The Molybdate-stabilized L-cell Glucocorticoid Receptor Isolated by Affinity Chromatography or with a Monoclonal Antibody Is Associated with a 90–92-kDa Nonsteroid-binding Phosphoprotein*

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We have previously reported that molybdate-stabilized cytosol prepared from 32P-labeled L-cells contains two phosphoproteins (a 90–92- and a 98–100-kDa protein) that elute from an affinity resin of deoxycorticosterone-derivatized agarose in a manner consistent with the predicted behavior of the glucocorticoid receptor (Housley, P. R., and Pratt, W. B. (1983) J. Biol. Chem. 258, 4630–4635). In the present work we report that both the 90–92- and 98–100-kDa 32P-labeled proteins are also extracted from molybdate-stabilized cytosol by incubation with a monoclonal antibody and protein A-Sepharose. Only the 98–100-kDa protein is specifically labeled when either L-cell cytosol or L-cell cytosol proteins bound to the affinity resin are labeled with the glucocorticoid binding site-specific affinity ligand [3H]dexamethasone 21-mesylate. The 98–100-kDa protein labeled with [3H]dexamethasone mesylate is adsorbed to protein A-Sepharose in an immune-specific manner after reaction with the monoclonal antibody. Sodium dodecyl sulfate-polyacrylamide gel analysis of the protein A-Sepharose-bound material resulting from incubating the monoclonal antibody with a mixture of 32P-labeled cytosol and [3H]dexamethasone mesylate-labeled cytosol demonstrates identity of the 98–100-kDa [3H]dexamethasone mesylate-labeled band with the 98–100-kDa 32P-labeled band and clear separation from the nonsteroid-binding 90–92-kDa phosphoprotein. The results of immunoblot experiments demonstrate that the 90–92-kDa protein is structurally distinct from the 98–100-kDa nonsteroid-binding protein. As the 90–92-kDa nonsteroid-binding phosphoprotein co-purified with the 98–100-kDa uncleaved form of the glucocorticoid receptor by two independent methods, one of which is based on recognizing a steroid-binding site and the other of which is based on recognizing an antibody binding site, we propose that the 90–92-kDa phosphoprotein is a component of the molybdate-stabilized, untransformed glucocorticoid-receptor complex in L-cell cytosol.

Three recent advances in technology have facilitated considerable progress in achieving an understanding of the molecular nature of glucocorticoid receptors. These advances include the development of [3H]dexamethasone 21-mesylate, a site-specific affinity label for the receptor (1), the synthesis of deoxycorticosterone-agarose (2), an affinity matrix that has facilitated rapid purification of the receptor, and the development of monoclonal antibodies to the receptor (3). Glucocorticoid receptors in a variety of cytosol preparations are subject to proteolytic cleavage (4), and reports of Mr, for glucocorticoid receptors identified by site-specific affinity labeling or by affinity chromatography have varied somewhat. Some laboratories have reported values for Mr of uncleaved receptor to be in the range of 97,000-90,000 (5–8) whereas others have reported an Mr of about 98,000 (9, 10). Most of the determinations of Mr for the glucocorticoid receptor have been made on receptors in cytosol prepared from normal rat liver or from transformed rat liver (HTC) cells (5–8, 10). The assignment of the Mr for the glucocorticoid receptor is important, as preparations of rat liver receptor that have been purified by affinity chromatography or deoxycorticosterone-derivatized agarose contain a major protein of about 90-kDa that migrates slightly faster than the phosphorylase marker (97-kDa) in denaturing gels (8). This 90-kDa protein, which behaves like the [3H]triamcinolone acetonide-bound receptor on further purification by chromatography on Bio-Gel A-1.5m and DEAE-cellulose in the presence of molybdate (8), is labeled with 32P when it is purified from the livers of rats injected with 32Porthophosphate prior to killing (11).

