Purification by affinity chromatography and immunological characterization of a 110kDa component of the chick oviduct progesterone receptor

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A 110kDa component of the chick oviduct progesterone receptor (PR) has been purified to homogeneity according to electrophoretic criteria and specific activity (assuming one progesterone-binding site/110kDa). The procedure involved affinity chromatography of 0.3M-KCl-prepared cytosol, followed by DEAE-Sephacel chromatography (elution at 0.2M-KCl). The final yield was about 12% in terms of binding activity. Properties of the 110kDa component indicate that it is identical with the 'B' subunit described previously [Stokes radius ~ 6.1 nm; sedimentation coefficient, ($s_{20,w}$) ~ 4 S; frictional ratio ~ 1.77]. It reacted with the IgG-G3 polyclonal antibody, but not with BF4 monoclonal antibody raised against the 8S molybdate-stabilized chick oviduct PR and reacting with its 90kDa component. Another progesterone-binding component, corresponding to the 'A' subunit, also previously described, was eluted from the DEAE-Sephacel column at ~ 0.08 M-KCl, and contained a peptide of molecular mass approx. 75–80kDa, which had $s_{20,w} \sim 4$ S in a sucrose gradient. This component was also recognized by IgG-G3, but not by BF4; it was very unstable in terms of hormone-binding activity.

We have recently purified to apparent homogeneity (Renoir *et al.*, 1981, 1982a) a molybdate-stabilized, non-activated form of the chick oviduct progesterone receptor (PR), sedimenting at ~ 8 S in density gradients. In such preparations, a single protein (~ 85 kDa) was detected by Coomassie Blue staining. Similar results were reported by Puri *et al.* (1982). However, during the past decade, two different forms, designated 'A' and 'B', have been characterized and purified (Kuhn *et al.*, 1975; Schrader *et al.*, 1977; Coty *et al.*, 1979). These two forms, which can be separated by ion-exchange chromatography, sediment at ~ 4 S in sucrose gradient, bind to nuclei and polyanions and are believed to be unique constituents of the native receptor. Their molecular masses are 79kDa for 'A' and 108kDa for 'B' (see recent review by Birnbaumer *et al.*, 1981).

Since our data obtained with the molybdate-

stabilized PR were in disagreement with those reported for the 'A' and 'B' forms, we have purified the chick oviduct PR in the absence of molybdate throughout the purification procedure and from high-ionic-strength-prepared [0.3M-KCl or 30%-satd.-(NH₄)₂SO₄] cytosol, a procedure yielding 4S molecule(s) of 'activated' ('transformed') steroid receptors (Milgrom *et al.*, 1973). We have also compared the purified components of PR immunologically by using polyclonal and monoclonal antibodies previously raised in this laboratory (Renoir *et al.*, 1982b; Radanyi *et al.*, 1983).

Materials and methods

Chemicals

[2,4,6,7-³H]Progesterone ([³H]P) (80–110 Ci/mmol) was from The Radiochemical Centre (Amersham, Bucks., U.K.). Its purity was verified by t.l.c. Non-radioactive cortisol and progesterone were $>95\%$ pure and were obtained from Roussel-Uclaf, Romainville, France. DEAE-Sephacel was from Pharmacia (Uppsala, Sweden), (NH₄)₂SO₄ from Schwartz–Mann (Orangeburg,

Abbreviations used: PR, progesterone receptor; [³H]P, [2,4,6,7-³H]progesterone; NADAC, N-12-aminododecyl-3-oxoandrost-4-ene-17 β -carboxamide; IgG, immunoglobulin G; SDS, sodium dodecyl sulphate.

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NY, U.S.A.). All other chemicals were of reagent grade and were obtained from Merck (Darmstadt, Germany) except where noted. Double-distilled water was used to prepare all buffers.

Buffers

Buffer A: 10 mM-Tris/1.5 mM-EDTA/10% (w/v) glycerol/12 mM-1 α -thioglycerol, pH 7.4 at 25°C. Na₂MoO₄ (20 mM) was added to buffer A to make buffer B.

