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CHARACTERIZATION OF STEROID HORMONE RECEPTORS WITH ION-EXCHANGE FAST PROTEIN LIQUID CHROMATOGRAPHY

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Summary—Androgen, estrogen and progesterone receptors have been characterized with anion exchange Fast Protein Liquid Chromatography (FPLC) on a Mono Q column (Pharmacia). In the presence of sodium molybdate androgen receptors in cytosols from rat prostate, rat epididymis and calf uterus eluted as a single sharp peak at 0.32 M NaCl with recoveries of approx 90%. The molybdate-stabilized form of the androgen receptor from rat prostate was purified about 75-fold. The receptor containing FPLC-peak fractions sedimented in high salt (0.4 M KCl) linear sucrose gradients at 3.6 S (prostate) and at 4.6 S (epididymis and calf uterus) respectively. Multiple forms of the androgen receptor were present in cytosols from rat prostate prepared in the absence of sodium molybdate, probably due to proteolytic breakdown of the native form. Calf uterine estradiol and progesterone receptors prepared in the presence of sodium molybdate (20 mM) eluted from the Mono Q column at 0.32 M NaCl. The molybdate-stabilized forms of the oestradiol and progesterone receptors were purified approx 70-fold and 30-fold respectively.

In the absence of molybdate the estradiol receptor dissociated into two major forms eluting at 0.23 M NaCl and 0.37 M NaCl. After heat induced transformation (30 min at 25°C) of the estradiol receptor one major peak was eluted at 0.42 M NaCl, indicating a change in the surface charge of the estradiol receptor as a result of the 4 S to 5 S transformation.

It is concluded that the FPLC anion exchange system is a powerful, fast tool for characterization and partial purification of steroid receptors. In addition this technique could be applied as a rapid procedure for the quantitative estimation of steroid receptors in small biological samples.

INTRODUCTION

"Activation" of steroid receptors is supposed to be an essential step in the mechanism of action of steroid hormones [1]. The activation process, which is defined as a steroid induced conformational change of the receptor, resulting in increased affinity of the steroid receptor complex for nuclei and DNA, appears to occur *in vivo* as well as in intact cells systems *in vitro* [2–4]. In addition to the non-activated and activated steroid receptor forms, a small receptor form (the so-called mero-receptor) has been identified [5, 6]. While the activation process is a common feature of all steroid receptor complexes, "transformation" of steroid receptors, which refers to a modification of the 4 S steroid receptor complex to a 5 S dimer appears to be a unique property of the estrogen receptor [7, 8]. Methods to discriminate between the activated and non-activated form of steroid receptors are based on differences in binding to nuclei or DNA. Sedimentation coefficients, Stokes Radii, dissociation kinetics as well as surface charge changes of steroid receptor complexes have been used as additional parameters for characterization of steroid receptor activation and transformation [5, 7, 9, 10]. These methods, however, are time consuming and laborious. Hence a rapid separation technique for the analysis of labile receptor proteins is essential for a proper characterization of various receptor forms. Recently high pressure liquid chromatography (HPLC) of steroid receptors has been introduced and was successfully applied for characterization and quantification of different steroid receptor forms [11–14]. Promising applications of the HPLC technique in establishing size and charge heterogeneity of steroid receptors were reported, using gel exclusion [11, 12], ion-exchange [14] as well as chromatofocussing [13]. The present report describes the introduction of "fast protein" liquid chromatography (FPLC) for the analysis of steroid hormone receptors. This novel approach to chromatography of labile biomolecules uses columns with uniform monodisperse spheres which allow a fast separation and fast flow characteristics at only a moderately increased pressure [15]. In the present investigation particular attention was given to the characterization of the non-activated, molybdate-stabilized form of androgen, estrogen and progesterone receptors by anion exchange chromatography. In addition, application of the FPLC technique showed the presence of two isoforms of the 4 S estradiol receptor in calf uterine cytosol.

EXPERIMENTAL

Materials

The synthetic androgen receptor ligand [^{3}H]17β-Hydroxy-17α-methylestra-4,9,11,-trien-3-one

([^{3}H]R_{1881}; sp. act. 87 Ci/mmol) and radioinert R_{1881} were purchased from NEN Chemicals GmbH (F.R.G.). [2,4,6,7-^{3}H]oestradiol (sp. act. 90 Ci/mmol) and the synthetic progesterone receptor ligand 16α-ethyl-21-hydroxy-19-nor[6,7-^{3}H]pregn-4en-3,20 dione (^{3}H-Org.2058; sp. act. 40 Ci/mmol) as well as radioinert Org.2058 were obtained from the Radiochemical Centre in Amersham (U.K.). The purity of the radiolabelled compounds was checked by thin-layer chromatography. All other steroids were purchased from Steraloids Inc. (Wilton, NH, U.S.A.). All chemicals and reagents used were of analytical grade.

