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PARTIAL PURIFICATION OF GLUCOCORTICOID RECEPTOR FROM RAT LIVER USING DNA–CELLULOSE

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SUMMARY

DNA–cellulose chromatography was used to purify the glucocorticoid receptor from rat liver. The heat-activated [^{3}H]-dexamethasone–receptor complex bound to DNA–cellulose in the presence of 0·1 M NaCl and could be eluted with 0·45 M NaCl. [^{3}H]-Dexamethasone–receptor complex that had not been heat-activated did not bind to DNA–cellulose. Using a single column of DNA–cellulose the receptor could be purified 40-fold. The partially purified [^{3}H]-dexamethasone–receptor complex was able to bind to isolated liver nuclei.

INTRODUCTION

Glucocorticoids bind to cytosol protein "receptors" in target tissues [1, 2, 3]. The GR complex* is then translocated into the nucleus [4, 5]. Nuclear uptake takes place only if the GR complex undergoes a temperature-dependent alteration referred to as heat activation [6].

Baxter *et al.*[7] observed that heat-activated (20°C, 1 h) GR complex could bind to double stranded DNA. They incubated heat-activated GR complex with DNA and showed that the GR complex eluted with DNA during gel filtration. Their observation suggested to us that DNA immobilized on cellulose (DNA cellulose) [8] could be used as a method for purifying the GR complex.

Beato *et al.* found that the heat activated GR complex from rat liver could bind to DNA–cellulose [9]. Proteins bound to DNA–cellulose can be eluted by NaCl [8]. We found that the heat-activated GR complex from rat liver could bind to columns of DNA–cellulose in the presence of 0·1 M NaCl and could be eluted by higher molarity (0·45–0·60 M) NaCl. The GR complex was thus separated from the cytosol proteins that did not bind to DNA–cellulose and was purified 40-fold.

The binding of the activated GR complex to the cell nucleus appears to be an important step in the biological action of glucocorticoids. The GR complex purified by DNA–cellulose chromatography was able to bind to isolated liver nuclei.

MATERIALS AND METHODS

Male Osborne–Mendel rats, weighing 100 to 120 *g*, were adrenalectomized and maintained on food *ad libitum* and drinking water supplemented with 1% dextrose and 0·5% NaCl. They were killed by decapitation 5–10 days after adrenalectomy. Livers were perfused *via* the portal vein with 10 ml of ice cold 0·9% NaCl, minced, and homogenized (Teflon–glass homogenizer) in 4 vol. of Tris–Mg buffer (20 mM Tris–Cl, pH 7·5, 1 mM $MgCl_2$) containing 0·1 M NaCl. Homogenates were centrifuged at 5000 *g* for 10 min at 4°C. Additions as indicated in the text were made to portions of the supernatant fraction, and the supernatants were then centrifuged (105,000 *g* for 1 h) to obtain a cytosol fraction which contains the glucocorticoid receptor [7]. Nuclei from livers of adrenalectomized rats were isolated by centrifugation through 2·2 M sucrose [10] and were suspended in 20 mM Tris–Cl (pH 7·5) containing 3 mM $MgCl_2$ and 0·25 M sucrose.

DNA–cellulose was prepared as described by Alberts and Herrick [8]. The preparation used in the present experiments, containing 0·7 mg of calf thymus DNA (Sigma, type V) per ml of packed cellulose (Whatman CF11 from Reeve Angel), was stored as a frozen slurry in 20 mM Tris–Cl buffer, pH 7·5, containing 0·1 M NaCl and 0·005 M EDTA. Thawed on the day of use, it was packed in 0·7 × 1 or 0·7 × 3 cm. columns (Bio-Rad) and washed with 20 ml of Tris–Mg buffer containing 0·1 M NaCl.

[^{3}H]-Dexamethasone (29 Ci/mmol) was obtained from Amersham–Searle, and unlabelled dexamethasone was obtained from Calbiochem. Sephadex G-25, obtained from Pharmacia, was equilibrated in Tris–Mg buffer containing 0·1 M NaCl. Protein was measured by the method of Lowry *et al.*[11], using crystalline bovine albumin (Armour Pharmaceuticals, Chicago, Ill.) as a standard. [^{3}H] was measured by dissolving 30 μl samples in 10 ml of Triton–toluene liquid scintillation fluid; counting efficiency was 30%.

