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Interaction of Estrogen Receptor of Calf Uterus with a Monoclonal Antibody: Probing of Various Molecular Forms†

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ABSTRACT: A monoclonal antibody to estrogen receptor (JS34/32) is able to recognize, in the calf uterine cytosol, a protein (~65 000 daltons) giving a single band on sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). Two molecules of this antibody are able to simultaneously interact with the native 8S form of the receptor present in the calf uterine cytosol ("twin antibody" assay). This indicates the presence of two antigenic determinants on the "low-salt" 8S form of the receptor. This form of the receptor shows an increase in M_r from 345 000 to 665 000 after interaction with the soluble antibody. Dissociating agents that induce the dissociation of the 8S form to smaller forms also induce the dissociation of the two antigenic determinants. The 4S "high-salt" form of the estrogen receptor has one determinant per molecule, appearing to be the smallest form of the receptor not containing repetitive structures associated with the steroid binding site. The nuclear receptor also shows the presence of more than one antigenic determinant on its molecule.

The estrogen receptor is present in the cytosolic fraction prepared in low ionic strength buffer from unstimulated target tissue as a large macromolecular complex (Toft & Gorski, 1966; Jensen et al., 1969; Stancel et al., 1973). This complex, defined by its sedimentation coefficient (8 S), is able to reversibly dissociate to a "high-salt" form that sediments in the 4–5S region of a sucrose density gradient (Korenmann & Rao, 1968). Furthermore, in a target tissue exposed to the hormone, the estrogen receptor is present mainly in the nuclei as a smaller 4.5–5S form (Puca & Bresciani, 1969; Puca et al., 1970). All the antibodies to the estrogen receptor so far described recognize both the cytoplasmic and the nuclear receptor (Greene et al., 1977, 1979, 1980a,b; Radanyi et al., 1979; Moncharmont et al., 1982); this further supports the postulated "two-step" mechanism of action (Jensen et al., 1972) for the steroid receptor. The physicochemical properties

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and the hormone binding characteristics of these forms of the receptor have been extensively described (Puca et al., 1972). The availability of monoclonal antibodies to the estrogen receptor (Greene et al., 1980a,b; Moncharmont et al., 1982) adds a new tool to further characterize such molecular forms. These monoclonal antibodies, by virtue of their specificity for one antigenic determinant (Köhler & Milstein, 1975), provide a highly specific recognition of receptor protein distinct from its estradiol binding site. They also form stoichiometric complexes with their antigen and therefore provide information on the number of antigenic determinants present on the receptor molecule. This report describes the application of such an approach to probe the different molecular forms of the estrogen receptor from calf uterus.

Materials and Methods

17β-[2,4,6,7-$^3H_4$]Estradiol (91.5 Ci/mmol), [^{14}C]ovalbumin, and ^{14}C-labeled bovine γ-globulin were purchased from New England Nuclear; Na^{125}I was from Atomic Energy of Canada. L-3,4-Dihydroxy[2,5,6-^{3}H]phenylalanine (40 Ci/mmol) was from Amersham. Reagents for sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE)[1] were from Bio-Rad. All the other reagents were of analytical grade and obtained from commercial sources. Protein concentrations were determined by the Coomassie blue method (Bradford, 1976). All the incubations were performed in duplicate at 4 °C unless stated otherwise.

Uteri were collected from immature calves, stripped from fat, ovaries, and ligament, and frozen at the local slaughterhouse. Cytosol was prepared by homogenization of the tissue in 2–3 volumes of TED buffer (20 mM Tris-HCl, pH 7.4, 1 mM EDTA, 1 mM dithiothreitol) with a Polytron, followed by centrifugation for 45 min at 150000g. The transformation of the cytosolic receptor to the nuclear form was performed in vitro by incubation for 1 h at 24 °C of a crude nuclear pellet from calf uterus with cytosol preincubated with 10 nM 17β-[^{3}H]estradiol. The nuclear receptor was then extracted from the nuclear pellet with TED buffer containing 0.4 M KCl. This technique has been previously described (Jensen et al., 1972; Moncharmont et al., 1982).