Animals

Adult (4–6 month old) rats from the Wistar Strain R-Amsterdam were used. Castration was performed through the scrotal route 24 h before the experiments. Calf uteri were obtained from a local slaughter house. After isolation the uterine tissue was immediately frozen in liquid nitrogen and stored at −80°C.

Preparation of cytosols

Prostatic and epididymal tissue was homogenized with an Ultra turrax in 3 vol of TEGD buffer (20 mM Tris–HCl, pH 7.7, 1.5 mM EDTA, 1.5 mM dithiothreitol, 0.6 mM phenylmethyl-sulfonylfluoride, 10% glycerol). The homogenate was centrifuged for 60 min at 105000 **g** and the supernatant was designated as cytosol. Calf uterine tissue was homogenized with an Ultra turrax in 4 vol of TEG buffer (20 mM Tris–HCl, pH 7.7, 1.0 mM EDTA, 10% glycerol, 1 mM 2,2′-dipyridyldisulfide). The homogenate was centrifuged for 10 min at 16000 ***g***. The fat layer and the pellet were discarded and the 16000 ***g*** supernatant was further centrifuged for 60 min at 105000 ***g*** after addition of 1-thioglycerol (final concentration 12 mM). The 105000 ***g*** supernatant was used as cytosol. In some experiments 20 mM sodium molybdate was included in the homogenization buffers.

Labelling of cytosols

Prostate cytosol was incubated for 2 h at 0°C with 10 nM[^{3}H]R_{1881}. In parallel incubations a 100-fold molar excess of R_{1881} was also added to measure non-specific binding. Epididymis cytosol was incubated for 1 h at 0°C with 10 nM[^{3}H]R_{1881}. Subsequently 1 μM testosterone was added and the cytosol was incubated for another hour to displace [^{3}H]R_{1881} bound by androgen binding protein. Free steroid was removed with Dextran-coated charcoal. Androgen receptors in calf uterine cytosol were labelled with 10 nM[^{3}H]R_{1881} for 18 h at 0°C after preincubation for 30 min at 0°C with 5 μM triamcinolone acetonide to prevent [^{3}H]R_{1881} binding by the progesterone receptor [16]. Estrogen and progesterone receptors in calf uterine cytosol were labelled for 60 min at 0°C with 10 nM [^{3}H]estradiol or with 10 nM [^{3}H]Org.2058 respectively.

Measurement of cytoplasmic receptors

Labelled cytosol (200 μl) was mixed briefly with 100 μl 0.5% Dextran-coated charcoal suspension (0.5% charcoal; 0.1% Dextran), allowed to stand for 15 min at 0°C and then centrifuged at 1500 ***g*** for 5 min to sediment the Dextran-coated charcoal with the adsorbed unbound steroid. Bound steroid was estimated in a 200 μl sample of the supernatant.

Chromatography

The FPLC system (Pharmacia, Sweden) consists of a gradient programmer controlling two reciprocating pumps—each pump delivers one buffer into a dynamic mixing chamber where the buffer then passes through a filter—and a manually operated valve for the introduction of samples via a 500 μl sample loop onto the anion-exchanger column (Mono Q [Pharmacia]). Fractions from the column were collected with an automated fraction collector system. The elution buffer was the same as for preparation of cytosols with addition of 0.35 or 0.50 M NaCl in the final buffer. Linearity of the salt gradient was assessed by measuring the conductivity of the collected fractions. In several experiments 20 mM sodium molybdate was also included in the buffer. All separations were carried out at 6°C in the cold room. The flow-rate was 1 ml/min at an operating pressure of 2.8 MPa. Before application on the column samples were centrifuged at 105000 ***g*** for 15 min in a Beckman Airfuge.

Sucrose gradient analysis

Sucrose gradient analysis (5–20% sucrose in TEDG buffer pH 7.7 containing 0.4 M KCl) was performed at 370000 g_{av} for 24 h in a Beckmann L5-65B ultracentrifuge with 250 μl cytosol fractions. γ-Globulin (7.2 S), ^{14}C-labelled bovine serum albumin (4.6 S) and ovalbumin (3.6 S) were used as sedimentation markers.

