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Enrichment of estradiol–receptor complexes in a transcriptionally active fraction of chromatin from MCF-7 cells

(chromatin binding/chromatin fractionation/mammary tumor cells/nucleosomes/steroid hormone)

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ABSTRACT **We have examined the interaction of the estradiol receptor molecule with chromatin in MCF-7 cells, a human breast tumor cell line responsive to estradiol. Receptor was found associated with the various nucleosomal products produced by digestion with micrococcal nuclease. In order to determine whether these receptor binding sites were distributed in a random or nonrandom manner within the chromatin, we have fractionated MCF-7 cell chromatin into transcriptionally active and inactive fractions by limited micrococcal nuclease digestion followed by Mg^{2+} precipitation. A comparison of the Mg^{2+}-soluble and insoluble chromatin fractions showed that the Mg^{2+}-soluble fraction: (*i*) was composed predominantly of mononucleosomes; (*ii*) was enriched in nonhistone proteins; (*iii*) apparently lacked histone H1; (*iv*) was enriched approximately 5-fold in transcribed sequences as measured by a cDNA probe to cytoplasmic poly(A)-RNA sequences; and (*v*) was depleted at least 5-fold of globin sequences, which is presumably a nontranscribed gene in these cells. When these cells were stimulated with β-[^{3}H]estradiol, the Mg^{2+}-soluble fraction showed a significant enrichment in chromatin-bound estradiol receptor: the Mg^{2+}-soluble mononucleosomes showed a 3- to 4-fold enrichment and the di- and trinucleosomes, a 7- to 19-fold enrichment, when compared to the corresponding subunits in the Mg^{2+}-insoluble chromatin fraction. This cofractionation of chromatin enriched in transcribed sequences and bound estradiol receptor indicated that receptor binding to MCF-7 cell chromatin was not random but, rather, occurred preferentially in specific regions of the chromatin.**

The site of action of steroid hormone–receptor complexes in nuclei of hormone target cells is unknown. It is generally postulated that once a steroid hormone enters such cells it binds to a cytoplasmic receptor protein (1), leading to activation of the complex (2) and its transport to the nucleus, where it exerts its control on gene expression (3). Various types of nuclear acceptor sites for the estradiol receptor have been proposed; they include DNA (4), chromatin (5), ribonucleoprotein complexes (6), nuclear matrix (7), and the nuclear membrane (8). Whatever the nature of these acceptor sites might be, their apparent abundance (9, 10) has led to the suggestion that they are mainly nonspecific (11). Were that correct, the detection of specific biologically functional sites would be extremely difficult (12).

Recently, we have shown that a large fraction of the nuclear estradiol receptor complex of rat uterus (13) and human mammary tumor cells (unpublished) is associated with nucleosomal subunits of chromatin after administration of hormone to intact tissues or cells. Because of the importance of the question of biological specificity, we have now attempted to determine whether these sites are distributed in a random or nonrandom manner within the chromatin. Several procedures have been developed that utilize nuclease digestion and differential solubility to fractionate chromatin into transcriptionally active and inactive fractions (14–16). We have applied a modification of these procedures to fractionate the chromatin of MCF-7 cells, a line of human mammary carcinoma cells that contain the estradiol receptor (17) and respond to estradiol with specific protein synthesis (18) and cell growth (19).

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MATERIALS AND METHODS

Cell Culture. MCF-7 cells were seeded in glass roller bottles in Dulbecco's modified Eagle's medium containing 0.2 mM glutamine, penicillin at 100 units/ml, streptomycin at 0.1 μg/ml, and 10% fetal calf serum. Three days prior to harvest the medium was changed to Dulbecco's modified Eagle's medium containing 0.2 mM glutamine, penicillin at 100 units/ml, streptomycin at 0.1 μg/ml, bovine insulin at 0.01 μg/ml, and 5% fetal calf serum that had been adsorbed two times at room temperature with 1 g of Norit A charcoal and 25 mg of dextran T110 per 100 ml of serum to remove endogenous steroids. In this medium endogenous estradiol was depleted within 2 days (unpublished observations).

