Characterization of the molybdate-stabilized glucocorticoid receptor from rat thymus

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The untransformed glucocorticoid receptor of rat thymus cytosol was characterized in the form of its complex with [1,2,4-³H]triamcinolone acetonide by ion-exchange chromatography and by gel filtration and sucrose-density-gradient ultracentrifugation at different ionic strengths. Molybdate (10 mM) was present throughout all experimental procedures and prevented receptor inactivation and degradation as well as transformation. At low ionic strength the molybdate-stabilized steroid–receptor complex was detected as a single highly asymmetric entity with a Stokes radius of 5.85 nm, a sedimentation coefficient of 9.6 S and an apparent molecular weight of 236 000. This form was converted into a smaller, even more asymmetric, form in increasing proportion as the ionic strength was increased. In the presence of 0.4 M-KCl, the smaller form had a Stokes radius of 4.95 nm, a sedimentation coefficient of 4.6 S and an apparent molecular weight of 95 500. It is concluded that the glucocorticoid–receptor complex exists at low ionic strengths as a homodimer or as a heterodimer in which only one subunit possesses a steroid-binding site, and that the process of dissociation into subunits brought about by increasing the ionic strength is a process distinct from, but possibly preceding, the transformation phenomenon responsible for conferring DNA-binding properties on the complex.

Glucocorticoid hormones bind to specific receptor proteins which, in the absence of hormone, are found in the soluble cytosol fraction of target cells (for a review, see Munck & Leung, 1977). Formation of the steroid–receptor complex is followed by a temperature- and/or ionic-strength-dependent process, variously termed ‘transformation’ or ‘activation’, that results in the complex exhibiting an increased affinity for binding sites in nuclear chromatin; this interaction with chromatin is believed to be responsible for the subsequent accumulation of specific mRNA molecules and the expression of the characteristic biological responses induced by these hormones.

Analysis of the structure of the untransformed form of the glucocorticoid–receptor complex and of the changes occurring during receptor transformation should assist in the elucidation of the

biochemistry of steroid-hormone action, and may also provide a rational basis for the design of synthetic steroids or other related compounds of pharmacological value. Studies of the physical characteristics of glucocorticoid–receptor complexes that have been reported (Koblinsky *et al.*, 1972; Carlstedt-Duke *et al.*, 1977; Middlebrook & Aronow, 1977; Litwack *et al.*, 1978; Cidlowski & Thanassi, 1979; Sherman *et al.*, 1979; Cidlowski, 1980; Lee *et al.*, 1981) have not been conducted under conditions where receptor transformation is prevented, and in some cases have given markedly divergent results. It is possible that the results obtained have been influenced by receptor transformation, occurring during characterization by procedures which utilize high ionic strengths, and perhaps also by receptor degradation, so that the assignment of physical parameters to particular forms or states of the glucocorticoid receptor must be viewed with caution. However, it has been shown that transformation of the glucocorticoid–receptor complex brought about by increased temperature or ionic strength can be inhibited in the presence of molybdate (Leach *et al.*, 1979). Molybdate also

Trivial and systematic names of steroids mentioned in the text: dexamethasone, 9 α -fluoro-11 β ,17,21-trihydroxy-16 α -methylpregna-1,4-diene-3,20-dione; triamcinolone acetonide, 9 α -fluoro-11 β ,16 α ,17,21-tetrahydroxypregna-1,4-diene-3,20-dione 16 α ,17-acetonide.

inhibits the inactivation process responsible for converting glucocorticoid receptors into a form incapable of binding steroid (Sando *et al.*, 1979; Jones & Bell, 1982) and may, like vanadate (Seglen & Gordon, 1981), inhibit protein degradation. The steroid-binding properties of the glucocorticoid receptor of rat thymus cytosol have been determined in the presence of molybdate (Jones & Bell, 1982), and we now report the physical characterization of this molybdate-stabilized untransformed glucocorticoid receptor in the form of its complex with the glucocorticoid agonist triamcinolone acetonide. Even in the absence of transformation, two forms of steroid-receptor complex have been detected, their relative proportions being dependent on ionic strength. Both are highly asymmetric, but differ in apparent molecular weight, Stokes radius and sedimentation coefficient.