Gel chromatography

Chromatography of the different receptor fractions was performed on ultrogel ACA-44 as previously decribed [17].

In situ *labelling of cytosol*

In some experiments minces of fresh prostatic tissue obtained from castrated rats were incubated for 1 h at 0 or 30°C respectively in Eagle's minimal essential medium with 10 nM [^{3}H]R_{1881}. Labelled cytosols were isolated according to the procedure described above.

RESULTS

Androgen receptors

Figure 1 shows the NaCl-gradient elution profile from a Mono Q anionexchange column for androgen receptor complexes from prostate cytosols incubated with [^{3}H]R_{1881}. Labelled cytosol receptors prepared in

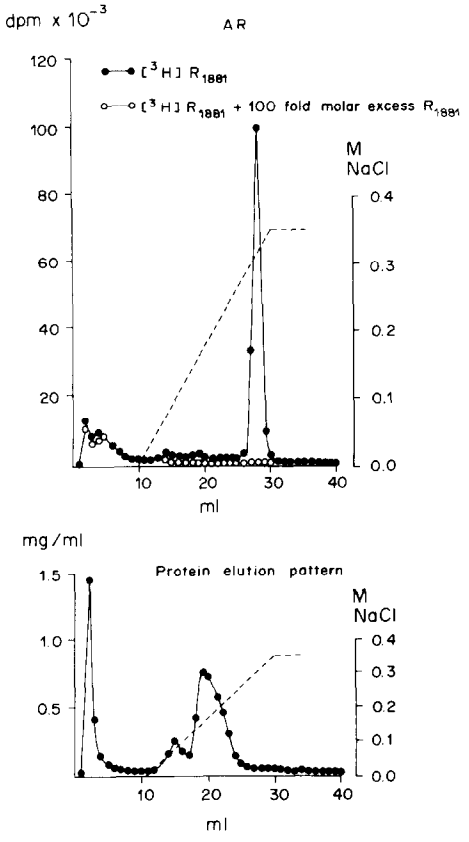

Fig. 1. FPLC-Chromatography of cytoplasmic androgen receptor complexes from rat prostates in the presence of 20 mM molybdate. Five-hundred μl Cytosol samples incubated with 10 nM [^{3}H]R_{1881} in the absence (●) or presence of 1 μM R_{1881} (○) were applied on a Mono Q column. The column was washed with 8 ml TEDG buffer to remove unbound complexes. Elution was carried out with a linear salt gradient [0–0.35 M NaCl] in TEDG buffer, pH 7.7 with 20 mM sodium molybdate (----). Each 1 ml fraction was assayed for total radioactivity and protein content.

the presence of 20 mM molybdate eluted as a single peak at 0.32 M NaCl. This peak of bound radioactivity could be completely suppressed with a 100-fold molar excess of R_{1881}. The radioactivity appearing in fractions 2 and 3, however, reflected non-specifically bound steroid, as was established by a chromatographic run of cytosol incubated with [^{3}H]R_{1881} in the presence of a 100-fold molar excess of non-radioactive R_{1881}. About 40% of the protein applied to the column eluted between fractions 17 and 24 and this might represent prostatic binding protein, which is abundant in the cytosol of rat ventral prostates [18]. All receptor activity was recovered in the fractions constituting the peak eluted at 0.32 M NaCl and a 75-fold purification of the androgen receptor was achieved in fraction 28 with a recovery of 71%. On sucrose gradients (high ionic strength) the FPLC-peak sedimented as 3.6 S entity, while a mol. wt of 48,000 was found after ACA-44 gel chromatography.

