Immunoochemical Characterization of the Rat Kidney Glucocorticoid Receptor

THE ROLE OF PROTEOLYSIS IN THE FORMATION OF CORTICOSTEROID BINDER IB*

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Glucocorticoid receptors of rat kidney and liver were compared by physicochemical and immunoochemical methods to investigate the role of proteolysis in the formation of corticosteroid binder IB. Kidney cytosol prepared in the presence of sodium molybdate contained receptor forms comparable to rat liver glucocorticoid receptor; [3H]triamcinolone acetonide-labeled receptors eluted from Sephacryl S-300 as a multimeric 6.1 nm component in the presence of molybdate and as a monomeric 8.7 nm component in the absence of molybdate. Both forms were recognized by the monoclonal antibody BUGR-1 which was raised against rat liver glucocorticoid receptor. When kidney cytosol was prepared in the absence of molybdate, labeled receptor complexes eluted from Sephacryl S-300 as a 5.8 nm component in the presence of molybdate. However, in the absence of molybdate, the receptor eluted as a smaller 3.4 nm component which was identical in size to that seen in activated kidney glucocorticoid receptor. Identification of the immunoreactive receptor subunit by Western blotting demonstrated that kidney cytosol prepared in the presence of molybdate contained a major 94-kDa immunoreactive component which co-migrated with rat liver glucocorticoid receptor, while cytosol prepared in the absence of molybdate contained principally a 44-kDa immunoreactive species. These results suggest that corticosteroid binder IB can be generated by in vitro proteolysis and does not represent a polymorphic form of the glucocorticoid receptor.

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that the smaller, atypical forms of the rat kidney glucocorticoid receptor seen in cytosols prepared in the absence of sodium molybdate are the result of proteolysis and do not represent the expression of tissue-specific receptors.

**EXPERIMENTAL PROCEDURES**

**RESULTS**

Interaction of BUGR-1 with Rat Liver and Rat Kidney Glucocorticoid Receptor—The interaction between the monoclonal antibody BUGR-1 (raised against rat liver glucocorticoid receptor) and rat liver receptor has previously been shown to result in an increase in the sedimentation coefficient of the steroid-receptor complex in sucrose gradients from 4 S to approximately 6 S (28). This interaction can also be detected by gel filtration of steroid-receptor complexes on Sephacryl S-300. Incubation of \(^{3}H\)TA-labeled rat liver receptor with BUGR-1 resulted in a shift in the elution position of labeled complexes from an apparent Stokes radius of \(\sim 7.1\) nm to \(\sim 8.5\) nm (Fig. 1A). The results presented in Fig. 1A presumably reflect the interaction of BUGR-1 with the multimeric, unactivated form of the steroid-receptor complex, since chromatography was performed in buffer containing 20 mM sodium molybdate. When chromatography was performed in high salt buffer in the absence of sodium molybdate, conditions shown to promote receptor subunit dissociation and steroid-receptor complex activation (32, 33), incubation with BUGR-1 resulted in a shift in the elution of \(^{3}H\)TA-labeled complexes from \(\sim 5.6\) nm to \(\sim 7.2\) nm (Fig. 1B). These results demonstrate that BUGR-1 reacts with both the multimeric and monomeric forms of the rat liver glucocorticoid receptor and that chromatography on Sephacryl S-300 can readily resolve steroid-receptor complexes to which the antibody is bound. They also suggest that the binding of BUGR-1 to the rat liver receptor does not interfere with receptor dissociation during chromatography in high salt in the absence of molybdate.

Examination of the interaction between BUGR-1 and rat kidney cytosol revealed that BUGR-1 also reacted with both the multimeric and monomeric forms of the kidney receptor. \(^{3}H\)TA-labeled kidney glucocorticoid receptor chromatographed in the presence of sodium molybdate eluted as a single component of \(R_c \sim 6.1\) nm, while incubation of labeled cytosol with BUGR-1 prior to chromatography resulted in the elution of the labeled complex as a larger component (Fig. 2A). Likewise, the monomeric kidney receptor seen after chromatography in the absence of molybdate (\(R_c \sim 5.7\) nm) was observed to elute as a larger species (\(R_c \sim 7.1\) nm) after cytosol was incubated with BUGR-1 (Fig. 2B).