We have recently reported similar observations for receptors in mouse L-cell cytosol (12). When L-cell cytosol was incubated with [3H]dexamethasone 21-mesylate, two peaks of specifically bound radioactivity were identified on subsequent separation of cytosol proteins by SDS-polyacrylamide gel electrophoresis. A minor species migrated slightly slower than the phosphorylase marker at about 100-kDa and a major species migrated slightly faster with an Mr in the range of 92,000. At the time this work was published, it was not clear if the minor 100-kDa species represented the intact receptor and the major 92-kDa species represented a cleavage product, or if the two species were products of different genes. We also reported (12) that cytosol prepared from L929 mouse fibro-

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1 The trivial names and abbreviations used are: dexamethasone, 9a-fluoro-16α-methyl-11β,17α,21-trihydroxyprogesterone-1,4-diene-3,20-dione; 87-kDa, 90-kDa, etc. refer to bands on sodium dodecyl sulfate-gel electrophoresis of 87,000, 90,000 kilodaltons, etc; triamcinolone acetonide, 9a-fluoro-16α-methyl-11β,17α,21-trihydroxyprogesterone-1,4-diene-3,20-dione; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; TES, N-tris(hydroxymethyl)methyl-2-amino-ethanesulfonic acid; DTT, dithiothreitol.
blasts cultured for 18 h in the presence of $^{32}$P orthophosphate contains a major phosphoprotein of $M_r \sim 92,000$ and a minor phosphoprotein of $M_r \sim 100,000$ that are eluted in a stereospecific manner from deoxycorticosterone-derivatized agarose. The 92-kDa $^{32}$P-labeled band migrates identically with a major protein in the affinity-purified receptor preparation that is demonstrable on SDS-polyacrylamide gels by staining with Coomassie Blue.

The observations made in both the mouse L-cell and rat liver systems have led to the proposal that the glucocorticoid receptor is a phosphoprotein with an $M_r$ of 90,000–92,000. It is critical that the relationship between the 90–92-kDa phosphoprotein and the glucocorticoid receptor be established in an unequivocal manner before studies of protein structure and phosphorylation are carried out. In this paper we use both affinity chromatography and a monoclonal antibody to demonstrate that the glucocorticoid receptor in mouse L-cell cytosol is a phosphoprotein of 98–100-kDa that is extracted from molybdate-stabilized cytosol by both the affinity resin and the antibody in association with a 90–92-kDa phosphoprotein that does not bind $[^3H]$dexamethasone mesylate. We would propose that the 90–92-kDa nonsteroid-binding phosphoprotein is part of the untransformed, molybdate-stabilized glucocorticoid-receptor complex. A similar 90-kDa nonsteroid-binding phosphoprotein appears to be a component of the molybdate-stabilized progesterone-receptor complex from chick oviduct (13, 14).

**RESULTS AND DISCUSSION**

**Purification of $^{32}$P-labeled L-cell Proteins by Steroid Affinity Chromatography**—In a previous study we reported that most of the specifically bound $[^3H]$dexamethasone mesylate in L-cell cytosol migrated as a 90–92-kDa species in SDS-polyacrylamide gels (12). As the major $^{32}$P-labeled protein eluted from deoxycorticosterone-agarose also migrated at 90–92-kDa, we suggested that this was the major form of the receptor. Accordingly, we wanted to purify the 90–92-kDa phosphoprotein so that we could study it in greater detail.

**As shown in Lane 1 of Fig. 1, molybdate-containing cytosol prepared from L-cells grown in the presence of $^{32}$P, contains two $^{32}$P-labeled bands that bind to deoxycorticosterone-agarose and migrate in the region of the phosphorylase marker (97-kDa) on SDS-polyacrylamide gel electrophoresis. When the material eluted from the affinity column is chromatographed on Bio-Gel A.5m in the presence of molybdate, the 90–92-kDa phosphoprotein is recovered in the fractions comprising the void volume peak (Fig. 1, Lane 2). The material in the Bio-Gel peak was then concentrated, adsorbed to DEAE-Sephasel, and eluted with a NaCl gradient. The 90–92-kDa, $^{32}$P-labeled material is eluted in the pooled fractions between 100 and 250 mM NaCl (Fig. 1, Lane 6). The $^{32}$P-labeled band on the autoradiogram superimposes on the only protein band in this fraction that is readily identified by staining with Coomassie Blue (stained bands not shown). Several questions are raised by the results presented in Fig. 1 and they will be addressed sequentially.