Experimental

Materials

[1,2,4-³H]Triamcinolone acetonide (20 Ci/mmol) was obtained from Amersham International, Amersham, Bucks., U.K. Dexamethasone, dithiothreitol, sodium molybdate, calf thymus DNA-cellulose, bovine serum albumin, bovine immunoglobulin G and catalase were supplied by Sigma Chemical Co., Poole, Dorset, U.K. DEAE-cellulose and phosphocellulose were from Whatman Biochemicals, Maidstone, Kent, U.K., and Sephadex G-25 and Sephacryl S-300 were from Pharmacia Fine Chemicals, London W.5, U.K. Calibration proteins for gel chromatography were obtained from Boehringer Corp., Lewes, East Sussex, U.K. All other reagents were supplied by BDH Chemicals, Poole, Dorset, U.K.

Preparation and assay of steroid-receptor complexes

Male Sprague-Dawley rats (8–12 weeks of age), bred in the Institute Animal Colony, were adrenalectomized 1–2 weeks before use. Animals were killed by decapitation under light ether anaesthesia. Thymus tissue was excised and homogenized in 5 vol. of TEDM buffer (1 mM-EDTA, 2.5 mM-dithiothreitol and 10 mM-Na₂MoO₄ in 50 mM-Tris/HCl buffer, pH 7.4 at 20°C) at 0–4°C. The homogenate was centrifuged at 105 000 *g* for 60 min at 4°C; the supernatant (cytosol) fraction was removed free of floating fat and used immediately. Steroid-receptor complexes for chromatographic studies were as a routine prepared by incubation of cytosol with 20 nM-[1,2,4-³H]triamcinolone acetonide for 2–3 h at 0°C. Unbound steroid was then removed by chromatography on small columns (bed volume 9.1 ml) of Sephadex G-25 that had been equilibrated with the appropriate buffer. All further

procedures were performed at 0°C. Steroid-binding studies were performed by using a dextran/charcoal competitive-binding assay or by chromatography on Sephadex G-25, as described by Jones & Bell (1980).

Assessment of transformation status of steroid-receptor complexes

Cytosol preparations that had been incubated with [1,2,4-³H]triamcinolone acetonide as described above were incubated further, for 2 h at 0°C in the presence or absence of 0.4 M-KCl. Salt was then removed from the preparations by chromatography on columns of Sephadex G-25 equilibrated with TEDM buffer, and portions of the salt-free eluates were applied to columns of DNA-cellulose or phosphocellulose (bed volume 1.0 ml) equilibrated in the same buffer. The columns were washed with TEDM buffer until the net *A*₂₈₀ of the eluate was zero; bound protein was subsequently eluted with TEDM buffer containing 0.4 M-KCl.

Cytosol containing [1,2,4-³H]triamcinolone acetonide-receptor complexes was also applied to a DEAE-cellulose column (bed volume 10.0 ml) in TEDM buffer. The column was washed with the same buffer until the eluate was protein-free; bound protein was eluted with a linear salt gradient (300 ml: 0–0.3 M-KCl) in TEDM buffer.

Gel filtration

Gel-filtration chromatography of preparations containing [1,2,4-³H]triamcinolone acetonide-receptor complexes was performed on a column of Sephacryl S-300 (void volume 192 ml). The column was equilibrated and subsequently developed with TEDM buffer containing 0, 0.1, 0.2, 0.3 or 0.4 M-KCl, at a flow rate of 20 ml/h. Columns were calibrated with ferritin, catalase, aldolase, bovine serum albumin and hen's-egg albumin (Stokes radii 6.1, 5.1, 4.5, 3.55 and 3.05 nm respectively) and with Blue Dextran.

