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HIGH-PERFORMANCE CHROMATOFOCUSING OF STEROID RECEPTOR PROTEINS IN THE PRESENCE AND ABSENCE OF STEROID

INVESTIGATION OF STEROID-DEPENDENT ALTERATIONS IN SURFACE CHARGE HETEROGENEITY

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SUMMARY

We have previously reported the development of high-performance chromatofocusing (HPCF) systems for rapid evaluation of the surface charge heterogeneity of steroid receptor proteins, each in the presence of its specific steroid ligand. However, the surface charge properties of ligand-free receptor proteins remain largely unknown. We have now employed HPCF to rapidly evaluate the surface charge properties of cytosolic estrogen receptor proteins in both the presence and absence of the ligand ([^{3}H]estradiol-17β). All operations were performed at 0–4°C. Cytosols prepared from immature calf uteri were preparatively analyzed by HPCF on a SynChropak AX-500 column (25 cm × 4.6 mm I.D.) either before or after incubation with 5–10 n*M* [^{3}H]estradiol-17β. Elution of receptor was by generation of internal pH gradients (pH 8.1 to 3.2) using Pharmacia Polybuffers 96 and 74. Postcolumn detection of previously unliganded receptor was accomplished by incubation of pH-neutralized (pH 7.4) fractions with 5 n*M* [^{3}H]estradiol-17β in the presence and absence of unlabelled competitor. Specifically bound steroid was determined in each fraction using an hydroxylapatite adsorption assay. Significant surface charge heterogeneity was observed for both unliganded receptor and the steroid–receptor complex. The heterodisperse pattern of receptor surface charge appeared to vary in a steroid-dependent manner. Preformed steroid–receptor complexes eluted primarily between pH 6.5–7 and between pH 5–6, with indications for heterogeneity within both regions. The surface charge distribution of unliganded receptor routinely revealed additional, more acidic eluting (pH 3.8–4.6) receptor forms. Sodium molybdate, a commonly used receptor-stabilizing agent, maintains receptors during HPCF as relatively acidic eluting forms (pH 3.8–5.0). The specific elution profile of molybdate-stabilized receptor also appears steroid-dependent. These data demonstrate that HPCF can be used preparatively to rapidly isolate unliganded receptor forms in a biologically active state.

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INTRODUCTION

The native structure of intracellular steroid receptor proteins remains unknown despite considerable efforts to elucidate it. These proteins vary considerably in their observed physicochemical properties. It has been argued that this heterogeneity is due primarily to their sensitivity to enzymatic processing and/or degradation[1–3]. However, by physical manipulation *in vitro*, steroid–receptor proteins can be induced to undergo significant, but discrete, alterations in size and in surface charge[3–14]. It is notable that certain of these changes in size are both steroid-dependent[8] and reversible[4–7,10].

We have suggested that *in vitro* transformation of steroid receptor proteins from their native (untransformed) state to their nuclear (or DNA) binding form, may occur in a reversible manner through nonenzymatic processes[5–8]. Transformation is generally thought to be a steroid-dependent event and appears to result in both a decreased molecular weight and increase in basic surface charge relative to the stabilized, untransformed estrogen-receptor complex[5–8,11,12,15,16]. It is important that investigations into the origin and significance of receptor heterogeneity attempt to distinguish the steroid-dependent transformation events from nonspecific degradation events.

We have previously reported the development of high-performance chromatofocusing (HPCF) systems for rapid evaluation of the surface charge heterogeneity of steroid receptor proteins[17,18]. Earlier analyses of untransformed or native steroid–receptor complexes were impeded by the proclivity of chromatographic techniques to cause receptor transformation[8,16,19]. Therefore, our recent studies by HPCF were conducted in the presence and absence of a receptor-stabilizing agent (sodium molybdate) thought to maintain the steroid–receptor complex in a native (at least untransformed) state[17]. With few exceptions[8], most investigations into the physicochemical nature of steroid receptor proteins have focused on the steroid–receptor complex. Physical descriptions of untransformed steroid–receptor complexes have largely been dependent upon the use of sodium molybdate. Any physical relationships between the molybdate-stabilized, untransformed steroid–receptor complex and the unliganded (by definition untransformed) receptor form are unknown. Both receptor forms are similar to some degree in their resistance to transformation *in vitro*. However, since the physical properties, including surface charge characteristics, of the unliganded (steroid-free) receptor form have not been reported the structural basis of this similarity is unclear.

The unliganded form of receptor, because of its innate resistance to transformation, may define the untransformed, native receptor configuration. We therefore feel it is important to characterize and describe its physical properties. In this preliminary report, estrogen receptors from immature calf uteri have been isolated preparatively by HPCF in both the liganded and unliganded forms. We have observed what appear to be both steroid-independent and steroid-dependent variations in surface charge. An acidic-eluting (pH 3.8–4.6) form(s) of receptor may be common to preparations of both molybdate-stabilized, steroid–receptor complexes and unliganded receptor preparations.

