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NUCLEASE SENSITIVITY OF ESTRADIOL-CHARGED ESTROGEN RECEPTOR BINDING SITES IN NUCLEI ISOLATED FROM NORMAL AND NEOPLASTIC RAT MAMMARY TISSUES

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Summary—The interaction of partially purified calf uterine estradiol-charged estrogen receptor ([^{3}H]ER) with rat nuclei was studied *in vitro*. We previously observed a significantly greater number of [^{3}H]ER binding sites (at saturation) in nuclei of R3230AC mammary tumors from intact vs ovariectomized (ovex) rats with no difference in the affinity of [^{3}H]ER binding for these nuclei. We now report on the nuclease sensitivity of [^{3}H]ER binding sites in nuclei from these tumors and from normal rat tissues. Digestion of tumor nuclei with deoxyribonuclease I (DNase I) prior to incubation with [^{3}H]ER *in vitro* resulted in a progressive loss of [^{3}H]ER binding capacity, which was not accompanied by alterations in the affinity of [^{3}H]ER for the nuclei (K_d = 1–3 nM). A significantly lower concentration ($P < 0.005$) of DNase I eliminated 50% of the [^{3}H]ER binding sites in nuclei of tumors from intact hosts (8 unit·min/ml) compared to tumors from ovex hosts (22 unit·min/ml). These results indicate that DNA regions capable of binding ER are more susceptible to DNase I digestion in tumors from intact rats than those from ovex hosts, suggesting that the endogenous hormonal milieu is responsible, at least in part, for maintenance of nuclease-sensitive DNA conformations in this hormone-responsive mammary tumor. The amount of DNase I required to eliminate 50% of [^{3}H]ER binding to nuclei from lactating mammary gland, liver, and kidney ranged from 14 to 56 unit·min/ml. Therefore, accessibility of [^{3}H]ER binding sites to nuclease digestion in normal rat tissue is generally less than that of R3230AC tumors.

INTRODUCTION

Eukaryotic DNA is highly condensed and packaged in the nucleus in a complex series of higher order forms that appear to relate to the functional state of each particular type of cell (reviewed in [1]). Changes in gene expression during proliferation and differentiation are preceded by alterations in the structure of chromatin as genes are readied for transcription. Steroid hormone action is manifested in target tissues by high affinity binding of the activated hormone–receptor complex to specific regions of the chromatin, called hormone responsive elements (HRE), thereby altering the expression of certain genes [2–6]. The unique characteristics of such binding loci for the hormone–receptor complex at regulated sites in nuclei of target tissues vs non target tissues have not been determined.

Exogenous nucleases, such as bovine pancreatic deoxyribonuclease I (DNase I), have been used to probe the chromatin structure in isolated nuclei (reviewed in [6–8]). When eukaryotic nuclei are partially digested with DNase I, the resulting DNA fragments form a continuum of sizes as a diffuse band of DNA on agarose gel electrophoresis [8, 11, 12]. DNase I, an endonuclease with little DNA sequence specificity, is thought to produce multiple nearby cleavages at accessible sites in chromatin effectively resulting in double strand breakage [7, 8]. At low concentrations DNase I preferentially digests genes that are, or have been, transcriptionally active (reviewed in [7–10]). This sensitivity to DNase I digestion is a general phenomenon that reflects the potential of a gene to be transcribed, but does not exactly correlate with the level of transcription [1, 10]. Using this enzyme, structural differences have been detected between transcriptionally inactive and active domains of chromatin [1, 7–14].

ER binding sites on chromatin in bovine uterine tissue were found to be particularly sensitive to

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Abbreviations: DNase I: deoxyribonuclease I, E.C.3.1.21.1; bp: base pairs; DCC: dextran-coated charcoal; E_2: 17β-estradiol; ER: estrogen receptor; F344: Fischer 344 rat; [^{3}H]ER: receptor charged with tritium labeled 17β-estradiol; kB: kilobases; MNase: micrococcal endonuclease, E.C.3.1.31.1; ovex: ovariectomized; PMSF: phenylmethylsulfonyl fluoride.

digestion by DNase I, micrococcal nuclease, or endogenous nucleases [15]. Brief digestion of calf uterine nuclei with DNase I, such that only hypersensitive sites were cleaved, yielded DNA fragments of 1–20 kB that were no longer capable of binding [^{3}H]ER [15]. Sites that are hypersensitive to digestion by DNase I were found within 1000 bp of the 5′ ends of genes that are active or that can be activated [7].