Production of the monoclonal antibody to estrogen receptor and its characterization and coupling to Mâtrex 102 (Mâtrex 102–JS34/32) have been described earlier (Moncharmont et al., 1982). For coupling to the microbeads as well as for iodination, the antibody from the ascites fluid was purified by ammonium sulfate precipitation, followed by DEAE chromatography. The radioiodination by the lactoperoxidase method (Marchalonis, 1969) of the antibody used in this study has been described in detail elsewhere (Moncharmont & Parikh, 1983).

Immunoprecipitation was performed in Eppendorf microcentrifuge tubes (1.5 mL). Calf uterus cytosol was prepared as described above in the following buffer: 50 mM potassium phosphate, pH 7.4, containing 1 mM EDTA and 0.2 mM phenylmethanesulfonyl fluoride. Aliquots of 200 μL of Mâtrex 102–JS34/32 or Mâtrex 102–mouse IgG suspension (40 mg of resin/mL) were each successively prewashed with 1 mL of homogenization buffer, 1 mL of 0.1 M sodium pyrophosphate (pH 5.0), and 1 mL of 0.1 N acetic acid and twice with 1 mL of homogenization buffer. The cytosol was previously preincubated with an equivalent amount of Mâtrex 102–mouse IgG for 20 min at 0 °C. The resin was then pelleted, and 1 mL of the supernatant (or 1 mL of homogenization buffer) was added to each of the prewashed pellets. Sodium deoxycholate (DOC) was added to each tube (50 μL of a 10% w/v solution). Following incubation for 2 h at room temperature and centrifugation for 2 min, the supernatant was decanted. The resin pellets were successively washed with the following buffers: twice with 1 mL of 50 mM potassium phosphate (pH 7.4) containing 0.5% DOC; once with 1 mL of 50 mM potassium phosphate (pH 7.4); twice with 1 mL of 0.1 M sodium pyrophosphate (pH 5.0); three times with 250 μL of 0.1 M acetic acid. The acetic acid washes were pooled, immediately lyophilized, and prepared for SDS–PAGE by dissolving in 50-μL sample buffer containing 10 mM Tris-HCl (pH 6.8), 10% glycerol, 0.5% β-mercaptoethanol, 3% SDS, and 0.05% Bromophenol blue and heating for 5 min at 90 °C. Polyacrylamide gel electrophoresis was performed according to Laemmli (1970) in a 3% stacking gel and 10% separating gel. The gel was stained with silver nitrate according to the procedure of Hancock (Wray et al., 1981). Alternatively, the gel was stained with 0.25% Coomassie blue R-250 solution in water–2-propanol–acetic acid (65:25:10) for 2 h and destained in water–2-propanol–acetic acid (80:10:10).

Gel exclusion chromatography was performed at 4 °C in a glass column (diameter 10 mm, 26 mL) packed with Ultrogel AcA22 (LKB) and equilibrated with TED buffer. Fractions were collected at a flow rate of 3.5 mL/min and their radioactivities determined. The elution profile of the protein used for calibration was determined by UV absorption at 280 nm. Stokes radius values for protein standards were from the literature (Siegel & Monty, 1966; Pagé & Godin, 1969).

Sucrose gradient analysis was performed by layering 0.2 mL of the sample on a 4.3-mL 5–20% (w/w) or 10–30% (w/w) sucrose (Schwarz/Mann Ultrapure) gradient in TED buffer (containing 0.4 M KCl where specified), followed by centrifugation in a SW60Ti Beckman rotor. After the centrifugation, gradients were fractionated by puncturing the bottom of the tube and fractions of 10 drops were collected. The data are plotted in the direction of increasing sucrose density. The samples applied to the Ultrogel column (and to the gradients) were pretreated for 5 min at 0 °C with the pellet of an equal volume of a charcoal suspension (1% charcoal, RIA grade, 0.1% Dextran T-70, 0.1% gelatin) in TED buffer and centrifuged at 3000 rpm for 10 min.

The twin antibody assay was performed by incubating aliquots of cytosol or aliquots of gradient fractions with a fixed amount of ^{125}I-labeled JS34/32 antibody for 2 h at 0 °C in a final volume of 225 μL in the presence of 10% human normal serum and PBS containing BSA (1 mg/mL) and 0.1% Triton X-100. An aliquot (25 μL) of Mâtrex 102–JS34/32 was then added. The Mâtrex 102–JS34/32 suspension (10 mg of resin/mL) had a capacity of approximately 2 pmol/mg of resin for calf estrogen receptor. At the end of the incubation, 4 mL of PBS (containing Triton X-100 and BSA) was added to each tube and the resin pelleted by centrifugation at 3000 rpm for 20 min. The pellet was then counted for radioactivity in a γ counter.

