Purification and Stabilization of Transcriptionally Active Glucocorticoid Receptor*

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A major obstacle to the purification of glucocorticoid receptor (GR) is the very high nonspecific surface adsorption of this protein. This phenomenon is a property of the GR itself and does not reflect overall protein concentration or buffer conditions. We have observed that the zwitterionic detergent 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonic acid (CHAPS) is unique in its ability to stabilize the receptor and largely eliminate loss to nonspecific adsorption. We have coupled this observation with a two-step purification method that allows efficient purification and stabilization of transcriptionally active glucocorticoid receptor. For this procedure, the GR first undergoes a major purification by anion exchange chromatography following hormone binding and on-column receptor transformation. Second, the GR is resolved to homogeneity utilizing a hydrophobic interaction chromatography step which consists of a 2.5 to 0 M NaCl gradient elution of contaminating proteins followed by displacement of GR by CHAPS. GR at both stages of purification was able to activate transcription from the glucocorticoid response element containing the promoter region of the long terminal repeat of the mouse mammary tumor virus. This simple and efficient methodology should be of considerable advantage for studies of the biology of the active, full-length GR.

The glucocorticoid receptor (GR) is the most studied and best understood member of the family of steroid hormone receptors (1). The GR and all members of this family act as transcription factors, either inducing or inhibiting gene expression upon hormone binding. Glucocorticoids represent an important class of therapeutic agents (2), and an understanding of the relationship between these agents and the receptor has broad implications in basic biology and the treatment of disease.

In its inactive state the GR resides in the cytoplasm in association with chaperone proteins, 2 molecules of the 90-kDa heat shock protein, 1 molecule of the 70-kDa heat shock protein, 1 molecule of the 56-kDa heat shock protein, and a 23-kDa protein (3). After hormone binding, some, and possibly all, of the chaperone proteins are lost (a process known as GR transformation), and GR is translocated to the nucleus (4). Once in the nuclear domain, the GR forms homodimers and binds to DNA at specific glucocorticoid response elements (GREs), leading to activation of transcription. The GR has also been shown to interact with other transcription factors, leading to modulation of its own functioning and that of the other factors (5). In the final step of this cyclical process, the GR is exported to the cytoplasm awaiting the next wave of activation (6).

Multiple mechanisms for transcriptional activation by the GR have been described. Characterization at the chromatin level indicates that GR functions by some mechanism to remodel the chromatin structure of genes, facilitating the loading of transcription factors required for induction of transcription (7, 8). GR also interacts directly, or through intermediary factors, with components of the basic transcriptional machinery (9). These mechanisms need not be mutually exclusive, although the architecture of certain gene systems suggests displacement of GR binding prior to initiation of transcription (10).

In vitro reconstitution studies with the intact, full-length GR have been difficult as no satisfactory protocol exists for the efficient purification of intact, stable, and biologically active GR from animal tissues and cells. Numerous approaches have been employed (for an overview, see Ref. (11)), including the use of almost all of the typical chromatographic media, as well as affinity-based applications utilizing hormone (12, 13), DNA (14), and antibodies (15, 16). Complete purification procedures have been demonstrated (14) and have contributed greatly to the understanding of GR. Nonetheless, these procedures have been hampered by low yields and instability of the purified GR.

Unfortunately, cloning of the GR has not provided an easy solution to these problems. Full-length GR is not well expressed in Escherichia coli (17). A baculovirus expression system for active GR has been described (18), but to date only partial purification of GR has been reported utilizing this system. Yeast systems may hold promise, as expression of a GR fusion product with glutathione S-transferase in Saccharomyces cerevisiae has been carried out (19), but purification of active GR from this system has not been reported. Unlike the full-length protein, domains have been expressed and purified. A functional DNA binding domain of the GR can be well expressed in E. coli and the protein purified (20). The steroid binding domain has also been expressed from a recombinant system (17). Unfortunately this fragment has a low affinity for hormone binding. A large portion of the in vitro functional studies in the literature have, accordingly, utilized partially purified GR or have conducted examinations utilizing only the DNA binding domain of the receptor. The relationship between...
the activities described in these studies and that of the holo-
receptor remains to be seen.