The molybdate-stabilized form of the androgen receptor in cytosols prepared from rat epididymis and calf uterus were eluted from the Mono Q column with a recovery of 85% and at the same ionic strength (0.32 M) as the prostatic androgen receptor. Further analysis of the eluted receptors on sucrose gradients and by ACA-44 gelchromatography resulted in sedimentation values of 4–5 S and mol. wt of 90,000,

In order to establish that the radioactivity eluted at 0.32 M NaCl really reflected the cytoplasmic androgen receptor, the following *in vitro* experiment was performed. Prostate tissue from castrated rats was minced and incubated with 10 nM [^{3}H]R_{1881} for 1 h either at 0 or 30°C. Cytosol prepared in the presence of 20 mM molybdate and assayed on the Mono Q column gave an elution pattern which was not different from that shown in Fig. 1. The amounts of receptor recovered in peak fractions 27–30 were 238 fmol (0°C incubation) and 96 fmol (30°C incubation) respectively. During incubation of prostatic tissue at 30°C activation occurs of androgen receptors resulting in an increase of receptors extractable from nuclei and a decrease in cytoplasmic receptor levels [19]. The 60% reduction of receptor levels found in cytosols after *in vitro* incubation of prostatic tissue at 30°C may indicate, therefore, that the radioactivity eluted at 0.32 M NaCl reflects the cytosolic form of the androgen receptor, which is susceptible to activation.

When prostate cytosol, prepared in molybdate-free buffer, was analyzed on FPLC, the total recovery of specifically bound [^{3}H]R_{1881} was reduced to about 80%. The elution profile on Mono Q became more complex, as a result of the formation of multiple forms of the androgen receptor in the absence of molybdate (Fig. 2). A three-fold reduction of the peak eluted at 0.32 M NaCl was observed (recovery 35%). In addition three other peaks of receptor-like

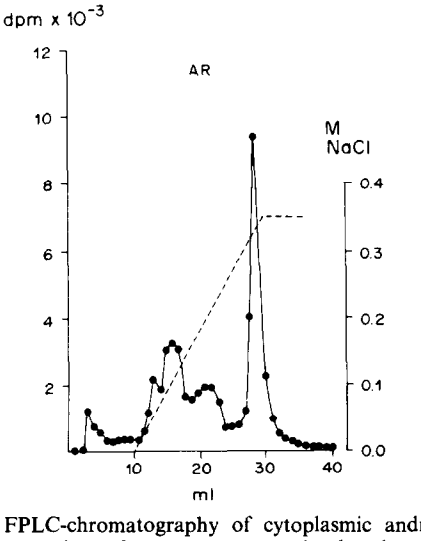

Fig. 2. FPLC-chromatography of cytoplasmic androgen receptor complexes from rat prostates in the absence of molybdate. Five-hundred μl Cytosol samples incubated with 10 nM [^{3}H]R_{1881} in the absence or presence of 1 μM R_{1881} were applied on a Mono Q column. All other conditions for elution were as described in the legend of Fig. 1. Only specific binding in each fraction is represented.

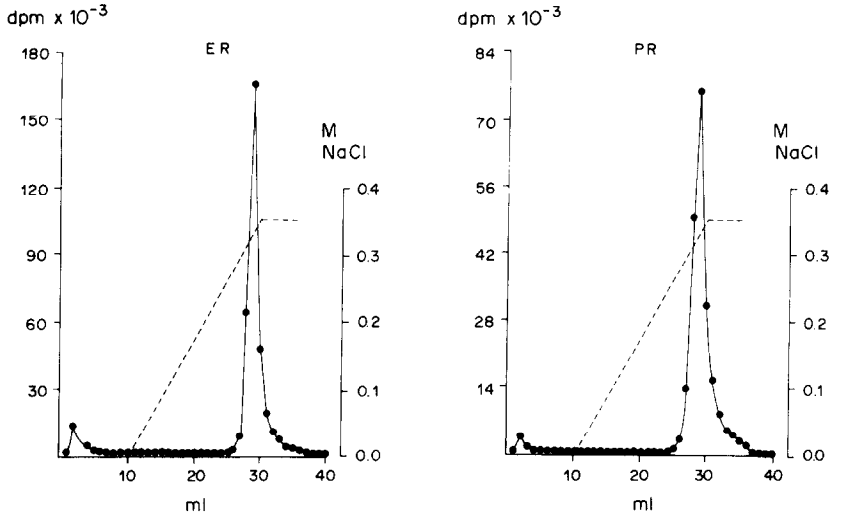

Fig. 3. FPLC-chromatography of cytoplasmic estrogen [ER] and progesterone [PR] receptor complexes from calf uterus in the presence of 20 mM molybdate. Five-hundred μl Cytosol samples, incubated either with 10 nM [^{3}H]oestradiol or 10 nM [^{3}H]Org.2058 were applied on a Mono Q column. All other conditions for elution were as described in the legend of Fig. 1.

androgen binding were found, which were eluted at 0.05, 0.10 and 0.20 M NaCl respectively.

