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PHYSICAL-CHEMICAL PROPERTIES OF THE ESTROGEN RECEPTOR SOLUBILIZED BY MICROCOCCAL NUCLEASE

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Summary—The physical-chemical properties of the nuclear estrogen receptor from MCF-7 cells were determined. The receptor was solubilized by micrococcal nuclease. Nuclei were isolated from cells previously exposed to 10 nM [^{3}H]estradiol. The amount of receptor released was parallel to the extent of chromatin solubilized, which suggested that the receptor is homogenously distributed on the chromatin. Following mild nuclease digestion the excised receptor sedimented as an abundant 6–7 S form and as a less abundant ~12 S species. The 6–7 S form represented the receptor excised in association with linker DNA, while the ~12 S may represent receptor bound to linker DNA which remained associated with the nucleosome. Increasing the extensiveness of digestion resulted in one receptor form sedimenting at 5.6 S. Additional digestion with DNase I did not affect the sedimentation coefficient of the receptor. Sedimentation of the micrococcal nuclease hydrolysate in a 0.4 M KCl sucrose gradient resulted in a 4.2 S receptor form. The same receptor form was extracted from undigested nuclei with 0.4 M KCl. Using Sephadex G-200 column chromatography we have determined the Stokes radii (Rs), molecular weight (Mr) and frictional ratio (f/fo) for the 5.6 S and 4.2 S receptor forms. For the 5.6 S form: Rs = 7.04 nm, Mr = 163,000 and (f/fo) = 1.80. For the 4.2 S receptor, Rs = 4.45 nm, Mr = 77,000 and (f/fo) = 1.46. The ability of the nuclease solubilized 5.6 S receptor to bind DNA was tested using DNA-cellulose column and highly polymerized DNA. About 40% of the applied receptor bound to the column and could be eluted by high salt concentrated buffer. The 5.6 S receptor form was sedimented on sucrose gradient by the highly polymerized DNA. These results suggested that the receptor is bound in chromatin as a dimer or as a monomer in association with other protein(s) which complexed it with DNA.

INTRODUCTION

Most of our knowledge of the physical-chemical properties of the estrogen-receptor were derived from studies on the receptor extracted from the cytoplasmic compartment of the cell [1]. The receptor which is found in the nucleus following exposure of the target cell to estradiol was less characterised. Most of the studies on this nuclear receptor were performed following extraction of the receptor by high salt concentrated buffers, usually 0.4 M KCl [2]. At this salt concentration the receptor may be completely dissociated from the chromatin components to which it was bound. Desalting of the nuclear extract resulted in an association of the receptor units into higher molecular weight forms [3].

Recently, evidence has been brought indicating that the estrogen receptor resides primarily in the target cell nuclei in the presence or absence of the steroid. The receptor recovered in the cytosolic fraction of a homogenate is an artifact representing the unoccupied receptor which is loosely associated with the nucleus. Exposure of the cell to estradiol leads to a receptor–steroid complex with a tighter association to the nucleus [4, 5].

In order to study the form and properties of the receptor in the nucleus and gain insight into its organization in chromatin, it is important to solubilize the chromatin bound receptor in a low salt containing buffer. This can be accomplished by digestion of the DNA component of the chromatin, thus solubilizing the receptor which may still be associated with other components of the chromatin [6].

In the present study we have used micrococcal nuclease and micrococcal nuclease followed by DNAse I to solubilize the chromatin bound estradiol receptor from MCF-7 cells [7]. Each of those nucleases showed a characteristic pattern of chromatin digestion. Micrococcal nuclease cleaves DNA preferentially at the linker region between the nucleosomes releasing mono, di, tri and poly-nucleosome particles [8]. DNAse I cleaves the DNA of both the linker and the nucleosome particles generating smaller DNA fragments [9].

Using this experimental approach we studied the distribution of the estrogen receptor on the chromatin fragments following mild micrococcal nuclease digestion and determined the Stokes radii (Rs), molecular weight (Mr), frictional ratio (f/fo), and the DNA binding ability of the receptor released by extensive micrococcal nuclease digestion.

EXPERIMENTAL

Cell culture

MCF-7 cells were generously provided by Dr Charles M. McGrath (Michigan Cancer Foundation,

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Detroit, MI, U.S.A.) and grown as previously described [10]. Confluent cells in Falcon flasks were exposed for 1 h to 10 nM 17β-[2,4,6,7-^{3}H] estradiol (sp. radioactivity 104 Ci/mmol; New England Nuclear Corp., Boston, MA, U.S.A.). The flasks were washed in cold phosphate buffered saline [11] and were stored 1–7 days at −80°C until assayed.