**Labeling of the 98–100-kDa Protein with $[^3H]$Dexamethasone Mesylate**—As shown in Fig. 2, the $[^3H]$triamcinolone acetonide-receptor complex in L-cell cytosol containing sodium molybdate is eluted from Bio-Gel A.5m in the void volume region and it elutes from DEAE-Sephasel as a single peak between 180 and 250 mM NaCl. Thus, the 90–92-kDa phosphoprotein eluted from deoxycorticosterone-agarose behaves according to both its size and charge characteristics in a manner that would be expected for the L-cell glucocorticoid-receptor complex under molybdate-stabilized conditions. In their purification of the molybdate-stabilized rat liver glucocorticoid receptor, Grandics et al. (8, 11) were able to elute the glucocorticoid receptor from deoxycorticosterone-agarose with $[^3H]$triamcinolone acetonide and show that the resulting noncovalent steroid-receptor complex eluted as a large species.
near the void volume on subsequent chromatography on Bio-Gel A1.5m using molybdate-containing buffer. When the Bio-Gel eluate was chromatographed on DEAE-cellulose, it eluted at 0.23–0.26 M potassium phosphate in the same manner as the 90-kDa phosphoprotein. As the [3H]triamcinolone acetonide-bound receptor behaved during the various separation procedures like the 90-kDa phosphoprotein, it was concluded that the 90-kDa phosphoprotein was the receptor. When we attempted to elute the L-cell receptor from the affinity matrix with [3H]triamcinolone acetonide, we were able to identify a small amount of [3H]triamcinolone acetonide bound to macromolecular components eluting from the Bio-Gel column, but after contraction of these fractions and application to theDEAE column, there was no radioactivity remaining in the bound form.

We reasoned that the only way we could directly demonstrate that the glucocorticoid receptor was present in the material recovered from the affinity column was to elute the column with [3H]dexamethasone mesylate and demonstrate the covalent complex on a denaturing gel. As shown in Lane 1 of Fig. 3, when the affinity matrix is eluted with 2.5 μM [3H]dexamethasone mesylate and subsequently chromatographed through Bio-Gel A.5m, two major tritium-labeled bands can be identified after gel electrophoresis and autoradiography. One band elutes just above the phosphorylase marker in the 98–100-kDa region and it is clearly different from the 90–92-kDa protein, which can be easily identified in the gel by Coomassie Blue staining (stained bands not shown). As shown in Lane 2, the 98–100-kDa band is not visible if the affinity column was eluted with [3H]dexamethasone mesylate in the presence of a 40-fold excess of nonradioactive dexamethasone. Because we have to use micromolar amounts of steroid to elute receptor from the affinity resin (12), we have used a very high concentration (100 μM) of nonradioactive competing steroid to try to demonstrate specific binding of [3H]dexamethasone mesylate to the receptor. The concentration of competing steroid is so high here that it could be producing nonspecific detergent effects. The mesylate function can react with sulfhydryl moieties in a variety of proteins other than the glucocorticoid receptor and it is possible that such a nonspecific reaction is responsible for producing the major band eluted below the 66-kDa marker protein. At least, it can be said from this experiment that [3H]dexamethasone mesylate is binding covalently to a 98–100-kDa component that binds to the affinity matrix and not to a 90–92-kDa component.

**Isolation of the [3H]Dexamethasone Mesylate-labeled 98-100-kDa Protein with Monoclonal Antibody and Protein A-Sepharose**—While we were proceeding with experiments designed to purify the 90–92-kDa phosphoprotein, we noted that we were recovering more 32P radioactivity in the 98–100-kDa region of the eluate from the affinity column than we had in our original studies (c.f. Lane 1 of Fig. 1 with Fig. 3 of Housley and Pratt (12)). This increase in intensity of the 98–100-kDa band was accompanied by some decrease in the intensity of the 32P-labeled in the 90–92-kDa band, suggesting to us that we might be having less cleavage of receptor from the 98–100-kDa to the 90–92-kDa species. In order to see if this shift towards a higher apparent M, was accompanied by a similar shift in the apparent M, of L-cell components labeled in a specific manner with [3H]dexamethasone mesylate, we again examined the binding of this site-specific affinity ligand in L-cell cytosol. To isolate the receptor-bound [3H]dexamethasone mesylate from other components of cytosol we used the monoclonal antibody GR49. We have described this antibody previously (3) and Table I shows its titre against the glucocorticoid-receptor complex in L-cell cytosol determined by a double antibody precipitation technique. As shown in Lane 1 of Fig. 4, we found that [3H]dexamethasone mesylate binds covalently to a 98–100-kDa protein that migrates slightly slower than the phosphorylase marker. If cytosol prebound with [3H]dexamethasone mesylate is incubated with the monoclonal antibody and adsorbed to protein A-Sepharose, the radioactivity bound to the 98–100-kDa protein becomes associated with protein A-Sepharose in an immune-specific manner. In the lanes containing the monoclonal antibody (Lanes 2,3, and 6 in this gel) there is no labeling of a 90–92-kDa component by [3H]dexamethasone mesylate but there is a Coomassie Blue-stained band (not shown) that migrates at 90–92-kDa.

