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SEPARATION OF TWO MOLECULAR FORMS OF HUMAN ESTROGEN RECEPTOR BY HYDROPHOBIC INTERACTION CHROMATOGRAPHY

GRADIENT OPTIMIZATION AND TISSUE COMPARISON

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SUMMARY

High-performance hydrophobic interaction chromatography (HPHIC) was used to separate and characterize two molecular forms of estrogen receptor with a SynChropak propyl hydrophobic column (300 Å pore size). The linear gradient utilized earlier with a polyether-bonded column (2 to 0 *M*) ammonium sulfate in 40 min, gave poor resolution with the propyl column. However, resolution was maximized with either an initial ammonium sulfate concentration of 1 *M* (40-min gradient) or with a two-phase gradient (2 to 0.5 *M* in 10 min, 0.5 to 0 *M* in 30 min). This indicated that the propyl column was more hydrophobic than the polyether column. Estrogen receptor separated into two isoforms, either in the presence [MI, retention time (t_R) = 13–14 min; MII, t_R = 20–21 min] or absence (I, t_R = 21–23 min; II, t_R = 31–33 min) of the estrogen receptor stabilizing reagent, sodium molybdate. Similar isoforms were observed in cytosols from human breast tumors, uterus, and MCF-7 breast cancer cells. Unlike others, MCF-7 estrogen receptor did not show MI. Since MCF-7 cells contain 90 000 dalton heat shock proteins (HSP-90), HSP-90 is probably not directly involved in MI formation. Sodium molybdate selectively interacted with isoform II and converted it to MI. All isoforms appeared to be high-molecular-weight proteins (> 60 Å) when subsequently analyzed by high-performance size-exclusion chromatography. Interestingly, when estrogen receptor was immobilized on the stationary phase, no change was detected in either hydrophobicity or steroid-binding capacity. After 16–18 h, immobilized receptor was eluted with a slightly longer t_R. During incubation on the column, component MI was converted into I and/or II. HPHIC appears to be a rapid, yet gentle procedure for isolating large receptor complexes in significant quantities with high recoveries. This allows one to discern the complicated structure–function relationships of estrogen receptor and associated non-receptor proteins and provides information about the on-column behavior of complex proteins.

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INTRODUCTION

Previous analyses of estrogen receptors from either human breast tumors or rat uterus by high-performance hydrophobic interaction chromatography (HPHIC) revealed the presence of two hydrophobic species of the ligand-binding form of the protein[1–8]. The two hydrophobic forms of receptors were detected either in the absence or presence of the receptor-stabilizing reagent, sodium molybdate[3,7]. Furthermore, one of the two isoforms of estrogen receptor detected contained an associated protein kinase activity[2] when immunoprecipitated with monoclonal Antibody D547, which was raised against the estrogen receptor molecule[9]. A thorough analysis of the detailed kinase reaction revealed that this is not an intrinsic property of receptor[3], although our earlier results suggested this[2,10,11]. In support of the latter observation, some estrogen receptor negative human breast tumors demonstrated the kinase reaction while other estrogen receptor positive tumors did not[3]. The consistent appearance of similar isoforms from several different samples of human breast tumors indicated that the presence of two molecular forms is not an artifact, nor are they dependent upon protein kinase activity.

Our analysis of estrogen receptor from a SynChropak propyl column revealed that both were hydrophobic isoforms of high molecular weight (> 65 Å), as judged by their elution in high-performance size-exclusion chromatography (HPSEC)[7,12,13]. Since the proteolyzed form of estrogen receptor did not aggregate, we concluded that the high-molecular-weight isoforms eluted from the hydrophobic column must contain the 60 000-dalton estrogen receptor molecule complexed with other cellular molecules, such as the heat-shock proteins and protein kinase(s)[2–4,7,13]. Similar evidence of this effect has been cited recently for androgen receptors[14].

In this paper we present data related to (a) optimization of the ammonium sulfate gradient conditions on a propyl-based hydrophobic column for the separation and resolution of estrogen receptor, based on their hydrophobicity; (b) the effect of sodium molybdate on selective interaction with the most hydrophobic form of estrogen receptor, converting it to the least hydrophobic form, (c) the effect of time on the HPHIC profile of the receptor, (d) the uniformity of hydrophobic elution patterns of estrogen receptor among different tissues, and (e) the usefulness of the hydrophobic column for storing receptors at 4°C in the immobilized form.

EXPERIMENTAL

Materials

Ammonium sulfate, (HPLC-grade) was obtained from Bio-Rad Labs. (Richmond, CA, U.S.A.). The ligand, [16α-^{125}I]iodoestradiol-17β (IE_2) (*ca.* 2200 Ci/mmol) was obtained from DuPont/New England Nuclear Products (Boston, MA, U.S.A.). Sodium molybdate, disodium ethylenediaminetetraacetic acid (EDTA) and glycerol were purchased from Fisher Scientific (Louisville, KY, U.S.A.). Unlabeled diethylstilbestrol (DES), which was used as an estrogen inhibitor, Norit A, Dextran T-70 and dithiothreitol (DTT) were obtained from Sigma (St. Louis, MO, U.S.A.).

