Biochimica et Biophysica Acta, 987 (1989) 21–28
Elsevier

BBAMEM 74663

Solubilization of nuclear steroid 5α-reductase from rat ventral prostate

Ulrike Enderle-Schmitt, Christian Neuhaus and Gerhard Aumüller

Institut für Anatomie und Zellbiologie, Marburg (F.R.G.)

(Received 31 May 1989)
(Revised manuscript received 5 September 1989)

Key words: 5α-Reductase; Solubilization; Nuclear membrane; (Rat ventral prostate)

Δ^4-Steroid-5α-reductase (3-oxo-5α-steroid: NADP$^+$ Δ^4-oxidoreductase, EC 1.3.1.22), is a membrane-bound enzyme. In the ventral prostate of the rat, its activity is found within the nuclear envelope. Solubilization of this enzyme can only be achieved in the presence of detergents. We studied the inhibitory effect of various detergents on 5α-reductase activity as a function of detergent concentration, of pH, of incubation time, of salt concentration and of additives to the buffer system. Four detergents (Lubrol WX, CHAPS, L-α-lysophosphatidylcholine and octyl D-glucopyranoside) were selected for subsequent solubilization studies. The overall recovery of solubilized enzyme activity was about 30% when compared to 100% of 5α-reductase activity found in freshly prepared nuclei. Up to 20–30% of the nuclear proteins were extracted during the solubilization procedure. Among the various treatments tested, a concentration of 3 mg/ml L-α-lysophosphatidylcholine per 10 mg/ml of nuclear protein in the presence of 5 mM $MgCl_2$, 0.1 M KCl, 0.1 M sodium citrate and 5 mM NADPH yielded the maximal enzymic activity of 56%, 15% of the nuclear proteins being solubilized in an active and stable form. The activity in these extracts could be kept stable for 2 days at 4°C with a recovery of 75% of enzymic activity. A 3-fold increase of specific 5α-reductase activity was obtained during solubilization under optimal conditions.

Introduction

The enzyme 3-oxo-5α-steroid: NADP$^+$ Δ^4-oxidoreductase (EC 1.3.1.22) of rat prostate is bound to the nuclear envelope and is responsible for the conversion of 4-ene-3-oxo-steroids into the corresponding 3-oxo-5α-dihydrosteroids. The physiological significance of nuclear prostatic 5α-reductase activity resides in the formation of the biologically active metabolite dihydrotestosterone from testosterone. A complete characterization of the enzyme is impeded by the low enzyme concentration in androgen target tissues, its instability in vitro, even in intact membrane preparations, and its structural integration into membranes rendering it highly hydrophobic [1] and requiring a solubilization for further characterization. Phospholipid – protein interactions are known to markedly affect 5α-reductase activity in rat epididymis [2] and rat prostate [1,3].

Methods have been described for the solubilization and partial characterization of the microsomal Δ^4-steroid 5α-reductase of rat liver, an enzyme with properties completely different from 5α-reductase in androgen target tissues [4,5]. Little is known about this enzyme in androgen target-dependent organs. Some progress has been made towards solubilizing [6] 5α-reductase in rat epididymis and human prostate [7]. Solubilization of 5α-reductase from rat prostate has been reported by use of digitonin and KCl [8]. This procedure has been difficult to reproduce in our hands. Therefore, we were forced to establish our own solubilization protocol. We report here the optimisation of conditions for the solubilization of rat nuclear 5α-reductase using various detergents.

Materials and Methods

Materials. [1,2,6,7-^{3}H]Testosterone (3.4 TBq/mmol) was obtained from Amersham Buchler, Braunschweig. Silica gel TLC-plates (Polygram Sil-GH) were obtained from Macherey & Nagel Co., Düren.

Detergents. Sodium deoxycholate was purchased from Serva KG, Heidelberg; CHAPS, Lubrol WX and L-α-lysophosphatidylcholine from Sigma, Munich; octyl

Abbreviations: CHAPS, 3-[(3-cholamido propyl)dimethylammonio]-1-propanesulfonate; DHT, 5α-dihydrotestosterone; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecylsulfate.

Correspondence: G. Aumüller, Department of Anatomy and Cell Biology, Philipps-University Marburg, D-3550 Marburg, F.R.G.