EXPERIMENTAL

Materials

Uteri from small, immature calves were obtained from local slaughterhouses. The uterine horns (7–15 g) were rinsed immediately after removal in ice-cold saline, cut into 1-g pieces, frozen in liquid nitrogen (or dry ice) and stored frozen at −85°C. SynChropak AX-500 high-performance anion-exchange columns (250 mm × 4.1 mm I.D.) used for chromatofocusing were kindly provided by SynChrom. Altex TSK-3000SW high-performance size-exclusion columns (600 mm × 7.6 mm I.D.) were purchased from Beckman. [^{3}H]Estradiol-17β (90–100 Ci/mmole) was purchased from New England Nuclear. Trizma base, diethylstilbestrol (DES), dithiothreitol (DTT) and glycerol were from Sigma. Disodium ethylenediaminetetraacetic acid (Na_2-EDTA) was from Fisher Scientific. Prepacked Sephadex G-25 (PD-10) columns (6 cm × 1.5 cm I.D.), Polybuffer 96 and Polybuffer 74 were obtained from Pharmacia.

Preparation of cytosol and labelling of estrogen binding proteins

All procedures were performed in a cold room at 0–6°C. Pieces of frozen uteri were sliced into small pieces before being homogenized in 2 volumes of 10 m*M* Tris–HCl buffer (pH 7.4–7.8 at 0°C) containing 1 m*M* DTT and 20% glycerol (by volume). Cytosol (10–15 mg protein/ml) was obtained by high speed centrifugation (100 000 *g*; 60 min) of the homogenate and labelled at 0°C with 5–10 n*M* [^{3}H]estradiol-17β in the presence (nonspecific binding) or absence (total binding) of a 100-fold molar excess of the radioinert competitor DES. Where noted, immediately before chromatofocusing or high-performance size-exclusion chromatography, radiolabelled steroid–protein complexes were quickly (< 5 min) separated from excess free steroid by rapid chromatography on small columns (9 ml) of G-25 Sephadex (Pharmacia PD-10 columns).

High-performance chromatofocusing

The development of HPCF for analysis of steroid–receptor complexes as well as details of this procedure were described earlier by Hutchens and co-workers [17,18]. High-performance AX-500 anion-exchange columns (250 mm × 4.1 mm I.D.) were equilibrated with 25 m*M* Tris–HCl buffer containing 1 m*M* DTT and 20% glycerol adjusted to pH 8.0 at 0°C. All chromatography was carried out in a cold room at 0–6°C. Samples of 250 μl to 3000 μl were loaded onto the columns and 1.0-ml fractions were collected at a flow-rate of 1.0 ml/min. Elution was with internally generated pH gradients developed using Pharmacia Polybuffers 96 and 74 (30:70) diluted 1:15 with 20% glycerol (pH 3–4 at 0°C). The pH values of alternate fractions were determined immediately after chromatography at 0°C using a Corning Model 125 pH meter equipped with a micro combination Calomel electrode. Radioactivity was determined (at 26–37% efficiency) by scintillation counting using a Beckman Model LS 250 scintillation counter.

High-performance size-exclusion chromatography

High-performance size-exclusion chromatography (HPSEC) was performed at 0–6°C using a Beckman Model 110A HPLC pump and an Altex TSK-3000SW column (600 mm × 7.6 mm I.D.) equipped with in-line frit filters and a 10-cm guard

column. Cytosolic estrogen binding proteins which were prelabelled with [^{3}H]estradiol-17β were cleared of excess free [^{3}H]estradiol-17β by rapid chromatography on small (9 ml) Sephadex G-25 columns (PD-10). Unliganded estrogen receptor preparations were treated similarly. Aliquots (500 μl) were injected with Rheodyne 7125-S injectors and 1.0-min fractions were collected at 0.380 ml/min. The column (TSK-3000SW) was equilibrated with 50 m*M* potassium phosphate buffer (pH 7.4 at 0°C) containing 1.5 m*M* Na_2-EDTA, 1 m*M* DTT and 10% glycerol (v/v) and calibrated with six purified proteins of known Stokes radii ranging from 18Å to 86Å. Column void volumes (V_0) were determined by exclusion of Blue-Dextran 2000 or thyroglobulin. The retention time of small molecules eluting in the inclusion volume was determined to be 73 min using β-mercaptoethanol and [^{3}H]estradiol-17β. The column was frequently cleaned with 1 *M* potassium chloride and stored in methanol when not in use. Column calibration was checked periodically and plots of log Stokes radii *versus* retention time (or elution volume) were linear with correlation coefficients (*r* values) typically better than 0.95.