After these experiments, it was clear that the site-specific affinity steroid had covalently labeled a 98–100-kDa protein in cytosol and that the same size component was labeled in the eluate from deoxycorticosterone-agarose (Fig. 3). Since our procedures for preparing L-cell cytosol and labeling with [3H]dexamethasone mesylate have not changed, it is not clear why only a minor portion of the receptor was previously recovered as the undegraded 98–100-kDa species and now most of the [3H]dexamethasone mesylate-labeled material is...
just ahead of the marker at 90-92-kDa and superimposes on
polyacrylamide gel electrophoresis and visualized by autoradiography
of the EN3HANCE gel. Antibody and Protein A-Sepharose—As shown in Fig. 5, when
protein A-Sepharose. L-cell cytosol prepared in Buffer B was
charcoal to remove free steroid. Aliquots (200 µl) were incubated with
10 µl of either monoclonal antibody (1.4 mg/ml) or nonimmune mouse
IgG (1.4 mg/ml) and adsorbed to protein A-Sepharose as described
under “Experimental Procedures.” Samples were resolved by SDS-
polyacrylamide gel electrophoresis and visualized by autoradiography
of the EN3HANCE gel. Lane 1, aliquot of untreated [3H]dexametha-
sone mesylate-labeled cytosol; Lane 2, monoclonal antibody sample
after protein A-Sepharose step where the protein A-Sepharose pellet
was washed as described under “Experimental Procedures” using
buffer in place of salt washes; Lane 3, same as Lane 2 except
that salt washes were included; Lane 4, nonimmune mouse IgG sample
washed without salt; Lane 5, same as Lane 4 except that salt washes
were included; Lane 6, monoclonal antibody sample precipitated with
rabbit antiserum IgG and washed as described in Table I, Lane 7,
nonimmune mouse IgG sample precipitated with second antibody as
in Lane 6.

recovered as the undegraded higher Mₙ species. It is curious
that a similar change in observed Mₙ has been reported for
the [3H]dexamethasone mesylate-labeled glucocorticoid recep-
tor in rat HTC cells. Simons et al. (7) originally reported
an apparent Mₙ of 89,900 for the HTC cell receptor. Recently,
however, the same laboratory has found that the [3H]dexametha-
sone mesylate-labeled HTC cell receptor is migrating
slightly slower than the phosphorylase marker, with an Mₙ of
98,600 ± 1,500 (S.D.) as determined for 28 separate prepara-
tions (10). It is useful to know that glucocorticoid receptors
in two mouse lymphoma cell lines (WEHI-7 and S49.1) have
been photoaffinity labeled with [3H]triamcinolone acetonide
and shown to migrate slightly slower than the phosphorylase
marker with an apparent Mₙ of 98,000 in SDS gels (9).

Isolation of Two ³²P-labeled L-cell Proteins with Monoclonal
Antibody and Protein A-Sepharose—As shown in Fig. 5, when
cytosol prepared from ³²P-labeled L-cells is incubated with
the monoclonal antibody, two phosphoproteins are subse-
quent bound to protein A-Sepharose. One phosphoprotein
migrates slightly slower than the phosphorylase marker in the
region of 98–100-kDa. The other phosphoprotein migrates
just ahead of the marker at 90–92-kDa and superimposes on
a Coomassie Blue-stained protein band (stained bands not
shown). There is much more of the ³²P-labeled and Coomassie
Blue staining material in Lanes 1 and 2 which contain the
monoclonal antibody than in Lane 3 which contains nonim-
mune mouse IgG. It is important to note, however, that the
90–92-kDa phosphoprotein is a major component of cytosol
and some of this material adsorbs to Protein A-Sepharose
even if no mouse IgG (i.e. neither monoclonal antibody nor
nonimmune IgG) is present (Lane 4).