Results

Monoclonal antibody JS34/32 is secreted by a hybridoma cell line produced by the fusion of a mouse myeloma cell line (SP2/0-Ag14) with the spleen cells of a mouse immunized with a purified preparation of calf uterus estrogen receptor (Moncharmont et al., 1982). This antibody, when covalently attached to an insoluble support (Mâtrex 102), was able to

[1] Abbreviations: DEAE, diethylaminoethyl; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; PBS, phosphate-buffered saline; BSA, bovine serum albumin; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; IgG, immunoglobulin G; DOC, sodium deoxycholate.

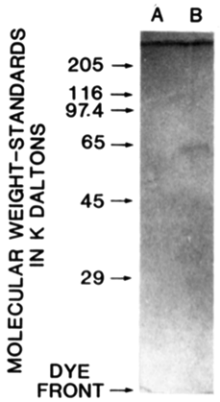

FIGURE 1: SDS–PAGE of the estrogen receptor immunoprecipitated with the antibody JS34/32 conjugate. Experimental conditions are described under Materials and Methods. The gel was stained with silver stain. Lane A: Control immunoprecipitation of calf uterine cytosol with Mâtrex 102–mouse IgG conjugate. Lane B: Immunoprecipitation of the cytosol with Mâtrex 102–JS34/32 conjugate. The molecular weight standards indicated by the arrows correspond to the migration of myosin, β-galactosidase, phosphorylase B, BSA, ovalbumin, and carbonic anhydrase.

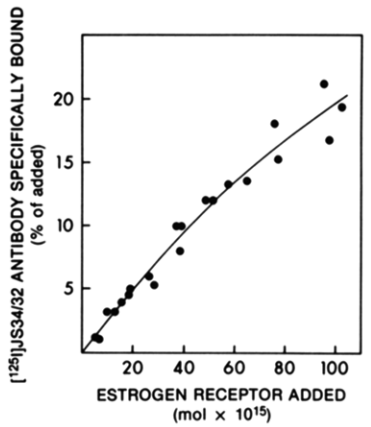

FIGURE 2: Binding of ^{125}I-labeled JS34/32 antibody to Mâtrex 102–JS34/32 in the presence of various concentrations of estrogen receptor. Experimental conditions are described under Materials and Methods. The total volume of cytosol added to each tube was kept constant by addition of cytosol deprived of estrogen receptor by preincubation with Sepharose–heparin or Sepharose–estradiol. The curve is the average of three separate experiments. The specific binding refers to the binding after subtraction of the blank value (incubation with receptor-deprived cytosol). The blank value varied between 7 and 10% of total bound radioactivity.

quantitatively remove the estrogen binding activity from the calf uterine cytosol. Because of the high affinity of the estrogen receptor with the immobilized antibody, it was possible to immunopurify on an analytical scale the protein recognized by this antibody. Figure 1 shows an SDS–PAGE profile of the immunopurified protein. The estrogen receptor migrates as a single band with an approximate M_r of 65 000.

After preincubation of the native receptor present in a low-salt cytosol with a nonsaturating amount of ^{125}I-labeled monoclonal antibody, it is possible to precipitate a significant percent of the added counts with an insoluble resin (Mâtrex 102) bearing covalently attached monoclonal antibody produced by the same clone (twin antibody). No radioactivity will be present in the pellet if the cytosol is omitted from the incubation or if the cytosol is devoid of estradiol binding activity. Figure 2 shows the dependency of the binding on increasing concentrations of the estrogen receptor present in calf uterine cytosol. The time course of the preincubation of the cytosol with radiolabeled antibody and the time course of the subsequent incubation with the insoluble resin at various