Preparation of purified nuclei

Purified nuclei were prepared as described previously [11, 12]. Briefly, the frozen cells were thawed and harvested in Tris–sucrose buffer (3 mM $MgCl_2$–0.33 M sucrose in 10 mM Tris–HCl buffer, pH 7.4). The cells were homogenized in a Dounce homogenizer (Kontes Co., Vireland, NJ, U.S.A.) using the B pestle (60–70 strokes), and centrifuged at 800 *g* for 10 min. The pellet was resuspended in Tris–sucrose buffer containing 2.2 M sucrose and centrifuged at 40000 *g* for 60 min. The pellet so obtained was resuspended in Tris–sucrose buffer containing 0.7% Triton X-100 and centrifuged at 800 *g* for 10 min. The pellet was washed twice with Tris–sucrose buffer and once with the micrococcal nuclease digestion buffer (10 mM Tris–HCl, pH 7.9, 0.1 mM $CaCl_2$, 1.5% sucrose).

Nuclease digestion

Micrococcal nuclease. Nuclei were resuspended in digestion buffer (800 μg/ml DNA) and incubated with 2–30 units/ml of micrococcal nuclease (Sigma Chemical Co., St Louis, U.S.A.) at 0°C for 5–120 min. Digestion was terminated by addition of EDTA to a final concentration of 5 mM. The hydrolysate was centrifuged at 6000 *g* for 5 min at 4°C.

DNase I. Nuclei were first digested with 30 units/ml of micrococcal nuclease at 0°C for 60 min and centrifuged at 6000 *g* for 5 min. $MgCl_2$ in a final 3 mM concentration was added to the supernatant and digestion continued with 300 units/ml of DNase I (DPFF, Worthington Biochemical Corp., New Jersey, U.S.A.) for 30 min at 0°C. The hydrolysate was pelleted at 12000 *g* for 10 min and EDTA, in a final concentration of 10 mM was added to the obtained supernatant. The pellet was resuspended in TED buffer (10 mM Tris–HCl, 1.5 mM EDTA and 0.5 mM dithiothreitol, pH 7.4) by vortexing. After 20 min the suspension was centrifuged at 12000 *g* for 10 min. Aliquots of the obtained supernatants were checked for radioactivity and DNA content, while the rest was usually applied to a sucrose gradient. The remaining pellets were extracted with ethanol for radioactivity and DNA was determined in the residual pellets. Radioactivity was counted in 5 ml scintillation fluid [11], and DNA was determined by the diphenylamine method of Burton[13].

Sucrose gradient centrifugation

Portions (500 μl) of nuclease hydrolysates were fractionated by centrifugation in a linear 5–20% sucrose gradient prepared in TED or TEDK (TED plus 0.4 M KCl) buffer. The tubes were centrifuged in a Beckman SW 41 rotor at 2°C. Fractions (seven drops) from the bottom of the tubes were collected, the absorbance at 260 nm was measured and the radioactivity was counted. Myoglobin (2S) and γ-globulin (6.6S) were used as external standards, while [^{14}C] bovine serum albumin (4.4S) and catalase (11.3S) were included in the gradient as internal standards.

Gel chromatography

Sephadex G-200 was swollen in TED or TEDK buffer at 4°C for 1–3 weeks. The gel was packed into columns of 1 × 30 cm and equilibrated at 4°C with the appropriate buffers.

Nuclei were digested with 30 units/ml of micrococcal nuclease at 0°C for 60 min. Digestion was terminated by the addition of EDTA to a final concentration of 5 mM and the supernatant was obtained following centrifugation at 100,000 *g* for 60 min. Aliquots (700 μl) of the supernatant were applied to the column (without or with adjusting the hydrolysates to 0.4 M KCl) and eluted with the appropriate buffer at a flow rate of 2 ml/h. Fractions of 600 μl were collected. Concurrently, aliquots of the hydrolysate supernatant were layered on a sucrose gradient and centrifuged at 185,000 *g* for 20 h, to determine the sedimentation coefficient. The distribution coefficients (K_d) were measured according to the equation $K_d = (Ve - Vo)/(Vt - Vo)$, where Vo, the void volume was determined with blue Dextran 2000; Vt, the total volume of the gel bed was measured with [^{3}H] leucine; and Ve is the elution volume of the receptor or the standard proteins. The protein standards, their concentrations and methods of determination were as follows: Ferritin in 2 mg/ml detected by absorption at 415 nm, Myoglobin in 2 mg/ml measured by absorption at 280 nm, Catalase in 0.5 mg/ml determined by the procedure of Goldblith and Proctor[14]. [^{14}C] bovine serum albumin (Sigma) applied in 20,000 cpm/ml. The last two standards were applied on the same column, simultaneously with the nuclease hydrolysates.