In the experiment of Fig. 6, L-cell cytosol labeled with [3H]
dexamethasone mesylate, or ³²P-labeled cytosol, or a mixture
of the two preparations was incubated with the monoclonal
antibody and protein A-Sepharose. Although it was difficult
to achieve the optimal proportions of each labeled cytosol
to react with antibody and put on the gel lane, there is a line of
continuity between the [3H]dexamethasone mesylate-labeled
band in Lane 2, a ³²P-labeled band in Lane 3, and the upper
band in the doublet recovered from the mixture of [3H]
dexamethasone mesylate-labeled and ³²P-labeled cytosols in
Lane 4. Again, by this method the dexamethasone mesylate-
labeled protein is distinct from 90–92-kDa phosphoprotein.

The Monoclonal Antibody Reacts Only with the 98–100-kDa
Steroid-binding Protein—It is clear from the immunoblot
shown in Fig. 7 that the monoclonal antibody reacts only with
the 98–100-kDa protein. The fact that the 90–92-kDa protein
is not immunoreactive in this procedure provides strong evi-
dence that it is structurally distinct from the steroid-binding
phosphoprotein with which it co-purifies. In Lane 7 of the gel
shown in Fig. 7 some L-cell cytosol that had been prepared several months earlier in the Marburg laboratory was unfrozen, boiled with SDS sample buffer, and directly applied to the gel. The main immunoreactive band in this sample has an apparent Mr of about 90-kDa and this partially degraded form of the receptor superimposes on the 90–92-kDa nonsteroid-binding phosphoprotein. Thus, it is easy to appreciate how the two proteins have been assumed to be identical.

**Immunoadsorption of the Two 32P-Labeled Proteins from the Affinity Column Eluate**—We have tried on several occasions to use the monoclonal antibody to immunoabsorb receptor from the affinity column eluate but have not been successful. We have a polyclonal rabbit antiserum prepared against the L-cell glucocorticoid receptor which is more effective than the monoclonal antibody in the immunoadsorption of receptor from cytosol. As shown in Lanes 3 and 4 of Fig. 8, the rabbit antiserum causes the immune-specific adsorption of both the 98–100-kDa and 90–92-kDa bands from the eluate of the affinity column. If molybdate is not present to stabilize the complex during incubation with antibody and washing of the protein A-Sepharose pellet, the rabbit antiserum does not cause immunoadsorption of the 90–92-kDa phosphoprotein.

**Other 32P-Labeled Components of L-cell Cytosol Present in the Affinity Column Eluate**—Molybdate stabilized, untransformed glucocorticoid receptor complexes have been reported
to behave as 9–10 S units with a molecular mass in the range of 300–350-kDa, whereas the transformed, DNA-binding state of the receptor has been reported to behave as a 4–5 S unit with a molecular mass in the range of 90–100-kDa (4, 8, 17, 20). Several investigators have speculated that the molybdate-stabilized untransformed unit may exist as a tetramer composed of four identical steroid-binding subunits (4, 8, 11). Therefore, we wanted to know if any of them were possible components of the molybdate-stabilized glucocorticoid-receptor complex. In this regard, Grandics et al. (8) have found a protein doublet at 40–41-kDa that co-purifies through chromatography on deoxytocorticosterone-agarose, Bio-Gel A1.5m, and DEAE-cellulose with the 90-kDa protein in rat liver cytosol. In addition, they have found a band at 24-kDa that elutes from DEAE-cellulose at a salt concentration higher than that of the bound radioactivity peak itself. When cytosol is preincubated with 50 nM triamcinolone acetonide to occupy receptor sites prior to adsorption with the affinity resin, the 40–41-kDa doublet and the 24-kDa band were missing from subsequent material purified through DEAE-cellulose. As saturation of specific binding sites with a high affinity glucocorticoid prior to purification resulted in a 95% decrease in the 90-kDa band and disappearance of the 40, 41, and 24-kDa bands, Grandics et al. (8) suggested that all four components are related as components of or degradation products of the molybdate-stabilized glucocorticoid-receptor complex of rat liver. Grandics et al. (11) later reported that the 24-kDa material is heavily phosphorylated, and does not stain with Coomassie Blue, and they have suggested that it is a small polynucleotide.