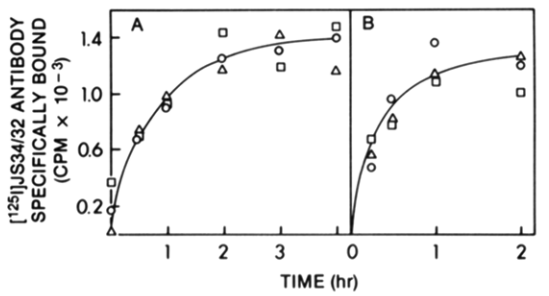

FIGURE 3: Time course of the interaction of ^{125}I-labeled JS34/32 antibody with the estrogen receptor (panel A) and of the ^{125}I-labeled JS34/32–receptor complex with Mâtrex 102–JS34/32 (panel B) at various temperatures. (A) Aliquots of calf uterine cytosol containing 0.15 pmol of estrogen receptor, preincubated for 1 h at 0 °C with 10 nM 17β-estradiol, were further incubated at temperatures and for time periods indicated with the radiolabeled antibody (100 000 cpm). At the end of the indicated time periods, Mâtrex 102–JS34/32 was added and the incubation continued for 1 h at 4 °C. The reaction was terminated by addition of buffer and centrifugation as described under Materials and Methods. (B) Aliquots of cytosol (same as panel A) were incubated at 4 °C for 2 h with ^{125}I-labeled JS34/32 antibody. Then Mâtrex 102–JS34/32 was added and the incubation continued at temperatures and for time periods indicated. The reaction was terminated by addition of buffer and centrifugation as described under Materials and Methods. Symbols: ○, 4 °C; □, 20 °C; △, 37 °C.

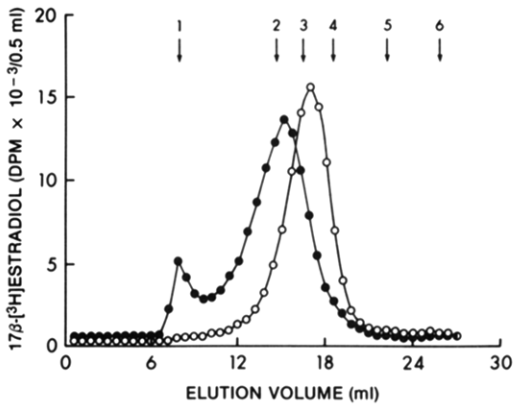

FIGURE 4: Determination of the elution volume of the estrogen receptor and of the estrogen receptor–JS34/32 antibody complex on a calibrated Ultrogel AcA22 gel filtration column. Aliquots of cytosol containing 0.7 pmol of estrogen receptor, preincubated with 10 nM 17β-[^{3}H]-estradiol for 2 h at 0 °C, were further incubated with (●) or without (○) monoclonal antibody JS34/32 (10 μg/mL) for 4 h at 0 °C. The arrows indicate the peaks of elution of (1) blue dextran, (2) thyroglobulin, (3) apoferritin, (4) catalase, (5) bovine γ-globulin, and (6) dihydroxy[^{3}H]phenylalanine.

temperatures are shown in parts A and B of Figure 3, respectively.

The dependency of the binding of the labeled antibody upon receptor concentration suggests that more than one antigenic determinant for antibody JS34/32 is simultaneously recognized by the labeled and by the immobilized monoclonal antibody on such receptor molecule. This hypothesis was verified by measurement of the increase in molecular weight of the estrogen receptor after it was complexed with antibody JS34/32. The gel chromatography elution profile of the estrogen receptor alone or after preincubation with an excess of antibody JS34/32 is shown in Figure 4. Similarly, the complex, as well as the receptor alone, was centrifuged through a sucrose gradient. The sedimentation profiles are shown in Figure 5. The standard protein gel filtration data were plotted according to the correlations of Porath: $K_d^{1/3} = \alpha - \beta a$, where a = Stokes radius (Porath, 1963). The distribution coefficient K_d was calculated from $K_d = (V_e - V_0)/(V_t - V_0)$, where V_e (elution volume) was the volume corresponding to the peak elution of