D-glucopyranoside from Riedel-De Haen, Hannover; and Nonidet P-40 from Fluka, Neu Ulm.

Chemicals. NADPH, unlabeled steroids, acrylamide and additional chemicals required for gel electrophoresis were obtained from Serva, Heidelberg and L-α-phosphatidylcholine (egg yolk Type V-E) from Sigma, Munich. All other chemicals were of analytical grade and supplied by Merck, Darmstadt.

Animals. Male Wistar rats, weighing 300–400 g, were obtained from Ivanovas, Kisslegg.

Preparation of purified prostatic nuclei. Purified nuclei were prepared from rat prostate as previously described [1]. Briefly, a homogenate was centrifuged at 1000 × *g* for 15 min according to Moore and Wilson [9]. The pellet was suspended in 2.0 M sucrose containing 0.5 mM $CaCl_2$ at a volume equal to 3 ml/g of starting tissue. Aliquots of this suspension were layered on 10 ml of 2.2 M sucrose containing 0.5 mM $CaCl_2$ and the tubes were centrifuged at 56000 × *g* for 90 min. This procedure was repeated twice. The 56000 × *g* pellet was again homogenized in 50 mM Tris-HCl buffer (pH 7.4) containing 5 mM $MgCl_2$ yielding 10 mg protein per ml suspension. This suspension was used for the experiments.

Assay of steroid 5α-reductase activity. Steroid 5α-reductase activity was assayed radiometrically by measuring the conversion of [^{3}H]testosterone to [^{3}H]dihydrotestosterone according to Enderle-Schmitt et al. [1]. The routine assay for the enzyme was carried out in a final volume of 30 μl containing 15 μl 50 mM Tris-HCl buffer (pH 7.0), 5 mM NaCl, 1 mM NADPH and 0.5 pmol testosterone including the tritiated testosterone (specific activity 3.4 TBq/mmol). The reactions were started by the addition of 15 μl of a 5α-reductase containing sample (15–75 μg of protein) and incubated for 15 min at 37°C. The reaction was stopped by addition of 50 μl ethanol/acetone (1 : 1, v/v). After shaking the mixture vigorously, precipitated protein was removed by centrifugation. The supernatant was applied to a TLC-plate and the plate was developed in dichloromethane/ether (8 : 2, v/v). Radioactivity on the plate was scanned and analyzed from quantitative plots printed by a TLC-analyzer (Berthold, Wildbad).

Detergent treatment. Sodium deoxycholate, L-α-lysophosphatidylcholine, octyl D-glucopyranoside, CHAPS, Lubrol WX and Nonidet P-40 were kept as stocks of 10 mg/ml in 50 mM Tris-HCl buffer (pH 7.4) at room temperature. Prior to membrane solubilization, the stock was diluted with appropriate buffer to the concentration desired for solubilization. Purified nuclei (50 μl of a suspension containing 2.5–5 mg protein/ml in inhibition experiments or 5–10 mg protein/ml in solubilization experiments) were precipitated by centrifugation (5 min, 10000 × *g*). The supernatant was discarded and the solubilization was initiated by adding 50 μl of the respective detergent to the pelleted nuclei. The resultant detergent-membrane sample was vortexed for 30 min at 4°C and centrifuged at 100000 × *g* for 1 h (Airfuge, Beckmann, 22 psi). The supernatant and pellet were collected separately. The pellet was suspended in detergent containing phosphate buffer. Activities released to the supernatant by this treatment were considered to be solubilized.

Solubilization in the presence of phosphatidylcholine. The phospholipid used in all experiments was L-α-phosphatidylcholine (egg yolk type V-E). The phospholipid was dissolved in chloroform/diethyl ether solution which then was evaporated and the phospholipid was resuspended twice prior to use. Phosphatidylcholine was suspended in phosphate buffer (pH 6.0) and sonicated to clarity before use. A stock solution of 50 mg/ml phosphatidylcholine was prepared, which was diluted for solubilization experiments. Using CHAPS as a detergent, the solubilization buffer contained 0, 1, 2, 3, 6 or 10 mg CHAPS with 0 or 12.5 mg phosphatidylcholine, respectively (higher phosphatidylcholine concentrations caused an unsoluble precipitate in the presence of CHAPS); using L-α-lysophosphatidylcholine as detergent, the solubilization buffer contained 0, 0.6, 1.5, 3, 6 or 10 mg detergent with 0, 12.5 or 25 mg phosphatidylcholine, respectively. A suspension of 1 mg nuclear protein in 50 μl of this buffer was incubated for 30 min at 4°C and subsequently centrifuged at 100000 × *g* for 1 h. 5α-Reductase activity was measured and specific activity was calculated.