Molecular weight determination

The method used for determining the molecular weights was originally described by Siegel and Monty[15]. By this method the Stokes Radii (Rs) determined by gel chromatography and the sedimentation coefficients (S) determined by sucrose gradient analysis are used together to estimate the molecular weight (Mr) and frictional ratio (f/fo) of a protein molecule.

Affinity chromatography

Double stranded calf thymus DNA-cellulose was obtained from Sigma. Columns containing 1 ml of packed DNA-cellulose were thoroughly washed and equilibrated with TED buffer. Aliquots (500 μl) of micrococcal nuclease hydrolysates prepared as de-

scribed previously for gel chromatography, were layered on the column and the resin washed with TED buffer. The DNA-cellulose bound receptor was eluted from the column with the same buffer containing 1 M KCl. Fractions containing 1 ml were collected and the radioactivity was counted.

DNA binding assay

Highly polymerized calf thymus DNA (Sigma) used in the experiment was first purified as described by Perlman *et al.*[6] and dissolved in 6 mg/ml buffer (10 mM Tris–HCl, pH = 7.4, 10 mM KCl). Nuclei were digested with micrococcal nuclease as described previously for gel chromatography, and divided into two portions. One portion (450 μl) received 50 μl of the dissolved DNA and the other received 50 μl buffer. Following 30 min incubation at 0°C each sample was centrifuged on a sucrose gradient.

RESULTS

Receptor solubilization

Figure 1 illustrates the correlation between the amount of the receptor released and the extent of chromatin solubilized following micrococcal nuclease digestion of nuclei isolated from MCF-7 cells 1 h after exposure to 10 nM [^{3}H]estradiol. At this concentration more than 90% of the cell receptors were found in the nucleus [11]. The extent of chromatin digestion was determined as the percentage of DNA solubilized. The radioactivity measured was mainly receptor bound as illustrated by the sedimentation profile of the [^{3}H]estradiol bound receptor in Fig. 2. It can be seen in Fig. 1 that the amount of the receptor released was parallel to the percentage of DNA solubilized. More than 90% of the receptor could be solubilized following micrococcal nuclease digestion.

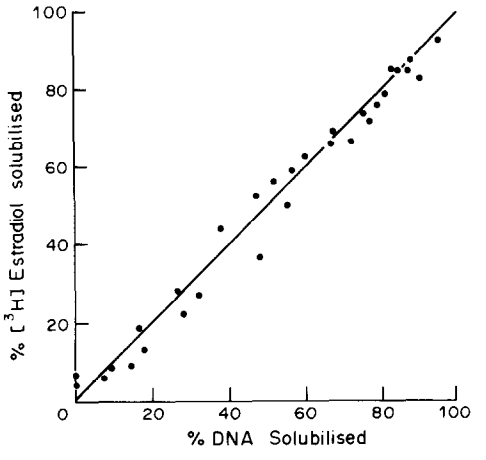

Fig. 1. Receptor released as a function of DNA solubilization. Nuclei were digested with micrococcal nuclease 0–30 units/ml at 0°C for 5 min. Radioactivity and DNA were determined in solubilized and nonsolubilized fractions as described under Experimental. The line drawn is that of identity (% receptor released = % DNA solubilized).

