Purified glucocorticoid–receptor complexes from rat liver cytosol preferentially bind in vitro to a homologous DNA fraction whose transcription is activated by cortisol

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The in vitro binding of 2000-fold purified rat liver glucocorticoid–receptor complexes to functionally differing homologous DNA fractions has been studied. The effectiveness of receptor binding to the transcriptionally active DNA, isolated from rat liver 4 h after cortisol administration, increased by 1.6–1.8-fold with simultaneous reduction in receptor affinity for the hardly extractable DNA fraction. The analogous DNA fractions from control animals did not differ in ability to bind the receptor. This suggests that sites of high affinity for the receptor are directly involved in the in vivo process of hormonal transcription activation.

Glucocorticoid receptor *DNA binding* *Cortisol treatment* *Rat liver* *Transcription regulation*

1. INTRODUCTION

The DNA-binding properties of steroid hormone receptor are now well-established [1]. A study of the interaction of highly purified rat liver glucocorticoid–receptor complexes with DNA and different polynucleotides has led us to the conclusion that, in addition to the sites of non-specific binding ($K_d \sim 10^{-3}$–10^{-4} M), mammalian DNA contains a relatively short sequence whose affinity for receptor proteins is higher by several orders of magnitude [2,3]. Recent studies showed preferential binding of purified glucocorticoid receptor to cloned MMTV DNA fragments, whose transcription is regulated by glucocorticoids [4–7]. Similarly, high-affinity sites for purified progesterone receptor were revealed within hormone-responsive genes [8–10]. Thus, the specificity of transcription modulation by receptors may be due, at least in part, to the recognition of a limited set of DNA sequences.

The aim of our investigation was to examine the distribution of hypothetical specific receptor binding sites within the functionally different homologous DNA fractions in intact as well as hormone-treated animals. The in vitro receptor–DNA binding was performed by an equilibrium competitive procedure with an excess of DNA–cellulose [2,3]. A transcriptionally active DNA fraction isolated from liver of cortisol-treated rats showed strongly increased affinity for the glucocorticoid receptor. This indicates that (i) numerical DNA sites with high affinity for glucocorticoid–receptor complexes are present in the rat genome and (ii) these sites are involved in the process of the transcription activation by glucocorticoids in vivo.

2. EXPERIMENTAL

Tritium-labeled glucocorticoid–receptor complexes were prepared by incubation of rat liver cytosol (100 000 × *g*, supernatant) with [^{3}H]triamcinolone acetonide (26 Ci/mmol, Amersham) [11,12]. For receptor purification, a two-step

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DNA–cellulose binding procedure alternating with ammonium sulphate precipitation was employed [11,12]. This purification procedure allows us to obtain about 2000-fold purified receptor quickly, and in a small volume.

The DNA fractions from rat liver were obtained by a modified [13,14] salt-phenol treatment procedure of intact liver cells [15]. DNA fraction I was extracted by a mixture of 1 M NaCl, 75% phenol (pH 6.4); DNA fraction II was subsequently extracted by a mixture of 0.14 M NaCl, 66% phenol (pH 8.5) and finally for DNA fraction III extraction, treatment of the interphase between phenol and water with pronase and SDS was needed [13,14]. The quantity of DNA fraction I was highly variable and correlated with the amount of active chromatin in the nucleus [15,16]. By specific hybridization tests, fraction I was shown to contain 15–20-times as much transcribed DNA sequences as DNA of other fractions [16,17]. This transcriptionally active DNA fraction I comprises about 20% of the total liver DNA. DNA fraction II represents the bulk (≥70%) of DNA and seems to be transcriptionally inactive. DNA fraction III amounts to about 10% of the genome, possesses low transcription capacity and is obviously associated with nuclear membrane components [13,14]. The purified DNA fractions were fragmented ultrasonically to pieces of an average length of 250–300 bp. These DNA fragments were additionally purified by hydroxyapatite chromatography and CTAB precipitation [18], and redissolved in a solution of 20 mM Tris–HCl (pH 8.1) and 1.5 mM EDTA. The base composition and main physico-chemical properties of the DNA fractions obtained were very similar. The binding of purified [^{3}H]triamcinolone–receptor complexes to DNA fractions was estimated by DNA–cellulose equilibrium competition experiments, as in [2,3]. The hydroxyapatite adsorption technique was also employed to determine the degree of decomposition of the hormone–receptor complexes [2,3]. Radioactivity was measured in a Mark III liquid scintillation spectrometer (Searle) with a counting efficiency of about 33%.