Gel exclusion chromatography. A 1 cm × 10 cm column of Sepharose 4B (Pharmacia) was equilibrated with 50 mM Tris-HCl (pH 7.4) containing 5 mM $MgCl_2$, 0.1 M KCl, 0.1 M sodium citrate, 1 mM NADPH and 1 mg/ml Lubrol WX. A 200 μl aliquot of solubilized nuclei in solubilization buffer containing 3 mg/ml Lubrol WX was chromatographed. Fractions of 600 μl were collected and tested for 5α-reductase activity. The column was calibrated with Dextran blue (void volume), bovine serum albumin (66 kDa), catalase (230 kDa), ferritin (450 kDa) and myosin (500 kDa).

Protein measurement. Protein concentrations were determined using the method of Bradford [11] with ovalbumin as standard. For determination of protein in solubilized enzyme preparations standard curves were prepared in the presence of equivalent amounts of solubilization medium.

Electrophoretic techniques. Gel electrophoresis on gradient gels of 7.5–20% polyacrylamide in the presence of SDS was performed according to Laemmli [12]. 30 μg protein per sample were applied to the gel and gels were stained with Serva blue G.

Results

Membrane fractionation

Rat prostatic 5α-reductase is pre-dominantly localized in the nuclear envelope [1,8,9,13]. During purifica-

tion of the nuclei, the specific 5α-reductase activity increased by about 15-fold when compared to the homogenate. Isolation of the nuclear envelope as further purification step again increased the specific activity about 150-fold when compared to the purified nuclei. Because of the considerable loss of absolute enzyme activity during preparation of the nuclear membranes, we simply used purified rat prostatic nuclei as source for the solubilizaiton studies of 5α-reductase. An electron microscopic examination of the nuclei revealed a negligible amount of residual microsomes. The outer nuclear membrane was seen nearly free of adherent microsomal residues (results not shown).

Preliminary studies indicated that 5α-reductase activity was not solubilized by simply resuspending the nuclei in buffer containing KCl concentrations up to 1.5 M [1]. This indicated that the enzyme was not a peripheral membrane protein requiring the use of a detergent for solubilization [14]. In a first set of experiments we studied the inhibitory effects of various detergents on 5α-reductase activity. Detergents which caused low inhibition, were selected for subsequent solubilization experiments.

Effect of Lubrol WX on 5α-reductase activity

The inhibitory effects on 5α-reductase activity of low concentrations of Lubrol WX (a nonionic detergent, which has been used previously for the solubilization of the epididymal steroid 5α-reductase [6]), was studied by using the enzyme assay. Lubrol WX was tested in a concentration range from 0.01 to 10 mg/ml. Concentrations above 1 mg/ml caused a concentration-dependent inhibition of 5α-reductase activity. 50% of the enzyme activity was inhibited at a concentration of 3 mg/ml Lubrol WX and 80% at a concentration of 10 mg/ml.

The influence of different parameters such as pH value, concentration of KCl, NADPH etc. on Lubrol WX-dependent decrease of 5α-reductase was studied in experiments starting with 3 mg/ml of Lubrol WX per incubation. The pH optimum of 5α-reductase activity (pH 6.0) was unchanged independent from the presence of 3 mg/ml Lubrol WX (Fig. 1A).

The effect of KCl concentrations increasing up to 1 M was assayed in phosphate buffer at pH 6.0 (250 μg of nuclear protein). 5α-Reductase activity was slightly increased by the addition of KCl when incubated without detergent. The addition of KCl in the presence of Lubrol WX, however, caused a concentration-dependent decrease of enzyme activity at concentrations above 0.1 M KCl (Fig. 1B).

Table I illustrates a slight increase of 5α-reductase activity in the presence of Lubrol WX, the incubation medium containing of either 1 mM $MgCl_2$ or a combination of 1 mM $MgCl_2$, 0.1 M KCl, 0.1 M sodium citrate and 10% glycerol. A significant effect (39% increase in enzyme activity when compared to a control without additional chemicals) was observed in a buffer system, where glycerol had been replaced by 1 mM NADPH (Table I: H).