We describe here a simple and efficient method for the pu-
rification and stabilization of the GR from mammalian cells.
The receptor produced by this procedure retains its biological
activity and is able to activate transcription from a GRE-driven
promoter. This procedure should be of a distinct advantage for
studies which require the entire GR in a stable and active form.

**EXPERIMENTAL PROCEDURES**

Cell Culture—WCL2 cells were kindly provided by Gordon Ringold
and were cultured as described previously (21). Cells were grown in
Dulbecco’s modified Eagle’s medium containing 10% iron-supplemented
fetal calf serum, 25 mM glucose, 1 mM methotrexate, and 350 mM
proline. Cells were plated in roller bottles and grown in a 5% CO2, 37°C
atmosphere under rotation; typically a group of 12–14 roller bottles
(40–50 ml of packed cells) were prepared (Cell Culture Center, Minne-
apolis, MN).

Preparation of Cytosol from WCL2 Cells—When the cells reached
80–90% confluence, they were washed with cold phosphate-buffered
saline and harvested from the bottles by scraping on ice. The cells were
then washed twice with phosphate-buffered saline by suspension and
centrifugal pelleting. The resulting pellet was then prepared or main-
tained on ice for shipping and next day preparation. The pellet was
suspending in 2 volumes of homogenization buffer; 20 mM Bis-Tris,
10 mM NaMoO4, 10% glycerol, 5 mM dithiorthreitol, pH 7.2, containing
protease inhibitors at the following concentrations: 1 mM 4-(2-amino-
ethyl)-benzene sulfonfyl fluoride hydrochloride (AEBSF), 10 μM leupep-
in, 10 μM E-64, and 10 μM bestatin. Cells were lysed by nitrogen cavitation (Parr Instrument Co., Moline, IL) following a 15 min pressure
step at 500 p.s.i. The lysate was then cleared by centrifugation at 100,000 × g for 1 h. The resulting cytosolic prepara-
tion was then aliquoted and quickly frozen in liquid nitrogen and stored
at −140°C until further use.

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**1. Preparation of Total Cytosolic GR Number—** Cytosolic receptor
number was determined as maximal [3H]Dexamethasone acetonide bind-
ing and was conducted as described previously (21). In brief, cytosol was
incubated in a volume of 250 μl for 90 min at 4°C with [3H]Dexam-
ethasone acetonide at concentrations of 50 and 100 nM in the presence
or absence of 100-fold excess unlabeled dexamethasone acetonide. Unbound
steroid was then removed by addition of a charcoal/dextran solution (3% Norit-A charcoal, 0.1% dextran 70) to a final charcoal concentration of
1% followed by gentle vortexing. The charcoal was pelleted by centrifu-
gation (12,000 × g, 5 min) after a 3-min incubation, and an aliquot of
the supernatant containing bound steroid scintillation was counted.
Specific binding was determined as the difference between total binding
(absence of unlabeled dexamethasone acetonide) and nonspecific binding
(presence of 100-fold excess labeled dexamethasone acetonide). Maximal bind-
ing was taken as the average specific binding of both concentrations
(triplicates); if the difference between the total binding at 50 and 100 nM
dexamethasone acetonide was greater than 10% of either, the sample was
reassayed. Total cytosolic GR number was determined as the maximal
specific binding/mg of protein in the assay. Protein added to the assay
was determined from the specific activity of [3H]Dexamethasone assuming
a hormone binding stoichiometry of one. Fractions with activity were stored at −140°C after rapid freezing in liquid
nigrogen.

**2. Hydrophobic Interaction Chromatography of Anion Exchange GR—**
[3H]Dexamethasone-containing fractions eluted from the anion exchange
column were then chromatographed to purity utilizing phenyl-Sepha-
rose hydrophobic interaction medium (Pharmacia). A 0.5-mm diameter
× 10-cm long column (2 ml total volume) was used with four buffer
solutions: Buffer A, 20 mM Bis-Tris, pH 7.2 NaCl, 5 mm diethiolethrol;
Buffer B, composition of Buffer A without NaCl; Buffer C, the com-
position of Buffer B but with 50 mM CHAPS and 100 mM NaCl; and Buffer
D, the same composition as Buffer A but containing 100 mM NaCl.