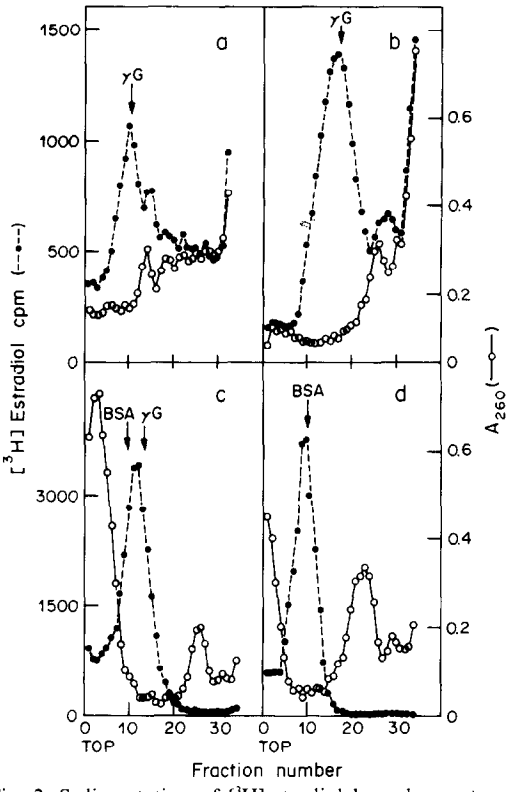

Fig. 2. Sedimentation of [^{3}H]estradiol bound receptor released by micrococcal nuclease digestion. Nuclei were digested with micrococcal nuclease at 0°C and the solubilized chromatin centrifuged for 20 h on a sucrose gradient. (a) 5 units/ml enzyme incubated for 5 min and centrifuged at 77,000 *g* (b) 7 units/ml enzyme incubated for 5 min and centrifuged at 185,000 *g*. 30 units/ml enzyme incubated for 60 min and centrifuged at 150,000 *g* in the absence (c) or presence of 0.4 M KCl (d). Arrows indicate the position of [^{14}C]bovine serum albumin (BSA) and γ-globulin (γG).

Sedimentation behaviour of the solubilized receptor

Figure 2a shows the sedimentation profile of the chromatin fragments obtained when about 80% of the receptors were solubilized. The u.v. absorbance shows the familiar pattern of DNA fragments separation into mononucleosomes (~11S), dinucleosomes (~10S) and trinucleosomes (~20S), while heavier molecular weight forms sedimented towards the bottom of the gradient[8]. Superimposed upon the absorbance profile in Fig. 2a is the radioactivity of each fraction. The [^{3}H]estradiol bound receptors sedimented predominantly as a 6–7S form and as a less abundant ~12S species which sedimented slightly more rapidly than the bulk of the mononucleosomes generated. Smaller peaks of radioactivity can be seen in the vicinity of the heavier molecular weight chromatin fragments. Figure 2b indicates that the [^{3}H]estradiol peak of ~12S is a discrete form and can be resolved from the abundant 6–7S receptor form. Centrifugation of the hydrolysate when less than 80% of the radioactivity was solubilized, did not show a distinct peak and most of it sedimented to the bottom of the tube. Increasing the extent of digestion resulted

in a further release of the slower sedimenting receptor, while a continuous disappearance of the ~12S form took place (results not shown). Furthermore, a stepwise decrease in the sedimentation coefficient of the 6–7S receptor occurred reaching 5.6S (see Fig. 1c). Adjusting the micrococcal hydrolysate to 0.4 M KCl and sedimenting it on a sucrose gradient containing 0.4 M KCl resulted in a receptor sedimenting at 4.2S (Fig. 1d). The same sedimentation coefficient (4.2S) was found for the nuclear receptor when extracted by 0.4 M KCl from undigested nuclei (results not shown).

In order to eliminate the possibility that the 5.6S receptor may contain DNA components which were not digested by the nuclease, we continued the digestion with DNase I. Since this enzyme needs Mg^{2+} for optimal activity, usually 3 mM $MgCl_2$, we investigated the effect of this Mg^{2+} concentration on the solubility of the micrococcal nuclease released receptor. Adjusting the micrococcal nuclease hydrolysate to 3 mM $MgCl_2$ resulted in a precipitation of the released receptor (results not shown). The small amount of radioactivity (about 10%) which remained in the supernatant did not sediment as a distinct receptor peak on a sucrose gradient (results not shown). The precipitated receptor could be resolubilized in TED buffer. As shown in Fig. 3b the sedimentation behaviour of the resolubilized receptor did not change following this treatment (5.6S). As shown in Fig. 3a, incubation of the micrococcal nuclease hydrolysate with DNase I did not affect the sedimentation behaviour of the receptor (5.6S). The effect of DNase I could be seen by the disappearance of the mononucleosome peak and the heavier chromatin fragments.