Human breast tumor tissues from patients were provided by the various surgeons and pathologists at the local hospitals, cooperating with the Hormone Receptor Laboratory. The tissues were brought to the laboratory on dry ice and kept frozen

at −86°C until analyzed. Only residual tissue from clinical receptor analyses was used in this study.

Residual tissue from human uteri was obtained following hysterectomy for a variety of clinical conditions through the cooperation of the Pathology Departments at Norton Kosair Children's Hospital and at Humana Hospital University (Louisville, KY, U.S.A.). These tissues were weighed and immediately placed on ice and transported to the laboratory. Samples were taken for histopathology, and the remainder was stored at −86°C until utilized. A pilot study conducted in our clinical laboratory determined the status of estrogen receptor and progesterone receptor in these tissues by the titration assay[15,16].

Cell culture

MCF-7 cells were grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal calf serum (FCS). Cells were cultured in a humidified atmosphere of 5% carbon dioxide in air at 37°C. Cultures were again fed the same medium at intervals of 2–4 days; cells were subcultured at weekly intervals. Cells were grown to a density of approximately 60–70% confluency and kept with DMEM-containing 1 mg/ml bovine serum albumin (BSA) for 24 h before the start of an experiment. Confluent cells were harvested with the addition of trypsin EDTA solution (Sigma) and collected after centrifugation. The cells were washed once with P_{10}EDG buffer [10 m*M* potassium phosphate (pH 7.4), containing 1.5 m*M* EDTA, 1 m*M* DTT and 10% (v/v) glycerol] and collected. The cells were resuspended in P_{10}EDG and sonicated for 2 × 10 s at a setting of 60 in a Fisher Sonicator Model 300. The supernatant from high-speed centrifugation (105 000 *g*) was then prepared as described below.

Preparation and labeling of soluble estrogen receptor

All subsequent procedures were performed at 4°C in a Puffer–Hubbard cold box (Ashville, NC, U.S.A.). Human breast tumors and uterine tissue (*ca.* 200–400 mg/ml) were homogenized in P_{10}EDG. Homogenization was performed with two 10-s bursts in a Brinkman Polytron homogenizer (Westbury, NY, U.S.A.).

Soluble fractions were prepared by centrifugation of the homogenate for 30 min at 40 000 rpm in a Beckman Ti 70.1 rotor (Palo Alto, CA, U.S.A.). The supernatant was removed carefully, avoiding the layer of fat at the top. The soluble fractions were labeled with 2–3 n*M* IE_2 in the presence and absence of a 200-fold excess of DES for 2–4 h at 4°C. Free steroid was removed with dextran-coated charcoal after centrifuging the sample for 5 min at 1000 *g*. Cytosol protein concentrations were determined by the method of Bradford[17], using BSA as the standard. The protein concentrations generally ranged from 4 to 8 mg/ml.

HPHIC

All buffers were filtered under vacuum through Millipore (Bedford, MA, U.S.A.) 0.45-μm HAWP filters before use. Free steroid or estrogen receptor complexes were applied to the 10 cm × 4.6 mm I.D. silica-based SynChropak propyl column, obtained from SynChrom (Lafayette, IN, U.S.A.), using an Altex Model 210 sample injection valve (Beckman Instruments, San Ramon, CA, U.S.A.). Elution was carried out with a Beckman Model 114 delivery module, including a Model 421 system controller.