Following packing, nonspecific binding to the column was blocked by
treatment with a 10-ml injection of a 10 mg/ml solution of protase-
and nuclease-free BSA (Calbiochem, La Jolla, CA) in Buffer A followed by
extensive washing with Buffer B. The column was initially equilibrated
with volumes of Buffer A, followed by a flow rate of 2 ml/min. Cytosol
loading NaCl was added to [3H]Dexamethasone-containing fractions to
produce a final NaCl concentration of 2.5 M. This material was then
loaded onto the column at a flow rate of 0.1 ml/min. The column was
washed with 5 ml of Buffer B and a gradient to 100% Buffer B (2.5 to 0
m NaCl) was run at a flow rate of 0.75 ml/min. The column was then
washed with 5 ml of Buffer B and 5 ml of Buffer D. [3H]Dexamethasone
was eluted with a 10-ml gradient from 0 to 100% Buffer C (0–50 mM
CHAPS) versus Buffer D. Aliquots of the 0.5-ml fractions were scintil-
lation counted to detect the presence of [3H]Dexamethasone. Active frac-
tions were either concentrated as described below or rapidly frozen in
liquid nitrogen and retained at −140°C.

**3. Concentration of GR Fractions—**[3H]Dexamethasone-containing solu-
tions were concentrated utilizing Centricon 30 centrifugal ultrafiltra-
tion units (Amicon Inc., Beverly, MA). Before use, nonspecific binding to
the surfaces of the concentrator was blocked by treatment with a
protein solution. The concentrator was filled with a 2 mg/ml solution of
protase and nuclease free BSA (Calbiochem) and incubated horizon-
tally on a rocking platform at room temperature for 30 min followed by
an 10 min centrifugation at 5000 × g and two full volume rinses with
phosphate-buffered saline. The sample was then loaded into the con-
centrator and concentrated by a 1–2 h centrifugation at 5000 × c, 4°C,
until maximum concentration was achieved (approximately a 50-μl
final volume). Proteins eliminated during the hydrophobic interaction
step were simultaneously dialyzed and concentrated utilizing a Micro-
Por Perfusion apparatus (Spectrapor, Laguna Hills, CA).

**Activation of Transcription in Vitro—**In vitro transcription reactions
were a modification of a G-free cassette-driven methodology described by
Tsai et al. (18) and contained the following in a 30-μl volume: 20 mM
DMSO (pH 7.9), 60 mM KCl, 2 mM dithiothreitol, 5 mM creatine
phosphate, 1 mM 3′-O-Methyl-GTP, 0.5 mM ATP, 0.5 mM CTP, 20
μM UTP, 5 mM MgCl2, 10 μCi of [γ-32P]UTP (400 Ci/mmol), 10% glycerol, and 10
units of RNase T1. All in vitro transcription reactions utilized HeLa cell
nuclear extracts (25) and were optimized for each preparation by vary-
ing the ratio of protein to DNA. Optimal conditions were typically 10 μg
protein and 100 to 1200 ng of negatively supercoiled DNA template.
Two DNA templates, each inserted in front of the 380-base pair G-free
cassette. DNA was inserted at 100 mM Bis-Tris, pH 6.7, 7.5 mM EGTA,
and two full volume rinses with