Since TA can also bind to mineralocorticoid receptors, it was important to demonstrate that the binding seen in Fig. 2 was the result of \(^{3}H\)TA binding to the glucocorticoid receptor. Cytosol was therefore labeled with \(^{3}H\)TA in the presence of 5 \(\mu\)M unlabeled RU28362. This synthetic steroid has high affinity for the glucocorticoid receptor, but does not bind to the mineralocorticoid receptor (34). When rat kidney cytosol labeled in the presence of RU28362 was chromatographed on Sephacryl S-300, no high molecular weight material was detected (Fig. 2). Thus, all of the high molecular weight steroid-receptor complexes seen in Fig. 2 represent the binding of...
[3H]TA to the rat kidney glucocorticoid receptor.

In some experiments with rat kidney cytosol, a small amount of a low molecular weight peak was also detected (Fig. 2). However, the labeling of this peak could not be competed with either RU28362 or aldosterone and therefore does not appear to represent the binding of [3H]TA to either the glucocorticoid or the mineralocorticoid receptor (data not shown).

Identification of Smaller Corticosteroid Binding Proteins in Kidney—In the experiments described above, cytosol was prepared from tissues homogenized in the presence of 20 mM sodium molybdate. However, binder IB and other smaller glucocorticoid binding forms have been identified principally in cytosols prepared in the absence of molybdate. We therefore prepared kidney cytosol in the absence of molybdate and examined the chromatographic profiles of [3H]TA-labeled cytosol on Sephacryl S-300 columns run in the presence or absence of molybdate. The results of these experiments indicated that receptor obtained from tissue homogenized in the absence of sodium molybdate appeared to be slightly smaller when chromatographed in the presence of molybdate (Rc ~ 5.8 nm, Fig. 3A) than receptor obtained from tissue homogenized in the presence of sodium molybdate (Rc ~ 6.1 nm, Fig. 2A).

A more striking difference was observed when [3H]TA-labeled cytosol prepared from kidneys homogenized in the absence of molybdate was chromatographed without molybdate. In this case, the [3H]TA-labeled material eluted as an Rc ~ 3.4 nm peak (Fig. 3B). This was considerably smaller than the apparent size of labeled complexes from cytosols prepared in the presence of molybdate (Fig. 2B) and is comparable to the size reported for the "activated" kidney receptor by Sherman et al. (35). All of the radioactivity in this peak appeared to be associated with glucocorticoid receptor since the labeling of this peak was completely abolished if an excess of unlabeled RU28362 was included during the labeling reaction (Fig. 3B).

Since gel filtration in the absence of sodium molybdate, especially in the presence of high salt, can lead to activation of agonist-receptor complexes (32, 33), the results obtained in Fig. 3B most probably reflect the activation of [3H]TA-receptor complexes. In order to confirm that this peak indeed represented activated steroid-receptor complexes, [3H]TA-labeled cytosol was activated at 25 °C for 20 min and then chromatographed on Sephacryl S-300 in the presence of molybdate (Fig. 4). Comparison of the data in Figs. 4 and 3B shows that activated steroid-receptor complexes chromatographed in the presence of sodium molybdate eluted in precisely the same position (Rc ~ 3.4 nm) as unactivated steroid-receptor complexes chromatographed in the absence of molybdate. Thus, it appears that kidney glucocorticoid receptor prepared in the absence of molybdate can be fully activated during chromatography without molybdate to a form which is significantly smaller than activated steroid-receptor complexes from rat liver.

DEAE and DNA Binding Characteristics of the Smaller Kidney-binding Protein—Because glucocorticoid binder IB has been described as the major form of the activated kidney glucocorticoid receptor, we felt that the 3.4 nm form seen in the kidney cytosols was comparable to binder IB. In addition to its smaller size, corticosteroid binder IB has been characterized in kidney and colon as a DNA-binding protein with a lesser affinity for DEAE-containing resins than typical activated receptors (20, 21). We therefore examined the DNA-cellulose and DEAE-cellulose binding properties of the activated and unactivated receptors in kidney cytosol preparations. Activation of kidney [3H]TA receptor complexes resulted in a marked increase in the binding of these complexes to DNA-cellulose; only 7.5% of unactivated complexes bound to DNA compared to 78% of the activated complexes (data not shown). The DEAE-cellulose binding properties of the 3.4 nm activated kidney receptor are shown in Figs. 5–7 of the Miniprint and are also comparable to those of binder IB. Thus, the smaller activated kidney receptor seen in cytosols prepared in the absence of molybdate has DNA- and DEAE-cellulose binding properties comparable to those previously described for corticosteroid binder IB.