Estradiol and progesterone receptors

Calf uterus cytosol containing 20 mM sodium molybdate and labelled with [^{3}H]oestradiol yielded one very sharp binding peak eluted at 0.32 M NaCl from the Mono Q column (Fig. 3). The proteins eluted in the peak fraction showed a limited capacity for binding of estrogen, since addition of a 200-fold molar excess diethylstilboestrol to the cytosol abolished the peak of bound radioactivity. Recovery of estradiol receptor binding in the peak fractions was 93% and a 70-fold purification was obtained for fraction 29 with a recovery of 50%. Similar results were obtained for the progesterone receptor in the calf uterus cytosol in the presence of molybdate (Fig. 3). Total recovery of the progesterone receptor in the peak fractions was 74% and progesterone receptor present in peak fraction 29 was purified 30-fold with a recovery of 37%.

The elution pattern of estradiol receptor from calf uterus cytosol was further investigated on FPLC in the absence of molybdate. Two distinct peaks of specific estradiol binding were observed; one eluted at 0.23 M (peak I) and one at 0.37 M NaCl (peak II)

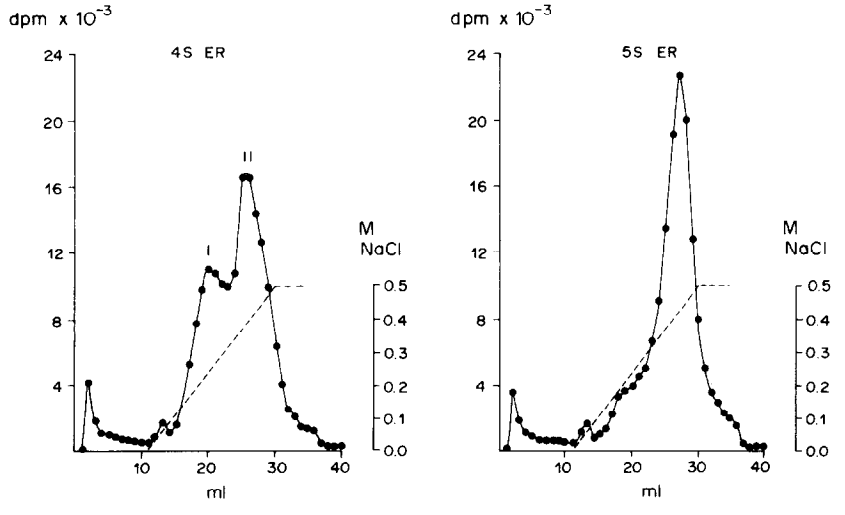

Fig. 4. FPLC-chromatography of cytoplasmic estrogen receptor complexes [ER] from calf uterus in the absence of molybdate. Five hundred μl Cytosol samples, prepared in Tris buffer pH 7.7, and labelled for 2 h with 10 nM [^{3}H]estradiol, were either kept at 0°C [4 S ER] or incubated for 30 min at 25°C [5 S ER] prior to application on a Mono Q column. Elution was accomplished with a linear salt gradient. All other conditions for elution were as described in the legend of Fig. 1.

(Fig. 4). Both forms of the receptor sedimented on a sucrose gradient on high salt (0.4 M KCl) with a coefficient of 4 S.

Brief warming of the cytosol for 30 min at 25°C resulted in a shift in the sedimentation coefficient from 4 to 5 S on sucrose gradients (high salt) and in the FPLC elution pattern one major peak was eluted at an ionic strength of 0.43 M NaCl (Fig. 4). The 5 S-form of the estrogen receptor eluted in five different experiments always at slightly higher ionic strength than peak II of the 4 S-form.

DISCUSSION

Anion exchange liquid chromatography on a Mono Q column of cytoplasmic androgen receptors prepared in the presence or absence of molybdate has allowed us to distinguish different forms of the androgen receptor. In the presence of molybdate a form was identified in prostate cystosol which elutes at 0.32 M NaCl and has the same ionic properties as the androgen cytosol receptor from epididymis and calf uterus. Radioactivity eluted at 0.32 M NaCl gives a proper reflection of the androgen receptor, because binding of the labelled ligand could be completely abolished by a 100-fold molar excess of unlabelled ligand. In addition a sedimentation coefficient of 3–4 S in high salt sucrose gradients was found and its level is decreased in cytosols obtained from prostatic tissue after incubation under conditions where activation occurs [19]. The fact that this form is also present in cytosols without molybdate, might suggest that it represents the non-activated receptor complex rather than a molybdate-induced artifact. Our present results with fractionation of prostate cytosol by FPLC on a Mono Q column in the presence of molybdate showed a substantial purification of the androgen receptor of approx 75-fold with a recovery of 71%.