Sucrose-density-gradient centrifugation

Steroid-receptor complexes were analysed on linear 5–20% (w/v) sucrose density gradients, prepared in TEDM buffer containing 0, 0.05, 0.1, 0.2 or 0.4 M-KCl. Gradients were centrifuged at 105 000 *g* for 18 h at 4°C and then fractionated by upward displacement with 40% (w/v) sucrose and analysed for radioactivity. Bovine serum albumin, bovine immunoglobulin G and catalase (*s*_{20,w} 4.6 S, 7.0 S and 11.3 S respectively) were included in a parallel gradient and located by their *A*₂₈₀.

General methods

Radioactivity was measured for aqueous samples in Packard emulsifying scintillant 299 (Packard Instrument Co., Caversham, Berks., U.K.) with a

Nuclear–Chicago Isocap liquid-scintillation spectrometer. Salt concentrations in the eluate from chromatography columns were determined by conductivity measurement with a Radiometer model CDM 83 conductivity meter, and protein concentrations were determined by the A_{280} or by the dye-binding method of Bradford (1976) with bovine serum albumin as standard.

Results

Binding parameters for [1,2,4-³H]triamcinolone acetonide

The specific, saturable binding of [1,2,4-³H]-triamcinolone acetonide to the molybdate-stabilized glucocorticoid receptor in rat thymus cytosol was assessed by incubating cytosol with a range of concentrations of the tritiated steroid for 72 h at 0°C, in the presence and in the absence of 10 μM-dexamethasone. Protein-bound steroid was determined by dextran/charcoal competitive binding assay and the data were analysed by the Scatchard (1949) method. The equilibrium dissociation constant for the interaction of [1,2,4-³H]triamcinolone acetonide with the receptor at 0°C was 0.68 nM; the concentration of receptor-binding sites for the steroid was 0.82 pmol/mg of cytosol protein.

Prevention by molybdate of receptor transformation

In the absence of molybdate, the glucocorticoid–receptor complex can be transformed by an increase in ionic strength to a form capable of binding to both DNA–cellulose and phosphocellulose (Milgrom *et al.*, 1979; Leach *et al.*, 1979). The binding of the molybdate-stabilized [1,2,4-³H]triamcinolone acetonide–receptor complex to DNA–cellulose and phosphocellulose was measured both before and after the exposure of preparations to 0.4 M-KCl, in order to determine whether the conditions to be used subsequently for gel filtration and density-gradient centrifugation would cause transformation of the receptor. When 10 mM-molybdate was present throughout, there was little binding of the complex that had not been exposed to KCl to

either matrix, and no significant increase in binding after exposure to 0.4 M-KCl in TEDM buffer (Table 1).

Glucocorticoid-receptor transformation is also associated with a decrease in the ionic strength required to elute the steroid–receptor complex from DEAE-cellulose; partially transformed preparations show two peaks of protein-bound radioactivity corresponding to transformed and untransformed forms (Sakaue & Thompson, 1977). Chromatography of the molybdate-stabilized [1,2,4-³H]triamcinolone acetonide–receptor complex on DEAE-cellulose, however, resulted in the elution of a single symmetrical peak of protein-bound radioactivity between 120 and 180 mM-KCl (Fig. 1).

Gel-filtration studies

Glucocorticoid–receptor complexes were characterized by gel filtration on Sephacryl S-300; 10 mM-molybdate was present throughout the experimental procedures. Chromatography of rat thymus cytosol containing [1,2,4-³H]triamcinolone acetonide–receptor complexes under low-salt conditions (TEDM buffer alone) resulted in the elution of a single symmetrical peak of radioactivity with an elution volume corresponding to a distribution coefficient, K_{av} , of 0.16 ± 0.02 (Fig. 2). Comparison with the K_{av} values of standard proteins of known Stokes radius indicated that, under these conditions, the glucocorticoid–receptor complex had a Stokes radius of 5.85 nm (Table 2).