Animals and oviduct cytosol preparation

The oestrogen-stimulation schedule of the Warren chickens was described previously (Wolfson *et al.*, 1980).

Cytosol of oestrogen-stimulated chicken oviduct was prepared as described by Renoir *et al.* (1982a), except that buffer A, without molybdate, was used instead of buffer B. Phenylmethanesulphonyl fluoride (0.3 mM final concn.) was added at homogenization in order to minimize proteolytic activity during subsequent operations.

Purification of 'transformed' receptor

1. *'High-salt' cytosol preparations.* Oviduct cytosol (300–400 ml) in buffer A was precipitated overnight at 0°C with 30%-satd. (NH₄)₂SO₄. After centrifugation (20 min; 12000g) in a Sorvall RC₅B centrifuge, the supernatant was removed and the pellet redissolved in one-third of the initial cytosol volume. Alternatively, crude cytosol was treated by incubation with 0.3 M-KCl for 2 h at 0–4°C. In both cases, unlabelled 1 μ M-cortisol was added in order to block the binding sites of either glucocorticosteroid receptor or plasma transcortin, which also bind to the affinity gel.

2. *Affinity chromatography.* The cytosol preparation was loaded on to 30–40 ml of NADAC-Sepharose 4B affinity-chromatography gel (Renoir *et al.*, 1981, 1982b) equilibrated in buffer A, at a flow rate of 5–10 ml/h. After loading, the gel was washed successively by 3 vol. of buffer A, 3 vol. of buffer A containing 0.3 M-KCl, 10 vol. of buffer A and 3 vol. of buffer A containing 3 M-urea, and was then re-equilibrated in buffer A. These washing steps were carried out as quickly as possible.

To elute the receptor bound to the affinity gel, one packed gel volume of 2 μ M-[³H]P (5–20 Ci/mmol) in buffer A was added, and the exchange reaction was performed in the column with gentle shaking for 18 h at 4°C.

3. *DEAE-Sepharcel chromatography.* DEAE-Sepharcel (0.5–1 ml) was equilibrated with buffer A and packed in a column (diameter 1 cm). The pooled fractions (40–80 ml) from the affinity-chromatography step, containing [³H]steroid–receptor complexes, were loaded on to the column and 1 ml

fractions were collected at a flow rate of ~30 ml/h. The column was washed with 10 ml of buffer A. Elution was carried out either with a linear 0–0.5 M-KCl gradient (total volume 50 ml), or first with a stepwise 0.08 M-KCl elution followed by a 0.08–0.5 M-KCl linear gradient.

Purification of 'non-transformed' receptor

For comparison studies, the (non-transformed) PR was purified by affinity chromatography as described previously (Renoir *et al.*, 1982a). Urea (2.5 M) was used to wash the gel prior to elution with [³H]P; PR remained in a 8S form (8S-PR).

Measurement of steroid receptor binding

The binding activities of the initial cytosol and of the redissolved (NH₄)₂SO₄ pellet were determined (after 10-fold dilution) by incubation with 20 nM-[³H]P. Non-specific binding was measured by carrying out a parallel incubation with radioactive progesterone plus a 200-fold excess of unlabelled progesterone. Dextran (0.025%)/charcoal (0.025%) adsorption was used to discriminate between the free and protein-bound steroid.

To estimate the amount of receptor-bound [³H]-steroid after elution from the affinity gel, samples (0.05 ml) were first incubated (10 min at 0°C) with an equal volume of 0.5%-charcoal/0.05%-dextran containing 2 mg of gelatin/ml. The suspensions were then centrifuged (800g; 5 min) and the supernatants mixed 1:10 (v/v) with a 5%-charcoal/0.5%-dextran suspension. After 1 min at 0°C, the suspensions were again centrifuged and the supernatants counted for radioactivity. The binding activity of the purified receptor after ion-exchange chromatography was determined by direct counting of 0.05 ml portions of the fractions.