In the absence of molybdate a lower yield of androgen receptor was obtained, while multiple forms appeared in the elution pattern each with different ionic properties. These multiple forms, eluted at a lower ionic strength, might represent androgen receptor complexes which became activated either during the homogenization procedure or during the ion exchange chromatography. In addition the high proteolytic enzyme activity present in prostate cytosol might have caused further degradation of the androgen receptor due to the relative instability of the activated complex. Even generation of a mero-type receptor cannot be excluded. Since no further characterization has been performed with respect to DNA-binding of these low-salt eluting forms a definitive conclusion with regard to their nature cannot be drawn. The molybdate stabilized forms of the estradiol and progesterone receptor from calf uterus cytosol showed the same ionic properties as the androgen receptor from rat prostate, rat epididymis and calf uterus and were eluted as well defined single peaks from the Mono Q column.

Activation of steriod receptors to a DNA-binding form has been shown to be accompanied by a change in the ionic properties of the complex. If both forms are present in a cytosol preparation, heterogenous elution profiles of cytoplasmic receptors would be expected on ionexchangers, like DEAE-cellulose [1, 2, 9, 20]. Molybdate can prevent the temperature induced activation of several steroid hormone receptors [21–23], and it is conceivable that the single peaks in the elution profiles, which we obtained represent the non-activated receptor complexes. Similarly only a single form of the glucocorticoid and estradiol receptor have been found in the presence of molybdate during ion exchange chromatography [4, 14, 23]. The present results show that in addition to estradiol and glucocorticoid receptors also androgen and progesterone receptors appear as single species which share common ionic properties during anion exchange chromatography in the presence of molybdate.

Recently Bailly *et al.* and Müller *et al.* have presented evidence that estrogen receptor activation does not necessarily involve receptor transformation [7, 8]. Their data strongly suggest that the 4 S estrogen receptor can exist in an activated and a non-activated state and it is conceivable that both states are present in a partial activated estradiol receptor preparation. In the present investigation two isoforms of the 4 S estradiol cytosol receptor were detected. No further characterization with respect to DNA binding of those two isoforms have been performed, but it is tempting to speculate that one form might be the activated 4 S estrogen receptor, while the other form represents the non-activated 4 S estrogen rector.

Estradiol receptor complexes can undergo a hormone-dependent transformation, which can be achieved *in vitro* either by temperature elevation or by exposure to salt [7, 8]. As a result a conformational change of the complex occurs which can be detected by the 4 to 5 S shift in the sedimentation coefficient in high salt sucrose gradients and by a decrease in the dissociation rate of the hormone from the receptor [9, 10]. In addition the ionic properties of the 5 S complex seem to differ from the 4 S estrogen receptor [9]. In the present investigation the transformed 5 S estrogen receptor complex eluted as a single form, which differs in ionic properties from both isoforms of the 4 S complex. These results confirm that formation of the 5 S dimer of the estrogen receptor is accompanied by a change in the surface charge of the complex [9].

The "fast protein" liquid chromatography system has allowed us to analyse 500 μl cytosol samples within 40 min with a high resolution power and reproducibility. The high recovery of the Mono Q column makes this technique a valuable tool in the quantitative analysis of the molybdate-stabilized

form of different steroid receptors in small biological samples.

