PARTIAL PURIFICATION OF THE GLUCOCORTICOID RECEPTOR FROM RAT LIVER: A RAPID, TWO-STEP PROCEDURE USING DNA-CELLULOSE

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SUMMARY: Glucocorticoid receptor from rat liver was purified 1800-fold by a rapid two-step procedure using DNA-cellulose. The procedure is based on increasing the affinity of the glucocorticoid-receptor complex for DNA by heating the complex. During a first chromatography step, unheated glucocorticoid-receptor complex is separated from cytosol proteins that bind to DNA-cellulose with high affinity. During a second chromatographic step, heat-treated glucocorticoid-receptor complex is separated from proteins with low affinity for DNA. The partially purified complex is functionally competent in that it is taken up by isolated rat liver nuclei.

INTRODUCTION

Glucocorticoids bind to protein "receptors" in the cytosol of target cells; the complex then enters the cell nucleus (1,2). Nuclear uptake of the glucocorticoid-receptor complex appears to involve DNA since the process can be inhibited by DNase treatment of nuclei (3). The complex also binds to purified DNA (3,4,5). However, both nuclear uptake and binding to purified DNA require "activation" of the complex by brief heating (20°C for 30 minutes). Receptor complex that has been formed and kept at a low temperature (4°C) does not bind to DNA (4).

The DNA binding property of the glucocorticoid receptor suggested that DNA could be used as an affinity ligand for its purification (6,7). We now describe a two-step procedure using DNA-cellulose chromatography which results in a 1800 fold purification of the glucocorticoid-receptor complex.

MATERIALS AND METHODS

Male Osborne-Mendel rats, weighing 100-120 gms, were adrenalectomized and maintained on food *ad libitum* and drinking water supplemented with 1% dextrose and 0.3% NaCl. They were killed by decapitation 5-10 days after adrenalectomy. Livers were perfused *via* the portal vein with 10 mls of ice-cold 0.9% NaCl, minced, and homogenized in 2 volumes of 10 mM Tris-Cl (pH 7.5) containing 50 mM NaCl, 1 mM EDTA, 5 mM β-mercaptoethanol, and 10% (v/v) glycerol at 4°C. Homogenates were centrifuged at 5000 g for 10 minutes at 4°C. The supernatant portion was then centrifuged (105000 g for 1 hr) to obtain a cytosol fraction which contains the glucocorticoid receptor. [^{3}H]-Triamcinolone acetonide (34 Ci/mmole) was obtained from Amersham-Searle, and was added, when indicated in the text, at a concentration of 60 nM to form the glucocorticoid-receptor complex. Columns containing DNA-cellulose (20 ml bed volume) were washed extensively with buffer containing 450 mM NaCl and then with buffer containing 50 mM NaCl before use. Nuclei from livers of adrenalectomized rats were isolated as previously described (6). DNA was measured by a mithramicin binding assay (9).

DNA-cellulose was prepared as described by Alberts and Herrick (8). Protein was measured by the method of Lowry *et al.*; crystalline bovine albumin was used as a standard. [^{3}H] was counted at 30% efficiency.

RESULTS

It has previously been demonstrated that the heat-activated glucocorticoid-receptor complex in rat liver cytosol binds to DNA-cellulose at a low molarity of NaCl, and that it can be released by increasing the molarity of NaCl (6,7). Since DNA-binding proteins in general can be eluted from DNA-cellulose with increasing NaCl concentrations (8), it is not surprising that other cytosol protein moieties that bind to DNA elute from the column with the glucocorticoid-receptor. We reasoned that the majority of these other DNA-binding proteins would bind to DNA-cellulose during chromatography of cytosol containing unactivated glucocorticoid-receptor and that initial chromatography of cytosol prior to heat activation of the glucocorticoid-receptor complex would substantially improve the purification of the complex.