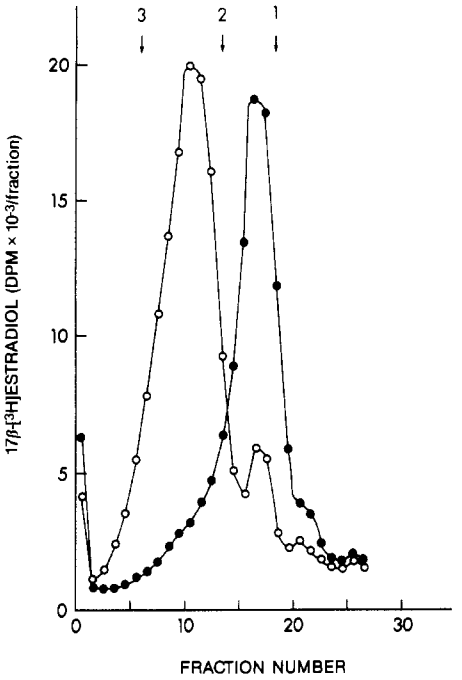

FIGURE 5: Determination of the sedimentation coefficients of the estrogen receptor and of the estrogen receptor–JS34/32 antibody complex by centrifugation on sucrose gradient. Aliquots of cytosol containing 0.7 pmol of the estrogen receptor, preincubated with 10 nM 17β-[^{3}H]estradiol for 2 h at 0 °C, were further incubated with (●) or without (○) monoclonal antibody JS34/32 for 4 h at 0 °C. The centrifugation was performed through a 5–20% (w/w) linear sucrose gradient in TED buffer at 35 000 rpm for 13.5 h. Arrows indicate the migration of standards: (1) thyroglobulin, (2) catalase, and (3) bovine γ-globulin.

the protein, V_0 (void volume) was the volume corresponding to the peak elution of blue dextran, and V_t (total volume) was the volume corresponding to the peak elution of the dihydroxyphenylalanine. By interpolation of the respective K_d's, Stokes radii of 63 and 79 Å were measured for the receptor and for the receptor–antibody complex, respectively. The sedimentation coefficient for the receptor, measured by centrifugation on a sucrose gradient, is 8 S whereas for the receptor–antibody complex it is 12.5 S. From the sedimentation coefficient (s) and from the Stokes radius value (a), an M_r of 345 000 can be calculated for the receptor alone from the equation $M_r = a(6\pi\eta N)s/(1 - \bar{v}\rho)$, in which η = viscosity, N = Avogadro's number and ρ = density of solution, assuming a partial specific volume ($\bar{v}$) of 0.734. Similarly, an M_r of 665 000 can be calculated for the receptor–antibody complex. The difference in M_r of 320 000, in conjunction with the ability of simultaneous binding to a soluble and an immobilized antibody, indicates that the native form of the estrogen receptor in the cytosol contains two determinants for antibody JS34/32 on the same macromolecular complex. The effect of dissociating agents on the conformational status of the receptor is shown in Table I. In the presence of heparin, KCl, or NaSCN the ability of the antibody to simultaneously recognize the two antigenic sites on the same molecular complex is lost. The dissociating effects of heparin (Shyamala, 1971), KCl (Korenmann & Rao, 1968), and NaSCN (Sica et al., 1976) on the estrogen receptor, at the concentrations used in the experiment, have been described. However, the three dissociating agents, at concentrations described in Table I, do not have any effect on the direct interaction of the receptor with the antibody, as examined by sucrose density gradient analysis (data not presented). The result presented in Table I, while further confirming the existence of two antigenic determinants on the

Table I: Effect of Dissociating Agents on the Twin Antibody Assay[a]

agents	^{125}I-labeled JS34/32 bound (cpm/mg of protein)
control	19 091
heparin (1 mg/mL)	983
KCl (0.5 M)	2 988
NaSCN (0.5 M)	656

[a] Aliquots of calf uterine cytosol were preincubated for 1 h at 0 °C at the conditions indicated. Aliquots of the preincubation mixture were assayed by the twin antibody assay as described under Materials and Methods. The dissociating conditions were kept constant during the incubation and the washing procedure.