Analytical Ultrogel AcA-34 filtration

Ultrogel AcA-34 was suspended in either buffer A or buffer B and packed into columns (volume 65 ml; diameter 1.6 cm). These columns were treated with cytosol as described by Soullignac *et al.* (1977) and Renoir *et al.* (1982a) to prevent receptor inactivation during filtration through freshly packed columns. The volume of chromatographed samples was about 1 ml. Flow rates were 8 ml/h for each column and calibration was carried out with the following proteins: transferrin, Stokes radius (R_s) = 3.6 nm; catalase, 4.5 nm; yeast alcohol dehydrogenase, 5.13 nm; ferritin, 6.15 nm; and pure 8S molybdate-stabilized PR (R_s = 7.1 nm; Renoir *et al.*, 1982a). Void volumes (V_0) were determined with Dextran Blue and total volumes (V_t) with potassium bichromate. Standard curves were plotted as described by Porath (1963): (K_D)^{1/2} against R_s , with $K_D = (V_e - V_0)/(V_t - V_0)$ where V_e is the elution volume of the protein being studied. The

Stokes radii were measured by reference to the calibration curve.

Sucrose-gradient centrifugation

Sucrose gradients (5–20%, w/v) were prepared in buffer B containing 0.15M-KCl as described previously (Wolfson *et al.*, 1980). Samples (200 μ l) containing the [3 H]steroid–receptor complexes mixed with marker enzymes and/or with 14 C-labelled bovine serum albumin ($s_{w,20} = 4.6$ S) were layered on top of preformed gradients, and the tubes were centrifuged in an SW 60 rotor (Beckman) at 0–2°C at 200000g for 16 h. Fractions were collected by piercing the bottom of the tubes, and sedimentation coefficients were determined as described by Martin & Ames (1962).

SDS/polyacrylamide-slab-gel electrophoresis

Electrophoresis were performed in a 7.5% or 10% (w/v) polyacrylamide slab gel (16 cm $\times$ 18 cm $\times$ 0.15 cm) containing 0.1% SDS as described by Laemmli (1970). Samples (0.2–4 μ g) of markers or of purified PR were layered on to the stacking gel. When necessary, samples were concentrated by precipitation with trichloroacetic acid [10% (w/v) final concn.] overnight at 25°C.

Electrophoresis was carried out at room temperature for about 4 h at 30 mA/gel. M_r values were determined by the method of Weber & Osborn (1969) by reference to a calibration curve obtained with the following standard proteins: myosin subunit from rabbit muscle, M_r 206000; β -galactosidase subunit from *Escherichia coli*, 130000; phosphorylase *b* subunit from rabbit muscle, 97400; bovine plasma albumin, 66000; ovalbumin, 45000; and carbonic anhydrase from bovine erythrocytes, 29000.

Gel staining

The gels were stained with the AgNO₃ technique (Wray *et al.*, 1981), which allows the detection of about 10 ng of protein per band.

Radioactivity measurements

Samples were vortex-mixed with 7 ml of Scintimix (0.4% in toluene) and counted for radioactivity in a Packard liquid-scintillation spectrometer (~50% counting efficiency).

Protein determinations

Protein concentrations were determined by the Amido Black method of Schaffner & Weissmann (1973), with bovine serum albumin as standard.

Detection of the receptor by protein blotting

The Western blotting technique (Burnette, 1980; Bowen *et al.*, 1980) was used. After SDS/polyacrylamide-gel electrophoresis in 10%-

polyacrylamide slabs, the proteins were transferred to nitrocellulose filters (Schleicher and Schüll; 0.45 μ m pore size). These were then incubated with anti-receptor antibodies, either polyclonal (IgG-G3) (Renoir *et al.*, 1982b) diluted to 5–10 mg/ml, or monoclonal (BF4) (Radanyi *et al.*, 1983) diluted to 1–3 μ g/ml. A second 125 I-labelled antibody [(5 $\times$ 10⁴)–10⁵ c.p.m./ml] was used to detect the PR–antibody complexes. Rabbit IgG species raised in the laboratory were used for this purpose. The filters were then placed for 3–20 h at –80°C with an X-Omat (XAR-5) film (Kodak).