Interaction of the 3.4 nm Receptor Form with BUGR-I—Previous reports have shown that binder IB does not react with polyclonal antisera raised against the rat liver glucocorticoid receptor (20). However, these and similarly prepared antisera seem to react with a specific portion of the receptor.
which is separated from the steroid-binding domain during proteolytic to smaller DNA-binding forms (9, 26, 27). In contrast, the monoclonal anti-rat liver receptor antibody BUGR-1 has been shown to react with the intact 94-kDa steroid-binding subunit of the rat liver receptor and with fragments of the receptor as small as 42 kDa containing both the DNA- and steroid-binding domains (29). We therefore used this antibody to determine if the 3.4 nm activated kidney receptor was immunologically related to the larger receptor forms seen in cytosols prepared in the presence of molybdate. Incubation of activated [3H]TA-labeled receptor with BUGR-1 prior to chromatography on Sephacryl S-300 shifted the elution position of labeled complexes from 3.4 nm to 5.4 nm (Fig. 8). In this experiment, not all of the receptor is shifted to the larger form after incubation with the antibody. However, incubation of the cytosol with increased amounts of antibody, or for longer times, resulted in an increase in the proportion of receptor shifted to the larger form (data not shown). It therefore appears that the 3.4 nm form of the kidney glucocorticoid receptor is immunochemically related to the larger forms of the receptor and that the lack of reactivity of the smaller kidney receptor forms with polyclonal antisera may be attributed to the different specificity of these antisera.

The ability of BUGR-1 to recognize the smaller 3.4 nm form of the kidney receptor suggested that this form may be derived by proteolytic cleavage of a larger receptor form. Alternatively, since the 3.4 nm form is seen only after activation of cytosols prepared in the absence of molybdate, it is possible that preparation of cytosol in the presence of molybdate results in the stabilization of the kidney receptor in such a manner that the 3.4 nm form cannot be detected during subsequent gel filtration. To discriminate between these two possibilities, samples of unactivated cytosol prepared in the presence or absence of molybdate were immunoadsorbed with BUGR-1 and then subjected to SDS-PAGE. The resolved proteins were electrophoretically transferred to nitrocellulose paper which was then incubated with BUGR-1 monoclonal antibody tissue culture supernatant. The sample was then chromatographed on Sephacryl S-300 in the absence of molybdate.

In order to ensure that immunoadsorption with BUGR-1 was not favoring the detection of certain receptor forms, a sample of activated kidney receptor from cytosols prepared in the absence of molybdate was partially purified by DNA-cellulose chromatography; lane D, liver receptor prepared in the presence of molybdate and immunoadsorbed with BUGR-1. The ~50-kDa band seen in lanes A, B, and D presumably represents the interaction of the 125I-protein A with IgG heavy chain. The letters to the right of the figure indicate the mobilities of the molecular weight markers myosin (M), 6-galactosidase (G), phosphorylase b (P), bovine serum albumin (B), and ovalbumin (O). ~94 kDa (Fig. 9, lane A), indistinguishable from the molecular weight of liver receptor prepared in the absence of molybdate (Fig. 9, lane D). In contrast, the major immunoreactive species seen from kidney cytosols prepared in the absence of sodium molybdate was only ~44 kDa (Fig. 9, lane B).

In order to ensure that immunoadsorption with BUGR-1 was not favoring the detection of certain receptor forms, a sample of activated kidney receptor from cytosols prepared in the absence of molybdate was partially purified on DNA-cellulose and also subjected to “Western blot” analysis. Again it was found that the predominant immunoreactive component was ~44 kDa (Fig. 9, lane C). Thus, it appears that the immunoreactive ~44-kDa, 3.4 nm, DNA-binding fragment is derived from proteolytic cleavage of a 94-kDa precursor.

**DISCUSSION**

Various attempts have been made to determine if the activated, DNA-binding form of the rat kidney glucocorticoid receptor (corticosteroid binder IB) represents a fragment of a normal, full-sized receptor protein, or is instead a distinct receptor form. The observation that formation of binder IB could not be prevented by a variety of protease inhibitors (36, 37) and the fact that antibodies raised against the activated form of the full-sized rat liver receptor failed to recognize kidney binder IB (20) suggested that binder IB is not derived from the larger protein. More recent studies, however, have shown that the antisera used to examine binder IB cross-reactivity, like most anti-glucocorticoid receptor antibodies, does not react with the major DNA-binding chymotryptic fragment of the rat liver receptor (20). It is possible, therefore, that binder IB may represent a receptor fragment, which, like the similar chymotryptic fragment, has lost the major immu-

**FIG. 8. Immunoreactivity of activated rat kidney glucocorticoid receptor.** Rat kidney cytosol (prepared without molybdate) was labeled with [3H]TA, activated at 25 °C for 20 min, and then incubated with BUGR-1 monoclonal antibody tissue culture supernatant. The sample was then chromatographed on Sephacryl S-300 in the absence of molybdate.