* Abbreviation is GR complex: glucocorticoid–receptor complex.

RESULTS

The GR complex was formed (4°C, in Tris–Mg buffer containing 0·1 M NaCl) by adding 25 nM [^{3}H]-dexamethasone to the supernatant fraction obtained from the initial contrifugation at 5000 *g*. After subsequent high speed centrifugation at 105,000 *g* for 1 h, 20 to 30% of the [^{3}H]-dexamethasone in the supernatant fraction (cytosol) was judged bound to macromolecules since it coeluted with protein in the void volume of a G25 Sephadex column (Table 1).

The cytosol was incubated at 20° for 1 h to activate the GR complex [6]. After cooling to 4°C, a portion of the heat activated cytosol was placed on a column of DNA–cellulose equilibrated with Tris–Mg buffer containing 0·1 M NaCl. The column was eluted with step increases in the concentration of NaCl in Tris–Mg buffer. In the experiments shown in Fig. 1, 30% of [^{3}H]-dexamethasone in the cytosol was retarded in the presence of 0·1 M NaCl and was sharply eluted by 0·6 M NaCl. No [^{3}H]-dexamethasone was eluted with subsequent increases in NaCl concentration (up to 2·1 M NaCl) and none remained bound to DNA–cellulose.

Cytosol that had *not* been heat activated was similarly eluted (Fig. 1b); little [^{3}H]-dexamethasone was bound to DNA–cellulose. ^{3}[H]-Dexamethasone (25nM) alone in Tris–Mg buffer did not bind to DNA–cellulose. The [^{3}H]-dexamethasone binding sites in cytosol could be saturated. Addition of 10 μM unlabelled dexamethasone with 25 nM [^{3}H]-dexamethasone to the 5000 *g* supernatant fraction completely inhibited subsequent binding of ^{3}[H]-dexamethasone to DNA–cellulose (data not shown).

The above experiments suggested that heat-activated GR complex could bind to DNA–cellulose. The choice of elution with NaCl was based on Alberts' and Herrick's observation [8] that proteins bound to DNA–cellulose could be eluted by NaCl. In order to determine if elution with NaCl actually released GR complex from DNA–cellulose, the 0·6 M NaCl elution fraction was passed through a column of G25 Sephadex: 29% of the [^{3}H]-dexamethasone in this fraction coeluted with protein in the void volume. Passage of the 0·1 M NaCl wash fraction through G25 Sephadex showed a marked depletion of GR complex when compared with the unchromatographed cytosol (Table 1).

Using the concentration of [^{3}H]-dexamethasone bound to macromolecules as a measure of GR complex in the 0·6 M NaCl fraction, a 40-fold purification of heat-activated GR complex was obtained by a single step elution from DNA–cellulose (Table 1).

The GR complex purified by DNA–cellulose chromatography was able to bind to isolated liver nuclei (Table 2).