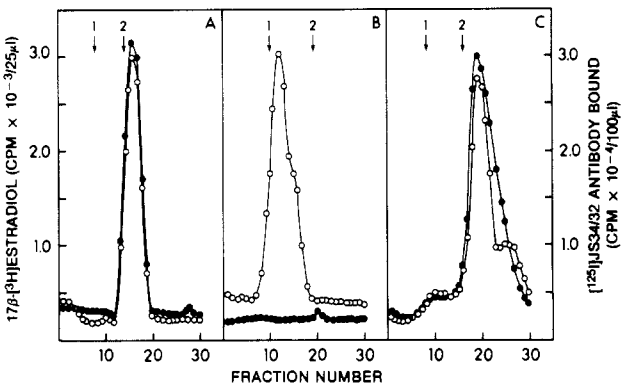

FIGURE 6: Comparative profiles of sucrose gradient analysis of the cytosolic 17β-[^{3}H]estradiol receptor complex and its reactivity in the twin antibody binding assay. Aliquots of cytosol containing 0.9 pmol of the estrogen receptor were preincubated with 10 nM 17β-[^{3}H]-estradiol for 2 h at 0 °C in TED buffer (A), in TED buffer containing 0.4 M KCl (B), or in TED buffer containing 0.4 M KCl followed by dialysis against TED buffer (C). The gradients were fractionated, and radioactivity in an aliquot of the fractions was determined in a liquid scintillation counter (○). Another aliquot of the fractions was incubated with the monoclonal antibody in the twin antibody assay as described under Materials and Methods (●). Arrows show the migration of (1) [^{14}C]ovalbumin and (2) ^{14}C-labeled bovine γ-globulin used as internal standards in all the gradients. Centrifugation was performed in a 10–30% (w/w) linear sucrose gradient in TED buffer (A and C) or in TED buffer containing 0.4 M KCl (B) for 15 h at 50 000 rpm.

native form of the receptor, suggests the presence of only one on the dissociated forms. The experiments shown in Figure 6 confirm this hypothesis. The sedimentation profile of the native (8S) form of the receptor (Figure 6A) and of the KCl-dissociated (4S) form (Figure 6B) is compared to the ability of the receptor present in each fraction to react simultaneously with the immobilized and with the radiolabeled antibody. The reversibility of the KCl-dissociating effect on the presence of two determinants on the native receptor form is shown in Figure 6C. After incubation of the cytosol with 0.4 M KCl, the salt was removed by dialysis, and the analysis by centrifugation on a sucrose gradient shows that the estrogen receptor reassociates in an 8S form as expected. The profile of the reactivity of the gradient fractions in the twin antibody assay shows that, after removal of the dissociating condition, the two antigenic determinants reassociate to a larger molecular complex. The nuclear form of the receptor was also analyzed by a similar approach. The nuclear receptor was extracted from nuclei prepared from calf uteri. Such nuclei were preincubated with labeled cytosol to induce nuclear translocation of the cytosolic receptor. The nuclear receptor migrates, as expected, as 5S on the sucrose gradient. The fractions of the gradient were assayed by the twin antibody method. The profiles of estradiol binding activity and of ^{125}I-labeled antibody binding are shown in Figure 7. The ability of the nuclear receptor to simultaneously interact with

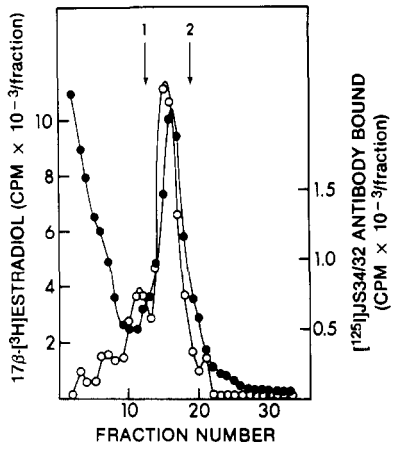

FIGURE 7: Comparative profiles of sucrose gradient analysis of the nuclear 17β-[^{3}H]estradiol receptor complex and its reactivity in the twin antibody binding assay. Aliquots of the nuclear extract containing 0.5 pmol of the 17β-[^{3}H]estradiol receptor complex were centrifuged on two 10–30% (w/w) sucrose gradients in TED buffer containing 0.4 M KCl for 15 h at 50 000 rpm. Radioactivity was determined in fractions of one gradient in a liquid scintillation counter (○). Each fraction of the other gradient was incubated with the monoclonal antibody in the twin antibody assay as described under Materials and Methods (●). Arrows show the migration of (1) [^{14}C]ovalbumin and (2) [^{14}C]bovine γ-globulin used as internal standards.

two antibody molecules indicates the presence of at least two antigenic determinants also on this form.