**FIG. 9. Immunoblot of kidney glucocorticoid receptor.** Rat kidney (lanes A-C) and rat liver (lane D) receptors were subjected to SDS-PAGE, transferred to nitrocellulose paper, and probed with BUGR-1 and 125I-Protein A as described under “Experimental Procedures.” Lane A, kidney receptor prepared in the presence of molybdate and immunoadsorbed with BUGR-1; lane B, kidney receptor prepared in the absence of molybdate and immunoadsorbed with BUGR-1; lane C, kidney receptor prepared in the absence of molybdate which was activated at 25 °C for 20 min and partially purified by DNA-cellulose chromatography; lane D, liver receptor prepared in the absence of molybdate and immunoadsorbed with BUGR-1. The ~50-kDa band seen in lanes A, B, and D presumably represents the interaction of the 125I-protein A with IgG heavy chain. The letters to the right of the figure indicate the mobilities of the molecular weight markers myosin (M), 6-galactosidase (G), phosphorylase b (P), bovine serum albumin (B), and ovalbumin (O).
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noreactive domain of the intact steroid-binding protein. In order to examine the homology between the kidney and liver receptor forms, we have used the anti-rat liver receptor monoclonal antibody BUGR-1. This antibody reacts with a 16-kDa receptor fragment that is in close proximity to the DNA-binding functional domain of the protein (29) and is likely therefore to react with DNA-binding receptor fragments.

All of the specifically labeled glucocorticoid receptor forms that were detected by gel filtration of rat kidney cytosol, including the smaller $R_c \sim 3.4$ nm activated kidney receptor, were recognized by BUGR-1. These results suggest that the smaller kidney receptor is not a distinct protein, but is related to or derived from a typical full-sized glucocorticoid receptor. The 3.4 nm component contained over 80% of the specifically labeled receptor in kidney cytosol that was activated either before or during chromatography and had DNA- and DEAE-cellulose binding properties comparable to those attributed to corticosteroid binder IB (20–22). Although our estimate of the Stokes radius of activated kidney receptor is in agreement with that of Sherman et al. (30), it is larger than that reported by Bastl et al. (21). It is likely that the discrepancy in size is due to the fact that the gel filtration matrices, column sizes, and elution buffers used in the latter study were different from the conditions that we employed.

The 3.4 nm form of the receptor was seen only after the activation of kidney cytosol that was prepared from tissue homogenized in the absence of molybdate. If the homogenization was performed instead with molybdate present, a larger receptor form ($R_c \sim 5.8$ nm) was seen after activation. Failure to detect the 3.4 nm form after activation of the kidney cytosol prepared with molybdate could reflect stabilization of higher forms of binder IB which do not readily dissociate when molybdate is removed. Alternatively, the presence of molybdate during homogenization may prevent receptor proteolysis. These two possibilities were readily distinguished by SDS-PAGE and "Western blotting." The major immunoreactive component in kidney cytosol prepared in the absence of molybdate was ~44 kDa. In contrast, kidney cytosol prepared in the presence of molybdate contained a full-sized (Mr, 94,000) receptor protein which was not seen in samples homogenized without molybdate. Because the 3.4 nm and 5.8 nm activated receptor forms share a common antigenic determinant, it appears that the smaller form is derived from the larger by proteolysis during homogenization without molybdate. This conclusion is consistent with the recent report of Webb et al. (11) in which rat kidney glucocorticoid receptor prepared with molybdate and purified by affinity chromatography was shown to have a molecular weight of ~90,000.