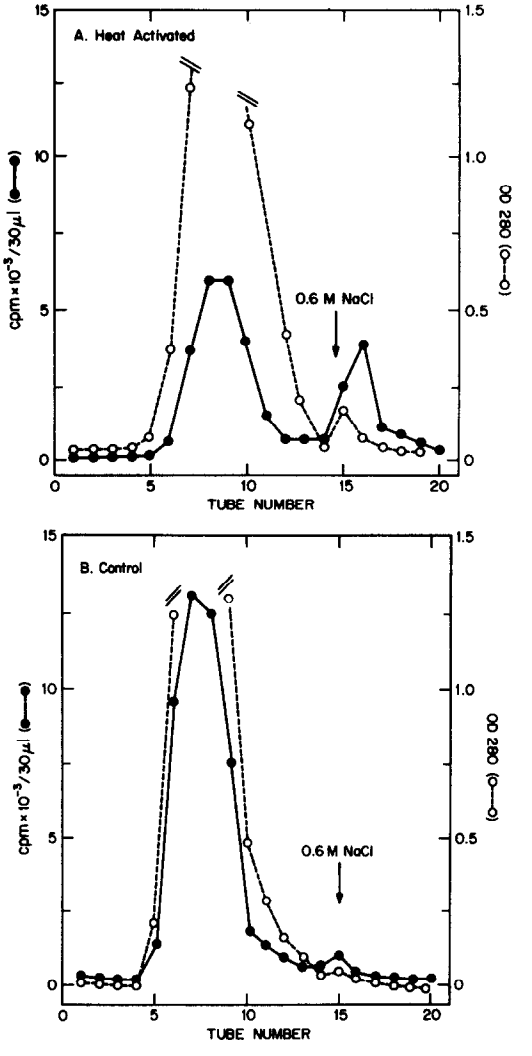

Fig. 1. [^{3}H]-Dexamethasone (25 nM) was added to the 5000 *g* supernatant. After centrifugation for 1 h (105,000 *g*) in the presence of 25 nM [^{3}H]-dexamethasone, cytosol was warmed to 20°C for 1 h (A) and then rechilled to 4°C, or kept at 4°C (B). Cytosol (1 ml) was placed onto a DNA–cellulose column (0·7 × 3 cm.), eluted with 2 bed vol. of 20 mM Tris–Mg buffer containing 0·1 M NaCl and then with 2 ml of 20 mM Tris–Mg buffer containing 0·6 M NaCl. Fractions (0·35 ml) were collected at a flow rate of 4 ml/h. $OD_{280\,nm}$ (1:10 dilution) and [^{3}H] (c.p.m./30 μl) were determined for each fraction.

Table 1. Gel Filtration (G25 Sephadex) of Fractions from DNA Cellulose Chromatography of Heat Activated Cytosol.

Sample	% [^{3}H]Dexamethasone Bound to Macromolecules	pMoles [^{3}H]Dexamethasone bound/mg protein
1. Heat activated cytosol	23	5
2. 0.1 M NaCl wash	8	1
3. 0.6 M NaCl eluate	29	197

Heat activated cytosol (1 ml) containing 25 nM [^{3}H]dexamethasone was placed onto a 0.7 x 3 cm column of DNA cellulose and eluted with 2.0 ml of 0.1 M NaCl and then with 2.0 ml of 0.6 M NaCl. 100 µl samples from the initial heat-activated cytosol and from peak fractions of the 0.1 M NaCl and 0.6 M NaCl washes were passed through G25 Sephadex columns (0.7 x 10 cm). Macromolecular-bound [^{3}H]dexamethasone and protein content were determined for each sample.

Table 2. Nuclear Binding of [^{3}H]Dexamethasone Receptor Complex.

Sample	cpm/50 µl	[^{3}H]Dexamethasone Bound to Nuclei (cpm) Blank	Sample
1. Heat activated cytosol	36,000	310	2900
2. 0.45 M NaCl eluate	4,090	115	1010

Heat-activated cytosol containing 40 nM [^{3}H]dexamethasone was fractionated on DNA cellulose as described (Figure 1), except that 0.45 M NaCl was used to elute the GR complex. In duplicate, 50 µl of sample or buffer (as blank) adjusted to the same [^{3}H]dexamethasone concentration were added to purified nuclei (2.2×10^7/ml) in 200 µl of Tris-Mg buffer containing 0.25 M sucrose and were incubated at 25° for 20 minutes. Reaction mixtures were chilled to 4°, and nuclei were separated by centrifugation (x2) in 3 ml of Tris-Mg buffer (0.25 M sucrose) at 1000 x *g* for 10 minutes. The nuclear pellet was resuspended in 100 µl H_2O and counted after solubilization in Triton-toluene. Each entry is the mean value of duplicate assays.

DISCUSSION

The elegant studies of Baxter[7] indicated that the glucocorticoid–receptor complex, subjected to temperature-dependent activation could bind to double-stranded DNA. Double-stranded DNA immobilized on cellulose is an effective ligand for the heat-activated GR complex from rat liver [9]. Since the GR complex can be eluted from DNA–cellulose by NaCl, DNA–cellulose chromatography provides a simple technique for separating the GR complex from other cytosol proteins that do not bind to DNA.

Most of the [^{3}H]-dexamethasone bound to macromolecules in heat-activated cytosol was retained by DNA–cellulose. Elution with 0·6 M NaCl released all of the [^{3}H]-dexamethasone bound to the column, but only 29% of this remained bound to macromolecules on the basis of gel filtration. The elution conditions could favor destruction of the receptor or, alternatively, dissociation of steroid from the receptor.