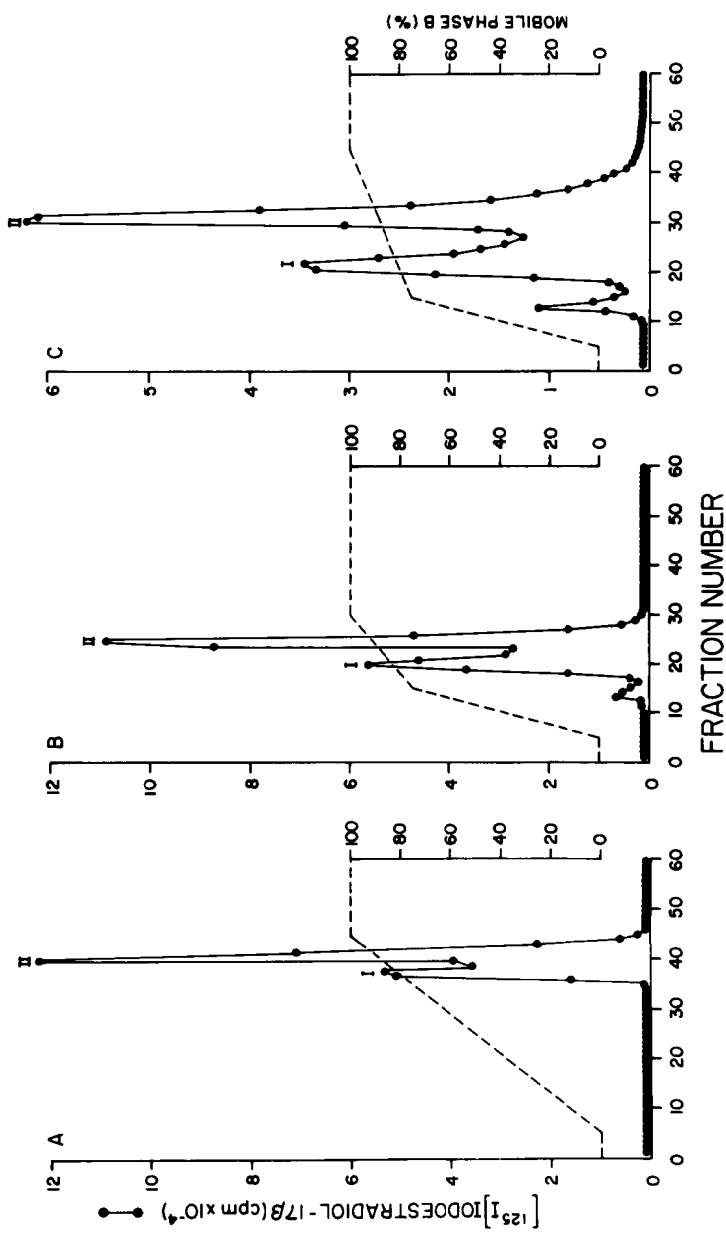

Fig. 1. Influence of gradient development time on HPHIC separation of estrogen receptor isoforms from human breast cancer. Dextran-coated charcoal-treated human breast tumor cytosol was injected into the SynChropak propyl column and chromatographed with gradient times reaching (A) 100% eluent B in 40 min, (B) 75% B in 10 min followed by 100% B in next 15 min and (C) 75% B in 10 min, followed by 100% B in 30 min. All samples were adjusted to 1.5 *M* ammonium sulfate prior to injection. Eluent A in this experiment was P_{10}EDG, containing 2 *M* ammonium sulfate; eluent B was P_{10}EDG. For clarity, only total cpm/fraction (●) are shown.

Unless otherwise stated, the gradient program for elution consisted of an starting eluent A (P_{10}EDG containing 2 *M* ammonium sulfate, pH 7.4) at a flow-rate of 1 ml/min and, following sample injection, a linear, descending salt gradient reaching 75% P_{10} EDG (eluent B) in 10 min, followed by 100% B in the next 30 min. Eluent B was maintained for the next 15 min before switching to 100% eluent A for reequilibration of the column.

In the initial phase of this study, other experimental gradients were developed. The conditions for these separations are listed in the individual figure legends.

Following chromatography, 1-ml fractions were collected, and free and protein-bound steroids were detected radiometrically in a Micromedics 4/600 gamma radioisotope detector (Rohm & Haas, Cleveland, OH, U.S.A.). The counting efficiency was 65%. Since the non-specific binding (radioactive steroid bound to cytosols labeled in the presence of DES) showed primarily base-levels, representing no more than 5–10% of the total binding, these are not shown in the figures. Recovery of specifically bound radioactive steroid was 80–100%.

HPSEC

Analytical size-exclusion columns (Spherogel TSK-3000 SW), particle size 10μm (600 × 7.5 mm I.D.) from Beckman Altex Instruments, were used for steroid receptor separation, as described previously[15,18]. HPLC was performed at 4°C with a Beckman 114 solvent delivery module, including a Model 421 system controller and injector block. Cytosol were applied in 100–200 μl volumes with a Hamilton syringe. The elution buffer (pH 7.4) at 4°C, was $PEDGK_{100}$ [10 m*M* potassium phosphate buffer (pH 7.4), 1.5 m*M* EDTA, 1 m*M* DTT, 10% (v/v) glycerol, 100 m*M* potassium chloride]. All buffers were filtered through a 0.45-μm filter (Millipore). Elution was carried out at a flow-rate of 0.7 ml/min. Fractions were collected at 0.5-min intervals in 12 × 75 mm tubes. Recoveries were in the range of 75–100%.

RESULTS AND DISCUSSION

The usefulness of HPHIC in the separation and analysis of proteins without denaturation has been discussed earlier[4,6,19] and we have applied this technique to the detailed characterization of estrogen receptor previously[1–8,11–13]. These analyses were performed on both a polyether-linked silica-based stationary phase, which is non-ionic in nature, and on a propyl column with a mobile phase containing organic solvent. To extend these studies and rule out the possibility of column-induced effects, a mobile phase without organic solvent was designed to be employed with a three-carbon chain (proprietary chemistry) hydrophobic column (SynChrom propyl).