Therefore, a phosphate buffer pH 6.0 containing 1 mM $MgCl_2$, 0.1 M KCl, 0.1 M sodium citrate and 1 mM NADPH was chosen for further experiments.

Effects of different detergents on 5α-reductase activity

The effects of six different detergents on enzyme activity were tested at a concentration range from 0.1 to 10 mg/ml (Fig. 2). The buffer system used for this set of experiments has been described above. Detergents tested belonged to different groups (for review see Ref. 15): Lubrol WX, Nonidet P-40 and octyl D-glucopyranoside are nonionic detergents. L-α-Lysophosphatidylcholine and CHAPS, a derivative of cholic acid [16], are zwitterionic detergents, and sodium deoxycholate is an anionic detergent. All detergents caused a concentration-dependent inhibition of activity. CHAPS, Lubrol WX, and octyl D-glucopyranoside had no effect on 5α-reductase activity over a concentration range up to 3 mg/ml, while Nonidet P-40 and sodium deoxycholate

TABLE I

Effects of various chemicals to the buffer system on 5α-reductase activity in the presence and absence of Lubrol WX

	$MgCl_2$ (1 mM)	KCl (0.1 M)	Sodium citrate (0.1 M)	Glycerol (10%)	NADPH (1 mM)	DHT satd.	Percentage of 5α-reductase relative to control value	
							no Lubrol WX	3 mg/ml Lubrol WX
A	−	−	−	−	−	−	±0	±0
B	+	−	−	−	−	−	+4.5	+14.4
C	−	+	−	−	−	−	+4.9	+8.0
D	−	−	+	−	−	−	+26.5	+9.7
E	+	+	−	−	−	−	+13.4	+5.4
F	+	+	+	−	−	−	+23.9	−0.9
G	+	+	+	+	−	−	+19.6	+13.7
H	+	+	+	−	+	−	+21.2	+39.2
I	+	+	+	+	−	−	+5.5	+2.5

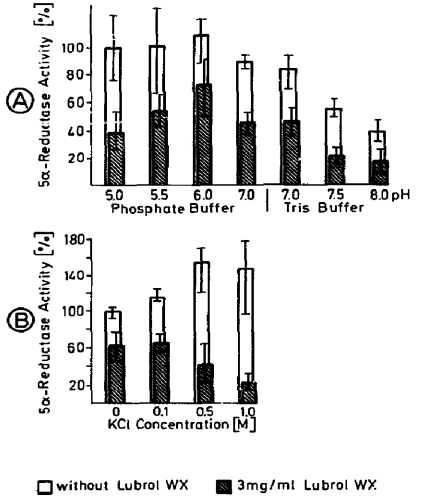

Fig. 1. 5α-Reductase activity in the presence of Lubrol WX as a function of pH, KCl concentrations and various chemicals. Activity of 5α-reductase (250 μg of protein, respectively) was assayed with and without the addition of 3 mg/ml Lubrol WX after an incubation time of 30 min at 4° C. The experimental protocol was varied according to the following parameters: (A) Effect of pH; (B) effect of KCl concentrations.

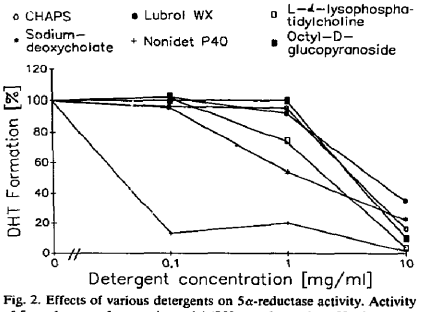

Fig. 2. Effects of various detergents on 5α-reductase activity. Activity of 5α-reductase of prostatic nuclei (250 μg of protein/150 μl, respectively) was assayed in the presence of various detergents in a concentration range up to 10 mg/ml.

inhibited 50% of the enzyme activity at concentrations of less than 0.1 mg/ml and 1 mg/ml, respectively. These data indicate that Lubrol WX, CHAPS, L-α-lysophosphatidylcholine and octyl D-glucopyranoside yield the highest recovery of 5α-reductase activity.