3. RESULTS AND DISCUSSION

With the purification procedure employed [11,12] one can obtain 2000-fold purified undegraded receptor. A 5–20% sucrose gradient sedimentation of the purified receptor in the presence of 0.4 M NaCl revealed a single narrow radioactivity zone migrating at about 4 S [11]. The purified [^{3}H]receptor yields a single peak when analyzed by gel filtration on Sephadex G-150 (fig.1) or DEAE–cellulose chromatography (not shown). This agrees well with the known characteristics of rat liver glucocorticoid receptor [19–22]. However, the purified glucocorticoid–receptor complexes were very unstable [4,11]: the radioactivity profile after gel-filtration (fig.1) or sucrose gradient sedimentation [11] revealed a substantial quantity of the released hormone.

Moreover, glucocorticoid–receptor complex decay was shown to be not entirely stochastic [3]. So, correction for the complex decay provides more accurate determination of their amount (%) bound to DNA–cellulose which is important for calculation of the relative affinity constant [2,3]. Besides, the accounting for hormone-receptor stability in each sample substantially reduces the role of occasional factors and thus decreases the deviation of the values obtained.

The affinity of the receptor for DNA fractions was measured by an equilibrium competitive assay procedure [2,3], which is sensitive enough to detect small differences in binding effectiveness. The

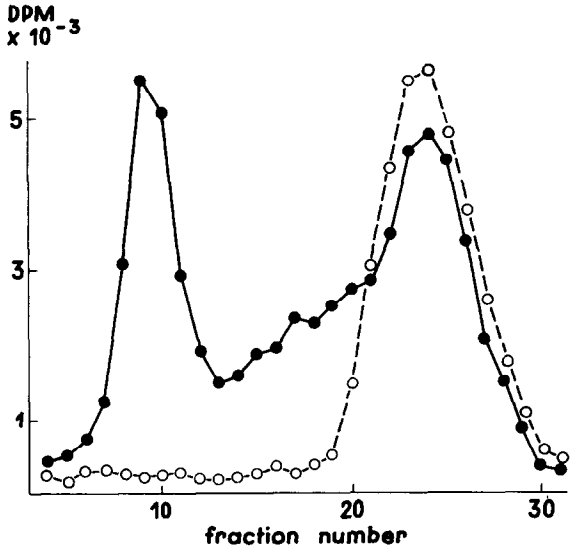

Fig.1. Sephadex G-150 column chromatography of purified rat liver. [^{3}H]Triamcinolone-receptor complexes (●) and free [^{3}H]triamcinolone acetonide (○).

advantage of this approach is that the receptor need not be as highly purified as for electron microscopy or nitrocellulose filter adsorption assay [4–6,8,10]. Our modification of the competition assay procedure with the use of a DNA–cellulose excess permits competition curves to be linearized in a graph of DPM_o/DPM_i and R_i (fig.2), where DPM_o is receptor radioactivity bound to DNA–cellulose in the absence of competitor, and DPM_i receptor radioactivity bound to DNA–cellulose at a given molar ratio R_i of free (competitive) and cellulose-coupled nucleic acids. From the slope of the straight lines one can readily obtain the relative equilibrium binding constant (α) for the nucleic acid–receptor interaction [2,3].