To investigate the effect of ionic strength on the chromatographic behaviour of the complex, steroid–receptor preparations were chromatographed in TEDM buffer containing 0.1, 0.2, 0.3 or 0.4 M-KCl. An increase in the elution volume of the previously identified form of the complex (form I) was observed as the concentration of KCl was increased, without any accompanying changes in the elution volumes of standard proteins such as catalase (Fig. 3). The distribution coefficient of this form of the complex changed from 0.16 ± 0.02 in the absence of KCl to 0.26 ± 0.02 in the presence of 0.4 M-KCl, corresponding to a change in Stokes radius from 5.85 nm

Table 1. Assessment of the transformation status of the [1,2,4-³H]triamcinolone acetonide–receptor complex in the presence of molybdate

Cytosol labelled with [1,2,4-³H]triamcinolone acetonide was incubated in TEDM buffer in the presence and in the absence of 0.4 M-KCl for 2 h at 0°C. The extent of binding of the steroid–receptor complex to DNA–cellulose and phosphocellulose was then determined as described in the text.

Conditions	DNA–cellulose			Phosphocellulose		
	Unbound complex (pmol)	Bound complex (pmol)	% bound	Unbound complex (pmol)	Bound complex (pmol)	% bound
–KCl	2.74	0.04	1.4	2.96	0.23	7.7
+KCl	2.60	0.07	2.6	3.18	0.27	8.4

Fig. 1. Chromatography of the [1,2,4-³H]triamcinolone acetonide-receptor complex on DEAE-cellulose in the presence of molybdate

Cytosol labelled with [1,2,4-³H]triamcinolone acetonide was absorbed on a column (bed vol. 10.0 ml) of DEAE-cellulose in TEDM buffer and subsequently eluted with a linear gradient of 0–0.3 M-KCl in the same buffer (----). Fractions (3.3 ml) were collected for the determination of ³H radioactivity (●) and A_{280} (▲). For full experimental details, see the text.

Fig. 2. Gel filtration of the [1,2,4-³H]triamcinolone acetonide-receptor complex on Sephacryl S-300 in the absence of KCl

Cytosol was labelled with [1,2,4-³H]triamcinolone acetonide in the presence of 10 mM-molybdate and free steroid was subsequently removed by chromatography on Sephadex G-25 as described in the Experimental section. The macromolecular fraction (5 ml) was applied to a column ($V_0 = 192$ ml) of Sephacryl S-300 and eluted with TEDM buffer at a flow rate of 20 ml/h. Fractions (3.25 ml) were collected; portions of each fraction were assayed for radioactivity (●) and A_{280} (▲). The positions of elution of ferritin, catalase and aldolase are shown by a, b and c, respectively.

to 5.50 nm. In addition, a second peak of protein-bound radioactivity appeared as the ionic strength was increased. The proportion of total protein-bound radioactivity in this second peak increased with increasing ionic strength, as did the elution volume and K_{av} . (Fig. 3). At 0.4 M-KCl the K_{av} of this second form of the complex (form II) corresponded to a Stokes radius of 4.95 nm (Table 2).

Conversion of form I into form II was incomplete, even in the presence of 0.4 M-KCl, when chromatography was performed immediately after labelling cytosol with [1,2,4-³H]triamcinolone acetonide (Fig. 4). In an attempt to achieve a complete conversion from one form into the other, preformed steroid-receptor complexes in cytosol were exposed to 0.4 M-KCl for 24 h at 0°C before chromatography on Sephacryl S-300. This procedure increased the proportion of the complex eluted as the smaller form (Fig. 4). Complete conversion was not always achieved, and often a small proportion of the larger species remained.

Sedimentation behaviour of the glucocorticoid-receptor complex

Rat thymus cytosol containing the glucocorticoid receptor complexed with [1,2,4-³H]triamcinolone acetonide was subjected to centrifugation on linear

Table 2. Physical parameters of the [1,2,4-³H]triamcinolone acetonide-receptor complex in the presence of molybdate

Stokes radii and sedimentation coefficients were determined as described in the text, in the absence of KCl for form I and in the presence of 0.4 M-KCl for form II. Apparent molecular weights were calculated as described by Siegel & Monty (1966) and frictional ratios as described by Notides & Nielsen (1974). The partial specific volume was taken to be 0.73 (Birnbaumer *et al.*, 1981) and the solvation factor to be 0.2 g of solvent/g of solute. Axial ratios were obtained from the tables of Schachman (1959). Results are expressed as mean values $\pm$ S.D. for at least three experiments.

