of protein within 30 min.

Kinetic parameters of purified 11β-HSD were calculated from experiments performed with substrate concentrations between 0.0125 mM and 0.4 mM of glucocorticoid. Time and enzyme protein concentrations were chosen so that reaction velocities were time linear which was found to be true at least for 4 h. pH values for glucocorticoid conversion were pH 9 for cortisol and corticosterone oxidation and pH 7.4 for cortisone and dehydrocorticosterone reduction, and were both based on the pH optimum of each reaction, respectively. Kinetic parameter estimations were made using the GraphPad InPlot kinetic computer software.

Metabolite determination by HPLC

After enzymatic conversion oxidized or reduced glucocorticoids were detected on a BioRad (Munich, Germany) reversed phase HPLC system, with an Octadecyl-Si 100 polyol (Serva, Heidelberg, Germany) matrix column (4.5 mm × 25 cm), a UV monitor and an HRLC integration software (BioRad). Using a methanol–H_2O (58:42, v/v) eluent and a flow rate of 0.5 ml/min the following retention times were achieved, cortisone: 16 min, cortisol: 20 min, corticosterone: 18 min, dehydrocorticosterone: 30 min. Glucocorticoids were monitored at 262 nm and concentrations were calculated referring to corresponding calibration curves ranging from 0.3 to 10 nmol of the respective glucocorticoid.

SDS–polyacrylamide gel electrophoresis

Sodium dodecyl sulphate (SDS)–polyacrylamide gel electrophoresis was carried out as described by Laemmli [16] using 10% acrylamide in the separating gel. Protein bands were visualized by staining with Coomassie blue or by applying the silver stain technique according to Ansorge [17].

Protein determination

Protein concentration was determined by the method of Lowry *et al.* [18] using bovine serum albumin as standard.

Glycoprotein staining

A highly sensitive silver stain method for the detection of carbohydrates in the homogeneous 11β-HSD preparation after SDS–polyacrylamide gel electrophoresis was performed according to the method of Dubray and Bezard [19]. In brief: after electrophoresis, the protein was fixed by incubating the gel overnight in 25% isopropyl alcohol and 10% acetic acid at room temperature. The gel was then washed for 30 min in 7.5% acetic acid and subsequently incubated for 1 h in 0.2% aqueous periodic acid at 4°C. After having washed the gel for 3 h in several changes of distilled

Table 1. Summary of purification of mouse liver 11β-HSD using corticosterone as substrate for 11-dehydrogenation

Step	Total protein (mg)	Total activity (μmol/30 min)	Specific activity (μmol/mg · 30 min)	Recovery (%)	Purification (fold)
Microsomes	254.48	14.91	0.059	100	1.0
Solubilized microsomes[a]	216.72	36.60	0.169	246	2.9
Octyl–sepharose CL-4B	10.03	29.68	2.959	199	50.2
DEAE–cellulose	0.95	7.30	7.686	49	130.3
Red sepharose A	0.49	4.84	9.880	32	167.5

11β-HSD activity was assayed in 50 mM sodium phosphate buffer, pH 9, 3.2 mM $NADP^+$ and 1 mM corticosterone as substrate for 11-dehydrogenation. Activity is expressed as μmol dehydrocorticosterone formed in 30 min per mg of protein.

[a]The solubilized microsomal suspension used in this reaction mixture did not contain sodium cholate.

water, it was incubated in 100 ml freshly made ammoniacal silver solution (1.4 ml of fresh NH_4OH were added to 21 ml of 0.36 NaOH; to this solution, 4 ml of 19.4% $AgNO_3$ were added slowly while agitating vigorously and completing it with H_2O). The gel was removed from the ammoniacal silver solution, washed for 2 min in distilled water and then developed in a freshly prepared solution containing 0.05% citric acid, 0.019% formaldehyde and 10% methanol.