Initial experiments revealed that other cytosol proteins as well as the glucocorticoid-receptor complex underwent temperature-dependent increases in DNA-affinity when heated to 20°C for 30 minutes (data not shown). In order to bind these proteins to DNA-cellulose during the initial chromatographic step, cytosol was heated to 20°C for 30 minutes

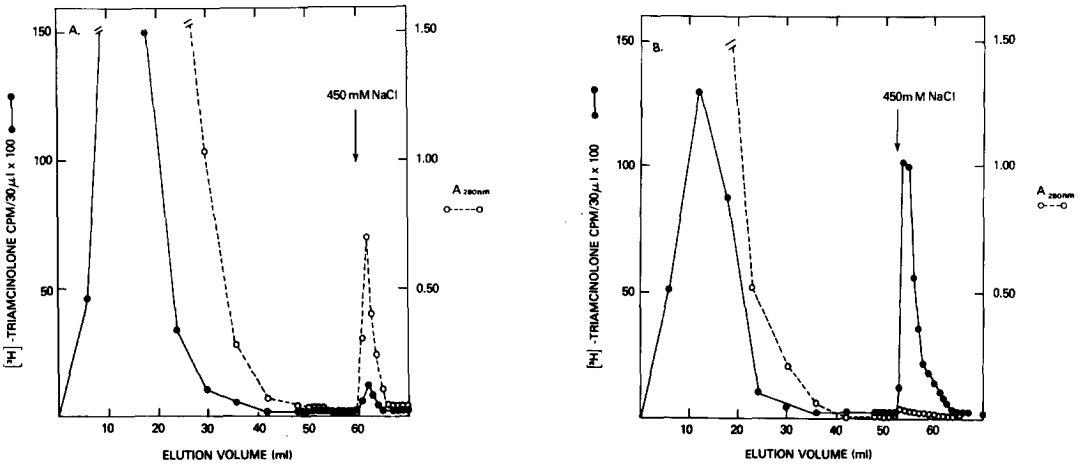

Fig. 1. DNA-cellulose chromatography of the glucocorticoid receptor.

A. Unactivated glucocorticoid-receptor complex. Cytosol (6 ml) prepared at 4°C was heated to 20°C for 30 minutes and then recooled to 4°C. [^{3}H]-triamcinolone acetonide (60 nM) was added. After an additional 1 hr at 4°C, the cytosol was applied to a 0.9 X 13 cm column of DNA-cellulose. The column was washed with 65 ml of buffer containing 50 mM NaCl; fractions (6.0 ml) were collected at a flow rate of 25 ml/hr. The column was then eluted with 20 ml of 450 mM NaCl; fractions (1.3 ml) were collected at a flow rate of 25 ml/hr. [^{3}H] and A_{280nm} were determined for each fraction. The position of the step increase in NaCl concentration was determined by conductivity measurement.

B. Heat activated glucocorticoid-receptor complex. Fractions containing the cytosol peak from step A (elution volume 6-24 ml) were pooled and heated to 20°C for 30 minutes. After rechilling to 4°C the material was applied to a DNA-cellulose column (0.9 x 13 cm) and washed with 45 ml of buffer containing 50 mM NaCl and then with 20 ml of buffer containing 450 mM NaCl. Fractions were collected as above; [^{3}H] and A_{280nm} were determined for each fraction.

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and then cooled to 4°C before [^{3}H]-triamcinolone was added. The glucocorticoid-receptor complex thus formed was in its unactivated state since heat-activation can take place only after the formation of the glucocorticoid-receptor complex (4). After 1 hr at 4°C, to allow formation of the [^{3}H]-triamcinolone-receptor complex, the cytosol was passed through a DNA-cellulose column. The receptor complex was not bound to the column at this stage, and it eluted with the bulk of cytosol protein in the 50 mM NaCl wash fractions. Cytosol DNA-binding moieties, including those that required heat treatment to bind to DNA, were bound

Table 1

Purification of the glucocorticoid-receptor complex.