Parameter	Form I	Form II (0.4 M-KCl)
Stokes radius (nm)	5.85 $\pm$ 0.10	4.95 $\pm$ 0.10
$s_{20,w}$ (S)	9.6 $\pm$ 0.2	4.6 $\pm$ 0.1
Apparent mol.wt.	236 000	95 500
Frictional ratio, f/f_0	1.32	1.51
Axial ratio	6	8–10

Fig. 3. Effect of KCl concentration on the distribution coefficient of the [1,2,4-³H]triamcinolone acetonide-receptor complex on Sephacryl S-300

The data presented were obtained from experiments similar to that shown in Fig. 2, except that the TEDM buffer used for elution of the Sephacryl column contained concentrations of KCl as indicated. Results are expressed as mean value $\pm$ range. Values for the distribution coefficient of form II of the complex in the absence of KCl were obtained from experiments in which receptor preparations that had been exposed to 0.4 M-KCl were subsequently desalted, as described in the Results section. ●, Form I; ○, form II; ▲, catalase.

Fig. 4. Gel filtration of the [1,2,4-³H]triamcinolone acetonide-receptor complex on Sephacryl S-300 in the presence of 0.4 M-KCl

Experimental procedures were as described in the legend to Fig. 2, except that the column had been equilibrated with, and was eluted with, TEDM buffer containing 0.4 M-KCl. The sample was applied to the column immediately after preparation (●) or after a further incubation at 0°C for 24 h in the presence of 0.4 M-KCl (○). The positions of elution of ferritin, catalase and aldolase are shown by a, b and c, respectively.

5–20% (w/v) sucrose density gradients containing 10 mM-molybdate and various concentrations of KCl. The sedimentation profiles showed a marked dependence on ionic strength (Fig. 5). Profiles obtained in the absence of KCl and with 0.4 M-KCl present showed minimal asymmetry and gave sedimentation coefficients of 9.6 S and 4.6 S respectively. Distinctly asymmetric profiles with intermediate sedimentation behaviour were obtained in the presence of 0.05, 0.1, 0.15 and 0.2 M-KCl (Fig. 5); this behaviour suggested that a slow salt-dependent dissociation or conversion process was being detected, identical with that observed in the gel-filtration studies. As expected, when form II of the complex was obtained by gel filtration after treatment of labelled cytosol with 0.4 M-KCl, and subjected to density-gradient centrifugation, it sedimented on gradients containing 0.4 M-KCl at 4.6 S. Apparent molecular weights and other parameters calculated from the data for the two forms of the complex are listed in Table 2.

Fig. 5. Sucrose-density-gradient centrifugation of $[1,2,4\text{-}^3\text{H}]$ triamcinolone acetonide-receptor complexes

Cytosol was labelled with $[1,2,4\text{-}^3\text{H}]$ triamcinolone acetonide and the macromolecular fraction was obtained by chromatography on Sephadex G-25 as described in the Experimental section. Portions of this fraction were sedimented on linear 5–20% (w/v) sucrose gradients containing (●) 0, (▲) 0.05 M-, (○) 0.1 M-, (□) 0.15 M-, (■) 0.2 M- or (△) 0.4 M-KCl. Gradients were centrifuged at 105 000 *g* for 18 h, then fractionated by upward displacement. The positions of sedimentation of bovine serum albumin, bovine immunoglobulin G and catalase ($s_{20,w}$ 4.6S, 7.0S and 11.3S, respectively) are shown by the arrows marked *a*, *b* and *c*, respectively. Sedimentation was from left to right.

Fig. 6. Effect of preincubation at elevated ionic strength on the sedimentation behaviour of the $[1,2,4\text{-}^3\text{H}]$ -triamcinolone acetonide-receptor complex

The labelled glucocorticoid-receptor complex was prepared as described in the legend to Fig. 5 and sedimented immediately on linear 5–20% (w/v) sucrose gradients containing no KCl (●), 0.1 M-KCl (○) or 0.4 M-KCl (△). The complex was also exposed to 0.1 M-KCl for 24 h at 0°C before sedimentation on sucrose gradients containing 0.1 M-KCl (■). The sedimentation positions of bovine serum albumin, bovine immunoglobulin G and catalase ($s_{20,w}$ 4.6S, 7.0S and 11.3S, respectively) are shown by the arrows marked *a*, *b* and *c* respectively. Sedimentation was from left to right.