Cell Labeling. MCF-7 cells were stimulated with 5 nM β-[^{3}H]estradiol (New England Nuclear, 152 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels) for 1 hr at 37°C in the medium containing 5% charcoal/dextran-adsorbed serum. In some experiments cells were uniformly labeled with [^{14}C]thymidine (New England Nuclear, 40.8 mCi/mmol) at 9 nCi/ml for 2 days prior to β-[^{3}H]estradiol addition.

Nuclear Isolation and Nuclease Digestion. All procedures were performed at ice/water temperature, and all glassware was siliconized. MCF-7 cell monolayers were collected by scraping into phosphate-buffered saline and resuspended in cell lysis buffer (10 mM Tris·HCl, pH 7.4/25 mM NaCl/5 mM $MgCl_2$/10 mM monothioglycerol/0.5% Nonidet P40/0.1 mM phenylmethylsulfonyl fluoride or kallikrein inactivator at 20 units/ml). The suspension was mixed frequently with a Vortex mixer and after 10 min was centrifuged at $250 \times g$ for 10 min. The crude nuclear pellet was resuspended in the cell lysis buffer two more times and then washed an additional two times in nuclear wash buffer (10 mM Tris·HCl, pH 7.4/25 mM NaCl/3 mM $MgCl_2$/10 mM monothioglycerol/0.1 mM phenylmethylsulfonyl fluoride or kallikrein inactivator at 20 units/ml). The final nuclear pellet was resuspended in this buffer at 20 A_{260} units/ml, $CaCl_2$ was added to 1 mM, and the nuclei were digested with micrococcal nuclease (Worthington).

Sucrose Gradient Centrifugation. Samples (0.4 ml) were layered onto 5–20% (wt/vol) sucrose gradients (10 mM Tris·HCl, pH 7.4/1 mM EDTA/10 mM NaCl) and centrifuged at 2°C in a Beckman SW41 rotor.

DNA Isolation. DNA was extracted from whole chromatin or chromatin fractions (see Fig. 1) by treating the samples with

proteinase K (EM Biochemicals) at 200 μg/ml in 0.5% NaDodSO$_4$ for 2 hr at 37°C. The S2 DNA was first digested with RNase (Calbiochem, grade A) at 20 μg/ml. The samples were extracted with phenol/chloroform/isoamyl alcohol (50:50:1, vol/vol) at room temperature and then precipitated with 2 vol of ethanol overnight at −20°C. Total DNA and DNA from the P1 and P2 chromatin fractions were heated in 0.3 M NaOH at 80°C for 90–120 min to hydrolyze the DNA to fragments with an average length of 250 nucleotides. All DNA samples were passed over Chelex 100. The A_{260}/A_{280} ratios were between 1.9 and 2.0.

Cytoplasmic Poly(A)-RNA Isolation. Total MCF-7 cell cytoplasmic RNA was isolated by a procedure described by T. Dolby (personal communication). Cells were resuspended in 50 mM Tris·HCl, pH 7.4/50 mM NaCl/5 mM $MgCl_2$/0.25 M sucrose/10 mM mercaptoethanol/0.5% Nonidet P40, incubated on ice for 10 min, and centrifuged at 300 × *g* for 5 min. This procedure was repeated on the crude nuclear pellet and the supernatants were combined and centrifuged at 15,000 × *g* for 15 min. The supernatant was brought to 330 μg of heparin per ml/1% NaDodSO$_4$/0.2 M NaCl/1.5 mM EDTA, extracted with phenol/chloroform/isoamyl alcohol (50:50:1) at room temperature, and precipitated with 2 vol of ethanol overnight at −20°C. The poly(A)-containing RNA fraction was isolated by using oligo(dT)-cellulose (P-L Biochemicals) chromatography as described by Aviv and Leder (20). The A_{260}/A_{280} ratio was greater than 2.0.