In order to understand the nuclear effects of hormonal perturbations *in vivo*, we developed a heterologous binding assay, which measures the binding of partially purified calf [^{3}H]ER to rat tissue nuclei *in vitro* [16, 17]. Conditions were established that demonstrate saturable, high affinity binding of calf uterine [^{3}H]ER to nuclei from rat tissues. With this system, we sought to determine the effects of ovariectomy and sex steroid administration *in vivo*, and observed that the number of these binding sites were changed after hormonal perturbation of the tumor-bearing host [16,17]. We now show that [^{3}H]ER binding sites are sensitive to DNase I digestion in both target and non-target tissues, and that the extent of loss of [^{3}H]ER binding capacity can be influenced by the hormonal status of the host.

EXPERIMENTAL

Animals

The conditions for animal maintenance and R3230AC mammary tumor transplantation in Fischer-344 rats have been described [18]. Lactating female Sprague–Dawley rats (150–200 g) were used as sources of mammary gland.

Treatments

When indicated, female Fischer 344 rats were ovariectomized one week prior to R3230AC tumor transplantation. All R3230AC tumors were obtained three weeks after initial tumor implantation. Estradiol valerate (Delestrogen, Squibb, Princeton, N.J.) or hydroxyprogesterone caproate (Delalutin, Squibb) were diluted in sesame seed oil as required for the indicated doses, and were administered (in 0.1 ml) subcutaneously on the dorsal surface.

Chemicals

All chemicals were reagent grade and were purchased from the commercial suppliers listed previously [16]. 17β-[2,4,6,7,16,17-^{3}H]Estradiol (158 Ci/mmol) was purchased from Amersham Corp., Arlington Heights, Ill.

Preparation of the estrogen receptor

Estrogen receptor was partially purified from calf uterus by ammonium sulfate fractionation [16, 19]. The receptor was dialyzed against TDP buffer (40 mM Tris–HCl, pH 7.5; 1 mM DTT: 0.5 mM PMSF) to remove salt, and sedimented at 20,000 ***g*** to remove denatured protein, prior to incubation with 10 nM [^{3}H]estradiol overnight at 4 C [20]. Unbound estradiol was removed by dextran-coated charcoal (DCC) treatment as previously described [15].

Isolation of nuclei and DNA determination

Nuclei were isolated from fresh rat tissues by a hypertonic sucrose method [21]. Briefly, the tissues were homogenized in 2.4 M sucrose (ultrapure) with 3.3 mM magnesium acetate, filtered through 4 layers of sterile grade 60 cheesecloth, and pelleted by centrifugation at 36,000 ***g***. Nuclei were free of cytoplasmic contaminants as assessed by phase contrast light microscopy. Nuclei were stored at a concentration of 4×10^7 nuclei/ml in storage buffer, consisting of 40 mM Tris–HCl, pH 7.5; 1 mM $MgCl_2$, 1 mM PMSF; 25% glycerol [16, 20]. DNA was extracted from nuclei using perchloric acid and DNA concentration was measured by the diphenylamine method using salmon sperm DNA as a standard [22].

Nuclear binding assay

The assay for binding of [^{3}H]estradiol-charged ER to nuclear sites has been described [16, 20].

Nuclease digestion of nuclei

Nuclei were resuspended in RSB^{+2} buffer (10 mM Tris–HCl, pH 7.5; 10 mM NaCl; 3 mM $MgCl_2$; 0.5 mM PMSF; and 0.1 mM $CaCl_2$) and incubated in a shaking water bath at 37°C for 5–15 min with 0–300 U/ml DNase I (2 Kunitz units/mg) as described [15, 16, 20]. The extent of digestion was expressed as unit-min/ml, i.e. time at 37°C multiplied by the number of units of nuclease/ml reaction mixture. The reaction was terminated by addition of EDTA to a final concentration of 3 mM and cooling in an ice bath prior to performing the nuclear binding assay.