Postcolumn detection (postlabelling) of estrogen receptor proteins: hydroxylapatite batch adsorption assay

All portions of this assay were conducted at 0°C. Fractions (1 ml) of estrogen receptor preparations (cytosol) analyzed by HPCF in the unliganded (unlabelled) form were collected into 1 ml of buffer containing 0.1 *M* HEPES (pH 7.6), 2 m*M* DTT, 20% glycerol (by volume) and 5–10 n*M* [^{3}H]estradiol-17β. To determine nonspecific binding, alternate fractions additionally contained a 100-fold molar excess of the estrogen-receptor-specific competitor, DES. The collected fractions (now 2 ml) were incubated for 2–24 h at 0°C before 500 μl of a 50% slurry of hydroxylapatite (Bio-Rad) were added to each fraction. The fractions were incubated with hydroxylapatite for 20–30 min. A volume of 2 ml of ice-cold distilled water was added before an additional 20–30 min incubation with hydroxylapatite. The hydroxylapatite was pelleted by centrifugation and resuspended in 4 ml of wash buffer containing 10 m*M* Tris–HCl (pH 7.4 at 0°C), 1 m*M* DTT, 20% glycerol (by volume) and 1% Tween-80. This wash procedure was performed four times (16 ml wash volume) or until the supernate contained negligible radioactivity. To extract bound radioactivity, the final pellet of hydroxylapatite was resuspended into 1 ml of ethanol and incubated for 30–45 min (25°C) with mixing every 5–10 min. The hydroxylapatite was pelleted by centrifugation and the ethanol supernate was decanted into scintillation vials. Atomlight scintillation cocktail (New England Nuclear) was added (3 ml) for liquid scintillation spectrophotometry. A Beckman Model LS-250 liquid scintillation system was used with a counting efficiency that averaged 25–26% for postlabelled HPCF–HPSEC fractions (due to ethanol) compared to 35–37% for prelabelled fractions. Profiles shown are uncorrected for quench.

RESULTS AND DISCUSSION

In the absence of stimulation by steroid hormone, the specific receptor proteins exist within their target cells in the unliganded state. Very little is certain about the precise subcellular location, structure or possible function of the unliganded receptor. Substantial evidence has accumulated to suggest that introduction of steroid hor-

mone in some way elicits the subcellular relocalization of receptor and induces, presumably through structural alterations, the receptor to mediate the biological effects associated with the hormonal stimulation. *In vitro*, the steroid-dependent process by which native receptor undergoes transformation and acquires an increased affinity for nuclei (or DNA) has been the subject of intense investigation and considerable speculation[2,16]. Sodium molybdate has been found to reversibly stabilize the steroid-receptor complex in a large[2,3], relatively acidic[16] untransformed configuration[10–12,16,17]. This has been considered, by some, a significant advance towards understanding receptor structure–function relationships[2,3,16,17,19]. Others have argued that molybdate is a nonphysiological agent which only creates more *in vitro* artifact[20]. We feel it significant that unliganded receptor, generally considered incapable of transformation, and molybdate-stabilized steroid–receptor complex share a certain resistance against *in vitro* perturbations known to transform the unstabilized steroid–receptor complex. Our investigations of glucocorticoid receptor structure suggest that the unliganded form of receptor is similar to the molybdate-stabilized glucocorticoid-receptor complex in size and surface charge properties[8].

One purpose of this study was to determine the surface charge properties of unliganded estrogen receptor in the presence and absence of sodium molybdate. A second purpose was to develop a rapid purification procedure to preparatively isolate unliganded receptor for subsequent purification and characterization. The estrogen receptor from immature calf uterus was chosen as a model system for these studies because calf uteri are readily available in large quantities, the estrogen–receptor complex is well characterized and at least the 60 000–70 000 daltons steroid binding por-

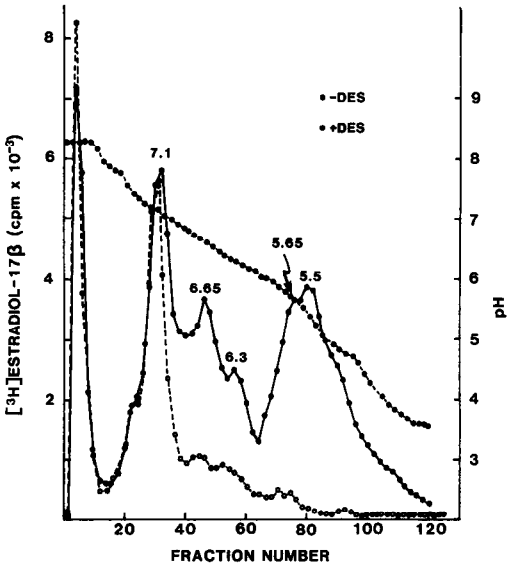

Fig. 1. HPCF of prelabelled estrogen receptors prepared from calf uterus. Cytosol was prelabelled with 10 n*M* [³H]estradiol-17*β* in the presence (nonspecific binding; O—O) and absence (total binding; ●—●) of a 100-fold molar excess of radioinert competitor, DES. Protein-bound and free [³H]estradiol-17*β* were separated just prior to HPCF using minicolumns (9 ml) of Sephadex G-25. Details of HPCF are provided in Experimental. The numbers shown above the elution profiles indicate the elution pH values.