cDNA Synthesis. A [^{3}H]cDNA probe for MCF-7 cell cytoplasmic poly(A)-RNA sequences was prepared according to a modification of the procedure described by Friedman and Rosbash (21). Reaction mixtures (100 μl) contained 50 mM Tris·HCl, pH 8.3, 60 mM KCl, 6 mM $MgCl_2$, 20 mM mercaptoethanol, $(dT)_{12-18}$ at 25 μg/ml, actinomycin D at 100 μg/ml, 0.5 mM each of dATP, dGTP, and dTTP (P-L Biochemicals), 0.25 mM [^{3}H]dCTP (New England Nuclear, 19.7 Ci/mmol), avian myeloblastosis virus reverse transcriptase (generously provided by the Division of Cancer Cause and Prevention, National Cancer Institute) at 718 units/ml, and poly(A)-RNA at 50 μg/ml. Incubation was at 43°C for 2 hr. The material excluded from Sephadex G-50 was hydrolyzed in 0.3 M NaOH at 100°C for 5 min, neutralized, and precipitated by ethanol in the presence of yeast tRNA (Sigma). cDNA specific activity was 5.9 × 10^6 cpm/μg of DNA. The cDNA probe had an average length of 500 nucleotides.

S1 Nuclease Digestion. S1 digestions were performed according to a modification of the procedure described by Weigand *et al.* (22). Each hybridization sample was incubated in 30 mM sodium acetate, pH 5.0/0.2 M NaCl/1 mM $ZnCl_2$/calf thymus DNA at 10 μg/ml, with and without 10 units of S1 nuclease (Sigma) per μg of DNA at 37°C for 1 hr. Hybrid formation was assayed as the percent S1-resistant trichloroacetic acid-precipitable radioactive material.

RESULTS

Chromatin Fractionation. The chromatin fractionation procedure that we used was a modification of the procedures described by Gottesfeld *et al.* (14) and Bloom and Anderson (16). All steps, including the nuclease digestion, were done at ice/water temperature to inhibit dissociation of hormone from receptor and hormone–receptor complexes from nuclear sites. The percentages of total nuclear A_{260} and β-[^{3}H]estradiol found in the chromatin fractions after limited micrococcal nuclease digestion are shown in Fig. 1. The nuclear supernatant fraction, S0, representing material that had leaked from nuclei during digestion, contained approximately 5% of the nuclear A_{260} and 4–6% of the total nuclear estradiol. The P1 fraction, which contained material not rendered EDTA-soluble by micrococcal nuclease digestion, was found to be slightly enriched in its estradiol content. However, the Mg^{2+}-soluble, or S2, fraction showed a significant enrichment, whereas the Mg^{2+}-insoluble, or P2, fraction was depleted in [^{3}H]estradiol content.

Fig. 2 shows sucrose gradient absorbance profiles of the S2 and P2 fractions after different amounts of nuclease digestion. With the least digestion (Fig. 2*A*), mono-, di-, and trinucleosomes were clearly distinguishable in the P2 fraction, with the larger oligonucleosomes predominating. However, the S2