Gradient optimization

When HPHIC was performed on the SynChrom propyl column under the same conditions as those used with the polyether-bonded phase, 2 to 0 *M* ammonium sulfate in 40 min, estrogen receptor was eluted with a longer retention time (t_R = 38 min for peak I and t_R = 41 min peak II) from the propyl column (Fig. 1A) than (t_R = 26 min for peak I and t_R = 34 min for peak II) from the polyether-based column[3]. Although the resolution on the propyl was not optimal under these conditions, the results were in agreement with previous experiments, where two receptor isoforms

were observed. This confirms that the propyl column is more hydrophobic in nature than the polyether-based column, yet estrogen receptor was not denatured.

To enhance resolution of the estrogen receptor isoforms, the gradient conditions were modified to reach 75% B in 10 min and, in a second phase, to reach 100% B in 15 min (Fig. 1B). This increased the relative resolution slightly. Also, a minor third peak (t_R = 13 min) was observed, which had been ignored initially. However, the majority of molybdate-stabilized receptor, which we have termed MI was eluted at this retention time (see Discussion below). A better resolution of receptor isoforms was observed when the second phase of the gradient was extended to reach 100% eluent B in 30 min (Fig. 1C).

Since the propyl column was more hydrophobic, the effect of starting the gradient at a lower ionic strength was analyzed on the basis of the retention of the estrogen receptor molecule. Fig. 2A demonstrates that when the initial ionic strength of ammonium sulfate was 1 *M*, the separation of estrogen receptor was comparable to that with 2 *M* ammonium sulfate as the starting buffer (Fig. 1C). The sample concentration was also adjusted to 1 *M* ammonium sulfate prior to injection. However, when the initial concentration of eluent A was lowered to 0.5 *M* ammonium sulfate, a considerable proportion of receptor protein was not bound to the bonded phase. This occurred regardless of whether the sample concentration was adjusted to 0.5 *M* ammonium sulfate (Fig. 2B) or to 1.0 *M* ammonium sulfate (Fig. 2C) prior to sample application. Therefore, it is imperative that a critical ammonium sulfate concentration be reached both in eluent A and in the receptor preparation to insure immobilization of the proteins on the bonded phase. This probably reflects the removal of water molecules associated with the receptor protein, which is known to be ionic[11,20] or water molecules from associated proteins.

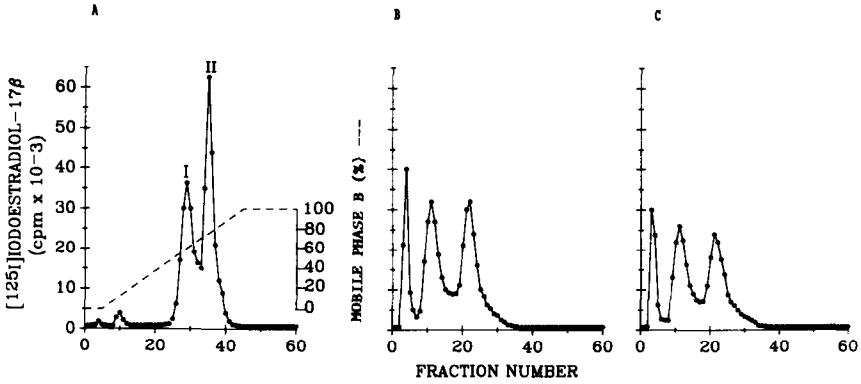

Fig. 2. Influence of the initial ionic strength on the separation of estrogen receptor isoforms in human breast tumor cytosol. Human breast tumor cytosol was injected into the SynChropak propyl column and chromatographed with a 1 to 0 *M* ammonium sulfate gradient in 40 min with prior adjustment of cytosol to 1 *M* ammonium sulfate (A). In (B) the same cytosol was chromatographed with a 0.5 to 0 *M* ammonium sulfate gradient with adjustment of the cytosol to 0.5 *M* ammonium sulfate. In (C) the conditions were the same as in (B), except that the cytosol was adjusted to 1.0 *M* ammonium sulfate prior to injection. ● = total cpm/fraction.

Effect of sodium molybdate on the hydrophobic properties of estrogen receptor

A previous study[3] indicated that once estrogen receptor was bound to the polyether-bonded hydrophobic column, only one of the two isoforms (isoform II) was displaced by molybdate-containing buffers and converted to MI. This behavior of isoform II was also observed for the propyl column (Fig. 3). It indicates that only one of the two receptor isoforms contains a molybdate-sensitive contact site, which may be near the DNA-binding domain. The sequence of estrogen receptor indicates that a small hydrophobic region exists in this domain[21]. It should be stressed that MI has been observed even in the absence of molybdate (*e.g.*, Fig. 3), suggesting that the receptor first exists in this least hydrophobic form on the column and then either dissociates into II or assumes another conformation (expressed as II), which is more hydrophobic.