As a control, to test for endogenous nuclease activity in each experiment, nuclei were incubated for 0, 5, 10 or 15 min at 37°C after which, EDTA was added, as above, prior to the nuclear binding assay. [^{3}H]ER binding to these nuclei was then assayed and no significant amount of endogeneous nuclease activity was observed. For instance, in a typical experiment, [^{3}H]ER binding/10^6 nuclei (in cpm) was, at 0 time = 9871, after incubation at 37°C for 5 min = 10,046, after 10 min = 9735 and after 15 min = 10,109.

Purification of DNA from nuclei

DNA was purified from control or nuclease-digested R3230AC tumor nuclei from intact or ovex rats by treatment with proteinase K, RNase T_1, RNase A and phenol/chloroform extraction [15, 23]. The DNA fragments were separated and analyzed by 0.8% agarose gel electrophoresis [23]. DNA in the agarose gel was visualized by ultraviolet illumination with ethidium bromide dye and photographed using Polaroid type 57 film (3000 ASA).

EcoRI digestion of DNA

Purified DNA, from control or nuclease-digested R3230AC tumor nuclei, was incubated with 3 units of EcoRI (New England Biolabs, Beverly, Mass)/g DNA under the assay conditions described in the New England BioLabs catalog. Agarose gel electrophoresis was carried out as described above.

RESULTS

Effect of DNase I digestion on [^{3}H]ER binding to nuclei

The concentrations of DNase I required to reduce the nuclear binding of 2 nM [^{3}H]ER by 50% of that observed in the absence of enzyme treatment were determined and compared for R3230AC mammary tumor nuclei from intact and ovex hosts. Results shown in Fig. 1 are representative of the decrease in [^{3}H]ER binding capacity of tumor nuclei after DNase I digestion. A significantly higher concentration of DNase I was required to eliminate 50% of the nuclear [^{3}H]ER binding sites in R3230AC tumors from ovex hosts vs intact hosts (Table 1). In fact, [^{3}H]ER binding sites in tumors from ovex hosts were more resistant to DNase I digestion over the entire range of DNase I levels examined.

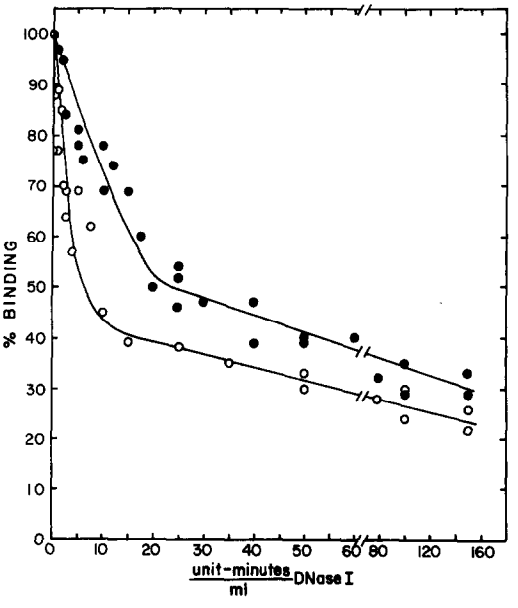

Fig. 1. R3230AC mammary tumor nuclei digested by DNase I show loss of [^{3}H]ER binding. Nuclei (10^7) were digested with varying concentrations of DNase I for 5, 10 or 15 min at 37°C. The reaction was terminated by addition of ice-cold EDTA to a final concentration of 3 mM and cooling in an ice water bath. [^{3}H]ER binding was determined using 2 nM [^{3}H]ER and R3230AC tumor nuclei (10^6) from intact (open circles) or ovex rats (closed circles) in TDP buffer containing 0.1 M KCl for 2 h at 4°C. The rinsed nuclear pellet was processed as described in Methods. Each point is the mean of triplicate determinations form which background counts have been substracted. This plot has been repeated four additional times (not shown) with essentially identical results. Points are plotted as percent of control binding vs amounts of DNase I × min at 37°C/ml digest. 100% = 15,400 cpm = 130 fmol for intact and 100% = 9486 cpm = 80 fmol for ovex.

[^{3}H]ER binding sites in nuclei from selected non-neoplastic rat tissues, including lactating mammary gland, showed decreased nuclease sensitivity compared to that observed in R3230AC tumors (Table 1). In contrast to tissue-specific differences in the number of [^{3}H]ER binding sites/nucleus, there were no significant differences in the concentration of DNase I that eliminated 50% of [^{3}H]ER binding sites for nuclei from liver, kidney, and lung. Interestingly, nuclei from lactating mammary gland were found to lose 50% of the [^{3}H]ER binding sites at lower concentrations of DNase I (14 ± 1 unit·min/ml) than did nuclei from other rat tissues (21–56 unit·min/ml).

