Physical Measurements of the Liver Glucocorticoid Receptor

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Physical measurements were made on the cytosolic form of the liver [^3H]dexamethasone receptor. These include a Stokes radius of 3.5 nm, determined by gel filtration, and sedimentation coefficients of 5.1 and 7–8 S, by sucrose-density-gradient centrifugation. From these measurements, the following physical properties were calculated: apparent mol.wt. 78000 (the 5.1 S form); $D_{\text{app.}}$ $6.1 \times 10^{-7} \text{ cm}^2 \cdot \text{s}^{-1}$; f/f_0 1.25; axial ratio 4.7; these indicate a globular protein. Measurements of sedimentation coefficients of cytosol steroid–receptor complexes previously subjected to various activating conditions gave different values and lead to the conclusion that the mechanism of activation *in vitro* enabling the steroid–receptor complex to bind to DNA is more complex than simple disaggregation to a uniform size.

The glucocorticoid receptor is one of six proteins in liver cytosol that bind either potent glucocorticoids or their metabolites. Three proteins bind unmetabolized glucocorticoids. Binder IB (Litwack & Rosenfield, 1975), a protein of diameter 2 nm that has a binding specificity somewhat similar to that of the dexamethasone receptor, but that binds natural glucocorticoids to nearly the same extent as does dexamethasone. It occurs in male adrenalectomized rat liver cytosol at about one-tenth of the concentration of Binder II (Litwack & Rosenfield, 1975). The second protein that binds potent glucocorticoids is Binder II, the glucocorticoid receptor, and the third is Binder IV, a fraction containing transcortin (Litwack *et al.*, 1973). Proteins that bind anionic metabolites of glucocorticoids are Binders IA, IIIA and IIIB. Binder IA is ligandin (Litwack *et al.*, 1971), Binder IIIA is a low-molecular-weight polypeptide (Morey & Litwack, 1969) and Binder IIIB is a newly discovered protein that binds primarily mono- and di-sulphates of cortisol (J. R. Steeger & G. Litwack, unpublished work). These proteins have been numbered in the order of their elution from a DEAE-Sephadex A-50 column (3 cm $\times$ 76 cm) and consequently reflect their pI values (Litwack, 1975).

In spite of this complexity in liver, it appears that there is only one glucocorticoid-receptor protein, Binder II, that translocates to the nucleus (Litwack *et al.*, 1973); however, Binder IB may be a distinct corticosteroid receptor (Litwack & Rosenfield, 1975). We have physically separated the [^3H]dexamethasone-labelled receptor of liver cytosol into non-activated and activated species by rapid

ion-exchange chromatography (Parchman & Litwack, 1977). In this same system, but with kidney receptors, natural glucocorticoids behave differently from dexamethasone (L. G. Parchman, R. D. Markovic & G. Litwack, unpublished work). In the present paper we report measured and calculated physical properties of the liver glucocorticoid receptor.

Materials and Methods

Liver cytosols were prepared from adrenalectomized male Sprague–Dawley rats by published methods (Cake & Litwack, 1975; Cake *et al.*, 1976). [$1,2,4\text{-}^3\text{H}$]Dexamethasone (20–40 Ci/mmol) was obtained from New England Nuclear Corp., Boston, MA, U.S.A. Ribonuclease-free sucrose was obtained from Schwarz/Mann, Orangeburg, NY, U.S.A. Other chemicals and biochemicals were of the highest purity obtainable from commercial sources.

Sucrose-density-gradient analysis was carried out as described by Martin & Ames (1961), with a 5–20% (w/w) linear sucrose gradient in 50 mM-Tris/HCl, pH 7.5, containing 30 nM-[^3H]dexamethasone throughout the gradient. Specifically bound radioactivity was determined on fractions obtained from the gradient by using a charcoal assay (Beato & Feigelson, 1972).

Conditions for binding the steroid–receptor complex to DNA–cellulose have been reported by Cake & Litwack (1975). Stokes radius (a) was determined on Sephadex G-100 (Pharmacia Piscataway, NJ, U.S.A. columns (1.8 cm $\times$ 60 cm), with the use of proteins of known Stokes radius to calibrate the relationship between elution volume and Stokes

radius (Siegel & Monty, 1966). Total volume of the column to the elution maximum of small molecules was determined with sodium dichromate and the void volume was determined with Blue Dextran (Pharmacia). Apparent molecular weight ($M_{app.}$) by gel filtration was determined by the method of Litwack *et al.* (1973).

Calculations of physical parameters were done by using experimentally determined constants. The apparent diffusion coefficient ($D_{app., 20, w}$) was calculated from the equation:

$$D_{app.} = \frac{RTs}{M(1-\bar{v}\rho)}$$

The buoyancy factor ($1-\bar{v}\rho$) was assumed to have a value of 0.26, calculated from the values of $\bar{v}$ based on amino acid analysis of a large number of proteins. The apparent molecular weight ($M_{app.}$) was calculated from the experimentally determined sedimentation coefficient (Martin & Ames, 1961) and Stokes radius by the equation:

$$M_{app.} = \frac{6\pi\eta Ns}{1-\bar{v}\rho}$$

Table 1. Measured and calculated physical values of the liver cytosol [3H]dexamethasone receptor

Physical constant	
Stokes radius (a)	3.5 nm
$s_{20, w}$	5.1 S; 7-8 S
$M_{app.}$ (from a and $s_{20, w}$)	78 000
$D_{app.}$	$6.1 \times 10^{-7} \text{ cm}^2 \cdot \text{s}^{-1}$
f/f_0	1.25
Axial ratio (prolate ellipsoid)	4.7