Solubilization of 5α-reductase using various detergents

In contrast to the aforementioned experiments which addressed the question of 5α-reductase inhibition by various detergents and selection of optimal incubation conditions, a second set of experiments checked the ability of detergents, of solubilizing nuclear 5α-reductase from rat prostate. The solubilization capacity of various detergents for 5α-reductase was studied by resuspending purified nuclei (5–10 mg of protein per ml) in the presence of detergent. After an incubation time of 30 min (longer incubation times causing inhibition of 5α-reductase activity), the samples were centrifuged at 100000 × *g* for 1 h. The 5α-reductase activity and protein concentrations were assayed in the pellets and supernatants, respectively. During initial experiments, a detergent concentration which resulted in a 50% decrease of 5α-reductase activity (see Fig. 2) was tested. Using sodium deoxycholate and Nonidet P-40, no 5α-reductase activity could be recovered in the solubilized supernatant. Other detergents left part of the enzyme active in the supernatants as well as in the pellets. For more detailed solubilization studies Lubrol WX, CHAPS, L-α-lysophosphatidylcholine and octyl D-glucopyranoside were selected. Using increasing concentrations of these detergents (up to 10 mg/ml), 5α-reductase activity and protein concentrations were analyzed in the 100000 × *g* supernatants and pellets. Results are shown in Fig. 3 and are summarized in Table II.

The maximum recovery of solubilized 5α-reductase activity was dependent on the detergent used reaching 23–56% of an untreated control pellet. Optimal solubilization was achieved at detergent concentrations of 2–3 mg/ml. This indicates an optimal detergent/protein ratio of about 1:4, independent of the nature of the detergent. Maximal protein concentration after solubilization varied between 20% to 30%. The addition of increasing detergent concentrations resulted in a concentration-dependent release of protein into the 100000 × *g* supernatant extracts at the solubilization optimum, i.e., at the point where maximum 5α-reductase activity is solubilized, the concentration of solubilized protein is below the value of the solubilization maximum. The highest recovery of 5α-reductase in the supernatant (56% of the control activity) was obtained using 3 mg/ml L-α-lysophosphatidylcholine in a phosphate buffer (pH 6.0) containing 5 mM $MgCl_2$, 0.1 M KCl, 0.1 M sodium citrate and 5 mM NADPH (nuclear protein concentration: 5 mg/ml). The activity in these

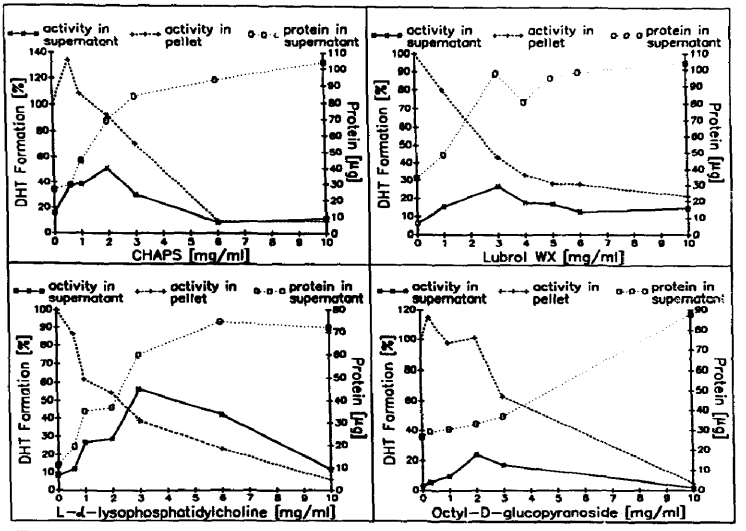

Fig. 3. Solubilization of 5α-reductase and protein with increasing concentration of detergent. Prostatic nuclei (50 µl of a suspension with 5–10 mg/ml nuclear protein) were incubated with CHAPS, Lubrol WX, L-α-lysophosphatidylcholine and octyl D-glucopyranoside, respectively, in a concentration-dependent manner for 30 min. After centrifugation (100000 × g, 1 h) the protein concentration and the 5α-reductase activity were analyzed in the pellets and supernatants. The solubilized protein in the supernatant is the percentage of the total protein concentration of the sample (pellet plus supernatant). Data represent mean values of three experiments.

extracts could be kept stable for 2 days at 4°C with a recovery of 75% of enzymic activity.