Effect of DNase I digestion on the size of DNA fragments isolated from tumor nuclei in intact vs ovex hosts

The next experiment was designed to determined whether the greater sensitivity of [^{3}H]ER binding sites to DNase I digestion in tumor nuclei from intact hosts is unique to these sites, or a phenomenon occurring throughout the chromosomal DNA. Tumor nuclei from either intact or ovex rats were digested with DNase I over a wide range of concentrations. The DNA was isolated and fractionated by

Table 1. Estrogen receptor binding to isolated nuclei following DNase I digestion. Nuclei were isolated from fresh rat tissues as described in the text

Tissue	n[a]	DNase I concentration to reduce [^{3}H]ER binding to 50% of control[b]
R3230AC tumor from:		
Intact host	8	8.6 ± 1.6
Ovex host	4	22.3 ± 1.7*
Ovex host + E_2[c,d]	3	27 ± 1.2*†
Ovex host + Pg[e]	3	18.3 ± 0.3*†
Other rat tissues:		
Liver		
Female F344	4	56 ± 17
Ovex F344	4	31 ± 1
Male F344	3	29 ± 6
Kidney		
Female F344	4	33 ± 15
Lung		
Female F334	2	51 ± 9
Mammary gland[f]	2	14 ± 1

Nuclei were incubated with 0–150 unit·min/ml DNase I prior to performing the nuclear binding assay using 2 nM calf uterine estrogen-charged estrogen receptor ([^{3}H]ER). Incubation was for 2 h at 4°C.

[a] n = number of samples, each represents tissue from one rat with the exception of kidney which was pooled from 2–4 rats.

[b] DNase I concentration = unit·min/ml; values are mean ± SEM.

[c] The indicated hormone was administered s.c. in sesame seed oil. Each ovex rat received 5 injections at the indicated dose over 2 weeks with the last dose administered 24 h prior to sacrifice.

[d] Estrogen dose = 0.1 mg.

[e] Progesterone dose = 2.0 mg.

[f] Mammary glands were from lactating Sprague–Dawley rats on day 15 after parturition.

*Significantly different ($P < 0.005$) from the value of the DNase I concentration to reduce [^{3}H]ER binding to 50% of control in R3230AC tumor from intact hosts.

†Significantly different ($P < 0.05$) from the value of the DNase I concentration to reduce [^{3}H]ER binding to 50% of control in R3230AC tumor from ovex hosts.

agarose gel electrophoresis. After digestion of nuclei using as little as 5 unit·min/ml of DNase I (Fig. 2), a spectrum of DNA fragment sizes from greater than 23 kB to approximately 500 bases in length was observed (lanes 2 and 10). More extensive DNase I digestion resulted in a progressive decrease in the length of DNA fragments (lanes 3 and 11, etc.). There was some minor variation in the intensity of DNA staining in the lanes. However, in every case, densitometric profiles of the photograph in Fig. 2 (data not shown) confirm that the size distribution obtained with nuclei from intact hosts was essentially identical to that obtained with nuclei from ovex hosts.

An additional experiment provided data to support the foregoing finding that the size of DNA fragments generated by identical DNase I digestions was equivalent for R3230AC tumor nuclei from intact vs ovex hosts. Nuclei initially digested with 0–100 units of DNase I were then incubated with 10 units of EcoRI restriction endonuclease, followed by ethanol precipitation of the DNA and subsequent agarose gel electrophoresis (data not shown). There were no differences in the size of DNA fragments generated by EcoRI digestion of these nuclease treated tumor nuclei from intact vs ovex rats. The EcoRI digestions of nuclease-digested rat tumor DNA revealed two prominent bands of approximately 2.3 and 1.8 kB, indicative of repetitive rat DNA [24]. Bands of this same size were seen in the tumor DNA from either intact or ovex rats, and their intensities faded equally with increasing DNase I digestion.