**4. Purification of Active GR—**

**1. Activation of GR in Vitro—**

The DNA template and GR (10–50 nM final con-
tention) were mixed and incubated 30 min at room temperature.
Following this, all other ingredients were added and incubated for 1 h at
30°C. Reactions were terminated by the addition of 300 μl of stop
solution (20 mM EDTA, 0.2 mM NaCl, 1% SDS, 10 μg of TRNA),
extracted with an equal volume of phenol-chloroform, followed by a
chloroform extraction and ethanol precipitation. Samples were then
applied to an 8% polyacrylamide sequencing gel, and following resolu-
tion and drying, analyzed using a Phosphorimage (model 422E, Mo-
lecular Dynamics, Sunnyvale, CA), permitting direct quantitation of
label incorporated into specific transcripts (ImageQuant, Molecular
Laboratories).
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FIG. 1. Typical elution profile of anion exchange chromatography of GR following on-column receptor transformation. GR was eluted from a Mono Q anion exchange column by a linear NaCl gradient from 0 to 500 mM NaCl. The calculated gradient would begin at fraction 8 and reach 500 mM from fraction 26 until completion of the procedure. The conductivity resulting from this gradient is shown by the filled circles. The filled squares represent the absorbance at 280 nm over the elution profile. The open circles show the cpm in 25 μl of each fraction arising from [3H]DexMes-GR. Preceding elution the Mono Q column was 1) loaded with NaMoO₄ stabilized cell cytosol of GR overexpressing WCL2 cells which had been incubated with [3H]DexMes for 16 h and brought to a final NaCl concentration of 260 mM, 2) washed with buffer containing 260 mM NaCl and 6 mM NaMoO₄, and 3) washed with buffer containing no NaCl or NaMoO₄. Additional details are given under “Experimental Procedures.”

FIG. 2. Silver staining and fluorography of anion exchange purified [3H]DexMes bound GR. Peak fractions from the anion exchange chromatography step were pooled, concentrated and resolved by SDS-PAGE electrophoresis. Following this the resulting protein bands were visualized by silver staining (A) or fluorography (B). The migration of molecular mass standards is indicated on the right side of the figure. GR has a molecular mass of 94 kDa (14).

Electrophoretic Mobility Shift Assay—For electrophoretic mobility shift assays hydrophobic interaction-purified GR (HI-GR) was incubated 30 min on ice in gel shift buffer (10 mM Tris-HCl (pH 7.4), 50 mM KCl, 5 mM MgCl₂, 1 mM EDTA, 1 mM dithiothreitol, 3% glycerol), with [32P]-end-labeled, double-stranded probe in a 20-μl volume. Typical assays contained HI-GR at a final concentration of 5 nM and GRE consensus probe at a concentration of 10 nM. Mobility shift probe was prepared by phosphorylation with T4 polynucleotide kinase in the presence of [γ-32P]ATP (5000 Ci/mmol), followed by inactivation of the kinase by a 15-min incubation at 90°C and separation of the probe from free label with a G-25 Quick Spin column (Boehringer Mannheim). The sequence of the GRE oligonucleotide probe used in the mobility shifts was 5'-GATCCGGTGACAATCGTCTTCA-3'. Samples were resolved in 5% acrylamide gels with a running buffer composed of 23 mM Tris borate and 1 mM EDTA. After drying, the gels were analyzed with the PhosphorImager (Molecular Dynamics).

RESULTS

On-column Transformation and Anion Exchange Chromatography of GR—This procedure was structured around the following three observations (23). 1) Untransformed hormone-bound GR (GR associated with its protein chaperones and bound to glucocorticoid, HSPn-GR) can be loaded onto a Mono Q anion exchange column stabilized with NaMoO₄ and eluted with a NaCl gradient; HSPn-GR elutes at a NaCl concentration of 325 mM. 2) Transformed hormone-bound GR (GR) can also be resolved on a Mono Q anion exchange column and elutes at a NaCl concentration of 180 mM. 3) Anion exchange bound HSPn-GR can be converted to anion exchange bound GR by elimination of NaMoO₄ from the running buffer. Utilizing these properties, the HSPn-GR in cytosol was loaded onto the anion exchange column at a salt concentration at which it binds to the column (260 mM NaCl) but above that at which the GR elutes. The HSPn-GR was then transformed to GR in the absence of NaCl and NaMoO₄. GR was eluted at a NaCl concentration (180 mM) below that of loading. Since the cytosol was loaded at a higher salt concentration than that of elution, the majority of contaminating cytosolic proteins which would normally elute from a Mono Q column at this salt concentration were not present.