Influence of time on HPHIC and HPSEC of estrogen receptor isoforms

In agreement with our observations with the polyether column[2], the hydrophobic properties of estrogen receptor from human breast cancer changed when this receptor was incubated overnight in the presence of IE_2 at 4°C (Fig. 4A and B). When analyzed after a short incubation with the steroid, the receptor was eluted as isoforms I and II. The longer incubation (18–24 h) at 4°C, converted isoform II into isoform I. Since the receptor may have been proteolyzed with time, resulting in the elution of receptors as the single peak I, receptor size was monitored by HPSEC. Trypsinized or proteolyzed receptor was eluted as a single, sharp peak at 25–30 Å which retained the steroid-binding domain[5]. The receptor is known to dissociate from other complex macromolecules, such as HSP-90[20,22] with time, to produce a non-proteolyzed form,

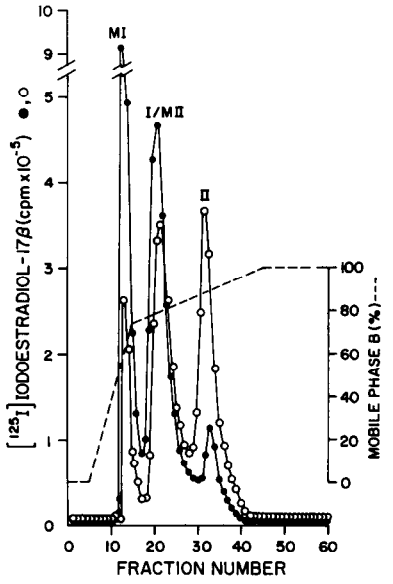

Fig. 3. Comparison of estrogen receptor isoforms separated by HPHIC in the absence and presence of 10 m*M* sodium molybdate. Breast cancer cytosol was prepared in buffer lacking molybdate. Samples were injected into the propyl column and eluted with buffers lacking (○) or containing (●) 10 m*M* sodium molybdate.

which is also eluted in the 25–30 Å range in HPSEC and is difficult to separate from the proteolyzed form[20]. This process can be accelerated with potassium chloride in buffers. Although some of the 65-Å receptor complex was transformed to the 30-Å species during a 24-h incubation (Fig. 4C and D), this conversion cannot be responsible for the extensive change in receptor hydrophobicity (Fig. 4A and B). This suggests that receptor size and hydrophobicity are unrelated.

Tissue distribution of estrogen receptor isoforms

Estrogen receptors from both human uterus and human breast cancer cells in culture were analyzed for their hydrophobic properties. Estrogen receptor separated into two isoforms, whether obtained from uterus (Fig. 5A) or from breast cancer cells (Fig. 5B). The retention times of the isoforms were the same as those of receptors separated from human breast tumor cytosols. This indicates that estrogen receptor in different tissues undergoes similar post-translational modifications and associates with similar proteins[20]. A protein known to interact with estrogen receptor is HSP-90, which has been detected in many different tissues[23]. Using monoclonal antibody raised against HSP-90 from chick oviduct, HSP-90 was detected only in the MI isoform[23].

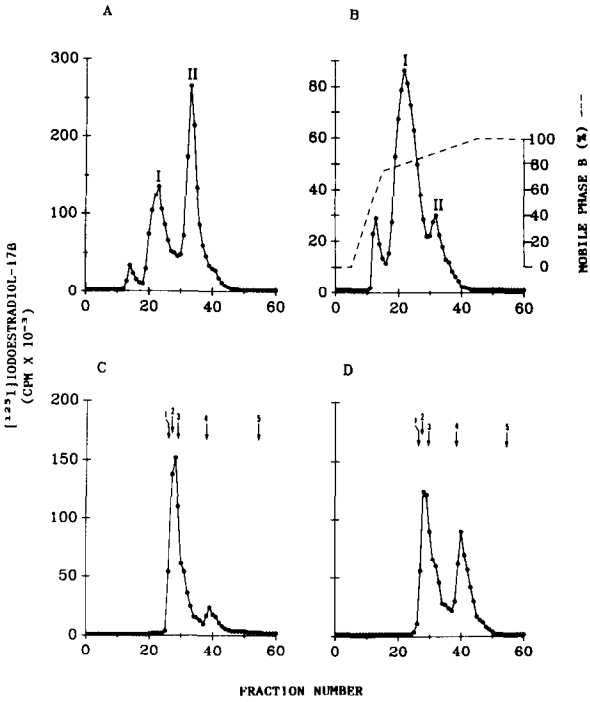

Fig. 4. Influence of time on HPHIC and HPSEC profiles of estrogen receptor isoforms. Human breast cancer cytosol was prepared as described in the Experimental section and incubated with IE_2. Following a 3-h incubation at 4°C, samples were injected simultaneously into (A) the propyl column and (C) the TSK-3000 SW size-exclusion column. A second sample was injected following a 24-h incubation of the cytosol into (B) the propyl and (D) the size-exclusion column. For details of methods see Experimental. The void volume is represented by (1). Markers used were ferritin (2), catalase (3), hemoglobin (4) and cytochrome *c* (5).