DNase I effects on the affinity of [^{3}H]ER binding to nuclei

We also examined the effects of nuclease digestion on [^{3}H]ER binding to the remaining sites, using saturation binding analysis. For these experiments, nuclei were predigested with 0, 1.0, 3.0, 5.0, 7.5, 15.0, 30.0, or 60.0 unit·min/ml DNase I before incubation with 0–5 nM [^{3}H]ER. A representative experiment showing the pattern of [^{3}H]ER binding is illustrated in Fig. 3. A progressive loss of [^{3}H]ER binding capacity was observed, but this loss was not attributable to an alteration in binding affinity (Table 2). Furthermore, administration of various doses (0.01–1.0 mg) of estradiol valerate to ovex rats did not alter the binding affinity of [^{3}H]ER for nuceli (data not shown).

We did observe a trend toward a slight increase in [^{3}H]ER binding affinity after digestion of R3230AC tumor nuclei with 30 unit·min/ml DNase I. An exception to this generalization was observed with mammary gland nuclei. This slight decrease in the K_d values may not have physiological significance. However, it is also possible that some proportion of higher affinity ER binding sites are exposed after nuclease cleavage of lower affinity binding sites. We plan further studies to address the role of chromatin

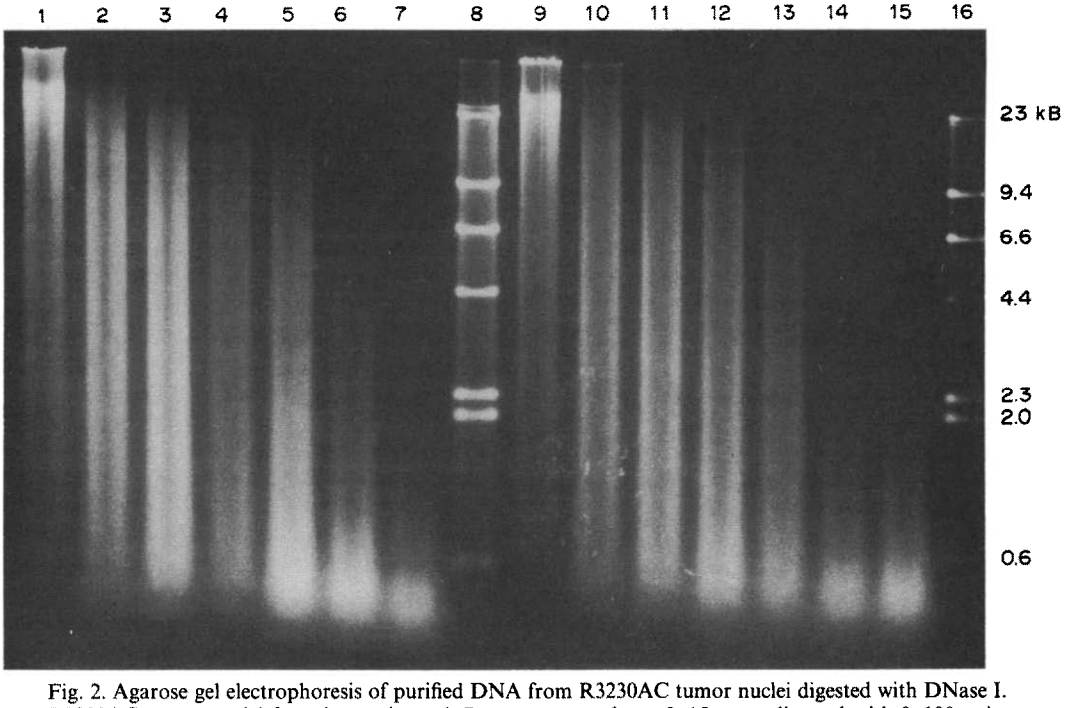

Fig. 2. Agarose gel electrophoresis of purified DNA from R3230AC tumor nuclei digested with DNase I. R3230AC tumor nuclei from intact, lanes 1–7, or ovex rats, lanes 9–15, were digested with 0–100 units of DNase I for 15 min at 37°C prior to purification of DNA and separation on a 0.8% agarose gel. The concentration of DNase I (in unit·min/ml) for each lane is as follows: lanes 1 and 9 = 0; lanes 2 and 10 = 5; lanes 3 and 11 = 10; lanes 4 and 12 = 15; lanes 5 and 13 = 30; lanes 6 and 14 = 50; lanes 7 and 15 = 100. The DNA molecular weight standard, lanes 8 and 16 is lambda phage DNA digested with Hind III. The gel was stained with ethidium bromide for 30 min prior to u.v. illumination and photography.