RESULTS

Purification of 11β-HSD

11β-HSD from mouse liver microsomes was solubilized by use of the nonionic detergent Emulgen 913 and resulted in a 3-fold increase in specific activity of corticosterone oxidation (Table 1) indicating that 11β-HSD is bound to the membranes of the endoplasmic reticulum in a latent state [20]. The addition of sodium cholate to the solubilized microsomes was critically important before being applied to the hydrophobic interaction chromatography on octyl–sepharose. On the one hand, sodium cholate turned out to decrease enzyme activity (data not shown) [21]. On the other hand, without sodium cholate no separation of 11β-HSD from microsomal monooxygenase components could be achieved on octyl–sepharose chromatography. Hydrophobic interaction chromatography resulted in a 50-fold increase in specific activity of 11β-HSD (Table 1).

On subsequent DEAE–cellulose chromatography 11β-HSD eluted without additional detergents by simply raising the ionic strength of the phosphate buffer to 40 mM. In this case detergent concentration was sufficient from the previous chromatographic step. Specific enzyme activity after DEAE–cellulose could be enhanced 130-fold compared to that in microsomes (Table 1).

During the last step of the purification procedure 11β-HSD was bound to red sepharose A and eluted with 1 mM NaCl, 2 mM $NADP^+$ and 0.1% Emulgen 913 in a single step as an apparently homogenous protein.

Table 1 summarizes the purification procedure of 11β-HSD with corticosterone as substrate for 11-dehydrogenation. Compared to microsomes the specific activity of homogenously purified 11β-HSD could finally be enhanced to about 168-fold. During the purification procedure respective enzyme preparations were also tested for 11-oxoreductase activity. Using dehydrocorticosterone as substrate for 11-oxoreduction the specific activity of corticosterone formation could likewise be progressively enhanced throughout the purification procedure and finally amounted 102-fold that of the crude microsomal fraction (Table 2). However, in this case the mere solubilization did not yield a marked increase in specific activity, which is possibly due to distinct dehydrogenation and reduction sites within the same enzyme molecule.

SDS–polyacrylamide gel electrophoresis of the purification steps

A sample taken from the various purification steps was subjected to SDS–polyacrylamide gel electro-

Table 2. Summary of purification of mouse liver 11β-HSD using dehydrocorticosterone as substrate for 11-oxoreduction

Step	Total protein (mg)	Total activity (nmol/30 min)	Specific activity (nmol/mg · 30 min)	Recovery (%)	Purification (fold)
Microsomes	254.48	1205.39	4.74	100	1.0
Solubilized microsomes[a]	216.72	1298.51	5.99	108	1.3
Octyl–sepharose CL-4B	10.03	1416.45	141.22	118	29.8
DEAE–cellulose	0.95	292.47	307.86	24	65.0
Red sepharose A	0.49	235.69	481.00	20	101.5

11β-HSD activity was assayed in 50 mM sodium phosphate buffer, pH 7.4, 3.2 mM NADPH and 1 mM dehydrocorticosterone as substrate for 11-oxoreduction. Specific activity is expressed as nmol corticosterone formed in 30 min per mg of protein.

[a]The solubilized microsomal suspension used in this reaction mixture did not contain sodium cholate.

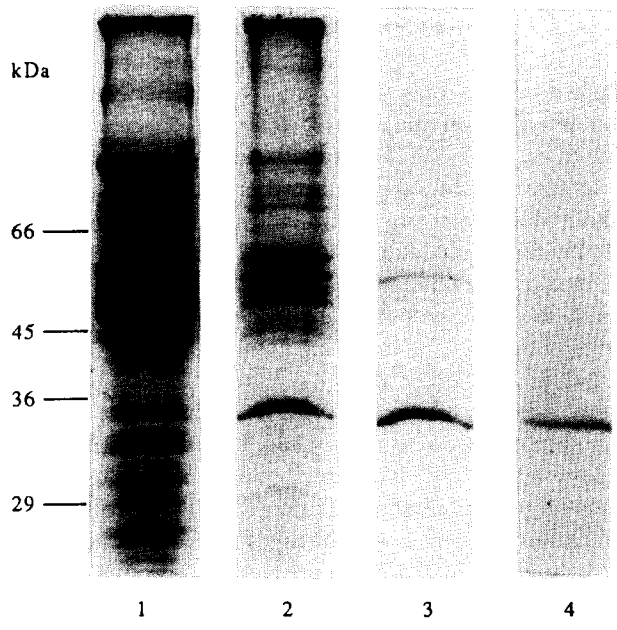

Fig. 1. SDS–PAGE of the purification steps of mouse liver 11β-HSD. Lane 1 = protein profile of mouse liver microsomes; lane 2 = after octyl–sepharose; lane 3 = after DEAE–cellulose; lane 4 = after red sepharose. Visualizing of protein bands was carried out by Coomassie blue staining.