Initial studies utilized the glucocorticoid [3H]triamcinolone acetonide as a ligand for following glucocorticoid binding/receptor throughout the purification. However, as problems developed with nonspecific surface interactions of the GR, it became advantageous to utilize a covalent ligand, which would allow direct tracking of GR. More specifically, one could equate loss of radioactivity with loss of receptor itself rather than dissociation and loss of labeled hormone. The procedure described utilized the dexamethasone derivative, [3H]DexMes to covalently label GR (24). A typical [3H]DexMes binding activity and A260 profile over the elution gradient is shown in Fig. 1. Protein immunoblotting verified that the first peak (fractions 13–18) contained GR and that the second peak (fractions 22–26) contained HSPn-GR (data not shown). This procedure, utilizing our preparations, resulted in a large purification in a single step but a homogeneous preparation of GR was not obtained (Fig. 2 and Table I). In addition, the receptor was not stabilized such that it could be handled without large losses due to nonspecific interactions.

Losses of the GR due to nonspecific interactions during this stage of the purification were largely controlled by addition of CHAPS at a final concentration of 7.5 mM to both of the running buffers (see next section). CHAPS also had the added benefit that it consistently led to increased transformation of HSPn-GR during the purification as reflected by the relative sizes of peaks 1 and 2 (data not shown). A shorter column of larger diameter and use of maximal flow rates during the elution gradient further minimized nonspecific loss of GR. An additional modification was the loading and washing of the column at a higher salt concentration (260 mM) to further eliminate contaminating proteins.
The purity of the preparations from this step is shown in Fig. 2. A silver staining of a SDS-PAGE gel of this material indicated the presence of 10–15 major proteins; the largest having a molecular mass of 94 kDa, the molecular mass of GR. At least four of these proteins are fragments of the GR, as indicated by fluorography of a SDS-PAGE gel of \(^{3}H\)DexMes labeled material (panel B). These fragments are the result of either proteolysis or expression of shorter than full-length GR. If they are the result of proteolysis, it most likely occurred prior to the chromatography as the amount of GR or the number and amount of the proteolytic fragments did not change following overnight incubation of concentrated anion exchange GR at room temperature (data not shown). Proteolysis, at least by serine proteases, during the chromatographic separation is also unlikely since GR or its shorter fragments were unchanged by the inclusion or exclusion of serine protease inhibitors in the running buffers as described in the original procedure.

Stabilization and Concentration of the GR—GR preparations in the absence of detergents or stabilizing protein exhibit very high levels of nonspecific adsorption. Loss of as much as 70% of \(^{3}H\)DexMes-GR could be seen following exposure to polypropylene tubes. Polyethylene tubes designed to minimize nonspecific adsorption gave similar losses. This effect was initially thought to be the result of low total protein concentrations, however, losses were changed only slightly by the addition of carrier insulin (1 mg/ml) or BSA (1–3 mg/ml). The nonspecific adsorption produced numerous complications as merely pipetting the material led to considerable losses and precluded more complicated manipulations such as concentration or further chromatography.

A number of detergents were examined for their ability to inhibit nonspecific adsorption of GR. A list of detergents examined includes Triton X-100, Tween 20, Nonidet P-40, Lubrol PX, octylglucoside, and CHAPS. Concentrations below, equal to, and above the critical micelle concentration were examined with and without the addition of salt and/or carrier protein. Mixtures of detergents which had been described to stabilize membrane proteins (26) were tested as well. The zwitterionic derivative of deoxycholate, CHAPS, proved to be exceptional for stabilization of GR. Addition of CHAPS to a final concentration of 10 mM minimized nonspecific loss to 20% allowing handling of the partially purified GR.

The high degree of nonspecific adsorption of the GR also made concentration of fractions difficult. GR bound avidly to dialysis membranes and concentration methods which used them resulted in complete loss of receptor under numerous conditions. Ultrafiltration apparatuses (Amicon 30; Amicon, Inc.) could be used for concentration if nonspecific binding to the membranes and polystyrene structure was blocked by BSA or insulin. In addition, CHAPS had to be present at a concentration of at least 5 mM but less than 30 mM. Utilizing these conditions, recoveries exceeding 80% could be achieved. Table I lists the ultimate yields and fold purification following the concentration for each of the purification steps.