Discussion

After extensive purification, the calf uterus estrogen receptor has been demonstrated to be a protein of M_r approximately 69 000 (Molinari et al., 1977; Sica & Bresciani, 1979). Monoclonal antibody JS34/32 recognizes all the estrogen binding activity present in the calf uterus cytosol (Moncharmont et al., 1982) and, as shown in Figure 1, specifically immunoprecipitates a protein that gives a single band of approximately 65 000 daltons on SDS–PAGE. However, we do not have any indication of the significance of this difference in molecular weight. In the crude cytosol, on the other hand, the receptor is present in a larger form usually indicated by its sedimentation coefficient, 8 S, that is able to reversibly dissociate by increasing ionic strength to a smaller 4S form. How the 65 000–69 000-dalton receptor protein is organized in these larger supramolecular complexes has not been elucidated yet. Various models have been presented so far that postulate either the existence of various proteic components of the cytosol which participate in the formation of the 8S form (Murayama et al., 1980a,b) or the repetitive assembly of the same subunit in a larger complex (Sica et al., 1976; Sica & Bresciani, 1979). The presence of more than one 65 000–69 000-dalton protein in the larger complex may be assessed by measuring the specific activity of the estradiol binding only in a highly purified preparation. Such purification of the 8S form of the receptor has not been achieved so far. As has been elegantly demonstrated for the acetylcholine receptor (Conti-Tronconi et al., 1981), monoclonal antibodies may be a useful tool to investigate the subunit structure of a protein in virtue of their specificity for only one antigenic determinant. The estrogen receptor has been subjected to similar studies in a previous publication (Moncharmont et al., 1982). The data presented here confirm the previous finding, though by a different experimental approach, that the high-salt 4S form of the receptor has only one antigenic determinant per molecule. This indicates that the 4S receptor is presumably the smallest form that does not contain any repetitive structure associated with the estradiol binding site. It is conceivable therefore that the 65 000-dalton receptor protein also has only one antigenic determinant per molecule. On the contrary, the ability of two antibody molecules to simultaneously interact with the 8S form of the receptor indicates that more than one antigenic determinant is present on this large molecular complex. An increment of about 300 000 in the molecular weight of the receptor is observed after it is complexed with the antibody in the presence of an excess of the latter. This suggests the presence of two antibody molecules in the complex and therefore two antigenic determinants for antibody JS34/32 on the 8S form. This finding is in disagreement with those previously reported (Moncharmont et al., 1982). The interpretation of the increase in size of the 8S receptor as a consequence of its interaction with the antibody by sucrose gradient analysis alone has been demonstrated to be erroneous as reported earlier (Moncharmont et al., 1982). The determination of the molecular weight performed here by gel chromatography and sedimentation analysis on a sucrose gradient (Siegel & Monty, 1966) appears to be undoubtedly more accurate and is in agreement with the results of the simultaneous binding of two antibodies. An apparent difference in the size of the estrogen receptor between the one determined by gel chromatography (Andrews, 1964) and the one determined by sedimentation on a sucrose gradient (Martin & Ames, 1961) has been previously reported (Puca et al., 1972; Auricchio et al., 1978) and can be ascribed to asymmetry of the molecule. In light of the above findings, the 8S form of the estrogen receptor in the calf uterine cytosol presumably contains two similar 4S molecular components in its molecule. The presence of other (proteic or nonproteic) component(s) in the 8S form appears to be very plausible. Such a "third component" of the 8S form may well be the one responsible for the interaction of the receptor with cytoplasmic components (Puca et al., 1981). Further investigation will probably provide meaningful insight about the physiological significance of this large complex in which the dimeric receptor molecule participates.

Finally, the nuclear receptor also simultaneously interacts with more than one antibody molecule. This finding strongly supports the hypothesis previously formulated about a homodimeric status of the 5S nuclear receptor (Notides & Nielsen, 1974; Nielsen & Notides, 1975; Notides et al., 1975, 1981). However, it is important to point out here that there is no evidence in the literature that the in vitro translocation is an accurate reproduction of the physiological phenomenon. Data presented here about the nuclear receptor should be reconfirmed with an in vivo translocated receptor; for obvious reasons, it was not possible to perform such experiments on the calf receptor used in this study.

Such homodimeric status of the receptor, in either its native or nuclear form, may suggest analogy with other receptors such as those for insulin and some insulin-like growth factor (Jacobs & Cuatrecasas, 1981), the benzodiazepine receptor (Doble & Iversen, 1982), the muscarinic receptor (Avissar et al., 1983), and the transferrin receptor (Schneider et al., 1982). The physiological significance of a homodimeric structure remains to be investigated. The homodimeric state of the estrogen receptor may also explain the positive cooperativity of the estrogen binding (Notides et al., 1981). However, further investigation is necessary to explore if both sites should be occupied in order to elicit the hormonal action and nuclear translocation phenomena.

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