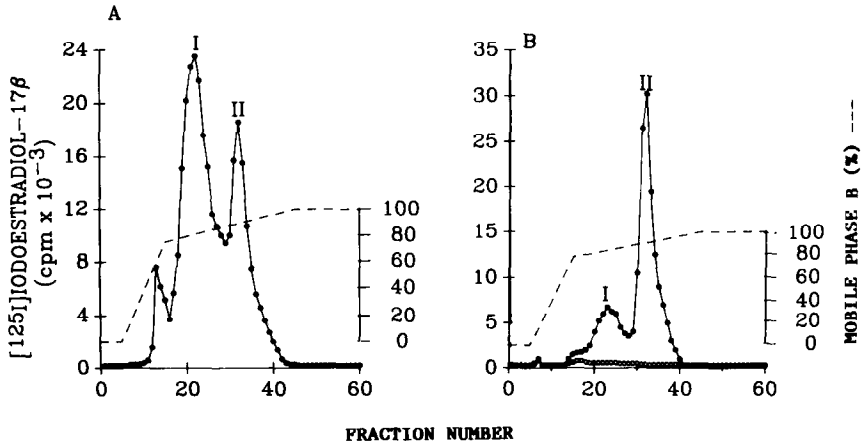

Fig. 5. HPHIC of estrogen receptor from human uterine tissue (A) and MCF-7 human breast cancer cells (B). Extraction and labelling conditions for estrogen receptor were as described in the Experimental section. Separation conditions were the same as described in the legend to Fig. 1.

We made the interesting observation that estrogen receptor, obtained from MCF-7 cells, exhibits little (5%) isoform MI when homogenized in the presence of sodium molybdate (Fig. 6). Since MCF-7 cytosol contained HSP-90[23], isoform MI was expected to be in the form of an estrogen receptor–HSP-90 complex[8]. It now appears that HSP-90 itself is not the contact domain for isoform MI. Rather, the receptor protein interacts with the stationary phase directly, or the equilibrium conditions for estrogen receptor–HSP-90 association in the MCF-7 cell cytosol are different. Nevertheless, hydrophobic heterogeneity of receptor isoforms from MCF-7 cells was observed. Collectively, these results suggest a common mechanism of steroid hormone receptor assembly in normal and neoplastic tissues, as judged by surface hydrophobicity.

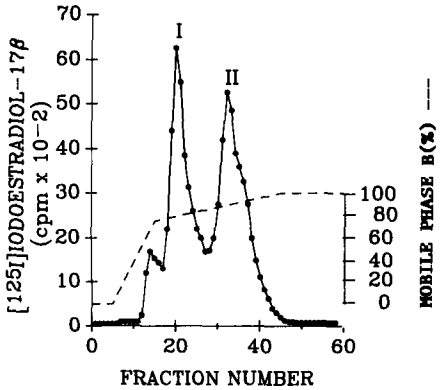

Fig. 6. Influence of sodium molybdate on HPHIC of estrogen receptor from human breast cancer cells. The extraction and labelling conditions for estrogen receptor were as described in the Experimental section, except that the extraction buffers contained 10 m*M* sodium molybdate. Samples were injected into the propyl column and eluted with buffers containing 10 m*M* sodium molybdate.

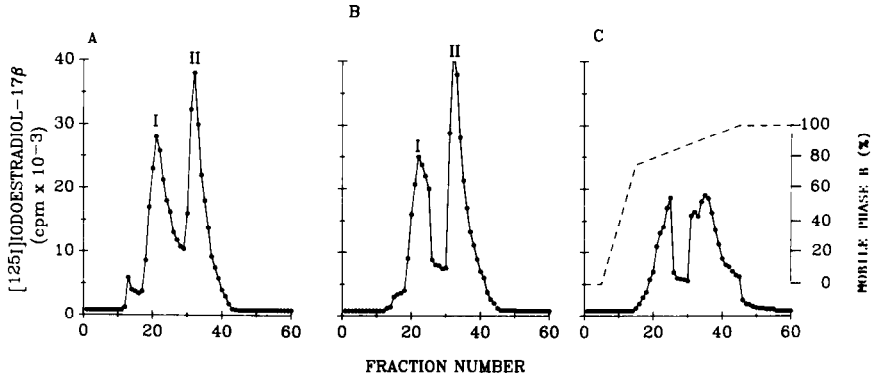

Fig. 7. Influence of contact time of stationary with estrogen receptor on HPHIC separation of estrogen receptor isoforms from human breast cancer cytosol. Dextran-coated charcoal-treated cytosol was injected into the propyl column and eluted with a 2 to 0 *M* ammonium sulfate gradient. (A) control, (B) elution after 90 min, (C) elution after 16 h contact with the stationary phase. After 16 h, the total loss of receptor concentration in the cytosol was 30% of the control. Recoveries of radioactive steroid in all the cases were 80–100%.