Gel chromatography analysis of the solubilized receptor

The micrococcal nuclease hydrolysate was chromatographed on a Sephadex G-200 column in a low (TED) or high salt concentrated buffer (TEDK). The column was calibrated with a series of proteins of known Stokes radii. The elution profile of the receptor and the position of the protein standards is shown in Fig. 4 (top) and the column calibration line in Fig. 4 (bottom). The receptor released by micrococcal nuclease digestion eluted as one peak between F and CA with a calculated Stokes radius of 7.04 nm in a low salt concentrated buffer. The same elution profile was found for the micrococcal nuclease hydrolysate with additional DNase I digestion (results not

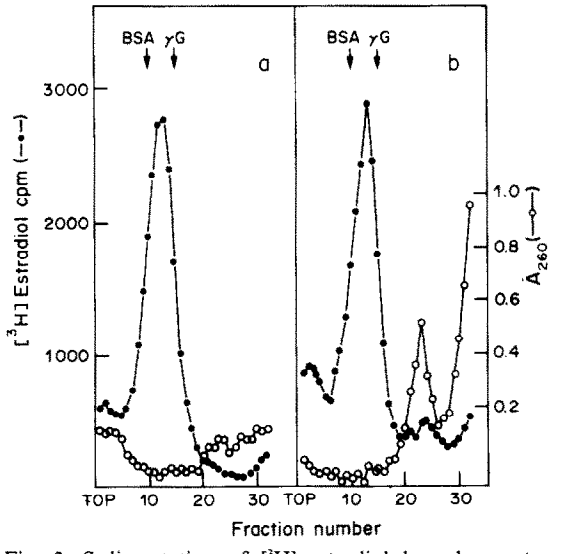

Fig. 3. Sedimentation of [^{3}H] estradiol bound receptor released by micrococcal nuclease and DNase I digestion. Nuclei were digested with micrococcal nuclease 30 units/ml at 0°C for 60 min. The supernatant obtained following centrifugation at 6000 *g* for 5 min, was adjusted to 3 mM $MgCl_2$ and divided into two parts. One part was digested with DNase I 300 units/ml (a) and the other part was used as a control (b). Following 30 min incubation the hydrolysates were centrifuged at 12,000 *g* for 10 min. The obtained pellets were resuspended in TED buffer for 20 min and centrifuged at 12,000 *g* 10 min. The supernatants were applied to a sucrose gradient and centrifuged at 185,000 *g* for 20 h. Arrows indicate the position of [^{14}C]bovine serum albumin (BSA) and γ-globulin (γG).

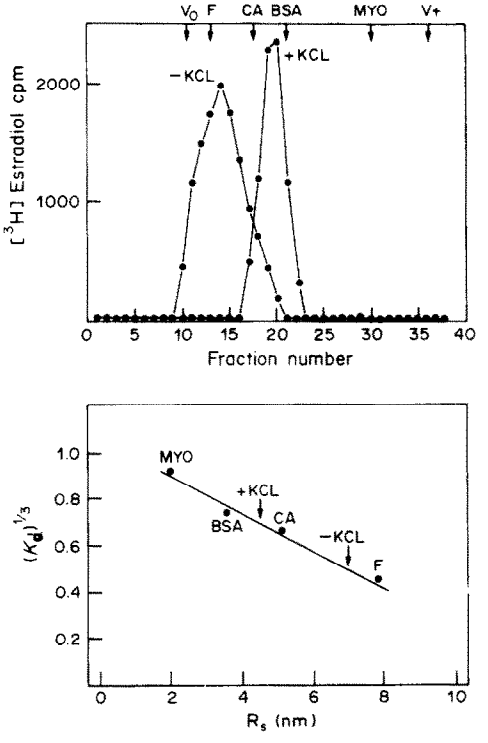

Fig. 4. Estimation of the Stokes radii of the solubilized receptor by Sephadex G-200 column chromatography. Micrococcal nuclease hydrolysates were prepared as described under Experimental and eluted on a Sephadex G-200 column in TED or TEDK buffer. Ferritin (F, Rs = 7.9 nm), Catalase (CA, Rs = 5.13 nm), [^{14}C]Bovine serum albumin (BSA, Rs = 3.59 nm) and Myoglobin (Myo, Rs = 2.01 nm) were chromatographed as standards. Top, the elution profile of the released receptor. Bottom, the relationships between Rs and $(K_d)^{1/3}$ for the protein standards and the solubilized receptor. The K_d values for each standard were identical under both buffer conditions.