Conversion of the complex from form I into form II was more complete after density-gradient centrifugation at high salt concentrations than after gel filtration under similar conditions, even though the two procedures were of similar duration. Nevertheless, the transition from the 9.6S form to the 4.6S form also depended on time as well as salt concentration; the effect of 0.1 M-KCl on the sedimentation coefficient of the steroid-receptor complex was increased by preincubation of steroid-labelled cytosol at this salt concentration for 24 h before centrifugation (Fig. 6).

To investigate the possible reversibility of the process responsible for conversion of the complex from form I into form II, cytosol labelled with $[1,2,4\text{-}^3\text{H}]$ triamcinolone acetonide was subjected to centrifugation on sucrose density gradients containing 0.4 M-KCl. Fractions containing the 4.6S species of complex (form II) were recovered, pooled and desalted on Sephadex G-25. Chromatography of the recovered steroid-receptor complex on Sephacryl S-300 in the absence of KCl yielded a single species with a K_{av} of 0.26 ± 0.02 , corresponding to a Stokes radius of 5.5 nm. Since form I of the complex had a Stokes radius of 5.85 nm (K_{av} , 0.16) in the absence of KCl, the process had not been reversed. Although form II of the complex was normally

absent from preparations that had not been exposed to KCl, the results obtained in this experiment presumably represent the distribution coefficient and Stokes radius of this smaller form under low-salt conditions, and are so depicted in Fig. 3.

Discussion

The characteristics of the glucocorticoid receptor that have been determined in the studies reported in this paper are believed to be those of the untransformed undegraded glucocorticoid-receptor complex. The inclusion of molybdate in all preparations and analytical systems prevented receptor transformation and inactivation and may also have inhibited protein degradation. The possibility of proteolysis was also diminished by using a tissue with low endogenous proteolytic activity and by using chromatographic systems with high flow rates and comparatively short run times. Under no circumstances were small forms of glucocorticoid-receptor complex detected similar to those reported to arise in liver or kidney cytosol as a result of proteolytic cleavage (Carlstedt-Duke *et al.*, 1979; Sherman *et al.*, 1979). However, it should be recognized that the properties determined for the glucocorticoid-receptor complex, even at low ionic

strength, may not necessarily be those of the native form as it exists in rat thymus cytosol; for example, a larger form, with additional subunits held together by relatively weak protein-protein interactions, could have dissociated during the course of the separation procedures used for characterization. Dissociation of the steroid ligand from the receptor was not a problem in these experiments, since the steroid used, triamcinolone acetonide, has a high affinity for the receptor and a correspondingly low dissociation rate constant (Jones & Bell, 1982).

Only one form of glucocorticoid-receptor complex was detected at low ionic strength, but this was converted into a second, smaller form in increasing proportion as the ionic strength was raised. The conversion process appeared to be irreversible under the conditions used. The salt concentration also influenced the elution volumes of both forms of complex from Sephacryl S-300, but had no effect on the elution volumes of the proteins used as standards for size determination. Because of this phenomenon it is difficult to make precise comparisons with results obtained by other workers. Nevertheless, it is clear that the molecular dimensions of the larger form of glucocorticoid-receptor complex detected in the present studies are greater than those reported in studies of the corresponding receptor from rat liver by Koblinsky *et al.* (1972) and Litwack *et al.* (1978). Values reported by these workers ranged from 3.5 to 4.5 nm for the Stokes radius and from 5.1S to 8S for the sedimentation coefficient. For the reasons discussed above, these results may have been influenced by receptor transformation and/or degradation. Carlstedt-Duke *et al.* (1977) characterized a form of the glucocorticoid-receptor complex from rat liver which had a Stokes radius of 6.1 nm and a sedimentation coefficient of 4.0S in the presence of either 0.15M- or 0.4M-KCl. These properties are similar to those of the smaller form of complex, denoted form II, detected in the present work. Whether the differences in molecular dimensions are real or not remains to be determined by comparative studies with thymus and liver in the presence and in the absence of molybdate.