	Macromolecular-bound [^{3}H]-triamcinolone (pmoles/ml)	Protein (mg/ml)	pmoles / mg protein
1. Cytosol*	5.4	15.0	0.36
2. 450 mM NaCl eluate	1.5	0.0026	580

Samples (200 µl) of cytosol and purified receptor complex were applied to G-25 Sephadex columns (0.7 x 20 cm) and were eluted with H_2O at a flow rate of 15 ml/hr. Fractions (1.0 ml) were collected; [^{3}H] and protein content in the void volumes are tabulated.

*Cytosol containing 60 nM [^{3}H]-triamcinolone acetonide plus 60 µM unlabelled triamcinolone acetonide was prepared and chromatographed as above. [^{3}H]-Triamcinolone acetonide eluting in the void volume represents nonspecific (low-affinity) binding and was subtracted from total cytosol binding.

to the column as evidenced by the large amount of protein eluting with 450 mM NaCl (Fig. 1).

Cytosol fractions from the first DNA-cellulose step containing unactivated glucocorticoid-receptor complex were pooled and then activated by heating at 20°C for 30 minutes. The fractions were then cooled to 4°C and applied to a second DNA-cellulose column. At this stage, 23% of the radioactivity was bound to the column and was released with 450 mM NaCl. The protein released from the column by 450 mM NaCl was considerably decreased from that of the first chromatographic step (Fig. 1).

Gel filtration (G-25 Sephadex) showed that 90% of the [^{3}H]-triamcinolone released by 450 mM NaCl was bound to macromolecules. The bound material was purified 1800 fold by the two step procedure (Table 1) with a yield of approximately 70%. The partially purified 3H-triamcinolone-receptor complex is taken up by isolated liver nuclei from adrenalectomized rats (Table 2).

Table 2

Nuclear binding of purified glucocorticoid-receptor-complex

Sample	cpm/50 µl	% cpm bound to nuclei
1. Control	18 000	3 %
2. 0.45 M NaCl eluate	18 000	48 %

Aliquots (50 µl) of the 0.45 M eluate fractions containing purified receptor complex or buffer containing the same concentration of [^{3}H]-triamcinolone (as control) were added to 250 µl of buffer containing purified nuclei (28 µg DNA/250 µl). The reaction mixtures were incubated for 20 minutes at 20° C, chilled to 4° C, and nuclei were separated by centrifugation for 1 minute with a Beckman 152-Microfuge. Nuclear pellets were washed 4 times with 250 µl buffer, resuspended in 200 µl H_2O and counted after solubilization in triton-toluene. Each entry is the mean of duplicate assays.

DISCUSSION

Synthetic 9α-fluorinated glucocorticoids bind in rat liver to a cytosol protein, termed "Binder II" (12) or "G" (11), which is taken up by liver cell nuclei following the *in vivo* administration of glucocorticoids (12). It is estimated that obtaining this moeity in homogenous form requires a 20000 fold purification (12), and standard protein separation techniques such as anion exchange and gel permeation chromotography afford to date a maximum purification of 200 fold (12). DNA affinity chromatography offers a promising alternative approach for purification of this glucocorticoid receptor (6).

Purification of the glucocorticoid-receptor complex which is achieved by a single step DNA-cellulose chromatography procedure is low (e.g. 40 fold) (6). However, the two-step procedure described in this report is highly selective for the glucocorticoid-receptor complex and permits 1800 fold purification. A similar procedure, which does not include heat-treatment of cytosol before addition of glucocorticoid, results in 100 fold purification of the receptor from hepatoma (HTC) cells (13).

As the receptor complex is increasingly purified, it is important to establish that it retains functional competence. That the partially purified [^{3}H]-triamcinolone-receptor complex binds to isolated liver nuclei from adrenalectomized rats is an indication that this important functional property of the receptor is preserved. Further purification and characterization of the glucocorticoid receptor will hopefully lead to a precise understanding of the way glucocorticoids control gene expression.

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