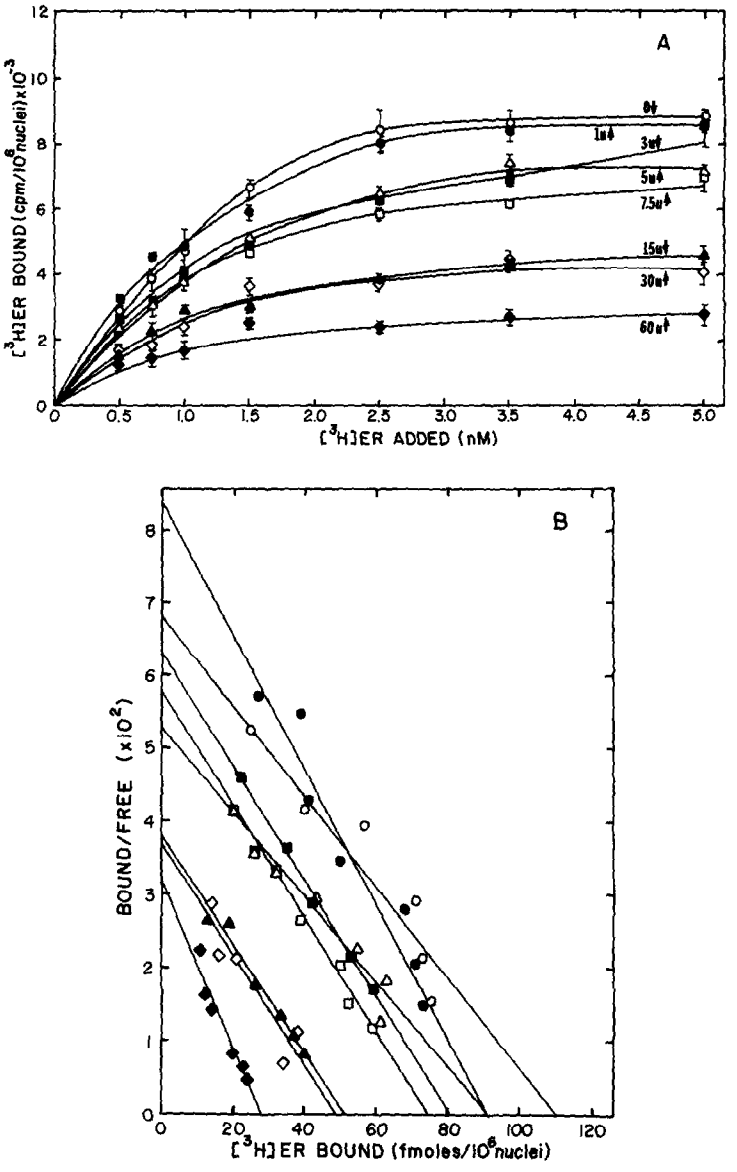

Fig. 3. Saturation analysis of [^{3}H]ER interaction with rat mammary tumor nuclei after nuclease digestion. (A) R3230AC tumor nuclei, for an ovex rat treated with 5 doses of 0.01 mg E_2 over 2 weeks with the last dose administered 24 h prior to sacrifice were digested with 0, 1, 3, 5, 7.5, 15, 30 or 60 units of DNase I, as indicated by arrows, at 37°C. The reaction was terminated by addition of ice-cold EDTA to a final concentration of 3 mM, followed by cooling in an ice water bath. The control or nuclease-digested nuclei (10^6) were incubated with 0–5 nM [^{3}H]ER in TDP containing 0.1 ,M KCl for 2 h at 4°C. Binding of [^{3}H]ER was determined (see Methods). Each point is the mean ± SEM of triplicate determinations from which background counts have been substracted. (B) Saturation analysis plotted according to the method of Scatchard [25]. The symbols correspond to those defined in A. The lines were calculated by linear regression analysis. These results are representative of the data presented in Table 2.

proteins in maintaining hormonally-regulated ER binding sites in this tumor system.

DISCUSSION

A heterologous nuclear binding assay was used to determine whether DNase I predigestion of nuclei isolated from lactating rat mammary gland or R3230AC mammary tumor would reveal differences in the ability of [^{3}H]ER to bind to nuclear sites that may be important in estrogen-dependent gene expression. Results presented here indicate that the accessibility of ER binding sites to digestion by DNase I, and presumably other proteins, is regulated by endogenous hormones. The altered accessibility may be part of a mechanism by which gene expression is regulated.