Results

Purification of the 110kDa component

1. *Affinity chromatography.* The high-salt (0.3M-KCl)-activated (transformed) PR can be purified by affinity chromatography on NADAC–Sephacrose. Purification was improved by using 3M-urea in the washing buffer. The purity of PR after elution was estimated to be 24–43% in three experiments, with a 25% average yield (Table 1a). The affinity eluates obtained with the molybdate-stabilized non-activated receptor showed a 90000- M_r band in SDS/polyacrylamide-gel electrophoresis analyses which was absent in the eluate of high-salt-cytosol chromatography (in the absence of molybdate) (Fig. 1a). A band at ~110000 M_r was present in both cases, corresponding to the B-subunit described by Schrader *et al.* (1977).

A slightly better purification could be afforded if the cytosol were first precipitated by 30%-satd. (NH₄)₂SO₄ (Table 1b). The yield of PR after affinity chromatography of the redissolved pellet was 8–10%. The purity at this stage was estimated to be ~40% on the basis of specific radioactivity and assuming one binding site per molecule of 110kDa. The same 110kDa component and the same absence of 90kDa polypeptide (Fig. 1b, lane 2) were observed as in the eluate of the KCl-activated PR shown in Fig. 1(a).

2. *DEAE-Sephacel chromatography.* When purification started from 0.3M-KCl cytosol, the affinity gel eluate chromatographed on DEAE-Sephacel column gave a single peak of receptor-bound radioactivity at 0.2M-KCl (results not shown). However, when the elution was performed stepwise with buffer A containing 0.08M-KCl, a first [3 H]P–PR complex was detected (Fig. 2), corresponding to the 'A' peak described by Schrader *et al.* (1972). During the following 0.08–0.4M linear KCl gradient, a second and larger peak was eluted with a maximum at 0.18M-KCl (form 'B') (Fig. 2). Both these 'A' and 'B' components sedimented at ~4S in a sucrose gradient (Figs. 3a and 3b), but a large proportion of the ligand bound to 'A' dissociated and remained at the top of the gradient. Form 'B'

Fig. 1. Electrophoretic studies of transformed PR
(a) Effect of receptor transformation by high salt on electrophoretic profile of affinity-chromatography-purified preparations. Cytosol (prepared in buffer A) was divided into two equal parts. One was treated for 1 h with 0.3M-KCl, whereas to the other, Na_2MoO_4 was added to 20mM final concentration. Both portions were purified by affinity chromatography as described in the Materials and methods section. After specific elution with ^3H IP from the affinity gel, each sample ($\sim 1.5\mu\text{g}$ of protein) was

Fig. 2. DEAE-Sephacel chromatography of purified PR from high-salt-treated cytosol

The affinity-chromatography eluates of the 0.3M-KCl-treated cytosol were loaded on to a 1 ml DEAE-Sephacel column. After washing with 10 ml of buffer A (starting fraction no. 18), the column was eluted first with 0.08M-KCl in buffer A and then with a linear KCl gradient between 0.08 and 0.4M (50 ml final volume). Fractions (1.2 ml) were collected and 30 μl portions were assayed for radioactivity (O). KCl concentration in the fractions was determined by measuring their conductivity (Δ). The fractions between the double-headed arrows were pooled and referred to as pools A ($\leftrightarrow$) and B ($\leftrightarrow$) respectively.

layered on top of a 7.5–15% acrylamide gradient gel. Lane 1, eluate from the column after chromatography of the KCl-treated cytosol; lane 2, eluate from the column after chromatography of molybdate-stabilized cytosol; lane 3, standard proteins. (b) SDS/polyacrylamide-gel electrophoresis of purified transformed PR components. Cytosol (in buffer A) was precipitated by 30% satd. $(\text{NH}_4)_2\text{SO}_4$. The re-dissolved precipitate was chromatographed on NADAC-Sephacel gel. Its ^3H IP eluate is shown on lane 2 (300 ng of protein). Cytosol in buffer A (+0.3M-KCl) was also chromatographed on NADAC-Sephacel and further purified by DEAE-Sephacel. Lane 3, pool B (see also Fig. 2) of DEAE-Sephacel (200 ng of protein). This preparation was $\sim 95\%$ pure on the basis of specific radioactivity. Lane 4, pool A (see also Fig. 2) of DEAE-Sephacel (400 ng of protein). This preparation was $\sim 25\%$ pure on a specific-radioactivity basis. SDS/polyacrylamide-gel electrophoresis was performed on 7.5% acrylamide gel. Lane 1, standard proteins.