Table 1. Molecular parameters of estrogen receptor from gel chromatography and sucrose gradient analysis*

Type of preparation	Buffer	Sedimentation coefficient (S)	Stokes radius† (nm)	Molecular weight‡	Frictional ratio§ (f/fo)	Axial ratio¶
Micrococcal nuclease digestion	TED	5.6 ± 0.08 (10)	7.04 ± 0.13 (5)	163,000	1.80	15.2
	TEDK	4.2 ± 0.07 (5)	4.45 ± 0.10 (4)	77,000	1.46	9.4
Salt extraction	TEDK	4.2 ± 0.08 (8)	4.45 ± 0.18 (5)	77,000	1.46	9.4

*Experimental values given as mean ± standard error of the mean with number of determinations indicated in parenthesis.
†Determined as described in Fig. 4.
‡Calculated by the equation of Siegel and Monty[15] assuming a partial specific volume of 0.725 cm^3 per g and a solution factor of 0.2 g of solvent per g of protein.
¶Calculated for prolate ellipsoids by Schachman tables [16].

shown). Adjusting the hydrolysate to 0.4 M KCl and elution in TEDK buffer resulted in a receptor which eluted between CA and BSA with a calculated Stokes radius of 4.45 nm. The same elution profile was found for the receptor extracted by 0.4 M KCl from undigested nuclei (results not shown). Utilizing the Stokes radii estimated from Sephadex G-200 column chromatography and the sedimentation coefficient determined from sucrose gradient analysis, the molecular weight and shape were calculated by the equation of Siegel and Monty[15] and are summarized in Table 1. The released micrococcal nuclease receptor, in low salt solution, has a calculated mol. wt or 163,000 and a frictional ratio of 1.80. In high salt solution a mol. wt of 77,000 and a frictional ratio of 1.46 were calculated for the micrococcal solubilised receptor as well as for the receptor extracted from undigested nuclei.

DNA Binding of the solubilized receptor

The binding ability of the micrococcal nuclease released receptor to DNA was studied by DNA-cellulose column chromatography and by ultracentrifugation on a sucrose gradient following incubation of the receptor with highly polymerized DNA. Figure 5 illustrates the binding of the receptor to DNA-cellulose column. About 40% of the receptor presented in the original sample were retained on the affinity resin and could be successfully eluted with high salt buffer. The eluted receptor sedimented as a 4.2S on a 0.4 M KCl sucrose gradient (results not shown). Figure 6 shows that incubation of the micrococcal nuclease released receptor with highly polymerized calf thymus DNA resulted in an association of the receptor with DNA as shown by the sedimentation of the receptor with the DNA to the bottom of the gradient.

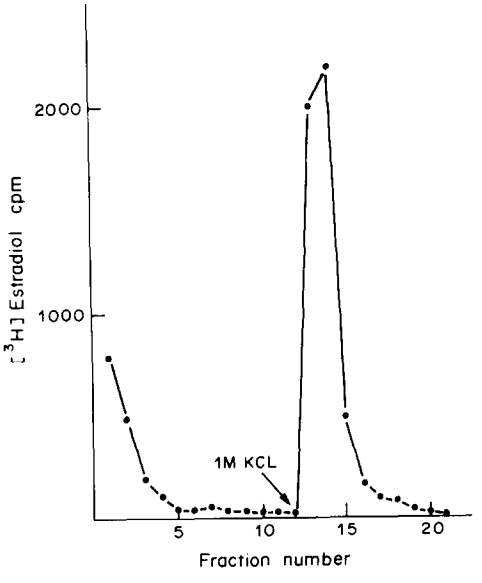

Fig. 5. Binding of the micrococcal nuclease solubilized receptor to a DNA-cellulose column. Micrococcal nuclease hydrolysate, prepared as described under Experimental, was chromatographed on a 1 ml DNA-cellulose column. The amount of the receptor bound to the column and eluted by 1 M KCl represented 41% of total receptors applied to the column.