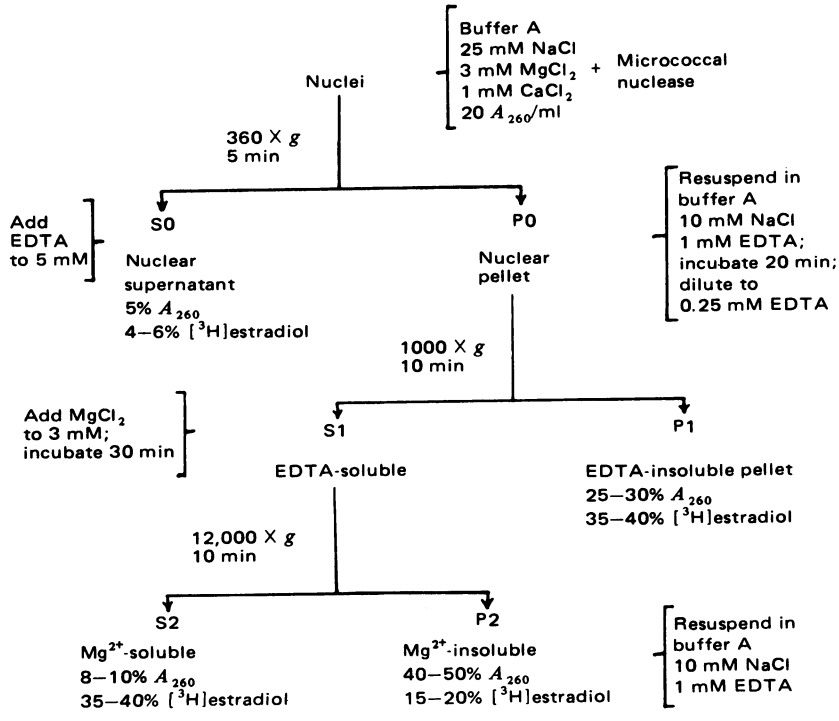

FIG. 1. Chromatin fractionation scheme. For determination of the percent distribution of β-[^{3}H]estradiol, MCF-7 cells were incubated with 5 nM β-[^{3}H]estradiol for 1 hr at 37°C. Nuclei were isolated and digested with 45 units of micrococcal nuclease per A_{260} unit for 10 min. Under these conditions, 6% of total nuclear A_{260} appeared as mononucleosomes. Buffer A: 10 mM Tris·HCl, pH 7.4/10 mM monothioglycerol/kallikrein inactivator at 20 units per ml or 0.1 mM phenylmethylsulfonyl fluoride.

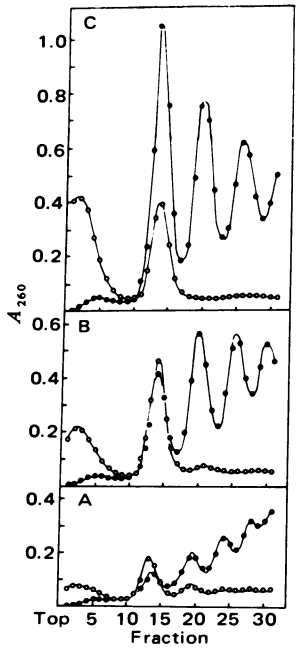

FIG. 2. Sucrose gradient absorbance profiles of S2 and P2 chromatin fractions. MCF-7 cell nuclei were digested for 10 min at 2°C with 15 (*A*), 45 (*B*), or 90 (*C*) units of micrococcal nuclease per A_{260} unit and fractionated as described in Fig. 1. The S2 (○) and P2 (●) fractions from each digestion were analyzed on sucrose gradients that were centrifuged at 34,000 rpm for 17 hr. The percentages of nuclear A_{260} that appeared as total mononucleosomes were 3.7% (*A*), 8.1% (*B*), 11.3% (*C*).

fraction contained primarily mononucleosomes, and at this stage of digestion there was a higher concentration of mononucleosomes in the S2 fraction than in the P2 fraction. This indicated a preferential production of Mg^{2+}-soluble mononucleosomes with limited micrococcal nuclease digestion. The level of S2 mononucleosomes, however, reached a maximum rather early in digestion, and the oligonucleosomes in this fraction became almost undetectable (Fig. 2 *B* and *C*). In contrast, there was a vast increase in the concentration of P2 mononucleosomes throughout digestion, with mononucleosomes predominating only at greater extents of digestion. Thus, the S2 and P2 fractions differed in the rate of formation and relative abundance of their nuclease digestion products.