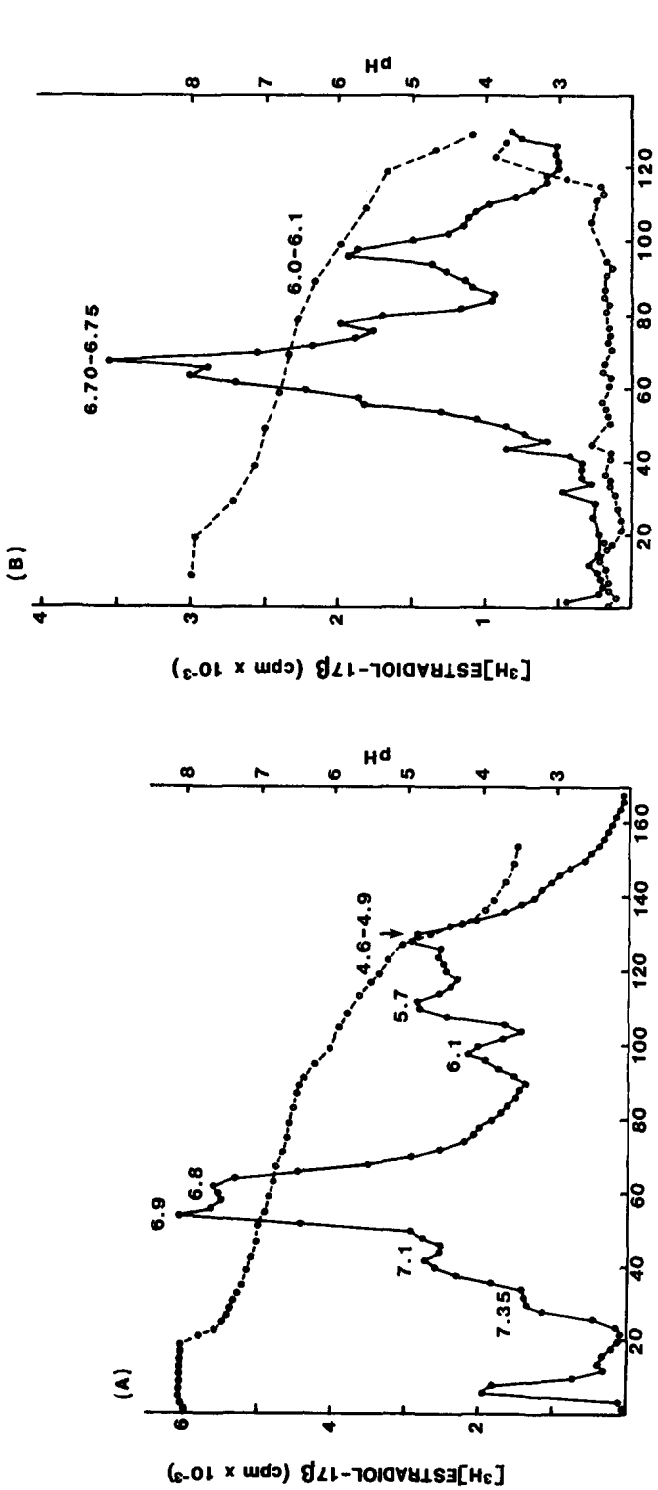

Fig. 2. HPCF of calf uterine estrogen receptors in the presence (A) and absence (B) of steroid. Cytosol was prepared from a single calf uterus. One portion of the cytosol was incubated with 10 n*M* [^{3}H]estradiol-17β for 2 h at 0°C. Protein-bound and free [^{3}H]estradiol-17β were separated immediately prior to HPCF using minicolumns (9 ml) of Sephadex G-25. A volume of 1 ml of the prelabelled estrogen–receptor complex was analyzed by HPCF and resulted in the profile shown in (A). The remaining portion of the cytosol was incubated in parallel at 0°C with no added steroid and passed through a Sephadex G-25 column just prior to analysis by HPCF. A volume of 1 ml of the unliganded receptor preparation was fractionated by HPCF. Postlabelling of the collected fractions with 5 n*M* [^{3}H]estradiol-17β (with or without DES) using the hydroxylapatite batch assay (see Experimental) resulted in the profile shown in (B). Both total (●—●) and nonspecific (○—○) binding are shown for profile (B). Details of HPCF are provided in Experimental. The numbers shown above the elution profiles indicate the elution pH values.

tion or subunit has been purified to apparent homogeneity. Additionally, it is a source of estrogen receptor relatively free of endogenous steroid and therefore available in the unliganded form.

Calf uterine estrogen receptor proteins were isolated preparatively by HPCF in both the liganded and unliganded forms. We have observed what appear to be both steroid-independent and steroid-dependent structural alterations in the presence and absence of molybdate.