Influence of contact time of receptor with the stationary phase

Steroid receptors are known to be labile proteins, found in very small amounts in target cells. This makes their isolation and study extremely difficult[4,6,8,16]. Properties of the hydrophobic stationary phase were investigated for separation and characterization of estrogen receptor isoforms and as a means of storing receptor protein without denaturation for short and long periods. Fig. 7 illustrates a representative experiment demonstrating that estrogen receptor eluted as isoforms I and II in control cytosol (Fig. 7A) and retains its chromatographic characteristics when immobilized on the stationary phase for 90 min at 4°C (Fig. 7B). However, when the receptor was in contact with the stationary phase for 16 h at 4°C, the peak characteristics and elution patterns were altered (Fig. 7C). Curiously, both isoform I and isoform II were eluted with t_R values that were 3 min longer than those observed for the control. Since the experimental conditions were identical, except for the holding time, these results suggest unfolding of the protein with an increase in hydrophobicity. It is intriguing that both receptor forms showed a similar change in hydrophobicity, suggesting a common event. Both receptor isoforms retained their ligand-binding properties, indicating that extensive denaturation did not occur.

CONCLUSION

In several of our previous publications[4,6,8,16] we discussed the usefulness of high-performance liquid chromatography (HPLC) for the rapid separation of steroid receptor isoforms. The synthesis of several hydrophobic bonded phases allows separating labile proteins, such as the estrogen receptor, without obvious denaturation[4]. Separation of two different high-molecular-weight proteins, based on their relative hydrophobicity, was a novel finding[7,13]. Although a single high-molecular-weight receptor species was observed by HPSEC[2,7], we demonstrated that this peak is composed of two distinct hydrophobic isoforms[7,12,13]. The basis of this polymorphism is of interest, since the sequence of the cloned gene predicts only a single 65 000-dalton

component[21]. Steroid receptors are known to be phosphoproteins[24]. Therefore, different degrees of phosphorylation would alter surface ionic properties, allowing the formation of different complexes, even with non-receptor proteins. Also, the modified receptors themselves may interact directly with the bonded phase. Further purification of individual isoforms is required to elucidate the nature of interaction of the receptor protein(s) with the bonded phase.

Another novel finding reported in this paper is that different human tissues, such as the breast, uterus, and MCF-7 breast cancer cells in culture show a remarkably similar hydrophobic isoform distribution in the absence of molybdate. Our previous analysis with rat uterine estrogen receptor gave identical results[2,3]. This indicates that (i) the post-translational modifications of estrogen receptor are common in different tissues and (ii) a single homologous gene is responsible for expression of estrogen receptor. The gene for estrogen receptor from several sources has been cloned and appears similar in sequence[21,25]. Although alternative initiation sites on DNA or alternate splicing of mRNA could give rise to different steroid receptor protein isoforms, accounting for receptor polymorphism, this has been ruled out in the case of the progesterone receptor[26]. It has been shown for the latter that proteolysis may account for these differences. Proteolysis does not appear to contribute to the formation of the two hydrophobic forms of estrogen receptor, since overnight incubation of receptor gives rise to the same isoforms (judged on the basis of size) as those observed in the control cytosol. Proteolyzed receptor is eluted from a size-exclusion column as a single, sharp peak[27] at 25–30 Å.

It is unclear why isoform II from human breast cancer cells was not converted to MI in the presence of sodium molybdate. In rat and human breast tumor cytosol, interconversion was consistent[2,3]. Since molybdate prevents DNA binding of the receptor, isoform II interconversion is attributed to the interaction of the oxy anion with the DNA-binding domain of the receptor. The transformation process should be a common event. The lack of MI in the HPHIC elution pattern of estrogen receptor from human breast cells is puzzling, particularly since MCF-7 cells contain HSP-90[23]. Perhaps molybdate associates with another domain on the receptor molecule, permitting the formation of different complexes.

Finally, immobilization of estrogen receptor from human breast cancer tissues on the hydrophobic column exhibited no change in the hydrophobicity of either isoform for up to 90 min. Longer retention (16–24 h) resulted in slight alteration of hydrophobicity of both receptor isoforms. With improved conditions, one may be able to transport the receptor in this immobilized form from one laboratory to another for analyses, such as protein sequencing.

An interesting observation was made here with respect to receptor interconversion. When the receptor is left overnight in solution, it is transformed into a single peak. In the immobilized form, this conversion does not take place even after 16–24 h of incubation. This indicates that a significant hydrophobic transformation takes place in solution due to an enzymatic process, such as protein phosphorylation/dephosphorylation, ribosylation, or acetylation, which is inhibited on the column. The inhibition of such an enzymatic activity could be due either to binding of the enzyme to the stationary phase with its catalytic site rendered inaccessible to substrate (receptor) or to the binding of the receptor to the column with the proteolytic domain hidden at the contact site.