The chromosomal protein compositions of the S2 and P2 fractions also showed significant differences. Mono- and dinucleosomes from each fraction were isolated from sucrose gradients and analyzed by $NaDodSO_4$/polyacrylamide gel

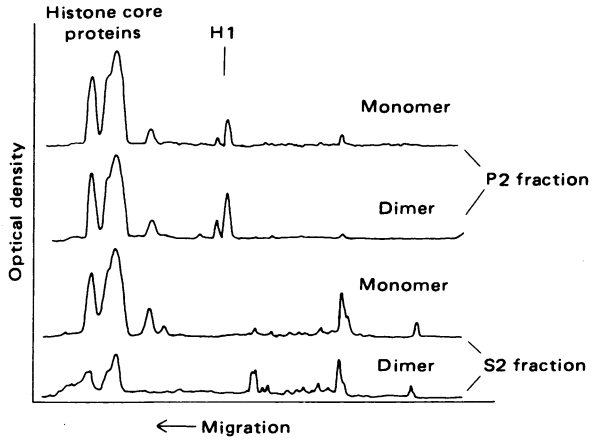

FIG. 3. Electrophoretic analysis of S2 and P2 chromosomal proteins. Mono- and dinucleosomes were isolated from sucrose gradients, dialyzed against two changes of water overnight, lyophilized, and electrophoresed on 12% $NaDodSO_4$/polyacrylamide gels at 16 mA constant current for 4 hr at room temperature. Optical density of stained gels was measured at 525 nm. H1 was identified by its comigration with the major doublet in a 0.25 M H_2SO_4/0.5 M KCl extract of MCF-7 cell chromatin. The S2 and P2 fractions were derived from a nuclear digest in which 9.5% of the total nuclear A_{260} appeared as mononucleosomes.

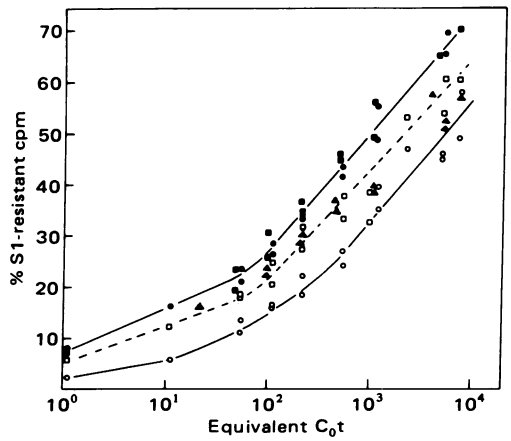

FIG. 4. Hybridization analysis of total, P1, P2, and S2 chromatin fraction DNAs with [^{3}H]cDNA probe to cytoplasmic poly(A)-RNA of MCF-7 cells. All hybridization reactions were in a total volume of 10 μl in sealed glass capillaries at 68°C in 10 mM Tris·HCl, pH 7.0/2 mM EDTA/0.6 M NaCl, with DNA at 3 mg/ml and 1000 cpm of [^{3}H]cDNA probe (specific activity, 5.9×10^6 cpm/μg of DNA). Hybridization was assayed as resistance of probe to S1 nuclease. Values were adjusted to give equivalent C_0t and were corrected for 5–7% probe self-hybridization. [C_0t is concentration of DNA (moles of nucleotide per liter) × time (seconds).] Driver DNAs were from total chromatin (▲) or chromatin fractions P1 (□), P2 (○), and S2 (■, ●; results from two different DNA preparations are shown).

electrophoresis. Optical scans of the Coomassie blue-stained gels are shown in Fig. 3. P2 nucleosomes contained mainly core histones, H1, and a few unidentified polypeptides. On the other hand, S2 nucleosomes showed a significantly higher ratio of nonhistone to histone protein. The S2 dinucleosomes showed the greatest enrichment for nonhistone protein. H1 appeared to be absent, or at least greatly depleted, in the S2 mononucleosomes and dinucleosomes; however, the low yield of S2 dinucleosomes made analysis of these particles difficult.