The surface charge heterogeneity of calf uterine estrogen receptors can be extensive and varied. As shown by the HPCF profiles in Figs. 1 and 2A, preparations of cytosolic estrogen receptor prelabelled with [^{3}H]estradiol-17β reveal significant surface charge heterogeneity in the absence of molybdate. Collectively, elution pH values for the major peaks of specific estrogen binding activity range from approximately pH 7 through pH 5.5. It can be seen that not all of the collectively observed receptor forms are present during analysis of cytosol prepared from a given uterus. However, the same preparation of cytosol used to generate the estrogen receptor profiles illustrated in Fig. 2 was also analyzed in parallel by chromatofocusing on Polybuffer Exchanger (PBE) 94 (Pharmacia). This set of experiments was performed twice using the same calf uterus. The profiles (not shown) were all similar in that the major peak of receptor eluted between pH 6.8–7.0 (n = 4). By dextran-charcoal analysis of specific [^{3}H]estradiol-17β binding activity in these cytosols, the quantity of nonspecific binding (determined in the presence of excess DES) was estimated to be 10–15% of total bound [^{3}H]estradiol-17β. When these cytosol preparations were analyzed by chromatofocusing for nonspecific estrogen binding activity, the low levels present eluted primarily in the flow-through fractions before initiation of the pH gradients. Similar characterizations of estrogen receptor surface charge heterogeneity were performed with cytosol preparations from each of four additional calf uteri. Chromatofocusing results were obtained using PBE 94. Parallel HPCF results were generated using two different types of Bakerbond polyethyleneimine (PEI) high-performance anion-exchange columns. These efforts consistently demonstrate that the major peak of calf uterine estrogen receptor elutes, as shown in Fig. 2A, at pH 6.8–7.0 (n = 12). Previous studies have shown that the principal form of uterine estrogen receptor from other animal species elutes during HPCF in this region. The estrogen receptors from normal (unpublished) and neoplastic [21] rabbit endometrium elute at pH 6.4 and 6.8. Human (both pre- and postmenopausal) uterine estrogen receptors elute in a heterogeneous manner but primarily at pH 6.6–6.7 (refs. 17 and 18). Cytosolic estrogen receptors from ovarian-dependent MTW9-D rat mammary tumors are reported to elute consistently (90% of tumors examined) at pH values of 7.5, 6.8 and 6.0 (ref. 22).

Fig. 2A illustrates an additional property of estrogen receptors from immature calf uteri. Even in the absence of molybdate, HPCF analyses of calf uterine estrogen–receptor complexes frequently (approximately 60%) reveal the presence of a receptor form(s) eluting at acidic pH values of 5 or below. The presence of specific estrogen binding activity eluting at or below pH 5 is not apparent in cytosol preparations from any of the several estrogen target organs we have analyzed, unless sodium molybdate is included[17,18,21]. Future studies will address the structural properties and possible relationship(s) of these acidic eluting receptor forms with the other more basic eluting receptor forms.

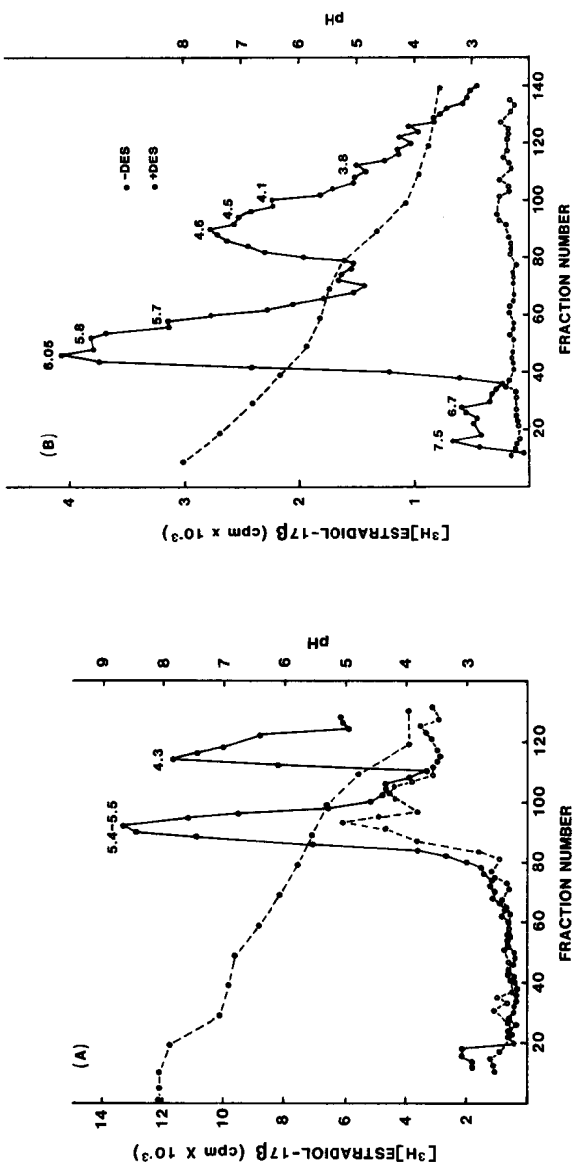

Fig. 3. HPCF of unliganded calf uterine estrogen receptors. Cytosol was prepared from two separate calf uteri. The estrogen receptor proteins were analyzed directly by HPCF in the unlabelled form. Postcolumn detection (postlabelling) of estrogen receptor proteins was as described in Experimental. Both total (●—●) and nonspecific (○—○) estrogen binding is shown. For the experiment shown in (A), postcolumn detection of estrogen receptor included a hydroxylapatite batch-adsorption of postlabelled receptor in which no Tween-80 (1%) was used. Note the indication of elevated nonspecific binding relative to that shown in (B) where 1% Tween-80 was added during the hydroxylapatite batch-adsorption assay. The numbers shown above the elution profiles indicate the elution pH values.