REFERENCES

1. Grody W. W., Schrader W. T. and O'Malley B. W.: Activation, transformation and subunit structure of steroid hormone receptors. *Endocr. Rev.* **3** (1982) 141–163.
2. Munck A. and Foley R.: Activation of Steroid hormone-receptor complexes in intact target cells in physiological conditions. *Nature* **278** (1979) 752–754.
3. Markovic R. D. and Litwack G.: Activation of liver and kidney glucocorticoid-receptor complexes occurs *in vivo*. *Archs biochem. Biophys.* **202** (1980) 374–379.
4. Holbrook N. J., Bodwell J. E., Jeffries M. and Munck A.: Characterization of nonactivated and activated glucocorticoid-receptor complexes from intact rat thymus cells. *J. biol. Chem.* **258** (1983) 6477–6485.
5. Sherman M. R., Pickering L. A., Rollwagen F. M. and Miller L. K.: Mero-receptors: proteolytic fragments of receptors containing the steroid binding site. *Fedn Proc.* **37** (1978) 167–173.
6. Wilson E. M. and French F. S.: Effects of proteases and protease inhibitors on the 4.5 S and 8 S androgen receptor. *J. biol. Chem.* **254** (1979) 6310–6319.
7. Bailly A., Le Fevre B., Savouret J. F. and Milgrom E.: Activation and changes in sedimentation properties of steroid receptors. *J. biol. Chem.* **255** (1980) 2729–2734.
8. Müller R. E., Traish A. M. and Wotiz H. H.: Estrogen receptor activation precedes transformation. *J. biol. Chem.* **258** (1983) 9227–9236.
9. Notides A. C., Lerner N. and Hamilton D. E.: Positive cooperativity of the estrogen receptor. *Proc. natn. Acad. Sci., U.S.A.* **78** (1981) 4926–4930.
10. Weichman B. M. and Notides A. C.: Estradiol-binding kinetics of the activated and nonactivated estrogen receptor. *J. biol. Chem.* **252** (1977) 8856–8862.
11. Pavlik E. J., van Nagell Jr J. R., Muncey M., Donaldson E. S., Hanson M., Kenady D., Rees E. D. and Talwalkar V. R.: Some practical aspects of the separation of estrogen and progesterone receptors by size exclusion high-performance liquid chromatography which are relevant to quantitation. *J. steroid Biochem.* **17** (1982) 553–558.
12. Kirdani R. Y., Pontes E. J., Murphy G. P. and Sandberg A. A.: A micromethod for the measurement of estrogen receptors using high pressure liquid chromatography. *J. steroid Biochem.* **17** (1982) 311–317.
13. Hutchens T. W., Wiehle R. D., Shahabi N. A. and Wittliff J. L.: Rapid analysis of estrogen receptor heterogeneity by chromatofocussing with high-performance liquid chromatography. *J. Chromatogr.* **266** (1983) 115–128.
14. Madhok T. C. and Leung B. S.: Characterization of uterine estrogen receptors by size-exclusion and ion-exchange high performance liquid chromatography. *Biochem. biophys. Res. Commun.* **115** (1983) 988–994.
15. Soderberg L., Bergstrom J. and Andersson K.: Physicochemical considerations in the use of monobeads for the separation of biological molecules. *Protides Biol. Fluids Proc. Colloq.* **30,** (1983) 629–634.
16. Zava D. T., Landrum B., Horwitz K. B. and McGuire W. L.: Androgen receptor assay with [^{3}H] methyltrienolone (R1881) in the presence of progesterone receptors. *Endocrinology* **104** (1979) 1007–1012.
17. Mulder E., Vrij A. A. and Brinkmann A. O.: DNA and ribonucleotide binding characteristics of two forms of the androgen receptor from rat prostates. *Biochem. biophys. Res. Commun.* **114** (1983) 1147–1153.
18. Heyns W. and de Moor P.: Prostatic Binding Protein. A steroid-binding protein secreted by rat prostate, *Eur. J. Biochem.* **78** (1977) 221–230.
19. Brinkmann A. O., Lindh L. M., Breedveld D. I., Mulder E. and van der Molen H. J.: Cyproterone acetate prevents translocation of the androgen receptor in the rat prostate. *Molec. cell. Endocr.* **32** (1983) 117–129.
20. Mainwaring W. I. P. and Irving R.: The use of deoxyribonucleicacid-cellulose chromatography and isoelectric focussing for the characterization and partial purification of steriod-receptor complexes. *Biochem. J.* **134** (1973) 113–127.
21. Leach K. L., Dahmer M. K., Hammond N. D., Sando J. J. and Pratt W. B.: Molybdate inhibition of glucocorticoid receptor inactivation and transformation. *J. biol. Chem.* **254** (1979) 11884–11890.
22. Nishigori H. and Toft D.: Inhibition of progesterone receptor activation by sodium molybdate. *Biochemistry* **19** (1980) 77–83.
23. Redeuilh G., Secco C., Baulieu E. E. and Richard-Foy H.: Calf uterine estradiol receptor. Effects of molybdate on salt-induced transformation process and characterization of a nontransformed receptor state. *J. biol. Chem.* **256** (1981) 11496–11502.