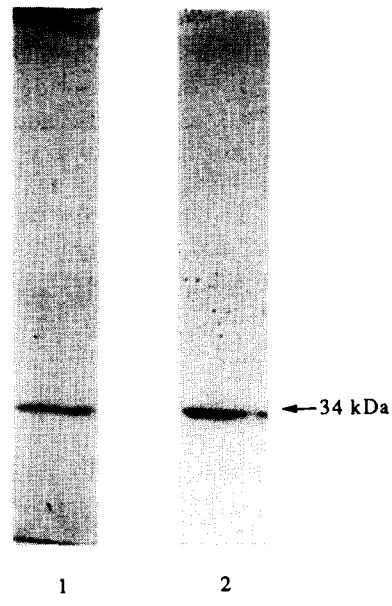

Fig. 2. Carbohydrate staining of purified 11β-HSD after SDS–PAGE according to the method of Dubray and Bezard [19], which is based on a periodic acid silver stain. For details cf. "Experimental". Lane 1 = Coomassie blue staining; lane 2 = carbohydrate staining.

phoresis followed by Coomassie blue or silver staining, as shown in Fig. 1. Lane 1 represents the total quantity of microsomal protein, which was progressively decreased by the subsequent chromatographic purification procedure. The last step yielded a single protein band in the 34 kDa region as compared to the protein standard, giving evidence that 11β-HSD was purified to homogeneity.

Carbohydrate staining

The presence of carbohydrate in 11β-HSD was determined according to the method of Dubray and Bezard [18], which is based on a periodic acid silver stain for 1,2 diol groups of glycoproteins in polyacrylamide gels. As shown in Fig. 2, the purified enzyme gives a strong positive carbohydrate staining after SDS–polyacrylamide gel electrophoresis in the 34 kDa molecular mass region, indicating that 11β-HSD is a glycoprotein.

Kinetic properties of 11β-HSD

The kinetic properties of homogenously purified 11β-HSD together with the estimated intrinsic clearance values of the respective glucocorticoids are presented in Table 3. These data also indicate that both 11-dehydrogenation and 11-oxoreduction can be catalysed by purified 11β-HSD. In detail, the 11β-hydroxy forms cortisol and corticosterone are oxidized to the respective 11-oxo forms in the presence of $NADP^+$ (or NAD^+) and, reversely, the 11-oxo forms cortisone and dehydrocorticosterone are reduced to the respective 11β-alcohols in the presence of the reduced pyridine nucleotides NADPH (or NADH). Accordingly, one and the same enzyme is able to catalyse the reversible oxidoreduction at carbon 11 of glucocorticoids, although intrinsic clearance values reveal that oxidation is favoured over reduction. These results thus support the one enzyme hypothesis of 11β-HSD.

As expected, corticosterone, the predominant glucocorticoid in rats and mice, has higher intrinsic clearance values than cortisol, which is the active glucocorticoid in man. Comparing the intrinsic clearance values

Table 3. Kinetic properties of purified mouse liver 11β-HSD

Substrate	Cosubstrate	V_{max}	K_m	V_{max}/K_m
Corticosterone	$NADP^+$	9.88	0.66	14.97
	NAD^+	13.42	1.00	13.42
Cortisol	$NADP^+$	3.24	0.34	9.53
	NAD^+	3.27	0.73	4.48
Dehydrocorticosterone	NADPH	0.160	0.22	0.73
	NADH	0.097	0.44	0.22
Cortisone	NADPH	0.149	0.184	0.81
	NADH	0.029	0.239	0.12