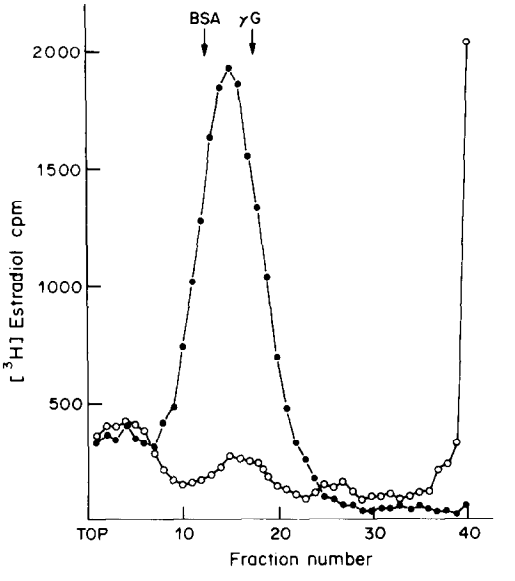

Fig. 6. Binding of the micrococcal nuclease released receptor to highly polymerized DNA. Micrococcal nuclease hydrolysates were prepared as described under Experimental. The hydrolysates were incubated for 30 min at 4°C in the presence (—○—) and in the absence (—●—) of highly polymerized DNA, and then centrifuged on a sucrose gradient at 185,000 *g* for 20 h.

DISCUSSION

The digestion of the MCF-7 cell nuclei with micrococcal nuclease at 0°C allowed us to extract the nuclear estradiol receptor under mild conditions. More than 90% of the receptor could be solubilized by this procedure and directly characterized in a low-salt medium. The amount of the receptor released was parallel to the extent of chromatin DNA solubilized which may indicate that the estrogen receptor is homogenously distributed throughout the chromatin domain and is not restricted to chromatin conformations which may be preferentially digested by this enzyme [17, 18].

Mild micrococcal nuclease digestion excised the receptor from the bulk of chromatin as an abundant 6–7S form and as a less abundant ~12S species which sedimented slightly more rapidly than the bulk of the mononucleosome generated (~11S). The ~12S species may represent the mononucleosome in association with the receptor, thus sedimenting faster than the bulk of mononucleosomes or the mononucleosomes in association with a part of the linker DNA, to which the receptor is associated. As this receptor form was rapidly digested by further nuclease treatment, although the mononucleosome fraction increased, the second assumption seemed to be more acceptable. Since more of the slower sedimenting receptor was released by further nuclease digestion, we would speculate that this form of receptor is bound to the linker region between the nucleosomal particles. Extensive digestion of the 6–7S receptor released a 5.6S form which may indicate that the 6–7S species contained DNA components. Continuing the digestion of the 5.6S receptor with DNase I did not affect its sedimentation behaviour indicating that this form may not contain DNA residues. Similarly, micrococcal nuclease digestion released the estradiol receptor from rat uteri [19], hen oviduct [20], MCF-7 cells [21] as well as the thyroid receptor from GH_1 cells [15] as two receptor forms, one associated with the nucleosomal particle and another slower sedimenting form.

In contrast to our results for the estradiol receptor, additional digestion of the slower sedimenting thyroid receptor (6.5S) with DNase I resulted in a receptor sedimenting at 3.8S, indicating that DNA may be a component of the 6.5S form. Another confirmation that the 5.6S estradiol receptor does not contain DNA components, was deduced from its ability to bind DNA, while the thyroid receptor binds DNA only in its 3.8S form. The sedimentation coefficient and molecular weight found by us for high salt dissociated receptor was similar to the results published for the high salt extracted nuclear MCF-7 cell estradiol receptor by Eckert *et al.*[22]. However, recently these authors published that extraction of the nuclear estradiol MCF-7 cell receptor in Tris–EDTA buffer instead of phosphate buffer used previously, resulted in a heavier receptor form sedimenting at 5.4S [23]. We could not confirm these results, although we used Tris–EDTA buffer in a 5-fold concentration as indicated by the authors [23].

The estimated molecular weight of the 5.6S receptor (Mr = 160,000) is approximately twice the molecular weight of the 4.2S form (Mr = 77,000). This suggests that the 5.6S form could be: (1) a dimeric form of the 4.2S receptor, (2) an association of the monomer receptor with other protein(s) which may bind the receptor to DNA (acceptor). The ability of the 5.6S receptor to bind DNA at 0°C led us to speculate about the second hypothesis. Additional studies are needed to identify the structure of this receptor form and its functional organization on the chromatin as a modulator of gene activation.