Table 2. Binding affinity of [^{3}H]ER to isolated nuclei following DNase I digestion

DNase I (unit·min/ml)	R3230AC tumor from Intact rats $n^b = 7$ K_d (nM)	Ovex rats $n = 3$ K_d (nM)	Lactating mammary gland[a] $n = 2$ K_d (nM)
0	1.9 ± 0.3	2.3 ± 0.7	2.1 ± 0.4
1.0	1.3 ± 0.2	2.2 ± 0.9	2.0 ± 0.5
3.0	1.6 ± 0.3	1.8 ± 0.5	2.0 ± 0.3
5.0	1.7 ± 0.4	1.9 ± 0.5	2.1 ± 0.3
7.5	1.6 ± 0.2	2.5 ± 0.6	2.1 ± 0.4
15.0	1.6 ± 0.3	2.3 ± 0.7	1.3 ± 0.2
30.0	1.1 ± 0.1*	1.4 ± 0.3	2.0 ± 0.9
60.0	1.1 ± 0.1*	1.5 ± 0.1	2.1 ± 0.7

Nuclei were isolated from fresh R3230AC tumors or normal lactating mammary gland as described in the text. Nuclei were preincubated with the indicated concentration of DNase I prior to saturation binding analysis using 0–5 nM partially purified calf uterine [^{3}H]estradiol-charged estrogen receptor ([^{3}H]ER). Incubation for the nuclear binding assay was for 2 h at 4°C. Saturation binding data were analyzed according to the method of Scatchard [25].

[a]Mammary glands were from lactating rats on days 13–15 after parturition. [b]n = number of samples, each represents tissue from one rat.

*Significantly different ($P < 0.05$) from the K_d value of [^{3}H]ER binding to nuclei from R3230AC tumor of intact rats without (0 unit·min/ml) DNase I digestion.

Examination of the chromatin of actively transcribed genes after partial DNase I digestion suggested the existence of gaps in the nucleosome structure on the chromatin fiber at unique sites that change in location with transcriptional activity [10]. Most of these sites have been mapped to the 5′ flanking regions of genes, although some are located near the 3′ termini and other sites are considerably removed from the nearest coding sequence [7–10, 26]. DNase I hypersensitivity has been correlated with other modifications in DNA, such as under-methylation [27–30]. Steroid hormone receptors were shown to bind to regions of chromatin that are digested preferentially by low concentrations DNase I [15, 20] and transcriptionally active genes regulated by steroid hormones have hormone-induced hypersensitive sites (reviewed in [10]).

The use of hormone-inducible cloned genes has facilitated characterization of the interaction of steroid hormone receptors with hormone regulatory elements (HREs). HREs are DNA sequences, generally conserved during evolution, that are located in proximity to promoter elements that act as orientation-independent but hormone-dependent enhancers of gene expression [3–6, 31]. The relationship between HREs and chromatin structure has been studied in the glucocorticoid-responsive system (reviewed in [31,32]) and the progesterone-responsive ovalbumin gene ([33], reviewed in [34]). Less information correlating DNase I sensitivity with receptor binding is available for estrogen-responsive systems. Burch and Weintraub characterized three types of hypersensitive sites in the estrogen-induced chicken vitellogenin gene [26].

The most significant finding reported here is that considerably more DNase I was required to decrease nuclear [^{3}H]ER binding by 50% in tumor nuclei from ovex vs intact hosts. Our results cannot distinguish whether the observed nuclease sensitivity represents an effect by endogeneous hormones solely at the specific ER binding sites (EREs), or whether other regions of hormonally regulated genes are affected as well. This result, however, was not attributable to an overall change in nuclease sensitivity of the chromatin, i.e. the size of chromosomal DNA segments remaining in nuclei from ovex vs intact hosts. As expected, DNA was reduced to its smallest size after digestion with the highest level of DNase I. However, at every level of DNase I digestion, the size profile of DNA, whether isolated from tumors of ovex or intact rats, was found to be the same. This demonstrates that the accessibility of a high percentage of the chromosomal DNA was not changed by the presence of endogenous ovarian hormones. Clearly, the structure of other non-hormonally regulated segments of chromatin may be affected by change in endogeneous hormone levels, although these regions must represent a small proportion of the chromosomal DNA or else they would have been observed in the agarose electrophoresis experiments. Therefore, ER interaction sites are part of some subset of chromosomal DNA, at which nuclease accessibility may be regulated by the hormonal status of the animal.