That the S2 and P2 fractions contained distinct classes of chromatin was confirmed by assaying their transcribed DNA sequence content. A cDNA probe prepared from MCF-7 cytoplasmic poly(A)-RNA was hybridized with an excess of total DNA or DNA extracted from the P1, S2, or P2 fractions. The resultant hybridization curves, shown in Fig. 4, indicated that the P1 and total DNA samples were virtually identical in their transcribed sequence content. However, the enrichment in transcribed sequence content of S2 DNA over P2 DNA, in several DNA and cDNA preparations, was reproducibly found to be approximately 5-fold.

When a ^{32}P-labeled cDNA probe prepared from human α and β globin mRNA was used in similar hybridization analyses, the $C_0t_{1/2}$ with P2 DNA was approximately 2000, but it was greater than 10,000 with S2 DNA (data not shown). Thus, the S2 DNA was enriched in transcribed sequences and depleted of at least one presumably nontranscribed sequence, whereas the P2 DNA showed the opposite properties.

Distribution of Chromatin-Bound Receptor. In order to distinguish estradiol receptor binding to chromatin from other possible nuclear binding sites, we measured the total radioactivity of β-[^{3}H]estradiol associated with nucleosomes of the S2 and P2 fractions. MCF-7 cells were incubated with labeled estradiol for 1 hr at 37°C, and their nuclei were isolated, digested, and fractionated as described in Fig. 1. The results are shown in Fig. 5. In both fractions, β-[^{3}H]estradiol was associated with the nucleosome absorbance peaks. As had previously been seen in a total digest of rat uterine nuclei (13), the estradiol radioactivities were displaced relative to each absorbance peak, consistent with the addition of hormone–receptor complexes

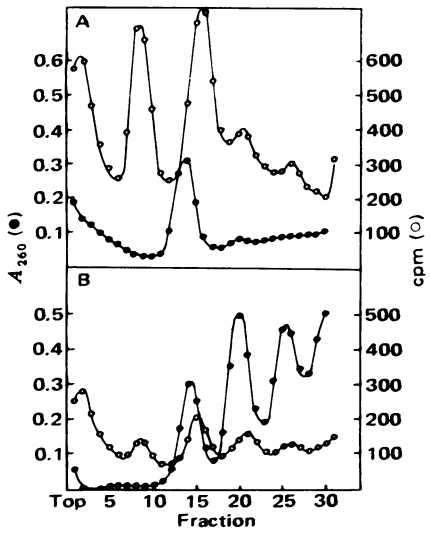

FIG. 5. Sucrose gradient centrifugation of S2 (*A*) and P2 (*B*) chromatin fractions from β-[^{3}H]estradiol-stimulated MCF-7 cells. MCF-7 cells were incubated for 1 hr with 5 nM β-[^{3}H]estradiol at 37°C. The nuclear digest contained 8.4% of its A_{260} as mononucleosomes. Sucrose gradients were centrifuged at 36,000 rpm for 15 hr; fractions were analyzed for A_{260} (●) and then radioactivities were measured in 3 ml of Aquasol II with 25% efficiency for β-[^{3}H]estradiol (○).

to a small fraction of the chromatin. There was also a radioactive peak in the 7S region of the gradients, associated with little if any A_{260}. This 7S component has also been reported in the rat uterine system (13), and a similar component was produced when nuclei from β-[^{3}H]estradiol-stimulated MCF-7 cells were digested with DNase II (unpublished results).

As shown in Fig. 5, the S2 nucleosomes were significantly enriched in bound estradiol when compared to the P2 nucleosomes. Measurement of the radioactivity associated with each of the absorbance peaks indicated that the S2 mononucleosomes were 3.4-fold enriched in bound β-[^{3}H]estradiol per A_{260} over the P2 mononucleosomes; the S2 dinucleosomes showed a 12-fold enrichment over the P2 dinucleosomes. Also, at this extent of digestion the specific activity (β-[^{3}H]estradiol/A_{260}) of the S2 mononucleosomes was about half that of the S2 dinucleosomes; this relationship was reversed in the P2 fraction.