Fig. 1. Analytical sucrose-density-gradient centrifugation of the [3H]dexamethasone receptor after various conditions of activation *in vitro*

The non-activated control (—) bound DNA-cellulose to the extent of 1.1% of specifically bound [3H]dexamethasone. Before centrifugation, activation by 0.1 M-NaSCN for 1 h at 0°C (---) led to binding of 34.5% of specifically bound [3H]dexamethasone to DNA-cellulose. Activation by 0.1 M-NaCl for 1 h at 0°C (····) resulted in 8.5% of specifically bound [3H]dexamethasone able to bind to DNA-cellulose. Sedimentation coefficients were calculated as described by Martin & Ames (1961) relative to the position of ovalbumin. Peaks in this Figure are not distorted by dissociation of the [3H]steroid during the run because binding was determined by the charcoal procedure on the fractions as described in Materials and Methods section.

The frictional coefficient (f/f_0) was calculated from the measured value of Stokes radius (a) by the equation:

$$f/f_0 = \frac{a}{(3\bar{v}M/4\pi N)^{1/3}}$$

For this calculation, an average value of 0.74 for $\bar{v}$ was used arbitrarily. The axial ratio was determined from Perrin's Rule from the calculated value of f/f_0

(Tanford, 1966); see Criss *et al.* (1970) for further explanation of physical symbols.

Results and Discussion

Physical measurements and calculated values for specific binding of [³H]dexamethasone are summarized in Table 1. On the basis of the sedimentation coefficients there appear to be three forms of the receptor. There is an aggregated form of mol.wt. higher than 200000 as shown on Sepharose 4B columns (Parchman *et al.*, 1977b) and by short-term sucrose-density-gradient centrifugation (G. Litwack & M. H. Cake, unpublished work). This form sediments to the bottom of the tube during conventional overnight sucrose-density-gradient-centrifugation experiments. The aggregate occurs in freshly prepared cytosol and becomes progressively disaggregated by dilution, directly or during centrifugation or column-chromatographic procedures. The physiological importance, if any, of this form is not clear. Two lower-molecular-weight forms are more easily documented; these have sedimentation coefficients of 7–8S and 5.1S (Fig. 1). The structural difference between these two forms on a molecular level is not yet clear. Calculation of the apparent molecular weight from the lowest sedimentation values obtained from sucrose-density-gradient centrifugation and by using a value of 3.5 nm for the Stokes radius (Fig. 2) gives a value of 78000, increasing the previous estimate of the cytosol cortisol-binding form from 67000. The lower estimate was made from direct measurement of molecular weights on Sephadex G-100 columns (Litwack *et al.*, 1973). Earlier measurements of sedimentation coefficient (4.4S) did not take into account stripping of radioactive ligand during sucrose-density-gradient centrifugation, a condition that tends to decrease the observed value of the sedimentation coefficient. The nucleoplasmic form of the cortisol–receptor complex has been estimated at 62000 by the same method, inferring a loss of molecular weight of 5000 from the cytoplasmic form in nuclear transfer. The nucleoplasmic form has not been reinvestigated under the present conditions (bound with dexamethasone and corrected for ligand dissociation during centrifugation). Other physical data in Table 1 suggest that the 5.1S form of the cytoplasmic steroid–receptor complex is globular.

Table 2 reports measurements of the sedimentation coefficient of the [³H]dexamethasone–receptor complex after various procedures known to cause activation enabling the complex to bind to DNA or liver nuclei (Milgrom *et al.*, 1973; Cake & Litwack, 1975, 1977; Parchman & Litwack, 1977; Goidl *et al.*, 1977). Although it is likely that there is some activation of the non-activated control under these

Table 2. *Effect of activation conditions on the sedimentation coefficient of the cytosol [³H]dexamethasone receptor* In all experiments the cytosol prepared from a 50% homogenate was used. The sucrose-gradient sedimentation was for 18 h. The numbers in parentheses refer to the number of determinations.

Activation procedure	DNA–cellulose binding (% of steroid–receptor complex bound)	Sucrose-binding (% of gradient sedimentation (<i>s</i> _{20,w}))
None (non-activated cytosol)	1.7 (4)	8S
Heat (25°C, 30 min)	28.5 (4)	8S
20 mM-Aminophylline (0°C, 90 min)	30.9 (2)	8S
0.1 M-NaSCN (0°C, 60 min)	33.1 (3)	5.1S
0.1 M-NaCl (0°C, 60 min)	8.8 (3)	5.1S

Fig. 2. *Determination of the Stokes radius of the liver glucocorticoid receptor on a column (3 cm × 70 cm) of Sephadex G-100*

The void volume (*V*₀) is 186 ml. Fractions of volume 3 ml were collected. The position of the receptor was measured by the location of specifically bound radioactivity. This measurement refers to the partially purified activated receptor (Binder II) which is the 5.1S form. The standards were: 1, lactate dehydrogenase; 2, bovine serum albumin; 3, ovalbumin; 4, chymotrypsinogen A; 5, myoglobin.

conditions (Parchman *et al.*, 1977a), it is clear from these results that specific activation procedures can result in steroid–receptor complexes with different sedimentation coefficients. On this basis, we conclude that the activation mechanism *in vitro* is more complex than a process of simple disaggregation.

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