HPLC analyses of unliganded (unlabelled) estrogen receptor also demonstrate surface charge heterogeneity. Fig. 2B shows the HPCF elution profile of unliganded receptor from the same cytosol preparation used to generate the control profile indicated in Fig. 2A. Postseparation analysis of eluted fractions for specific estrogen binding activity reveals an elution profile for unliganded receptor which is distinct from that of the estrogen–receptor complex (Fig. 2A). This would suggest that receptor surface charge distribution varies in a steroid-dependent manner. The major peak of unliganded receptor elutes at pH 6.70–6.75 with a significant peak of receptor eluting at pH 6.0–6.1. Fig. 3 further demonstrates the surface charge heterogeneity of unliganded receptor. Fig. 3A and B represent preparations of unliganded receptor from two different calf uteri. Unliganded receptor was observed to elute in a heterodisperse manner at pH values primarily equal to or less than pH 5.8–6.05, 5.4–5.5, 4.6 and 4.3. We feel it important to note that in comparison to results presented for the estrogen-receptor complex (Figs. 1 and 2A), there is little or no evidence for unliganded receptor eluting at or above pH 6.8–7.0. In general, but not in absolute terms, the surface charge distribution of estrogen–receptor complexes seems relatively more basic (*i.e.* pH 6.8–7.0) than that observed for the unliganded receptor (pH $\leqslant$6.7). Fig. 3 also makes an important point about the technical details of these experiments. The inclusion of a detergent (1% Tween-80) during the hydroxylapatite adsorption assay effectively eliminates background (nonspecific) radioactivity (Fig. 3B) otherwise observed in the area of estrogen receptor elution (Fig. 3A). Large amounts of nonspecific radioactivity in the acidic region of the pH gradient hindered earlier efforts to monitor specific receptor eluting in this region.

Fig. 4A is yet another example of the surface charge distribution and heterogeneity of unliganded (unlabelled) estrogen receptor. Thje profile presented here is partially similar to that offered in Fig. 2B in that a major form of unliganded receptor elutes at pH 6.7. However, a peak of nearly equal magnitude eluting at pH 5.2 and two small peaks eluting at pH 3.8 and 4.1 clearly distinguish this profile from any of those ever obtained for the estrogen–receptor complex. The origin of unliganded receptor heterogeneity is unclear but may be related to the occasional presence of low levels of endogenous estrogens or estrogen agonists. Another possibility for this variation is proteolytic degradation of receptor. However, when the same receptor preparations were analyzed in parallel by HPSEC whether in the liganded (Fig. 4B) or unliganded forms (Fig. 4C), no indication of size heterogeneity was observed. There was a conspicuous absence of smaller (degraded) receptor forms. The HPSEC results shown here are typical of the majority of calf uterine estrogen receptors analyzed, in the presence or absence of steroid[23]. One interesting possibility that we can not exclude at this time is the differential stability of individual receptor forms in the unliganded *versus* liganded state. Upon postlabelling, the yield of receptor varies from approximately 30–60%. The HPCF column recovery of prelabelled estrogen–receptor complexes is significantly greater (80–100%) with less variation. We have no reason to suspect that bound steroid improves the elution efficiency of receptor from the HPCF columns. We do suspect that the apparently low recoveries may reflect the inefficiencies of our postlabelling technique, particularly the hydroxylapatite adsorption assay. We believe that the difference in elution profile of liganded and unliganded receptor does not reflect a generalized pH effect on all receptor species (*i.e.* pH 6.7 to pH 3.8) since we have demonstrated specific interactions of receptor