Little is known about the chemical basis for the binding of heat-activated GR complex to DNA. Milgrom *et al.*[12] have recently shown that heat activation increases the affinity of the GR complex to various polynucleotides, such as homologous and heterologous DNA and tRNA, and also to carboxymethyl and sulfopropyl–Sephadex. Homologous and heterologous double stranded DNA, however, were best able to compete with nuclei for the binding of heat-activated GR complex.

Rat liver contains several protein moeities that can bind natural glucocorticoids such as corticosterone and polar metabolites of natural glucocorticoids. The ability to bind the synthetic glucocorticoid dexamethasone, however, seems to be restricted to a single protein moeity [13, 14]; anion exchange chromatography using DEAE–cellulose [15], or DEAE–Sephadex [14] has been used to purify the dexamethasone-binding moeity from rat liver. Litwack *et al.*[14] provided immunological evidence that this dexamethasone-binding protein (Binder II) enters the cell nucleus following glucocorticoid treatment *in vivo* and hence is presumably the receptor involved in mediating the biological effects of glucocorticoids.

It is of interest that the androgen-receptor complex binds to DNA–cellulose and can be eluted by KCl; both the 8S form and the heat-activated 4S form bind [16]. Similarly, both the 8S and 4S forms of the estrogen–receptor complex bind to DNA cellulose [16, 17]; although Puca *et al.*[18] could demonstrate estrogen-receptor complex binding to DNA–cellulose only after the addition of basic protein to the DNA–cellulose.

DNA–cellulose chromatography may thus prove useful in defining the nature of the nuclear acceptor sites for various steroid receptor complexes.

ADDENDUM

Following completion of this study, Colman P. and Kalimi M.: Fedn Proc. **33** (1974) 1360, reported that the heat-activated glucocorticoid–receptor complex from rat liver binds to DNA–cellulose and can be eluted by NaCl.

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REFERENCES

1. Baxter J. D. and Tompkins G. M.: *Proc. natn Acad. Sci. U.S.A.* **65** (1970) 709–715.
2. Munck A. and Brinck-Johnsen T.: *J. biol. Chem.* **243** (1968) 5556–5565.
3. Beato M., Biesewig D., Braendle W. and Sekeris C. E.: *Biochim. biophys. Acta* **192** (1969) 494–506.
4. Wira C. and Munck A.: *J. biol. Chem.* **245** (1970) 3436–3438.
5. Rousseau G. G., Baxter J. D., Higgins S. J. and Tompkins G. M.: *J. molec. Biol.* **79** (1973) 539–554.
6. Higgins S. J., Rousseau G. G., Baxter J. C. and Tompkins G. M.: *J. biol. Chem.* **248** (1973) 5866–5872.
7. Baxter J. D., Rousseau G. G., Benson M. C., Garcea R. L., Ito J. and Tompkins G. M.: *Proc. natn. Acad. Sci. U.S.A.* **69** (1972) 1892–1896.
8. Alberts B. and Herrick G.: *Meth Enzymol.* **XXI** (1971) 198–217.
9. Beato M., Kalemi M., Konstam M. and Feigelson P.: *Biochemistry* **12** (1973) 3372–3378.
10. Zieve F.: *J. biol. Chem.* **247** (1972) 5987–5995.
11. Lowry O. H., Rosebrough N. J., Farr A. L. and Randall R. J.: *J. biol. Chem.* **193** (1951) 265–279.
12. Milgrom E., Alger A. and Baulieu E.-E.: *Biochemistry* **12** (1973) 5198–5205.
13. Beato M. and Feigelson P.: *J. biol. Chem.* **247** (1972) 7890–7896.
14. Litwack G., Fuller R., Rosenfield S. A., Lichtash N., Wishman C. and Singer S.: *J. biol. Chem.* **248** (1973) 7481–7486.
15. Koblinsky M., Beato M., Kalemi M. and Feigelson P.: *J. biol. Chem.* **247** (1973) 7897–7904.
16. Mainwaring W. I. P. and Irving R.: *Biochem. J.* **134** (1973) 113–127.
17. Clemens L. E. and Kleinsmith L. J.: *Nature New Biol.* **237** (1972) 204–206.
18. Puca G. A., Sica V. and Nola E.: *Proc. natn Acad. Sci. U.S.A.* **71** (1974) 979–983.