Criteria of complete solubilization

Razin [17] has established three criteria for solubilization of membrane-bound proteins. We have monitored our detergent-treated 100000 × *g* supernatants with respect to these criteria: (1) Solubilized proteins should not be pelleted by a 100000 × *g* centrifugation for 1 h. The supernatant formed after long-term, high-speed centrifugation (1 h, 100000 × *g*) of the detergent-treated nuclear proteins contained 5α-reductase activity. (2) Solubilized proteins should be retained on a Sepharose 4B column. Using the 100000 × *g* supernatant of Lubrol WX-treated nuclei for gel exclusion chromatography on a Sepharose 4B column (equi-

TABLE II

Solubilization of 5α-reductase with different detergents. Determination of the solubilization optimum

Detergent	Detergent concentration at the solubilization (mg/ml)	Solubilized 5α-reductase activity [a] (%)	Membrane-bound 5α-reductase activity [b] (%)	Solubilized protein [c] (%)
CHAPS	2	55.4	84.1	17.3
Lubrol WX	3	26.9	43.1	25.3
L-α-Lysophosphatidylcholine	3	56.3	38.3	14.8
Octyl D-glucopyranoside	2	23.1	97.9	6.2

[a] Percentage of 5α-reductase activity in the 100000 × *g* supernatant relative to the activity of a non-detergent-treated 100000 × *g* pellet (control activity 100%).

[b] Percentage of 5α-reductase activity in the 100000 × *g* pellet relative to the activity of a non-detergent-treated 100000 × *g* pellet.

[c] Percentage of solubilized protein (100000 × *g* supernatant) relative to the total protein concentration of the sample (pellet + supernatant).

TABLE III

Solubilization in the presence of phosphatidylcholine

	Phosphatidylcholine (mg/ml)	Maximum solubilized 5α-reductase activity [a] (%)	Detergent concentration at the solubilization optimum (mg/ml)	Protein concentration at the solubilization optimum [b] (%)
CHAPS	0	2	55.4	17.3
	12.5	3	50.2	9.4
L-α-Lysophosphatidylcholine	0	3	56.3	14.8
	12.5	6	29.6	17.3
	25	10	18.5	9.7

[a] Percentage of 5α-reductase activity in 100000 × *g* supernatant relative to the activity of a non-detergent treated 100000 × *g* pellet.
[b] Percentage of solubilized protein (100000 × *g* supernatant) relative to the total protein concentration of the sample (pellet plus supernatant).

librated with the solubilization buffer containing 1 mg/ml Lubrol WX), 5α-reductase activity was eluted immediately after the void volume of the column. According to the calibration of the column the molecular size of this fraction was about 500 kDa (data not shown). (3) No membrane fragments should be detectable in the solubilized sample by electron microscopy.

Electron microscopic examination of negatively stained 100000 × *g* supernatant of CHAPS-solubilized nuclei failed to reveal any membranous structures. Instead, particles of about 30 nm in diameter were present (results not shown).

Solubilization in the presence of phosphatidylcholine

Phosphatidylcholine was added to the solubilization buffer containing CHAPS or L-α-lysophosphatidylcholine at detergent concentrations ranging from 1–10 mg/ml; phosphatidylcholine concentrations tested were 0, 12, 5 and 25 mg/ml, respectively. The results are summarized in Table III.

With increasing amounts of phosphatidylcholine, the solubilization optimum was shifted to higher detergent concentrations (CHAPS: from 2 mg/ml to 3 mg/ml; L-α-lysophosphatidylcholine: from 3 mg/ml to greater than 10 mg/ml). 5α-Reductase activity decreased after solubilization in the presence of phosphatidylcholine especially when L-α-lysophosphatidylcholine was used as a detergent (Table III). Concentration of solubilized protein were likewise decreased. In conclusion, the addition of phosphatidylcholine to the solubilization buffer resulted in a shift of the solubilization optimum to higher detergent concentrations. Solubilized 5α-reductase activity, however, was not stabilized, it even was decreased. Therefore, the presence of phospholipids as protectives during solubilization is not required, but must instead be avoided.