The mechanism by which molybdate protects the rat kidney glucocorticoid receptor from degradation during homogenization is unclear. Molybdate could be acting directly as a protease inhibitor (19) or indirectly by maintaining the steroid-binding protein in a configuration which is inaccessible to protease activity. It has been suggested that smaller receptor forms in kidney are generated by a membrane-associated protease (19). This is consistent with our finding that kidney receptor prepared in the presence of molybdate is not degraded after the molybdate is removed and with the observation that there is no activity in kidney cytosol capable of cleaving rat liver receptor (41). The molecular weight of the major immunoreactive component in kidney cytosols prepared in the absence of molybdate was comparable to that of fragments of the rat liver receptor generated by in vitro proteolysis with chymotrypsin or lysosomal proteases (24, 25). It is also comparable to the size of the receptor fragments seen in the glucocorticoid-resistant P1798 and S49 cells (14, 39) as well as fragments seen in cytosols of mouse thymocytes which seem to be produced by a calpain-like protease (40). Thus, the kidney glucocorticoid receptor appears to be a particularly protease-sensitive region.

Sodium molybdate is often used in studies of steroid receptors because it increases the stability of the native un dissociated receptor complex (38). This stabilization presumably accounts for the chromatographic behavior of the glucocorticoid receptor in cytosol prepared with molybdate; chromatography in the presence of molybdate resulted in elution of a 7.1 nm species while chromatography without molybdate resulted in elution of a 5.6 nm species (Fig. 1). In addition, molybdate can apparently stabilize higher order forms of the receptor even if the steroid-binding protein has been cut by protease, since "Western blotting" of cytosol prepared in the absence of molybdate demonstrated that the receptor had been cut to a 44-kDa species, while gel filtration chromatography of the same preparations in the presence of molybdate resulted in elution of a larger 5.8 nm form. Thus, proteolytic cleavage of the 94-kDa receptor during homogenization did not result in the immediate dissociation of the receptor complex; subsequent activation was required for the release of the 3.4 nm fragment. These results also suggest that molybdate is not required for maintaining higher order structure during tissue homogenization.

The question of whether smaller receptor forms are generated in vivo and, if so, whether they may be physiologically significant, remains unresolved. It has been suggested that binder IB is indeed generated in vivo (22) and that this form of the glucocorticoid receptor may play a role in the regulation of sodium transport (21). Further, in vivo, studies will be required to answer this question.

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HEDC buffer (10 mM HEPES, 1 mM EDTA, 0.1 mM DTT, and 10% glycerol; pH 7.6) was applied to a column of Sephadex G-25, and the column was washed with HEDC containing 350 µM H2O2. 125I-labeled samples (1.0 ml) were applied in 3.0 ml columns of DMSO-cellulose pre-equilibrated with TEG buffer containing 0.5 M NaCl. The columns were washed with 30 ml of this buffer and boiled receptor was then eluted with 5 ml of TEG containing 0.5 M NaCl used for radioactivity. If samples were used for SDS-PAGE, the DNA-bound material was collected in 1 ml fractions and precipitated with 10% trichloroacetic acid for one hour. The resulting precipitates were dissolved in 10 mM HCl and frozen in 25 ml sample buffer (3:1) at -20°C.

PREPARATION OF CORTISONE- Antisera were killed by decapitation, and livers and kidneys were perfused in situ with cold phosphate buffered saline. Tissues were homogenized in two volumes of TEG buffer (2 M NaF, 1 mM EDTA, 10% glycerol: pH 8.3) containing 50 mM NaCl, with or without 0.5 mM sodium molybdate. The homogenate were subjected to centrifugation at 105,000 g for 1 h and the resulting supernatant were frozen and stored in liquid nitrogen.

Labeling of Glucocorticoid Receptor- Cortisone was incubated at 0-4°C in the presence of 50 nM [3H]TA for 3 hours. Macromolecular binding was evaluated by measuring the radioactivity in the supernatant of labeled cortisone (200 µl) treated with 5 µg of dextran coated charcoal (10 mg dextran sulfate per gm of activated charcoal) and subjected to centrifugation for 10 min at 2200 g x g. Specific binding to glucocorticoid receptor was determined as the portion of total macromolecular [3H]TA binding which was subsequent in the presence of 5 nM unlabeled 202562.