The observations that Grandics et al. (8, 11) have made in rat liver cytosol are very similar to our observations in mouse L-cell cytosol except that we have not been able to eliminate the low M_r, 32P-labeled components from the affinity resin eluate by preincubating cytosol with 50 nM triamcinolone acetonide. If the small M_r phosphorylated bands shown in Figs. 1 and 9 are components of the molybdate-stabilized, untransformed receptor complex, then they should bind to protein A-Sepharose when 32P-labeled cytosol containing molybdate is incubated with the monoclonal antibody. As presented in Fig. 10A, the only 32P-labeled components of L-cell cytosol that are associated with protein A-Sepharose in a manner that is specific for the monoclonal antibody are the 90–92-kDa and 98–100-kDa phosphoproteins. The salt wash of the protein A-Sepharose pellet contains the broad intensely 32P-labeled band in the 21–25-kDa region shown in Figs. 1 and 9. This material is present in the salt wash of protein A-Sepharose regardless of whether the monoclonal antibody, nonimmune mouse IgG, or no immunoglobulin is present in the system (Fig. 10, A and B). Thus, we would conclude that this 32P-labeled band represents a prominent phosphorylated component of L-cell cytosol that binds to the affinity resin and to protein A-Sepharose in a manner that is not specific for the molybdate-stabilized, untransformed glucocorticoid receptor. We have extracted the Bio-Gel material with phenol-chloroform-isooamyl alcohol and found that the broad 32P-labeled band that migrates at 21–25-kDa in SDS-polyacrylamide gels co-migrates with marker yeast tRNA in a 1.1% agarose gel under denaturing conditions (not shown).

One observation presented in Fig. 1 that we have not yet explained is the loss of the 32P-labeled 98–100-kDa band that occurs during subsequent purification. The intensity of the 98–100-kDa band decreases during the Bio-Gel procedure and is always lost by the DEAE-Sephacel step of purification. As suggested perhaps by the presence of [3H]dexamethasone mesylate-labeled bands that are smaller than 98–100-kDa in the experiment shown in Fig. 3, receptor cleavage may be occurring during the relatively long time it takes to run the Bio-Gel column and contract the macromolecular fractions. The degree of loss of the 98–100-kDa 32P radioactivity during the Bio-Gel step is variable. In the experiment of Fig. 9, for

![Fig. 9. Protease and ribonuclease sensitivity of 32P-labeled components of the Bio-Gel eluate.](image-url)
were resolved by SDS-polyacrylamide gel electrophoresis and autoradiography. Part A: Lane 1, monoclonal antibody-protein A-Sepharose pellet washed as described under “Experimental Procedures” using buffer washes in place of salt washes; Lane 2, nonimmune mouse IgG pellet washed as in Lane 1; Lane 3, monoclonal antibody pellet washed with salt as described under “Experimental Procedures”; Lane 4, nonimmune mouse IgG pellet washed with salt as in Lane 3; Lane 5, salt wash of monoclonal antibody-protein A-Sepharose pellet shown in Lane 3 after concentration with a Centricron-10 (Amicon); Lane 6, salt wash of nonimmune IgG pellet shown in Lane 4. Part B: Lane 1, protein A-Sepharose pellet of 32P-labeled cytosol with no immunoglobulin addition after washing without salt; Lane 2, same as Lane 1 but washed with salt; Lane 3, salt wash of protein A-Sepharose pellet shown in Lane 2. Samples were run on 10% SDS-polyacrylamide gels.

example, there was little loss of radioactivity in the 98–100-kDa region, even when the samples were incubated at 30 °C for 30 min. It is possible that some of the 98–100-kDa protein is dissociating from a large complex during Bio-Gel chromatography and it is possible that dephosphorylation of the 98–100-kDa protein occurs during purification. It will probably be necessary to elute 32P-labeled receptors from the affinity resin with [3H]dexamethasone mesylate and further purify the double-labeled material to determine whether dephosphorylation or proteolysis, etc., is occurring. As we have previously recovered the majority of the [3H]dexamethasone mesylate-bound material coincident with the 90–92-kDa Coomassie Blue-stained phosphoprotein band (12), it is entirely possible that the DEAE-purified material shown in Lane 6 of Fig. 1 contains both the 90–92-kDa phosphoprotein and cleaved receptor.