REFERENCES

1. King R. J. B. and Mainwaring W. I. P.: *Steroid–Cell Interaction*. University Park Press, Baltimore (1974) p. 202.
2. Notides A. C. and Nielsen S.: The molecular mechanism of the *in vitro* 4S to 5S transformation of the uterine estrogen receptor. *J. biol. Chem* **249** (1974) 1866–1873.
3. Rochefort H. and Baulieu E. E.: Estradiol neonuclear receptor in the rat uterus: Sedimentation constant and conditions of formation. *Biochimie* **54** (1972) 1303–1317.
4. King W. J. and Greene G. L.: Monoclonal antibodies localize oestrogen receptor in the nuclei of target cells. *Nature* **307** (1984) 745–747.
5. Welshons W. V., Lieverman M. E. and Gorski J.: Nuclear localization of unoccupied oestrogen receptors. *Nature* **307** (1984) 747–749.
6. Perlman A. J., Stanley F. and Samuels H. H.: Thyroid hormone nuclear receptor evidence for multimeric organization in chromatin. *J. biol. Chem.* **257** (1982) 930–938.
7. Brooks S. C., Locke E. R. and Soule H.: Estrogen receptor in a human cell line (MCF-7) from breast carcinoma. *J. biol. Chem.* **248** (1973) 6251–6253.
8. Liew C. C. and Chan P. K.: Identification of nonhistone chromatin proteins in chromatin subunits. *Proc. natn. Acad. Sci., U.S.A.* **73** (1976) 3458–3462.
9. Burgoyne L. A., Mobbs J. D. and Marshal A. J.: Chromatin structure: A property of the higher structure of chromatin and the time curve of its formation during chromatin replication. *Nucleic Acids Res.* **3** (1976) 3293–3304.
10. Geier A., Cocos M., Ginzburg R., Haimsohn M. and Lunenfeld B.: Estradiol binding to nuclear receptors in human breast cancer tissue (MCF-7 cell line) and in Dimethylbenz (a) anthracene-induced mammary carcinoma. *J. clin. Endocr. Metab.* **49** (1979) 34–39.
11. Geier A., Haimsohn M., Malik Z. and Lunenfeld B.: Unoccupied binding sites for oestrogen in nuclei of a breast tumor cell line (MCF-7). *Biochem. J.* **200** (1981) 515–520.
12. Geier A., Haimsohn M. and Lunenfeld B.: Evidence for the origin of the unoccupied oestrogen receptor in nuclei of a human breast cancer cell line (MCF-7). *Biochem. J.* **202** (1982) 687–691.
13. Burton K. H.: A study of the conditions and mechanism of diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. *Biochem. J.* **62** (1956) 315–323.
14. Goldsmith S. A. and Proctor B. E.: Photometric determination of catalase activity. *J. biol. Chem.* **187** (1950) 705–709.
15. Siegel L. M. and Monty K. J.: Determination of

molecular weights and frictional ratios of proteins in impure systems by use of gel filtration and density gradient centrifugation. Application to crude preparations of sulfite and hydroxylamine reductases. *Biochim. biophys. Acta* **112** (1966) 346–362.
16. Schachman H. K.: *Ultracentrifugation in Biochemistry*. Academic Press, New York (1959) p. 239.
17. Bloom K. S. and Anderson J. N.: Fractionation of hen oviduct chromatin into transcriptionally active and inactive regions after selective micrococcal nuclease digestion. *Cell* **15** (1978) 141–150.
18. Bloom K. S. and Anderson J. N.: Conformation of ovalbumin and globin genes in chromatin during differential gene expression. *J. biol. Chem.* **254** 10532–10539.
19. Senior M. B. and Frankel F. R.: Evidence for two kinds of chromatin binding sites for the estradiol–receptor complex. *Cell* **14** (1978) 857–863.
20. Massol N., Lebean M. C. and Baulieu E. E.: Estrogen receptor in hen oviduct chromatin digested by micrococcal nuclease. *Nucleic Acids Res.* **5** (1978) 723–738.
21. Scott R. W. and Frankel F. R.: Enrichment of estradiol–receptor complexes in a transcriptionally active fractions of chromatin from MCF-7 cells. *Proc. natn. Acad. Sci., U.S.A.* **77** (1980) 1291–1295.
22. Eckert R. L. and Katzenellenbogen B. S.: Physical properties of estrogen receptor complexes in MCF-7 human breast cancer cells: Differences with antiestrogen and estrogen. *J. biol. Chem.* **257** (1982) 8840–8846.
23. Miller M. A., Greene G. L. and Katzenellenbogen B. S.: Estrogen receptor transformation in MCF-7 breast cancer cells: Characterization by immunochemical and sedimentation analysis. *Endocrinology* **114** (1984) 296–298.