Sepharose 4B Cellulose Partition Chromatography- [3H]TA-labeled samples (1.0 ml) were applied to 1.6 x 60 cm columns of Sepharose 4B equilibrated in 10 mM HEPES, 1 mM EDTA, 0.1 mM DTT, and 10% glycerol: pH 7.6 containing 420 mM NaCl, and 50 mM MgCl2. Alternatively, chromography in the absence of molybdate was performed in HEDC buffer containing 0.5 M NaCl. Ascending chromatography was performed at 4°C at a flow rate of 10 cm h-1 and 1.0 ml fractions were collected for measurement of radioactivity. The apparent Stokes’ radius of eluted complex was determined from standard curves of [125I]hormone bound to the protein standards bovine thyroid thyroglobulin (5.0 x 104 S), horse spleen ferritin (4.5 x 104 S), bovine liver catalase, aldolase, bovine serum albumin and ovalbumin (4.8 x 104 S). The DNA bound material was collected in 1 ml fractions and precipitated with 10% trichloroacetic acid for one hour. The resulting precipitates were dissolved in 10 mM HCl and frozen in 10 ml sample buffer (3:1) at -20°C.

DNA-Cellulose Chromatography- DNA-cellulose was prepared essentially as described by Alberts and Herrick (30) except that high molecular weight self thymus DNA was dissolved in 10 mM HEPES, 1 mM EDTA: pH 7.6 at a concentration of 3 mg/ml. After addition of cellulose and extensive mixing, the paste was dried overnight, dispersed into a fine powder and lyophilized for 40 h. The powder was washed extensively in 10 mM HEPES, 1 mM EDTA: pH 7.6 and stored frozen at -20°C in buffer containing 0.5 M NaCl. DNA-cellulose prepared in this manner contained ~ 325 µg DNA/ml of packed cellulose. [3H]TA-labeled samples (1.0 ml) were applied in 3.0 ml columns of DNA-cellulose pre-equilibrated with TEG containing 50 mM NaCl. The columns were washed with 30 ml of this buffer and boiled receptor was then eluted with 5 ml of TEG containing 0.5 M NaCl used for radioactivity. If samples were used for SDS-PAGE, the DNA-bound material was collected in 1 ml fractions and precipitated with 10% trichloroacetic acid for one hour. The resulting precipitates were dissolved in 10 mM HCl and frozen in 25 ml sample buffer (3:1) at -20°C.

DNA-Cellulose Chromatography- [3H]TA-labeled samples (1.0 ml) were first applied to a column of Sepharose 4B (10 mg packed bed) eluted with HBSG buffer (10 mM HEPES, 10 mM EDTA, 10 mM MgCl2, pH 7.6) to remove excess salt and to separate steroid-receptor complexes from free steroid. A portion (1.75 ml) of the peak eluted fraction was then applied to a column of DNA-cellulose (1 ml packed bed) pre-equilibrated with HBSG buffer. The column was washed with 33 ml of HBSG buffer and the first 6 ml of unretained material was collected in 1 ml fractions. Several [3H]TA-receptor complexes were eluted with a linear gradient of HBSG buffer containing 0 to 400 mM KC1. One ml fractions were collected and assayed for radioactivity. The conductivity of the eluted fractions was measured with a Radiometer CSM 3 conductivity meter (Radiometer, Copenhagen) using a 0.1% cell, and KC1 concentrations were determined from a KC1 calibration curve.
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DEAE-Cellulose Binding Properties of Activated Kidney Glucocorticoid Receptor. The DEAE-cellulose elution patterns for unactivated and activated rat kidney receptors are shown in Figs. 3 and 4. Unactivated kidney [2H]A receptor complexes were found to elute at ~ 92 M KCl (Fig. 3a). This was virtually identical to the concentration of KCl at which unactivated rat liver [2H]A receptor complexes were found to elute (Fig. 3b), and is characteristic of the high salt eluting unactivated steroid-receptor complex (4,7). It should be noted that approximately 15% of the radioactivity applied to these columns was not retained, and probably represents a small amount of free steroid not removed prior to chromatography. After activation of liver cytosol, there was a large increase in the proportion of labeled material eluting in the column wash (Fig. 3a). The radioactivity in this fraction is closely associated with the 5.6 M component since chromatography of this material on Sephacryl 300 in the presence of molybdate revealed a single 5.6 M peak (Fig. 7). A much smaller proportion of labeled complexes eluted at 0.04 M KCl, the position characteristic of the low salt eluting activated steroid-receptor complexes of rat liver (Fig. 6a) and other tissues (4,7). When rat liver cytosol is heated at 25°C for 20 min, not all of the receptor is "activated" to the DNA binding form. This is consistent with the partial conversion to the low-salt eluting receptor form seen in Figure 6a. The small increase in the amount of labeled complex seen in the column wash after activation of liver cytosol (Fig. 6b) is consistent with previous reports of the existence of a small amount of binder 1B in liver cytosols (22).