Fig. 3. Sucrose-gradient (5–20%) analysis of PR components obtained from high-salt-treated cytosol. Aliquots (200 μ l) of each 'A' and 'B' radioactive pool from the DEAE-Sephacel chromatography column were ultracentrifuged in 5–20% sucrose gradients (see the Material and methods section). [14 C]Bovine serum albumin (14 C-BSA) was added as internal marker. After centrifugation, gradients were collected and counted for radioactivity. Sedimentation was from right to left.

was about 95% pure as judged by specific radioactivity (Table 1a), and contained a single protein detectable by silver staining of the SDS/polyacryl-

amide slab gel (Fig. 1b, lane 3), migrating at ~ 110 kDa. Its Stokes radius was found to be 6.1 nm by filtration on an Ultrogel AcA-34 column (result not shown), and the calculated frictional ratio (f/f_0) was 1.77. Other authors have reported conflicting data for these parameters; for instance, $R_s = 5.05$ nm and $f/f_0 = 1.55$ (Sherman *et al.*, 1976), as against $R_s = 6.3$ nm, $f/f_0 = 1.9$ (Birnbauer *et al.*, 1981). The 'A' peak never represented more than 25% of total radioactivity recovered from the DEAE-column, and it was unstable upon storage. It was ~ 25 –35% pure (~ 1000 -fold overall purification) on the basis of specific radioactivity (Table 1a).

When purification started with $(\text{NH}_4)_2\text{SO}_4$ precipitation, DEAE-Sephacel chromatography with a linear ionic-strength gradient of the [^3H]P affinity eluate gave a 'B' peak that was eluted at ~ 0.2 M-KCl (results not shown). It contained the 110 kDa protein at $\sim 95\%$ purity, but the yield was low (2–3%; see Table 1b). No peak 'A' was observed when the ionic-strength gradient was used, but some specifically bound radioactivity was found when the column was first eluted with ~ 0.1 M-KCl.

By SDS/polyacrylamide-gel electrophoresis analysis, it was always difficult to detect a 75–80 kDa band in eluates from affinity columns (Fig. 1a, lanes 1 and 2; Fig. 1b, lane 2) and from DEAE-Sephacel (Fig. 1b, lane 4). The detection by antibodies was more successful (see below).

Table 1. Purification of PR components from high-salt-prepared chick oviduct cytosol

	Volume (ml)	Protein (mg/ml)	$10^{-6} \times$ Binding activity* (c.p.m.) (corrected)	$10^{-6} \times$ Specific radioactivity (c.p.m./mg of protein)	Purification (fold)	Yield (%)
(a)						
Cytosol (0.3 M-KCl)	295	13	974	0.254	1	100
Affinity chromatography†	120	0.008	228	237.5	935	24
DEAE-Sephacel‡						
'A'	3.4	0.0272	32.8	355	1396	3.4
'B'	16	0.0068	113.5	1047	4107	12
(b)						
Cytosol (buffer A)	310	11.8	880	0.24	1	100
P ₃₀ §	80	1.4	270	2.4	10	31
Affinity chromatography§	60	0.0032	69	359	1496	8
DEAE-Sephacel	8.8	0.00192	18.3	1084	4516	2.2

* The binding activity was determined as described in the Materials and methods section and is expressed in terms of [^3H]P taking into consideration the isotopic dilution at the affinity-chromatography elution step on the basis of 104 Ci/mmol.

† A 56 ml portion of NADAC affinity gel was loaded with 295 ml of 0.3 M-KCl-treated cytosol. The specific radioactivity of radioactive progesterone used at the elution step was 8.25 Ci/mmol (isotopic dilution 12.6-fold).