Dilution of solubilized 5α-reductase

Solubilized prostatic nuclei (8 mg/ml nuclear per protein, 2 mg/ml CHAPS) were diluted 1 : 1, 1 : 3 and 1 : 9 with a detergent-free phosphate buffer. After a 100000 × *g* centrifugation for 1 h, enzyme activities and protein concentrations in the pellets and supernatants were assayed. No significant loss of 5α-reductase in the supernatant was obtained, if the detergent concentrations was decreased to 1 mg/ml or even 0.5 mg/ml (10% loss of enzyme activity). Contrary, a dilution to 0.2 mg/ml caused a 50% loss of solubilized 5α-reductase activity. The higher the degree of dilution was, the more 5α-reductase activity was precipitated in a protein-lipid-detergent complex. A 2-fold increase of specific 5α-reductase activity was obtained in the 1 : 3 diluted precipitate. In purification experiments of the solubilized enzyme, a dilution exceeding 1 : 3 should be avoided to prevent precipitation.

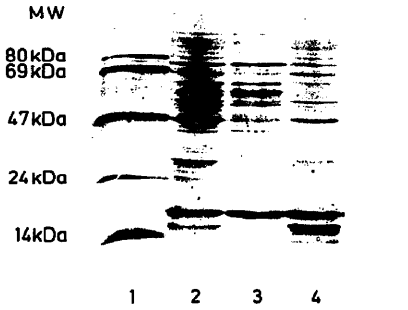

Fig. 4. SDS-PAGE of CHAPS-solubilized prostatic nuclei. Nuclei and CHAPS (2 mg/ml) treated nuclei (100000 × *g* supernatant and 100000 × *g* pellet) were applied to a 7.5–20% gel. Lane 1, molecular weight markers: transferring (80 kDa, human serum albumin (69 kDa) ovalbumin (47 kDa), chymotrypsinogen (24 kDa), lysozyme (14 kDa). Lane 2, prostatic nuclei. Lane 3, CHAPS-treated nuclei, supernatant. Lane 4, CHAPS-treated nuclei, pellet.

SDS-PAGE of CHAPS solubilized nuclei

An electrophoretic analysis of the polypeptide components was performed after CHAPS treatment (2 mg/ml) of prostatic nuclei (100000 × *g* supernatant and 100000 × *g* pellet) and compared to the polypeptide pattern of intact nuclei (Fig. 4). The 100000 × *g* pellet, representing the residual membranous structures and the chromatin, contained most of the histone proteins. Its distribution pattern of polypeptides was similar to that of intact nuclei. In contrast, the solubilized fraction showed enrichment of several polypeptides, predominantly in the molecular weight range of 40 kDa to 80 kDa. This indicates a selective solubilization of some of the nuclear (membrane) proteins, including 5α-reductase. Increased specific 5α-reductase activity in the solubilized fraction might be due to the enrichment of proteins in the 100000 × *g* supernatant of CHAPS-treated nuclei.

Discussion

Recent data indicated that protein–phospholipid interactions are important factors for 5α-reductase activity in androgen target tissues such as rat epididymis [2] and rat ventral prostate [1]. We have studied the effects of low concentrations of detergents on enzyme activity prior to attempting the solubilization of a membrane-bound enzyme. Among the detergents used in this study only CHAPS, Lubrol WX, octyl D-glucopyranoside and L-α-lysophosphatidylcholine were suitable for further solubilization studies. An inhibitory effect of detergents on 5α-reductase activity was only considered in the paper of Houston et al. [7], reporting of solubilization studies on human prostatic 5α-reductase. It has not been mentioned by others [6,8]. Thus, the amount of a given detergent concentration is decisive for the amount of solubilized enzyme, but simultaneously also for the degree of inactivation of the enzyme [18].

The following mixture was found to be suited for the solubilization of maximum 5α-reductase activity from nuclei of rat ventral prostate: 3 mg/ml L-α-lysophosphatidylcholine in phosphate buffer (pH 6.0) containing 5 mM $MgCl_2$, 0.1 M KCl, 0.1 M sodium citrate and 5 mM NADPH. Under such conditions we succeded in solubilization of 14.8% of the nuclear protein and were able to preserve 56% of the enzymic activity in soluble form. At high detergent concentrations enzyme activity of the supernatant is lower than expected. This is due to the concentration dependent inactivation of 5α-reductase by the detergent. Thus, 5α-reductase activity recovered at a certain detergent concentration is a function of both the amount of enzyme solubilized at that detergent concentration and the degree of inactivation occurring at the same concentration. Specific enzyme activity increased by about 3-fold when compared to the specific 5α-reductase activity in purified nuclei.