Purification of the GR to Homogeneity by Hydrophobic Interaction Chromatography—Nonspecific binding of the GR led to extremely low yields of receptor following chromatography using appropriate formats for gel filtration regardless of the medium source or additives. Anion exchange chromatography, at pH values above and below the pI of GR, was also ineffective. In an effort to utilize a component of the nonspecific binding to advantage, chromatography of the GR utilizing hydrophobic interaction medium was examined. Use of this medium was not without its problems, but eventually gave way to a useful methodology. The GR bound well to hydrophobic interaction medium (phenyl- or butyl-Sepharose) in the presence of high salt concentrations, but was not eluted by a typical protocol of salt elimination. Further, receptor was not eluted from either medium by gradients of decreasing polarity such as 0 to 30% ethylene glycol or 0 to 50% isopropanol. However, CHAPS was able to displace the receptor from these media and was chosen for this procedure. The final method consisted of loading of GR in 2.5 mM NaCl buffer, followed by a gradient/wash to no NaCl and elution of the receptor by a 0 to 50 mM CHAPS gradient in the presence of 100 mM NaCl. A typical chromatographic profile is shown in Fig. 3. Utilizing this methodology, GR was purified to homogeneity as seen in the single 94-kDa band following SDS-PAGE and silver staining or fluorography of \(^{3}H\)DexMes.
bound receptor (Fig. 4). The typical recovery from this step following concentration was 37% giving an overall yield of 20% (Table I).

Activation of Transcription in Vitro by the Purified GR Fractions—Retention of biological activity of the purified GR preparations was assessed by its ability to activate transcription in vitro from a GRE-driven promoter (Fig. 5). Nonspecific activation of transcription was assessed with a template containing only the MMTV TATA box and the 8 bases immediately upstream (TATA template). Fig. 5 shows the results of these studies. Addition of anion exchange-purified GR (panel A) led to a progressive increase in transcription of the GRE template. A 14-fold induction was observed at a GR concentration of 40 nM. This induction was not without background activity as transcription of the TATA template was increased 4-fold over the same range. The background activity was not the result of components of the buffer system or the stabilizing detergent, CHAPS. Equivalent amounts of the buffer alone were included in the 0 nM GR examinations, and separate experiments demonstrated little or no change in GRE or TATA template transcription over an equivalent range of CHAPS concentrations.

HI-GR also activated transcription from the GRE template, although less vigorously than the anion exchange purified fractions (Fig. 5, panel B). A progressive increase in transcription was observed reaching a level of 2.3-fold induction at a 50 nM GR concentration. Non-GRE driven transcription was not induced by the addition of the homogeneous GR to a level of 50 nM. Again, no effect was observed with the buffer system used.

Preliminary experiments show that addition of proteins eliminated during the hydrophobic interaction step was able to partially restore activity lost during this stage (Fig. 6). A combination of the HI-GR and the salt gradient fractions (SGF), following their dialysis and concentration, led to increased DNA binding to a GRE-containing template, as monitored by electrophoretic mobility shift assay analysis (panel A), and to an increase in GRE-driven transcriptional activation (panel B). The SGF alone caused no change in DNA mobility and had an inhibitory effect on transactivation of the TATA template. The results cannot be explained by nonspecific protein stabilization, as they were not duplicated by the addition of carrier proteins (data not shown). These initial findings suggest that factors important for GR DNA binding and/or transcriptional activation are lost during HI chromatography.

DISCUSSION
Presented here is a methodology for the rapid and efficient purification of stable and transcriptionally active GR. It is a two-step procedure utilizing a first step of on-column transfor-
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mation and anion exchange chromatography followed by hydrophobic interaction chromatography with CHAPS elution. The overall recovery of this procedure is 20% of the starting GR with a yield of 5–10 μg of protein. Although the procedure was carried out utilizing tissue culture cells as the protein source, other sources in which the GR is found untransformed in association with heat shock proteins could also be used.