Fig.2 demonstrates the competition of functionally different fractions of rat liver DNA plotted in the traditional as well as the proposed manner.

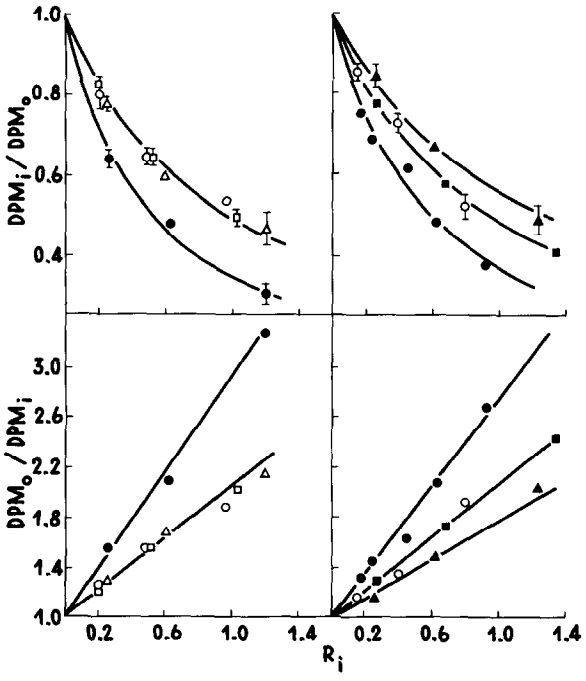

Fig.2. Competition of rat liver DNA fractions and DNA–cellulose for purified glucocorticoid receptor binding. The competition of DNA fraction I (○,●), fraction II (□,■), and fraction III (△,▲) of control (open symbols) and cortisol-treated (solid symbols) rats is presented in the traditional (upper) as well as in the linearized (lower) manner (for details see text and [3]). The right and left graphs represent different series of experiments.

The liver DNA fractions were obtained by sequential salt-phenol extraction of rat liver cells (see section 2). Three main fractions of high-M_r DNA were isolated by this method: fraction I, enriched by transcriptionally active DNA sequences; fraction II, representing the bulk of cellular DNA; and fraction III, apparently associated with some membrane components [13–17].

Fig.2 (left) shows that all 3 DNA fractions, isolated from untreated rats, compete to a similar extent for receptor binding with DNA–cellulose. In contrast, transcriptionally active fraction I, isolated from cortisol-treated animals, is 1.6–1.8-fold more effective as a competitor than the other DNA fractions ($p < 0.01$). This means that cortisol-responsive genome loci are enriched in specific binding sites for immediate inducer, i.e., glucocorticoid–receptor complexes. Since glucocorticoid-induced RNA synthesis in liver proceeds without any DNA template amplification [18], specific receptor-binding sites should translocate to fraction I from DNA of other fractions, i.e., most of all for fraction III, whose binding capacity is reduced after hormone administration (fig.2, right). These results are in agreement with the observed hormone-induced transfer of some middle repetitive DNA sequences from fraction III to fraction I [14,16]. RNA/DNA hybridization experiments also demonstrated the relative increase in intensity of the repeated DNA sequence transcription after cortisol administration [23]. So, it is reasonable to assume that the rat genome contains multiple high-affinity binding sites for glucocorticoid–receptor complexes. These sites are localized more or less uniformly in the DNA fractions of control rats (fig.2), perhaps close to moderately repeated DNA sequences. Under cortisol treatment, the receptor-binding sites are found mostly in an actively transcribed DNA/chromatin fraction, whose affinity for glucocorticoid–receptor complexes is therefore enhanced (fig.2). This is obviously a result of some changes in chromatin organization under the in vivo hormone action, primarily involving high-affinity receptor-binding sites and surrounding DNA sequences, including hormone-responsive structural genes. Thus, the experimental data here are to be considered as a direct indication of the involvement of specific receptor-binding DNA sites in the in vivo process of hormone-induced gene activation.

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