‡ 'A' was eluted from the DEAE-Sephacel column by 0.08 M-KCl and form 'B' by a subsequent 50 ml linear 0.08–0.4 M-KCl (see the text).

§ A 30 ml portion of NADAC affinity gel was loaded with 80 ml of redissolved 30%-satd. $(\text{NH}_4)_2\text{SO}_4$ precipitate fraction of cytosol (P₃₀). The specific radioactivity of radioactive progesterone used at the elution step was 19.8 Ci/mmol (isotopic dilution 5.25-fold).

Fig. 4. Western blotting of purified PR with polyclonal (a) and monoclonal (b) antibodies. Blotting with either the goat IgG-G3 polyclonal antibodies (a) or the monoclonal BF4 antibody (b) was performed as indicated in the Materials and methods section. Lane 1, total cytosol in buffer A (10 μ l); lane 2, 30%-satd.-(NH₄)₂SO₄-precipitated cytosol fraction redissolved in one-third the initial volume (10 μ l); lane 3, 110 kDa 'B' from the DEAE-Sephacel chromatography step performed after affinity chromatography of (NH₄)₂SO₄-precipitated cytosol ($\sim$ 5 μ g, 95% pure); lane 4, eluate of affinity chromatography of the (NH₄)₂SO₄ precipitate ($\sim$ 5 μ g of protein).

Detection of PR components with specific antibodies

Protein blots were revealed with polyclonal IgG-G3 and monoclonal BF4 antibodies.

In the original cytosol the polyclonal antibodies IgG-G3 revealed major bands at 110 kDa and 90 kDa. A number of other bands, probably not related to the PR, were also recognized by these antibodies (Fig. 4a, lane 1), namely at $\sim$ 45 kDa (position of ovalbumin), 82 kDa (conalbumin), $\sim$ 70 kDa (serum albumin) and $\sim$ 206 kDa (myosin). Precipitation with 30%-satd. (NH₄)₂SO₄ enriched the 110 kDa band, whereas other bands became weaker, particularly at 90 kDa (Fig. 4a, lane 2). In the affinity-chromatography eluate,

whether after KCl treatment or (NH₄)₂SO₄ precipitation of the cytosol (Fig. 4a, lane 4), the signal at 90 kDa disappeared almost completely, whereas that at 110 kDa remained strong. There was also a band at 75–80 kDa, already visible in the (NH₄)₂SO₄-precipitated preparation, and reinforced after affinity chromatography.

The 'B' peak of DEAE-Sephacel chromatography after the affinity step, which gave a single 110 kDa band on silver-stained gel, showed, in addition to the 110 kDa signal, several lower-*M_r* bands revealed by IgG-G3 (Fig. 4a, lane 3). These smaller polypeptides may have been contaminants present in amounts too low to be detected by staining but sufficient for immunological detection. It is

also possible that these bands represent partial degradation of the receptor during storage.

In blots with the BF4 monoclonal antibody, a strong signal was observed at 90kDa in the crude oviduct cytosol (Fig. 4b, lane 1). However, in the $(\text{NH}_4)_2\text{SO}_4$ precipitate (Fig. 4b, lane 2) and in purified 'B' fraction, no signal was produced by the BF4 monoclonal antibody (Fig. 4b, lanes 4 and 3). Similarly, no BF4-positive signal was detected in purified 'B' fractions obtained from KCl-treated cytosol.

Discussion

According to previous reports from our laboratory (Renoir *et al.*, 1981, 1982a), purification of the non-transformed, molybdate-stabilized 8S PR leads to isolation of a hormone-receptor complex containing a single detectable polypeptide chain of 85–90kDa, detected by Coomassie Blue. Similar data were published by Puri *et al.* (1982). The results reported here demonstrate that the 'B' and 'A' components of the PR (Schrader *et al.*, 1977; Birnbaumer *et al.*, 1981) can also be purified by using the same affinity gel with which we have purified 8S PR. We find M_r values of ~ 110000 and 75000 – 80000 respectively for these two forms obtained when starting from a cytosol preparation in which PR has been transformed by exposure to high KCl concentration or by $(\text{NH}_4)_2\text{SO}_4$ precipitation. No molybdate was used in these high-salt preparations, since it would preclude transformation of the receptor (Wolfson *et al.*, 1980).