11β-HSD activities were assayed as described in the Experimental section. Kinetic parameters of purified 11β-HSD were calculated from three individual enzyme preparations and were performed with substrate concentrations between 0.0125 mM and 0.4 mM of glucocorticoid. Standard deviations did not exceed values of 20%. Time and enzyme protein concentrations were chosen so that reaction velocities were time linear. pH values for glucocorticoid conversion were pH 9 for cortisol and corticosterone oxidation and pH 7.4 for cortisone and dehydrocorticosterone reduction. Kinetic parameter estimations were made using the GraphPad InPlot kinetic computer software. V_{max}: (μmol/30 min/mg); K_m: (mM).

estimated with both pyridine nucleotide forms NADP(H) or NAD(H) it turns out that, in the oxidative direction, both pyridine nucleotides can function as cosubstrate. In detail, using corticosterone and cortisol as substrates, intrinsic clearance values with NAD^+ were 90 and 47%, respectively, compared to those with $NADP^+$. In the reductive direction, using dehydrocorticosterone and cortisone as substrates, only weak intrinsic clearance values were obtained with NADH compared to those with NADPH (30 and 15%, respectively).

DISCUSSION

The enzyme 11β-HSD (EC 1.1.1.146) is assumed to protect the nonselective mineralocorticoid (Type I adrenocorticoid) receptor in mineralocorticoid target tissues from exposure to excess of cortisol or corticosterone by converting the much higher levels of active 11-hydroxyglucocorticoids to the receptor inactive 11-oxoglucocorticoids cortisone and 11-dehydrocorticosterone, respectively [1]. The resulting inactive 11-oxo metabolites have a much lower affinity to the mineralocorticoid receptor thus allowing preferential binding of aldosterone [2, 3]. Congenital deficiency of 11β-HSD, the syndrome of apparent mineralocorticoid excess (AME), is associated with severe hypertension, hypokalaemia, and suppression of plasma aldosterone and plasma renin activity [3]. Similarly liquorice and carbenoxolone, which are potent inhibitors of 11β-HSD cause activation of mineralocorticoid receptors by corticosterone or cortisol in animals and man, respectively [5, 6].

All body organs have the capacity to metabolize glucocorticoids, but the liver is recognized as the most metabolically active tissue for the interconversion of 11-oxocorticosteroids and 11β-hydroxycorticosteroids by the action of 11β-HSD [7, 8]. It has long been assumed that the 11β-HSD system comprises 2 kinetically distinct microsomal enzyme activities, 11-dehydrogenase and 11-oxoreductase, because the oxidative and reductive behavior of 11β-HSD could not be explained by the concept of a unique reversible oxidoreductase [1, 9, 10, 22]. Other possible patterns of the 11β-HSD system which have been considered are a single multimeric enzyme containing distinct dehydrogenase and reductase sites or a single-site enzyme that undergoes conformational changes, permitting the enzyme to shuttle between 11β-hydroxy and 11-oxosteroid specific configurations.

Lakshmi and Monder [12] have purified the 11-dehydrogenase component of the 11β-HSD system from rat liver microsomes, which they showed to be devoid of any 11-oxoreducing activity. However, the same authors demonstrated that expression of the 11-dehydrogenase cDNA in CHO cells results in an enzyme with both dehydrogenase and reductase activities [13, 14] which means that both reside within the same primary amino acid sequence, but behave as if they were distinct enzymes.

It is likely that 11-oxoreductase of 11β-HSD from rat liver microsomes is more labile than 11-dehydrogenase, which may be successively destroyed during the purification procedure. Possibly 11-oxoreductase activity of 11β-HSD is strictly dependent on a favourable surrounding architectural structure of the phospholipid bilayer, or, in the case of a purified enzyme, of the artificial detergent embedding. Therefore, 11-oxoreductase activity of purified rat liver 11β-HSD could not be demonstrated under *in vitro* conditions until today.

In the present study we have purified 11β-HSD from mouse liver microsomes. In order to preserve 11-oxoreducing activity of 11β-HSD during the purification procedure we developed a new purification method which affords a gentle membrane protein solubilization as well as providing a favourable detergent surrounding during the subsequent chromatographic steps.