The major problem encountered working with the GR was the inherent instability of transformed GR following any significant purification. In this regard, possibly the most significant result described here is the development of conditions for stabilization of the GR. Judging from our experience, this instability is likely to have been a major barrier to the study of holo-GR in the past. In the absence of stabilizer, the GR exhibited very high nonspecific binding to the surfaces of any containers with which it came into contact. The seriousness of this problem can be readily assessed by the fact that losses as high as 70% can result from mere pipetting of the receptor, using a typical polypropylene pipette tip. This effect was not simply a hydrophobic interaction or the result of dilute protein solutions. Stabilization experiments (data not shown) revealed that minor stabilization could be accomplished by the addition of carrier protein, salt, or detergent, indicating the involvement of hydrophobic, as well as hydrophillic, elements of the protein itself. Ultimately, addition of the zwitterionic detergent, CHAPS, allowed inhibition of this interaction such that handling and study of the intact protein was possible.

CHAPS also proved to have other benefits in addition to its stabilizing properties. First, its inclusion in the anion exchange stage led to increased yields. The described methodology (23), which we used for the initial step of the purification, incorporated 2% acetonitrile in the buffer systems to aid in the washing of unbound steroid from the column. We did observe some stabilization of GR by acetonitrile and we would suggest that it decreased nonspecific adsorption of hormone-bound GR. These studies also revealed that use of CHAPS, in lieu of acetonitrile, resulted in larger yields of receptor (data not shown). This increase appeared to be the result of an increase in on-column transformation of the GR, as well as a decreased loss of GR due to nonspecific adherence. Finally, CHAPS proved to be very useful in the displacement of the GR from the hydrophobic interaction column used in the final stage of the purification.

CHAPS, in the ranges necessary for GR stabilization, did not inhibit the biological activity of the receptor as reflected by activation of transcription. Transcriptional activation was observed following the inclusion of as much as 15 mM CHAPS. This is a considerable level given that the critical miscellation concentration of this detergent is approximately 1 to 3 mM at the salt concentrations used in these assays (27). CHAPS is known as a mild nondenaturing detergent and has a planar structure containing a hydrophobic and a hydrophillic side. CHAPS micelles are sandwich-like in nature with aggregation numbers of less than 10 members. Interestingly, this detergent has also been described to have a unique activity for increasing binding of a number of DNA-binding proteins in gel mobility retardation assays (28). However, there is a critical range of CHAPS concentration which can be utilized in this protocol. Step elution of the GR from the hydrophobic interaction column by CHAPS concentrations greater than 50 mM led to irreversible loss of GR transactivation activity (data not shown).

Although CHAPS was able to significantly stabilize the GR-containing fractions, it was not sufficient by itself to allow the concentration of samples using ultrafiltration devices. GR in fractions from either stage of the purification was significantly lost to nonspecific adsorption in these devices. Increases in the CHAPS concentration did little to decrease this effect but blocking of the nonspecific adsorption could be achieved by treatment of the membranes and polystyrene structures of these units with BSA. Such pretreatment led to recovery of 70–90% of the GR from either stage of the purification. Nonetheless, inclusion of CHAPS remained necessary for recovery. However, inclusion of CHAPS at concentrations greater than 30 mM led to a significant decrease in recovery from the concentrators. Possibly the effect resulted from solubilization of the passivating proteins and re-exposure of sticky surfaces. Furthermore, treatment with BSA solutions above 5 mg/ml also decreased recovery; potentially, this resulted from competition for available CHAPS micelles during the latter part of the concentration process.

A substantial difference in the transcriptional activation was observed between the anion exchange and the hydrophobic interaction-purified GR preparations. The less pure anion exchange fractions had approximately 6-fold the activity of the homogeneous hydrophobic interaction fractions. Two potential explanations exist; either the protein is being denatured during the final purification step, or a GR-associated activation factor is missing from the homogeneous preparations. Preliminary experiments suggest the involvement of an accessory factor. Addition of proteins eliminated during the final purification step both increased GR binding to DNA and stimulated transcriptional activation. In addition, single examinations with higher concentrations of GR at both stages of the purification demonstrate achievement of maximal activation of transcription over the range of GR concentrations shown here (data not shown). This suggests that both preparations exhibit their full range of activity over a similar GR concentration span and that they differ in their intrinsic activity. This result may also be explained by the presence of an accessory protein. Procedures are currently being modified to allow the production of GR at higher concentrations and isolation of proteins eliminated during the hydrophobic interaction step for a definitive answer to this question.

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