The properties of the larger form of steroid-receptor complex characterized in the studies reported here, form I, are similar to those reported by Cidlowski (1980), also for the rat thymus receptor but in the absence of molybdate. He detected a complex with a Stokes radius of 5.6 nm and a sedimentation coefficient of approx. 8S. In contrast with the present observations, however, this form was unstable and was converted into a 1.8 nm meroreceptor form on extended incubation. The comparison with our observations constitutes the strongest evidence for the inhibition by molybdate of the proteolytic cleavage of the glucocorticoid receptor.

The results of both the gel-filtration studies and the density-gradient-centrifugation analyses indicated that the conversion of the glucocorticoid-receptor complex from the larger into the smaller form was dependent on the salt concentration and on the duration of exposure. Nevertheless, there were quantitative differences between the two procedures. In particular, conversion into the smaller form was always complete after centrifugation on density gradients containing 0.4M-KCl, but was incomplete after chromatography on Sephacryl S-300 at the same salt concentration. This difference in behaviour could not be attributed to a difference in the duration of the two procedures; instead it seems likely that the greater degree of conversion on sucrose density gradients was due to the disruptive effect on subunit interactions of the hydrodynamic pressure generated during centrifugation. The same explanation has been invoked to account for similar effects observed in the study of oestrogen receptors (Auricchio *et al.*, 1978; Sica & Bresciani, 1979). Some variation in the extent of conversion into the smaller form of the complex that could not be attributed to the influence of time or salt concentration was also observed in the gel-filtration studies. A similar variability has been noted by Lee *et al.* (1981) and may be related to the influence of other factors such as the total protein concentration or the receptor concentration of the different preparations used.

Our interpretation of the results obtained in the studies reported here is that the untransformed glucocorticoid-receptor complex exists at low ionic strength as an asymmetric molecule composed of two subunits (form I). The subunits themselves are even more asymmetrical, possibly rod-shaped, and are aligned in a side-by-side configuration in the dimer. Dissociation of the dimer by exposure to increased ionic strength generates a highly asymmetric monomer (form II) which has a Stokes radius that is only slightly less than that of the dimer but which has a much decreased sedimentation coefficient. This model has similarities to that proposed for the progesterone receptor (Schrader *et al.*, 1975) except that no evidence has been obtained, either in the present investigation or in any other studies, for the existence of two different monomeric units, each with a steroid-binding site. Thus the dimeric form of the untransformed glucocorticoid-receptor complex appears to be either a homodimer or a heterodimer in which only one subunit possesses a steroid-binding site. The molecular-weight estimates obtained from the Stokes radii and sedimentation coefficients can only be regarded as approximations and cannot be relied on to distinguish between a homo- and a hetero-dimer. However, the molecular weight estimated for the presumed monomeric untransformed steroid-binding unit, 95 500, is in

reasonable agreement with values of 87000 and 85000 determined by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis after affinity labelling of glucocorticoid-receptor preparations (Nordeen *et al.*, 1981; Simons & Thompson, 1981).

Conversion of the glucocorticoid-receptor complex into the smaller monomeric form by exposure to increased ionic strength in the presence of molybdate did not confer DNA-binding properties on the complex. Dissociation into subunits must therefore be distinct from receptor transformation, although it is likely that it accompanies the transformation process that is induced by salt in the absence of molybdate (Leach *et al.*, 1979). It is possible that the dissociation process is in fact a necessary prelude to transformation. The transformed glucocorticoid-receptor complex that has been purified from rat liver cytosol has been reported to have a molecular weight of 89000 by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis, a Stokes radius of 6.0nm and a sedimentation coefficient of 3.4S (Wrange *et al.*, 1979). These values are comparable with those obtained for the monomeric untransformed complex in the work reported in the present paper, and suggest that the transformation process causes only a small overall change, if any, in the molecular dimensions of the glucocorticoid-receptor complex.

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