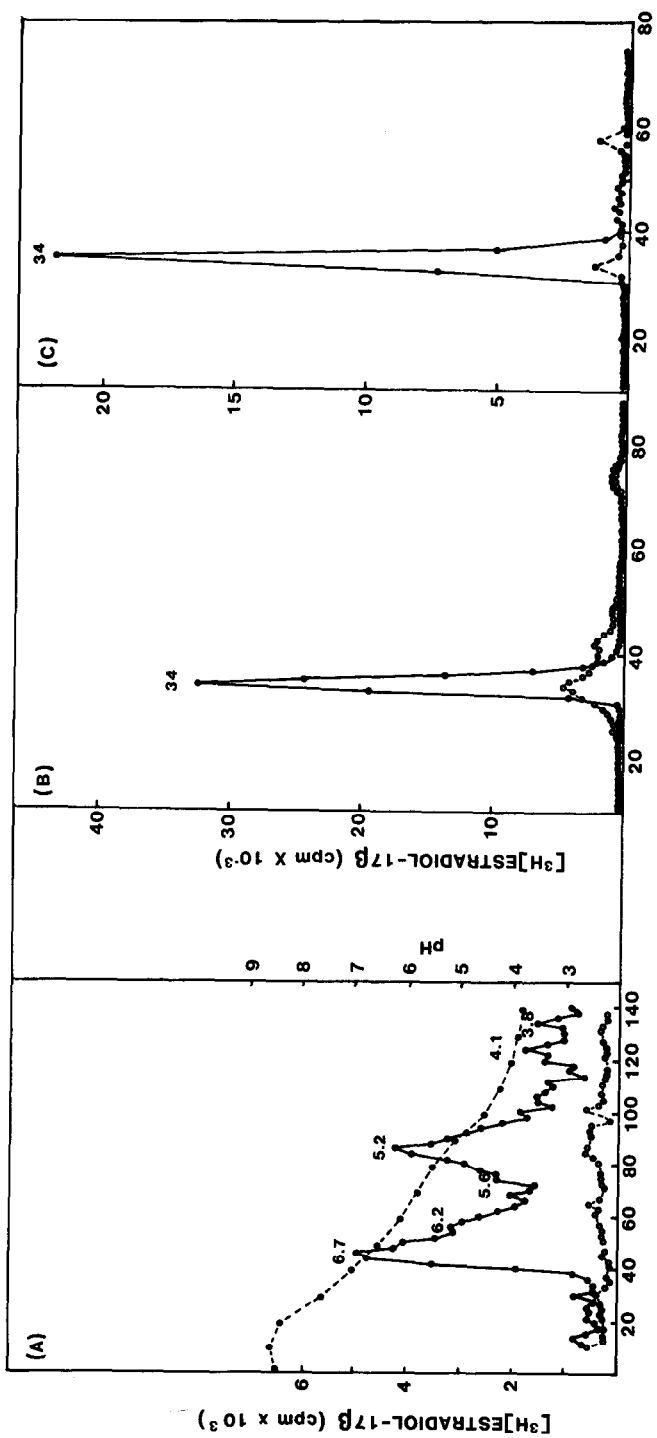

Fig. 4. HPCF and HPSEC of unliganded calf uterine estrogen receptor. A single cytosol preparation (from portions of one calf uterus) was analyzed directly by HPCF (A) and HPSEC (C) for estrogen receptor in the (unliganded) unlabelled form. For comparison, a portion of this cytosol was prelabelled with 10 n*M* [^{3}H]estradiol-17β in the presence (nonspecific binding; ○—○) and absence (total binding; ●—●) of a 100-fold molar excess of radioinert competitor (DES) before analysis by HPSEC (B). Total and nonspecific binding of [^{3}H]estradiol-17β are also indicated for the postlabelling analyses (A and C). Details of HPCF and HPSEC as well as the postcolumn detection (postlabelling) of estrogen receptor proteins are provided in Experimental. The numbers shown above the elution profiles indicate the elution pH values.