Our overall recovery of solubilized enzyme (up to 58%) exceeds that reported by several previous authors. Houston et al. [7] report a recovery of about 30% using a related detergent, Lubrol PX for solubilizing 5α-reductase from human prostatic tissue. Scheer and Robaire [6] four that 21% of the 5α-reductase activity in rat epididymal membranes could be solubilized by incubation in the presence of 0.1 M KCl and 0.1 M citrate in the absence of detergent. The solubilization ratio of 5α-reductase could only slightly be increased to 25% in the presence of Lubrol WX. Neither in the hands of Houston and co-workers [7], nor in our hands, the presence of salt alone did result in any solubilization of 5α-reductase activity. This might be due to species specificity or it could be that the results of Scheer and Robaire [6] were due to the stabilization of a small amount of 5α-reductase simply released by resuspending the membranes. Moore and Wilson [8] report a 35% recovery of solubilized 5α-reductase from rat ventral prostate using digitonin in the presence of KCl. Using the experimental protocol of Moore and Wilson [8], we were unable to reproduce their result. We found a detergent-protein ratio of 1 : 4 at the solubilization optimum, being in the same order of magnitude for all four detergents tested.

All three criteria for solubilization of membrane-bound proteins claimed by Razin [17] have been fulfilled. It seems reasonable to conclude that enzyme-containing membranes have been disrupted by the agents and that 5α-reductase has been solubilized. Stringent evidence, however, for solubilization (proof of separation from membrane lipids, separation of the enzyme from the detergent) has not been obtained. Because of the low amount of 5α-reductase activity and the instability of the enzyme in the ventral prostate of the rat, complete purification does not appear feasible as yet.

Acknowledgements

We thank Mrs. E. Völck-Badouin for excellent technical assistance and Mrs. K. Gerbig for typing the manuscript. The work was supported by the Deutsche Forschungsgemeinschaft (SFB 215, B5).

References

1 Enderle-Schmitt, U., Völck-Badouin, E., Schmitt, J. and Aumüller, G. (1986) J. Steroid Biochem. 25, 209–217.
2 Cooke, G.M. and Robaire, B. (1985) J. Biol. Chem. 260, 7489–7495.
3 Neuhaus, C., Enderle-Schmitt, U. and Aumüller, G. (1987) Anat. Anz. (Jena), in press.
4 McGuire, J.S. and Tomkins, G.M. (1960) J. Biol. Chem. 235, 1634–1638.
5 Golf, S.W. and Graef, U. (1978) J. Steroid. Biochem. 9, 1087–1092.
6 Scheer, H. and Robaire, B. (1983) Biochem. J. 211, 65–74.
7 Houston, B., Chisholm, G.D. and Habib, F.K. (1985) J. Steroid. Biochem. 22, 461–467.
8 Moore, R.J. and Wilson, J.D. (1974) Biochemistry 13, 450–456.

9 Moore, R.J. and Wilson, J.D. (1981) Meth. Enzymol. 36, 466–474.
10 Spiess, E., Zimmermann, H.P. and Lünsdorf, H. (1987) in Electronmicroscopy in Molecular Biology, A Practical Approach (Sommerville, J. and Scheer, U., eds.) 147–166, IRL-press, Oxford.
11 Bradford, M.M. (1976) Anal. Biochem. 72, 248–254.
12 Laemmli, U.K. (1970) Nature (Lond.) 227, 680–685.
13 Hudson, R.W. (1981) J. Steroid Biochem. 14, 579–584.
14 Brown, D.A. and Simoni, R.D. (1974) Proc. Natl. Acad. Sci. USA 81, 1674–1678.
15 Helenius, A. and Simons, K. (1975) Biochim. Biophys. Acta 415, 29–79.
16 Hjelmeland, L.M. (1980) Proc. Natl. Acad. Sci. USA 77, 6368–6370.
17 Razin, S. (1972) Biochim. Biophys. Acta 256, 241–296.
18 Hjelmeland, L.M. and Chrombach, A. (1984) Meth. Enzymol. 104, 305–318.