In another experiment, cells were uniformly labeled with [^{14}C]thymidine prior to the 1-hr incubation with β-[^{3}H]estradiol, and specific activity was calculated as the ratio of β-[^{3}H]estradiol to [^{14}C]thymidine. The results are shown in Table 1. The specific activities of the S2 mononucleosomes were 3–4 times greater than those of the P2 mononucleosomes, whereas the specific activities of the S2 dinucleosomes were as much as 19 times greater than those of the P2 dinucleosomes. Also, S2 monomers had lower specific activities than S2 dimers, whereas P2 monomers had higher specific activities than P2 dimers. These results showed that limited digestion of MCF-7 cell nuclei with micrococcal nuclease preferentially released Mg^{2+}-soluble nucleosomes that were enriched in bound estradiol. The S0 fraction has been reported by Bloom and Anderson to contain Mg^{2+}-soluble mononucleosomes that are enriched in transcribed sequences (16). As seen in Table 1, when detected, these mononucleosomes were also enriched in bound estradiol.

That the β-[^{3}H]estradiol cpm associated with nucleosomes actually represented chromatin-bound estradiol receptor was supported by the following experiment. MCF-7 cells, when growing at a reduced rate in the absence of estradiol, have been reported to contain a large fraction of their estradiol receptor population as a hormone-free species in the nucleus (23). Therefore, nuclei were isolated from unstimulated MCF-7 cells that had been depleted of their endogenous steroid hormone (see *Cell Culture*) and incubated with β-[^{3}H]estradiol or β-[^{3}H]estradiol plus a 200-fold excess of unlabeled estradiol for 1 hr at 2°C. The washed nuclei were then digested with micrococcal nuclease and fractionated as described in Fig. 1. The sucrose gradient profiles of the S2 and P2 fractions from the nuclei incubated with only β-[^{3}H]estradiol were similar to those seen in Fig. 5. However, in the S2 and P2 fractions isolated from nuclei incubated with excess unlabeled estradiol, all of the nucleosome-associated β-[^{3}H]estradiol, as well as the 7S component, was absent (data not shown). This limited capacity of estradiol binding to chromatin implied that it was mediated by the estradiol receptor (24).

DISCUSSION

We have found that limited micrococcal nuclease digestion of MCF-7 cell nuclei followed by Mg^{2+} precipitation resulted in a soluble chromatin-fraction that was approximately 5-fold enriched in DNA sequences complementary to cytoplasmic poly(A)-RNA. This transcriptionally active fraction also contained chromatin that was depleted in histone H1 and enriched in nonhistone chromosomal proteins. In these ways it was similar to fractions generated by others, using DNase II rather than micrococcal nuclease (25, 26) or using NaCl rather than $MgCl_2$ to precipitate the inactive fraction (27). In addition, we found that our active fraction was depleted more than 5-fold of a sequence that apparently is not transcribed in these cells.

By assaying directly for chromatin association and using conditions that should minimize receptor migration (digestion at 2°C, low salt), we found that the Mg^{2+}-soluble, transcriptionally active fraction was also 3- to 19-fold enriched in chromatin-bound estradiol receptor. Furthermore, 40% of the total nuclear estradiol receptor could be recovered in this fraction, which contained less than 10% of the DNA. This cofractionation of chromatin enriched in transcribed sequences

Table 1. β-[^{3}H]Estradiol specific activities in the S2 and P2 chromatin fractions during micrococcal nuclease digestion

Sample	Extent of digestion as % monomer	S0 fraction	S2 fraction			P2 fraction			S2/P2	
		Monomer	Monomer	Dimer	Monomer/dimer	Monomer	Dimer	Monomer/dimer	Monomer	Dimer
1	0.6	ND	1.24	1.44	0.861	NA	NA	NA	NA	NA
2	1.9	ND	0.915	1.05	0.871	0.340	0.144	2.36	2.69	7.29
3	5.6	0.596	0.683	1.18	0.541	0.206	0.076	2.70	3.10	17.1
4	13.1	0.569	0.483	1.32	0.366	0.110	0.070	1.57	4.39	18.9