As shown in the results, the mere solubilization of microsomal mouse liver 11β-HSD by use of the nonionic detergent Emulgen 913 already resulted in a 3-fold increase of the 11-dehydrogenation activity indicating that 11β-HSD is bound to the membranes of the endoplasmic reticulum in a latent state. The addition of sodium cholate to the solubilized microsomes was critically important before being applied to the hydrophobic interaction chromatography on octyl–sepharose. On the one hand, sodium cholate turned out to decrease enzyme activity, which could be due to an enzyme inhibition because of an affinity of sodium cholate to the substrate binding site of 11β-HSD. On the other hand, without sodium cholate no separation of 11β-HSD from microsomal monooxygenase components could be achieved on octyl–sepharose chromatography. Using corticosterone as substrate for 11-dehydrogenation the enzyme purification yielded 168-fold the specific activity of homogenous 11β-HSD compared to that of the crude microsomal fraction.

After each chromatographic step respective enzyme preparations were also tested for 11-oxoreducing activity. In this case, the mere solubilization of microsomes did not yield a comparable increase in specific activity. The different behaviour of the two activities may be due to distinct dehydrogenation and reduction sites within the same enzyme molecule. However, following dehydrocorticosterone 11-oxoreduction by 11β-HSD throughout the entire purification almost the same pattern of progressively increasing specific activity was obtained.

Consequently, deducing from the coincidentally increasing specific activities of both oxidation and reduction during the purification procedure the conclusion can be drawn that homogenously purified mouse liver 11β-HSD is able to mediate both 11-dehydrogenation and 11-oxoreduction, which means that both activities reside within the same protein. Probably, our purification method does not dramatically alter the architectural and 3-dimensional structure

of mouse liver 11β-HSD, thus preserving 11-oxo reducing capability of the purified enzyme. A comparison of 11-dehydrogenation and 11-oxoreduction during the whole purification procedure reveals a nearly constant ratio between the two activities indicating that the interconversion dynamics of both reactions remain nearly constant.

In addition to the gentle purification procedure the reason for the preserved 11-oxoreducing activity of the homogenous enzyme could well be that mouse liver 11β-HSD is more stable than that of the rat liver.

The purity of 11β-HSD enzyme preparation after the last chromatographic step was checked, on the one hand, by SDS–PAGE followed by Coomassie blue or silver staining, yielding a single band in the 34 kDa molecular mass region, which parallels the molecular mass value of the rat liver enzyme [12]. On the other hand, N-terminal aminoacid sequence analysis revealed a sequence homology to rat liver 11β-HSD [13] of 54% and thus simultaneously proved the homogeneity of our enzyme preparation. Corresponding to rat liver 11β-HSD [12] also that of mouse liver is a glycoprotein, as shown by carbohydrate staining in the polyacrylamide gel. This finding was further evidenced by deglycosylating the purified 11β-HSD which resulted in a lowered molecular mass in the 32 kDa region (data not shown). Unlike rat liver 11β-HSD that of mouse liver is also able to use NAD(H) as cosubstrate in addition to NADP(H), which is mainly true for the oxidative direction.

In addition to catalysing the reversible oxidoreduction of corticosterone into dehydrocorticosterone, 11β-HSD of mouse liver is also capable of mediating the reversible oxidoreduction of cortisol into cortisone, the predominant glucocorticoids in man.

In summary, we have purified 11β-HSD from mouse liver microsomes by a new purification method. This is the first report providing evidence that homogenously purified 11β-HSD retains reversibility of 11-dehydrogenation, namely reduction of 11-oxo glucocorticoids. Moreover, whereas $NADP^+$ is described to be the preferred cosubstrate of rat liver 11β-HSD, the mouse liver enzyme also accepts NAD^+ as cosubstrate for glucocorticoid 11-dehydrogenation. NADH as cosubstrate for 11-oxoreduction plays only a minor role compared to that with NADPH. Corresponding to rat liver 11β-HSD, mouse liver 11β-HSD is a glycoprotein with a molecular mass unit of 34 kDa which prefers corticosterone as substrate, rather than cortisol, the predominant glucocorticoid in man.

In conclusion, our results provide evidence that the oxidative and reductive behaviour of 11β-HSD can be explained by the concept of a unique, reversible oxidoreductase thus conclusively disproving the two enzyme theory.

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