The complexity of steroid receptor proteins with multiple binding domains for steroid, DNA and associated proteins and their labile nature hinder their investigation in small specimens, such as cultured cells. HPHIC, as developed in our laboratory, using estrogen receptor as a model protein, provides a sensitive means of working with femtomolar quantities of a specific regulatory protein without destroying its biological activity. This study shows that surface properties of proteins may be used to distinguish subtle alterations in receptor configurations which may relate to the mechanism of action of steroid receptors. Changes in receptor hydrophobicity appear to reflect several events in the natural history of the receptor as it is synthesized on the endoplasmic reticulum, during the process of receptor activation which brings about its ability to bind DNA and to initiate gene transcription, its dissociation from the DNA, and its subsequent turnover.

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REFERENCES

1 S. M. Hyder, R. D. Weihle, D. W. Brandt and J. L. Wittliff, *J. Chromatogr.*, 327 (1985) 237.
2 S. M. Hyder, N. Sato and J. L. Wittliff, *J. Chromatogr.*, 397 (1987) 251.
3 S. M. Hyder, N. Sato, W. Hogancamp and J. L. Wittliff, *J. Steroid Biochem.*, 29 (1988) 197.
4 S. M. Hyder and J. L. Wittliff, *BioChromatography*, 2 (1987) 121.
5 S. M. Hyder and J. L. Wittliff, *J. Chromatogr.*, 44 (1988) 225.
6 S. M. Hyder and J. L. Wittliff, in F. Ausubel, R. Brent, R. Kingsdon, D. Moore, J. A. Smith, J. Seidman and K. Struhl (Editors), *Current Protocols in Molecular Biology*, Greene Publishing, New York, 1987, p. 10.15.1.
7 S. M. Hyder and J. L. Wittliff, *J. Steroid Biochem.*, (1989) in press.
8 J. L. Wittliff, J. Allegra, T. Day, Jr. and S. M. Hyder, in V. K. Moudgil (Editor), *Steroid Receptors in Health and Disease*, Plenum Press, New York, 1988, p. 287.
9 G. L. Greene, C. Nolan, J. P. Engler and E. V. Jensen, *Proc. Natl. Acad. Sci. U.S.A.*, 77 (1980) 5115.
10 A. Baldi, D. M. Boyle and J. L. Wittliff, *Biochem, Biophys. Res. Commun.*, 135 (1986) 597.
11 J. L. Wittliff, R. D. Wiehle and S. M. Hyder, in A. Kerlavage (Editor), *Receptor Biochemistry and Methodology*, Alan R. Liss, New York, in press.
12 S. M. Hyder and J. L. Wittliff, *70th Endocrine Society Meeting, New Orleans, LA, 1988*, p. 348.
13 S. M. Hyder, N. Heer and J. L. Wittliff, presented at *8th International Symposium on HPLC of Proteins, Peptides and Polynucleotides, Copenhagen, 1988.*
14 Y. Ohara-Nemoto, T. Nemoto, N. Sato and M. Ota, *J. Steroid Biochem.*, 31 (1988) 295.
15 S. M. Hyder, F. P. Kohrs and J. L. Wittliff, *J. Chromatogr.*, 397 (1987) 269.
16 J. L. Wittliff, in W. Donegan and J. Spratt (Editors), *Steroid Receptor Analysis, Quality Control and Clinical Significance*, W. B. Saunders Co., Philadelphia, PA, 1988, p. 303.
17 M. M. Bradford, *Anal. Biochem.* 72 (1976) 248.
18 R. D. Wiehle, G. E. Hofmann, A. Fuchs and J. L. Wittliff, *J. Chromatogr.*, 307 (1984) 39.
19 N. T. Miller, B. Feibush, K. Corina, S. P. Lee and B. L. Karger, *Anal. Biochem.*, 148 (1985) 510.
20 S. M. Hyder, N. Shahabi and J. L. Wittliff, *BioChromatography*, 3 (1988) 216.
21 R. M. Evans, *Science (Washington, D.C.)*, 240 (1988) 889.
22 W. P. Sullivan, B. T. Sullivan, V. J. Vroman, R. K. Bauer, R. M. Puri, G. R. Pearson and D. O. Toft, *Biochemistry*, 24 (1985) 6586.
23 D. Toft, personal communication.
24 V. K. Moudgil (Editor), *Steroid Receptors in Health and Disease*, Plenum Press, New York, 1988.
25 L. A. Shepel and J. Gorski, *BioFactors*, 1 (1988) 71.
26 M. Misrahi, H. Loosfelt, M. Atger, C. Merid, V. Zerah, P. Dessen and E. Milgram, *Nucleic Acids Res.*, 16 (1988) 5459.
27 N. Sato, S. M. Hyder, L. Chang, A. Thais and J. L. Wittliff, *J. Chromatogr.*, 359 (1988) 475.