It is not yet clear how endogenous hormone levels change the number or accessibility of ER binding sites. Sequence analysis of 5′-regions of several estrogen regulated genes revealed repeated ER binding sites [35–37]. It is possible that if several nearby sites in one gene are bound by ER, the binding of ER to one site may facilitate ER binding at an adjacent site and induce changes in chromatin that enhance nuclease sensitivity at other sites.

We find a higher number of [^{3}H]ER binding sites/tumor nucleus, 94,500 and 70,400 from R3230AC from intact and ovex rats, respectively, [17] compared to reports on a variety of normal animal tissue. The higher values may derive from two differences: (1) R3230AC tumor is polyploid and (2) we use 0.1 M KCl for all our saturation binding experiments. This salt concentration was chosen because it is the lowest that allows us to detect saturation of [^{3}H]ER binding to isolated nuclei *in vitro* [16, 17]. Although sequenced estrogen responsive genes have multiple ER binding sites, these still may not account for the number of sites we observe. If so, not all of the sites we measure are necessarily involved in regulation of estrogen responsive transcripition. However, there is essentially no information on the possible role of nuclear binding sites for ER other than those associated with promoter induction.

Treatment of ovex tumor-bearing rats with pharmacologic doses of estrogen or progesterone modestly altered the amount of DNase I required to reduce [^{3}H]ER binding by 50%, but neither treatment returned nuclease sensitivity to that seen in nuclei from intact hosts. These results, along with those of

our earlier studies [16, 17] emphasize the complexity of discerning the contribution of individual hormones in the regulation of [^{3}H]ER binding sites that may be specific for estrogen regulated gene transcription vs those regions of chromatin that appear to bind [^{3}H]ER with identical affinity but whose role in nuclear functions in unknown.

Nuclease sensitivity of ER binding sites differed between R3230AC tumor and normal rat tissues. ER binding sites in normal tissues appear to be less sensitive to DNase I digestion than those in tumors from intact hosts. In general, nuclease sensitivity was more comparable between tumors from ovex or hormone-treated, ovex rats and many of the normal tissues examined. ER binding sites in lung appeared to be somewhat more resistant to DNase I digestion than other normal tissues examined. In contrast, ER binding sites in lactating mammary gland were the most sensitive to nuclease digestion among the non-neoplastic tissues, and were more comparable to that of sites in tumors from intact rats than to any of the other rat tissues sampled. No differences were detected in the nuclease sensitivity of [^{3}H]ER binding sites in liver from intact ovex, or male rats. The large standard error in DNase I concentration required to eliminate 50% of binding sites in liver may be due to differences in estrous cycle between intact rats that were not examined here.

If the nuclease sensitivity of ER binding sites reflects transcriptional activity, our findings may indicate that there is less estrogen-regulated transcription in lung than in other rat tissues, whereas, the greater nuclease sensitivity of ER binding sites in actively replicating R3230AC tumor from rats and lactating mammary gland (which, at day 15, is during the peak of hormone-responsive transcriptional activity, [38–40]) may reflect the importance of estrogenic regulation of these processes. As measured here, however, nuclease sensitivity *per se* does not allow us to distinguish between specific estrogen-regulated genes and other transcriptionally active regions in the genome.

Our results are in agreement with a report by Schoenberg and Clark that only a portion of endogenous ER was associated with rat uterine chromatin and that ER associated with chromatin regions that were preferentially sensitive to DNase I digestion [40]. Moreover, their report also indicated that the actual percentage of ER released by DNase I digestion was dependent on the prior hormonal treatment of the rat. For example, DNase I treatment (50 U/ml) of uterine nuclei from estrogen-treated ovex rats, one hour after estrogen treatment, resulted in a release of 30% of ER and 1–2% of the DNA, within 5 min.

Although the mechanism remains to be defined, our results demonstrate that the hormonal milieu is responsible, at least in part, for the number and nuclease assessibility of ER binding sites in this hormone responsive tumor.

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