MCF-7 cells continuously labeled with [^{14}C]thymidine for 2 days prior to the 1-hr β-[^{3}H]estradiol stimulation were fractionated as described in Fig. 1. The S2 and P2 fractions were centrifuged through sucrose gradients and the specific activities of the nucleosomes were calculated by dividing the total cpm of β-[^{3}H]estradiol by the total cpm of [^{14}C]thymidine in each of the nucleosomal peak regions. ND, not detected; NA, not assayed.

and estradiol receptor indicated that receptor binding to MCF-7 cell chromatin was not random but, rather, occurred preferentially in specific regions of the chromatin.

The S2 (Mg^{2+}-soluble) fraction was composed predominantly of mononucleosomes, with only small amounts of di- and trinucleosomes, whereas the P2 (Mg^{2+}-insoluble) fraction contained mainly higher oligomers of nucleosomes. This may reflect the fact that chromatin in regions of transcriptional activity has a conformation that makes it more accessible to nuclease, resulting in its rapid digestion to mononucleosomes (28). We previously showed that chromatin associated with estradiol receptor complexes was preferentially digested to mononucleosomes by micrococcal nuclease (13). Thus it is possible that a large fraction of the nuclear estradiol receptor population is associated with transcriptionally active chromatin.

Whether the estradiol receptor is bound directly to the transcriptionally active chromatin or to some other minor Mg^{2+}-soluble chromatin population cannot be determined from our present data. Recently, we have found that the fractionation procedure devised by Levy-Wilson and Dixon (29), which is dependent on the differential solubility of transcriptionally active regions of chromatin in 0.1 M NaCl, provides a fraction from MCF-7 cell chromatin containing nucleosomes that are 3- to 15-fold enriched in estradiol–receptor complexes. These results support the general validity of our observations, and, in addition, suggest that the nature or concentration of cations is not important in generating the distribution of receptor that we see.

Interestingly, the S2 di- and trinucleosomes had significantly higher specific activities of [^{3}H]estradiol per unit DNA than the S2 mononucleosomes; the P2 di- and trinucleosomes had lower specific activities than the P2 mononucleosomes. Furthermore, it was the di- and trinucleosomes of the S2 and P2 fractions that showed the most striking differences in specific activity. These observations might be accounted for as follows. The S2 and P2 mononucleosomes may have been cross-contaminated to a much greater extent than the oligonucleosomes, thereby artifactually lowering the specific activity of the S2 mononucleosomes later in digestion and raising the specific activity of the P2 mononucleosomes early in digestion (see also ref. 29). In addition, and more interesting, the di- and trinucleosomes of the S1 fraction may have bound additional estradiol receptor in the linker region between nucleosomes—at sites apparently not present or accessible on the P2 oligonucleosome linker regions. Binding at these sites not only would have raised the specific activity of the S2 di- and trinucleosomes but would have generated a local region of chromatin rich in receptor, much like that predicted in the hypothesis of Yamamoto and Alberts (12).

Several attempts to examine the chromosomal distribution of steroid hormone receptors have been reported previously. Studies in which mechanical shear had been used to fractionate chromatin (30, 31) are difficult to interpret because shear forces have been shown to disrupt native chromatin structure (32). In studies using nuclease digestion, preferential binding of the estradiol receptor in presumed transcriptionally active chromatin has been reported in the hen oviduct (33) in agreement with our results; however, no preferential distribution of the glucocorticoid nuclear receptor was found in GC cells (34), a rat pituitary cell line. The apparent conflict between the estradiol and glucocorticoid nuclear receptor distributions may result from the use of different chromatin association assays.

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