The 110kDa protein obtained using the NADAC affinity gel followed by ion-exchange chromatography, was nearly homogeneous on the basis of electrophoretic analysis and specific radioactivity of bound hormone, which was not the case in previous reports (Kuhn *et al.*, 1975; Schrader *et al.*, 1977). The preparative method using KCl-prepared cytosol, affinity chromatography and DEAE-Sephacel enabled us to obtain about 120–150 μg of pure 110kDa protein in 3 days.

The 75–80kDa 'A'-PR could not be purified to more than 20–30%, and was very unstable in terms of binding activity (as already observed by Schrader *et al.*, 1972). Recently, after injection of the 110kDa 'B' protein into a rabbit, we obtained specific antibodies (P. Tuohimaa, J.-M. Renoir, J. Mester & E.-E. Baulieu, unpublished work). The 110kDa and the 75–80kDa proteins do not react with the BF4 antibody, but do react with IgG-G3 polyclonal antibodies raised in the goat after injection of purified non-transformed progesterone receptor.

A clear difference between the electrophoretic profile of the PR purified by affinity chromato-

graphy of the 'activated' receptor (the present paper) or of the non-transformed molybdate-stabilized 8S receptor (Renoir *et al.*, 1982b) is the presence of the 90kDa band in the latter case (Fig. 1a). The results suggest that the 90kDa protein is a non-hormone-binding component of the 8S PR molecule. This is in contradiction with our previous report (Renoir *et al.*, 1982a), when we found only one Coomassie Blue-stained band of M_r 85000, and concluded that the purified non-transformed PR may be composed only of 85kDa subunits. This conclusion was wrong, possibly due to differential staining of different proteins in quantitative assays, as already suggested by Puri *et al.* (1982). Our recent data (Baulieu *et al.*, 1983) show that the 8S-PR molecules are heterogeneous, containing the 'A' and/or 'B' subunit(s) plus the 90kDa protein. We have recently observed that the BF4 monoclonal antibody interacts with the progesterone-binding 8S PR (whether in presence of molybdate or not), but after salt-induced transformation of the receptor it only interacted with a component which does not bind progesterone (Joab *et al.*, 1983) and is a 90kDa polypeptide (Fig. 4). The association between this 90kDa polypeptide and the hormone-binding proteins (110kDa and 75–80kDa) in 8S PR, is apparently strong, since it is resistant to conditions such as high ionic strength (Yang *et al.*, 1982) or 2.5M-urea (Buchou *et al.*, 1983) when stabilized by 20mM- Na_2MoO_4 . It is unlikely that the 8S PR complexes could be an artefact due to addition of molybdate to the cytosol, since they are also found in absence of molybdate in cytosol and, when complexes are dissociated, for instance by high-salt treatment, they do not re-form by dialysis against low-salt, molybdate-containing buffer (Yang *et al.*, 1982). The fact that the 90kDa protein belongs to the untransformed 8S PR is confirmed by a recent experiment showing no 90kDa protein in the eluate of affinity chromatography of molybdate-containing cytosol preincubated with 2 μM -progesterone (Baulieu *et al.*, 1983). This indicates that its presence in the purified 8S PR depends on receptor binding to the steroid residues of the affinity column. Finally the observations obtained with IgG-G3 antibodies lead us to conclude that the 'A' and 'B' forms were present in the purified 8S molybdate-stabilized PR preparations used to immunize the goat.

Altogether these results demonstrate the purification of the hormone-binding 110kDa 'B' subunit of the chick oviduct PR, and suggest that the non-progesterone-binding 90kDa component is only present in the non-transformed 8S form of PR. It is further suggested that the progesterone-binding and non-progesterone-binding components of the PR are immunologically unrelated.

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