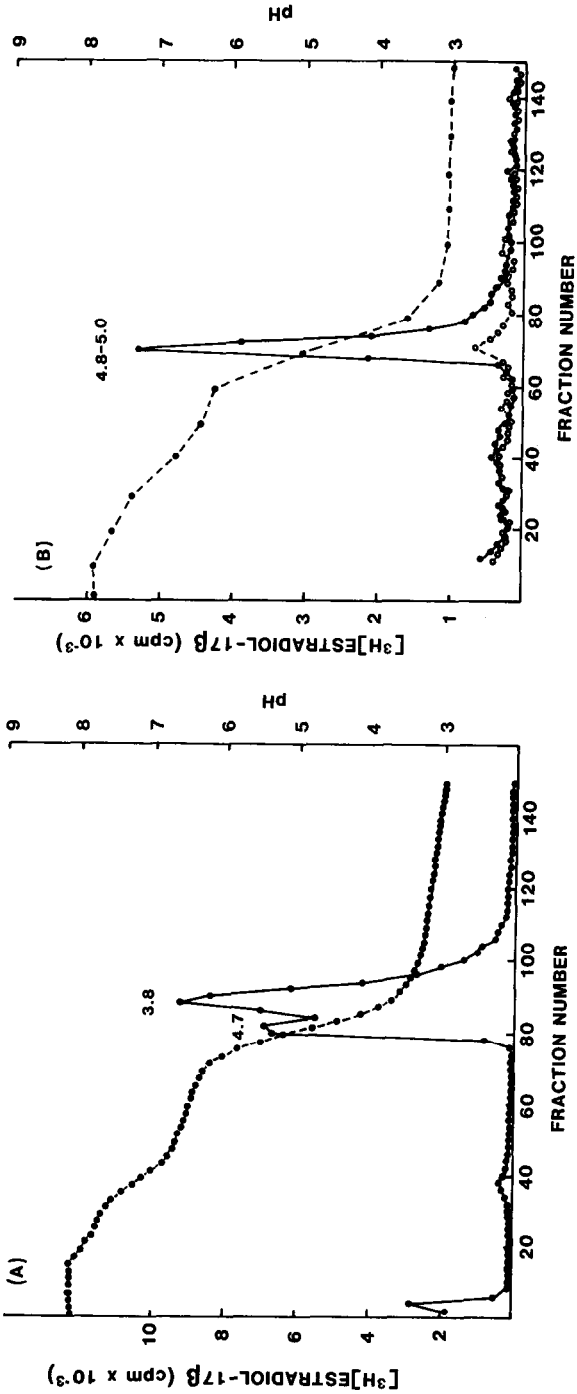

Fig. 5. HPCF of molybdate-stabilized estrogen receptor in the presence (prelabelled) and absence (postlabelled) of steroid ligand. Cytosol was prepared in buffers containing 10 m*M* sodium molybdate and analyzed by HPCF (with molybdate) either with (A) or without (B) prelabelling of receptor with 10 n*M* [^{3}H]estradiol-17β. Nonspecific binding (○—○) as shown in (B) is absent or negligible relative to total binding (●—●) when molybdate-stabilized estrogen receptor preparations are analyzed by HPCF. Details of HPCF and postcolumn detection (postlabelling) techniques are provided in Experimental. The numbers shown above the elution profiles indicate the elution pH values.

with steroid after elution at pH 6.7 and at pH 3.8. We are currently attempting to augment receptor–steroid binding capacity to maximize our recoveries. Our ability to rapidly and preparatively isolate the various liganded and unliganded receptor forms should ultimately allow the question of differential receptor stabilities to be addressed directly.

The HPCF elution profile of molybdate-stabilized estrogen receptor preparations further suggests possible steroid-dependent alterations in surface charge heterogeneity. As illustrated in Fig. 5, when analyzed by HPCF in the liganded (prelabelled) form, two acidic species of molybdate-stabilized receptor are observed (Fig. 5A). This has been a consistent observation with calf uterine estrogen receptor complexes ($n = 6$) and estrogen receptors from several other sources[17,21]. These observations made initially by HPCF[17] have now been confirmed for calf uterine estrogen receptors by another group of investigators using DEAE-Sephadex in an ion-exchange mode[13,14]. In contrast to results obtained for the molybdate-stabilized estrogen–receptor complex, only a single acidic receptor form is seen when the same receptor preparations are analyzed in the unliganded (unlabelled) form (Fig. 5B). The investigators using DEAE-Sephadex[14] were unable to demonstrate any steroid-dependence of the molybdate effects. The reported inability of these investigators to elute their receptor preparations from DEAE-Sephadex in the absence of molybdate precluded a more complete analysis of steroid effects on receptor elution behavior from this resin. Using HPCF to analyze receptor both in the presence or absence of ligand, we have observed that sodium molybdate consistently has a dramatic influence on the absolute surface charge as well as the relative charge distribution of estrogen receptors.

CONCLUSIONS

Preparations of calf uterine estrogen receptor prelabelled with [^{3}H]estradiol-17β show extensive surface charge heterogeneity. HPCF analyses of unliganded (unlabelled) estrogen receptor also reveal surface charge heterogeneity. However, the surface charge distribution of unliganded receptor appears to be altered (generally more acidic). Receptors in the unliganded form elute consistently at or below pH 6.7. In contrast, the surface charge profile of estrogen–receptor complexes is more heterodisperse with the major peak of receptor typically eluting at approximately pH 6.8–7.0. Significant quantities of prelabelled receptor, but not unliganded receptor, elute as more basic proteins with elution pH values of 7.1 to 7.35. The surface charge heterogeneity of both liganded and unliganded estrogen receptors shows tissue dependent variations. The origin(s) of these variations are currently unknown.

While HPCF suggests that the surface charge heterogeneity of calf uterine estrogen receptors is extensive, parallel analyses by HPSEC reveal little or no size heterogeneity[23]. This would argue against the surface charge heterogeneity of calf uterine estrogen receptor being due to proteolytic activity.

The molybdate-treated estrogen receptor shows molybdate-induced stabilization of acidic surface charge. Interestingly, this effect also appears to be steroid-dependent.

Our physicochemical analyses of the estrogen–receptor complex from immature calf uteri have suggested that this receptor is possibly more stable in its relatively

large, acidic, untransformed state than the estrogen receptor in other target tissues we have examined. The acidic-eluting (pH 3.8–4.8) form(s) of receptor occasionally observed in relatively small amounts when liganded receptors are analyzed are more common to preparations of both unliganded receptor and molybdate-stabilized, steroid–receptor complexes.

The preparation of different molecular forms of biologically active, unliganded estrogen receptor by HPCF should help further our detailed investigations into the molecular mechanism(s) by which steroid hormones exert their receptor-mediated effects on target cells.

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