A Two Component Structural Model of the Receptor—As the 90–92-kDa, nonsteroid-binding phosphoprotein co-purifies with the receptor by two independent methods, one of which is based on recognizing a steroid-binding site and the other on recognizing an antibody binding site, we would propose that the 90–92-kDa protein is a component of the molybdate-stabilized, untransformed glucocorticoid-receptor complex in L-cell cytosol. This conclusion agrees with that of Mendel2 who has used a different monoclonal antibodydirected against the glucocorticoid receptor to identify a 90-kDa nonsteroid-binding phosphoprotein which co-purified with the [3H]dexamethasone mesylate-labeled glucocorticoid receptor from molybdate-stabilized mouse thymus cell (WEHI-7) cytosol. The proof is not absolute, as preincubation of cytosol with a receptor-saturating concentration of triamcinolone acetonide prior to affinity chromatography does not completely eliminate the 32P-labeled 90–92-kDa band (8, 12) and as a small amount of 90–92-kDa protein is bound to protein A-Sepharose when nonimmune mouse IgG or even no IgG is present (Fig. 5). Nevertheless, when taken together, the observations presented in this paper provide strong evidence that the molybdate-stabilized, untransformed glucocorticoid receptor must exist in some kind of association with a 90–92-kDa, nonsteroid-binding phosphoprotein that appears to be present in cytosol in rather large amounts. If this proposal is correct, a model that describes the untransformed glucocorticoid receptor as a tetramer of four identical steroid-binding subunits cannot be correct.

It is important to note that a 90-kDa, nonsteroid-binding phosphoprotein has been shown to co-purify with the molybdate-stabilized avian progesterone receptor. Dougherty et al. (14) have purified two 8 S forms of the molybdate-stabilized progesterone receptor from chicken oviduct by affinity chromatography using deoxycorticosterone-agarose. Form I contains a 75–79-kDa steroid-binding protein, Form II contains a 110-kDa steroid-binding phosphoprotein, and both forms contain a 90-kDa nonsteroid-binding phosphoprotein. Radanyi et al. (22) have prepared a monoclonal antibody to the molybdate-stabilized chicken oviduct progesterone receptor and have shown that it reacts with the molybdate-stabilized 8 S form of the chicken oviduct receptor labeled with either [3H]progesterone or with [3H]ORG 2058 (a high-affinity synthetetic progestin). This monoclonal antibody is directed against the 90-kDa nonsteroid-binding protein and does not react with the purified 75–79- or 110-kDa progesterone-binding units (13). Similarly, a polyclonal antibody raised against the purified 110-kDa protein, which recognizes both the 110- and 75–79-kDa progesterone-binding proteins, reacts with the molybdate-stabilized 8 S form of the progesterone receptor but does not interact with the 90-kDa nonsteroid-binding component (23).

There is a great deal of similarity between the observations made with the chick oviduct progesterone receptor and the data on the mouse fibroblast glucocorticoid receptor presented in this paper. The observations support a general model in which the molybdate-stabilized, untransformed state of both glucocorticoid and progesterone receptors contains a 90-kDa nonsteroid-binding phosphoprotein in addition to a steroid-binding phosphoprotein. The stoichiometry of the two phosphoproteins in the complex is not known and it is possible that other nonsteroid-binding proteins that have not yet been identified are also components of the untransformed receptor complex.

Acknowledgments—We would like to thank Dirk Mendell and Allan Munck for their helpful advice during the course of this work.

REFERENCES

2 D. Mendel, personal communication.
A Glucocorticoid Receptor-associated 90-kDa Phosphoprotein


Supplementary Material

Table I

Immunoprecipitation of the mouse L-cell glucocorticoid receptor with monoclonal antibody and rabbit antiserum 90

| Antibody | Saline-Controlled | Adipose-Receptor Complex | 90 kDa Phosphoprotein
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<td>None</td>
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<td>Mouse 90</td>
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<td>Rabbit 90</td>
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Figure 2. Chromatography of the [3H]triamcinolone-acetonide-bound L-cell receptor on Bio-Gel A.5m and Sephacryl S-300. L-cell cytosol was incubated with [3H]triamcinolone-acetonide plus insulin (0.05 nM) or 0.037 nM tritiated glucocorticoid receptor (0.2 nM) at 4°C for 4 h to form tritiated receptor complexes. The complexes were adsorbed to Bio-Gel A.5m (A) or Sephacryl S-300 (B) columns and fractionated at 0°C. Data are mean values ± SD.